DISSERTATION

IMPACTS OF BLACK SOLDIER FLY LARVAL FRASS ON VEGETABLE CROP PRODUCTION

Submitted by
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ABSTRACT

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The "insects as food and feed" movement is gaining considerable momentum as a novel way to provide protein to human and animal diets. Insects require significantly fewer resources, such as water and land, to produce, process and distribute as a food source. Food, service, and restaurant partners often donate their waste to insect producers. This converts landfill destined waste into high protein food sources. The left-over waste product from mass rearing insects, known as frass, creates a problem for insect producers. However, across the food system numerous industries are involved, and this research examines how waste from the hospitality and insect industries can be utilized in vegetable crop production. Peat is the most common medium for plant growth in greenhouse and gardening operations; however, peat extraction has severe environmental consequences for marsh ecosystems, surrounding environments, and climate change. Partially replacing peat with insect frass could reduce industrial waste from insect and food producers and decrease peat consumption. Greenhouse studies were designed to investigate the use of frass in vegetable production. In pot studies with arugula, lettuce, and tomato different ratios of peat and vermicompost and peat and insect frass were compared to a 100% peat control. Arugula and lettuce grown in distillery grain frass, all BSFL treatments were equal or better in yield than the 100% peat control, regardless of season. For brewery grain frass, most treatments in arugula and lettuce were worse or comparable to the peat control in yield, regardless of season. The diet of the larvae (distillery grain vs. brewery grain) was a significant factor in determining the impacts on vegetable yield. Arugula and lettuce leaf tissues were analyzed for nutrient concentrations. Primary macronutrient (NPK) concentrations were higher in frass treatments for both crops. Secondary macronutrients and micronutrients did not show clear trends on the effect of distillery or brewery grain frass. Tomatoes did not produce any significant differences across insect frass treatments, though average individual fruit weight was significantly higher in low percentages of vermicompost. Emergence, germination, and seedling vigor of arugula, lettuce, and tomato seeds were negatively impacted by brewery grain frass in the absence of vermicompost. In the presence of vermicompost, low concentrations of insect frass in a germination mixture produced comparable results to a 100% peat control. There are many components of insect frass yet to be fully explored, such as the impact on cation exchange capacity and microbial activity. More research to understand the physical, chemical, and microbial components of the medium will be essential in pushing the field forward, improving the material as an amendment, and closing gaps in the cycle of production.

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CHAPTER ONE

THE SUSTAINABILITY OF INDUSTRIAL INSECT MASS REARING FOR FOOD AND FEED PRODUCTION: ZERO WASTE GOALS THROUGH BY-PRODUCT UTILIZATION

1. Introduction

Waste streams are the left-over substrates from production systems. Food waste is a growing problem and can be mitigated by reducing, reusing, and recycling materials at various levels of the food chain (Ojha *et al.,* 2020). One example of waste treatment in the industry includes incineration, but this is known to have detrimental environmental effects. The bioconversion of food waste is one of the most notable benefits of insect production systems (Salomone *et al.,* 2017). Reallocating waste from the food industry as a feed for larvae better supports a circular system. For example, 900 kg of food waste requires 0.06 kg of black soldier fly, *Hermetia illucens,* Linneaus (Dipera: Stratiomyidae) larvae to digest the material, resulting in 60 kg of frass and 9.57 kg of black soldier fly (BSF) larvae, representing a 92.27% conversion (Pleissner and Smetana, 2020). This varies by operation, for example, from 1000 kg of food waste, other insect production plants may produce 335 kg of frass and 30 kg of larvae, producing at 63.50% conversion (*Salomone et al.,* 2017). The prepupae are a valuable source of protein and are the primary output for insect farmers. Livestock manure and municipal biowaste (*i.e.,* human waste) produce higher protein larvae than those larvae that are reared on food waste. [1]However, the economic value of the insects and the feed stocks they may consume vary based on the regulations from one country to another (Smetana *et al.*, 2016). Insect production is a simpler

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[1] Chavez, M. 2021. "The sustainability of industrial insect mass rearing for food and feed production: zero waste goals through by-product utilization". *Current Opinion in Insect Science* 48: 44-49.

process than other methods of bioconversion, such as algal operations, and can be more cost effective as well (Pleissner and Smetana, 2020).

Insect production for food and feed is a growing industry. The movement is gaining a considerable amount of momentum as a novel way to provide protein for human and animal consumption (Van Huis, 2020). Mass rearing of insects as a protein source to augment other forms of livestock has many notable benefits (Jantzen da Silva Lucas *et al.,* 2020). Insects require considerably fewer resources, such as land and water, to produce, process, and distribute as a food source (Ojha *et al.,* 2020; Salomone *et al*., 2017). BSF larvae are especially ideal as livestock feed because of their high protein content (Miranda *et al*., 2019). Finding ways to utilize the waste stream from the insect industry can further increase its sustainability and profitability. Like any successful venture, this growth has caveats. Diversion of the left-over substrates and by-products from industrial insect mass rearing is an area of concern for producers.

A major goal of many insect producers is to sustain a zero-waste system. Zero waste efforts emphasize reusing and recycling as many byproducts as possible, sending essentially no waste to landfills (Romano *et al*., 2019). Insect larvae can reduce manure dry matter up to 50%, indicating significant digestive capabilities (Miranda *et al*., 2019). However, even small companies, such as EVO Conversion Systems, can produce up to 11,000 kilograms of waste in one year (JA Cammack, personal communication). At a larger scale, such as the country of China, the estimated live larvae production per day is around 250 tons, and considering conversion rate of 20%, these accounted for 1,250 tons of waste being eaten by BSFL per day (F Yang, personal communication). The left-over substrates and by-products (depending on the operation) are typically composed of frass, chitin, and lipids. This article aims to synthesize and analyze the practice and application of these products while considering management for zero waste goals.

2. Life cycle assessments

For propriety reasons, very few companies are willing or able to share their data, but several life cycle assessments have been conducted to investigate insect production systems. These life cycle assessments are generated to provide insight into the general process and the potential impacts of the industry being observed (Smetana *et al*., 2016). Insect production is an important alternative protein source for feed stock because it requires significantly less land than other livestock production (Salomone *et al.,* 2017; Chia *et al*., 2020). Similar to food waste, the accumulation of livestock manure has associated problems that can be addressed by insect bioconversion (Chavez and Uchanski, 2021; DiGiacomo and Leury, 2019). However, some studies show increases in energy use during the larval drying process and increased greenhouse gas emissions during transportation when compared to other protein alternatives, such as rapeseed and soy (Salomone *et al*., 2017). Yet, when compared to conventional livestock production, most studies show lower greenhouse gas emissions and energy use in insect production (Ojha *et al.,* 2020).

The production of protein through mass insect rearing also presents the potential to improve nutrient cycles in industrial systems (Chia *et al*., 2019). For example, during mass production BSF larvae are reared on an existing waste stream, such as spent brewery grains waste. When waste streams accumulate, they have the potential to create negative environmental consequences such as leaching into surrounding environments and emitting greenhouse gases (Salomone *et al*., 2017). Reducing waste streams through bioconversion decreases the potential of these negative impacts (Bosch *et al*., 2019). This redirection of nutrients complies with circular economy principles, promoting a closed loop system (Ojha *et al*., 2020). To create a truly closed loop system insect rearing by-products should be reused as well. The incorporation of insect left-over substrates as a nitrogen fertilizer replacement is one of the major contributing factors to improved environmental impacts as a result of mass insect production (Salomone *et al*., 2017).

3. Frass

Many producers currently donate or sell their frass to local crop growers and farmers (Ojha *et al*., 2020). Frass is the left-over substrate or excrement from insects during the rearing process. Insect production provides an opportunity to create and utilize biofertilizers that are similar to composts often used in conventional production (Chavez and Uchanski, 2021). Compost application has been shown to improve soil fertility. The added organic matter can improve overall soil health and enhance beneficial microbial populations. Composts contain significant and balanced nitrogen, phosphorus, and potassium concentrations (Swiader and Ware, 2002). These are the three macro nutrients required for plant growth, potentially reducing the need for inorganic fertilizer inputs, while still maintaining maximum yields. Insect biofertilizers are also of interest for growers concerned with preventing eutrophication and soil degradation (Xiao *et al*., 2019). Bioconverted by-products from insect production have a fairly balanced and complete contribution of nitrogen (1.49%), phosphorus (0.98%), and potassium (1.03%). Additionally, they have low moisture content (~25%) and these dry products are more ideal for producers due to shipping costs (Salomone *et al*., 2017). However, nutrient concentrations and moisture content vary considerably by insect species and waste composition (Chavez and Uchanski, 2021).

BSF larvae frass may be a potential peat replacement in various crops. A study found that peat can be supplemented with 10% insect frass to provide comparable yields as a 100% peat substrate (Setti *et al.,* 2019). Maize grown with BSF larvae frass applications produced similar yields to organic fertilizer treatments tested in the study (Gärttling *et al*., 2020). Other studies producing maize with BSF frass applications observed higher yields than the standard inorganic fertilizer control (Beesigamukama *et al*., 2020). Shallot yields treated with bioconverted brewery waste by-products were significantly higher than those treated with conventional fertilizers. Bioconverted poultry manure also produced statistically comparable shallot yields to the conventional practices (Quilliam *et al*., 2021). Other studies with BSF larvae treatments observed improvements in lettuce productivity (Putra *et al*., 2017). Though these results are promising, the scalability of these systems as insect rearing expands remains unclear.

There has been evidence demonstrating mealworm (*Tenebrio molitor*) Linnaeus (Coleoptera: Tenebridae) frass may be important in mitigating environmental stress conditions. Under salinity, drought, and flooding stress, crops grown with frass fertilizer amendments produced yields similar to conventionally fertilized crops (Poveda *et al*., 2019). Mealworm frass has also produced similar promising results with barley (Houben *et al*., 2020). Additionally, evidence of plant disease and pathogen resistance have been observed in frass application experiments (Klammsteiner *et al*., 2019; Choi *et al*., 2019). Gut isolated bacteria from BSF larvae supplemented into the insect biofertilizers may improve the frass as a soil amendment in crop production (Xiao *et al*., 2019). Chinese cabbage germination increased with the gut bacteria supplements, and also saw larger reductions in manure biomass and moisture and increases in reduction rate, producing a more ideal compost composition for farmers (Xiao *et al.,* 2019).

Though we understand there are positive benefits of frass and chitin applications, the mechanism is yet to be fully explored and understood.

A new area of research for frass is evaluating the suitability of frass as a protein supplement for cattle consuming low-quality forage. So far, preliminary results show that frass has the potential to become a protein source when compared to conventional cottonseed meal (S Maggitt, personal communication).

4. Chitin

Chitin is found in the exoskeletons of insects and other arthropods. During growth, exoskeletons are shed and left in the insect's digestive residues, also known as frass (Sharp, 2013). Chitin sourced from shrimp production has shown improvements in crop production (Fatima *et al*., 2018; Xu *et al*., 2018; Spiegel *et al*., 1986). Since shrimp and insect waste have similar percentages of chitin, it is likely insect waste could have similar positive effects (Chavez and Uchanski, 2021). Insect samples range from 2-36% chitin, while shrimp are 5-32% (Hahn *et al*., 2020). Chitin may also improve pathogen and disease resistance in crop plants as well. Yields in chitin treated crops are higher when inoculated with pathogens than those that are not treated with chitin (Spiegel *et al*., 1986; Bell *et al*., 1998; Sid Ahmed *et al*., 2003). From what we understand about chitin, it is likely that it is the substance driving this process in the frass studies as well (see previous section).

Chitin is difficult to separate from frass and the benefits of doing so remain unclear, with one exception. Purely isolated chitin and chitin derivatives may have human medicinal properties. Chitosan is the ideal derivative due to its water-soluble properties (Hahn *et al*., 2020). Antimicrobial and antioxidant properties of marine crustacean derived chitin have been successfully used to treat various ailments such as cancer and rheumatoid arthritis (Zheng *et al*., 2012). Research indicates that these results are conducive with chitin found in fungal species as well. Similar to shrimp and insects, fungal chitin ranges from 2-42% depending on the species (Zheng *et al*., 2012). Chitin-glucan complex, found in fungal cell walls, has shown to have antioxidant, anti-inflammatory, antibacterial, and wound healing properties (Hong *et al*., 2019). Hydrolysis of this complex can result in chitin and chitosan derivatives, which have been shown to exhibit antioxidant, anti-tumor, and anti-inflammatory properties as well (Hong *et al*., 2019). Extraction of chitin from marine crustaceous waste is not environmentally friendly due to the resulting emissions (Zheng *et al*., 2012). Unfortunately, it is likely chitin extracted from insect exoskeletons would encounter similar issues. Other methods of chitin purification and chitosan derivation are underway to improve this process (Zheng *et al*., 2012).

5. Lipids

Once larvae have matured, they are harvested as protein rich products. Second to protein, lipids are the largest compound present in insect bodies and are a valuable by-product from insect production (Jantzen da Silva Lucas *et al*., 2020). Depending on the species, this can be 10-50% of their dry matter (Hong *et al*., 2019). A recent exploration for biodiesel production utilizes the by-products from insect production. Biodiesels are fuels produced from natural, organic (carbon based) substrates. For example, a common biodiesel is made from soybean oil. Motivations to improve biodiesel production and innovation has increased with awareness around renewable, clean energies (Xiaoming *et al*., 2010). Lignocellulosic substates are often found in agricultural waste streams, such as crop residues, and are difficult to break down (Olofsson *et al*., 2017). However, insects are capable of utilizing these substances as nutrition and bioconverting them into more ideal biodiesel products during the digestive process (Jonathan *et al*., 2010) The use of insect waste-based biodiesels can reduce environmental pollution by redirecting waste streams and reducing emissions found in other systems (Zheng *et al*., 2012).

Fatty acids can be extracted from BSF larvae tissues and converted into biodiesel through esterification and transesterification. Studies have shown the oil derived from BSF larvae has a fatty acid profile suitable for producing high quality biodiesels (Surendra *et al*., 2016). Specifically, BSF larvae have comparable fuel properties to rape seed oil, meeting standards for European production (Li *et al*., 2011). For example, studies have shown that BSF larvae raised on restaurant waste serve as a potential biodiesel system (Xiaoming *et al*., 2010). However, insects can be reared on a variety of waste to achieve these profile goals and different diets influence the composition of their body fat chemistry (Pinzi *et al*., 2014). Thus, the biodiesel yield depends on which waste material the insects are reared (Li *et al*., 2011), which is likely due to the insects fatty acid profile.

Additionally, using insects to bioconvert agricultural waste products into biodiesel is a promising endeavor. For example, rice straw is a lignin heavy waste residue and when accumulated is problematic for many areas of the world (Manurung *et al*., 2016). Additionally, some countries cannot afford to produce crop-based biodiesels and insect left-over substrates provide a cheaper alternative, but increasing efficiencies is an ongoing process (Li *et al*., 2011). Methods to improve insect-based biodiesel products have been investigated. Similar to biofertilizers, additional microbes can be added to the digestive process of insect rearing to improve the properties for biodiesel production. Rid-X is the current commercial commodity available with microbial properties for these improvements (Xiaoming *et al*., 2010). Multi-insect biorefineries utilize two different insect species, such as BSF larvae and mealworms, at different steps in the process. This eliminates the need for harmful chemical pretreatments that are used in single insect refineries (Wang *et al*., 2017). Therefore, this practice produces adequate conversion rates and yields and can also promote environmentally friendly practices.

Insect sourced lipids provide potential for other research and application as well. Fatty acids present in insect larvae are oils such palmitic and oleic acids (Ekpo *et al*., 2009). Oleic acids are known to have mammalian immunity benefits. These additional nutrients have been successfully applied to the diets of immunocompromised and chronically ill patients to improve their health (Pontes-Arruda, 2009). Some farmers are adding lipids to their livestock's diets to improve their overall health. Post-partum cows with fatty acid supplemented diets saw an increase in milk fat, protein, and lactose yields, as well as an increase in body condition of the mother (de Souza *et al*., 2020). Medicinal application for lipids and lipid derivatives is a promising avenue of research for insect by-products.

6. Conclusions

The research presented above provides many promising avenues for left-over substrate redirection, direct utilization, or extraction. The overall generation of food waste involves agriculture, processing, and consumption at many faceted levels. Incorporating methods for reduction and redirection are necessary as the waste continues to increase; currently 50% of production is wasted, and the emissions from the process continue to rise (Ites *et al.,* 2020). Not only can insect producers maintain a near zero waste system, but they could also potentially profit from their own waste streams. From current research we understand that the efficiency of composting practices relies heavily on the type of waste that is incorporated (Ites *et al.,* 2020). So further research optimizing waste products for bioconversion will be needed to better appreciate this process. Most of our understanding about the potential benefits from insect by-products comes from research in other animals. Moving forward more research will need to focus on the properties of insect specific chitin and lipids, especially in measuring their impacts on immunity and disease resistance. Though insect protein is often more sustainable than other sources of animal protein, it has some environmental consequences not seen in plant-based proteins, such as an increase in carbon dioxide emissions and electrical usage. However, there is a decrease in land use from insect production that will be especially important as populations continue to grow rapidly (Salomone *et al*., 2017; Smetana *et al*., 2019). Questions that remain involve completing supply chains in a real-world setting and scaling up as insect rearing increases. New approaches to provide direction for insect producers in waste redirection and resource utilization will improve the overall impact profile of their operations (Smetana *et al*., 2019).

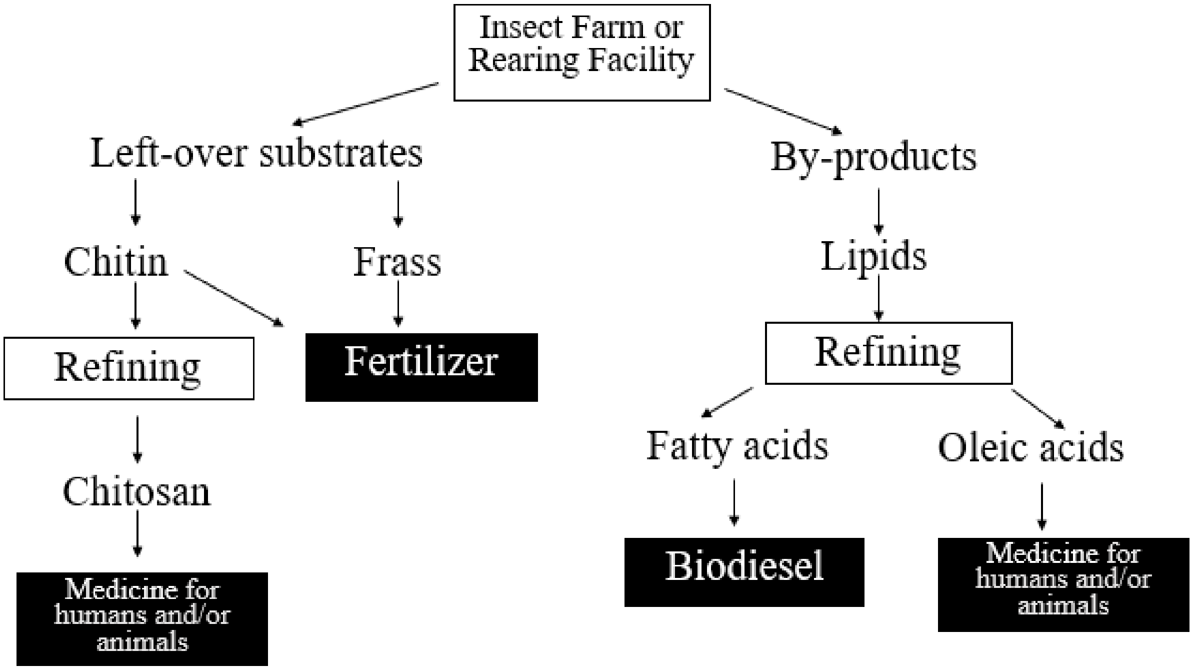

Figure 1.1: The utilization of left-over substrate and by products of insect mass rearing: frass, chitin, and lipids. White boxes outlined in black are facilities. White unlined boxes are products. Black filled boxes are final applications

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CHAPTER TWO

INSECT LEFT OVER SUBSTRATE AS PLANT FERTILIZER [2]

1. Introduction

Entomophagy is defined as the consumption of insect protein for human and animal nutrition (Table 2.1). The practice of eating insects is a well-known tradition in many areas of the world and is beginning to become more common in the United States (Durst *et al.,* 2010). Currently the main source of protein in the modern American diet comes from livestock, such as cattle, poultry, and swine. Animal agriculture is responsible for the largest extractions and loss of water in the environment, occupies large amounts of land, and is one of the highest producers of atmospheric methane, which is a potent greenhouse gas (Sims *et al.,* 2005). Additionally, accumulated manure may result in environmental degradation, known as eutrophication, when bacteria and nutrients runoff into surrounding streams and lakes. Infiltration into the groundwater from these same sources can also occur, causing complications for the industry (Gay and Knowlton, 2005). Insects can provide an alternative protein source that has the potential to improve consumer health and reduce the negative impacts on the environment compared to other sources of fertility (Chia *et al.,* 2019).

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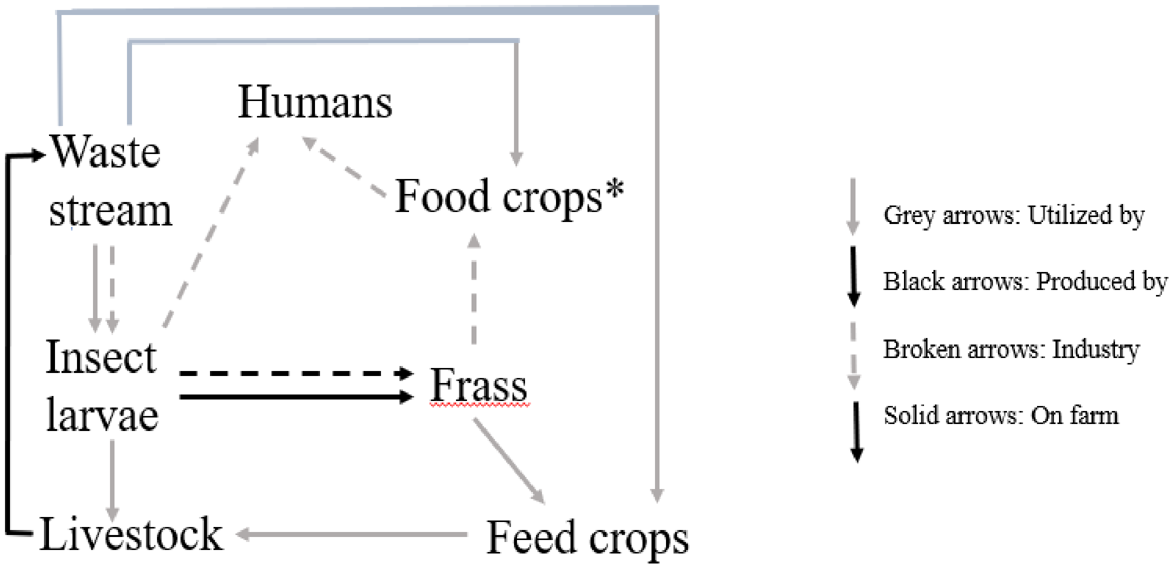

Figure 2.1: Production and Entomophagy: Rearing insects for consumption On the farm, farmers feed manure to insect larvae to reduce manure accumulation. The insects are then harvested at larval stage for livestock (poultry, cattle, swine, etc..). The frass produced by the larvae is used to fertilize agronomic crops that are then fed to the livestock. During industrial insect rearing procedures, waste streams, such as livestock manure, are brought to the facility and consumed by larvae. The insect larvae are harvested alive and sent to farmers and other consumers in need. Insects may also be produced in a similar way for human consumption. The rearing process also results in left over frass. Traditionally raw manures are used to fertilize crops. *The frass produced by industrial insect production may be useful as an amendment in horticultural food crop production. Frass contains chitin, which has additional fertility and pathogen resistance properties.

Though the entomophagy industry provides a possible solution to many agricultural problems, it has also resulted in a few of its own. One of particular concern is the waste created during the process of insect rearing. Currently, this digested product does not have any widely adopted practices of application. As a result, the digestive residue, also known as frass or digestate, is mainly discarded as a waste product. Due to its concentration of nitrogen and phosphorus, this product has the potential to utilize existing waste streams to create a fertility amendment for food crop production (Schmitt, 2020). These additional nutrients from frass may serve as a supplement or substitute for the inorganic fertilizers that are applied during typical crop production (Figure 1). Decreases in inorganic fertilizer use can reduce the subsequent environmental problems, such as eutrophication.

Table 2.1: Definition of key terms

Term	**Definition**	**Citation**
Bioconversion	Digestion of organic material into a more enriched material via animal intake and excrement.	Putra *et al.,* 2017
Biofertilizer	Bioconverted waste material via insect digestion.	Xiao *et al.,* 2018
Chitin	Polysaccharide found in insect exoskeletons.	Sharp, 2013
Decomposition	Modification of detritus in the soil.	Chapin *et al.,* 2011
Digestate	Type of frass produced during insect production, often black soldier flies.	Temple *et al.,* 2013
Entomophagy	The practice of eating insects, especially by people.	Durst *et al.,* 2010
Frass	Insect excrement (solid).	Kagata and Ohgushi, 2012a
Fertility amendment	Additional supplement to provide nutrients for crops.	Schmitt, 2020
Herbivory	Consumption of plant material by animals.	Chapin *et al.,* 2011

Crops require a combination of resources for survival such as carbon dioxide, mineral nutrients, heat, and light (Swiader and Ware, 2002). The most important mineral nutrients include nitrogen, phosphorus, and potassium. These are abundant on earth in their elemental forms but must be present in bioavailable forms in order to be absorbed and utilized during the vegetative growth of plants. Unfortunately, bioavailable nutrients are considerably more limited and sparser within our biosphere. Because of this cultivated food crop production requires special soil fertility management in order to meet nutritional needs (Swiader and Ware, 2002).

There are several large challenges with the conventional system of fertility management. Many of the resources required are limited and non-renewable. For example, phosphorus is mined in several areas throughout the world for inorganic liquid fertigation. However, these sources are being depleted at extremely high rates, complicating the likelihood that our current reserves can sustain future production (Koppelaar and Weikard, 2012). Peat is also in high demand by agricultural producers and is found only in limited and sensitive wetland areas. Mining peatlands causes dramatic losses in biodiversity and cultural services. Additionally, drained peatlands are large producers of greenhouse gas emissions (Couwenberg *et al.,* 2011). Others concerns for agricultural production include nutrient infiltration and runoff into surrounding landscapes. This occurs because farmers apply nutrients in higher dosages than is actually accessible to the plant (Chapin *et al.,* 2012). When nutrients are not absorbed by the plant, they leach into the soil or across land surfaces and are carried into the surrounding environments. The nutrient pollution to the system increases algae and plant growth and decreases oxygen levels. This results in environmental degradation, known as eutrophication, and leads to large decreases in biodiversity (Chapin *et al.,* 2012). Though nitrogen is the most common problem due to its frequent over application in fertilizer regimes, phosphorus run-off is a large detriment to natural systems as well (Eghball and Gilley, 1999). Phosphorus run-off increases with soil erosion, a problem that is common on agricultural land. (Daverede *et al.,* 2004).

Additionally, ammonia emissions from wet manure and compost applications to production fields have considerable impacts on the environment (Fuchs *et al.,* 2008). Volatized ammonia can travel large distances and be deposited as nitrogen pollution in surrounding lakes and oceans, escalating eutrophication in those systems. It can also be deposited in soil increasing acidity and reducing plant growth (Gay and Knowlton, 2005). All of these reasons provide further justification for the increasing demand and interest in alternative amendments for crop fertility, or fertility amendments (Table 2.1).

Frass is solid insect waste material that has been converted to a microbially rich substance through digestion. This results in a product higher in organic matter and lower in nitrogen and phosphorus content than its' parent source (Kagata and Ohgushi, 2012a). Once digested, nutrients such as nitrogen, phosphorus, and potassium become more bioavailable for plants (Table 2.1); Total nutrients are reduced and the leaching of these nutrients outside of the system decrease as well. The ratio of nitrogen to phosphorus decreases after biological digestion, or bioconversion (Table 2.1) and provides a more balanced product for plants than the traditional amendments, since most composts are very high in nitrogen and low in phosphorus (Swiader and Ware, 2002).

Chitin is the predominant substance in the shed exoskeletons of insects and is often found mixed in their left over substrate, such as waste material and digestate. It naturally occurs in large abundances, making it ideal for application in resource-intensive crop production. As such, over the past few decades, chitin has gained interest as a potential biofertilizer, and is mostly sourced from salt water crustacean exoskeletons (Sharp, 2013). For example, shrimp waste that is high in nitrogen and phosphorus, also contains abundant chitin (Fatima *et al.,* 2018).

Studies have demonstrated that crop production increases in the presence of chitin. Lettuce leaf number, leaf area per plant, leaf dry weight, leaf fresh weight, and chlorophyll index increased in the presence of chitosan applications that were 0.05%, 0.10%, 0.15%, and 0.02% of soil mixtures (Xu and Mou, 2018). Another study by Spiegel *et al.,* (1987) observed that chitin applications increased fresh root weight of bean, corn and tomato plants. However, more observations in economic yield improvement will be important for this field going forward. Chitin may also improve pathogen and disease resistance in plants. A study by Bell *et al.,* (1998) observed that 3 mg/ml of chitin soil applications reduced both incidence and severity of fusarium yellows (*Fusarium oxysporum*) in celery petioles, while celery with chitosan root dips experienced less severe incidences of yellows. Chitin applications in 0.5% of soil mixtures have been shown to reduce incidence of the root-knot nematode, *Meloidogyne fava*, in bean, corn, and tomato plants (Spiegel *et al.,* 1987). Chitin can enhance the antifungal action of bacterial isolates in root rot disease (Ahmed *et al*., 2003). Chitin that has been deproteinized and demineralized form shrimp samples ranges from 5-32% of their dry weight. Chitin from insect samples range from 2-36% (Hahn *et al*., 2020). Since shrimp chitin has proved to produce plant growth changes related to fertility and pathogen protection, the similarities in the chitin concentration of insects reared for entomophagy may drive similar processes as well.

Insects contribute to nutrient diversion through decomposition and herbivory (Table 2.1) (Chapin *et al.,* 2011). Though ninety-five percent of decomposition occurs by microbes, such as bacteria and fungi, insects and other arthropods can also influence decomposition in several different ways, both directly and indirectly. Insects can also influence the chemical alteration stage of decomposition. Certain species act directly as decomposers by eating dead organic matter that is coated in bacteria. Insects ingest the bacterial coated material and recycle the organic matter as a waste product that is higher in carbon and nitrogen (Chapin *et al.,* 2011). Decomposers may also have specialized mechanisms for consuming and processing plant material. This can include microbial populations in their gut to digest recalcitrant lignin and cellulose compounds (Kagata and Ohgushi, 2012a).

Herbivory can help increase decomposition rates (Chapman *et al.,* 2003). Specifically, insect herbivores contribute to decomposition by consuming live plant material and converting it into frass. Frass is rich in bioavailable compounds that are beneficial to plants and other smaller decomposers. Additionally, this process may injure leaves that are still attached to the stem, forcing early abscission of damaged leaves and their deposition on the soil floor (Chapin *et al.,* 2011). Frass has been shown to decompose more quickly than leaf litter resulting in large releases of total nitrogen (Kagata and Ohgushi, 2012b). The impact of insect solid waste excrement (frass) on soil nutrient dynamics has been well studied over the course of the past few decades. Frost and Hunter (2004) analyzed how frass deposition influenced soil carbon and nitrogen. Northern red oak (*Quercus rubra)* Linnaeus (Fagales: Fagaceae) plants were used to create an artificial system to observe the herbivory of the eastern tent caterpillar (*Malacosoma americanum*) Fabricius (Lepidoptera: Lasiocampidae). Herbivory of *Q. rubra* decreased soil and nitrogen in the soil, while additions of *M. americanum* frass increased inorganic soil nitrogen and carbon.

Insect herbivore diet selection can affect litter quality and subsequently nutrient cycling. Changes in nutrient cycling alter composition of plant species and communities. Therefore, nitrogen availability and plant abundance increase with the presence of insects, such as grasshoppers (Belovsky and Slade, 2000). Entomophagy seeks to produce insect-based protein and take advantage of these processes to reduce environmental impacts for production.

2. Entomophagy

Entomophagy is the animal consumption of insects (Durst *et al.,* 2010). A variety of insects are grown for the purpose of animal consumption, and each has their own benefits and challenges. Insect production requires minimal land, supports a closed nutrient cycle, benefits a more circular economy, and provides alternative sources for feed stock (Figure 2.1) (Chia *et al.,* 2019). We will briefly examine a few insects that are of interest for frass utilization.

Black soldier fly larvae (*Hermetia illucens*), Linnaeus (Dipera: Stratiomyidae), have become a species of particular interest for research in the past few decades. Black soldier fly larvae are efficient in reducing and metabolizing waste, such as animal manure. Studies have shown they can reduce manure dry matter by 30 to 50 percent (Miranda *et al.,* 2019) and metabolize approximately 17.6 to 32.5 percent of poultry waste fed (Diener *et al.,* 2009). Additionally, the protein and fat content of the larvae is high and depends on the insects' diet (Table 2.2) (Miranda *et al.,* 2019). They are a valuable resource to livestock farmers who accumulate considerable amounts of wet manure. Wet manure is problematic for farmers as it is expensive and labor intensive to manage.

Farmers can use black soldier flies to reduce accumulated manure moisture and dry matter, while also harvesting the larvae themselves as a protein rich food source for other livestock, such as poultry. Black soldier fly larvae are also known to provide additional benefits such as reducing the oviposition of house flies (Bradley and Sheppard, 1984), *E. coli* populations (Liu *et al.,* 2008), and odor causing compounds in manure (Beskin *et al.,* 2018). Black soldier fly larvae are reared on already existing waste streams and can possibly decrease the ecological consequences of food production (Bosch *et al.,* 2019).

A less adopted decomposer of interest are beetle larvae, or mealworms, Linnaeus (Coleoptrea: Tenebridae). Mealworms are either fed to livestock, mostly poultry, or processed as a flour for human consumption. There are three species of edible meal worms: *Tenebrio molitor, Zophobas atratus,* and *Alphitobius diaperinus*. Conversion efficiency of feed by larvae depends on species and diet, ranging from 6.36 to 34.37 percent of dry matter (Van Broekhoven *et al.,* 2015). Like black soldier fly larvae, mealworms are also high in protein and fat (Table 2.2). Like black soldier fly larvae, mealworms also produce less greenhouse gas emissions and require less land than other animal livestock (Ooinix and Boer, 2012).

House flies (*Musca domestica*), Linneaus (Dipera: Muscidae), have similar fat and protein content to mealworms. They are typically acknowledged as a nuisance to livestock farmers, but efforts have been made to demonstrate that their larvae could be useful as livestock feed as well. House flies reared on gibberellin fermentation produce similar protein and fat concentrations to mealworms and black soldier fly larvae (Yang *et al.,* 2015b). Poultry waste bioconverted by house fly larvae have resulted in large reductions of moisture content (75 percent to 50 percent). The final products were also greatly reduced in biomass (80 percent) and become an odorless, granular substance (Boushy, 1991). Improvements in livestock body conditions have also been observed. Broiler chickens raised on diets supplemented with house fly larvae were significantly heavier than control groups that were not fed larvae (Hwangbo *et al.,* 2009).

Table 2.2: Protein and fat content of insect larvae reared for entomophagy

Insect	**Crude protein (%)**	**Crude fat (%)**	**Citation**
Hermetia illucens (Black soldier fly)	42-48	28	Miranda *et al.,* 2019
Tenebrio molitor (Mealworm)	47	25	Van Broekhoven *et al.,* 2015
Zophobas atratus (Mealworm)	40	38	Van Broekhoven *et al.,* 2015
Alphitobius diaperinu (Mealworm)	64	19	Van Broekhoven *et al.,* 2015
Musca domestica (Housefly)	56	22	Yang *et al.,* 2015

All three of these insects provide promise for altering the typical framework of protein production and introducing novel food sources into human and livestock diets.

3. Use of insect frass as fertilizer

Horticultural food production often demands large quantities of inorganic fertilizers to promote plant growth (Swiader and Ware, 2002). The value of insect production for protein sources is recognized, but what is less understood is how nutrient diversion during this process could be utilized as potential plant biofertilizer. The bioconversion of waste streams by insects in the entomophagy industry is an exciting opportunity for food crop production and biofertilizer utilization. Biofertilizers are an interesting fertility option in alternative agriculture and may provide a more sustainable option for farmers concerned with eutrophication and soil degradation (Xiao *et al.,* 2018). Waste from insect production could also potentially serve as a peat replacement substrate or as an additional amendment to a reduced inorganic fertilizer regime. A few studies have been conducted to investigate uses of insect frass for application in horticultural production.

The frass of various insects has been analyzed to determine their nutrient profile (Table 2.3). These profiles are useful as a comparison to conventional manures and composts. Manure that has not been composted tends to have the highest carbon, nitrogen, and phosphorus concentrations (Table 2.3). Insect digested manure and traditionally composted manure tend to have similar carbon, nitrogen, and phosphorus concentrations (Table 3). For example, a study by Zhang *et al.,* (2012) monitored the impacts of manure quality on bioconversion by houseflies. Manure treated with houseflies saw decreases in total nitrogen and phosphorus, while bioavailable nitrogen and phosphorus increased. Temple *et al.,* (2013) conducted an incubation trial to assess the nutrient profile and availability of black soldier fly larvae digestate compared to other organic amendments commonly applied in the vegetable crops produced. Zhu *et al.,* (2015) used house flies to compost swine manure and analyzed the left-over substrates. Larvae compost was highest in total phosphorus and potassium concentrations and lowest in total nitrogen, creating a more balanced nutrient regime for plant growth. Larvae treated compost also had the highest levels of micronutrients such as zinc, copper, iron, and cadmium. Zhu *et al.,* (2012) observed decreases in total nitrogen and water-soluble carbon when manure was treated with houseflies as compared to naturally composted manure. Detoxification and pH were also higher in fly treated manure.

Plant utilization of nutrients may also be improved by frass application. Putra *et al.,* (2017) observed differences in nutrient utilization depending on treatment. Nitrogen utilization was highest in raw manure treatments. However, phosphorus and potassium utilization were highest in in the black soldier fly digestate treatments. Improving nutrient utilization of a quickly depleting resource, such as phosphorus, may provide one solution to reducing phosphorus inputs and improving plant growth. Companies that produce insect for food and feed are also highly interested in utilizing the waste stream they create during this process. Enterra Feed Corporations conducted a study to test their black soldier fly larvae digestate product in field, seedling, and incubation trials (Temple *et al.,* 2013).

Frass may also improve soil properties, improving the overall environment for crop production. Houben *et al.,* (2020) assessed the nutrient profile of mealworm frass and revealed similar concentrations of nitrogen, phosphorus, and potassium to raw manure. The frass in this experiment mineralized quickly, resulting in increased rates of decomposition. This can reduce nutrient leaching into surrounding environments. Aligned with these observations, soluble phosphorus concentration was significantly lower in frass treatments, continuing to decrease the likelihood of leaching and infiltration. Klammsteiner *et al.,* (2019) fed black soldier fly larvae different diets and utilized their resulting frass as separate treatments. They monitored the impacts of each frass treatment on ryegrass productivity. They did not see any differences in soil total carbon and nitrogen or biomass. However, overall, in the black soldier fly larvae treatments there was a significant increase in phosphorous bioavailability, similar to what had been seen by Temple *et al., (*2013). Zhan and Quilliam (2017) observed improvements in soil organic matter from all treatments, but most consistent improvements occurred in the black soldier fly larvae digestate treatments. It seems that at lower levels black soldier fly larvae can produce comparable results to traditional compost and conventional inorganic fertilizers, while also improving soil organic matter. Increases in organic carbon were also observed in house fly treatments. Gibberellin fermentation residue digested by houseflies contained 1.3 percent potassium, 3.2 percent total nitrogen, 2.0 percent inorganic phosphorus, and 91.5 percent organic matter (Yang *et al.,* 2015b). These concentrations are appropriate proportions to what is often required for crop productivity and further indicate housefly waste as a potential biofertilizer. Other characteristics of waste material can be improved through bioconversion. The mechanical conversion of waste could also be helpful in improving the waste sources as amendments in crop production. House fly larvae feed on a variety of waste materials and can efficiently convert animal manures into odorless, coarse substrates (Čičková *et al.,* 2015). Zhang *et al.,* (2012) also saw a decrease in moisture, fecal coliforms, and odor causing compounds. Zhu *et al.,* (2012) also observed increases in compost maturation time, detoxification, and pH and decreases in percent moisture when manure was treated with house flies as compared to naturally composted manure.

Table 2.3: Insect larvae frass composition

Waste treatment	**pH**	**EC (dS/m)**	**Moisture (%)**	**C (%)**	**N (%)**	**P (%)**	**K (%)**	**S (%)**	**Ca (%)**	**Mg (%)**	**Citation**
Mealworm	-	-	-	38.90	2.92	1.53	1.86	0.18	0.10	0.54	Poveda *et al.,* 2019
Mealworm	-	-	-	38.80	2.67	1.44	1.97	0.17	0.09	0.52	Poveda *et al.,* 2019
Mealworm	-	-	-	42.44	7.75	1.02	1.15	0.28	0.11	0.34	Poveda *et al.,* 2019
Black soldier fly	5.50	44.0	-	42.90	4.54	1.23	2.44	0.49	0.64	0.13	Temple *et al.,* 2013
Black soldier fly	-	-	-	31.10	1.27	0.46	2.79	-	-	-	Rosmiati *et al.,*2017
Black soldier fly	8.84	8.50	51.40	35.20	4.40	5.20	4.1	-	4.50	0.80	Setti *et al.,* 2019
House fly	7.78	-	18.55	3.36	4.66	2.70	1.3	-	-	10.55	Zhu *et al.,* 2015
House fly	8.50	-	29.80	78.23	3.20	2.0		-	-	0.0	Yang *et al.,* 2015
Traditional compost	7.30	11.00	-	40.70	2.80	1.81	2.24	0.65	3.69	0.66	Temple *et al.,* 2013
Untreated manure	6.59	-	72.42	84.80	6.23	3.72	2.40	-	-	10.55	Zhu *et al.,* 2015
Peat	6.10	1.30	-	-	0.15	-	-	-	-	-	Setti *et al.,* 2019

Frass can serve various purposes in crop production systems (Table 2.4). Most studies have been conducted in pots within a controlled environment setting, such as a greenhouse or growth chamber. Some studies have considered it as a growth medium replacement for peat. Setti *et al.,* (2019) applied black soldier fly larvae digestate in lettuce, tomato, and basil crops. They analyzed the chemical characteristics of the black soldier fly larvae digestate (Table 2.3). From these results, we can determine that a modest decrease in peat utilization, supplemented with black soldier fly larvae digestate, can sustain the current conventional growth parameters of crop production. Over time this ten percent reduction in peat extraction, if widely adopted, could result in some conservation of peat resources.

Table 2.4: Utilization of insect frass in crop production

Use	**Citation**
Replacement for peat	Setti *et al.,* 2019
Improved waste streams for fertility amendment	Putra *et al.,* 2017; Rosmiati *et al.,* 2017
Reduced plant pest, pathogens, and disease	Choi and Hassanzadeh, 2019; Klammsteiner *et al.,* 2019; Vickerson *et al.,* 2016;
Improved resistance to stress	Poveda *et al.,* 2018
Increased crop productivity and yield	Beesigamukama *et al.,* 2020; Buenvinida and Tamban, 2016; Choi and Hassanzadeh, 2019; Houben *et al*., 2020; Poveda *et al.,* 2018; Putra *et al.,* 2017; Rosmiati *et al.,* 2017; Setti *et al*., 2019; Temple *et al.,* 2013; Xiao *et al.,* 2018; Zhan and Quilliam, 2017; Zhu *et al.,* 2012

Frass can also be used to reduce existing waste streams and convert those streams into useful plant amendments. Coffee husks, a coffee waste product, are abundant in tropical production areas around the world. The organic waste material created from this accumulation has little value, but can possibly be improved by bioconversion, or the intake and excrement of organic material by animals. Putra *et al.,* (2017) and Rosiamti *et al.,* (2017) analyzed coffee husks digested by black soldier fly larvae for their nutrient profile, effectiveness as a fertilizer treatment, and nutrient utility. The black soldier fly larvae digestate had lower carbon, nitrogen and potassium compared to the undigested coffee husks, but higher amounts of phosphorus. However, based on these results, major modifications need to be made in coffee husk bioconversion before it can be considered a valuable replacement for raw manure.

Insect frass may also reduce incidences of plant pathogen and disease. Several studies show the relationship between the use of insect frass as promoters of resistance to biotic and abiotic stressors. For example, black soldier fly larvae frass has been proposed to have insecticidal qualities. Insecticides are natural compounds that can decrease pest insect populations. It was shown that when applied as an insecticide through this method, the frass reduced wireworm populations (Vickerson *et al.,* 2016).

Choi and Hassanzadeh, 2019 also demonstrated that black soldier fly larvae frass inhibited the growth of *Fusarium oxyspourm* and *Rhizoctonia solani*. Additionally, treatments including the frass also reduced disease prevalence in bean plants inoculated with *Trichoderma*. A study by Klammsteiner *et al.,* (2019) revealed black soldier fly larvae frass produced from chicken feed, fresh produce, and grass cutting diets entirely eliminated *Salmonella* population in the treatments. *E. coli* populations were reduced in the fresh produce and grass cutting diets, but not the chicken manure. This indicates that bioconversion of animal wastes could decrease risks of human pathogen transmission as well as improve crop production.

Poveda *et al.,* (2018) was able to demonstrate the role of meal worm frass *(T. molitor*) in lettuce resistance to abiotic stresses under field conditions. Three types of meal worm frass were produced from different diets. These frass treatments were exposed to salinity, drought, and flooding stress. Typically, fertilization improved survival and performance in stress conditions. Two of the three diets saw improved dry weight, root length, and aerial part length compared to the negative unfertilized control and equal to the fertilized positive control. This indicates that meal worm frass can provide equivalent support for crops under stress conditions as compared to a conventional fertilizer.

Table 2.5: Influence of insect larvae frass and other amendments on crop productivity in pot studies

Crop	Amendment	Application (% media)	Germination Index (%)	Height (cm)	Weight (g)	Citation
Baby lettuce	Black soldier fly	10.00	95.40	11.70	0.90	Setti *et al.,* 2019
Baby lettuce	Black soldier fly	20.0	70.70	10.30	0.70	Setti *et al.,* 2019
Lettuce	Black soldier fly	50.0	-	-	21.0	Putra *et al.,* 2017
Lettuce	Black soldier fly	33.33	-	-	11.78	Putra *et al.,* 2017
Lettuce	Black soldier fly	50.00	-	14860.00	-	Rosmiati *et al.,* 2017
Lettuce	Black soldier fly	33.33	-	12390.00	-	Rosmiati *et al.,* 2017
Chard	Mealworm	2.00	-	-	70.00	Poveda *et al.,* 2019
Basil	Black soldier fly	10.00	120.10	11.80	0.15	Setti *et al.,* 2019
Basil	Black soldier fly	20.00	143.40	8.30	0.09	Setti *et al.,* 2019
Tomato	Black soldier fly	10.00	122.0	18.10	0.70	Setti *et al.,* 2019
Tomato	Black soldier fly	20.00	113.30	18.60	0.50	Setti *et al.,* 2019
Spring onion	Black soldier fly	5.00	-	359.70	1.00	Zhan and Quilliam, 2017
Spring onion	Black soldier fly	10.0	-	250.60	1.10	Zhan and Quilliam, 2017
Spring onion	Black soldier fly	15.00	-	66.70	1.10	Zhan and Quilliam, 2017
Green bush bean	Black soldier fly	1.50	-	56.00	11.83	Choi and Hassanzadeh, 2019
Barley	Mealworm	50.00	-	-	3.00	Houben *et al.,* 2020
Barley	Mealworm	100.00	-	-	3.50	Houben *et al.,* 2020
Maize	Black soldier fly	0.19	-	95.40	6.83	Gaarttling *et al.,* 2020
Maize	Black soldier fly	1.09	-	96.70	6.61	Gaarttling *et al.,* 2020

Baby Lettuce	Peat	100.00	78.40	8.10	0.40	Setti *et al.,* 2019
Baby Lettuce	Peat and fertilizer	100.00	94.90	11.70	0.70	Setti *et al.,* 2019
Basil	Peat	100.00	97.50	5.30	0.11	Setti *et al.,* 2019
Basil	Peat and fertilizer	100.00	100.00	9.80	0.16	Setti *et al.,* 2019
Tomato	Peat	100.00	98.70	14.90	0.20	Setti *et al.,* 2019
Tomato	Peat and fertilizer	100.00	91.30	18.30	0.60	Setti *et al.,* 2019

Various crops have been studied to determine the impact of frass on productivity (Table 2.5). Most of these studies have been pot studies in controlled environments. Lettuce amended with black soldier fly larvae digestate at a 90:10 ratio (peat: digestate) produced increases of height, stem diameter, leaf dry weight, and leaf area that were statistically comparable, or higher than the control (Setti *et al.,* 2019). Putra *et al.,* (2017) also treated lettuce with black soldier fly larvae digestate. The digestate treatments achieved significantly higher wet and dry weights than the undigested coffee husks, though the highest dry and wet weights of lettuce vegetative growth were seen in the raw manure treatments. A related study conducted by Rosmiati *et al.,* (2017) showed similar results with applications of black soldier fly larvae bioconverted coffee husks on lettuce production. Plant height, number of leaves, leaf area, and chlorophyll content of lettuce were all highest in the raw manure treatments, similar to Putra *et al.,* (2017).

A study by Poveda *et al.,* (2018) revealed that mealworm (*T. molitor*) frass may have some success with improving chard yield. The fresh weight, length aerial part, width of basal stem, and chlorophyll content of the leaves all indicated significant increases with application of meal worm frass when compared to the unfertilized negative control. Statistically the fertilized positive control and the frass treatments produced comparable measurements indicating that frass could be a substitute for inorganic commercial fertilizer.

Basil experienced increases in height, stem diameter, leaf number, leaf dry weight, stem dry weight, total dry weight, and leaf area that were statistically comparable or higher to the control when black soldier fly larvae digestate was applied in a 90:10 (peat: digestate) mixture (Setti *et al.,* 2019). One study collected lab raised caterpillar frass and added the frass to basil plants in different quantities. The greatest plant heights occurred in the treatments with the largest amount of frass, 15 gram and 10 gram applications. Leaf width was greatest in the two higher frass treatments as well (Buenvinida and Tamban, 2016). Setti *et al*., (2019) applied black soldier fly larvae digestate to tomato plants. Height, leaf number, leaf dry weight, stem dry weight, total dry weight, and leaf area were highest in the 90:10 mixture and statistically comparable, or higher than the control.

Zhan and Quilliam (2017) investigated the influence of black soldier fly larvae frass on spring onion plants. Plant height and weight were similar across treatments. The lowest heights and weights were observed in the highest-level frass treatment. Choi and Hassanzadeh demonstrated the use of black soldier fly larvae digestate as a fertilizer for green bush beans. They found that digestate in combination with humic acid produced the statistically highest height, biomass, and nitrate concentrations.

Houben *et al*., (2020) used mealworm frass to investigate its impact on barley productivity. Barley treated with mealworm frass applications had similar biomass, nitrogen, phosphorus, and potassium concentrations in comparison to inorganic fertilizer treatments. Maize treated with black soldier fly larvae digestate produced statistically comparable yields to other organic fertilizers, though these yields were significantly lower than the standard inorganic fertilizer (Gaarttling *et al.,* 2020). These studies provide further evidence that application in a controlled environment can provide similar benefits to currently adopted fertilizer regiments.

Table 2.6: Influence of black soldier fly larvae frass on crop productivity in field studies

Crop	**Waste**	**Application Rate (kg/m^2)**	**Yield (kg/m^2)**	**Citation**
Lettuce	Enterra Feed Corp	0.50	2.70	Temple *et al.,* 2013
Lettuce	Enterra Feed Corp	1.00	3.30	Temple *et al.,* 2013
Bok choi	Enterra Feed Corp	0.50	5.90	Temple *et al.,* 2013
Bok choi	Enterra Feed Corp	1.00	7.30	Temple *et al.,* 2013
Bean	Enterra Feed Corp	0.50	5.10	Temple *et al.,* 2013
Bean	Enterra Feed Corp	1.00	5.90	Temple *et al.,* 2013
Potato	Enterra Feed Corp	0.50	3.40	Temple *et al.,* 2013
Potato	Enterra Feed Corp	1.00	3.10	Temple *et al.,* 2013
Chinese Cabbage	-	1.21	0.28	Choi *et al.,* 2009
Maize	Brewery	3.00	0.52	Beesigamukama *et al.,* 2020
Maize	Brewery	6.00	0.60	Beesigamukama *et al.,* 2020
Maize	Brewery	10.0	0.61	Beesigamukama *et al.,* 2020
Shallots	Poultry	0.25	0.21	Quilliam *et al.,* 2020
Shallots	Brewery	0.25	0.22	Quilliam *et al.,* 2020

Studies conducted in the field indicate that bioconversion of waste material can improve its ability to benefit plant production, regardless of the production setting (Table 2.6). Enterra Feed Corporation applied black soldier fly larvae digestate at zero, five, and ten tons per hectare to lettuce crops in a field setting. Fresh weight and mortality of lettuce was statistically higher than the controls and improved by a second application of digestate (Temple *et al.,* 2013). They applied these same condition to bok choi crops in the field. Bok choi fresh weight was highest and morality was the lowest in the ten t/ha treatment (Temple *et al*., 2013). Black soldier fly larvae digestate was applied to potato fields. Potato tubers produced the highest yield at five ton/ha compared to the other treatments. From these results, they recommend that digestate should be applied to each crop at a rate of five tons per hectare, twice per season (Temple *et al*., 2013).

Additional studies have been done that support these findings (Table 6). Choi *et al.,* (2009) applied black soldier fly digestate to Chinese cabbage and saw statistically similar results to the standard fertilizer control. Beesigamukama *et al.,* (2020) applied black soldier fly frass in three quantities of Nitrogen content (30, 60, and 100 kg) and compared their impact on maize yield when a standard fertilizer was applied at the same rates. 30 and 60kg of black soldier fly larvae frass produced significantly higher maize yields than their corresponding standard fertilizer. The highest yields were produced at 100kg and were statistically comparable to the corresponding standard fertilizer. Quilliam *et al.,* (2020) utilized two different origins of waste on shallot crops. The statistically highest yields were produced when treated with brewery biofertilizer. Poultry waste fertilizer was also statistically comparable to a standard fertilizer treatment. These results indicate that black soldier fly larvae left over substrates can sufficiently support yields comparable to a standard fertilizer regiment. These adaptations to conventional practices in the field may also lead to less negative impacts that are associated with commonly used organic amendments, such as raw manure.

Different stages of crop growth can be useful in determining the impact of frass applications. Temple *et al*., (2013) conducted seedling trials with several different crops to assess black soldier fly larvae digestate as a growing medium for seeds in the field. Bok choi, onion, bean, and tomato seedlings saw highest dry yield when applied at 15 t/ha. Lettuce and squash saw highest dry yield at ten t/ha. These results and their value for commercial production are highly dependent on crop and adaptation practice. Zhu *et al.,* (2012) measured the germination index of Chinese cabbage and cucumber seeds treated with fly digested composts. The composts exhibited higher percentages of germination than those treated with naturally composted manure.

Setti *et al*., (2019) also saw increases in germination index of lettuce when black soldier fly larvae digestate was applied to lettuce. In the same study, basil experienced increased rates of emergence after digestate applications. This indicates that fly bioconversion of manure can improve emergence time in crop production.

Certain considerations may be required to improve the efficacy of frass application. A study by Xiao *et al.,* (2018) combined the effects of a naturally found digestive bacteria from within black soldier fly larvae gut contents and black soldier fly larvae frass to analyze their synergistic impact on plant productivity. The bacteria and larvae treated manure saw higher reductions in manure biomass and increases in rate of reduction compared to the non-bacterial treated control. There were also lower levels of moisture and nutrients in the digested manure, which is desirable to livestock farmers. The bacteria treatments increased the germination index of Chinese cabbage and rape seeds compared to the control. Though this process is not entirely realistic for commercial production, gut isolated bacteria could provide further insights in improving bioconversion efficiency for biofertilizer production.

Vermicomposting is a common practice in small scale, organic production and the literature supporting its usefulness as a fertilizer for high value specialty crops is voluminous. Earthworms are added to manure in order to break down carbon-based materials, and this biological conversion creates several positive impacts. Compared to other fertilizers, vermicompost results in lower runoff and infiltration rates (Elliot *et al.,* 2007), and may improve yield, organic matter content, and soil fertility (Paul and Metzger, 2005). Though earthworms are not insects the process of bioconversion undergone by the worms is similar to the digestive processes of insects (Chapin *et al.,* 2011). Because of these similarities, it is reasonable to anticipate that they are capable of decomposing similar food sources and that insect frass may provide many of the same benefits as worm castings to soil fertility and crop production. Similar to insect frass, several studies have produced significant increases in vegetable crops total yields when vermicompost was applied in combination with inorganic fertilizers (Alam *et al.,* 2007; Ansari, 2008; Arancon et *al.,* 2003; Atiyeh *et al.,* 2000; Gutiérrez-Miceli *et al.,* 2007; Narayan *et al.,* 2013; Nongmaithme and Pal, 2001; Yourtchi *et al.,* 2013). Within these studies vermicompost treatments also produced consistent increases in seedling growth, plant height, stem diameter, leaf area, germination, leaf number, shoot length, emergence, branch number, total biomass, and fruit, leaf, shoot, and root weight for many species of vegetable crops. Typically, the most effective treatments were supplemented with 10 to 50 percent vermicompost applications by volume, or 10:90 and 50:50 vermicompost to peat mixtures.

Vermicompost studies have also shown that the substitution of biofertilizers can decrease pathogens and disease in crop production. Vermicompost application can reduce the occurrence of blossom end rot of tomatoes (Surrage *et al.,* 2013). Decreases in pathogens such as nematodes (Renčo and Kováčik, 2015), pea leafminers (Suryawan and Reyes, 2016), and scab (Singhai *et al.,* 2011) have occurred when applied to potatoes.

4. Conclusions, special considerations, and future directions

Insect waste streams present a product with possible applications to food crop production. Many of these studies show that insect frass in combination with inorganic fertilizer actually produce the best results concerning crop productivity, and pathogen and disease resistance. The most effective treatments typically include 10 to 40 by volume percent frass applications; this varies greatly by crop, insect, waste source, treatment, and environment (*e.g.* field vs greenhouse). The addition of non-traditional amendments, such as humic acid (Choi and Hassanzadeh, 2019) and bacterial isolates (Xiao *et al.,* 2018) may serve as a further supplement or alternative of inorganic conventional fertilizers used for production of food crops.

One of the consistent concerns mentioned in this research refer to the sodium content and electrical conductivity levels of the soil after application. This may require that the industry monitors the diet of industrially produced insects so that their frass does not have negative repercussions on plant productivity and soil health (Temple *et al.,* 2013). For example, compared to inorganic conventional fertilizers, black soldier fly larvae frass has a much higher electrical conductivity (Zhan and Quilliam, 2017) than traditional composts. Additionally, there is some evidence that starting diet composition affects the bacterial populations present in fly larvae frass, which further complicates the system (Klammsteiner *et al.,* 2019). House fly frass also showed higher levels of heavy metals such as lead and arsenic (Zhu *et al.,* 2015). Each of these issues are important considerations for larval production and biofertilizer utilization in the future. Studies that show high levels of bioavailable phosphorus in insect frass are especially promising for agricultural areas limited in phosphorus moving forward. As described, phosphorus is a non-renewable resource and prospects to replace it should be of high priority for future research and adoption of practices. Lastly, common units should be adopted to express relative quantities of insect digestate applied across different environments. For example, kg/ha for field applications and liter: liter for greenhouse use. These factors should be noted and considered as this body of literature is expanded through additional studies.

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CHAPTER THREE

THE IMPACTS OF BLACK SOLDIER FLY LARVAE FRASS ON THE PRODUCTION OF SHORT CYCLE, COOL SEASON CROPS, ARUGULA AND LETTUCE

1. Introduction

The “insect as food and feed” movement is propelling research for insect protein in various directions. This includes several different insect species, but the focus of this work is black soldier fly larvae (*Hermetia illucens*), Linneaus (Dipera: Stratiomyidae). The larvae are of interest to insect producers because they are efficient at reducing and metabolizing waste streams (Diener *et al.,* 2009). Because of black soldier fly larvae’s (BSFL) protein content, 42 to 48 percent (Miranda *et al*., 2019), they are also valuable as feed to livestock producers. Compared to beef, which can range from 13 to 16 percent (Leheska *et al.,* 2009), this content is extremely high. One major issue during insect production is the leftover substrates generated by the larvae after they have been fed. Due to its concentration of nitrogen and phosphorus, this product has the potential to provide agriculturally valuable nutrients derived from existing waste streams and reduce inorganic fertilizer application during crop production (Schmitt, 2020).

Additionally, this material could serve as a partial replacement for peat (Setti *et al*., 2019). Peat is the compacted, below ground debris found in marsh ecosystems (Bloom, 1964). It is comprised of partially decomposed plants, minerals, and water (Cameron *et al*., 1989). Peat has become the most common media for controlled environment and gardening operations (Niu and Masabni, 2018). However, peat extraction has severe consequences for the environment and surrounding landscapes. As controlled environment agriculture becomes more popular, the rate that peat is being mined is faster than the peatlands can be naturally replenished. After extraction, peatlands are left barren and emit large amounts of methane, a potent greenhouse gas. Additionally, peat marshes support diverse and abundant wildlife that is disrupted during extraction events, leading to large decreases in cultural and biological diversity (Couwenberg *et al.,* 2011).

However, this creates an exciting opportunity to redirect multiple waste streams and reduce the consumption and extraction of a valuable natural resource. Food and restaurant partners often donate their waste to insect producers. This converts what would essentially go to the landfill into food for high quality protein sources. By reusing the waste and left over substrates from industrial insect production as a partial replacement for peat, growers can reduce costs and inputs, while improving yield and environmental consequences (Chavez, 2021).

Studies have been conducted to investigate the uses of insect frass for application in horticultural production. Setti *et al.* (2019) used BSFL digestate as a growth medium replacement for peat in tomato, basil, and lettuce crops. They found that at 10:90% BSFL digestate and peat mixture, respectively, could adequately replace a 100% peat based medium. Additionally, Enterra Feed Corporations conducted a field study to test their BSFL frass product, known as Enterra Natural Fertilizer. They found that the fertilizer was an effective amendment for production of potatoes, bok choi, and lettuce at a rate of 5 tons per hectare or 500 grams per square meter (Temple *et al*., 2013). Coffee husks digested by BSFL were used as a fertilizer treatment for lettuce and compared with raw manure and undigested coffee husks. The highest lettuce dry and wet weights were found in the raw manure treatments, though the frass treatments did achieve significantly higher wet and dry weights than the undigested coffee husks (Putra *et* *al.,* 2017). Another amendment similar to BSFL frass, but with a much more robust body of literature, is vermicompost.

Vermicompost is the reuse and digestion of a waste product by earthworms. The recycled and digested carbon materials from the waste provides an improved soil amendment for plant growth (Tammam *et al*., 2022). For example, Rocky Mountain Soil Stewardship raises their worms on horse manure. This is a popular treatment for small scale, organic, and/or sustainable agricultural producers. It is reasonable to anticipate that insects will be capable of decomposing similar food sources as worms and that insect frass may provide many of the same benefits as worm castings to soil fertility and crop production (Chapin *et al.,* 2011). The use of frass as a horticultural soil amendment can provide application for a growing (potentially local) waste stream in combination with inorganic fertilizers. It can provide growers access to a beneficial amendment that is more affordable than vermicompost, while also decreasing peat consumption.

Leafy vegetables are global staples because of their high fiber content. Lettuce (*Lactuca sativa*), Linnaeus (Asterales: Asteraceae), has important commercial value for the United States and can be successfully grown on a diversity of soil types, making it an ideal crop for this study. Additionally, romaine lettuce is known to thrive well in irrigated environments, like the western United States. Lettuce is considered a cool season crop because optimal temperatures do not rise above 23^{o}C (Swiader and Ware, 2002). Arugula (*Eruca sativa*), Linnaeus (Brassicales: Brassicaceae), is a less commercially important crop than lettuce but offers more nutritional and culinary value (Shubha *et al*., 2019). Many of the cultivation and harvest techniques are similar as well.

A greenhouse study was conducted from June to July of 2021, March to April of 2022, and July to August of 2022 that included two different leafy, cool season vegetable crop species:

arugula and lettuce. Our major objective was to investigate the impact of BSFL frass on vegetable yield and greenness. We compared the performance of crops grown in peat amended with insect frass and vermicompost in a greenhouse setting. Additionally, we compared the differences in impact of two different starting diets for the BSFL. We conducted three studies with two corresponding questions and hypotheses. Hypothesis were deduced based on existing black soldier fly larvae data and the vermicompost literature described in Chapter 2.

Question 1: How does distillery grain frass impact arugula and lettuce yield?

Hypothesis: BSFL frass applications will produce comparable or better arugula and lettuce fresh and dry yields than the peat control and vermicompost treatments.

Question 2: How does distillery grain digested frass impact arugula and lettuce greenness?

Hypothesis: BSFL frass treatments will produce greener arugula and lettuce plants than the peat control and vermicompost treatments.

Question 3: How does brewery grain-digested frass impact arugula and lettuce yield?

Hypothesis: BSFL frass applications will produce comparable or better arugula and lettuce fresh yields than the peat control and vermicompost treatments.

Question 4: How does brewery grain-digested frass impact arugula and lettuce greenness?

Hypothesis: BSFL frass treatments will produce greener arugula and lettuce plants than the peat control and vermicompost treatments.

Question 5: How do the impacts of distillery grain digested frass and brewery grain digested frass amendments differ as it relates to arugula and lettuce yield?

Hypothesis: Fresh weight of BSFL digested brewery grain treatments will have a similar result as BSFL digested distillery grain treatments.

Question 6: How do the impacts of distillery grain digested frass and brewery grain digested frass amendments differ as it relates to arugula and lettuce greenness?

Hypothesis: Greenness of BSFL digested brewery grain treatment will have a similar result as BSFL digested distillery grain treatment.

Table 3.1: Layout of the experiments that were completed across 2 different years (2021 and 2022) and seasons (Summer and Spring): 2 crops (lettuce and arugula) were grown in 3 different amendments (distillery grain frass (DGF), brewery grain frass (BGF), and vermicompost (VC) to address 3 different questions per trial (fresh weight, dry weight, and SPAD). Highlighted spaces indicate which amendments were evaluated in that season.

	DGF vs. VC	BGF vs. VC	DGF vs. BGF
Summer 2021	🟪		
Spring 2022	🟪	🟪	🟪
Summer 2022		🟪	

2. Materials and Methods

Preliminary studies were conducted in 2020 (Appendix I) and produced the information needed to design the set of experiments that took place in 2021 and 2022. This series of experiments were conducted in Fort Collins, CO at the CSU Horticulture Center greenhouses, elevation 1,525.22 meters (en-us.topographic-map.com). They spanned the duration of June of 2021 to August of 2022. The experiments (Table 3.1) were carried out as complete randomized designs (CRD) using 7 levels total (three treatments of each amendment and 100% commercial peat control), two crop species, and 10 replicates/level over the three growing periods. These amendments were: BSFL distillery grain-digested frass (BSFL-DGF), BSFL brewery grain-digested frass (BSFL-BGF), and vermicompost (VC). Both BSFL frass amendments were sourced from Evo Conversions System (College Station, TX), the vermicompost was from

Rocky Mountain Soil Stewardship (Fort Collins, CO), and the peat was a Berger B6 mix (Saint-Modeste, QC). Each amendment type was mixed with commercial peat at different percentages of amendment to peat (40%:60%, 20%:80%, 10%:90%), in addition to a 0%:100% peat control (Table 3.2). Inorganic fertilizer (24-8-16 NPK) Miracle Gro (Marysville, OH) was applied at the label recommended rate (3.7 mL per 0.95 L water) for each crop, every other week. Productivity was assessed by weighing the vegetative biomass, or the leaves and petiole of the crop. Greenness was measured with a SPAD (soil plant analysis development) meter.

Lettuce (‘Salvius MT0’), and arugula (‘Apollo’) were started as plugs in 288 cell trays with an organic germination mix: 3.79L peat, 0.47L vermicompost, 29.57mL blood meal, and 29.57mL bone meal. They were grown on warming benches for two to four weeks in the greenhouse, and one to three plugs (depending on season and crop) were transplanted into “1-gallon” pots (2L in volume). In the summer of 2021, seedlings were transplanted into pots on June 23. Harvest and data collection took place on July 30. In the spring of 2022, seedlings were transplanted into pots on March 13. Harvest and data collection took place on April 10. Summer of 2022, seedlings were transplanted into pots on July 20. Harvest and data collection took place on August 11.

Table 3.2: Amendment treatment summary for 2021 greenhouse vegetable studies. This design applied to arugula and lettuce: vermicompost (VC) and black solider fly larvae (BSFL) frass.

Black soldier fly larvae frass treatments				**Vermicompost treatments**			
Peat %	**Peat (L)**	**BSFL %**	**BSFL (L)**	**Peat %**	**Peat (L)**	**VC %**	**VC (L)**
60	4.8	40	3.2	60	4.8	40	3.2
80	6.4	20	1.6	80	6.4	20	1.6
90	7.2	10	0.8	90	7.2	10	0.8
100	8.0	0	0.0	100	8.0	0	0.0

Yield

Crops were grown in pots placed on the greenhouse floor at 20-25ºC. Four to five weeks after being transplanted, arugula and lettuce were harvested by cutting plants above the soil surface. Above ground biomass was weighed, and the lant material was put in paper bags and left in the Horticulture Center drying oven at 70ºC. Dried leaves were removed from the oven and dry weights were taken one week later for one week. Weights were analyzed based on the average of the observation units, plants, within the experimental unit, the pot.

SPAD Analysis

Soil plant analysis development (Konica Minolta: Ramsey, NJ) measurements were taken once before harvest. SPAD measures the difference of red and infrared light transmitted through the plant, and is used as an indicator of greenness. The upper right side of the tallest leaf was measured. Each plant in the pot was measured separately, and the average for each pot was analyzed.

Statistical Analysis

Lettuce and arugula plants, the observational units, were treated as averages within the pot, or experimental replicates. Statistical analysis was conducted using JMP®, Pro 16 (SAS Institute Inc., Cary, NC, 2022). The Anderson-Darling test for normality and the Levene's test for equal variance were conducted to determine if the assumptions for an ANOVA and Pooled T Test were met. When they were not met, non-parametric Kruskal Wallis and Mann Whitney U rank sums tests were conducted in addition to the parametric tests. A threshold of $p < 0.05$ was considered as significant. Tukey HD and Dunn method for Joint Ranks pair wise comparisons were conducted to assess and test for differences between levels. Mean and statistical summary tables can be found in Appendix II.

3. Results

Distillery grain digested frass (DGF)- Arugula

Summer of 2021 (Figure 3.1a.) had a clear trend that the BSFL treatments produced significantly higher arugula fresh yields (33.8 – 28.4g) than the control (13.8g) and the vermicompost treatments (10.5-12.8g). Yield by dry weight (Figure 3.2a.) showed similar results. DGF 20% (3.35g) was significantly higher than a few of the vermicompost treatments, VC 10% (1.7g) and 40% (1.2g), and statistically comparable to the other treatments and the control. Arugula greenness (Figure 3.3a.) showed some variability, but statistically most of these treatments were similar. DGF 10% (44.79g) was greener than the higher concentrations of vermicompost, 20% (36.65g) and 40% (37.17g).

Arugula yield in spring 2022 (Figure 3.1b.) showed less consistent trends for distillery grain frass treatments than the summer study. The main differences observed in this study were between the highest concentrations of frass (1.74g) and vermicompost (6.07g). VC 40% was significantly higher than BSF 40%, though statistically similar to all the other treatments and the control. Dry weights produced different trends compared to the fresh yields (Figure 3.2b.); there were no statistically significant treatment differences. However, BSF 20% produced the greatest dry weights and DGF 40% produced the lowest. Arugula greenness in the spring seasons also produced variable results. Distillery grain digested frass (35.93-36.72) appeared to produce greener plants than the control (24.56) and the VC 10% treatment (24.12) (Figure 3.3b.).

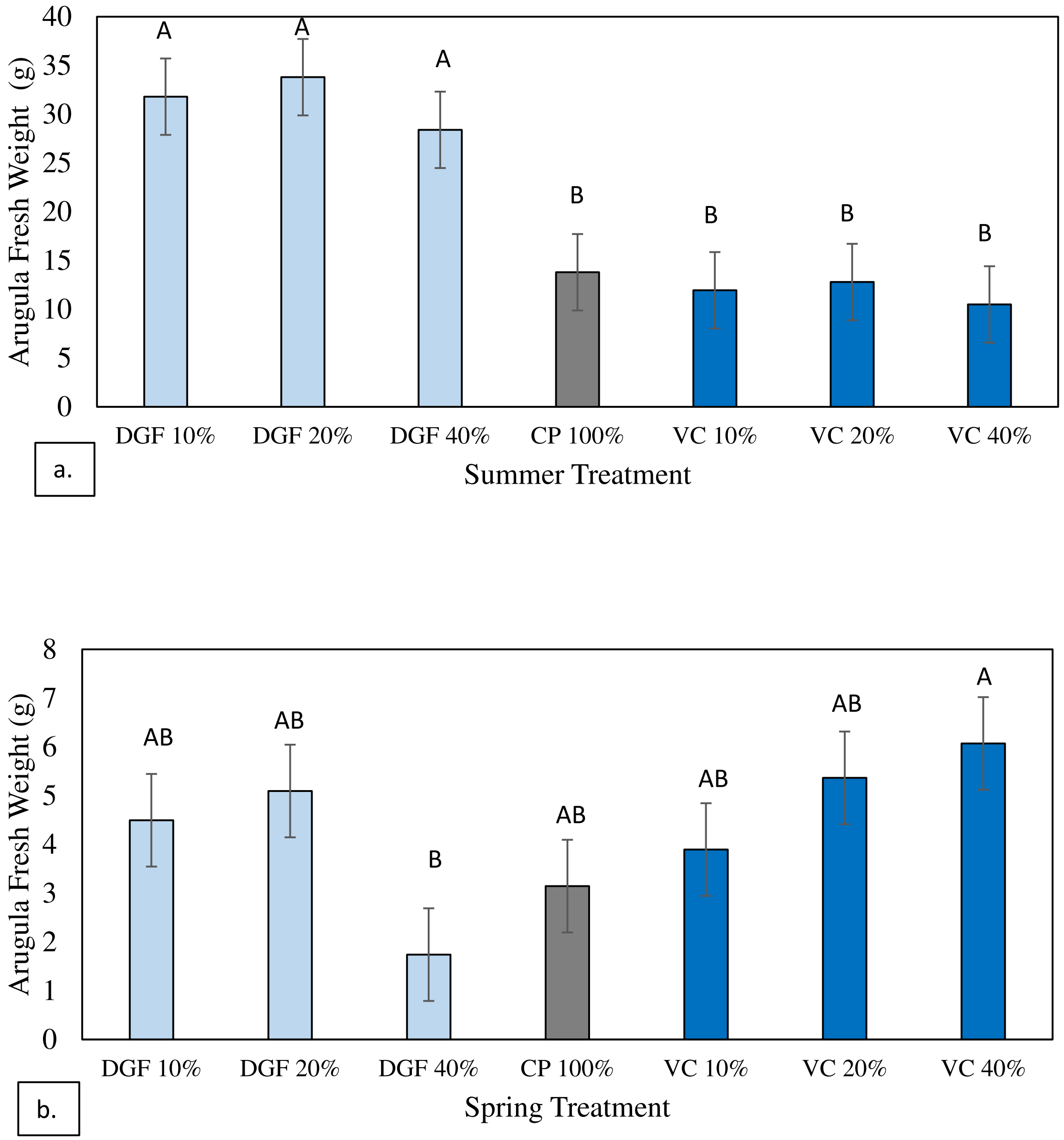

Figure 3.1: ***Fresh weight (g) of arugula grown in BSFL- DGF amended peat a greenhouse, a. summer 2021 and b. spring 2022.*** Arugula was grown with mixtures of commercial peat and distillery grain black soldier fly larvae frass (DGF: light blue columns) or vermicompost (VC: sky blue columns). Commercial peat (100%) served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p < 0.0001$/F= 16.65, b. p = 0.038/F=2.40).

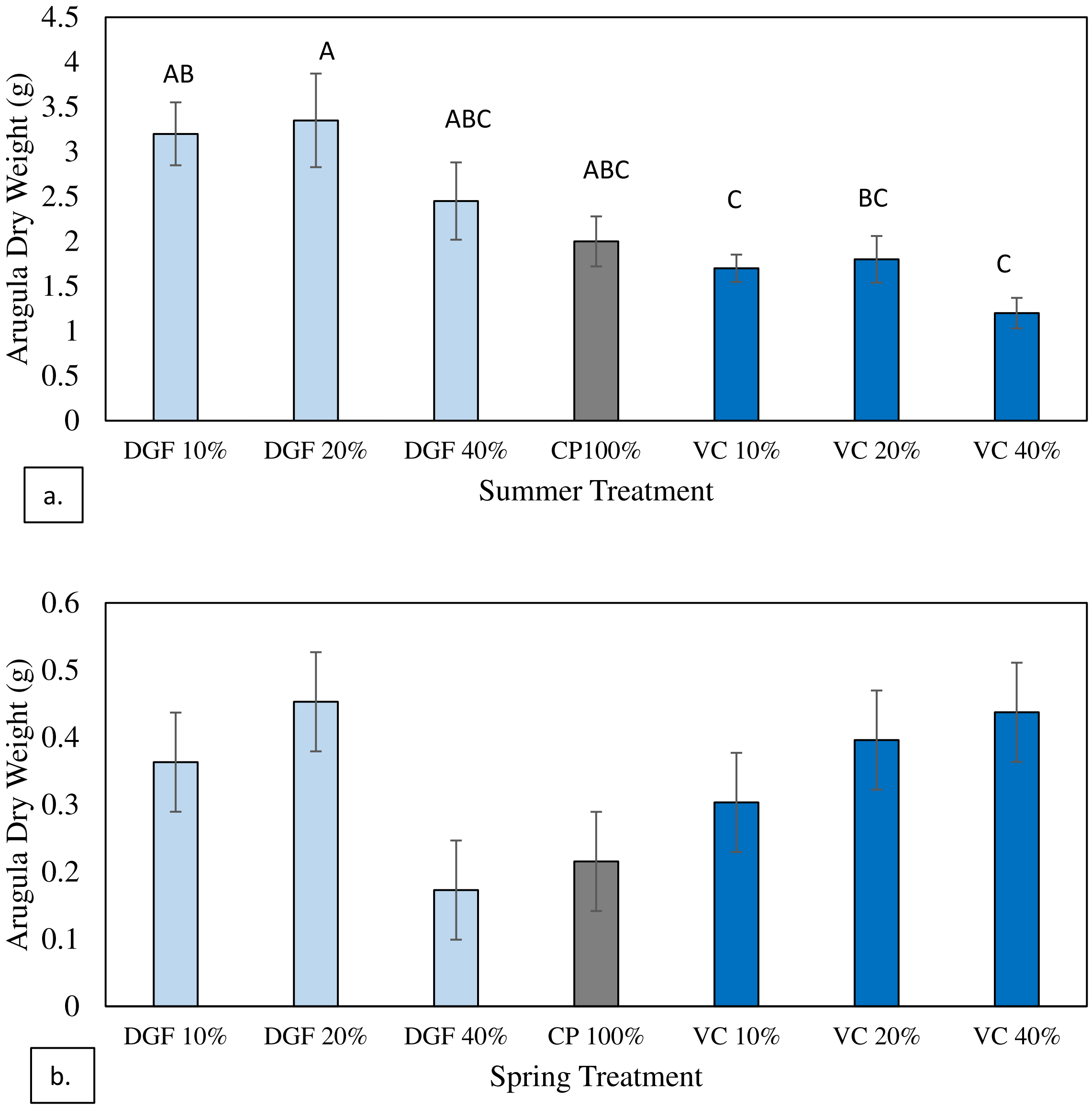

Figure 3.2: ***Arugula dry weight (g) of BSFL- DGF amended peat grown in a greenhouse, a. summer 2021 and b. spring 2022.*** Arugula was grown with mixtures of commercial peat (CP) and distillery grain frass (DGF) or vermicompost (VC). Commercial peat (100%) served as the control, df = 6 (a. $p < 0.0001$/ F=5.72; b. $p = 0.059$/F=2.16).

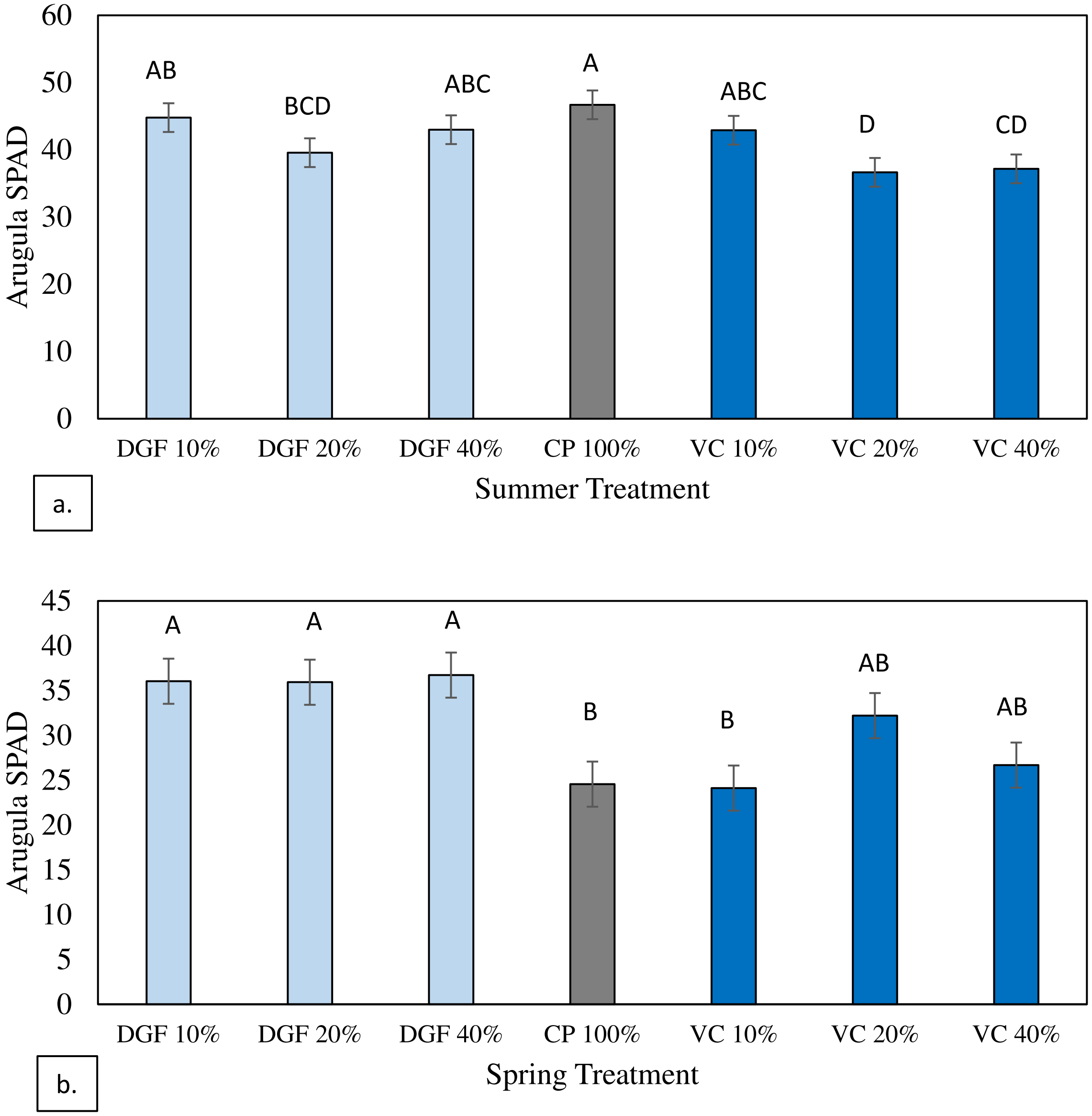

Figure 3.3: ***Arugula SPAD measurements of BSFL- DGF amended peat grown in a greenhouse, a. summer 2021 and b. spring 2022.*** Arugula was grown with mixtures of commercial peat (CP) and distillery grain frass (DGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (p = 0.0082/F=3.21; p = 0.0003/F=5.03)

Distillery grain digested frass – Lettuce

Lettuce yield in the summer of 2021 (Figure 3.4a.) produced similar results to arugula in the same season. However, unlike most of the results, the highest concentration of frass, DGF 40%, produced the highest yield (72.6g). This was also evident in the dry weight results (Figure 3.5a.). DGF 40% (9.97g) was statistically the highest treatment, following BSF 20% (6.5g) and BSF 10% (6.92g), while the control (2.52g) and VC treatments (1.82-2.7g) were the lowest. Lettuce grown in summer 2021 produced similar SPAD results as arugula (Figure 3.6a.). There were some significant differences in greenness between frass treatments (44.32-46.23) and vermicompost treatments (35.78-37.78). Overall, the frass treatments were the greenest while BSF 10% (46.23) was statistically the greenest.

For spring 2022, lettuce yields (Figure 3.4b.) were more variable than in the summer study. The 10% frass treatment produced the highest fresh yield (13.97g), and the 40% frass treatment (3.2g) produced the lowest. Otherwise, frass and vermicompost were statistically similar to the control peat. The dry weights showed similar results (Figure 3.5b.). DGF 10% (0.74g) was significantly higher than DGF 40% (0.3g), which was the lowest overall. Lettuce greenness was significantly higher in the distillery grain-digested treatments (35.5-41.69) than the vermicompost treatments (26.28-29.61) and control (29.30) (Figure 3.6b.).

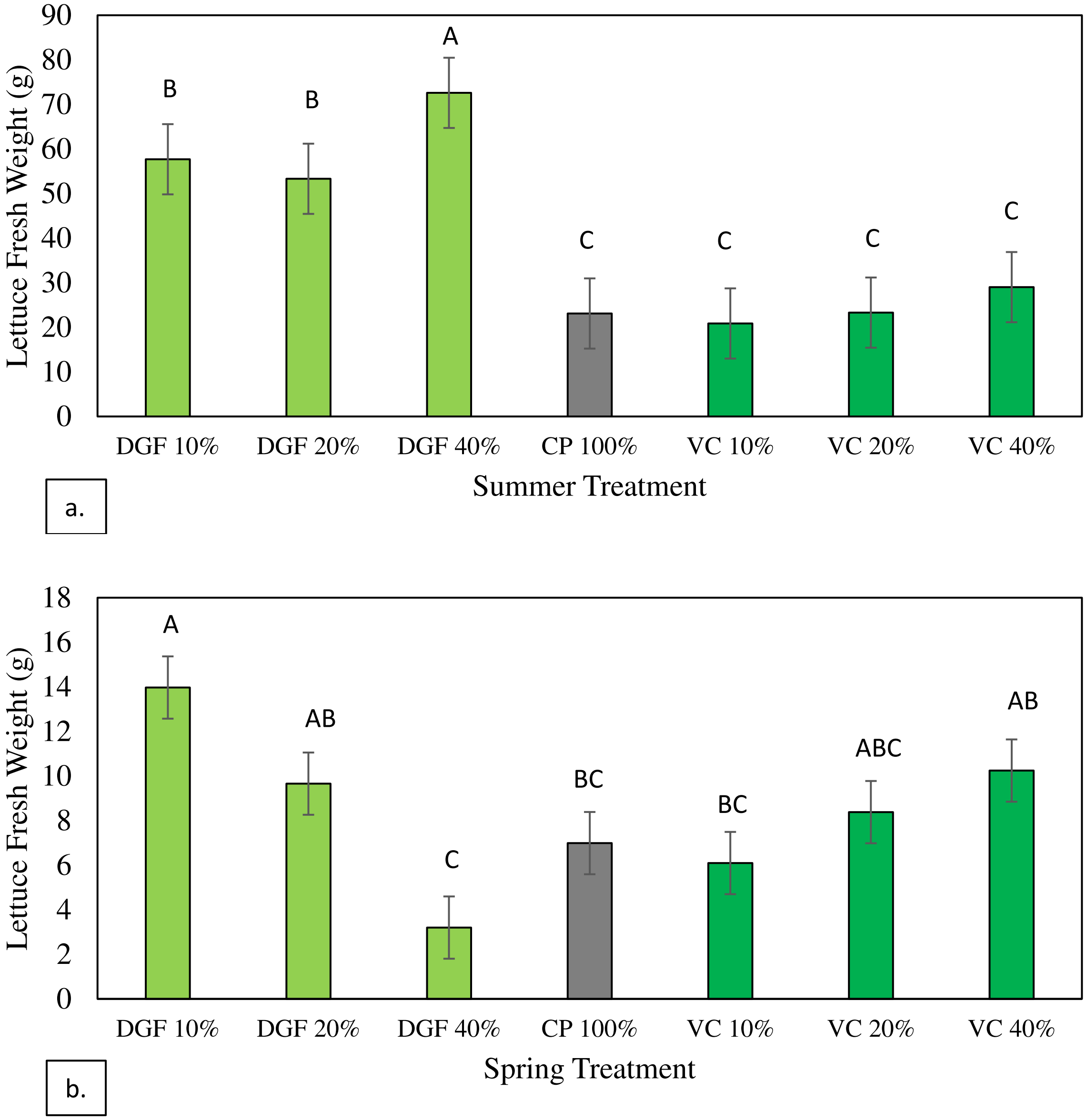

Figure 3.4: ***Fresh weight (grams) of lettuce grown in BSFL- DGF amended peat in a greenhouse, a. summer 2021 and b. spring 2022.*** Lettuce was grown with mixtures of commercial peat and distillery grain black soldier fly larvae frass (DGF: light green columns) or vermicompost (VC: Kelly green columns). Commercial peat (100%) served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p < 0.0001$/F=56.16; b. $p < 0.0001$/F=6.01).

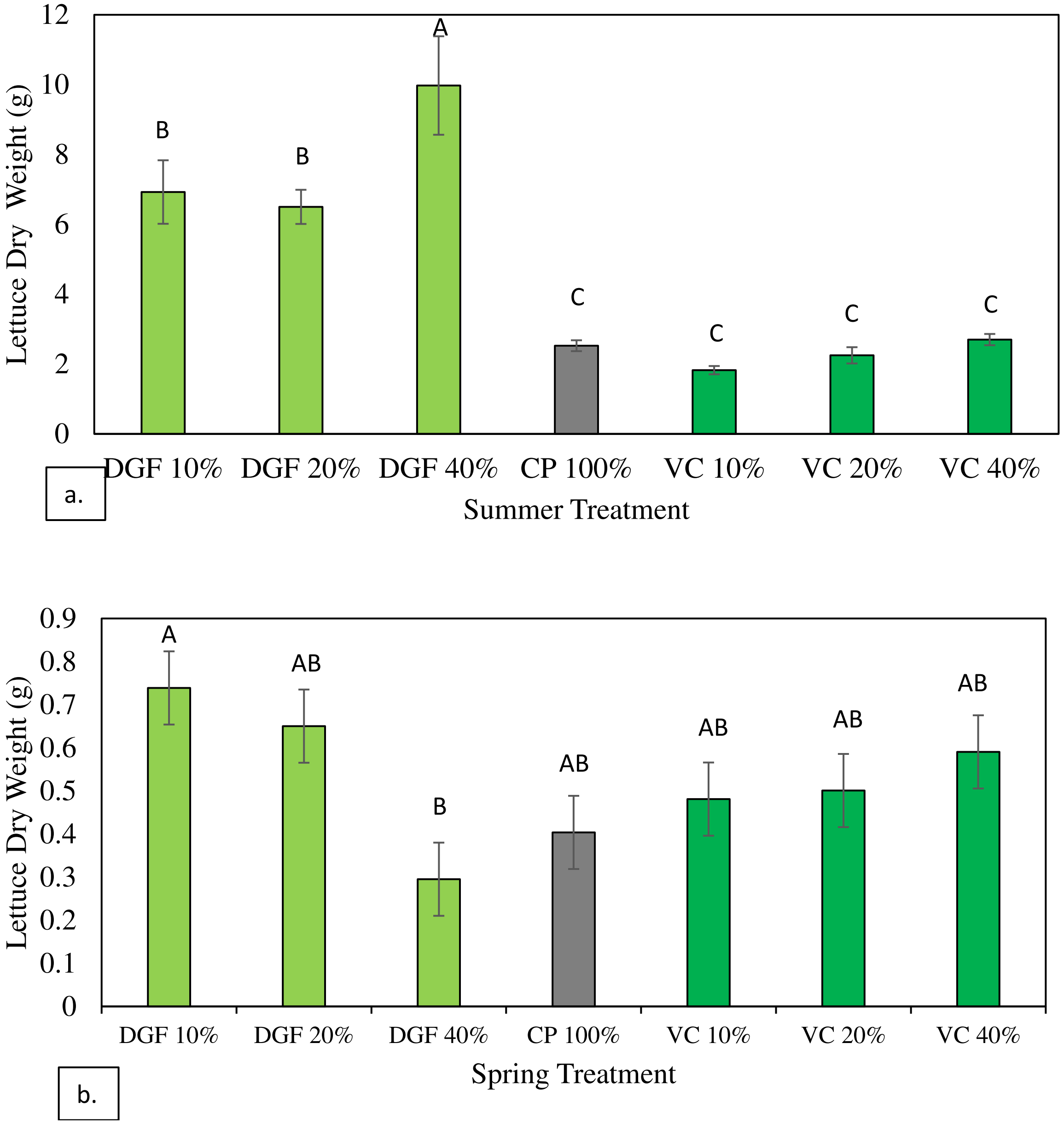

Figure 3.5: ***Lettuce dry weight (grams) of BSFL- DGF amended peat grown in a greenhouse, a. summer 2021 and b. spring 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and distillery grain frass (DGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (a.p < 0.0001/F=56.16; b. p = 0.0094/F=3.14).

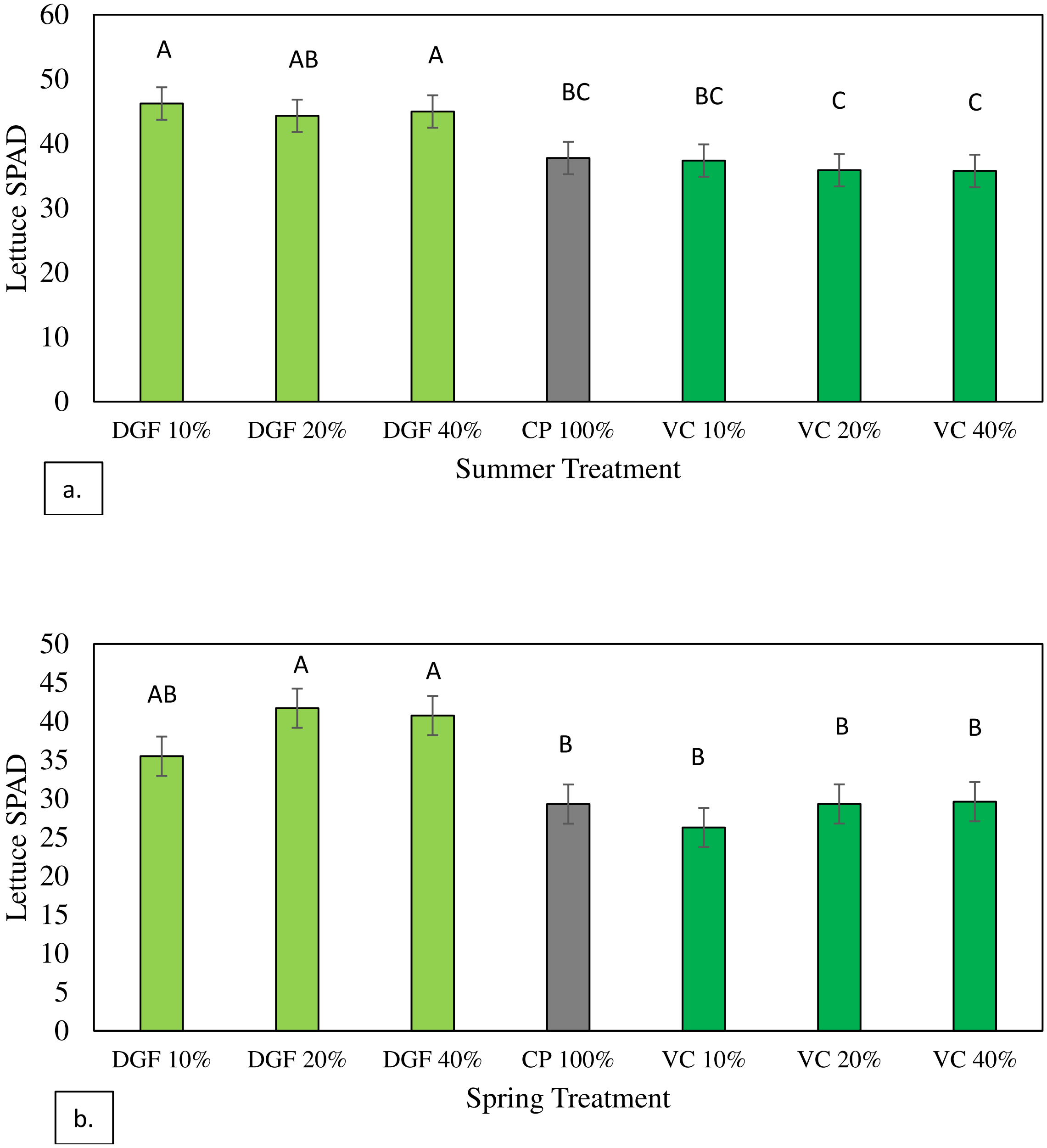

Figure 3.6: ***Lettuce SPAD of BSFL- DGF amended peat grown in a greenhouse, a. summer 2021 and b. spring 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and distillery grain frass (DGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (a. $p = 0.006$/ F=3.37; b. $p < 0.0001$/F=5.86).

Brewery grain digested frass (BGF) - Arugula

In spring 2022, a clear trend emerged in the arugula fresh yield data. The vermicompost treatments were significantly higher than the brewery grain digested frass (BGF) treatments (Figure 3.7a.). All three were comparable to the control (3.14g), but BGF 40% produced the lowest numerical yield (0.63g), while VC 40% was the highest (6.07g). The fresh weight results were confirmed by the dry weight analysis (Figure 3.8a.). Notable statistical differences occurred in both the BGF 20% (0.091g) and BGF 40% (0.067g) treatments, which produce the lowest yields. In the spring, there were no statistically significant differences in arugula greenness based on treatment (Figure 3.9a.).

The summer produced similar arugula fresh yield results as those grown in the spring study, but all frass treatments (0.33-3.85g) were significantly lower than the control and the vermicompost treatments (6.8-9.42g) (Figure 3.7b.). This was also observed in the dry weight analysis (Figure 3.8b.). For both wet and dry weights, BGF 40% produced the lowest yields (0.082g). There were also no significant differences in arugula greenness based on treatment in the summer trials. However, BGF 10% produced the greenest plants on average (43.77) (Figure 3.9b.).

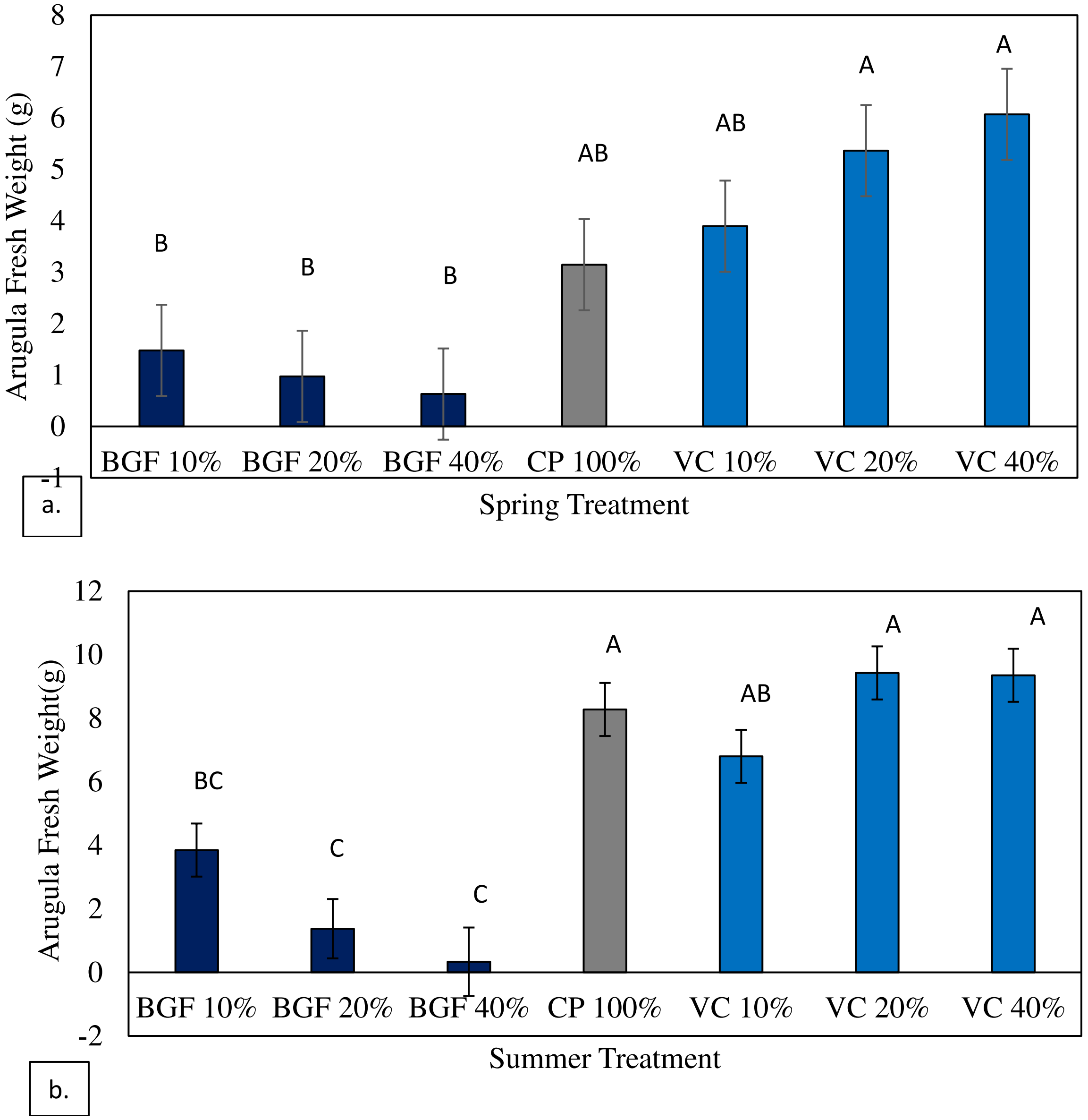

Figure 3.7: ***Fresh weight of arugula grown in BSFL- BGF amended peat in a greenhouse, a. spring 2022 and b. summer 2022.*** Arugula was grown with mixtures of commercial peat and brewery grain black soldier fly larvae frass (BGF: dark blue columns) or vermicompost (VC: sky blue columns). Commercial peat (100%) served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p < 0.0001$/F=5.89 ; b. $p < 0.0001$ /F=16.66).

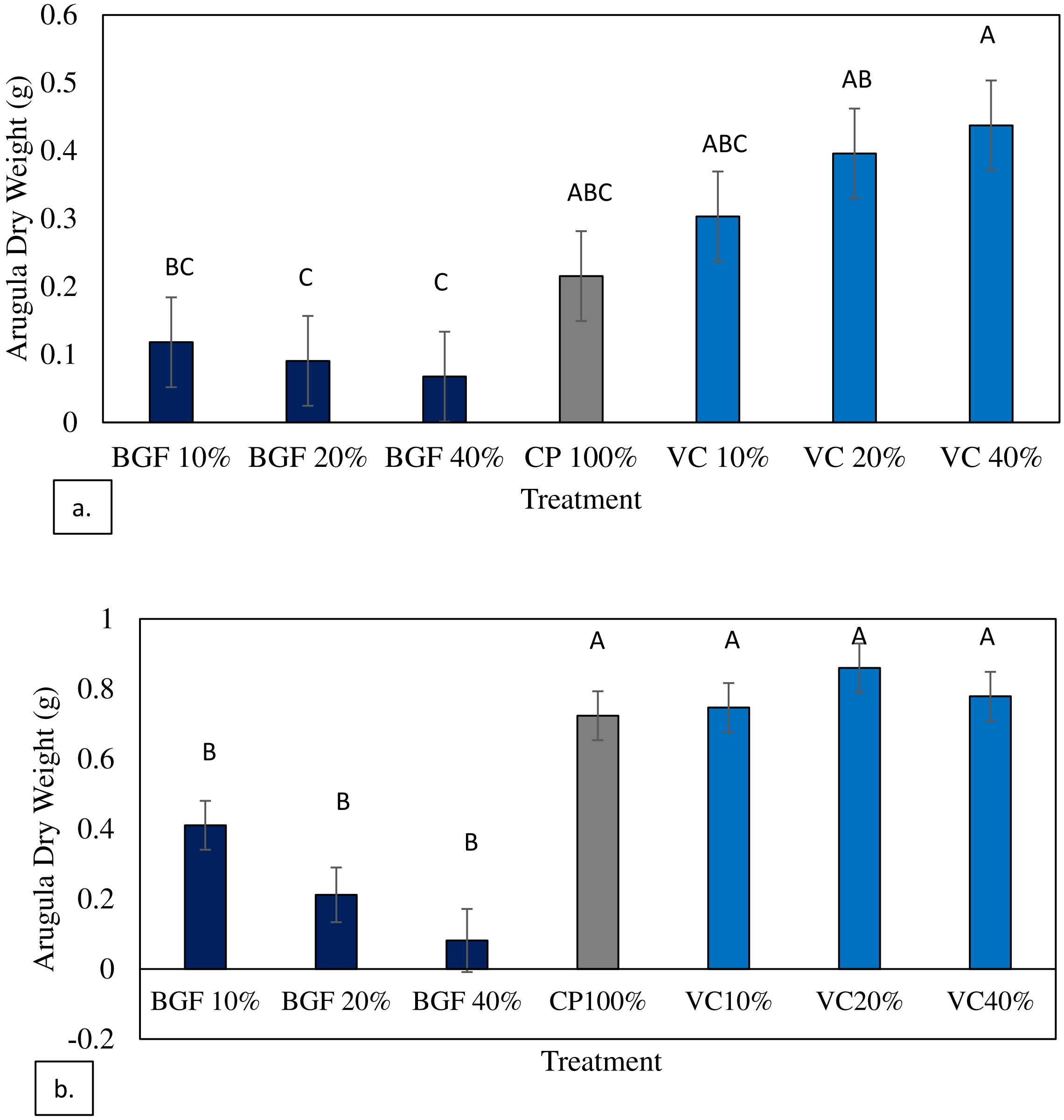

Figure 3.8: ***Arugula dry weight of*** **BSFL-** ***BGF amended peat grown in a greenhouse, a. spring 2022 and b. summer 2022.*** Arugula was grown with mixtures of commercial peat (CP) and brewery grain frass (BGF) or vermicompost (VC). Commercial peat (100%) served as the control pot differences, df = 6 (a. $p = 0.0002/F=5.12$; b. $p < 0.0001/F=15.47$).

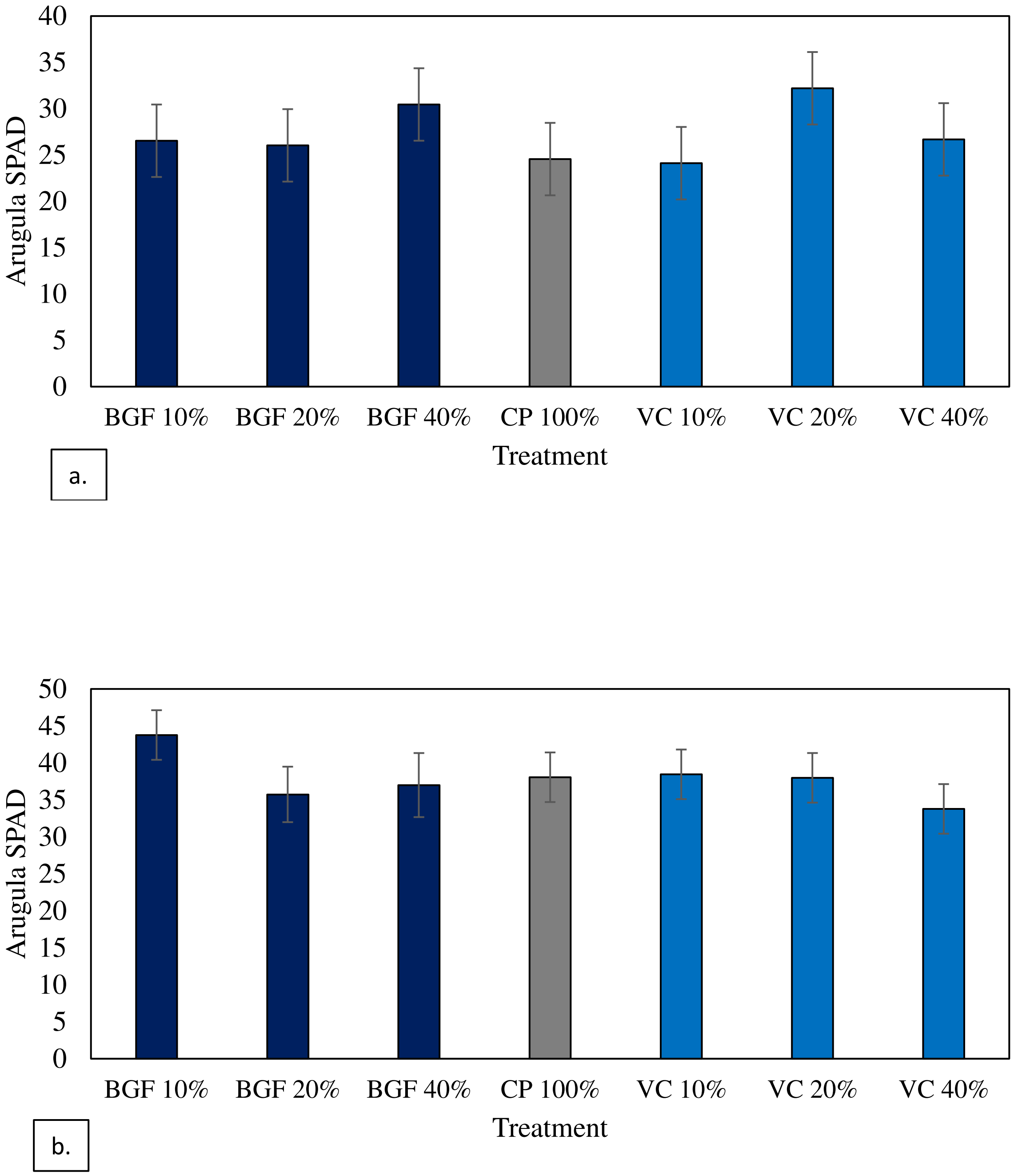

Figure 3.9: ***Arugula SPAD measurements of BSFL- BGF amended peat grown in a greenhouse, a. spring 2022 and b. summer 2022.*** Arugula was grown with mixtures of commercial peat (CP) and brewery grain frass (BGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (a. p = 0.74/F=0.59; b. p = 0.55 /F=0.83).

Brewery grain-digested frass- Lettuce

Lettuce media amended with BGF in the spring produced slightly higher fresh and dry yield results than arugula grown in the same season (Figure 3.10a. and 3.11a.). For both fresh and dry weights, BGF 40% (1 g; 0.10g) produced the lowest yield, VC 40% (10 g; 0.5 g) produced the highest yield, while all other treatments and the control were statistically similar to one another. There were some differences in lettuce greenness to note during the spring (Figure 3.12a.). For example, BGF 10% (38) and BGF 20% (37) produced the highest SPAD measurements, while BGF 40% (25) produced the lowest.

Lettuce yield was lower in the summer compared to the spring (Figure 3.10b. and 3.11b.). The BGF treatments yields were significantly lower than the control and vermicompost treatments, for both wet and dry weights. BGF 40% produced the lowest fresh and dry yield (2 g; 25 g), while the VC treatments produced the highest yields (35 – 45 g; 26 – 30 g). Significant differences in SPAD measurements indicate that BSF 40% (25) produced the least green plants (Figure 3.12b.).

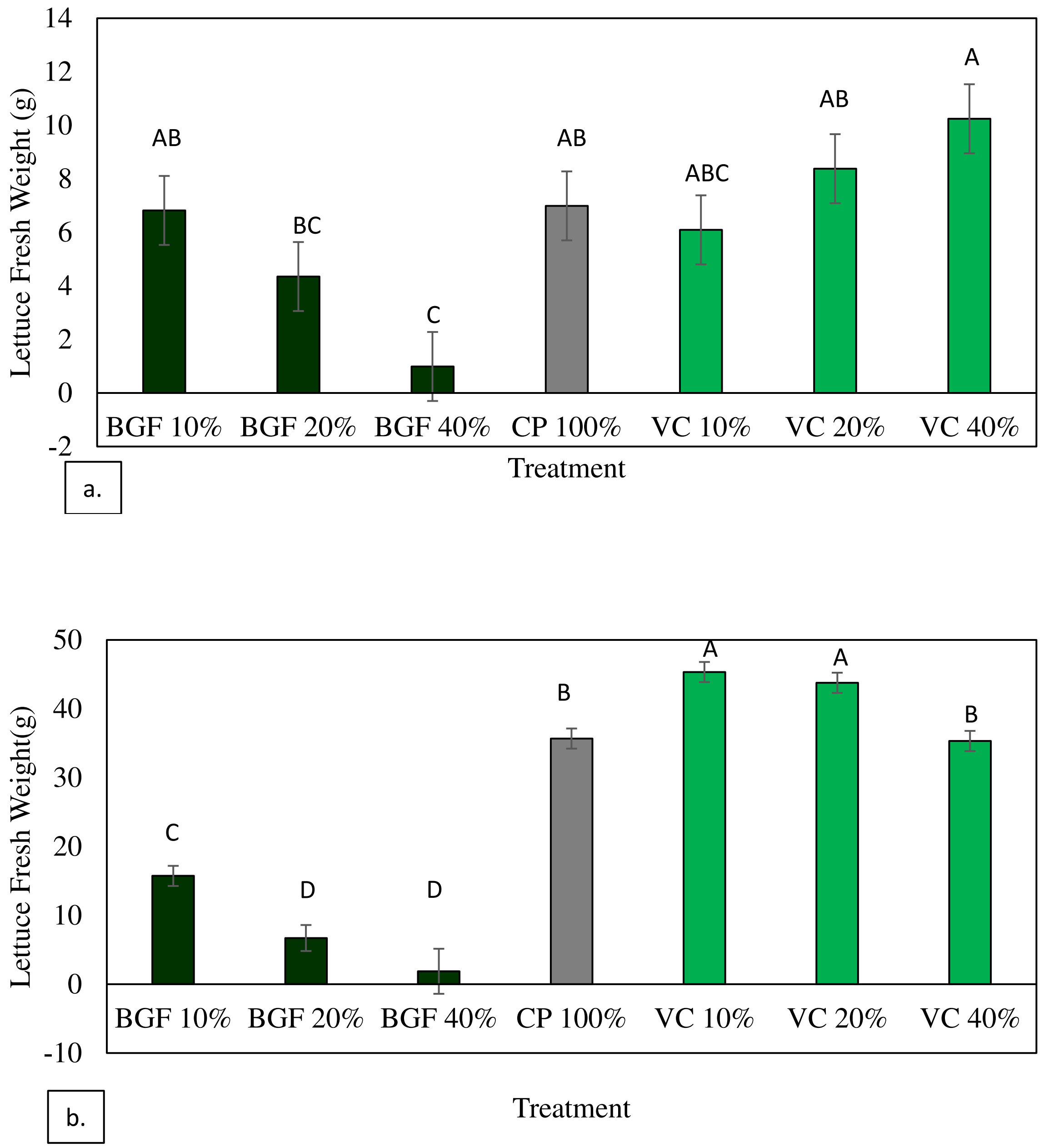

Figure 3.10: ***Fresh weight (grams) of lettuce grown in BSFL- BGF amended peat in a greenhouse, a. spring 2022 and b. summer 2022.*** Lettuce was grown with mixtures of commercial peat and brewery grain black soldier fly larvae frass (BGF: dark green columns) or vermicompost (VC: Kelly green columns). Commercial peat(100%) served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. p = 0.0002/F=5.28 ; b. p < 0.0001/F=90.79).

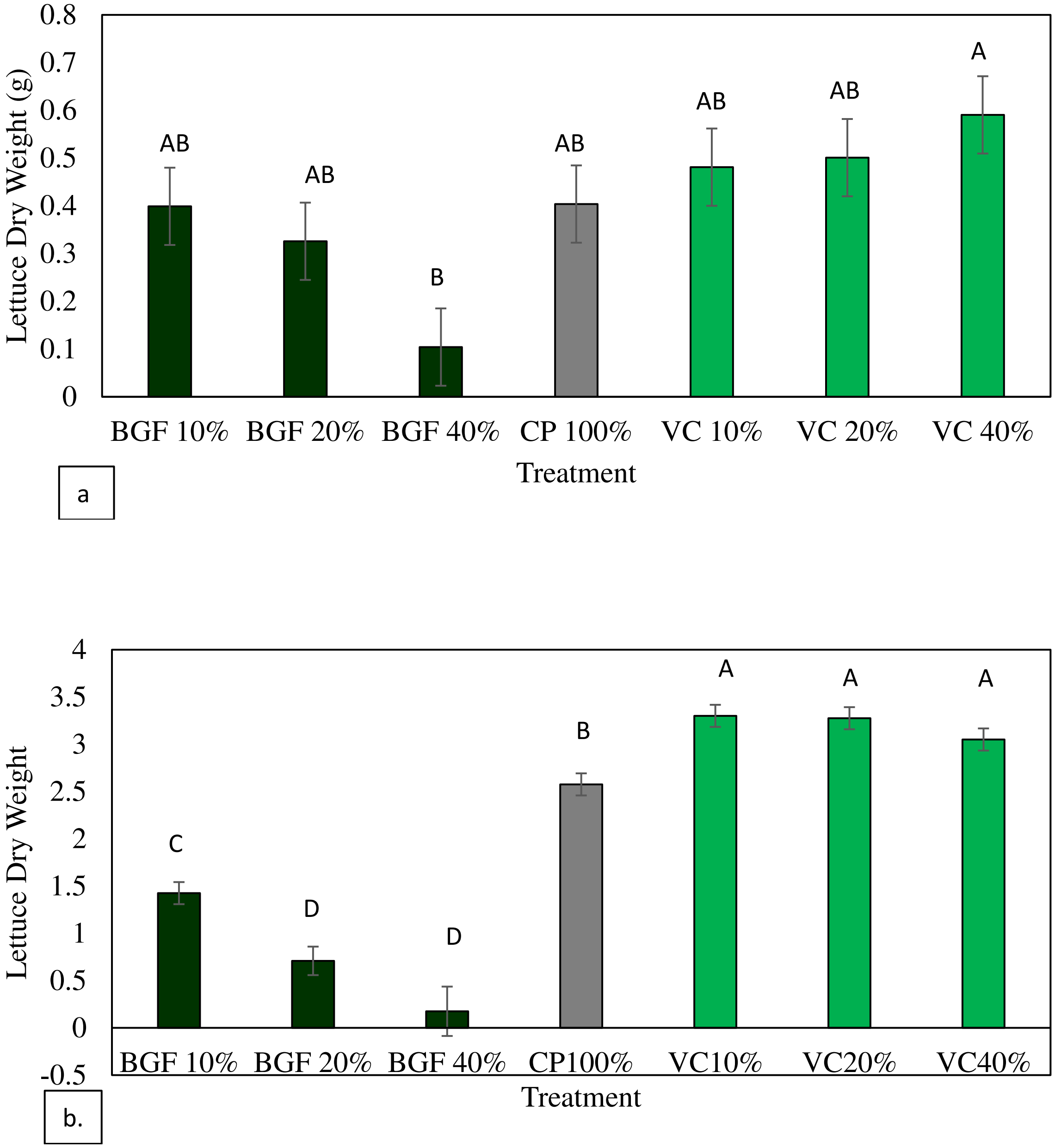

Figure 3.11: ***Lettuce dry weight (grams) of BSFL- BGF amended peat grown in a greenhouse, a. spring 2022 and b. summer 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and brewery grain frass (BGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (a .p = 0.0032/F=3.72 ; b. p < 0.0001)/F=69.42).

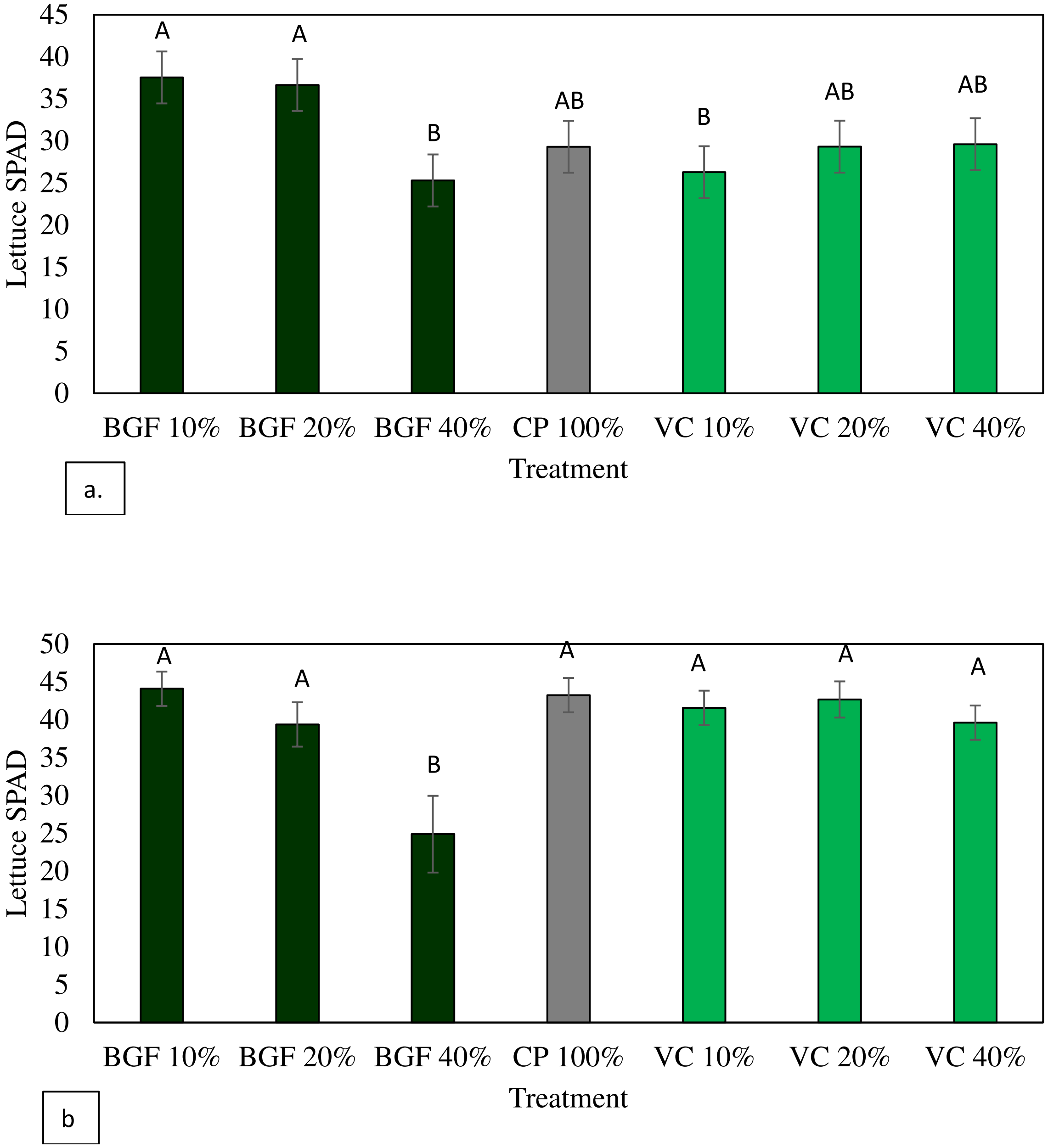

Figure 3.12: ***Lettuce SPAD measurements of BSFL- BGF amended lettuce grown in a greenhouse, a. spring 2022 and b. summer 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and brewery grain frass (BGF) or vermicompost (VC). Commercial peat (100%) served as the control pot, df = 6 (a. p = 0.040/F=2.37; b. p = 0.045/F=2.35).

Distillery grain frass (DGF) vs brewery grain digested (BGF) frass Spring 2022

For arugula yield in the spring of 2022 (Figure 3.13) it was evident that distillery grains produce significantly higher fresh yields than brewery grains. The only treatment not comparable to the control (3 g) was BGF 40% (2 g). The frass 40% treatment was also the lowest yield of the three DGF treatments. The dry weight analysis revealed that lower DGF 20% (0.5 g) was significantly higher than the BGF treatments (0.07 - 0.12g) and control (0.22 g) (Figure 3.14). DGF 10% (0.36 g) was significantly higher than the BGF treatments, but comparable to the control. The ANOVA results for SPAD reveal there were no differences in arugula greenness based on treatment (Figure 3.15). However, this data failed the tests for normality and equal variances. Follow up nonparametric Wilcoxon tests were conducted for further analysis. The χ^2 (14.20) results indicate a significant difference between treatments (p = 0.028). Dunn ranks for multiple comparisons confirmed a difference between DGF 40% (37) and the control (25).

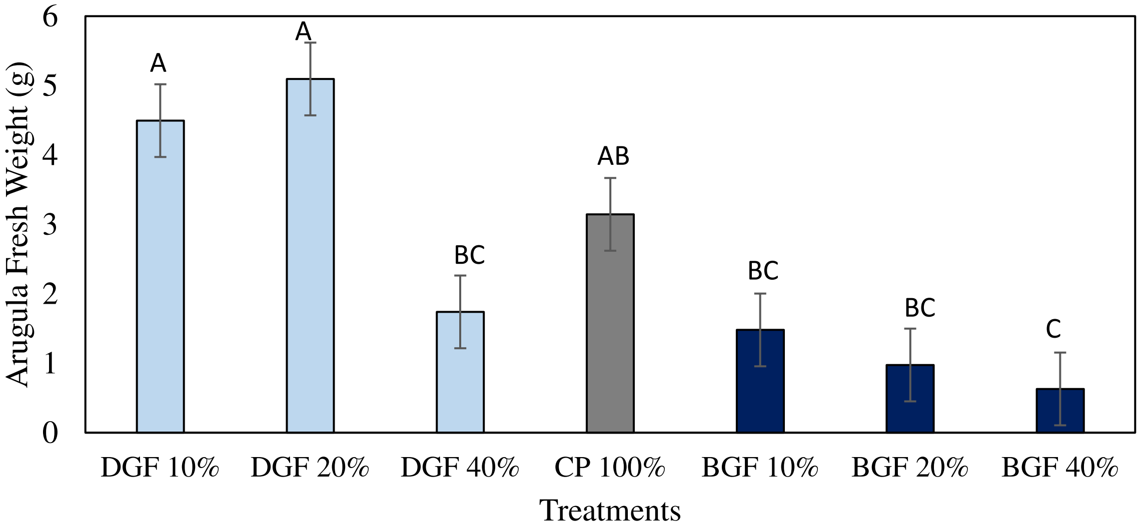

Figure 3.13: ***Fresh weight (g) of arugula grown in DGF and BGF amended peat in a greenhouse, spring 2022.*** Arugula was grown with mixtures of commercial peat and black soldier fly larvae frass (DGF: light blue columns) or (BGF: dark blue columns). Commercial peat served (100%) as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (p < 0.0001/F=11.28).

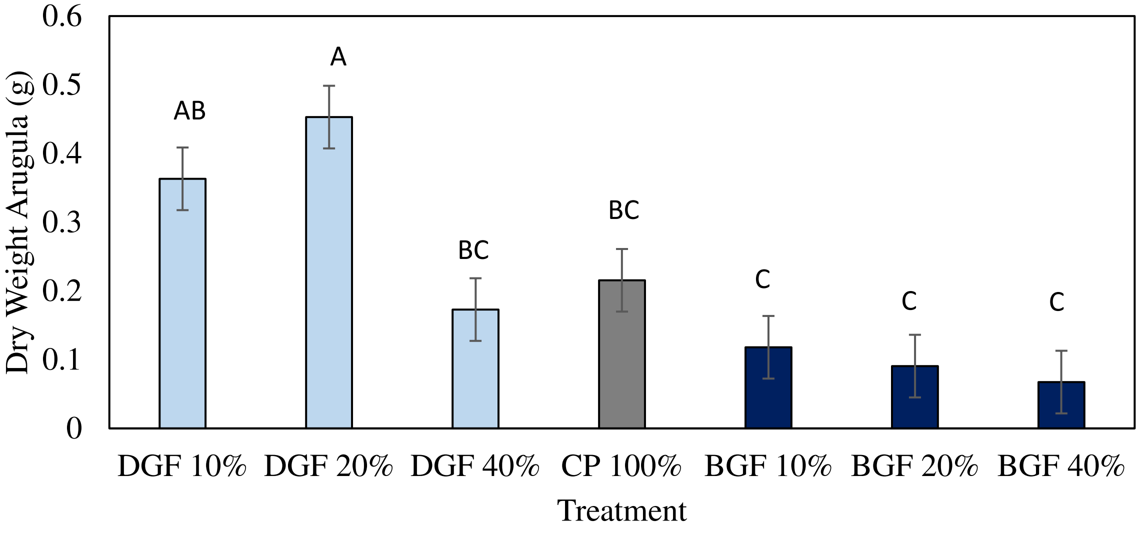

Figure 3.14: ***Arugula dry weight (g) of DGF- BGF amended peat grown in a greenhouse, spring 2022.*** Arugula was grown with mixtures of commercial peat (CP) and distillery grain (DGF) or brewery grain (BGF) larvae frass. Commercial peat (100%) served the control pot, df = 6 ($p < 0.0001$/F=10.19).

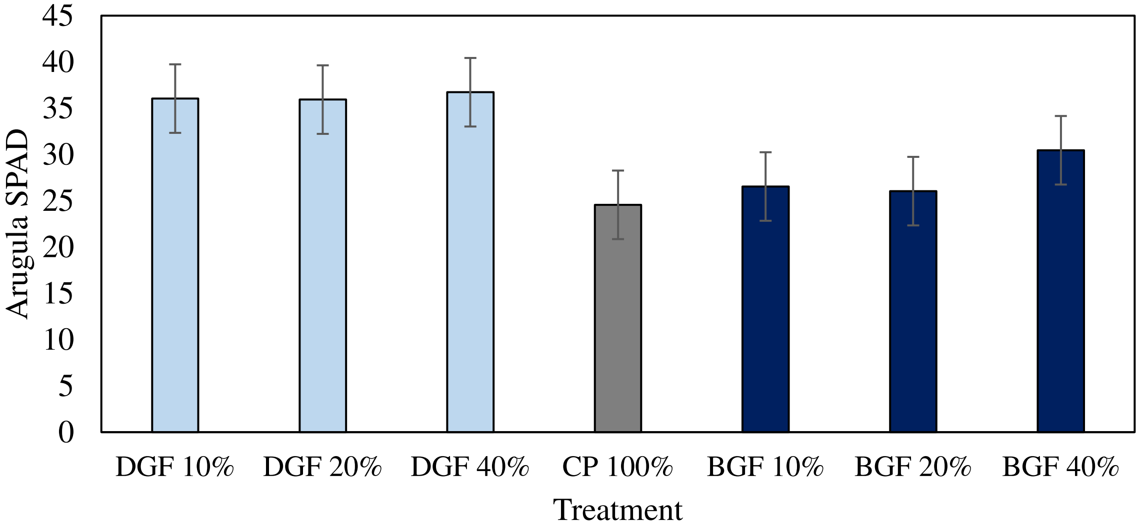

Figure 3.15: ***Arugula SPAD measurements of DGF and BGF amended peat grown in a greenhouse, spring 2022.*** Arugula was grown with mixtures of commercial peat (CP) and distillery grain (DGF) or brewery grain (BGF) larvae frass. Commercial peat (100%) served as the control pot, df = 6 ($p = 0.072$/F=2.05).

The fresh yield results for lettuce were similar to the arugula yield results (Figure 3.16). The DGF 10% treatment (14 g) was significantly higher than the control (7 g). The BGF 40% treatment (1 g) produced the lowest fresh yields. Dry weights for DGF 10% (0.74 g) were significantly higher than all of the BGF treatments (0.096-0.40 g) and the peat control (0.40 g) (Figure 3.17). BGF 40% produced the lowest dry yield. There were some differences in lettuce greenness based on treatment. Most notably, the BGF 40% treatment had the lowest SPAD measurements (26) (Figure 3.18).

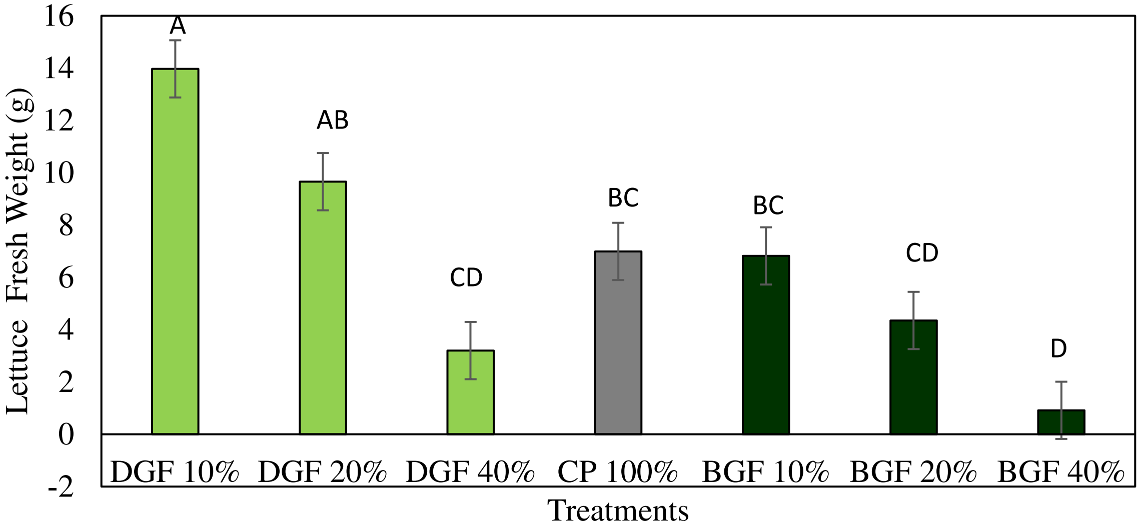

Figure 3.16: ***Fresh weight (grams) of lettuce grown in DGF and BGF amended peat in a greenhouse, spring 2022.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (DGF: light green columns) or (BGF: dark green columns). Commercial peat (100%) served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 ($p < 0.0001$/F=15.70).

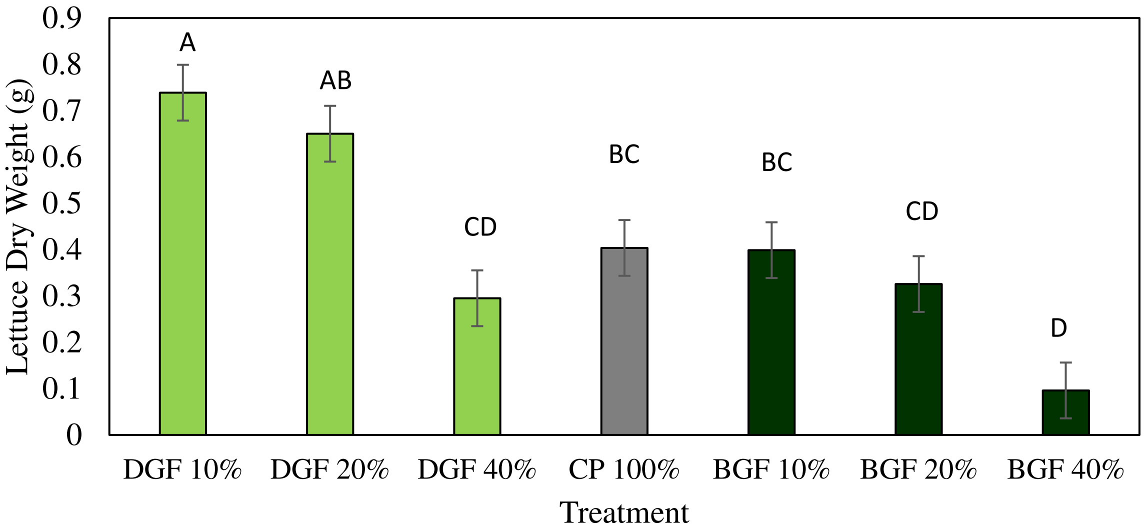

Figure 3.17: ***Lettuce dry weight (grams) of DGF and BGF amended peat grown in a greenhouse, spring 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and distillery grain (DGF) or brewery grain (BGF). Commercial peat (100%) served as the control pot, df = 6 (p < 0.0001/F=13.08).

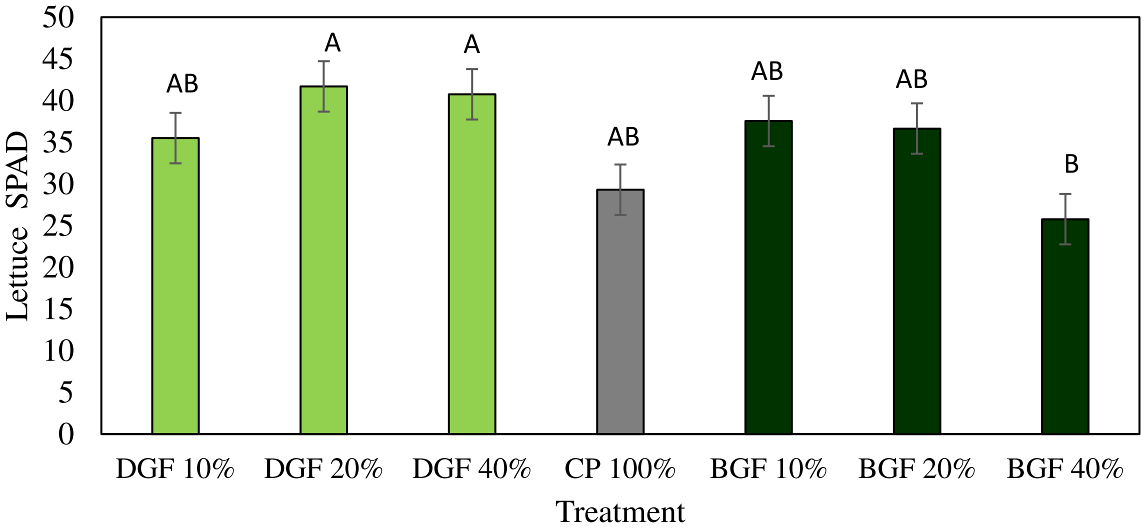

Figure 3.18: ***Lettuce SPAD measurement of DGDF and BGF amended peat grown in a greenhouse, spring 2022.*** Lettuce was grown with mixtures of commercial peat (CP) and distillery grain (DGF) or brewery grain (BGF). Commercial peat (100%) served as the control pot, df = 6 (p = 0.0032/F=3.72).

4. Discussion

Distillery grain digested frass

Crop impact depended on the season. For example, all BSFL-DGF treatments for both crops produced significantly higher fresh yields during the summer of 2021. In the spring of 2022, DGF 40% produced the lowest yields in both crops. The impacts of frass on greenness appeared to vary more depending on crop. BSFL frass treatments produced higher SPAD measurements in lettuce, regardless of season, and in arugula during the spring.

Brewery grain digested frass

BSFL-BGF treatments produced the lowest fresh yields in both crops and in both seasons. BGF 40% produced the lowest fresh and dry weights in all studies. VC treatments produced the highest fresh and dry yields and were consistently higher or comparable to the control. Like in the distillery grains, the impacts of frass on greenness varied noticeably by crop. Arugula did not produce significant differences in SPAD measurements based on treatment. In both seasons lettuce crops amended with BGF 40% produced the lowest SPAD measurements. The vermicompost treatments were greener or comparable to the control.

Distillery grain vs brewery grain digested frass

Diet seemed to have a significant impact on greenness, and especially on yield for both crops grown. Brewery grains treatments resulted in lower fresh and dry yields than distillery grains treatments. BSFL 40% applications for both frass amendments was the lowest in their respective treatments.

5. Conclusions

For distillery grains, all BSFL treatments in arugula and lettuce were equal to or better than the 100% peat control, regardless of season. For brewery grains, most treatments in arugula and lettuce showed lower or comparable to the peat control, regardless of season. This indicates that the diet of the larvae seems to be a significant factor in determining the impacts on vegetable yield. Potential differences in nutrient concentrations of the amendments could be responsible for this response. The nutrient profile of the three different amendments and control peat were characterized, discussed in Chapter 4, to improve the understanding of this impact.

Arugula rarely produced differences in greenness regardless of season, year, or treatment. Lettuce greenness tended to be improved by low concentrations of frass, 10-20%. SPAD is a relative metric to indicate differences in greenness, which can often be correlated with nitrogen and chlorophyl concentrations. Studies with romaine lettuce grown in high tunnels revealed that an increase in SPAD measurements also resulted in increases in leaf nitrogen and chlorophyl concentrations (Mendoza-Tafolla *et al.,* 2019). The plant tissue analysis, Chapter 4, was conducted to determine if there were differences in nutrient concentrations based on treatment.

Overall lettuce seems to be more responsive to frass treatments, in both yield and greenness, compared to arugula. Lettuce is a conventionally successful crop because of its ubiquitous acclimation to different soil conditions (Swiader and Ware, 2002). Lettuce, is not only a more domesticated crop, but also a more completely researched one (Koukounaras *et al.,* 2020), which highlights the importance of including both crops in this study. The difference in seasonal productivity could be simply explained by day length and intensity. It could also be attributed to frass age, local weather patterns, or source materials. Arugula harvested in field trials during July resulted in lower post-harvest quality compared to those harvested during cooler months (Koukounaras *et al.,* 2020). Though we saw better yields in our first summer season than the spring, it is possible that other quality parameters, like post-harvest shelf life are also impacted by season. Future research would benefit from standardizing season and manure age and replicating over several years. Restricting our research to an even more controlled environment, like growth chambers, could eliminate seasonal effects that may have impacted some of the results in this study.

A study by Fernández-Romero *et al*., (2016) observed the impacts of BSFL frass on lettuce production and found increases in soil organic matter and residual nutrient content. This increase in soil organic matter could indicate potential reductions in nutrient run-off (Fernández-Romero *et al.,* 2016), like those seen in vermicompost (Elliot *et al.,* 2007). Differences in enzymatic activity of the soil amendments have also been observed (Esteves *et al.,* 2022), which could also lead to future improvements in soil health and function. By reducing peat consumption, redirecting waste streams, and improving local landscapes, the effects of frass adoption could have longer term impacts than just the potential improvements in crop yield. The research on insect frass is sparse and variable. However, it is still a worthy field of research in order explore novel improvements of the food system. Documented improvements in yield, vegetable quality, and soil health could be an incentive for growers to mitigate some of the harmful consequences of horticultural production. Recommendation to growers interested in adopting insect frass in their potting mixes is to first consider larval diet and start with a 10% peat replacement.

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CHAPTER FOUR

THE IMPACTS OF BLACK SOLDIER FLY LARVAE FRASS ON THE NUTRIENT CONCENTRATIONS OF ARUGULA AND LETTUCE

1. Introduction

Plant growth requirements vary by species, but certain requirements like light, water, and nutrients are ubiquitous necessities across the plant kingdom (Swiader and Ware, 2002). Important macronutrients that are often limiting for plants and considered primary are: nitrogen (N), phosphorus (P), and potassium (K). Less limiting macronutrients, or secondary nutrients, are: calcium (Ca), magnesium (Mg), and sulfur (S). Micronutrients that are also required for plant health but needed in relatively small amounts are boron (B), iron (Fe), manganese (Mn), copper (Cu), Nickel (Ni), and zinc (Zn). Other nutrients like sodium (Na) and aluminum (Al) are only required by certain plants and under certain conditions (Chapin *et al.,* 2011).

Frass is the waste material and shed exoskeletons of insects. The original waste material (such as feed stock) fed to the larvae is altered by their gut microbiota and improved during insect digestion (Kagata and Ohgushi, 2012). After digestion, N, P, and K become more bioavailable for potential plant uptake during development. Overall, total nutrients are reduced and any potential leaching and loss to neighboring environments may also be reduced (Kagata and Ohgushi, 2012). Due to its concentration of N and P, frass may serve as a supplement or substitute for inorganic fertilizers that are typically applied during the crop production cycle (Schmitt, 2020). Decreases in inorganic fertilizer application can reduce subsequent environmental problems, such as soil degradation and eutrophication (Xiao *et al*., 2018).

Including frass as an amendment for fertility also decreases the amount of peat required in greenhouse production, reducing the overall necessity for peatland disruptions.

Soil characteristics may also experience alterations with frass applications (Houben *et al.*, 2020) The nutrient profiles of raw manure and mealworm frass from Ÿnsect (ŸnFrass, Paris, France) were compared. Similar concentrations of N, P, and K were observed. Additionally, nitrogen mineralization and decomposition in the frass treatments occurred more quickly than in raw manure (Houben et al., 2020). Lower soluble P concentrations were also observed in the frass treatments. Increases in P bioavailability of black soldier fly larvae frass treated samples has been reported in several studies (Klammsteiner *et al.*, 2019; Temple *et al.*, 2013). BSFL frass treatments have also produced improvements in soil organic matter (Zhan and Quilliam, 2017).

Insect frass may also improve plant nutrient utilization. Insect frass has been studied mostly for the purpose of understanding its impacts on yield and other plant growth parameters, but only a few studies have monitored the impact on plant nutrition. The nutrient concentration of lettuce leaves grown in a greenhouse study were observed (Esteves *et al.*, 2022). Treatments were comprised of a varying blend of mineral fertilizer and BSFL frass fertilizers to complete a 100% N profile. There were no differences in K utilization, and N, Fe, and Mg concentrations were highest in the mineral fertilizer only control. However, P was lowest in the high frass treatments and manganese was highest in the high frass treatments. Na, Ca, Cu, and Zn were highest in the unfertilized control and the low frass treatment.

A study compared raw manure with regular coffee husks and coffee husks that had been digested by BSFL (Putra *et al.*, 2017). Differences in lettuce nutrient utilization depending on treatment were observed. N utilization was highest in the raw manure treatments. However, P and K utilization were highest in the BSFL treatment. Another study analyzed cabbage tissues after the application of insect frass compost compared to a commercial fertilizer (Choi *et al.,* 2009). Contrary to the results from the coffee husk study, P was the only mineral content measured that produced significantly lower P concentrations in the insect frass compost treatment (Choi *et al.,* 2009).

Another greenhouse pot study measured the NPK concentrations of barley grown with a conventional fertilizer treatment, an insect frass treatment, a 50%frass/50% fertilizer treatment, and an unfertilized control (Houben et al., 2020). There were no significant differences between the three treatments, but all NPK concentrations of barley were higher in the treatments than the control (Houben *et al.,* 2020). Maize grown in insect frass, insect larval skins, insect adult bodies, organic fertilizers, and inorganic fertilizers was harvested and analyzed for nutrient content (Gaarttling *et al.,* 2020). N concentrations were lowest in the frass and larval skin treatments, and highest in the adult body and inorganic fertilizer treatments. The opposite was observed for P concentrations; percent P was highest in frass treatments and lowest in adult body and organic fertilizer treatments (Garttling *et al.,* 2020).

Vermicompost amendments have produced promising results for nutrient uptake in vegetable crops. Humic substances extracted from vermicompost improved yields and an increase in protein and nitrates in field grown lettuce (Hernandez *et al.,* 2015). In another study, vermicompost was added to a greenhouse potting medium at increasing percentages. Tomato seedling tissues, with or without conventional fertilizers, produced increases in N concentration when also grown in high vermicompost treatments (Atiyeh *et al.,* 2001). Additionally, fertilizer combined with vermicompost can increase the NPK content of potato tubers in the field (Yourtchi *et al.,* 2013). In addition, the NPK uptake of field grown rosemary was highest in a conventional fertility treatment, but second highest in a slightly reduced fertilizer treatment supplemented with 0.4 kilograms per square meter vermicompost amendment (Singh and Wasnik, 2013).

This improved nutrient utilization by various crops indicates greater uptake of nutrients from the soil media. Ultimately, all of these alterations may lead to decreases in leaching and loss to the environment**. Improved nutrient utilization and nutrient bioavailability indicate that an improvement in the quality of crops treated with frass applications could be anticipated.** A greenhouse study was conducted from June to July of 2021 and March to April of 2022 that included two different vegetable crop species: arugula (*Eruca sativa*) and lettuce (*Lactuca sativa*). Our main objective was to investigate the impact of insect frass on vegetable nutrient concentrations and subsequent measurements in edible crop tissues.

Question: How does distillery grain-digested frass impact nutrient concentrations of arugula and lettuce?

Hypothesis: Distillery grain digested frass treatments will have greater concentrations of nutrients in harvested tissues than vermicompost treatments and the control.

Question: How do the impacts of distillery grain-digested frass compare to the impacts of brewery grain-digested frass on arugula and lettuce nutrient concentrations?

Hypothesis: Nutrient concentrations of BSFL digested distillery grain frass treatments will be higher than those in the peat control and BSFL brewery grain-digested frass treatments.

2. Materials and Methods

These experiments were conducted in Fort Collins CO, at the CSU Horticulture Center greenhouse, elevation 1,525.22 m (en-us.topographic-map.com), from June of 2021 to April of 2022. Both experiments were conducted as completely randomized designs (CRD) using 7 levels total (three treatments of each amendment and a 100% commercial peat control), two crop species, and 10 replicates/level over the three growing periods (Table 4.1).

Table 4.1: Amendment treatment summary for 2021-2022 greenhouse vegetable studies. This design applied to arugula and lettuce: vermicompost (VC) and black solider fly larvae (BSFL) frass**.**

Black soldier fly larvae frass treatments				**Vermicompost treatments**			
Peat %	**Peat (L)**	**BSFL %**	**BSFL (L)**	**Peat %**	**Peat (L)**	**VC %**	**VC (L)**
60	4.8	40	3.2	60	4.8	40	3.2
80	6.4	20	1.6	80	6.4	20	1.6
90	7.2	10	0.8	90	7.2	10	0.8
100	8.0	0	0.0	100	8.0	0	0.0

Lettuce (‘Salvius MT0’), and arugula (‘Apollo’) were started as plugs in 288 cell trays with an organic growing mix of the following composition: 3.79 L peat, 0.47 L vermicompost, 29.57 mL blood meal, and29.57 mL bone meal. They were grown on warming benches for two to four weeks in the greenhouse, and then one to three plugs were transplanted into “1-gallon” pots (2L in volume) with the amendment treatments in the greenhouse at 20-25°C. The amendments were: black soldier fly larvae (BSFL) frass from distillery grains, BSFL frass from brewery grains, and vermicompost (VC). The amendments were mixed into a traditional growing peat media at percentages of amendment of 40%, 20%, 10%, in addition to a 100% peat control (Table 4.1). Inorganic fertilizer (24-8-16 NPK) Miracle Gro (Marysville, Ohio) was applied at the label recommended rate (3.7 mL per 0.95 L) for each crop, every other week.

In the summer of 2021, seedlings were started on the bench and transplanted into 2 liter pots on June 23. Harvest took place on July 30 and fresh weights were collected. In the spring of 2022, seedlings were started on the bench and transplanted into pots on March 13. Harvest took place on April 10.

Plant Tissue Analysis

Only two of the three studies were analyzed for plant tissue concentrations. The summer 2021 distillery grain-digested frass study and the Spring 2022 distillery grain-digested frass compared to the brewery grain-digested frass study were chosen. After harvest, arugula and lettuce were dried in the drying oven at 70ºCelsius. The samples were homogenized, ground, and sent to Brookside Labs (New Bremen, OH) for plant tissue analysis on a dry weight basis. In the summer 2021 there was sufficient material total to produce three replicates per treatment to be used for statistical analysis. Spring 2022 only produced enough ground dried material for one replicate per treatment and was not statistically analyzed but is characterized later in this chapter.

Compost Analysis

There were four sources used in the potting mixes: distillery grain digested frass, brewery grain digested frass (EVO Conversions Systems: College Station, TX), vermicompost (Rocky Mountain Soil Stewardship: Fort Collins, CO), and peat (Berger B6: Saint-Modeste, QC) One quart of each, unaltered material was sent to Brookside Labs (New Bremen, OH) for a standard compost analysis.

Statistical Analysis

Statistical analysis was conducted using JMP®, Pro 16 (SAS Institute Inc., Cary, NC, 2022). Percent data was converted to proportions and transformed with the logit function before analysis. The Anderson-Darling test for normality and the Levene’s test for equal variance were conducted to determine if the assumptions for an ANOVA were met. When they were not met, non-parametric Wilcoxon rank sums tests were conducted in addition to the parametric tests. A threshold of a $p \leq 0.05$ was considered significant. Tukey HD and Dunn method for Joint Ranks pair wise comparisons were conducted to assess and test for differences between each level. Mean and statistic summary tables can be found in Appendix II.

3. Results

Compost Analysis

The different amendments were characterized based on nutrient concentration in Table 4.2. Both frass diets, distillery grain (3.83%) and brewery grain (3.97%), had relatively high concentrations of N compared to vermicompost (1.88%) and peat (0.54%). Distillery grain also had the highest concentration of P (2.25%) and K (6.11%). These three elements are the primary macronutrients required for plant growth and could produce increases in yield. Brewery grain frass had the highest levels of sodium (1.15%) which can be disruptive to plant growth, while distillery grain frass had the highest concentration of B (52.1%). Though B is an important element for plants, it can become toxic at large concentrations. Fe (1068.62%) was extremely high in distillery grain frass and could also contribute to plant growth seen in Chapter 3. Notably, distillery grain frass had the highest pH, or was the most basic, while peat had the lowest pH and was the most acidic.

Table 4.2 Analysis of amendments used to create treatments 2021-2022. Distillery grain frass (DGF), brewery grain frass (BGF), and vermicompost (VC) were the treatments and peat was the control.

	DGF	BGF	VC	Peat
Nitrogen (%)	3.83	3.97	1.88	0.54
Phosphorous (%)	2.25	1.18	0.69	0.03
P2O5 (%)	5.16	2.71	1.58	0.06
Potassium (%)	6.11	1.32	1.35	0.13
K2O (%)	7.35	1.59	1.63	0.15
Calcium (%)	1.27	0.34	3.2	1.23
Magnesium (%)	0.98	0.43	0.71	0.37
Sodium (%)	0.52	1.15	0.05	0.22
Sulfur (%)	0.66	0.5	0.32	0.16
Boron (ppm)	52.1	18.27	39.7	14.39
Carbon (%)	37.49	41.56	214.60	38.35
Iron (ppm)	1068.62	367.03	11.320	851.52
Manganese (ppm)	157.32	57.31	0.436	49.25
Copper (ppm)	131.29	17.87	0.049	11.72
Zinc (ppm)	130.32	114.94	0.204	33.99
pH (%)	9.52	8.59	7.29	6.22
C/N (%)	9.79	10.48	10.08	71.15

Distillery grain digested frass vs vermicompost: plant tissue analysis (on a dry weight basis)

Primary Macronutrients: Nitrogen, Phosphorus, and Potassium

N concentrations of both arugula (7%) (Figure 4.1a.), and lettuce (5%) (Figure 4.2a.) tissues were significantly higher in the BSF 40% treatment. The other BSF treatments (20% and 10%) were also significantly higher in N than the control and vermicompost treatments. In both crops, the vermicompost treatments were comparable to the control. This aligns with the results from the compost analysis. Distillery grains had high levels of N, vermicompost had intermediate levels, while peat was very low in N.

P concentrations of arugula (Figure 4.1b.) were comparable across all BSFL and VC treatments, though all treatments were significantly higher than the control (0.34%). Lettuce P concentrations were significantly higher in the BSFL treatments (0.74-0.84%) (Figure 4.2b.), and the vermicompost treatments (0.46-0.54%) were significantly higher than the control (0.32%).

K concentrations in arugula and lettuce were very similar (Figures 4.1c. and 4.2c.). BSF 20% produced the highest concentrations in arugula (7%) and BSF 40% was highest in lettuce (8%), while the control peat produced the lowest K concentrations in both crops. Additionally, the BSFL treatments were significantly higher than most of the vermicompost treatments as well.

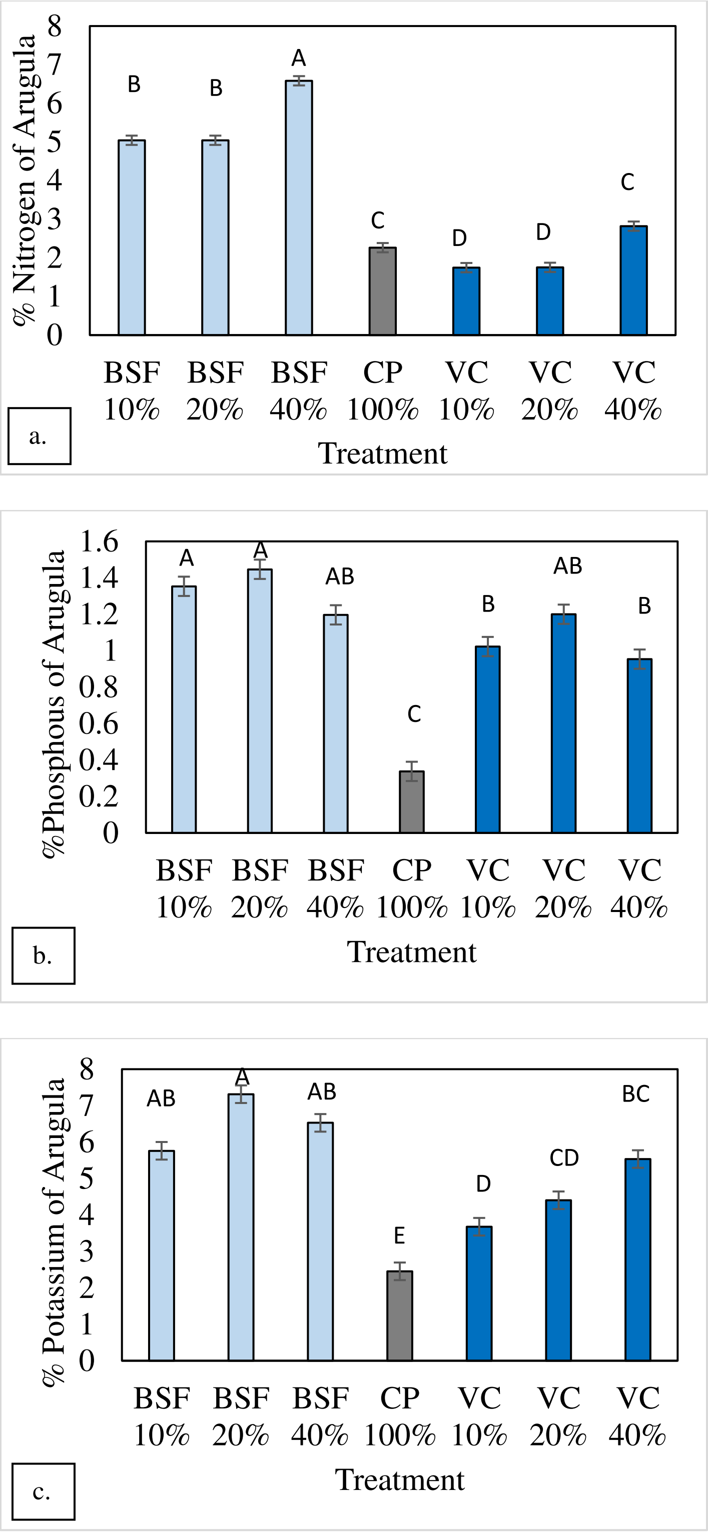


Figure 4.1: ***Concentration of primary macronutrients (a. N; b. P; c. K) of BSFL amended arugula grown in a greenhouse.*** Arugula was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light blue columns) or vermicompost (VC: sky blue columns). 100% commercial peat served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p < 0.0001$/F=109. 41; b. $p < 0.0001$/F=105.07; c. $p < 0.0001$/F=54.06).

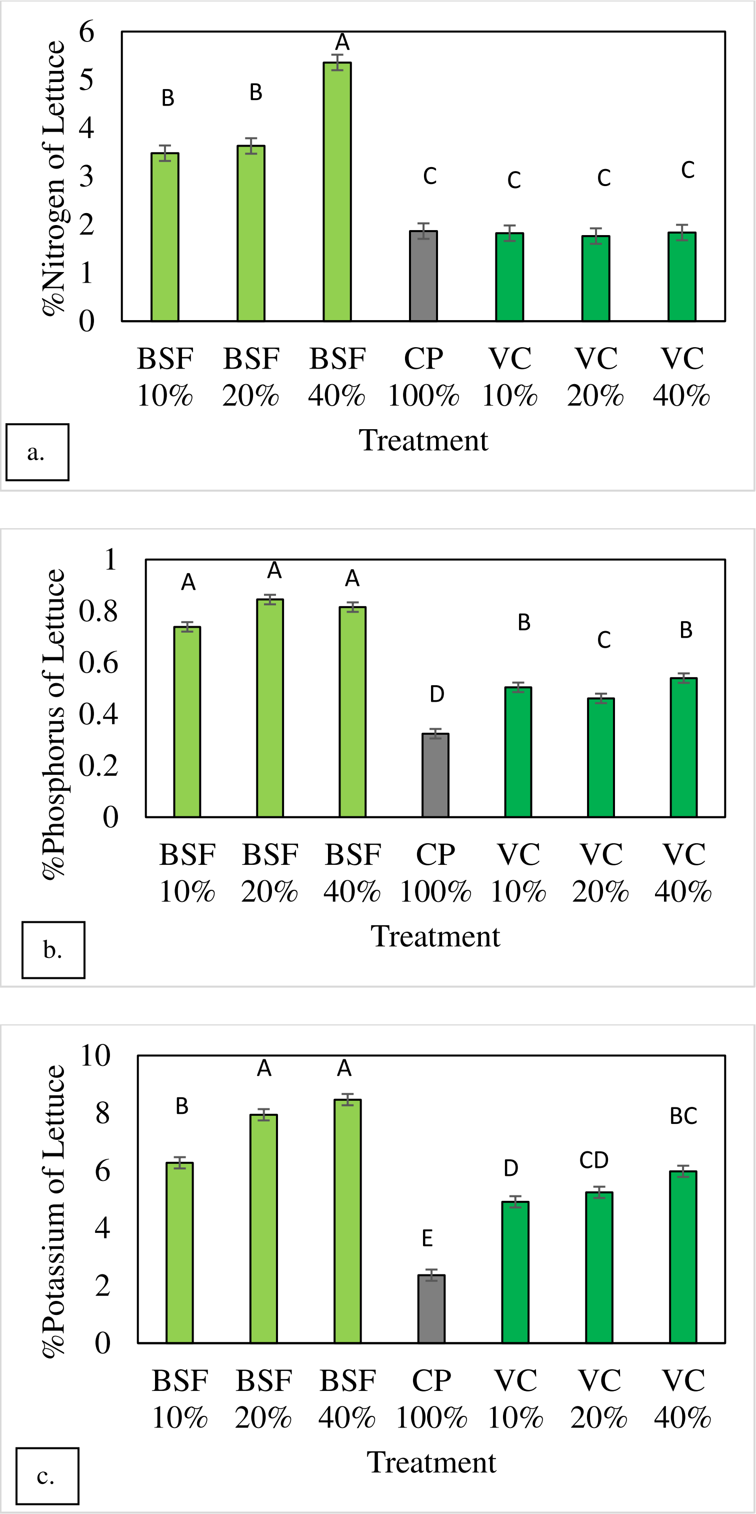


Figure 4.2: ***Concentrations of primary macronutrients (a. N; b. P; c. K) of BSFL amended lettuce grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light green columns) or vermicompost (VC: Kelly green columns). 100% commercial peat served as the control pot (CP: grey column), df = 6 (a. $p < 0.0001$/F=54.66; b. $p < 0.0001$/F=135.44; c. $p < 0.0001$/F=174.62).

Secondary Macronutrients: Calcium, magnesium, and sulfur

In arugula plant tissues there were not many differences in percent Ca (Figure 4.3a.). The ANOVA results indicated significant differences, but the data did not pass the test for normality. The peat control (1%) had the lowest concentrations of calcium while BSF 10% (2%) had the highest. The BSF and VC treatments were all statistically comparable. A non-parametric test was conducted to further evaluate the results. The χ^2 (12.26) analysis indicated no significant differences between treatments ($p = 0.056$). The opposite effect appears in lettuce, where the control produced the greatest percentages of Ca (1%) (Figure 4.4a.). This was statistically comparable to all the vermicompost treatments, but higher than all of the BSF treatments. This information is slightly confounding to what we understand about the amendments, considering peat has the lowest amount of Ca and vermicompost has the highest amount (Table 4.2).

Arugula (Figure 4.3b.) and lettuce (Figure 4.4b.) amended with BSFL frass contains significantly higher percentages of Mg compared to the control and VC treatments. For arugula (1.1%) and lettuce (0.5%) crops, BSF 40% was significantly greater than all the other treatments and control. In arugula leaves Mg is lowest in the control (0.3%), while in lettuce the lowest is VC 20% (0.31%) and VC 40% (0.31%). This corresponds to higher percentages of Mg in the frass (Table 4.2).

S concentration in arugula leaves (Figure 4.3c.) were greatest in the vermicompost treatments, VC 20% (1%) and VC 40% (1%), and was lowest in the BSF 10% treatment (0.5%). Lettuce tissue analysis produced different results (Figure 4.4c.). The BSFL frass treatments (0.24-0.27%) were all significantly higher in percent S than the control (0.14%) and vermicompost treatments (0.13-0.14%). The VC treatments were all comparable to the control.

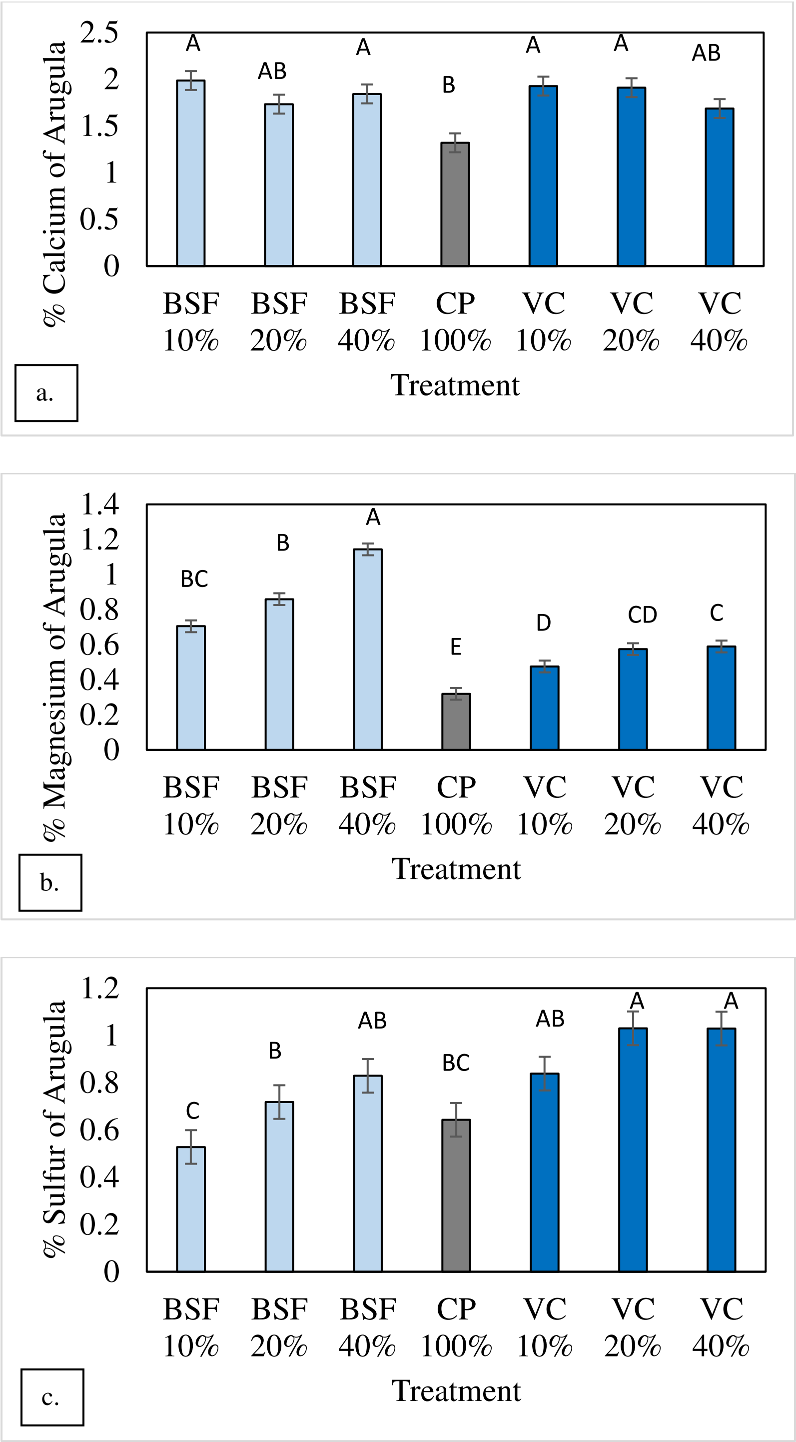


Figure 4.3: ***Concentrations of secondary macronutrients (a. Ca; b. Mg; c. S) BSFL amended arugula grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light blue columns) or vermicompost (VC: sky blue columns). 100% commercial peat served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p = 0.0027/F=6.025$; b. $p < 0.0001/F=87.27$; c. $p < 0.0001/F=20.53$).

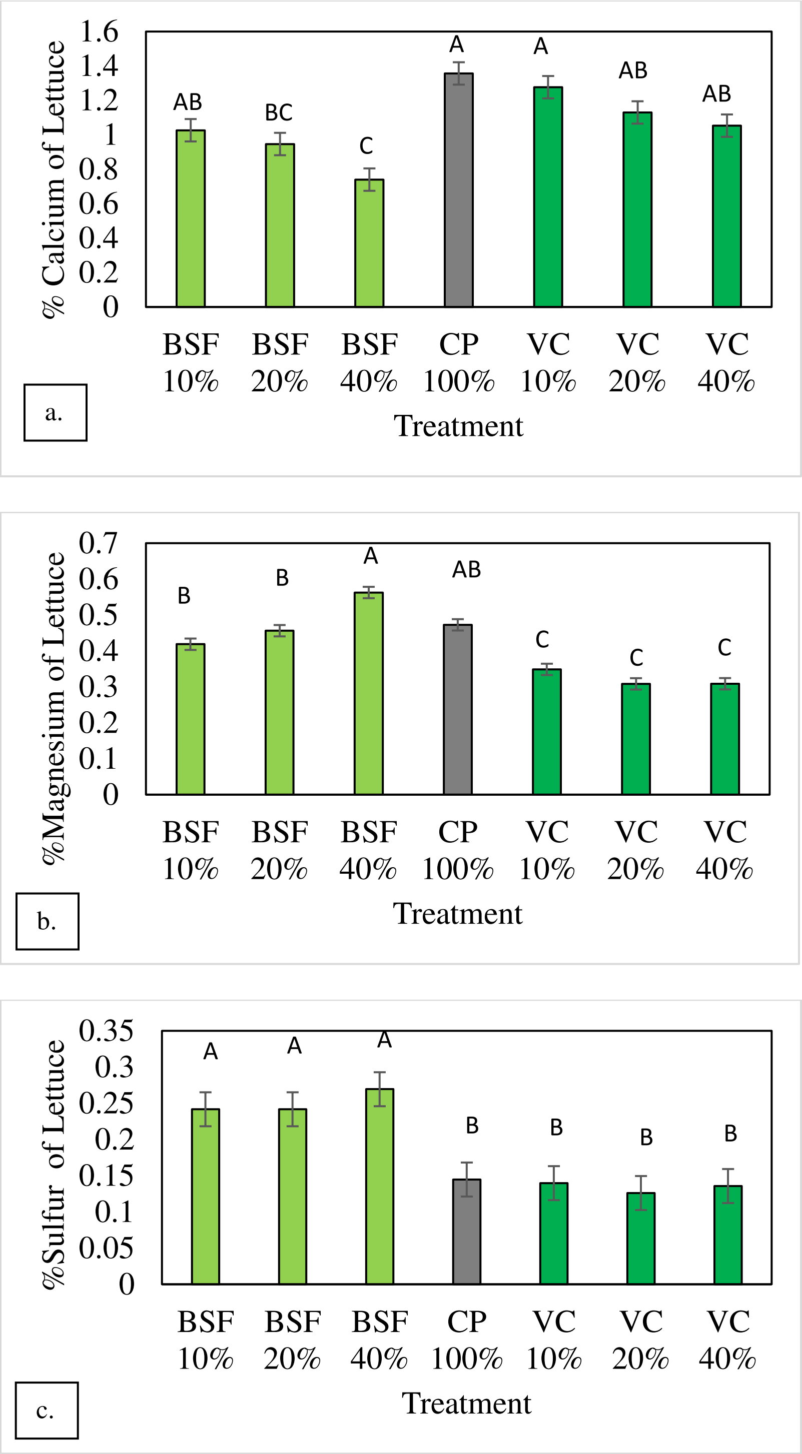


Figure 4.4: ***Concentrations of secondary macronutrients (a. Ca; b. Mg; c. S) in BSFL amended lettuce grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light green columns) or vermicompost (VC: Kelly green columns). 100% commercial peat served as the control pot (CP: grey column), df = 6 (a. p = 0.0001/F=11.084; b. p < 0.0001/F=37.34; c. p < 0.0001/F=121.60).

Micronutrients: Boron, Iron, Manganese, Copper, and Zinc

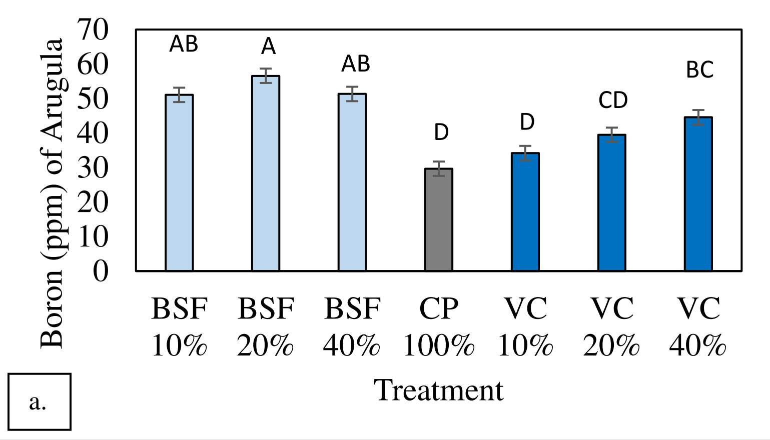
Arugula plant tissues produced the greatest amounts of B when grown in BSFL frass (51 - 57 ppm) (Figure 4.5a.). These treatments were significantly higher than the control (29.67ppm) and most of the vermicompost treatments (34 -45 ppm). The control peat and VC 10% produced the lowest concentrations of B. B concentrations in lettuce tissue produced similar results (Figure 4.6a.). The BSFL treatments (30 -33 ppm) are all significantly higher than the control (21.67ppm) and vermicompost treatments (20 -23 ppm). This corresponds with the compost analysis that revealed high amounts of boron in distillery grain digested frass.

There were no treatment differences in arugula Fe tissue concentrations (Figure 4.5b.). Though not statistically significant, vermicompost treatments produced the greatest concentrations of Fe in arugula tissues. However, in lettuce tissues, the insect frass treatments (73 – 67 ppm) produced significantly higher concentrations of iron compared to the control (50 ppm) and vermicompost treatments (38.73-45.03) (Figure 4.6.b.). This both confirms and contradicts the compost analysis. Distillery grains had much higher levels of Fe than the other three materials.

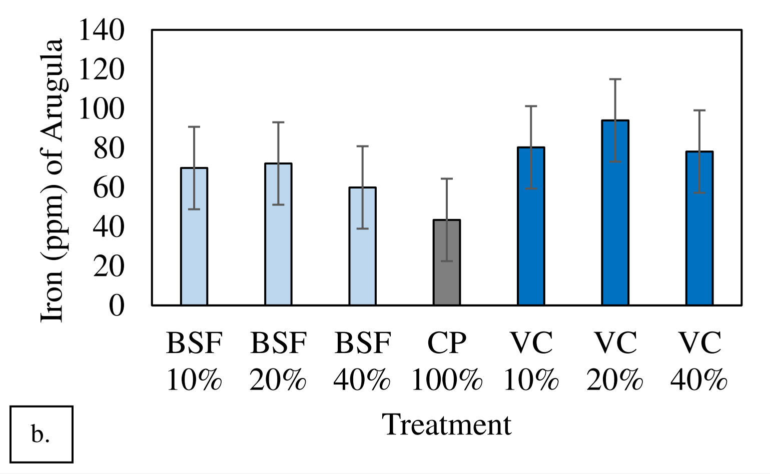
Mn concentrations in arugula (Figure 4.5c.) was highest in the BSFL treatments and lowest in the vermicompost treatments. BSF 40% (234 ppm) was significantly higher than the control (96.27ppm) and all other treatments. Additionally, Mn concentrations in the control peat were significantly higher than the VC treatments in arugula tissue. In lettuce tissues (4.6c.), Mn concentrations were significantly higher in the control (200 ppm) and the BSFL treatments, BSF 40% (167 ppm) and BSF 20% (202 ppm), compared to the VC treatments, which produced the lowest Mn concentrations. This corroborates the compost analysis that revealed higher concentrations of Mn in the distillery grain digested frass (Table 4.2). However, there were low concentrations of Mn that were found in the peat.

Cu concentrations in arugula (Figure 4.5d.) and lettuce (Figure 4.6d.) tissues did not produce any significant differences based on treatment. This cannot be explained by the compost analysis (Table 4.2) alone because the Cu levels are fairly variable depending on the material.

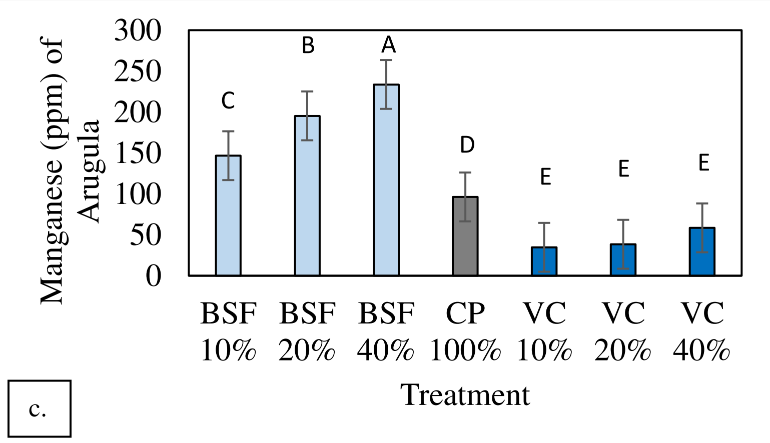
Arugula tissues were highest in Zn (Figure 4.5e.) when grow in the BSF 40% treatments (105 ppm) and lowest when grown in the control (59). BSF 40% was significantly higher than all the treatments and control, except BSF 20% (86 ppm). In lettuce (Figure 4.6e), BSF 40% (53 ppm) and BSF 20% (52%) also produced the significantly higher concentrations of Zn. However, VC 40% (0.5%) and VC 20% (23%) contain the least amount of Zn. This corresponds with the compost analysis (Table 4.2) that revealed high amounts of percent Zn in the distillery grain frass.


70
60
50
40
30
20
10
0
Boron (ppm) of Arugula
AB
A
AB
D
D
CD
BC
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

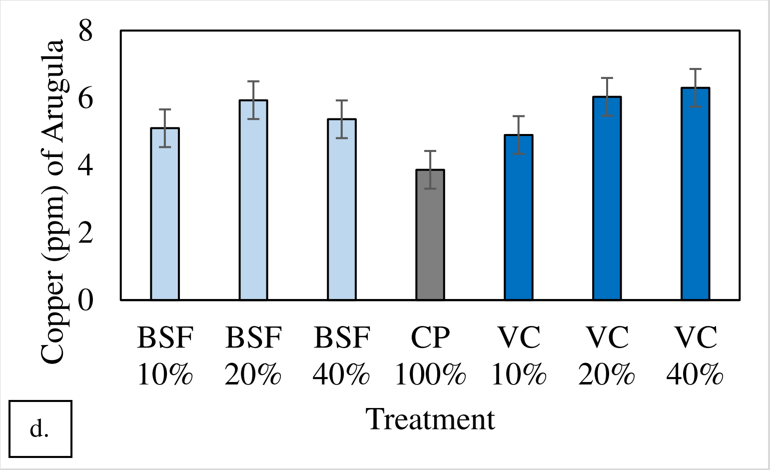
a.


140
120
100
80
60
40
20
0
Iron (ppm) of Arugula
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

b.


300
250
200
150
100
50
0
Manganese (ppm) of Arugula
C
B
A
D
E
E
E
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

c.



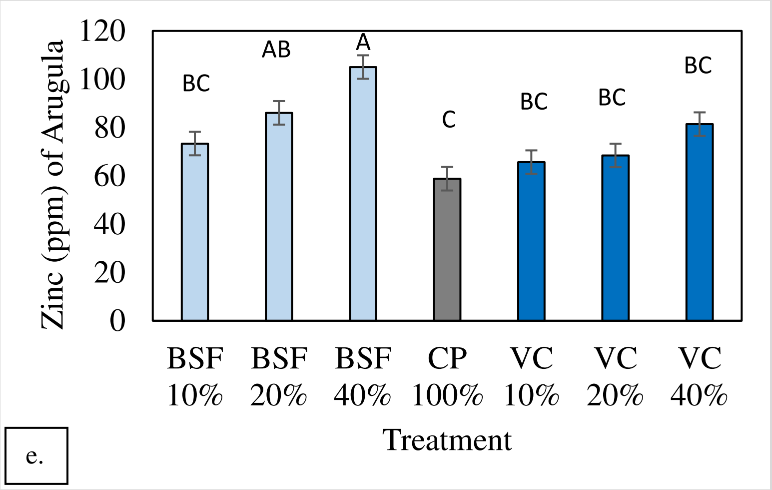
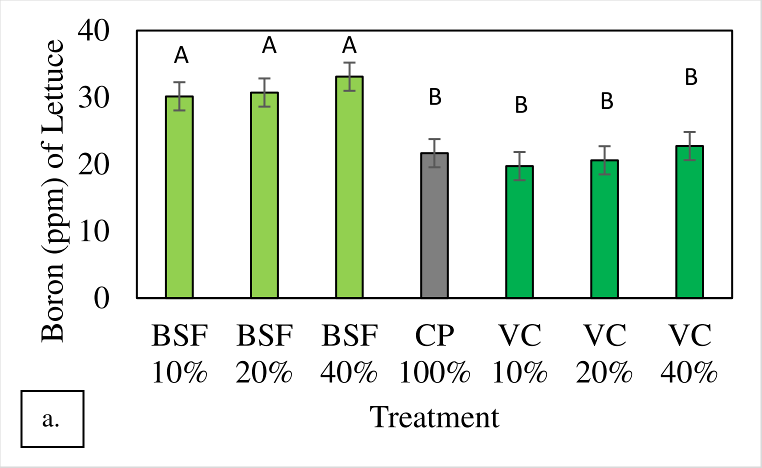
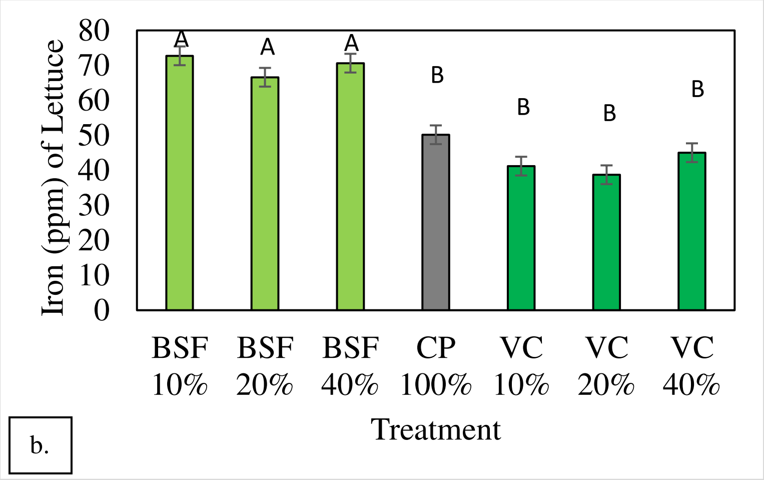


Figure 4.5: ***Concentrations of micronutrients (a. B; b. Fe; c. Mn; d. Cu; e. Zn) in BSFL amended arugula grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light blue columns) or vermicompost (VC: sky blue columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise differences, df = 6
(a. $p < 0.0001$/F=22.42; b. $p = 0.73$/F=0.59; c. $p < 0.0001$/F=131.71; d. $p = 0.10$/F=2.22; e. $p = 0.0002$/F=10.07).



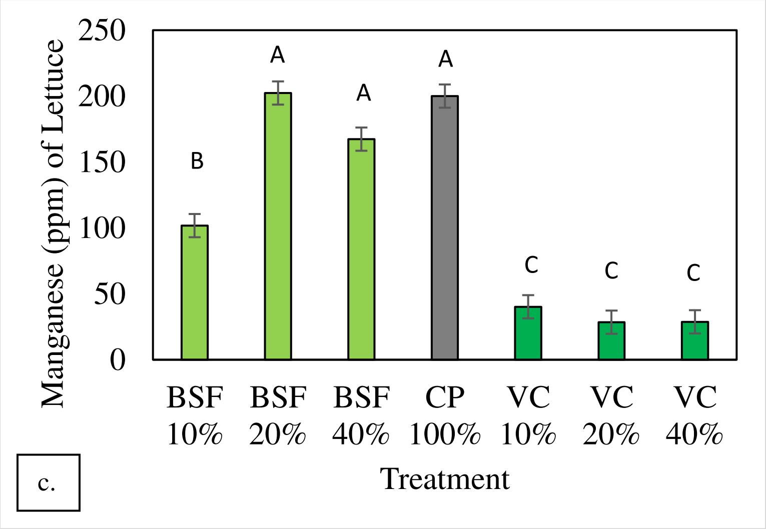
Boron (ppm) of Lettuce
40
30
20
10
0
A
A
A
B
B
B
B
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

a.



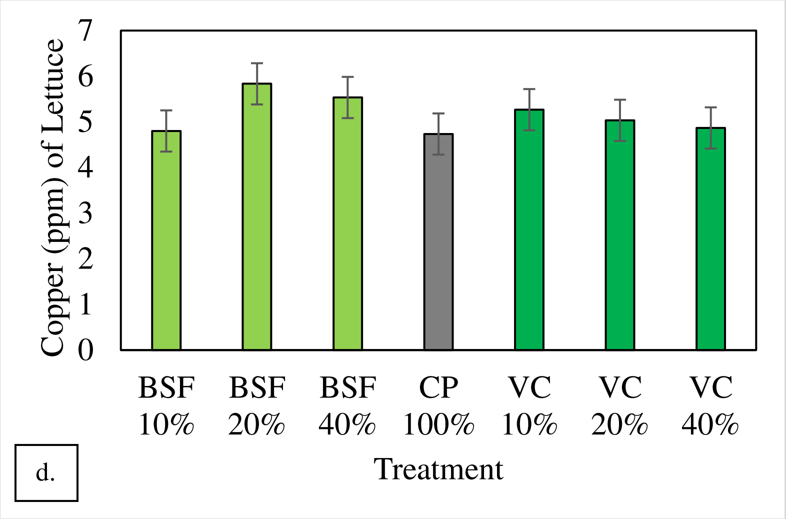
Iron (ppm) of Lettuce
80
70
60
50
40
30
20
10
0
A
A
A
B
B
B
B
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

b.



Manganese (ppm) of Lettuce
250
200
150
100
50
0
B
A
A
A
C
C
C
BSF 10%
BSF 20%
BSF 40%
CP 100%
VC 10%
VC 20%
VC 40%
Treatment

c.



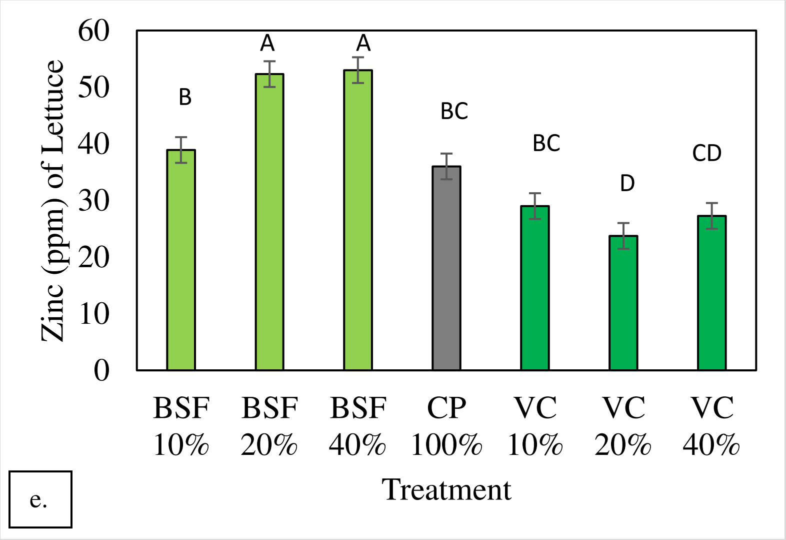


Figure 4.6: ***Concentrations of micronutrients (a. B; b. Fe; c. Mn; d. Cu; e. Zn) in BSFL amended lettuce grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light green columns) or vermicompost (VC: Kelly green columns). 100% commercial peat served as the control pot (CP: grey columns), df = 6. (a. $p < 0.0001/F=27.43$; b. $p < 0.0001/F=29.52$; c. $p < 0.0001/F=81.70$; d. $p = 0.56/F=0.83$; e. $p < 0.0001/F=26.75$).

Other elements: Aluminum and Sodium

Al concentrations in both arugula (Figure 4.7a.) and lettuce (4.8a.) did not differ statistically based on treatment. In lettuce, Al was much higher in the vermicompost treatments, though not statistically significant. Al was not measured in the compost analysis and cannot be used to discuss these results.

Na concentrations were significantly greater in BSFL treatments (446-1173 ppm) in arugula (Figure 4.7b.). The control produced the lowest levels of Na (162 ppm). In lettuce tissues, sodium was highest in the control (2460 ppm) and lowest in the vermicompost treatments (587-876ppm) (Figure 4. 8b.) Distillery grains did have the highest level of Na, and vermicompost had the lowest (Table 4.2), which validates the findings in the plant tissues.

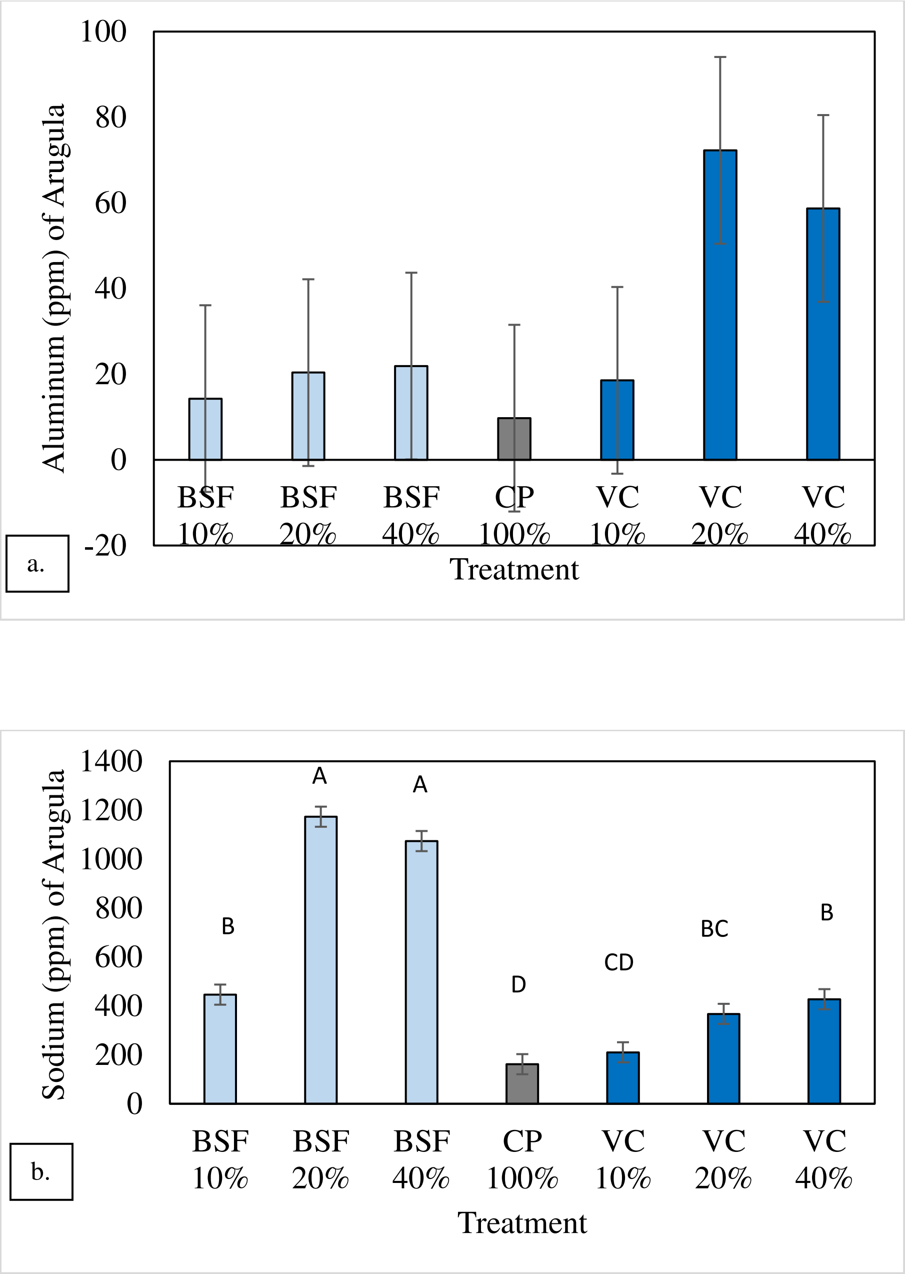
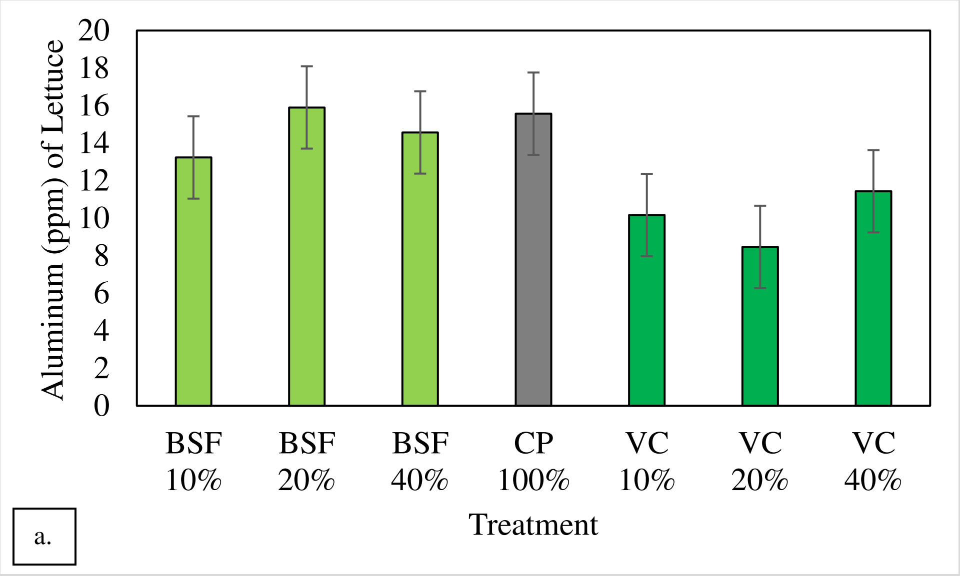


Figure 4.7: ***Concentrations of a. aluminum and b. sodium in BSFL amended arugula grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light blue columns) or vermicompost (VC: sky blue columns). 100% commercial peat served as the control pot (CP: grey column). Columns with different letters indicate pairwise differences, df = 6 (a. $p = 0.34$/$F = 1.25$; b. $p < .0001$/$F = 97.35$).



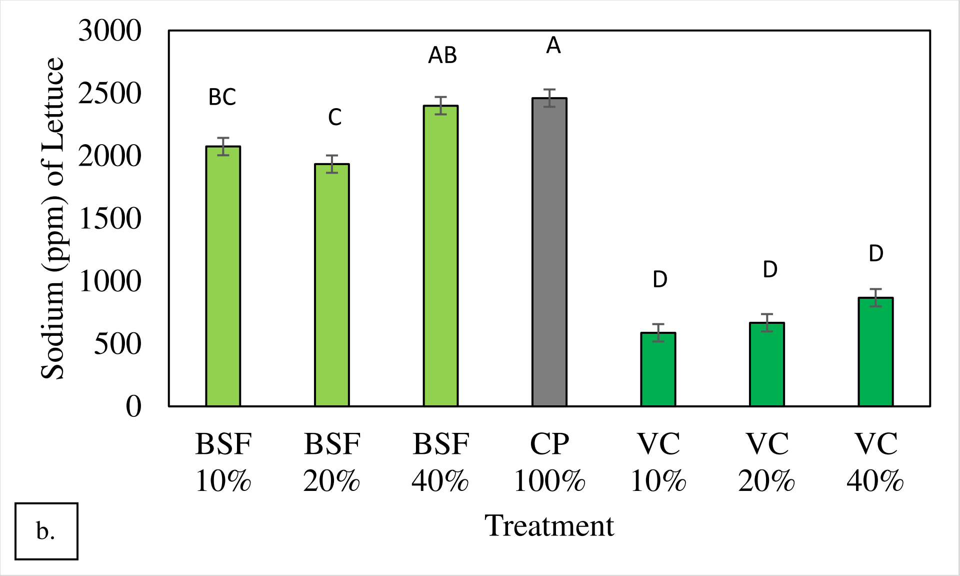


Figure 4.8: ***Concentrations of a. aluminum and b. sodium in BSFL amended lettuce grown in a greenhouse.*** Lettuce was grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: light green columns) or vermicompost (VC: Kelly green columns). 100% commercial peat served as the control pot (CP: grey column), df = 6 (a. p = 0.20/F = 1.67; b. p < .0001/F = 143.31).

Distillery grain vs brewery grain digested frass (on a dry weight basis)

The nutrient profile of arugula (Table 4.3) tissues grown in the Spring 2022 study was characterized based on the materials available and not statistically analyzed. Na was highest (10%) in the BGF 20% treatment and lowest in the control peat (7%). P was highest (0.85%) in BGF 20% and lowest (0.70%) in the control peat. K was highest (8.11%) in the BGF 40% and lowest (3%) in the BGF 20% treatment. Ca was highest (1%) in the control and lowest (0.79%) in the DGF 20% treatment. Mg was also highest (0.34%) in the DGF 20% and lowest (0.24%) in the BGF 10%. S was highest (1%) in DGF 10% and lowest (1%) in the DGF 40% treatment.

Table 4.3: ***Nutrient characterization of arugula leaf tissues*** Treatments of distillery grained digested frass (DGF) and brewery grain digested frass (BGF) and the control peat (CP) are in columns.

	DGF 10%	**DGF 20%**	**DGF 40%**	**CP 100%**	**BGF 10%**	**BGF 20%**	**BGF 40%**
Nitrogen (%)	8.4	8.75	10.14	7.37	9.87	10.22	8.47
Phosphorus (%)	0.75	0.81	0.72	0.70	0.73	0.85	0.71
Magnesium (%)	0.29	0.34	0.25	0.28	0.24	0.29	0.34
Potassium (%)	7.13	7.85	5.13	6.30	5.95	2.78	8.11
Calcium (%)	0.98	0.79	0.98	1.23	1.08	0.96	0.80
Sulfur (%)	1.20	1.19	0.98	1.14	1.02	0.99	1.17
Boron (ppm)	28.80	29.90	24.30	31.00	25.80	34.50	34.30
Iron (ppm)	148.00	114.00	129.00	118.00	128.00	119.00	115.00
Manganese (ppm)	49.30	64.90	51.10	55.70	49.90	102.00	56.10
Copper (ppm)	13.20	13.90	9.20	7.90	7.70	13.90	16.20
Zinc (ppm)	56.60	52.80	65.70	57.70	57.00	59.80	58.30
Aluminum (ppm)	63.80	49.90	31.90	39.60	30.20	40.90	45.20
Sodium (ppm)	2220.00	3030.00	5620.00	1280.00	3970.00	9610.00	3180.00

The nutrient profile of lettuce tissues grown in the Spring of 2022 study was characterized and is presented in Table 4.4. N was highest (8%) in the BGF 20% treatment and lowest in the DGF 10% treatment (7%). P was highest (%) in DGF 20% and lowest (0.72%) in the BGF 40% treatment. K was highest (9%) in the DGF 20% and lowest (5%) in the peat control. Ca was highest (0.71%) in the control and lowest (0.25%) in the DGF 20% treatment. Mg was also highest (0.34%) in the control and lowest (0.25%) in the DGF 40%. S was highest (0.37%) in DGF 20% and lowest (0.31%) in the DGDF 40% treatment.

Table 4.4: ***Nutrient characterization of lettuce leaf tissues*** Treatments of distillery grained digested frass (DGF) and brewery grain digested frass (BGF) and the control peat (CP) are in columns.

	DGF 10%	**DGF 20%**	**DGF 40%**	**CP 100%**	**BGF 10%**	**BGF 20%**	**BGF 40%**
Nitrogen (%)	6.81	7.03	6.87	7.02	7.11	7.57	6.91
Phosphorous (%)	0.96	0.99	0.77	0.82	0.92	0.88	0.72
Magnesium (%)	0.32	0.31	0.25	0.34	0.34	0.32	0.27
Potassium (%)	7.59	8.67	8.84	5.46	5.80	5.55	5.62
Calcium (%)	0.58	0.44	0.29	0.71	0.66	0.58	0.53
Sulfur (%)	0.36	0.37	0.36	0.33	0.33	0.34	0.31
Boron (ppm)	30.20	34.50	40.50	25.40	28.30	28.10	27.50
Iron (ppm)	127.00	124.00	99.20	112.00	128.00	132.00	105.00
Manganese (ppm)	89.50	101.00	87.60	99.80	93.20	92.00	101.00
Copper (ppm)	5.90	7.30	7.20	5.20	5.20	5.10	5.40
Zinc (ppm)	40.40	44.10	40.70	40.10	39.60	38.00	31.10
Aluminum (ppm)	44.70	40.60	24.60	19.80	37.60	35.80	27.50
Sodium (ppm)	2100.00	3790.00	4410.00	1140.00	3710.00	4790.00	8190.00

4. Discussion

Distillery grain digested frass

Nutrient concentrations were often higher in the BSFL frass treatments for both crops. In the summer 2021, higher fresh arugula and lettuce yields were produced in all the BSFL treatments compared to the vermicompost treatments and control (Chapter 3). Lettuce SPAD measurements reported in Chapter 3 correspond with the results of the compost and plant tissue analysis, which also supports the significantly higher concentrations of macronutrients N, P, K, and Mg in both crops. Al concentrations observed in arugula tissues were significantly higher in the vermicompost treatments. At low levels of pH (<5.5), high levels of Al can be toxic and damage root systems (Smith and Smith, 2015). That may explain the lower yields of arugula in the vermicompost treatments seen in Chapter 3. In the distillery grain treatments, there was a decrease in Ca concentrations of lettuce, compared to the peat control, despite the high Ca concentrations observed in distillery grain frass. The opposite trend was seen in the arugula, so response could vary by crop.

Distillery grain vs brewery grain digested frass

Based on the low quantity of samples provided for this study, it is difficult to make conclusions based on the nutrient characterization. However, overall, there is not as much variation among treatments based on what might have been expected. In Chapter 3, we saw much higher lettuce yields and SPAD measurements in the DGDF treatments compared to the BGDF, which are often related to increases in chlorophyll and nitrogen content of lettuce (Mendoza-Tafolla *et al.,* 2019) and tomato plants (Jiang *et al.,* 2017). However, nitrogen concentrations varied less than a percent across all six treatments and the control (6.81-7.57%). Sufficiency levels of lettuce and arugula, reported by Brookside labs, indicate that other studies have shown that the effect of season had greater influence on lettuce nutrient uptake than fertilizer treatments (Fallovo *et al.,* 2009).

Though some vermicompost studies report increases in nutrient concentrations of plant tissues, other studies have observed that humic acid did not improve nutrient uptake in vegetable crops (Hartz and Bottoms, 2010). The effect seems to vary based on the crop and growth environment. For example, tomatoes did not show any differences in total percent nitrogen when vermicompost was applied to the soil (Gutiérrez-Miceli *et al.,* 2007). However, nutrient presence in the soil or media does not equate to nutrient availability and there a variety of other edaphic and biotic factors that could be contributing to potential nutrient use (Chapin *et al*., 2011)

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5. Conclusions

The ion exchange capacity of the soil, or the number of charged sites present on soil particles, is determined by soil texture and acidity. This controls how well cations (K+, Ca2+ and Mg2+) and anions (NO3-, H2PO4-, and SO42-) can stay in the soil solution, remaining available for plant uptake (Smith and Smith, 2015). The lower pH of the peat (6.22) could explain the lower nutrient concentrations often observed in the control plants. As soil acidity increases and pH decreases, it increases binding affinity for Al and decreases binding to other important cations like Ca and Na (Smith and Smith, 2015). This could explain the observations in Figure 4.7b., where the sodium concentration of arugula is significantly lower than all the other treatments. The same trend is not observed in lettuce, so the response may vary per species.

Though arugula and lettuce seem to retain most macronutrients in a similar manner; there was considerable variation in their response to micronutrients as well. Na levels in arugula were highest in the frass treatments, while Na concentrations in lettuce were highest in the control. Additionally, arugula did not seem to respond to Fe, while lettuce grown in the frass treatments produced significantly higher tissue Fe levels than the control and other treatments.

Past studies have been done to indicate that vermicompost can alter the soil microbiome and increases the plant growth regulators present (Ravindran *et al*., 2020). Plant growth regulators are essential in signaling development during growth. Given the similar process of bioconversion, it is possible that an increase in PGR and an altered microbiome may be responsible for some of the results found in frass experiments, although this was not measured in this study. More experiments are warranted as this could explain why increases in yield were observed, but nutrient concentrations were not.

Overall, the addition of BSFL digested distillery grains can not only improve yield, but can also improve vegetable quality, for most macronutrients of concern. For the other macronutrients, additional steps could be considered to improve the frass as a growth amendment and fertility product. The addition of nutrient containing amendments, such as frass, could increase nutrient leaching from the growth media. A study by Boharaa *et al.* (2019), observed increased nutrient concentrations of leachate after the application of poultry litter. Soils amended with pine biochar and poultry litter + pine biochar produced a significant decrease in nutrient leaching. Incorporating biochar into frass could improve the overall value as a peat replacement and fertilizer and provides another consideration for future studies.

6. References

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CHAPTER FIVE

THE IMPACTS OF BLACK SOLDIER FLY LARVAL FRASS ON THE PRODUCTION OF A LONG CYCLE, WARM SEASON TOMATO CROP

1. Introduction

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae or nightshade family. They can be grown in open or controlled environments for the purposes of being eaten fresh or processed (Heuvelink, 2018). Today, tomatoes are one of the most important vegetable crops in the world (Swiader and Ware, 2002). Most cultivars require high temperatures and frequent water regiments (Heuvelink, 2018). Completing their full life cycle can take several months, distinguishing them from other vegetable crops such as lettuce, that only require a few weeks to reach full maturity (Swiader and Ware, 2002). SPAD has been a positive indicator for chlorophyll and nitrogen concentrations in tomato leaves (Jiang *et al.*, 2017; Soval-Villa *et al.*, 2002).

Many studies have been conducted to evaluate alternative sources of fertility or amendments for tomatoes grown in greenhouses. In several studies vermicompost has shown synergistic improvements in yield when combined with a fertility regime (Arancon *et al.,* 2003; Atiyeh *et al.,* 2000; Gutiérrez-Miceli *et al.,* 2007; Joshi and Vig, 2010; Surrage *et al.,* 2010; Yang *et al.,* 2015; Zaller, 2007). It has also been shown to reduce the occurrence of blossom end rot (Surrage *et al.,* 2010) and, depending on water regime, improve soil fertility (Yang *et al.,* 2015). Several studies also observed consistent increases in seedling growth, plant height, stem diameter, leaf area, germination, leaf number, shoot length, emergence, peel firmness, branch number, total biomass, and fruit, leaf, shoot, and root weight when using vermicompost as an amendment in tomato crops (Atiyeh *et al.,* 2000; Atiyeh *et al.,* 2001; Bachman and Metzger, 2008; Gutiérrez-Miceli *et al.,* 2007; Joshi and Vig, 2010; Roberts *et al.,* 2007; Tringovska and Dintcheva, 2012; Zaller, 2007; Zucco *et al*., 2015).

Black soldier fly larvae (BSFL) applications have been studied in tomatoes; however, publications are limited. A study by Setti *et al.* (2019) showed germination, leaf number, and emergence in a 10% BSFL amended crops were all statistically higher than the 100% peat control. In contrast, stem diameter, height, SPAD, leaf area, and leaf, stem, and total fruit weight were all statistically the same as the peat control. This indicates that the replacement of peat with BSFL can result in comparable or better tomato growth based on some early development crop parameters than those grown in 100% peat.

Preliminary Studies 2020

Though the highest BSFL frass and vermicompost treatments (100%, 80%, and 60%) were not lethal to tomato plants in 2020, they were removed from future studies due to their low yields and subsequent financial constraints to growers. Blossom end rot (BER) was also very prevalent in the 2020 tomato screening experiments. BER is a physiological disorder that causes the blossom end of the tomato fruit to become necrotic (Taylor and Locascio, 2004). This could have been due to the cultivar selected, ‘Brandywine’. A less susceptible, more common greenhouse cultivar, ‘Jet Star’, was used in the set of experiments conducted in 2021, described in this chapter. Additionally, a tomato specific fertilizer, which contained calcium, replaced the general vegetable fertilizer utilized the year before. Calcium is known to reduce rates of BER commonly seen in greenhouse tomatoes (Taylor and Locascio, 2004). Hypothesis were deduced based on the findings of the preliminary studies and the vermicompost experiments detailed in Appendix I.

Question 1: Does tomato fruit yield vary between treatments and control?

Hypothesis: BSFL frass amended peat treatments will produce greater or equal yield when compared to the vermicompost treatments and control.

Question 2: Does tomato leaf greenness vary between treatments and control?

Hypothesis: SPAD measurement will indicate a higher leaf greenness in BSFL frass amended treatments when compared to vermicompost treatments and the control.

Question 3: Does tomato fruit BER vary between BSFL amended treatments and control?

Hypothesis: BER will be less prevalent in BSFL frass treatments when compared to the vermicompost treatments and 100% peat control.

Question 4: Does tomato vegetative biomass vary between BSFL amended treatments and control?

Hypothesis: Biomass after final harvest will be greatest in BSFL frass when compared to vermicompost treatments and the control.

Question 5: Does tomato fruit sugar content vary between BSFL amended treatments and control?

Hypothesis: BRIX measurements will indicate a higher sugar concentration in BSFL frass treatments when compared to vermicompost and 100% peat control.

2. Materials and Methods

This study took place in Fort Collins, CO at the Colorado State University Horticulture Center greenhouses, elevation 1,525.22 meters (en-us.topographic-map.com). Experiments ran from June to November of 2021. Tomatoes seeds were started on the misting bench on May 28, 2021. They were germinated in a peat (3.79 L), vermicompost (0.47 L), blood meal (29.57 mL), and bone meal mixture (29.57 mL). Once the tomatoes had sprouted and grown one set of true leaves, they were transplanted into plastic pots, eight liters in volume, on June 22, 2021. The crop was grown in a greenhouse, at 20-25^{o}Cs. Treatments consisted of Berger B6 (Saint-Modeste, QC) peat mix and two different amendments: BSFL frass reared on distillery grains (sourced from Evo Conversions System) and vermicompost (sourced from Rocky Mountain Soil Stewardship). The amendments, BSFL frass and vermicompost (VC), were mixed into a traditional growing peat media (CP) at the following percentages: 40%, 20%, and 10%, and the 100% CP (see Table 5.1). This experiment was carried out as a randomized complete block design (RCBD) using 7 levels total (3 treatments of each amendment and a 100% commercial peat control), 1 crop, and 10 replicates/level over the growing period. The fertilizer applied was Jack's Tomato FeED (NPK - 12:15:30), JR Peters, Inc: Allentown, PA. Label guidelines were followed and fertilizer was applied at recommended rate (3.7 mL fertilizer per 0.95 L water) per plant every other week. Tomatoes were watered once to twice daily, depending on crop needs.

Table 5.1: Amendment treatment summary for 2021 greenhouse tomato studies: vermicompost (VC) and black solider fly larvae (BSFL) frass.

Black soldier fly larvae frass treatments				**Vermicompost treatments**			
Peat %	**Peat (L)**	**BSFL %**	**BSFL (L)**	**Peat %**	**Peat (L)**	**VC %**	**VC (L)**
60	4.8	40	3.2	60	4.8	40	3.2
80	6.4	20	1.6	80	6.4	20	1.6
90	7.2	10	0.8	90	7.2	10	0.8
100	8.0	0	0.0	100	8.0	0	0.0

Yield

Tomatoes began to ripen on September 4, 2021 (Days after planting (DAP): 74) and were harvested once fully red. Ripe tomatoes were removed from the plant weekly and weighed. They were checked for any defects, such as BER, labelled marketable or unmarketable, and weighed. A subset of tomatoes, three per plant, were collected from each replicate and frozen for BRIX analysis. Final harvest was collected on November 20, 2021.

SPAD Analysis

On November 18, 2020, two days before final harvest, tomato leaves were measured for greenness using a soil plant analysis development (Konica Minolta: Ramsey, NJ) meter. The right side of the third highest leaf of each plant was chosen for measurement.

BRIX analysis of Tomato Fruits

BRIX analysis was conducted based on the methods described by Mitchell (2018). Three representative tomato fruits were harvested from each replicate. The samples were sealed in plastic bags and stored in a freezer at 30°F. After being thawed, the fruits were smashed and blended to obtain an aggregate measure. The pulp was strained with cheesecloth, and the juice was assessed using a refractometer. Soluble solid content was measured and recorded for each of the 7 treatments that were analyzed in 2021. An acceptable range for fresh market tomatoes in Colorado is 3.5 to 5.3 BRIX. BRIX degrees were used to assess harvest readiness and to determine quality in the field and during processing. BRIX is a measurement of dissolved solids and can indicate a product's potential sweetness and flavor (Kleinhenz and Bumgarner, 2015).

Statistical Analysis

Statistical analyses were conducted in RStudio (R Core Team, 2022). Packages (version 3.4.2) include The R Base, Datasets, Graphics, Graphics Devices and Support for Colours and Fonts, Stats and Utils Packages, and Formal Methods and Classes. One-way ANOVAs (95% confidence interval) with block effects were used to analyze the differences in means of each treatment. A threshold of $p < 0.05$ was considered as significant. Tukey HD pair wise comparisons were conducted to assess and test for differences between levels. Mean and statistic summary tables can be found in Appendix II.

3. Results

Yield

The only analysis that revealed statistically significant differences, was the average individual fruit weight of tomato. As seen in Figure 5.1, the 10% vermicompost treatment produced heavier individual tomato fruits (161 g) than the 100% peat control and the 20% and 40% BSFL frass treatments (ranging between 134 and 137 g). Parametric and non-parametric tests revealed no statistical differences in treatments in the total weight of tomato fruits produced per plant (Figure 5.2). BSFL 20% produced the most tomatoes per weight (3,045 g) and 10% BSFL frass treatment produced the least tomatoes per weight (2,322 g), but this was not significantly different.

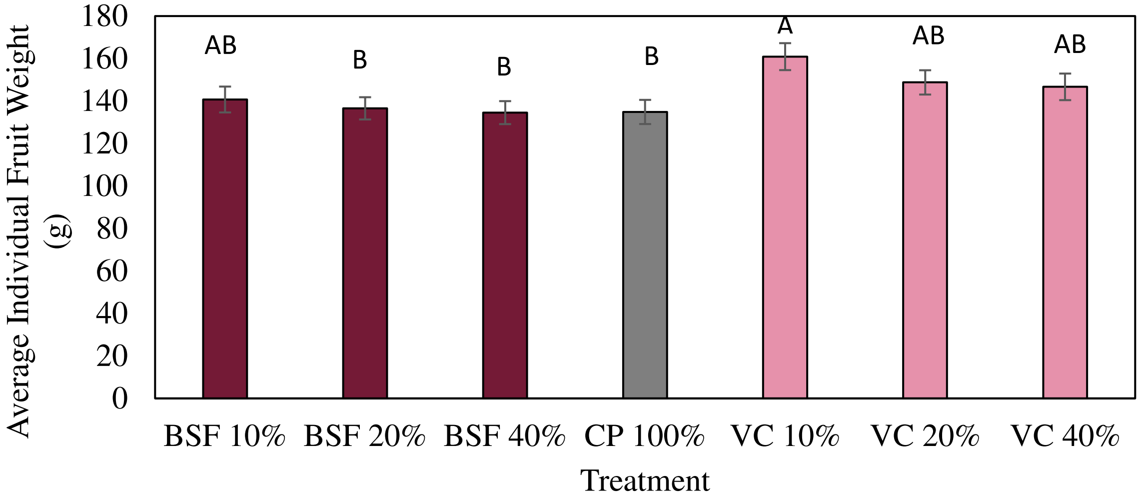

Figure 5.1: ***Average individual fresh weight (grams) of tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: dark pink columns) or vermicompost (VC: light pink columns). 100% commercial peat served as the control (CP: grey column). Letters indicate pair wise differences, df = 6 (p = 0.020/F = 2.51).

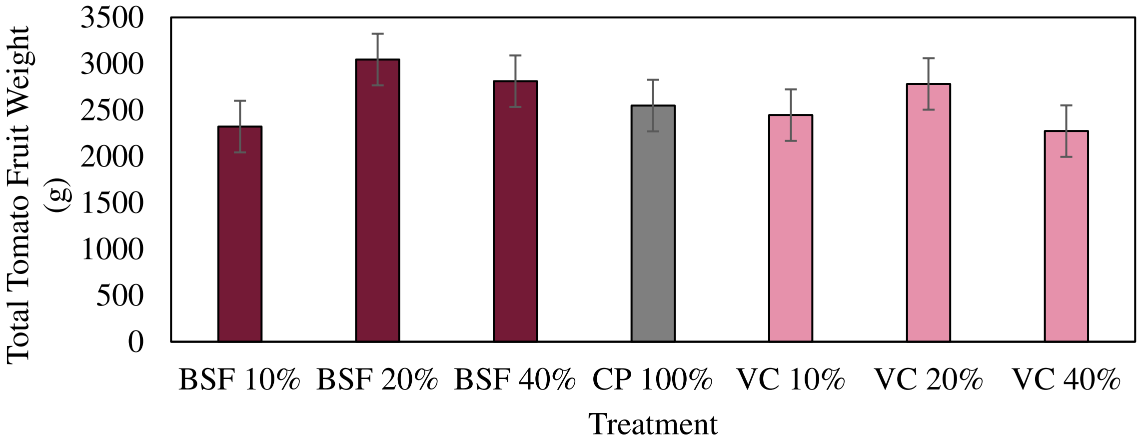

Figure 5.2: ***Total fresh weight (grams) of tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly (BSF) larvae frass or vermicompost (VC). 100% commercial peat (CP) served as the control, df = 6 (p = 0.53/F = 0.85)

Greenness

SPAD analysis (Figure 5.3) revealed no significant differences in greenness between the black soldier fly larvae treatments, vermicompost treatments, and peat control. There was very little variation in the numerical differences of the SPAD per treatment mean, although VC 10% and BSF 10% had the highest numerical SPAD readings.

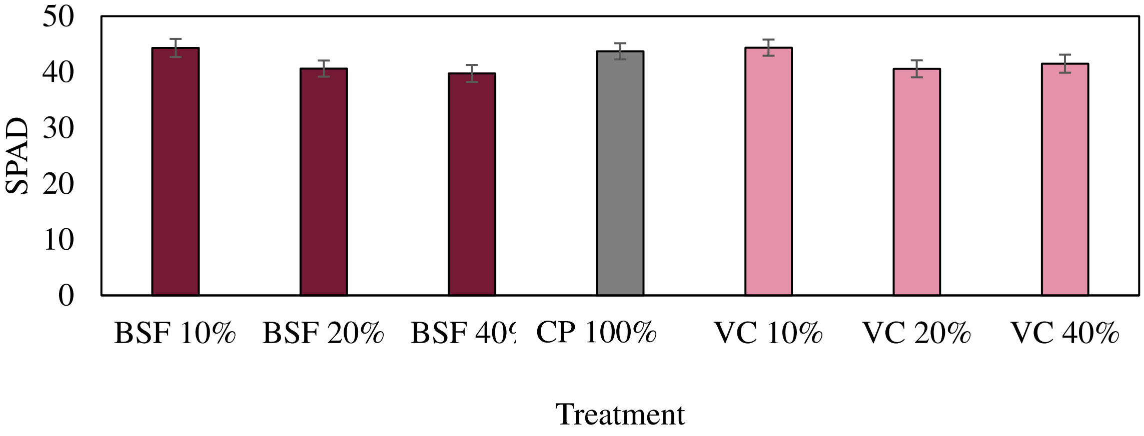

Figure 5.3: ***SPAD measurements of tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly (BSF) larvae frass or vermicompost (VC). 100% commercial peat (CP) served as the control pot, df = 6 (p = 0.66/F

Blossom End Rot Infection

The average individual fruit weight and total weight per plant of BER fruits were not statistically significant (Figures 5.4-5.5). The total BER weight per tomato plant was lower in the vermicompost treatments. The peat control produced the least amount of BER fruit by weight (196g). It is also important to note that VC treatments had the lowest number of fruits with BER.

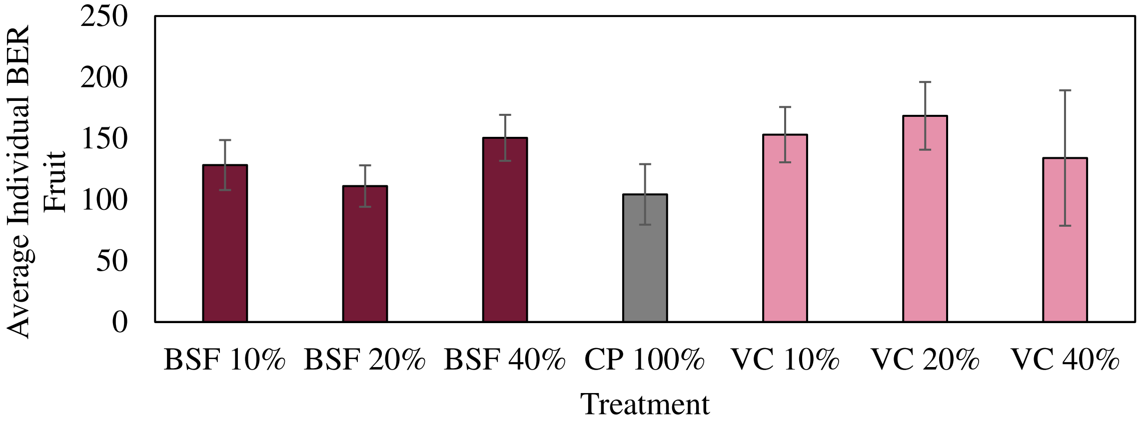

Figure 5.4: ***Average individual fruit weight (g) of blossom end rot (BER) tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly (BSF) larvae frass or vermicompost (VC). 100% commercial peat (CP) served as the control, df = 6 (p = 0.35/F = 1.13).

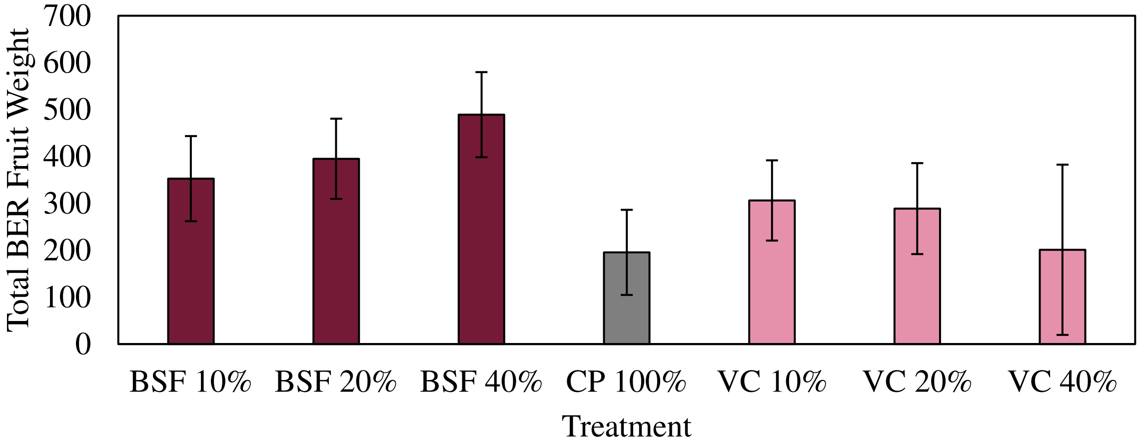

Figure 5.5: ***Total fruit weight (g) of BER tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat (CP) and black soldier fly (BSF) larvae frass or vermicompost (VC). 100% commercial peat served as the control, df = 6 (p = 0.39/F =

Final Plant Vegetative Biomass

The total weight of the tomato plants after final harvest was not significantly different among treatments (Figure 5.6). BSF40% had the numerically lightest plants (570 g), while VC10% had the heaviest plants (758 g).

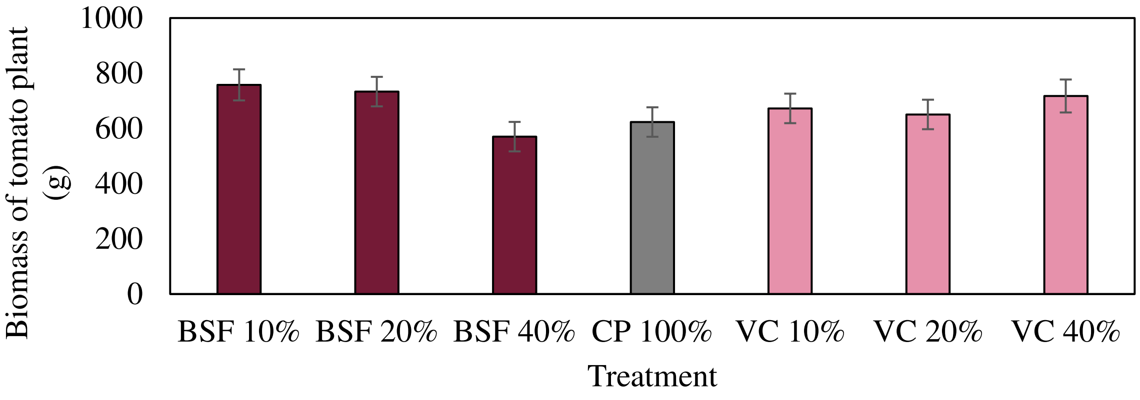

Figure 5.6: ***Final biomass (g) measurements of tomato plants grown in the greenhouse*****.** Tomatoes were grown with mixtures of commercial peat (CP) and black soldier fly larvae (BSF) frass or vermicompost (VC). 100% commercial peat served as the control pot, df = 6 (p = 0.20/F = 1.48).

BRIX

BRIX analysis revealed no differences in dissolved solids of fruits among treatments and controls (Figure 5.7). All treatment means fell within the acceptable range for Colorado tomato fruits.

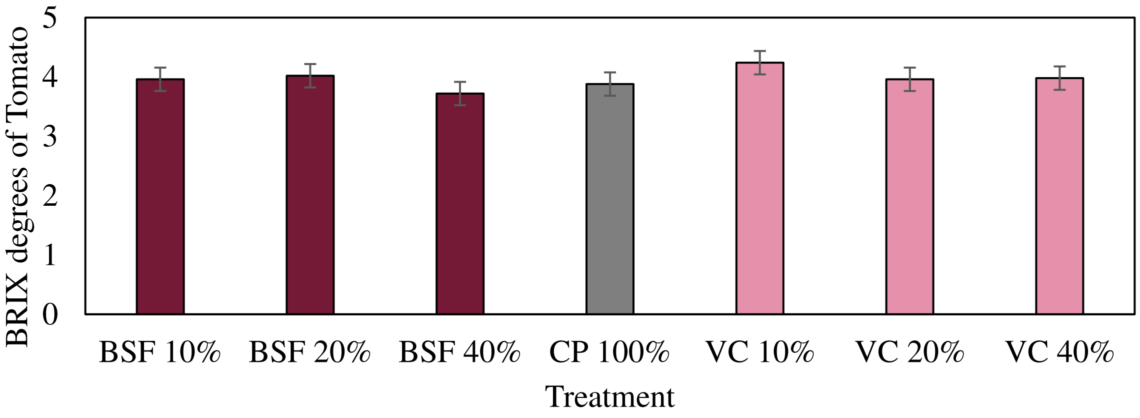

Figure 5.7: ***BRIX measurements of tomato fruits grown in the greenhouse*****.** Tomatoes were grown with mixtures of commercial peat (CP) and black soldier fly (BSF) larvae frass or vermicompost (VC). 100% commercial peat served as the control pot, df = 6 (p = 0.75/F = 0.57).

4. Discussion

Yield

The yield results seen in the 'Jet Star 'cultivar was fairly similar to what was observed in the 'Brandywine' trials. The individual fruit weight of the tomatoes was highest when grow in low concentrations of vermicompost, VC 10%. All other treatments were comparable to the control. Additionally, the other treatments of vermicompost (20% and 40%) and BSF 10% were all statistically equal to the VC 10%. There is plenty of evidence in the literature that confirm vermicompost is a beneficial amendment for yield in tomato crops (Arancon *et al.,* 2003; Atiyeh *et al.,* 2000; Gutiérrez-Miceli *et al.,* 2007; Joshi and Vig, 2010; Surrage *et al.,* 2010; Yang *et al.,* 2015; Zaller, 2007). All the studies above that detail the worm diet, expect for Zaller, 2007, use a vermicompost that is produced from pig, cow, or sheep manure. The vermicompost in this study was produced from horse manure and the frass was produced from distillery grains. Zaller, 2007 utilized frass from larvae reared on cotton gin waste and they did observe any significant differences in yield. As seen in Chapter 3, larval and worm diets may be one of the largest influences in treatment differences.

Greenness

SPAD measurements were not taken in the 2020 'Brandywine' trial, but the lack of differences across treatments indicates that soil amendments did not affect greenness. These results indicate that, though BSFL amendments might not improve greenness, but it does not negatively influence it either. Since SPAD has been an indicator for nitrogen concentrations in tomato leaves (Jiang *et al.*, 2017; Soval-Villa *et al.*, 2002), these results could also indicate consistent nitrogen concentrations in the plant leaves across treatments. Statistically similar nitrogen concentration within the tomato leaves would also indicate similar fruit yields across treatments, since N is an essential macronutrient for plant growth and fruit development (Swiader and Ware, 2002).

Blossom End Rot Incidence

BER was much lower in 2021 compared to 2020, but similar to 2020, it does not seem to be influenced significantly by different soil mixtures. The issues with blossom end rot in 2020 could have been a result of cultivar and nutrient applications. By addressing these in our design, we may have eliminated any relevance to the study. The calcium applications in our fertilizer are likely a large contributor as it is associated with reducing BER rates in greenhouse grown tomatoes (Taylor and Locascio, 2004). Though other studies observed improvements in BER (Surrage *et al.,* 2010; Roberts, 2007), these studies utilized vermicompost with pig manure.

Final Plant Biomass

Similar to the 2020 ‘Brandywine’ trials, there were no differences in plant biomass of tomatoes between the treatments and control. Plant biomass is generally accumulated through nitrogen productivity. As indicated in our SPAD results, lack of differences in greenness would also indicate lack of differences in overall biomass.

BRIX

BSFL and VC amendments do not appear to affect BRIX measurements of dissolved solid concentrations of tomato fruits when compared to a peat control. Studies have indicated that insoluble solids increase with sheep manure (Gutiérrez-Miceli *et al.,* 2007). More experimentation with different larval and diet types may prove useful in improving fruit quality results. Though BRIX is one indicator of potential sweetness and flavor, there are other methods to indicate fruit quality. Sensory panels are another common approach to addressing these parameters and could be useful in future research.

5. Conclusion

Overall, it seems that tomatoes regardless of treatment, do not respond significantly to the addition of an insect frass soil amendment. Amendments were applied only during the transplanting stage. It is possible that the life cycle of this crop is too long to see any observable differences with a single application approach. Since the insect frass and vermicompost amendments were only added to the peat mixture at the beginning of the experiment, longer cycle crops like tomatoes may need a second dressing of insect frass or horse based vermicompost to see treatment differences.

Additionally, other studies indicate positive impacts on germination, emergence, and seedling growth that occurs early in the plant life cycle (Atiyeh *et al.,* 2001; Setti *et al.,* 2019; Zaller, 2007). To address some of these questions, we designed germination experiments examined in Chapter 6. This will allow observations to be made in early emergence and growth of the tomato plant.

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CHAPTER SIX

THE IMPACTS OF BLACK SOLDIER FLY LARVAL FRASS ON THE GERMINATION AND SEEDLING VIGOR OF THREE VEGETABLE CROP SPECIES (ARUGULA, LETTUCE, AND TOMATO)

1. Introduction

There is currently a limited amount of insect frass literature available investigating its effectiveness as a media amendment for seedling germination (Chavez and Uchanksi, 2021). Setti *et. al.* (2019) grew three different vegetable crops in growing media comprised of different ratios of peat to black soldier fly larvae (BSFL) frass. They saw increases in the germination index (speed of germination percentage), rate of emergence, and percent emergence of lettuce when grown in a 10% BSFL frass, 90% peat treatment. Similar results were observed in basil, where 10% BSFL frass treatments produced the highest emergence rate and percent emergence, and BSFL 20% produced the highest germination index. Though tomatoes saw no differences in percent emergence, the germination index was highest in the BSFL 10% treatment, and the emergence rate was highest in BSFL 20%. Other studies have shown similar results. For example, the germination index of cabbage and cucumber seeds was improved when grown with applications of housefly larvae (*Musca domestica*) treated compost (Zhu *et al*., 2012). Seedling trials with several different crops were conducted to assess BSFL frass as a growing medium for seeds in the field (Temple *et al*., 2013). Bok choi, onion, bean, and tomato seedlings produced highest dry matter yields at 1.5 kg/m^2 frass applications, while lettuce and squash had the highest dry matter yield at the 1 kg/m^2 BSFL frass application rate (Temple *et al*., 2013).

Vermicompost (VC) is a similar product to frass, and it serves as a useful standard comparison in our experimentation. Consistent increases in seedling growth, germination, and emergence are reported across the literature. Depending on tomato cultivar, percent emergence may be highest in 100% vermicompost treatments (Zaller, 2007). Other tomato studies have shown greater benefits at lower percentages, such as 15% vermicompost (Joshi and Vig, 2010). A study observed an increase in germination rates when tomatoes were grown with standard fertilizer applications and 20-40% (by volume) vermicompost (Atiyeh *et al.,* 2000). Seedling weight was also higher in 10-50% vermicompost treatments. Another study also reported greater seedling growth at 25-50% vermicompost when applied with standard fertilizers (Atiyeh et al., 2001). Additionally, another study using tomatoes observed the highest germination rates in the treatments amended with 20% vermicompost (Roberts *et al.*, 2007).

Potting media replaced with vermicompost at different percentages was analyzed for impacts on growth and germination (Bachman and Metzger, 2008). Vermicompost 20% produced greater shoot and root weight of seedlings, but overall germination was not impacted by treatments. However, seedlings in the vermicompost treatments performed better after transplantation into the field compared to those without vermicompost (Bachman and Metzger, 2008).

Vegetable crop germination preliminary experiments were conducted in August of 2022. Poor germination was observed in high frass treatments, especially in the absence of vermicompost. A follow up greenhouse study was then conducted in September of 2022 that included three different vegetable crops: arugula, lettuce, and two tomato cultivars. Our major objective was to investigate the impact of insect frass on vegetable seed germination and seedling vigor in the greenhouse. Our hypotheses were deduced based on the preliminary studies.

Question 1: Does partially replacing peat as a media amendment with BSFL frass impact germination and seedling vigor of vegetable crops?

Hypothesis: Treatments amended with BSFL frass will produce seedlings comparable to the control.

Question 2: Is the germination and seedling vigor of vegetable crops impacted by replacing vermicompost with BSFL frass as a fertility source in a starting medium?

Hypothesis: BSFL frass treatments will perform more poorly than the vermicompost control.

2. Materials and Methods

This study took place in Fort Collins, CO at the Colorado State University Horticulture Center greenhouses, elevation 1,525.22 meters (en-us.topographic-map.com). On September 12, 2022, two tomato cultivars ('Brandywine' and 'Jet Star') were sown at 0.64 cm deep, and lettuce ('Salvius MT0') and arugula ('Apollo') seeds were sown at 0.32 cm deep. These crops were grown at 20-25°Celsius, in flats, and arranged on greenhouse benches. The experiment was carried out as a completely randomized design (CRD) using four levels total (three treatments of each amendment and a 100% standard media mix), four vegetable crops, and five replicates/level. Ten seedlings of each crop, the observational units, were sown in each tray or flat, which were treated as the experimental units or replicates (Figure 6.1). Seedlings were harvested fourteen days after planting on September 26, 2022.

Standard organic media mixture for CSU Specialty Crops Program was comprised of peat (3.79 L), vermicompost (0.47 L), blood meal (29.57 mL), and bone meal (29.57 mL). This recipe served as our control medium. Seedlings were also grown in three different treatments: 1. standard mixture with the replacement of vermicompost with BSFL frass, 2. standard mixture (with vermicompost) and 10% of the peat replaced with BSFL frass, and 3. standard mixture with (vermicompost) and 20% of the peat replaced with BSFL frass (Table 6.1). Seedlings were watered daily with no additional fertilizer applications. The day each seedling emerged was recorded. After two weeks, the seedlings in each tray were clipped at ground level and weighed as is, and the total number of seedlings emerged was recorded. Percent emergence was calculated based on the number of seedlings that emerged [(Seedlings emerged/Seeds planted) x 100%].

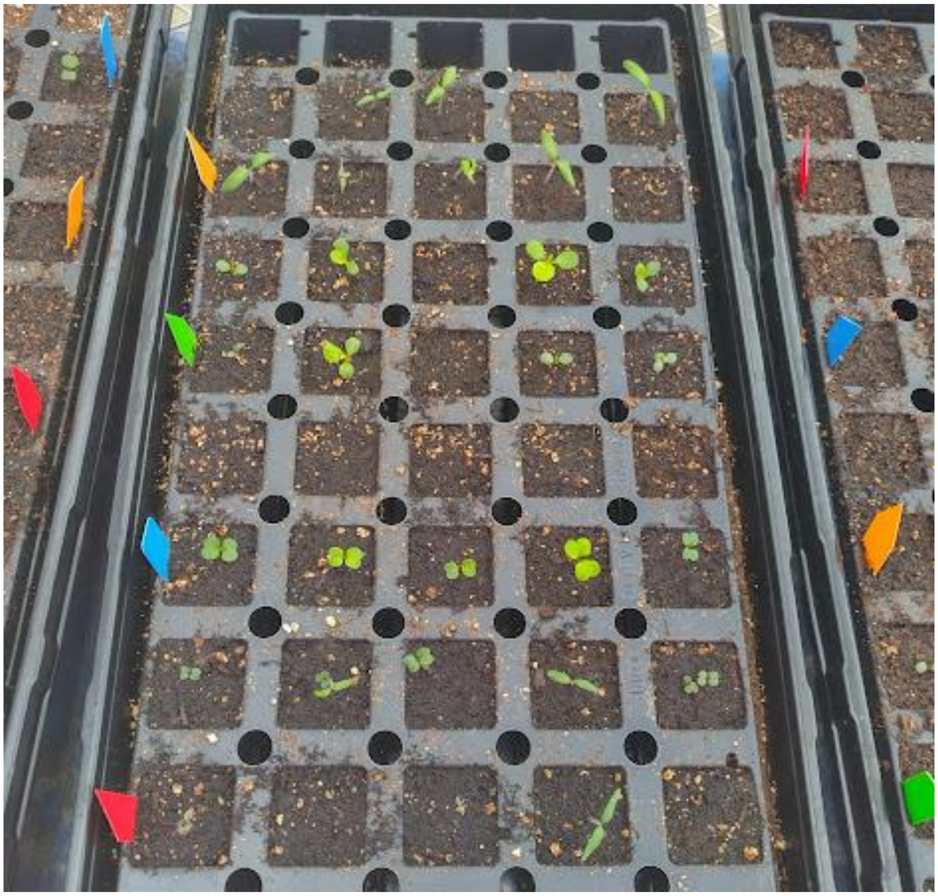


Figure 6.1: ***Seedlings (observational units) planted in the treatment flats (experimental replicates).*** Arugula (blue stakes), lettuce (green stakes), 'Brandywine' tomatoes (red stakes), and 'Jet Star' tomatoes (orange stakes) were grown in treatment mixtures of commercial peat and black soldier fly larvae frass and/or vermicompost. Vegetable species were randomly placed in each flat/replicate.

Table 6.1: Standard mixture for seed starting media in 2022 greenhouse vegetable studies. This design was applied to two cultivars of tomato, lettuce, and arugula.

	Control	**BSFL frass**	**BSFL 10 %**	**BSFL 20%**
Peat	3.79 L	3.79 L	3.46 L	3.072 L
Blood meal	29.57 mL	29.57 mL	29.57 mL	29.57 mL
Bone meal	29.57 mL	29.57 mL	29.57 mL	29.57 mL
Vermicompost	0.47 L	0	0.47 L	0.47 L
BSFL frass	0	0.47 L	0.38 L	0.77 L

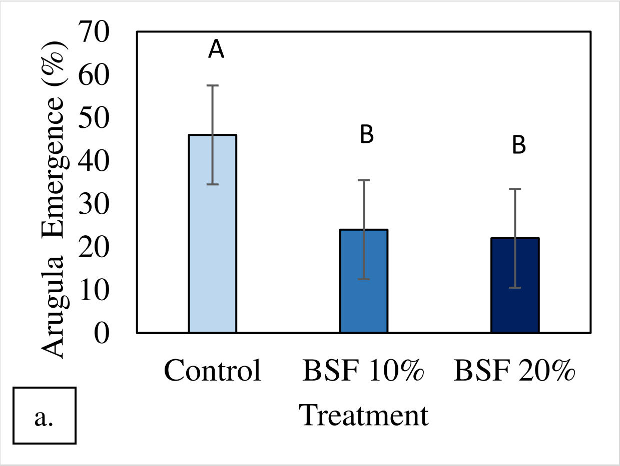
Statistical Analysis

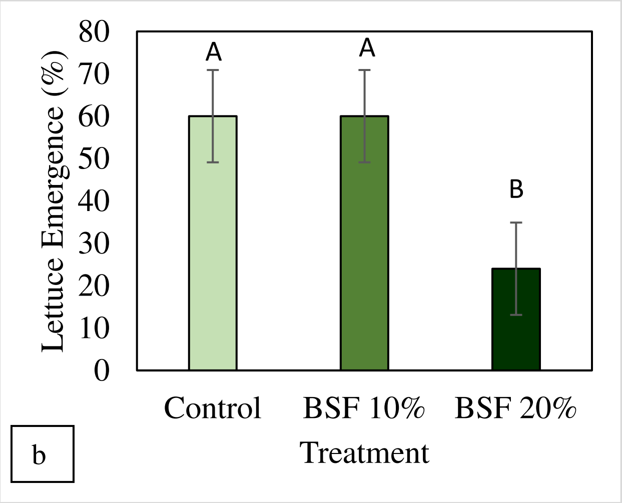
Statistical analysis was conducted using JMP®, Pro 16 (SAS Institute Inc., Cary, NC, 1989–2022). To evaluate days of emergence and seedling vigor, the Anderson-Darling test for normality and Levene's test for equal variance were conducted to determine if the assumptions for an ANOVA and Pooled T Test were met. When they were not met, non-parametric Wilcoxon and Mann Whitney U rank sums tests were conducted in addition to the parametric tests. A threshold of $p < 0.05$ was considered to be significant. Tukey HD and Dunnet pair wise comparisons were conducted to assess and test for differences between each level. The likelihood of emergence for each plant was examined with an odds ratios using a nominal logistic fit for outcome. Mean and statistic summary tables can be found in Appendix II.

3. Results

BSFL frass as a growth germination medium: percent emergence

Average percent emergence varied based on the crop (60% - 18%), but ultimately there were no significant differences based on treatment in Brandywine tomatoes (Figure 6.2). BSF 20% produced the lowest numerical emergence in all four varieties. In arugula (46%), lettuce (60%), and 'Jet Star' tomatoes (46%), the control produced the highest numerical emergence. In lettuce (60%) and ‘Jet Star’ tomatoes (38%), the BSF 10% treatment was comparable to the control, while significantly higher than the BSF 20% treatment.





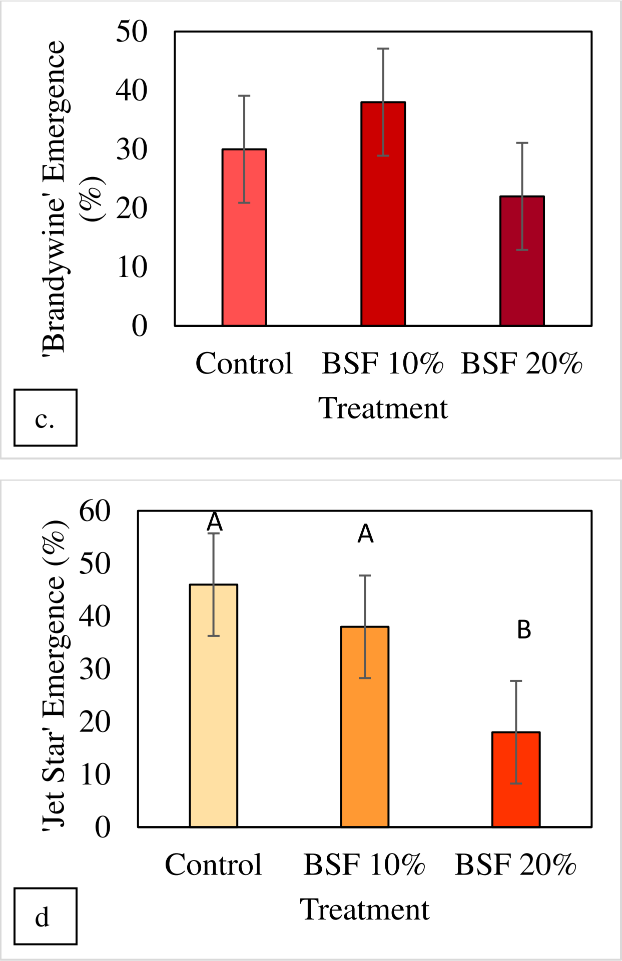
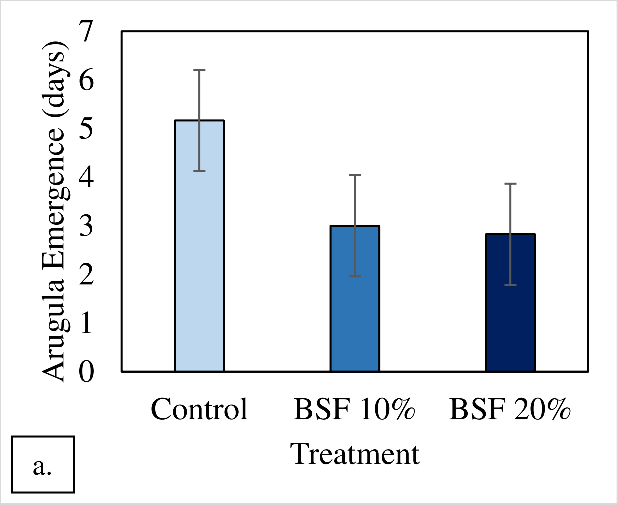
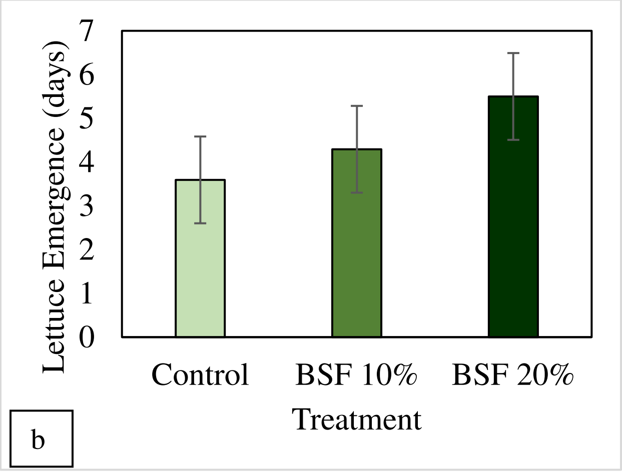


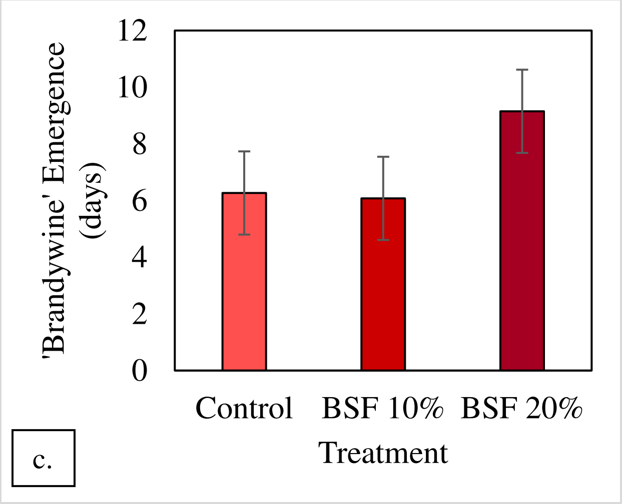
Figure 6.2: ***Percent emergence of vegetable crop seeds: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jetstar'.*** Arugula, lettuce, and tomatoes were grown in a standard starting media and the same starting media (Control: lightest column) with black soldier fly larvae frass utilized as a partial peat replacement (BSF treatments: darker columns), df=2 (a. $p = 0.017/\chi^2 = 8.13$; b. $p = 0.0001/\chi^2 = 17.99$; c. $p = 0.21/\chi^2 = 3.076$; d. $p = 0.0076/\chi^2 = 9.77$).

BSFL frass as a germination medium: days to emergence

There were no statistically significant differences in days to emergence based on treatment in any of the four crops (Figure 6.3). In lettuce and the tomato cultivars, the control plants emerged the earliest. Arugula was the only crop to experience numerically earlier emergence in the frass treatments (three days after sowing).


Arugula Emergence (days)
0
1
2
3
4
5
6
7
Control
BSF 10%
BSF 20%
Treatment
a.


Lettuce Emergence (days)
0
1
2
3
4
5
6
7
Control
BSF 10%
BSF 20%
Treatment
b


'Brandywine' Emergence (days)
0
2
4
6
8
10
12
Control
BSF 10%
BSF 20%
Treatment
c.

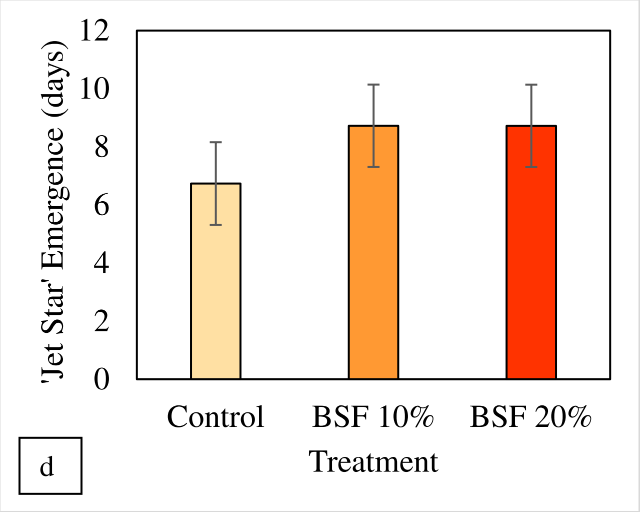
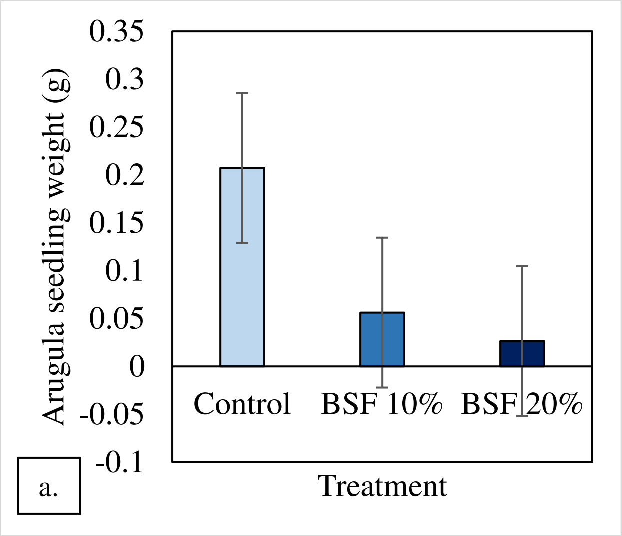
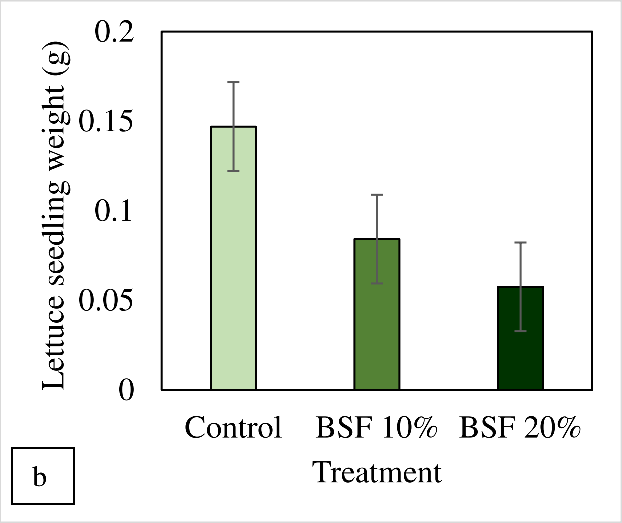


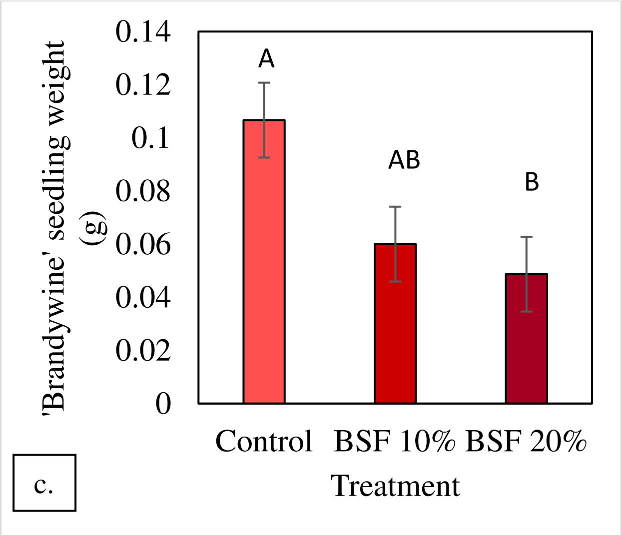
Figure 6.3: ***Days to emergence for vegetable crops: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jet Star'.*** Arugula, lettuce, and tomatoes were grown in a traditional starting media (Control: lightest columns) and the same starting media with black soldier fly larvae frass as a partial peat replacement (BSF treatments: darker columns), df=2 (a. p = 0.25/F = 1.57; b. p = 0.42/F = 0.95; c. p = 0.29/F = 1.38; d. p = 0.54/F = 0.65).

BSFL frass as a germination medium: seedling vigor by weight

According to the ANOVA results, arugula (Figure 6.4a.) and lettuce (Figure 6.4b.) seedling weight did not vary significantly by treatment, though in both crops the control produced the heaviest seedlings, and the BSF 20% produced the lightest seedling on average. Arugula data did not pass the test for normality or equal variances, so non-parametric tests were conducted for further inference. According to the χ^2 (7.33) analysis, there are significant differences (p = 0.03) in treatments based on rank sums. From this it can be further emphasized that the control (0.21g) produced the largest seedlings. In both tomato cultivars, 'Brandywine' (Figure 6.4c) and 'Jet Star' (Figure 6.4d.), the control (0.11g) was significantly heavier than the BSF 20% treatment, and the BSF 10% was comparable to both.


Arugula seedling weight (g)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
Control
BSF 10%
BSF 20%
Treatment
a.


Lettuce seedling weight (g)
0.2
0.15
0.1
0.05
0
Control
BSF 10%
BSF 20%
Treatment
b


'Brandywine' seedling weight (g)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
A
AB
B
Control
BSF 10%
BSF 20%
Treatment
c.

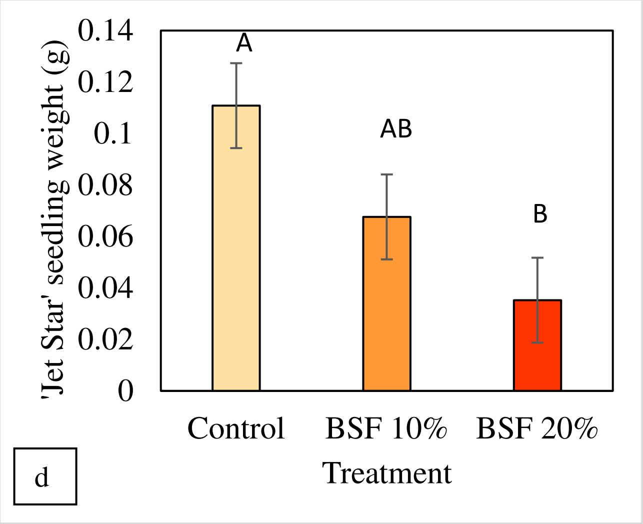
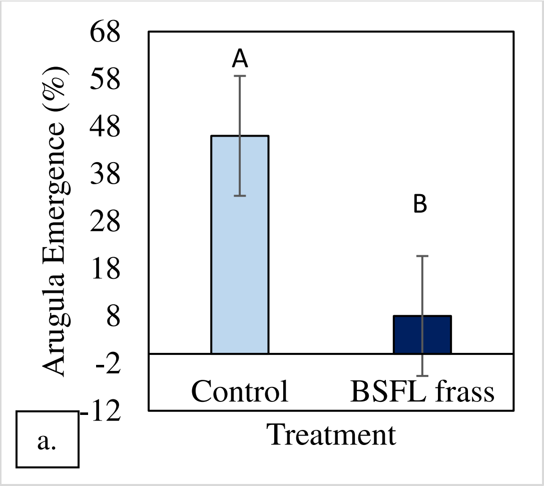
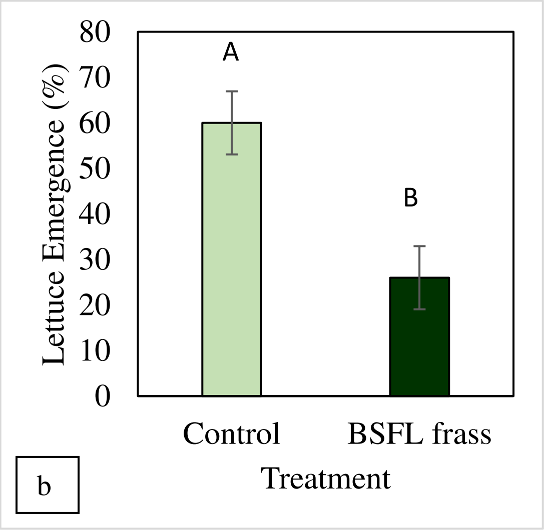


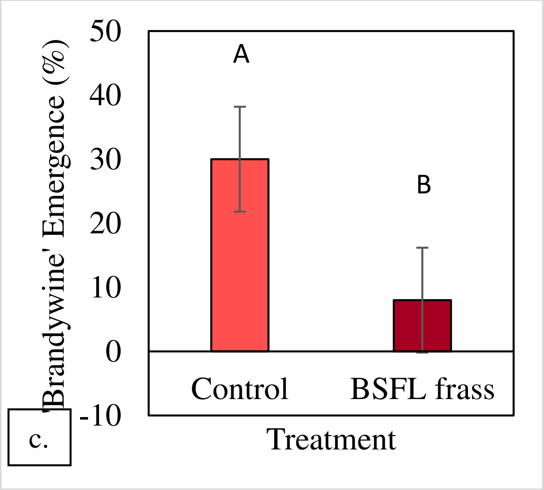
Figure 6.4: ***Seedling weight of vegetable crops: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jet Star'.*** Arugula, lettuce, and tomatoes were grown in a traditional starting media (Control: lightest columns) and the same starting media with black soldier fly larvae frass instead partial peat replacements (BSF treatments: darker columns). Different letters indicate pairwise differences, df=2 (a. p = 0.25/F =1.57; b. p = 0.066/F = 3.44; c. p = 0.03/F = 4.76; d. p = 0.023/F = 5.29).

BSFL frass a fertility replacement: percent emergence

There were significant differences in percent germination of arugula (Figure 6.5a), lettuce (Figure 6.5b), 'Brandywine' (Figure 6.5c) tomato and 'Jet Star' (Figure 6.5d) tomato based on treatment. The control in arugula (46%), lettuce (60%), 'Brandywine' (30%), and 'Jet Star' (46%) produced a significantly higher germination emergence compared to the BSFL frass treatment.


68
58
48
38
28
18
8
-2
-12
Arugula Emergence (%)
A
B
Control
BSFL frass
Treatment
a.


80
70
60
50
40
30
20
10
0
Lettuce Emergence (%)
A
B
Control
BSFL frass
Treatment
b


50
40
30
20
10
0
-10
'Brandywine' Emergence (%)
A
B
Control
BSFL frass
Treatment
c.

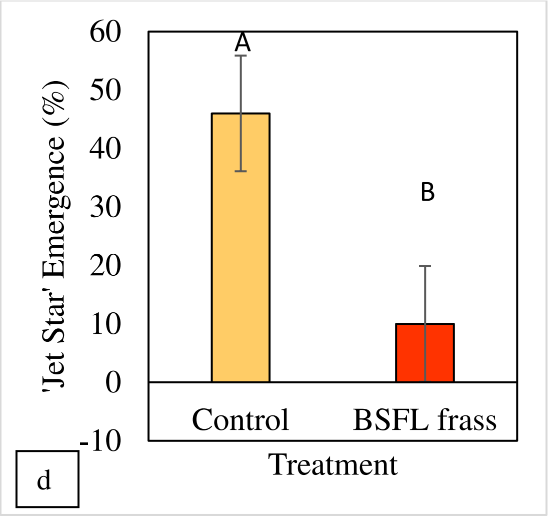
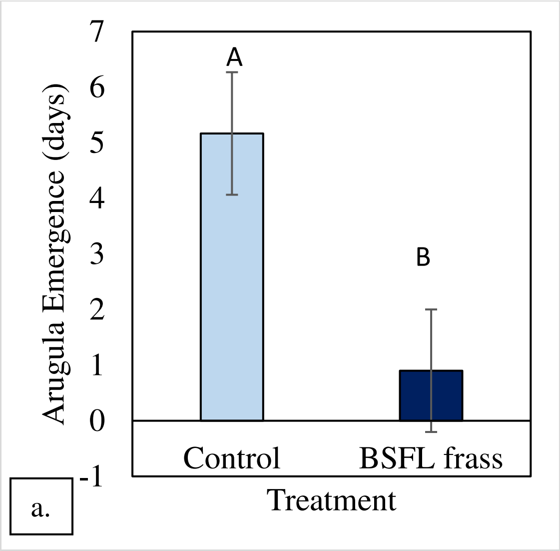
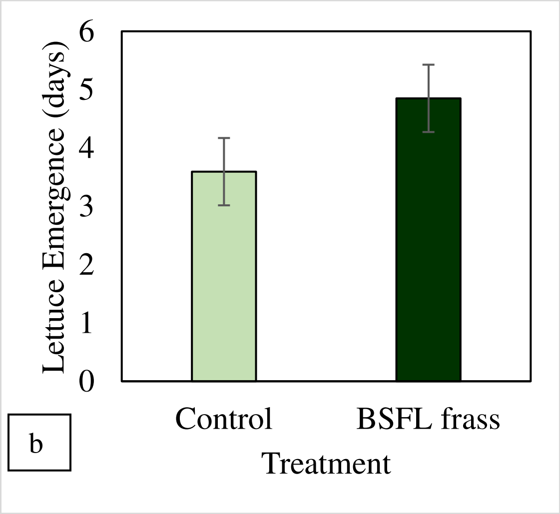


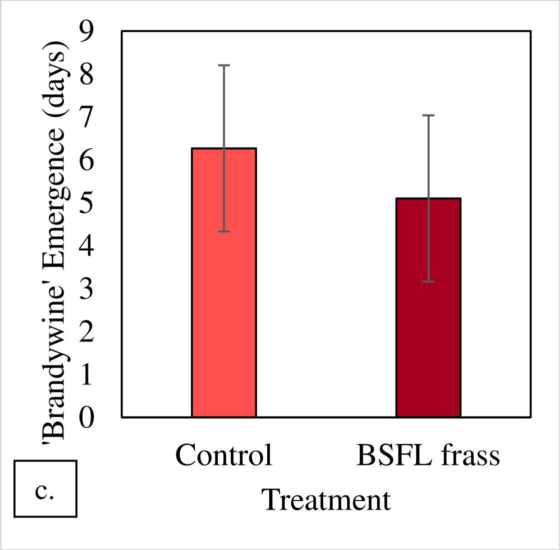
Figure 6.5: ***Percent emergence of vegetable crop seeds: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jet Star'.*** Arugula, lettuce, and tomatoes were grown in a traditional starting media with vermicompost (Control: lightest columns) and the same starting media with black soldier fly larvae frass instead of vermicompost (BSFL frass: darker column), df=1 (a. $p < 0.0001/\chi^2 = 19.78$; b. $p = 0.0005/\chi^2 = 12.056$; c. $p = 0.004/\chi^2 = 8.28$; d. $p < 0.0001/\chi^2 = 17.058$).

BSFL frass a fertility replacement: days to emergence

Most crops did not experience significant differences in the days that it took for emergence to occur (Figure 6.6). In arugula (Figure 6.6a), the control plants (5 days) emerged significantly later than the BSFL frass (1 day). This was also true in the two tomato cultivars, though not statistically significant. The opposite was observed in lettuce; the control plants emerged sooner on average.


A
B
Arugula Emergence (days)
7
6
5
4
3
2
1
0
-1
Control
BSFL frass
Treatment
a.


Lettuce Emergence (days)
6
5
4
3
2
1
0
Control
BSFL frass
Treatment
b


'Brandywine' Emergence (days)
9
8
7
6
5
4
3
2
1
0
Control
BSFL frass
Treatment
c.

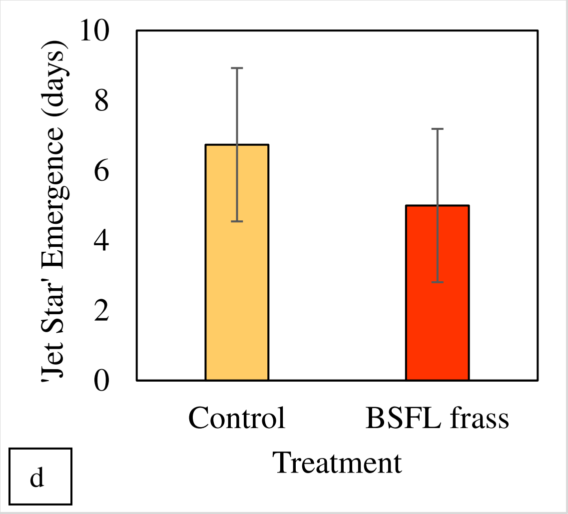
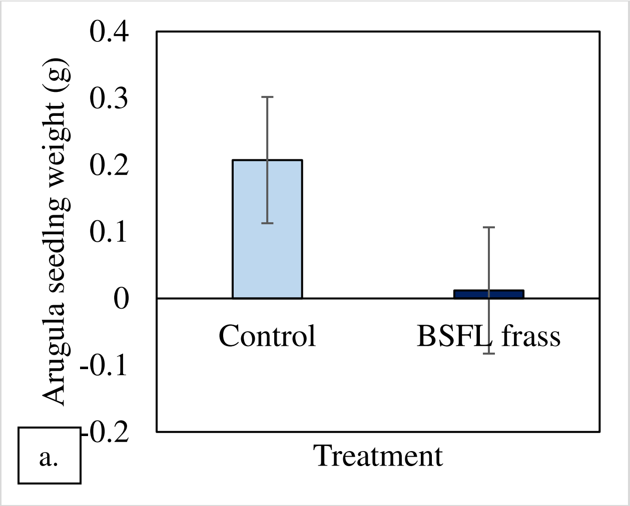
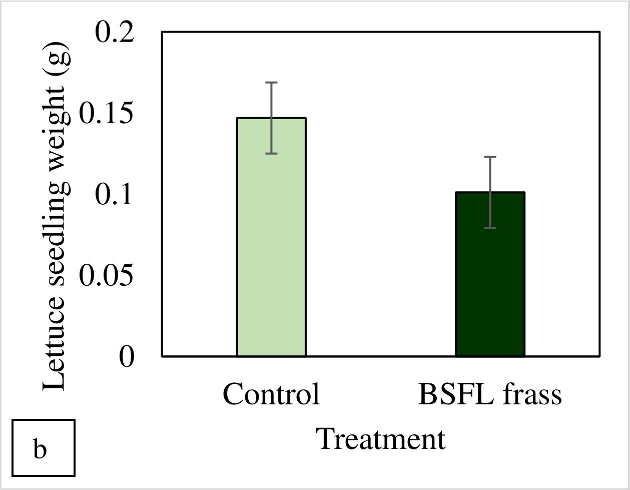


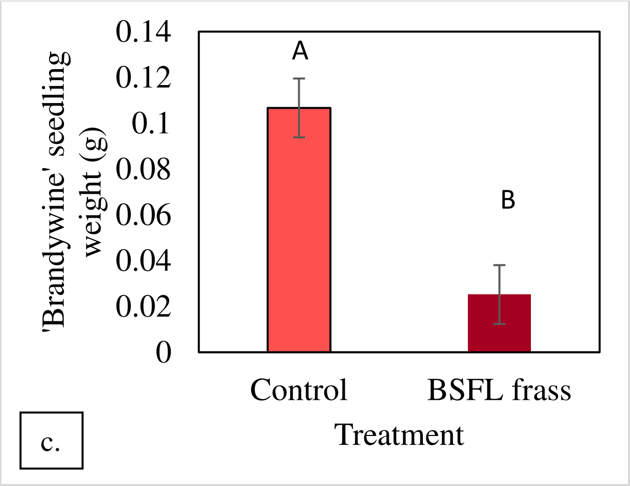
Figure 6.6: ***Days to emergence for vegetable crops: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jet Star'.*** Arugula, lettuce, and tomatoes were grown in a traditional starting media with vermicompost (Control: grey columns) and the same starting media with black soldier fly larvae frass instead of vermicompost (BSFL frass: black column), df=1 (a. p = 0.026/T = 2.74; b. p = 0.16/T = -1.54; c. p = 0.69/T = 0.43; d. p = 0.60/T = 0.56).

BSFL frass a fertility replacement: seedling vigor by weight

There were no significant differences in lettuce seedling vigor (weight) in the BSFL frass treatment compared to the VC control (Figure 6.7b.). 'Brandywine', and 'Jet Star' seedlings grown without vermicompost were significantly lighter than those grown in the traditional starting mix (Figure 6.7c.,d.). The arugula seedling weight ANOVA concluded that there were no significant differences between the control and BSFL frass treatment (p = 0.22). However, the data did not pass the assumptions for normality, so a Mann Whitney U test was performed to assess the differences. A chi square analysis generated a significant difference between the control and treatment (p = 0.0095). Therefore, we can use the information to speculate on existing differences within this crop species.


0.4
0.3
0.2
0.1
0
-0.1
-0.2
Arugula seedlng weight (g)
Control
BSFL frass
Treatment
a.


0.2
0.15
0.1
0.05
0
Lettuce seedling weight (g)
Control
BSFL frass
Treatment
b


0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
'Brandywine' seedling weight (g)
A
B
Control
BSFL frass
Treatment
c.

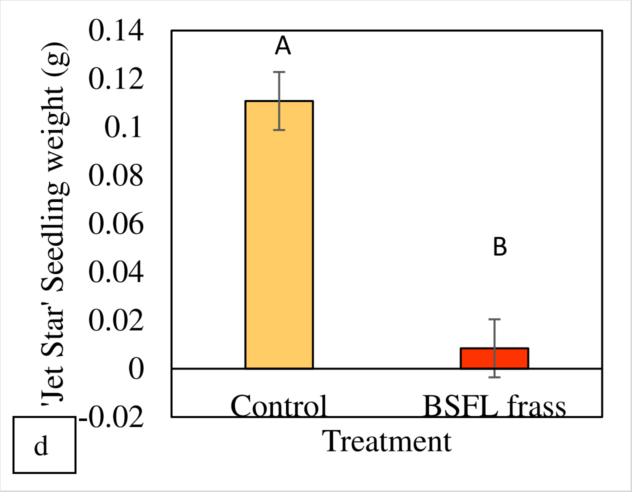


Figure 6.7: ***Seedling weight of vegetable crops: a. arugula, b. lettuce, c. 'Brandywine', d. 'Jet Star'.*** Arugula, lettuce, and tomatoes were grown in a traditional starting media with vermicompost (Control: lightest columns) and the same starting media with black soldier fly larvae frass instead of vermicompost (BSFL frass: darker column), df=1 (a. p = 0.22/T = 1.46; b. p = 0.18/ T = 1.48; c. p = 0.0021/ T = 4.48; d. p = 0.002/T = 6.03).

4. Discussion

BSFL frass as a vegetable seed germination medium

The percent of seedlings emerged for lettuce, arugula, and 'Jet Star' was statistically higher in the control compared to the BSF 20% treatment. BSF 10% in all crops was equal to the control, indicating a small amount of BSFL frass replacement would not influence emergence positively nor negatively. Setti *et al* (2019) saw similar results in germination with a 10% frass growing media. Several studies showed similar results in germination when applied with low amounts of vermicompost (Bachman and Metzger, 2008; Joshi and Vig, 2010; Roberts et al., 2007; Zaller, 2007).

The average days to germination were not statistically different across treatments when a peat-based germination mixture was amended with BSFL frass. This indicates that frass did not increase the timing of emergence, but it did not impede it either. Seedling vigor in the tomato cultivars, the BSF 10% treatment was comparable to the control, but BSF 20% was significantly lower. This indicates that seedling vigor could be negatively impacted at higher percentages of insect frass. The insect frass utilized in this experiment was from a brewery grain feed stock. Observations in Chapters 3 and 4 indicate that brewery grains were not an ideal amendment and further research incorporating other feedstocks should be conducted before making any final conclusions.

BSFL frass a fertility replacement

Replacing vermicompost with brewery grain digested frass appears to produce negative outcomes. Across all four crops the BSFL frass treatment produced lower percent emergence and seedling weight than the control, though not always statistically significant. This could be due to the lack of vermicompost in the growing media. Vermicompost has proven useful in reducing the negative impacts of environmental stressors (Tammam *et al*., 2022). Contradictory to those results, BSLF treatments experienced earlier emergence on average. The only exception was lettuce, which emerged later in the BSFL treatment than the control, but not significantly later.

Tomato varieties seemed to be more sensitive to the growing conditions created by the BSFL frass amendments than the lettuce and arugula. Most studies assessing early-stage growth in tomato seedlings saw the best results with low percentages of vermicompost in the growing media (Bachman and Metzger, 2008; Joshi and Vig, 2010; Roberts et al., 2007; Zaller, 2007). Lettuce and arugula are short cycle crops that had mostly positive or neutral results to frass applications, as reported in chapters 3 and 4. Negative impacts early in the lifespan of a long cycle crop may be harder to address in full season studies if the effects are washed out over time. Crops with longer life cycles often have larger demands for resources, such as water and nutrients (Martin-Gorriz *et al.,* 2020). Fertilizer, organic or synthetic, was not added after the initial growing media. So, it is possible the larger tomato plants reached nutrient depletion more quickly, inhibiting growth across all treatments.

5. Conclusions

Overall, leafy, cool season, short cycle crops, lettuce and arugula, appear to respond better to the BSFL frass applications in the germination mixture than the warm season, longer cycle crop (tomatoes). This aligns with the yield data from our studies in Chapters 3 and 5. Arugula and lettuce grown in BSFL frass treatments often produced higher yield and SPAD measurements than the control and vermicompost treatments. In most cases the tomatoes did not produce yield or quality differences regardless of treatment. The micronutrients present in higher amounts may not be as useful to tomatoes as it is to arugula and lettuce. Lettuce is known to have a more positive response to Mn and Cu than tomatoes, which exhibit a more moderate response (Swiader and Ware, 2002). It is also possible that arugula and lettuce have higher tolerance for salt, which were more present in the BSFL frass compared to the VC. Modern lettuce cultivars and their wild relatives have exhibited a wider range of salt tolerances compared to other crops (Shannon and Grieve, 1998). Arugula cultivars are known to produce high amounts of sugars, proline, and amino acids, which have been correlated with high sodium tolerance (Shannon and Grieve, 1998). A review by Foolad (2004) explored the genetic traits that result in tomato's sensitivity to salinity.

We do not recommend replacing vermicompost with insect frass in the starting media based on this study. The BSFL used in this germination study were reared on brewery grains, which in Chapter 4 we discovered was high in sodium and often had negative impacts on yield compared to other diet types. It is possible that VC provides additional benefits to the starting mix that allowed the seedlings in the BSF 10% treatment to endure salt and heat stress. Vermicompost is known to alleviate the detrimental impacts of environmental stressors (Tammam *et al*., 2022). A study by Hosseinzadeh and Ahmadpour (2018) observed increases in carotenoids, chlorophyll, leaf and root calcium, leaf and root potassium, carbon dioxide assimilation, and transpiration rate of lentils grown in vermicompost applications under no, moderate, and severe water stress. In addition, humic acid and vermicompost applications can improve maize yield when grown in coastal saline soils (Liu *et al.,* 2019).

Depending on the species and feed stock, this effect could also be seen when using insect frass amendments. A study by Poveda *et al*. (2019) investigated meal worm (*T. molitor*) frass treatments in lettuce crops exposed to salinity, drought, and flooding stress. They observed increases in dry weight and root length compared to the negative unfertilized control and results were comparable to the fertilized positive control. This suggests that insect frass could provide support for crops under a variety of stress conditions.

It should be noted that some studies produced negative results when frass was used at the germination growth phase (Kawasaki *et al*., 2020). This may have been due to pathogens present in the frass. Edible insects have been categorized and analyzed for microbial properties (Garofalo *et al.,* 2019). Based on these results and our understanding of bioconversion, it is likely that the microbes observed in insect guts will be found in their fecal excrements as well. Osimani *et al.* (2018) documented this by observing the gut of mealworms and their frass simultaneously. For lactic acid bacteria and mesophilic aerobic bacteria there were no significant differences between frass and larvae. It is important to note that mesophilic aerobic bacteria typically have negative consequences for agricultural production, but other studies have shown that food pathogens are often inhibited by lactic acid bacteria (Lewus *et al.,* 1991). Subsequently, the harmful pathogens, *Listeria* and *Salmonella,* were absent in both the larvae and the frass (Osimani *et al.,* 2018).

Insect frass is allowed in USDA certified organic operations when certain parameters are met. According to Organic Materials Review Institute (OMRI.org, 2022), insect frass cannot contain more than 1,000 Most Probable Number (MPN) fecal coliform/gram and/or more than 3 MPN Salmonella/4 grams sampled. Following the USDA organic guidelines for frass application may be wise for all growers until more is understood about this novel product.

6. References

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2. Bachman, G. R., and Metzger, J. D. 2008. "Growth of bedding plants in commercial potting substrate amended with vermicompost." *Bioresource technology* 99 (8): 3155-3161.
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CHAPTER SEVEN

ECOLOGICAL IMPACTS OF INSECT INTEGRATION INTO THE FOOD SYSTEM

1. Introduction

One-third of all food produced in the United States is eventually discarded as waste. This equates to 1.3 billion tons of food per year (FAO, 2011). Insect production as food and feed provides an outlet to redirect waste streams from certain food partners. Large insect production companies, like Protix, digest around 10-100 metric tons per day. Smaller companies, like EVO Conversion Systems, are more likely to digest around one metric ton per week (Jeff Tomberlin, personal communication). If 50% of the available food waste was used as feed for insects, 1.5 million tonnes of insect protein could be produced globally by 2030 (Elleby *et al.,* 2022).

The major caveat with this system is that insect rearing creates a waste product of its own, primarily insect frass mixed with left-over feed stock. EVO Conversion Systems can produce up to 11,000 kilograms of waste in one year (JA Cammack, personal communication). At a larger scale, such as the country of China, the estimated live larvae production per day is around 250 metric tons, and considering conversion rate of 20%, these accounted for 1,250 metric tons of waste being eaten by BSFL per day (F Yang, personal communication). Recently, research and attention have been brought to repurposing this waste product (frass), as well. Peat replacement in controlled environment agriculture (CEA) is currently a popular option (Niu and Masabni, 2018).

Peat is the decaying plant matter found in peat marsh ecosystems (Bloom, 1964). It is harvested for horticultural production because of its excellent water holding capacity and loamy texture. This is technically a renewable resource because it accumulates from living plant matter and edaphic characteristics (Brioche, 2020). However, it is being mined more quickly than can be naturally replenished, resulting in local and large-scale detriments to the ecosystem (Couwenberg *et al*., 2011). Due to harvesting and land use change, peatlands have been decreasing by 0.05% globally each year. (Brioche, 2020). Peat is used mostly in agriculture and energy production. Around 30,000 thousand metric tons of peat are mined each year, and 12,000,000 thousand metric tons are currently in reserves (Brioche, 2020). About 0.0005% of the extracted peat is utilized in horticultural production, typically for growing media in controlled environment agriculture (CEA) (IPS, 2019). This equates to 6,015 thousand metric tons of peat each year. Alternatively, there are around four million km^2 of peatland on earth, most of which is located in undisturbed arctic and subarctic peat marshes. Roughly half a million (572,000) km^2 is used for agroforestry purposes in total, and 2,000 km^2 of that area is specifically designated for growing media (IPS, 2019). This equates to 2.86% of agroforestry production that is designated for growing media.

A 2019 International Peat Society (IPS) report delineates horticultural growing media and agriculture as two separate categories and does not explicitly define agriculture. Based on this vague reporting, it can be assumed that these values included in the agriculture category are peatlands that are drained for large scale agronomic and horticultural crop production.

CEA is the production of plants in structures such as high tunnels, greenhouses, growth chambers, or indoor vertical farming (Niu and Masabni, 2018). CEA is an alternative approach to provide food production with protection from natural environmental factors, such as pests, disease, and extreme weather events. Greenhouses are popular in urban areas with less land for crop production (Walker et al., 2019). Additionally, environments with extreme climate factors, such as cold and aridity, rely on greenhouses for access to locally grown food all year.

Greenhouse production was mostly developed and refined in the Netherlands, which is the source of most of the imported technology around the world. Greenhouse products make up around 1-2% of all agricultural production. Recently, however, the number of greenhouse producers has increased by 71%, and these numbers are expected to increase (Maureira *et al.,* 20).

This project utilized an existing waste stream, black soldier fly larvae frass sourced from EVO conversion systems, to partially replace peat in a greenhouse vegetable potting media. BSF larvae had been reared on either distillery or brewery grains as a feedstock. The research from this study, and several others, indicates that insect frass can safely and effectively replace about 10% rate by volume of the peat in a growing media. Reducing peat-based growing media by 10% would result in 602 fewer thousand metric tons of utilized peat and 200 km^2 fewer disturbed peatlands. Because insect companies can produce up to 250 metric tons of frass a day, they could easily support a 10% reduction in peat based growing media.

As observed in this research, some starting materials for larval diets are more suitable than others for growing media substitutes. Navigating this will require planning by food waste donators, insect producers, and crop growers to maintain consistent testing during implementation. Coordinating the burdens of production across these three shareholders will strengthen our circular economy (Chavez, 2021).

2. Limitations

The major limitations of this work were the inability to replicate the same frass type in the same season. Due to supply issues, we had to switch the source material for the frass halfway through. This allowed us to address different questions, but also introduced new variables.

Additionally, pot studies can only provide so many answers. Pots studies produce isolated environments for plant growth. Therefore, there is a limited scope in the interpretation of research questions and inference. We treated frass as a soil amendment, instead of a fertilizer. Multiple applications of a modified frass as a fertilizer may be more effective than treating it as a substrate, especially in long cycle crops like tomatoes.

The negative results with the brewery grain frass (Chapters 3, 4, and 6) may have been the result of microbial contamination or simply the feedstock characteristics. Other studies also produced negative results when frass was used for germination (Kawasaki *et al*., 2020). Various microbes, harmful and beneficial, have been discovered in the guts of edible insects (Garofalo *et al.,* 2019). It is likely that the microbes observed in insect guts will be found in their fecal excrement, frass, as well (Osimani *et al.* 2018). The Organic Materials Review Institute (OMRI.org, 2022) states that insect frass cannot contain more than 1,000 Most Probable Number (MPN) fecal coliform/gram and/or more than 3 MPN Salmonella/4 grams sampled. These USDA organic guidelines may be beneficial for all growers and researchers interested in frass applications. We did not test for MPN of Salmonella in this work.

Though greenhouses are a relatively standardized environment, fluctuations in seasonal temperatures and other climatic factors can impact results. This work took place over two seasons: spring and summer. The greenhouse temperatures were set to 20-25^{o}C. However, the average outdoor temperatures in the spring months were much lower, 4.4 - 6.1^{o}C, than the summer months, 20.6 - 23.3^{o}C (CoAgMet.com), which could have placed some pressure on plants grown during the spring. Utilizing other controlled environment technology could provide more discrete results throughout the year. Growth chambers provide precise control of the growing conditions for plants and provide a convenient solution for controlling variables that may influence productivity (Potvin and Tardif, 1988), but are not typically used for food production due to size and costs.

3. Future Work

Through this project we can only account for one edible insect species, sourced from one company, applied to three vegetable crops. There are many more questions left to be explored. Ideally, we would evaluate a wider range of crops in different environments using different sources of frass.

Some of the largest users of peat are ornamental and backyard growers. Focusing the use of frass to satisfy their needs will be important to maintaining relevancy, increasing adoption, and maximizing impact. Additionally, insect producers are interested in discarding their frass waste at relatively large volumes. Field applications would be the quickest way to manage large amounts of material. More studies focused on impacts on field crops and long-term influences of frass application at a landscape level are essential to truly mitigating waste stream accumulations and improving circular economies.

The characteristics of frass itself remains somewhat unexplored. Cation exchange capacity, bulk density, field capacity, water holding capacity, plant growth regulators, and enzymatic activity have only been sparsely explored and will require more research. More microbial, chemical, and physical analyses of frass and its interactions with natural soil, water, fertilizer, and other substances will provide an underlying foundation for this research and advancing our understanding of the material and its potential uses.

We observed increases in yield and nutrient concentrations of lettuce and arugula leaves when those crops were grown in distillery grain frass amendments. However, the addition of nutrient-containing amendments, such as frass, could increase nutrient leaching from the growth media as well. Soils amended with pine biochar and poultry litter + pine biochar produced a significant decrease in nutrient leaching (Boharaa *et al.,* 2019). Therefore, incorporating biochar into frass could improve its overall value as a peat replacement and fertilizer.

Sensory panels to evaluate the flavor differences in fruits and vegetables grown in frass amendments could provide beneficial insight to produce quality and consumer preferences. In one study, human sensory panels were trained to evaluate the differences in ecologically (grown by organic/natural methods) and conventionally grown tomatoes that were informed of the distinction in growing conditions preferred the ecologically grown tomatoes (Johansson *et al.,* 1999). This indicates that marketing focused on crop production practices may have an influence on consumer preference and could be an area of investment for insect producers and vegetable growers.

4. Conclusions

Utilizers of peat-based growing media inherit the smallest portion of responsibility in reducing peat consumption in agroforestry practices. However, this does not mean their practices should not be improved. Creating awareness of the large-scale global impacts of peat extraction and altering production systems to reduce impact allows CEA producers the opportunity to lead by positive example. Our actions in comparison may seem immaterial, but they are not. Accountability from all sectors, must be addressed and using the momentum of peat consciousness currently in the field can put pressure on other industries to mitigate their impact as well.

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APPENDIX I

PRELIMINARY SCREENING STUDIES OF THE IMPACT OF BLACK SOLDIER FLY LARVAE FRASS ON VEGETABLE CROPS

1. Introduction - 2020 Summary Experiments

A greenhouse study was conducted from June – November 2020 that included three different vegetable crop species: arugula, radishes, and tomatoes. Our major objective was to investigate the impact of insect frass on vegetable yield. Peat potting media was amended with six different frass volumes and compared to an unamended control (100% peat) and a commercially adopted amendment, vermicompost.

Questions: How will yield compare across frass amendment treatments, the vermicompost amendment treatments, and the peat control? In initial screening, is there a lethal dose?

> **Hypothesis**: Frass amendment treatments will positively influence yield up until a certain concentration. Black soldier fly larvae (BSFL) frass 10 and 20% will have the greatest impact on yield compared to vermicompost amendments and the peat control.

2. Materials and Methods

This study took place in Fort Collins, CO at the Colorado State University Horticulture Center greenhouses. Experiments ran from June to November of 2020. Treatments were comprised of either amendments mixed and incorporated into the Berger OM peat potting media, black soldier fly larvae frass (sourced from Evo Conversions System,) and vermicompost (sourced from Rocky Mountain Soil Stewardship, Fort Collins, CO); in addition to the unamended peat control. The screening experiment was conducted as a completely randomized design (CRD) using 13 levels total (6 treatments of each amendment and a 100% commercial peat control), three crop species, and five replicates/level over the growing period. The amendments, black soldier fly larvae (BSFL) frass or vermicompost (VC), were mixed into a traditional growing peat media (control peat, CP) at the following percentages of amendment of 100%, 80%, 60%, 40%, 20%, and 10%, and the 100% CP (see Table 1). The three crops selected were arugula, radishes, and tomatoes based their length of life cycle and temperature needs. The powdered solid fertilizer had the analysis 12:16:4 (Jirdons Premium Vegetable Fertilizer, Alliance, NE) was applied at the labelled rates specific to each crops needs.

Table I.1: Amendment treatment summary for 2020 greenhouse vegetable studies. This design applied to arugula and radish crops, and both amendment types: vermicompost (VC) and black solider fly larvae (BSFL) frass. Tomato percentages were consistent with this table, but they were grown in a pot with a volume 4 times the size of the arugula and radish, so the volumes were 4 times higher.

Black soldier fly larvae frass treatments				**Vermicompost treatments**			
Peat %	**Peat (L)**	**BSFL %**	**BSFL (L)**	**Peat %**	**Peat (L)**	**VC %**	**VC (L)**
0	0.0	100	2.0	0	0.0	100	2.0
20	0.4	80	1.6	20	0.4	80	1.6
40	0.8	60	1.2	40	0.8	60	1.2
60	1.2	40	0.8	60	1.2	40	0.8
80	1.6	20	0.4	80	1.6	20	0.4
90	1.8	10	0.2	90	1.8	10	0.2
100	2.0	0	0.0	100	2.0	0	0.0

Arugula

Arugula (*Eruca sativa* 'Apollo') was planted on June 19, 2020. These were direct seeded and grown in plastic pots, 2 liters in volume. Arugula needs lower temperatures; so they were grown at 15-22ºC. They have short life cycles and were ready to harvest in approximately four weeks, July 22, 2020 (DAP: 33). Powered solid fertilizer was applied once during initial planting according to crop needs, 5.35 g of Jirdon Premium Vegetable Fertilizer (NPK- 12:16:4) incorporated into the potting media. Crops were watered once daily.

Radish

Radishes (*Raphanus sativus* 'Early Scarlet') were planted on June 19, 2020. These were directly seeded and grown in plastic pots, 2 liters in volume. Like arugula, radishes need lower temperatures, and were grown in the same greenhouse, at . They also have short life cycles and were ready to harvest on the same date as the arugula, July 22, 2020 (DAP: 33). Fertilizer was applied once during initial planting according to fit crop needs, 5.35 g (roughly 0.5 tablespoons) of Jirdon Premium Vegetable Fertilizer per plant (NPK- 12:16:4). Crops were watered once daily.

Tomato

Tomatoes were started as seedlings on a misting bench on June 17, 2020. They were grown in a peat, vermicompost, blood meal, bone meal mixture. Once the tomato transplants had grown one set of true leaves, they were transplanted into plastic pots, 2 liters in volume, on July 10, 2020. Several weeks after that, they were then transplanted into "3 gallon" plastic pots, 8 liters in volume, on August 7, 2020. This crop was grown in a warm temperature greenhouse, at

20-25^{o}Celsius. Tomatoes began to ripen on September 9, 2020 (DAP: 82). Tomatoes have a long life cycle and fertility management was adjusted for this reason. Jirdon Premium Vegetable Fertilizer (NPK – 12:16:4) was applied biweekly every other week and depending on crop needs. Tomatoes were initially irrigated by hand once daily while in the 1 pots, but were then watered twice daily once transplanted into the larger pots. Tomatoes were harvested once fully red. Ripe tomatoes were removed from the plant and weighed. They were checked for any disease or physiological disorders, such as blossom end rot (BER), marked marketable or unmarketable, and weighed. Final harvest and tomato plant biomass was collected on November 23, 2020.

Statistical Analysis

Lettuce and arugula plants, the observational units, were treated as averages within the pot, or experimental replicates. Statistical analysis was conducted using JMP®, Pro 16 (SAS Institute Inc., Cary, NC, 1989–2022). The Anderson-Darling test for normality and the Levene's test for equal variance were conducted to determine if the assumptions for an ANOVA and Pooled T Test were met. When they were not met, non-parametric Kruskal Wallis and Mann Whiteny U rank sums tests were conducted in addition to the parametric tests. A threshold of a $p > 0.05$ was considered as significant. Tukey HD and Dunn method for Joint Ranks pair wise comparisons were conducted to assess and test for differences between each level. Mean and statistic summary tables can be found in Appendix II.

3. Results

Arugula

Fresh (Figures I.1-2) and dry (Figures I.3-4) weights from arugula experiments were assessed as a mean total per replicate (Figures I.1 and I.3) and the mean average within each replicate (Figures 2 and 4). In arugula, the three highest frass treatments (100%, 80%, 60%) were lethal to the plants. Zero inflated results are difficult to analyze, and we have utilized these findings mostly in the efforts to plan future experiments. Since all analysis failed equal variance and normality, we followed all of our ANOVAS with non-parametric, Wilcoxon tests and Dunn method for Joint Ranking to in order to sufficiently analyze the treatment differences (Table I.2). Though there were some numerical differences, there were no statistically significant differences between non-zero treatments (Figures I.1-4). Leaf area of arugula was also averaged for each treatment (Figure I.5). There was considerable variability in the data and despite some large numerical differences, there was no statistical evidence to suggest leaf area varied per treatments.

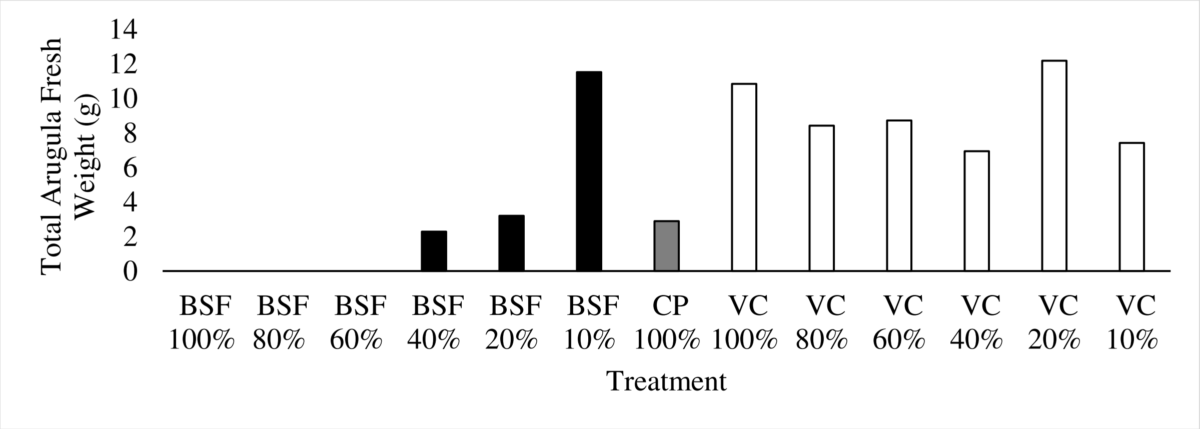

Figure I.1: ***Total fresh weight (grams) of amended arugula grown in the greenhouse.*** Arugula were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey column). 100%, 80%, and 60% BSF larvae frass were a lethal concentration and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking.

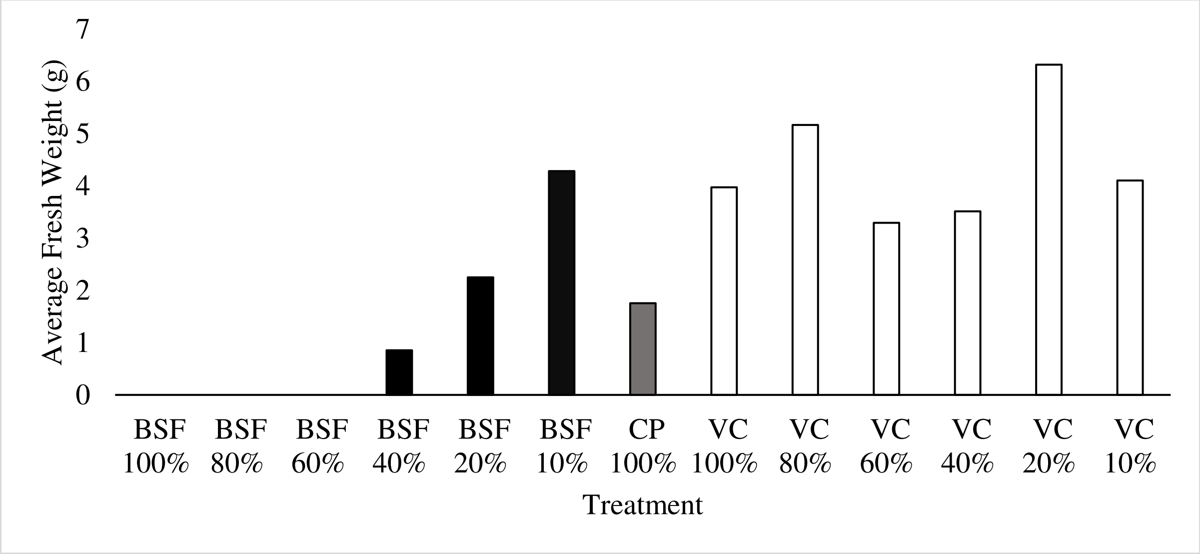

Figure I.2: ***Average fresh weight (grams) of amended arugula grown in the greenhouse.*** Arugula were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey column). 100%, 80%, and 60% BSF larvae frass were a lethal concentration and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by Dunn method for Joint Ranking.

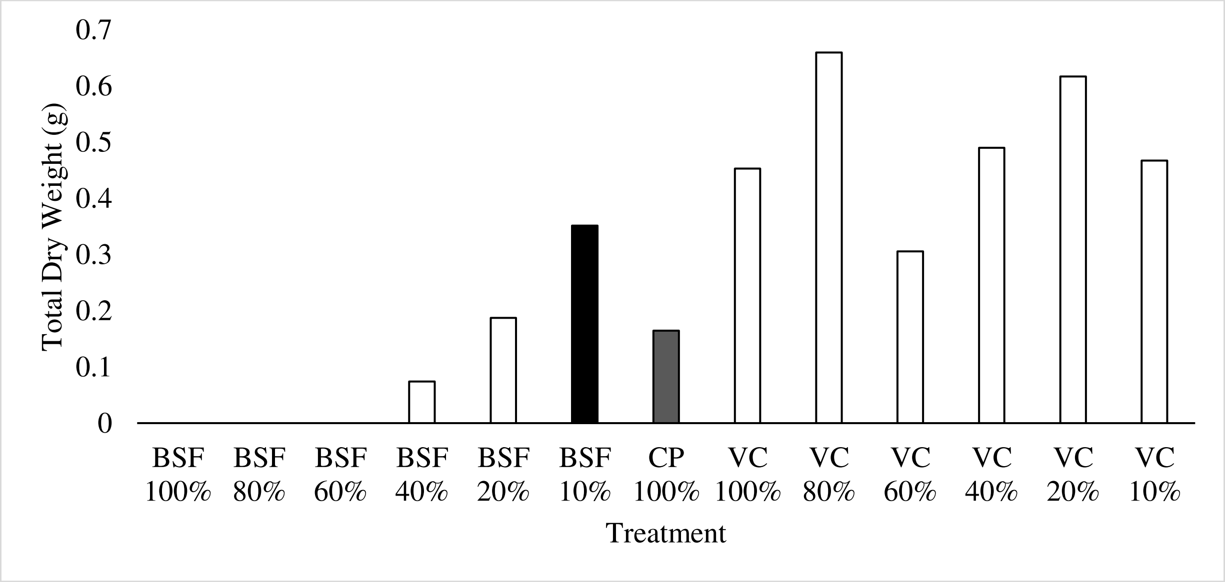

Figure I.3: ***Total dry weight (grams) of amended arugula grown in the greenhouse.*** Arugula were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey column). 100%, 80%, and 60% BSF larvae frass were a lethal concentration, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking.

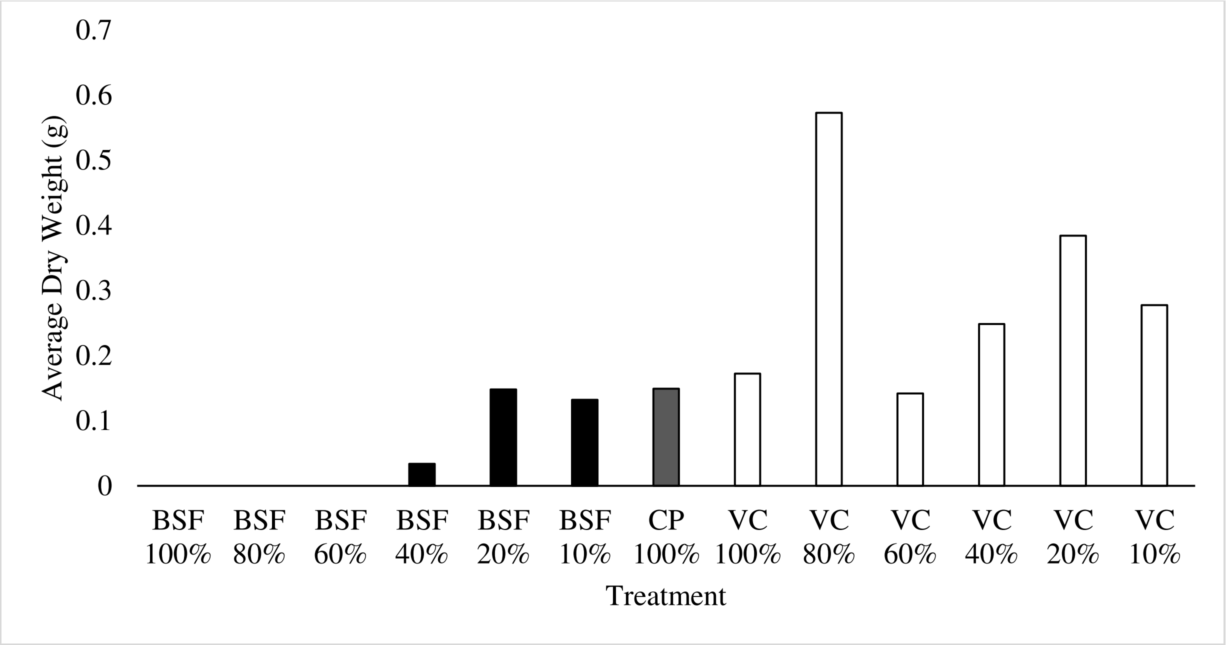

Figure I.4: ***Average dry weight (grams) of amended arugula grown in the greenhouse.*** Arugula were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey column). 100%, 80%, and 60% BSF larvae frass were a lethal concentration and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn

Radishes

Fresh (Figures 6-8) and dry (Figures 9-11) weights from radish experiments were assessed as a total weight (Figures 6 and 9), above ground weight (Figures 7 and 10), and below ground weight (Figures 8 and 11) of each replicate. Similar to arugula, the 3 highest frass treatments (100%, 80%, 60%) were lethal to the radish plants and several treatments were zero inflated. Parametric and non-parametric tests were utilized to interpret results. Though there are some numerical differences, there were no statistically significant differences between the non-zero treatments (Figures 6-11).

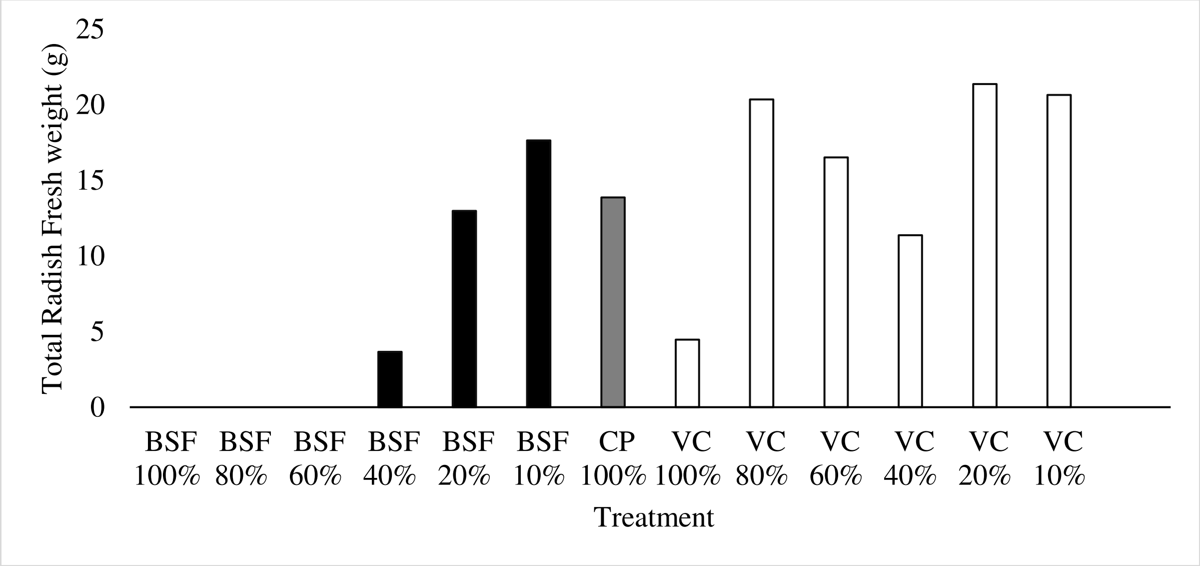

Figure I. 5: ***Total fresh weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

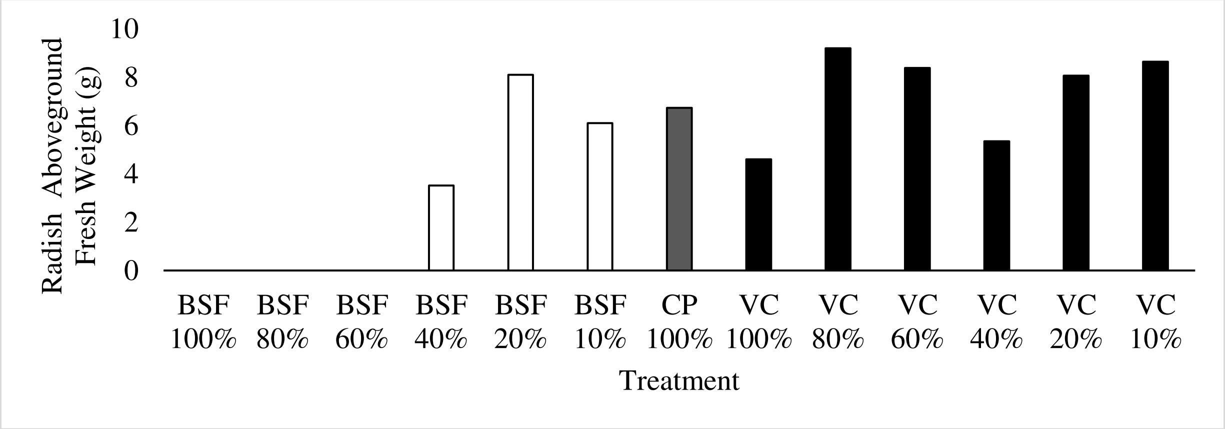

Figure I.6: ***Above ground fresh weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

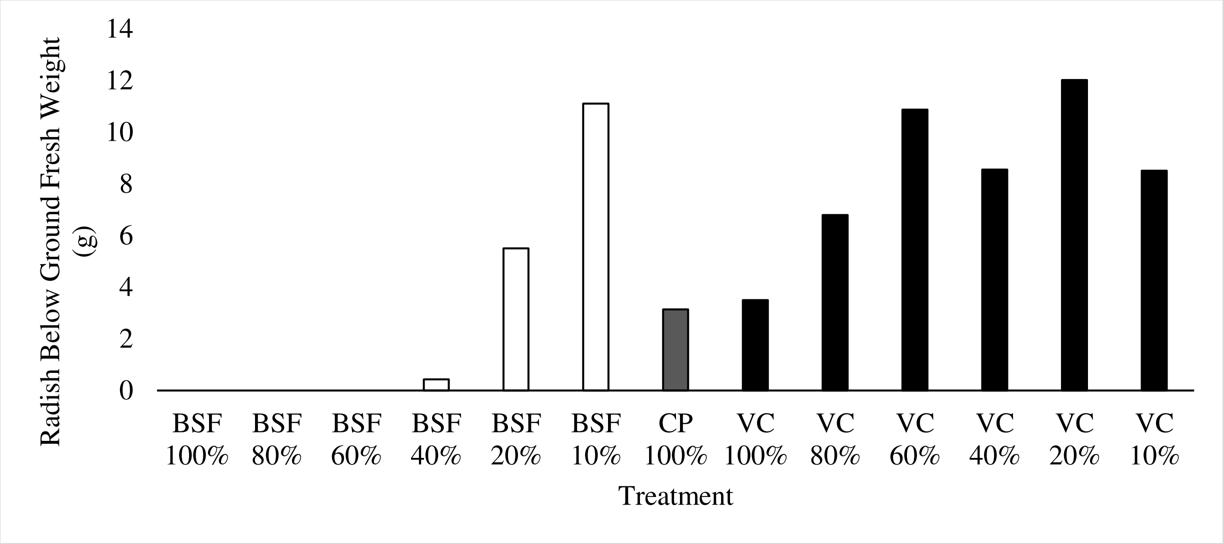

Figure I.7: ***Below ground fresh weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

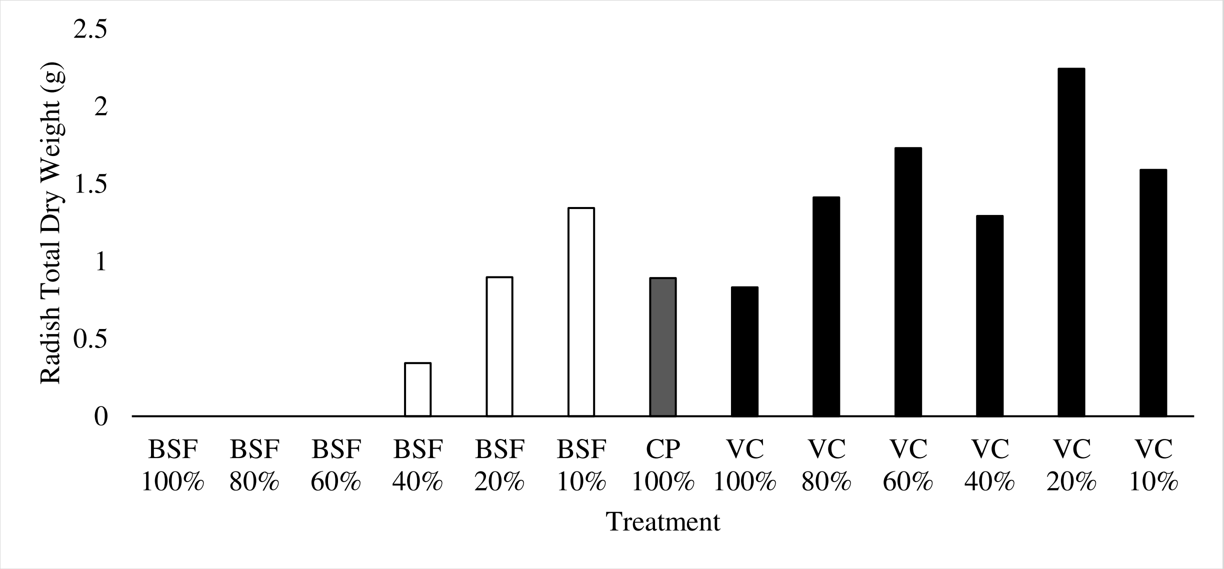

Figure I.8: ***Total dry weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

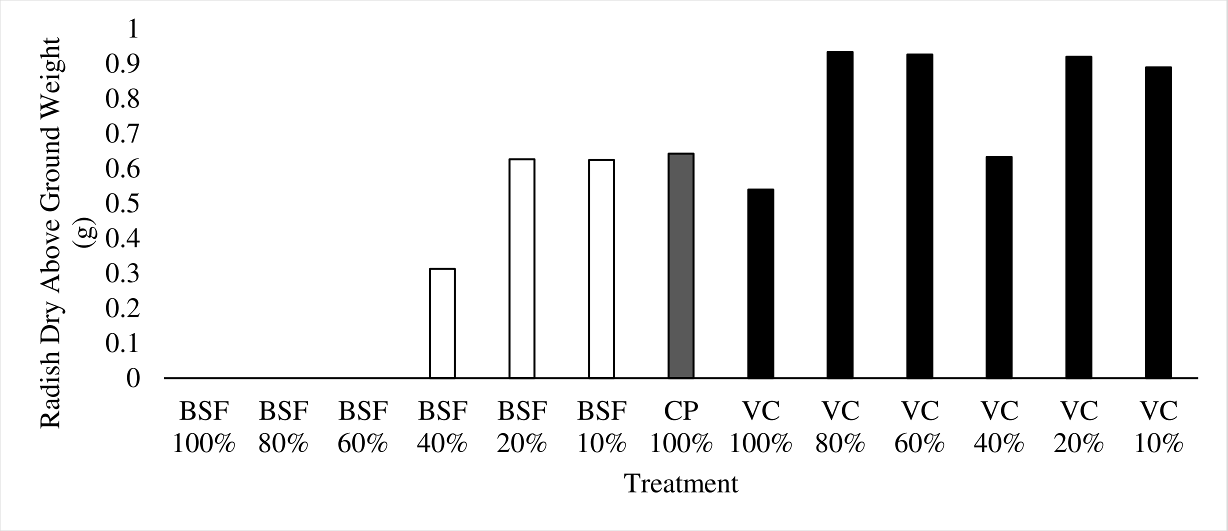

Figure I.9: ***Above ground dry weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

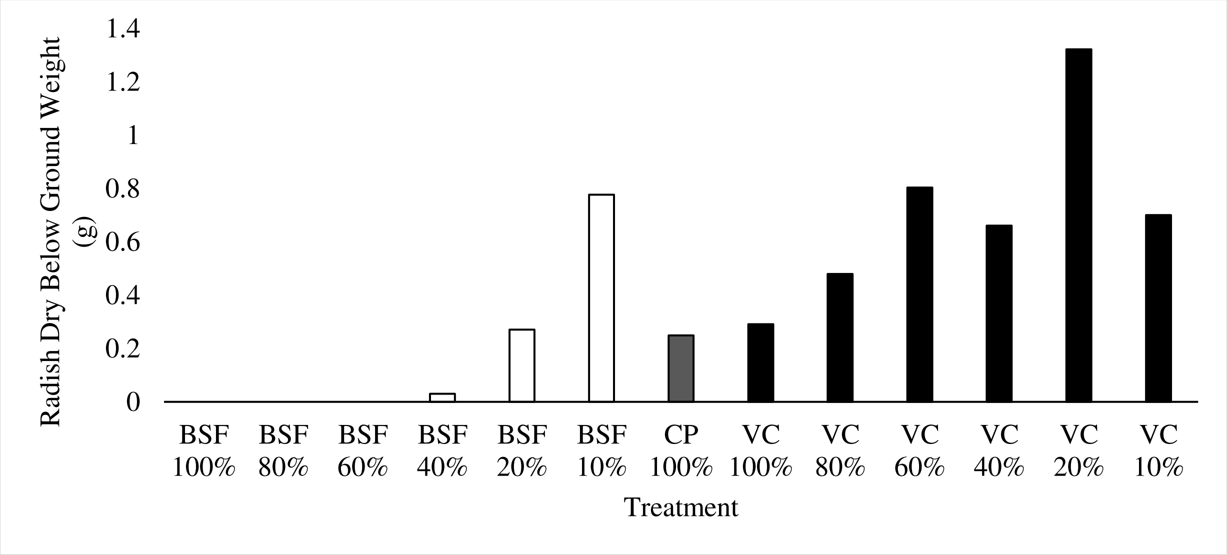

Figure I.10: ***Below ground dry weight (grams) of amended radishes grown in the greenhouse.*** Radishes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). 100%, 80%, and 60% BSF larvae frass were lethal concentrations, and those treatments were screened out of future experiments. There were no significant statistical differences between the remaining treatments as confirmed by the Dunn method for Joint Ranking

Tomatoes

In tomatoes similar trends emerged as the arugula and radishes, though there were statistically significant results. Extremely high standard errors, and failures in normalcy and equal variance presented problems for analysis and non-parametric tests were utilized. Total fresh weight of fruits produced per plant (Figure 12) and average fruit fresh weight of each plant (Figure 13) were assessed. 100% vermicompost was notably the best treatment for total tomato fruit production (1616 grams). 100% BSF larvae frass and the 100% peat control were the worst (< 400g total fruit produced). The average individual fruit weights were highest in the 20% vermicompost treatment (129.5 grams) and lowest in the 100% BSF frass treatment (15.57 grams).The biomass of the left over plants after the experiment (Figure 14) and the total number of fruits per plant (Figure 15) were also analyzed. 60%, 40%, and 20% vermicompost treatments had the highest aboveground vegetative biomass (all above 1000 grams) while the 100% peat control had the lightest (641.61 grams). The Levene's and Anderson Darling tests revealed that the number of fruits per plant was the only variable that passed both tests equal variances and normality. Because of the irregularity of this data overall, non-parametric tests were conducted to verify the parametric results. 100% vermicompost produced the most fruits per plant (11 fruits) and 100% BSF frass and 100% peat control produced the least fruits (2 and 4 fruits respectively).

Blossom end rot (BER) was heavily prevalent in the tomato screening experiments, which is a notable problem for the cultivar Brandywine grown in the greenhouse. This disease could explain our irregular results. The presence of BER in tomato fruits was analyzed, but there were significant differences between treatments. For the total and average individual fruit weight of BER prevalence, 80% BSF frass was the lowest (355.54 and 46.78 g respectively). 80%

Vermicompost produced the largest average individual fruits with BER (109.356 grams) and the greatest average largest total of fruits with BER (962.34 g).

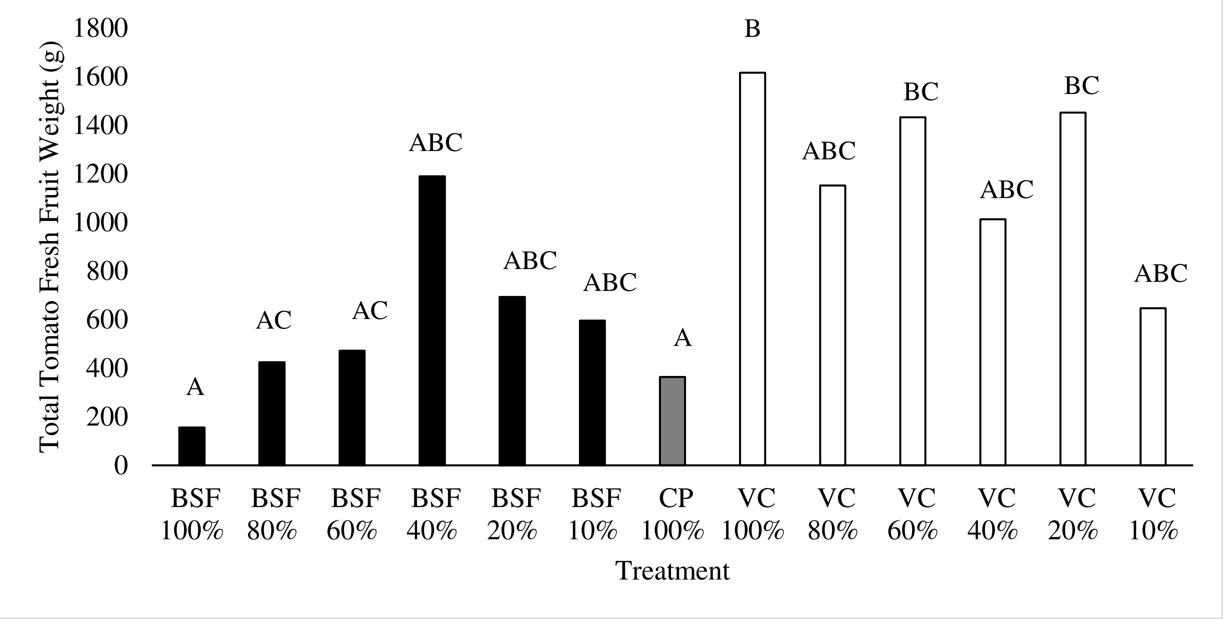

Figure I.12: ***Total fresh fruit weight (grams) of amended tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise significant statistical differences ($p < 0.05$) between the treatments as confirmed by the Dunn method for Joint Ranking.

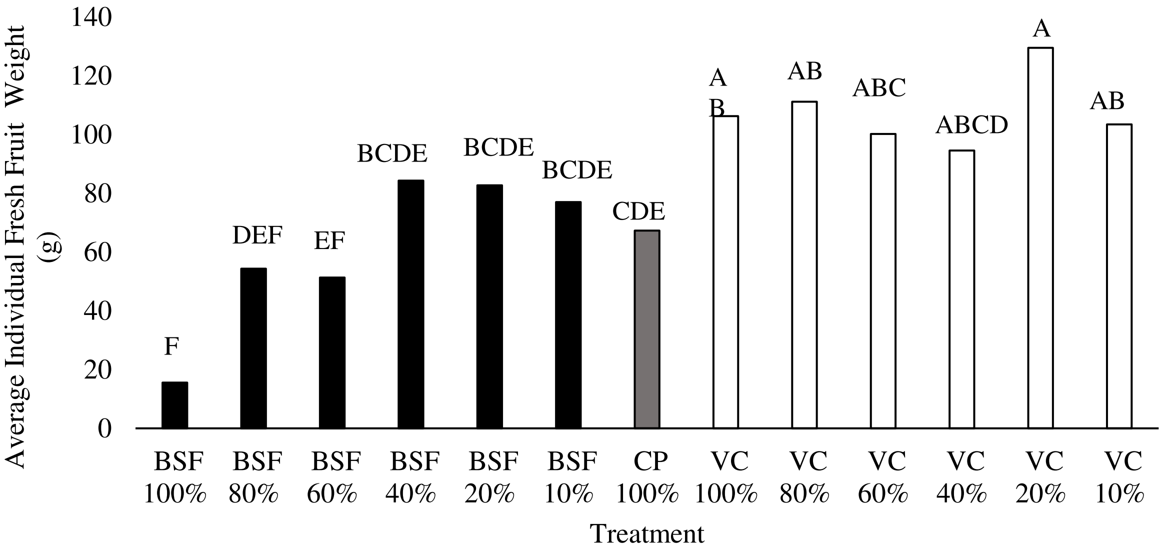

Figure I.13: ***Average individual fresh weight (grams) of amended tomatoes grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise significant statistical differences ($p < 0.05$) between the treatments as confirmed by the Dunn

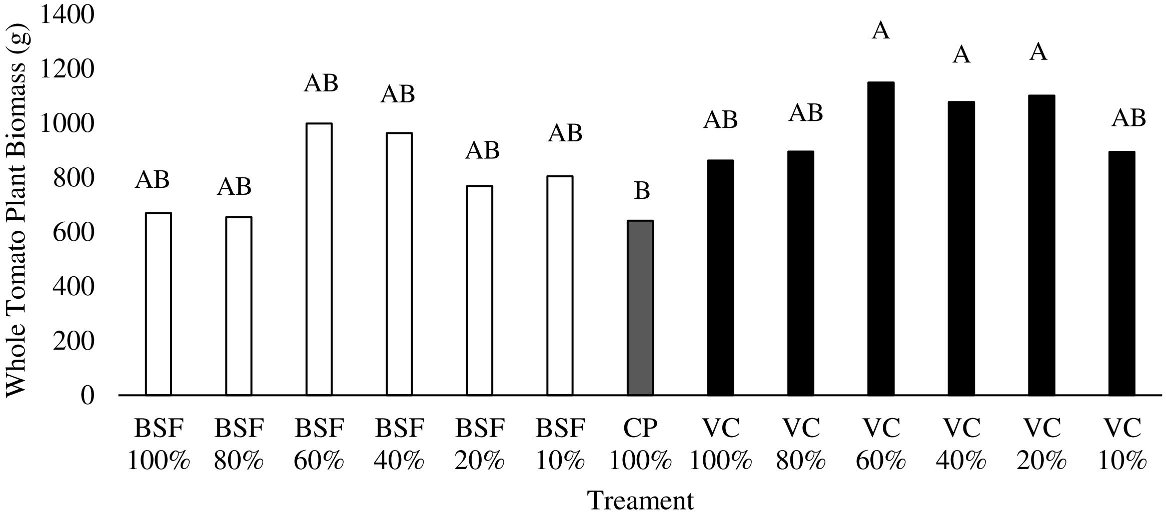

Figure I.14: ***Total biomass (grams) of amended tomato plants grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise significant statistical differences ($p < 0.05$) between the treatments as confirmed by the Dunn method for Joint Ranking.

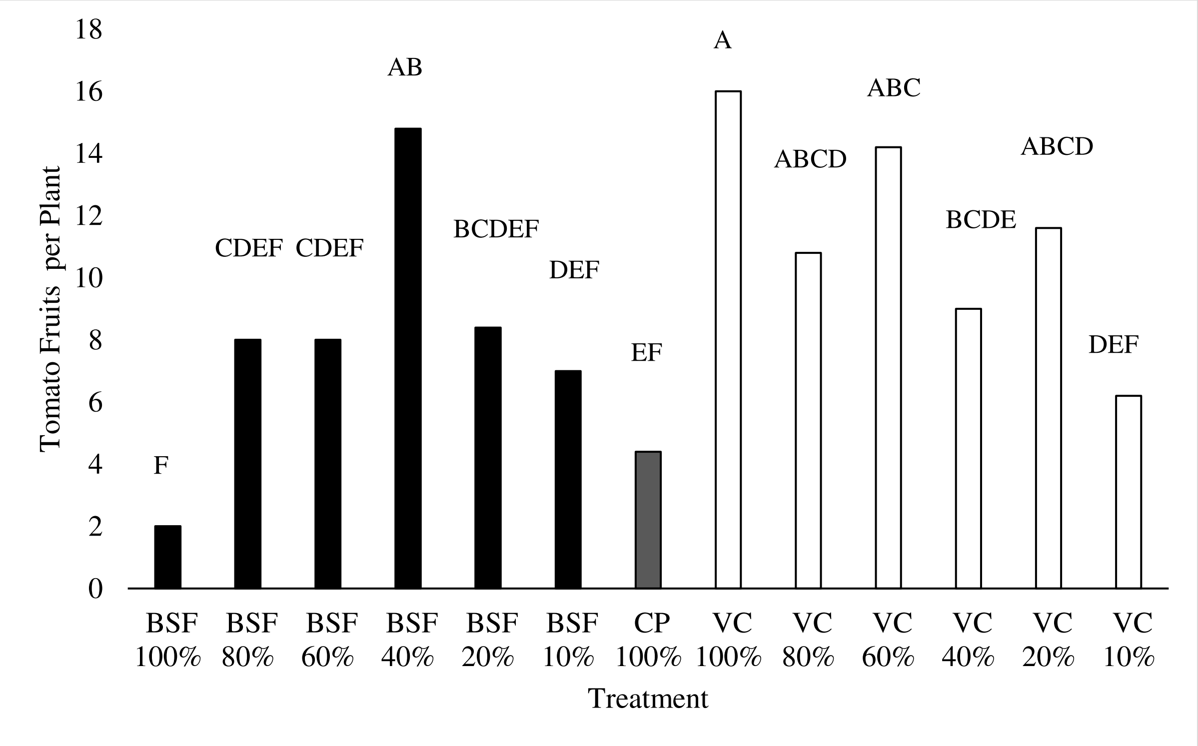

Figure I.15: ***Total number of amended tomato plants grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise differences ($p < 0.05$).

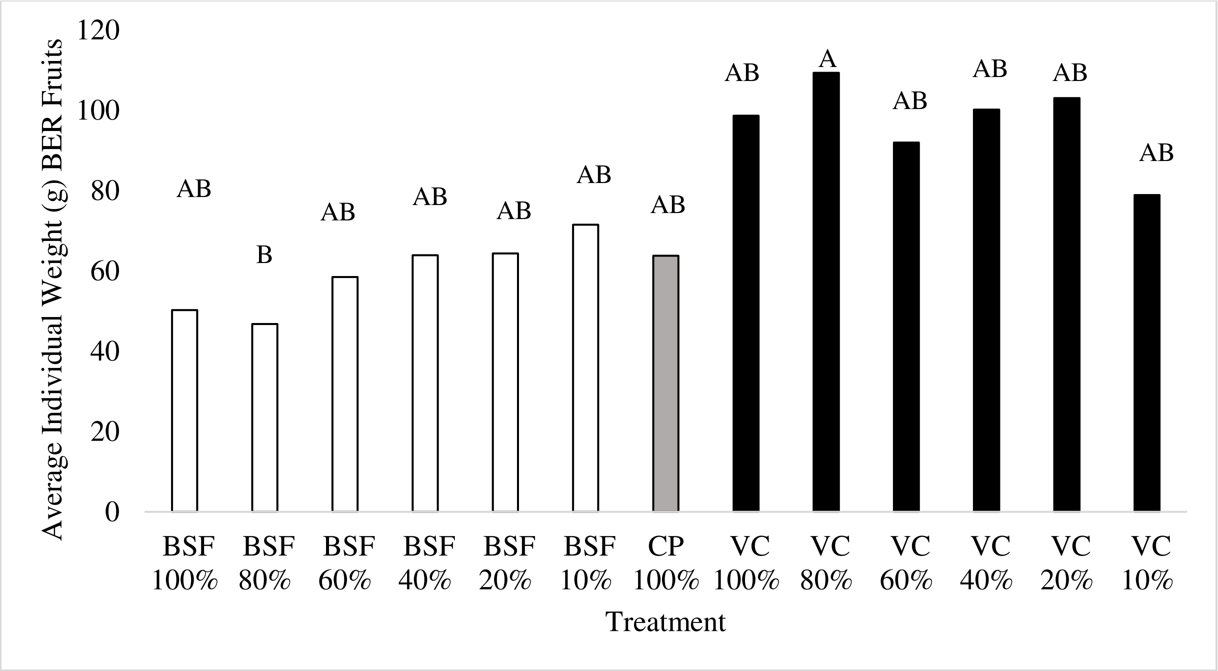

Figure I.16: ***Average individual weight of BER tomato fruits grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). Columns with different letters indicate pairwise significant statistical differences ($p < 0.05$) between the treatments as confirmed by the Dunn method for Joint Ranking.

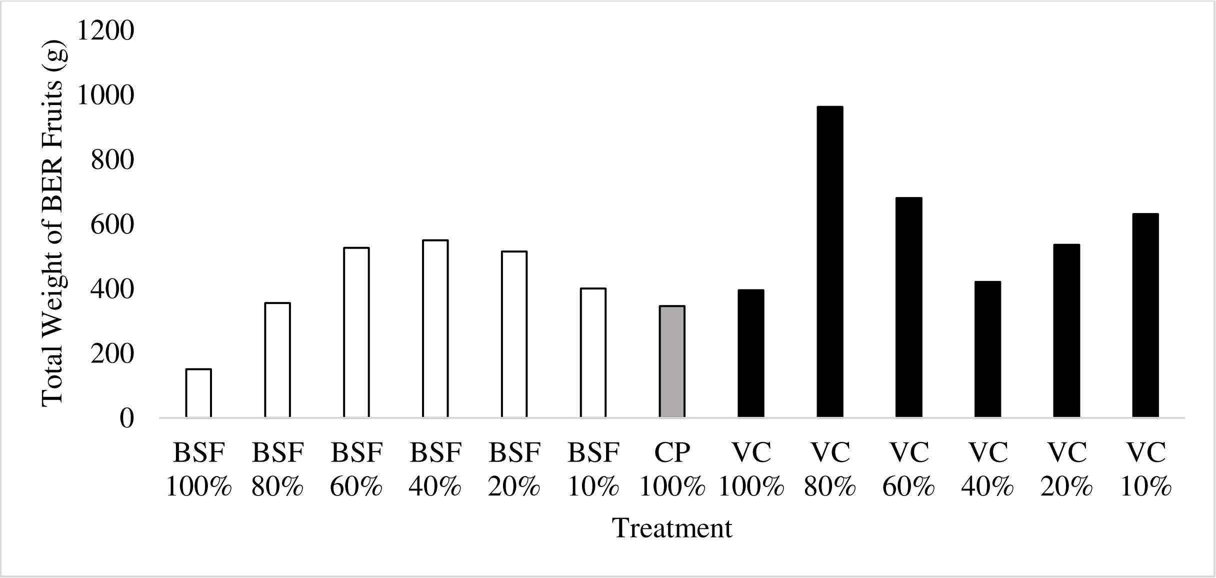

Figure I.17: *Total* ***weight (g) of BER fruits grown in the greenhouse.*** Tomatoes were grown with mixtures of commercial peat and black soldier fly larvae frass (BSF: black columns) or vermicompost (VC: white columns). 100% commercial peat served as the control pot (CP: grey columns). There was no significant statistical differences ($p < 0.05$) between treatments.

4. Discussion

Arugula

In arugula the three highest frass treatments (100%, 80%, 60%) were lethal to the crop. In the screening phase of this experiment these high percentages were included but were removed as treatments in subsequent experiments. The three highest vermicompost treatments (100%, 80%, 60%) produced variable results, but were screened out as well. The results were not consistent with what has been normalized in past literature and more importantly, it would be cost prohibitive to commercial growers to use this high of vermicompost concentrations. Vermicompost at market cost is around two to ten dollars per pound (personal communication Rocky Mountain Soil Stewardship). Frass is often donated by industrial producers to growers (e.g., EVO Conversion Systems), but small insect growers (such as Organic Nutrients) are selling their frass for around three-six dollars per pound.

The lack of statistically significant differences between treatments may have been due to a low sample size. Five replications per treatment is the standard presented by the small amount of research done in this field so far and is a common replication size in greenhouse studies generally. But to improve statistically accuracy the replications was increased in later experiments to double the original, 10 replications per treatment. The fertilizer used in this phase was a standard organic powdered fertilizer. For the next phase we chose a standard conventional liquid fertilizer to supplement to each experiment, (24-8-16 NPK) Miracle Gro (Marysville, Ohio). Additionally, a non-organic peat substrate was chosen for future studies as well; Berger B6 instead of Berger OM (Saint-Modeste, QC).

Radish

Similar to arugula the three highest frass treatments (100%, 80%, 60%) were lethal to the radish plants and the three highest vermicompost treatments (100%, 80%, 60%) were also variable and inconsistent with past findings. We chose not to continue with the radish crop for the next phase of the experiment and replaced it with lettuce. Lettuce has a short life cycle like arugula and radish and is also a more commonly grown cool season crop, with higher economic value in many areas of the world.

Tomato

Though the highest BSFL frass and vermicompost treatments were not lethal to tomato plants, they were removed from future studies. They generally produced the lowest yields and provide similar financial constraints to growers as described for arugula. Blossom end rot (BER) was very prevalent in the tomato screening experiments. BER is a physiological disease that causes the blossom end of the tomato fruit to become necrotic (Taylor and Locascio, 2007). This could have been due to the cultivar, Brandywine. A less susceptible cultivar, Jet Star, was used in the next set of experiments (Chapter 5). Additionally, for the next experiments we chose a tomato specific fertilizer, (analysis, Tom’s Tomato, manufacturer, location), which contained calcium. Calcium is known to reduce rates of BER commonly seen in greenhouse tomatoes (Taylor and Locascio, 2007).

5. Conclusions

Insect frass is a novel medium with potential benefits and challenges. Overall our greatest interests for evaluating insect frass are to close gaps in the circular economy, reduce inorganic fertilization, reduce natural resource extraction, and improve soil microbiomes. Reusing materials as waste from one system to improve another system reduces the need to source more materials from natural systems (Chavez, 2021). For example, peatlands are being extracted at alarming rates, resulting in increased greenhouse gas emissions, and diminishing cultural values of the marsh landscapes. Inorganic fertilizers are applied in excessive and have detriment to surrounding landscapes. Utilizing frass as a fertilizer, not only reduces the need for artificial supplements, but improves the soil microbiome, improving the overall quality and health of the soil (Chavez and Uchanski, 2021).

Because this medium is so novel, and the data regarding its impact of plant productivity is sparse and an initial screening step was necessary. This experiment was the first of three phases and has provided us with the preliminary information to continue forward. Overall, yield was not impacted by vermicompost or frass treatments in all three crops: arugula, radishes, and tomatoes. Progressing further, we made several additional alterations to our experimental design to reduce variability, as described in the next chapter. Adjustments to increase replications, change fertilizers, adjust crop cultivars, reduce treatment numbers, and change peat type will improve results. In addition, we gained a better understanding of how the BSFL frass breaks down in the first one to two weeks of each experiment. After being potted and watered regularly, the frass appeared to congeal, shrink, and pull away from the plastic pots. This is different from the peat and vermicompost, which is more consistent in texture and its physical properties did not change.

6. References

1. Chavez, M. 2021. “The sustainability of industrial insect mass rearing for food and feed production: zero waste goals through by-product utilization”. *Current Opinion in Insect Science* 48: 44-49.
2. Chavez, M. and Uchanski, M., 2021. “Insect left-over substrate as plant fertilizer”. *Journal of Insects as Food and Feed* 1-12.
3. Taylor, M. D., and Locascio, S. J. 2007. “Blossom-end rot: a calcium deficiency”. *Journal of Plant Nutrition* 27(1): 123-139.

APPENDIX II

STATISTICAL SUMMARY TABLES

1. Chapter three

Distillery grain digested frass

Arugula Summer 2021

Table II.1: Summary statistics for fresh weight (g) of greenhouse grown arugula (Figure 3.1a) in 2021, Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	16.65	**F ratio**	5.57
P > F	<.0001	**P > F**	0.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	41.89	**A2**	3.47
P > χ2	<.0001	**P > A2**	<.0001

Table II.2: Mean summary for fresh weight (g) of greenhouse grown arugula (Figure 3.1a) in 2021
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	31.8	2.68
BSF 20%	33.8	3.80
BSF 40%	28.4	4.42
CP 100%	13.8	1.0033
VC 10%	11.95	0.69
VC 20%	12.8	1.29
VC 40%	10.5	0.89

Table II.3: Summary statistics for dry weight (g) of grown in the greenhouse arugula in 2021 (Figure 3.2a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.72	**F ratio**	3.0053
P > F	<.0001	**P > F**	0.012
Wilcoxon		**Anderson Darling test for Normality**	
χ2	26.86	**A2**	1.57
P > χ2	0.0002	**P > A2**	0.001

Table II.4: Mean summary for dry weight (g) of greenhouse grown arugula in 2020 (Figure 3.2a) in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	Mean	Standard Error
BSF 10%	3.2	0.35
BSF 20%	3.35	0.52
BSF 40%	2.45	0.43
CP 100%	2	0.28
VC 10%	1.7	0.15
VC 20%	1.8	0.26
VC 40%	1.2	0.17

Table II.5: Summary statistics for SPAD of greenhouse grown arugula in 2021 (Figure 3.3a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.21	**F ratio**	2.76
P > F	0.0082	**P > F**	0.019
Wilcoxon		**Anderson Darling test for Normality**	
χ2	19.50	**A2**	0.34
P > χ2	0.0034	**P > A2**	0.509

Table II.6: Mean summary for SPAD of greenhouse grown arugula (Figure 3.3a) in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	44.79	2.14
BSF 20%	39.57	2.14
BSF 40%	42.99	2.14
CP 100%	46.69	2.14
VC 10%	42.91	2.14
VC 20%	36.65	2.14
VC 40%	37.17	2.14

Distillery grain digested frass

Arugula Spring 2022

Table II.7: Summary statistics for fresh weight of greenhouse grown arugula in 2022 (Figure 3.1b), Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	2.40	**F ratio**	4.43
P > F	0.038	**P > F**	0.0008
Wilcoxon		**Anderson Darling test for Normality**	
χ2	15.77	**A2**	1.35
P > χ2	0.015	**P > A2**	0.001

Table II.8: Mean summary for fresh weight of arugula (Figure 3.1b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	4.49	0.95
BSF 20%	5.095	0.95
BSF 40%	1.74	0.95
CP 100%	3.14	0.95
VC 10%	3.89	0.95
VC 20%	5.36	0.95
VC 40%	6.07	0.95

Table II.9: Summary statistics for dry weight (g) of greenhouse grown arugula in 2022 (Figure 3.2b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.16	**F ratio**	3.98
P > F	0.059	**P > F**	0.0019
Wilcoxon		**Anderson Darling test for Normality**	
χ2	13.72	**A2**	1.49
P > χ2	0.033	**P > A2**	0.001

Table II.10 : Mean summary for dry weight of greenhouse grown arugula (Figure 3.2b) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	0.36	0.074
BSF 20%	0.45	0.074
BSF 40%	0.17	0.074
CP 100%	0.22	0.074
VC 10%	0.30	0.074
VC 20%	0.40	0.074
VC 40%	0.44	0.074

Table II.11: Summary statistics for SPAD measurements of greenhouse grown arugula in 2022 (Figure 3.3b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.03	**F ratio**	3.59
P > F	0.0003	**P > F**	0.004
Wilcoxon		**Anderson Darling test for Normality**	
χ2	30.18	**A2**	3.078
P > χ2	<.0001	**P > A2**	<.0001

Table II.12: Mean summary for greenhouse grown arugula SPAD measurements (Figure 3.3b) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	36.04	2.52
BSF 20%	35.93	2.52
BSF 40%	36.72	2.52
CP 100%	24.56	2.52
VC 10%	24.12	2.52
VC 20%	32.20	2.52
VC 40%	26.68	2.52

Distillery grain digested frass

Lettuce Summer 2021

Table II.13: Summary statistics for fresh weight (g) of greenhouse grown lettuce in 2021 (Figure 3.4a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	56.16	**F ratio**	6.78
P > F	<.0001	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
$\chi2$	56.45	**A2**	2.66
P > $\chi2$	<.0001	**P > A2**	<.0001

Table II.14 : Mean summary for fresh weight (g) of greenhouse grown lettuce (Figure 3.4a) in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	57.7	2.18
BSF 20%	53.32	2.71
BSF 40%	72.6	5.75
CP 100%	23.1	1.40
VC 10%	20.86	1.12
VC 20%	23.3	2.13
VC 40%	29.01	1.10

Table II.15: Summary statistics for dry weight (g) of greenhouse grown lettuce in 2021 (Figure 3.5a), Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	56.16	**F ratio**	7.34
P > F	<.0001	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	52.34	**A2**	4.80
P > χ2	<.0001	**P > A2**	<.0001

Table II.16 : Mean summary for dry weight (g) of lettuce (Figure 3.5a)) in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	6.92	0.91
BSF 20%	6.5	0.49
BSF 40%	9.97	1.41
CP 100%	2.52	0.16
VC 10%	1.82	0.12
VC 20%	2.2	0.23
VC 40%	2.7	0.16

Table II.17: Summary statistics for SPAD measurements of greenhouse grown lettuce in 2021 (Figure 3.6a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.37	**F ratio**	1.51
P > F	0.006	**P > F**	0.19
Wilcoxon		**Anderson Darling test for Normality**	
$\chi2$	31.41	**A2**	1.33
P > $\chi2$	<.0001	**P > A2**	0.004

Table II.18: Mean summary for SPAD of greenhouse grown lettuce (Figure 3.6a) in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	46.23	2.52
BSF 20%	44.32	2.52
BSF 40%	44.99	2.52
CP 100%	37.78	2.52
VC 10%	37.38	2.52
VC 20%	35.89	2.52
VC 40%	35.78	2.52

Distillery grain digested frass

Lettuce Spring 2022

Table II.19: Summary statistics for fresh weight (g) of greenhouse grown lettuce in 2022 (Figure 3.4b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	6.0078	**F ratio**	1.066
P > F	<.0001	**P > F**	0.39
		Anderson Darling test for Normality	
		A2	0.75
		P > A2	0.051

Table II.20: Mean summary for fresh weight (g) of lettuce (Figure 3.4b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	Mean	Standard Error
BSF 10%	13.97	1.40
BSF 20%	9.66	1.40
BSF 40%	3.2	1.40
CP 100%	6.99	1.40
VC 10%	6.095	1.40
VC 20%	8.38	1.40
VC 40%	10.24	1.40

Table II.21: Summary statistics for dry weight (g) of greenhouse grown lettuce in 2022 (Figure 3.5b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.14	**F ratio**	1.38
P > F	0.0094	**P > F**	0.24
		Anderson Darling test for Normality	
		A2	0.44
		P > A2	0.31

Table II.22: Mean summary for dry weight (g) of greenhouse grown lettuce (Figure 3.5b) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

TreatmenT	**Mean**	**Standard Error**
BSF 10%	0.74	0.085
BSF 20%	0.65	0.085
BSF 40%	0.30	0.085
CP 100%	0.40	0.085
VC 10%	0.48	0.085
VC 20%	0.50	0.085
VC 40%	0.59	0.085

Table II.23: Summary statistics for SPAD measurements of greenhouse grown lettuce in 2022 (Figure 3.6b), Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	5.86	**F ratio**	1.85
P > F	<.0001	**P > F**	0.10
Wilcoxon		**Anderson Darling test for Normality**	
χ2	28.98	**A2**	1.48
P > χ2	<.0001	**P > A2**	0.001

Table II.24: Mean summary for SPAD measurements of greenhouse grown lettuce (Figure 3.6b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	35.5	2.53
BSF 20%	41.69	2.53
BSF 40%	40.75	2.53
CP 100%	29.30	2.53
VC 10%	26.28	2.53
VC 20%	29.32	2.53
VC 40%	29.61	2.53

Brewery grain digested frass

Arugula Spring 2022

Table II.25: Summary statistics for fresh weight (g) of greenhouse grown arugula in 2022 (Figure 3.7a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.89	**F ratio**	7.73
P > F	<.0001	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	29.31	**A2**	4.057
P > χ2	<.0001	**P > A2**	<.0001

Table II.26: Mean summary of fresh weight of greenhouse grown arugula (Figure 3.7a) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	1.48	0.89
***BSF 20%**	0.97	0.89
***BSF 40%**	0.63	0.89
CP 100%	3.14	0.89
VC 10%	3.89	0.89
VC 20%	5.36	0.89
VC 40%	6.07	0.89

Table II.27: Summary statistics for dry weight of greenhouse grown arugula in 2022 (Figure 3.8a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.12	**F ratio**	6.84
P > F	0.0002	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
$\chi2$	25.11	**A2**	3.75
P > $\chi2$	0.0003	**P > A2**	<.0001

Table II.28: Mean summary for dry weight of greenhouse grown arugula (Figure 3.8a) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	0.12	0.066
***BSF 20%**	0.091	0.066
***BSF 40%**	0.067	0.066
CP 100%	0.22	0.066
VC 10%	0.30	0.066
VC 20%	0.40	0.066
VC 40%	0.44	0.066

Table II.28: Summary statistics for SPAD measurements of greenhouse grown arugula in 2022 (Figure 3.9a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	0.59	**F ratio**	5.24
P > F	0.74	**P > F**	0.0002
Wilcoxon		**Anderson Darling test for Normality**	
χ2	6.38	**A2**	1.33
P > χ2	0.38	**P > A2**	0.003

Table II.30: Mean summary for SPAD measurement of greenhouse grown arugula (Figure 3.9a) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	26.54	3.91
***BSF 20%**	26.04	3.91
***BSF 40%**	30.45	3.91
CP 100%	24.56	3.91
VC 10%	24.12	3.91
VC 20%	32.20	3.91
VC 40%	26.68	3.91

Brewery grain digested frass

Arugula Summer 2022

Table II.31: Summary statistics for fresh weight (g) of greenhouse grown arugula in 2022 (Figure 3.7b), Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	16.66	**F ratio**	2.10
P > F	<.0001	**P > F**	0.067
Wilcoxon		**Anderson Darling test for Normality**	
χ2	39.33	**A2**	1.16
P > χ2	<.0001	**P > A2**	0.004

Table II.32: Mean summary for fresh weight (g) of greenhouse grown arugula (Figure 3.7b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	3.85	0.84
BSF 20%	1.37	0.93
BSF 40%	0.33	1.078
CP 100%	8.27	0.84
VC 10%	6.8	0.84
VC 20%	9.42	0.84
VC 40%	9.35	0.84

Table II.33: Summary statistics for dry weigh (g) of greenhouse grown arugula in 2022 (Figure 3.8b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	15.47	**F ratio**	1.89
P > F	<.0001	**P > F**	0.098
Wilcoxon		**Anderson Darling test for Normality**	
χ2		**A2**	0.73
P > χ2		**P > A2**	0.053

Table II.34: Mean summary for dry weight (g) of greenhouse grown arugula (Figure 3.8b) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	Mean	Standard Error
BSF 10%	0.41	0.070
BSF 20%	0.21	0.078
BSF 40%	0.082	0.090
CP 100%	0.72	0.070
VC 10%	0.75	0.070
VC 20%	0.86	0.070
VC 40%	0.78	0.070

Table II.35: Summary statistics for SPAD measurements of greenhouse grown arugula in 2022 (Figure 3.9b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	0.83	**F ratio**	1.27
P > F	0.55	**P > F**	0.29
Wilcoxon		**Anderson Darling test for Normality**	
χ2	7.80	**A2**	1.74
P > χ2	0.25	**P > A2**	<.0001

Table II.36: Mean summary for SPAD measurements of arugula (Figure 3.9b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	43.77	3.35
BSF 20%	35.74	3.75
BSF 40%	37	4.33
CP 100%	38.06	3.35
VC 10%	38.45	3.35
VC 20%	37.98	3.35
VC 40%	33.78	3.35

Brewery grain digested frass

Lettuce Spring 2022

Table II.37: Summary statistics for fresh weight (g) of greenhouse grown lettuce in 2022 (Figure 3.10a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.28	**F ratio**	3.36
P > F	0.0002	**P > F**	0.0062
Wilcoxon		**Anderson Darling test for Normality**	
χ2	26.85	**A2**	1.18
P > χ2	0.0002	**P > A2**	0.004

Table II.38: Mean summary for fresh weight (g) of greenhouse grown lettuce (Figure 3.10a) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	6.82	1.29
***BSF 20%**	4.35	1.29
***BSF 40%**	0.99	1.29
CP 100%	6.99	1.29
VC 10%	6.09	1.29
VC 20%	8.38	1.29
VC 40%	10.24	1.29

Table II.39: Summary statistics for dry weight (g) of greenhouse grown lettuce in 2022 (Figure 3.11a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.72	**F ratio**	2.99
P > F	0.0032	**P > F**	0.012
Wilcoxon		**Anderson Darling test for Normality**	
χ2	21.19	**A2**	0.83
P > χ2	0.0017	**P > A2**	0.027

Table II.40: Mean summary for dry weight (g) of greenhouse grown lettuce (Figure 3.11a) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	0.40	0.081
***BSF 20%**	0.33	0.081
***BSF 40%**	0.10	0.081
CP 100%	0.40	0.081
VC 10%	0.48	0.081
VC 20%	0.50	0.081
VC 40%	0.59	0.081

Table II.41: Summary statistics for SPAD measurements of greenhouse grown lettuce in 2022 (Figure 3.12a), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.37	**F ratio**	0.99
P > F	0.040	**P > F**	0.44
Wilcoxon		**Anderson Darling test for Normality**	
$\chi 2$	13.85	**A2**	0.85
P > $\chi 2$	0.031	**P > A2**	0.024

Table II.42: Mean summary for SPAD measurements of greenhouse grown lettuce (Figure 3.12a) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
***BSF 10%**	37.54	3.09
***BSF 20%**	36.64	3.09
***BSF 40%**	25.3	3.09
CP 100%	29.30	3.09
VC 10%	26.28	3.09
VC 20%	29.32	3.09
VC 40%	29.61	3.09

Brewery grain digested frass

Lettuce Summer 2022

Table II.43: Summary statistics for fresh weight (g) of greenhouse grown lettuce in 2022 (Figure 3.10b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	90.79	**F ratio**	2.11
P > F	<.0001	**P > F**	0.068
Wilcoxon		**Anderson Darling test for Normality**	
χ2	46.59	**A2**	2.014
P > χ2	<.0001	**P > A2**	<.0001

Table II.44: Mean summary for fresh weight (g) of greenhouse grown lettuce (Figure 3.10b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	Mean	Standard Error
BSF 10%	15.72	1.46
BSF 20%	6.71	1.89
BSF 40%	1.87	3.27
CP 100%	35.67	1.46
VC 10%	45.33	1.46
VC 20%	43.77	1.46
VC 40%	35.32	1.46

Table II.45: Summary statistics for dry weight (g) of greenhouse grown lettuce in 2022 (Figure 3.11b), Df = 6

ANOVA		**Levene’s test for Equal Variance**	
F ratio	69.42	**F ratio**	1.50
P > F	<.0001	**P > F**	0.20
Wilcoxon		**Anderson Darling test for Normality**	
$\chi 2$	44.38	**A2**	2.35
P > $\chi 2$	<.0001	**P > A2**	<.0001

Table II.46 : Mean summary for dry weight (g) of greenhouse grown lettuce (Figure 3.11b) in 2022
Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	Mean	Standard Error
BSF 10%	1.42	0.12
BSF 20%	0.71	0.15
BSF 40%	0.17	0.26
CP 100%	2.57	0.12
VC 10%	3.3	0.12
VC 20%	3.27	0.12
VC 40%	3.05	0.12

Table II.47: Summary statistics for SPAD measurements of greenhouse grown lettuce in 2022 (Figure 3.12b), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.35	**F ratio**	5.98
P > F	0.045	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	8.90	**A2**	0.94
P > χ2	0.18	**P > A2**	0.017

Table II.48: Mean summary for SPAD measurements of greenhouse grown lettuce (Figure 3.12b) in 2022 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (*BSF) or vermicompost (VC)

Treatment	**Mean**	**Standard Error**
BSF 10%	44.08	2.27
BSF 20%	39.37	2.93
BSF 40%	24.87	5.069
CP 100%	43.23	2.27
VC 10%	41.56	2.27
VC 20%	42.67	2.39
VC 40%	39.60	2.27

Distillery grain digested frass vs. Brewery grain digested frass

Arugula Spring 2022

Table II.49: Summary statistics for fresh weight (g) of greenhouse grown arugula in 2022 (Figure 3.13), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	11.28	**F ratio**	3.11
P > F	<.0001	**P > F**	0.0098
Wilcoxon		**Anderson Darling test for Normality**	
χ2	37.65	**A2**	2.35
P > χ2	<.0001	**P > A2**	<.0001

Table II.50: Mean summary of greenhouse arugula fresh weight (Figure 3.13) in 2022 Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	4.49	0.52
BSF 20%	5.09	0.52
BSF 40%	1.74	0.52
CP 100%	3.14	0.52
***BSF 10%**	1.48	0.52
***BSF 20%**	0.97	0.52
***BSF 40%**	0.63	0.52

Table II.51: Summary statistics for dry weight of greenhouse arugula in 2022 (Figure 3.14), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	10.19	**F ratio**	4.94
P > F	<.0001	**P > F**	0.0003
Wilcoxon		**Anderson Darling test for Normality**	
χ2	33.69	**A2**	2.45
P > χ2	<.0001	**P > A2**	<.0001

Table II.52: Mean summary for dry weight of greenhouse arugula (Figure 3.14)) in 2022 Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	0.36	0.046
BSF 20%	0.45	0.046
BSF 40%	0.17	0.046
CP 100%	0.22	0.046
***BSF 10%**	0.12	0.046
***BSF 20%**	0.091	0.046
***BSF 40%**	0.067	0.046

Table II.53: Summary statistics for SPAD measurements of greenhouse arugula in 2022 (Figure 3.15), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.051	**F ratio**	8.066
P > F	0.072	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	14.20	**A2**	2.35
P > χ2	0.028	**P > A2**	<.0001

Table II.54: Mean summary SPAD measurements of greenhouse arugula (Figure 3.15)) in 2022
Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	36.04	3.70
BSF 20%	35.93	3.70
BSF 40%	36.72	3.70
CP 100%	24.56	3.70
***BSF 10%**	26.54	3.70
***BSF 20%**	26.04	3.70
***BSF 40%**	30.45	3.70

Distillery grain digested frass vs. Brewery grain digested frass

Lettuce Spring 2022

Table II.55: Summary statistics for fresh weight (g) of greenhouse lettuce in 2022 (Figure 3.16), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	15.70	**F ratio**	2.72
P > F	<.0001	**P > F**	0.021
Wilcoxon		**Anderson Darling test for Normality**	
χ2	43.38	**A2**	1.60
P > χ2	<.0001	**P > A2**	0.001

Table II.56: Mean summary for fresh weight (g) of greenhouse lettuce (Figure 3.16)) in 2022
Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	13.97	1.094
BSF 20%	9.66	1.094
BSF 40%	3.2	1.094
CP 100%	6.99	1.094
***BSF 10%**	6.82	1.094
***BSF 20%**	4.35	1.094
***BSF 40%**	0.91	1.094

Table II.57: Summary statistics for dry weight (g) of greenhouse lettuce in 2022 (Figure 3.17), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	13.08	**F ratio**	1.64
P > F	<.0001	**P > F**	0.15
Wilcoxon		**Anderson Darling test for Normality**	
		A2	0.67
		P > A2	0.081

Table II.58: Mean summary for dry weight (g) of greenhouse lettuce (Figure 3.17) in 2022
Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	0.74	0.060
BSF 20%	0.65	0.060
BSF 40%	0.30	0.060
CP 100%	0.40	0.060
***BSF 10%**	0.40	0.060
***BSF 20%**	0.33	0.060
***BSF 40%**	0.096	0.060

Table II.59: Summary statistics for dry weight (g) of greenhouse lettuce in 2022 (Figure 3.18), Df = 6

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.72	**F ratio**	1.24
P > F	0.0032	**P > F**	0.30
Wilcoxon		**Anderson Darling test for Normality**	
$\chi2$	16.36	**A2**	1.82
P > $\chi2$	0.012	**P > A2**	<.0001

Table II.60: Mean summary for dry weight (g) of greenhouse lettuce (Figure 3.18) in 2022 Treatments include mixtures of control peat (CP) and brewery grain frass (*BSF) or distillery grain frass (BSF)

Treatment	**Mean**	**Standard Error**
BSF 10%	35.5	3.025
BSF 20%	41.69	3.025
BSF 40%	40.75	3.025
CP 100%	29.30	3.025
***BSF 10%**	37.54	3.025
***BSF 20%**	36.64	3.025
***BSF 40%**	25.78	3.025

2. Chapter Four

Table II.61: Summary statistics for transformed primary macronutrients of arugula (Figure 4.1), Df = 6 Nitrogen (N), Phosphorus (P), and Potassium (P) were tested for concentration in greenhouse grown arugula leaf tissues.

ANOVA	**N**	**P**	**K**	**Levene's**	**N**	**P**	**K**
F ratio	109.41	105.07	54.06	**F ratio**	2.86	2.28	1.59
P > F	<0.0001	<0.0001	<0.0001	**P > F**	0.05	0.095	0.22
Wilcoxon	**N**	**P**	**K**	**Normality**	**N**	**P**	**K**
χ2	18.95	18.34		**A2**	0.94	2.35	0.60
P > χ2	0.0042	0.0054		**P > A2**	0.012	< 0.0001	0.10

Table II.62: Mean summary for primary macronutrients of arugula (Figure 4.1) Nitrogen (N), Phosphorus (P), and Potassium (P) were tested for concentration in greenhouse grown arugula leaf tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean			**Standard Error**		
Treatment	**N**	**P**	**K**	**N**	**P**	**K**
BSF 10%	5.043	1.35	5.76	0.12	0.053	0.24
BSF 20%	5.043	1.45	7.31	0.12	0.053	0.24
BSF 40%	6.58	1.20	6.53	0.12	0.053	0.24
CP 100%	2.26	0.34	2.45	0.12	0.053	0.24
VC 10%	1.75	1.023	3.68	0.12	0.053	0.24
VC 20%	1.75	1.2	4.40	0.12	0.053	0.24
VC 40%	2.82	0.95	5.53	0.12	0.053	0.24

Table II.63: Summary statistics for transformed primary macronutrients of lettuce (Figure 4.2), Df = 6 Nitrogen (N), Phosphorus (P), and Potassium (P) concentrations were tested in greenhouse grown lettuce leaf tissues

ANOVA	**N**	**P**	**K**	**Levene's**	**N**	**P**	**K**
F ratio	54.66	135.44	174.62	**F ratio**	2.85	2.07	1.62
P > F	<.0001	<.0001	<.0001	**P > F**	0.050	0.12	0.21
Wilcoxon	**N**	**P**	**K**	**Normality**	**N**	**P**	**K**
$\chi 2$	16.14		18.91	**A2**	1.20	0.033	1.064
P > $\chi 2$	0.013		0.0043	**P > A2**	0.0040	0.05	0.0052

Table II.64: Mean summary for primary macronutrient of lettuce (Figure 4.2) Nitrogen (N), Phosphorus (P), and Potassium (P) concentrations were tested in greenhouse grown lettuce leaf tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean			**Standard Error**		
Treatment	**N**	**P**	**K**	**N**	**P**	**K**
BSF 10%	3.48	0.74	6.27	0.16	0.018	0.20
BSF 20%	3.63	0.84	7.94	0.16	0.018	0.20
BSF 40%	5.36	0.82	8.47	0.16	0.018	0.20
CP 100%	1.87	0.32	2.36	0.16	0.018	0.20
VC 10%	1.82	0.50	4.91	0.16	0.018	0.20
VC 20%	1.76	0.46	5.24	0.16	0.018	0.20
VC 40%	1.84	0.54	5.97	0.16	0.018	0.20

Table II.65: Summary statistics for secondary macronutrients of arugula (Figure 4.3), Df = 6 Calcium (Ca), Magnesium (Mg), and Sulfur (S) concentrations were tested in greenhouse grown arugula tissues

ANOVA	**Ca**	**Mg**	**S**	**Levene's**	**Ca**	**Mg**	**S**
F ratio	6.025	37.27	20.53	**F ratio**	1.29	1.68	2.66
P > F	0.0027	<0.0001	<0.0001	**P > F**	0.32	0.20	0.062
Wilcoxon	**Ca**	**Mg**	**S**	**Normality**	**Ca**	**Mg**	**S**
$\chi 2$	12.26		18.016	**A2**	0.77	0.21	0.37
P > $\chi 2$	0.056		0.0062	**P > A2**	0.039	0.83	0.38

Table II.66: Mean summary for secondary macronutrients of arugula (Figure 4.3) Calcium (Ca), Magnesium (Mg), and Sulfur (S) concentrations were tested in greenhouse grown arugula tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean			**Standard Error**		
Treatment	**Ca**	**Mg**	**S**	**Ca**	**Mg**	**S**
BSF 10%	1.99	0.0043	0.53	0.10	0.034	0.049
BSF 20%	1.73	0.0043	0.72	0.10	0.034	0.049
BSF 40%	1.84	0.0043	0.83	0.10	0.034	0.049
CP 100%	1.32	0.0043	0.64	0.10	0.034	0.049
VC 10%	1.93	0.0043	0.84	0.10	0.034	0.049
VC 20%	1.91	0.0043	1.03	0.10	0.034	0.049
VC 40%	1.69	0.0043	1.029	0.10	0.034	0.049

Table II.67: Summary statistics for transformed secondary macronutrients of lettuce (Figure 4.4), Df = 6 Calcium (Ca), Magnesium (Mg), and Sulfur (S) concentrations were tested in greenhouse grown lettuce tissues

ANOVA	**Ca**	**Mg**	**S**	**Levene's**	**Ca**	**Mg**	**S**
F ratio	11.084	37.34	121.60	**F ratio**	1.73	0.21	4.26
P > F	0.0001	<.0001	<.0001	**P > F**	0.19	0.97	0.012
Wilcoxon	**Ca**	**Mg**	**S**	**Normality**	**Ca**	**Mg**	**S**
$\chi 2$			17.88	**A2**	0.16	0.34	1.84
P > $\chi 2$			0.0065	**P > A2**	0.95	0.48	<0.0001

Table II.68: Mean summary for secondary macronutrients of lettuce (Figure 4.4) Calcium (Ca), Magnesium (Mg), and Sulfur (S) concentrations were tested in greenhouse grown lettuce tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean			**Standard Error**		
Treatment	**Ca**	**Mg**	**S**	**Ca**	**Mg**	**S**
BSF 10%	1.027	0.42	0.24	0.065	0.016	0.0061
BSF 20%	0.95	0.46	0.24	0.065	0.016	0.0061
BSF 40%	0.74	0.56	0.27	0.065	0.016	0.0061
CP 100%	1.36	0.47	0.14	0.065	0.016	0.0061
VC 10%	1.28	0.35	0.14	0.065	0.016	0.0061
VC 20%	1.13	0.31	0.13	0.065	0.016	0.0061
VC 40%	1.053	0.31	0.14	0.065	0.016	0.0061

Table II.69: Summary statistics for micronutrients of arugula (Figure 4.5), Df = 6 Boron (B), Iron (Fe) Magnesium (Mn), Copper (Cu), and Zinc (Zn) concentrations were tested in greenhouse grown arugula tissues

ANOVA	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**	**Levene's**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
F ratio	22.42	0.59	131.71	2.22	.0002	**F ratio**	1.053	6.14	1.42	4.52	2.6
P > F	<.0001	0.73	<.0001	0.10	10.07	**P > F**	0.43	0.0025	0.28	0.0094	.06
Wilcoxon	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**	**Normality**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
$\chi 2$		6.37	19.29	12.07		**A2**	0.36	1.67	0.80	0.44	.57
P > $\chi 2$		0.38	0.0037	0.061		**P > A2**	0.44	<.0001	0.03	0.27	.13

Table II.70: Mean summary for micronutrients of arugula (Figure 4.5) Boron (B), Iron (Fe) Magnesium (Mn), Copper (Cu), and Zinc (Zn) concentrations were tested in greenhouse grown arugula tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean					**Standard Error**				
Treatment	**B**	**Fe**	**Mn**	**Cu**	**Zn**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
BSF 10%	51.067	69.8	146.67	5.1	73.37	2.084	20.95	6.88	0.56	4.87
BSF 20%	56.6	72.1	195.33	5.93	86.067	2.084	20.95	6.88	0.56	4.87
BSF 40%	51.33	59.93	233.67	5.37	105.033	2.084	20.95	6.88	0.56	4.87
CP 100%	29.67	43.43	96.27	3.87	58.8	2.084	20.95	6.88	0.56	4.87
VC 10%	34.2	80.3	34.7	4.9	65.67	2.084	20.95	6.88	0.56	4.87
VC 20%	39.5	94	38.433	6.033	68.43	2.084	20.95	6.88	0.56	4.87
VC 40%	44.6	78.17	58.5	6.3	81.4	2.084	20.95	6.88	0.56	4.87

Table 11.71: Summary statistics for micronutrients of lettuce (Figure 4.6), Df= 6 Boron (B), Iron (Fe), Manganese (Mn), Copper (Cu), and Zinc (Zn) concentrations were tested in greenhouse grown lettuce tissues

ANOVA	**B**	**Fe**	**Mn**	**Cu**	**Zn**	**Levene's**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
F ratio	27.43	29.51	81.70	0.83	26.75	**F ratio**	0.36	3.57	3.59	2.85	1.91
P > F	<.0001	<.0001	<.0001	0.56	<.0001	**P > F**	0.89	0.023	0.023	0.05	0.15
Wilcoxon	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**	**Normality**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
$\chi 2$	16.61	17.79	18.37			**A2**	0.93	1.059	1.33	0.15	0.68
P > $\chi 2$	0.011	0.0068	0.0054			**P > A2**	0.017	0.008	0.0028	0.97	0.062

Table II.72: Mean summary for micronutrients of lettuce (Figure 4.6) Boron (B), Iron (Fe) Magnesium (Mn), Copper (Cu), and Zinc (Zn) concentrations were tested in greenhouse grown lettuce tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean					**Standard Error**				
Treatment	**B**	**Fe**	**Mn**	**Cu**	**Zn**	**Bo**	**Fe**	**Mn**	**Cu**	**Zn**
BSF 10%	30.17	72.73	101.73	4.8	38.9	1.065	2.68	8.82	0.45	2.27
BSF 20%	30.73	66.6	202.3	5.83	52.3	1.065	2.68	8.82	0.45	2.27
BSF 40%	33.1	70.63	167.33	5.53	53	1.065	2.68	8.82	0.45	2.27
CP 100%	21.67	50.17	200	4.73	36	1.065	2.68	8.82	0.45	2.27
VC 10%	19.73	41.2	40.17	5.27	29	1.065	2.68	8.82	0.45	2.27
VC 20%	20.6	38.73	28.47	5.033	23.73	1.065	2.68	8.82	0.45	2.27
VC 40%	22.73	45.03	28.7	4.87	0.45	1.065	2.68	8.82	0.45	2.27

Table II.73: Summary statistics for other nutrients of arugula (Figure 4.7), f = 6 Aluminum (Al) and Sodium (Na) concentrations were tested in greenhouse grown arugula tissues

ANOVA	**Al**	**Na**	**Levene's**	**Al**	**Na**
F ratio	1.25	97.35	**F ratio**	10.35	1.28
P > F	0.34	<.0001	**P > F**	0.0002	0.33
Wilcoxon			**Normality**		
χ2	13.021	17.76	**A2**	3.77	1.56
P > χ2	0.043	0.0068	**P > A2**	<.0001	<.0001

Table II.74: Mean summary for other nutrients of arugula (Figure 4.7) Aluminum (Al) and Sodium (Na) concentrations were tested in greenhouse grown arugula tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean		**Standard Error**	
Treatment	**Al**	**Na**	**Al**	**Na**
BSF 10%	14.3	446	21.80	41.13
BSF 20%	20.367	1173.33	21.80	41.13
BSF 40%	21.9	1073.67	21.80	41.13
CP 100%	9.73	161.67	21.80	41.13
VC 10%	18.567	210.33	21.80	41.13
VC 20%	72.267	367.33	21.80	41.13
VC 40%	58.7	427.33	21.80	41.13

Table II.75: Summary statistics for other nutrients of lettuce (Figure 4.8), Df = 6 Aluminum (Al) and Sodium (Na) concentrations were tested in greenhouse grown lettuce tissues

ANOVA	**Al**	**Na**	**Levene's**	**Al**	**Na**
F ratio	1.67	143.31	**F ratio**	4.16	2.3493
P > F	0.20	<.0001	**P > F**	0.013	0.88
Wilcoxon			**Normality**		
χ2	10.36	18.59	**A2**	0.38	1.45
P > χ2	0.11	0.0049	**P > A2**	0.38	0.0008

Table II.76: Mean summary for other nutrients of lettuce (Figure 4.8), df = 6 Aluminum (Al) and Sodium (Na) concentrations were tested in greenhouse grown lettuce tissues. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

	Mean		**Standard Error**	
Treatment	**Al**	**Na**	**Al**	**Na**
BSF 10%	13.23	2073.33	2.19	69.41
BSF 20%	15.9	1933.33	2.19	69.41
BSF 40%	14.57	2400	2.19	69.41
CP 100%	15.57	2460	2.19	69.41
VC 10%	10.17	587	2.19	69.41
VC 20%	8.47	667	2.19	69.41
VC 40%	11.43	867	2.19	69.41

3. Chapter Five

Table II.77: Summary statistics for average individual fresh fruit weight (g) of 2021 greenhouse tomatoes (Figure 5.1), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	2.51	**F ratio**	1.80
P > F	0.020	**P > F**	0.095
Block		**Anderson Darling test for Normality**	
F ratio	1.23	**A2**	27.170
P > F	0.27	**P > A2**	<.0001

Table II.78: Mean summary for average individual fresh fruit weight (g) of tomatoes (Figure 5.1) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	140.72	6.09
BSF 20%	136.56	5.24
BSF 40%	134.53	5.41
CP 100%	134.85	5.69
VC 10%	160.90	6.34
VC 20%	148.77	5.72
VC 40%	146.67	6.26

Table II.79: Summary statistics for total fresh fruit weight (g) of 2021 greenhouse tomatoes (Figure 5.2), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	0.85	**F ratio**	2.53
P > F	0.53	**P > F**	0.029
Block		**Anderson Darling test for Normality**	
F ratio	2.43	**A2**	2.12
P > F	0.12	**P > A2**	<.0001

Table II.80: Mean summary for total fresh fruit weight (g) of tomatoes (Figure 5.2) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	2321.85	278.05
BSF 20%	3045.3	278.05
BSF 40%	2811.74	278.05
CP 100%	2548.7	278.05
VC 10%	2445.6	278.05
VC 20%	2781.93	278.05
VC 40%	2272.95	278.05

Table II.81: Summary statistics for SPAD measurements of 2021 greenhouse tomatoes (Figure 5.3), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	0.69	**F ratio**	2.024
P > F	0.66	**P > F**	0.0768
Block		**Anderson Darling test for Normality**	
F ratio	0.00	**A2**	2.83
P > F	0.99	**P > A2**	<.0001

Table II.82: Mean summary for SPAD measurements of tomatoes (Figure 5.3) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	44.31	1.62
BSF 20%	40.61	1.45
BSF 40%	39.74	1.52
CP 100%	43.71	1.45
VC 10%	44.36	1.45
VC 20%	40.58	1.52
VC 40%	41.49	1.62

Table II.83: Summary statistics for Average Individual fruit weight of BER 2021 greenhouse tomatoes (Figure 5.4), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	1.13	**F ratio**	2.87
P > F	0.35	**P > F**	0.012
Block		**Anderson Darling test for Normality**	
F ratio	0.52	**A2**	3.44
P > F	0.47	**P > A2**	<.0001

Table II.84: Mean summary for Average Individual fruit weight of BER tomatoes (Figure 5.4) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	128.24	20.43
BSF 20%	111.078	16.94
BSF 40%	150.48	18.79
CP 100%	104.27	24.74
VC 10%	153.083	22.58
VC 20%	168.46	27.66
VC 40%	134	55.32

Table II.85: Summary statistics for total fruit weight of BER 2021 greenhouse tomatoes (Figure 5.5), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	1.083	**F ratio**	1.57
P > F	0.39	**P > F**	0.18
Block		**Anderson Darling test for Normality**	
F ratio	0.075	**A2**	1.17
P > F	0.78	**P > A2**	0.003

Table II.86: Mean summary for total fruit weight (g) of BER tomatoes (Figure 5.5) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	352.66	90.73
BSF 20%	394.94	85.54
BSF 40%	489.063	90.73
CP 100%	195.5	90.73
VC 10%	306.17	85.54
VC 20%	288.79	96.99
VC 40%	201	181.45

Table II.87: Summary statistics for final biomass measurements (g) of 2021 greenhouse tomato plants (Figure 5.6), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	1.48	**F ratio**	0.61
P > F	0.20	**P > F**	0.72
Block		**Anderson Darling test for Normality**	
F ratio	1.79	**A2**	0.50
P > F	0.19	**P > A2**	0.21

Table II.88: Mean summary for final biomass measurements (g) of tomato plants (Figure 5.6) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	757.78	56.31
BSF 20%	733.45	53.42
BSF 40%	570.2	53.42
CP 100%	623.2	53.42
VC 10%	672.55	53.42
VC 20%	650.65	53.42
VC 40%	717.56	59.73

Table II.89: Summary statistics for BRIX measurements (g) of 2021 greenhouse tomato fruits (Figure 5.7), Df = 6

Treatment		**Levene's test for Equal Variance**	
F ratio	0.57	**F ratio**	0.25
P > F	0.75	**P > F**	0.96
Block		**Anderson Darling test for Normality**	
F ratio	0.60	**A2**	0.29
P > F	0.45	**P > A2**	0.61

Table II.90: Mean summary for BRIX measurements (g) of tomato plants (Figure 5.7) grown in the greenhouse in 2021 Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 10%	3.96	0.20
BSF 20%	4.02	0.20
BSF 40%	3.72	0.20
CP 100%	3.88	0.20
VC 10%	4.24	0.20
VC 20%	3.96	0.20
VC 40%	3.98	0.20

4. Chapter Six

Table II.91: Summary statistics for percent emergence of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.2). Arugula (A), Lettuce (L), 'Brandywine' (BW) Tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

χ2	**A**	**L**	**BW**	**JS**	**P > χ2**	**A**	**L**	**BW**	**JS**
	8.13	17.99	3.08	9.77		0.02	0.001	0.21	0.008

Table II.92: Mean summary for percent germination of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.2). Arugula (A), Lettuce (L), 'Brandywine' (BW) Tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	46	60	30	46	11.49	10.89	9.092	9.73
BSF 10%	24	60	38	38	11.49	10.89	9.092	9.73
BSF 20%	22	24	22	18	11.49	10.89	9.092	9.73

Table II.93: Summary statistics for days to germination of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.3). Arugula (A), Lettuce (L), 'Brandywine' (BW) Tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

ANOVA	**A**	**L**	**BW**	**JS**	**Levene's**	**A**	**L**	**BW**	**JS**
F ratio	1.57	0.95	1.38	0.65	**F ratio**	0.75	6.85	6.56	2.61
P > F	0.25	0.42	0.29	0.54	**P > F**	0.49	0.010	0.012	0.11
Wilcoxon	**A**	**L**	**BW**	**JS**	**Normality**	**A**	**L**	**BW**	**JS**
χ2	0.88	5.60	3.14		**A2**	0.87	1.36	0.39	0.36
P > χ2	0.64	0.061	0.21		**P > A2**	0.21	0.0004	0.34	0.45

Table II.94: Mean summary for days to germination of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.3). Arugula (A), Lettuce (L), 'Brandywine' (BW) Tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	5.17	3.59	6.27	6.74	1.040	0.99	1.47	1.42
BSF 10%	3	4.29	6.077	8.72	1.040	0.99	1.47	1.42
BSF 20%	2.83	5.5	9.15	8.72	1.040	0.99	1.47	1.42

Table II.95: Summary statistics for seedling weight of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.4). Arugula (A), Lettuce (L), 'Brandywine' (BW) Tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

ANOVA	**A**	**L**	**BW**	**JS**	**Levene's**	**A**	**L**	**BW**	**JS**
F ratio	1.57	3.44	4.76	5.29	**F ratio**	5.19	0.73	1.016	1.45
P > F	0.25	0.066	0.03	0.023	**P > F**	0.023	0.50	0.39	0.27
Wilcoxon	**A**	**L**	**BW**	**JS**	**Normality**	**A**	**L**	**BW**	**JS**
$\chi 2$	7.33				**A2**	3.22	0.24	0.17	0.15
P > $\chi 2$	0.026				**P > A2**	<.0001	0.75	0.94	0.97

Table 11.96: Mean summary for seedling weight of vegetable crops grown in BSF frass as partial peat replacement (Figure 6.4). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	0.21	0.15	0.11	0.11	0.078	0.024	0.014	0.016
BSF 10%	0.056	0.084	0.06	0.068	0.078	0.024	0.014	0.016
BSF 20%	0.026	0.058	0.049	0.035	0.078	0.024	0.014	0.016

Table II.97: Summary statistics for percent emergence of vegetable crops grown in BSFL frass fertility replacement (Figure 6.5). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

χ2	**A**	**L**	**BW**	**JS**	**P > χ2**	**A**	**L**	**BW**	**JS**
	19.78	12.06	8.28	17.06		<0.0001	0.0005	0.004	<0.0001

Table II.98: Mean summary for percent germination of vegetable crops grown in BSFL frass fertility replacement (Figure 6.5). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	46	60	30	46	12.65	6.93	8.19	9.90
BSF 10%	8	26	8	10	12.65	6.93	8.19	9.90

Table II.99: Summary statistics for days to germination of vegetable crops grown in BSFL frass fertility replacement (Figure 6.6). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

T test	**A**	**L**	**BW**	**JS**	**Levene's**	**A**	**L**	**BW**	**JS**
T ratio	2.74	-1.54	0.43	0.56	**F ratio**	0.47	3.35	12.70	59.46
P > F	0.026	0.16	0.69	0.60	**P > F**	0.51	0.10	0.0074	<0.0001
Wilcoxon	**A**	**L**	**BW**	**JS**	**Normality**	**A**	**L**	**BW**	**JS**
χ2		4.036			**A2**	0.61	1.59	0.16	0.46
P > χ2		0.045			**P > A2**	0.084	0.0004	0.94	0.22

Table II.100: Mean summary for days to germination of vegetable crops grown in BSFL frass fertility replacement (Figure 6.6). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	5.17	3.59	6.27	6.74	1.10	0.58	2.19	1.94
BSF 10%	0.9	4.85	5.1	5	1.10	0.58	2.19	1.94

Table II.101: Summary statistics for of seedling weight of vegetable crops grown in BSFL frass fertility replacement (Figure 6.7), Df = 6. Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022.

T test	**A**	**L**	**BW**	**JS**	**Levene's**	**A**	**L**	**BW**	**JS**
T ratio	1.46	1.48	4.48	6.027	**F ratio**	5.82	2.29	0.0088	11.53
P > t	0.22	0.18	0.0021	0.002	**P > F**	0.042	0.17	0.93	0.0094
Wilcoxon	**A**	**L**	**BW**	**JS**	**Normality**	**A**	**L**	**BW**	**JS**
$\chi 2$	6.7325				**A2**	2.19	0.43	0.19	0.50
P > $\chi 2$	0.0095				**P > A2**	<.0001	0.26	0.87	0.18

Table II.102: Mean summary for seedling weight of vegetable crops grown in BSFL frass fertility replacement (Figure 6.7). Arugula (A), Lettuce (L), 'Brandywine' (BW) tomatoes, and 'Jet Star' tomatoes (JS) seedlings were grown in the greenhouse in 2022. Treatments include mixtures of control peat and black soldier fly larvae frass (BSF).

	Mean				**Standard Error**			
Treatment	**A**	**L**	**BW**	**JS**	**A**	**L**	**BW**	**JS**
Control	0.21	0.15	0.11	0.11	0.095	0.022	0.013	0.012
BSF 10%	0.012	0.10	0.025	0.0085	0.095	0.022	0.013	0.012

5. Preliminary Studies

Table II.103: Summary statistics for total fresh weight (g) of 2020 greenhouse arugula (Figure I.1), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	4.62	**F ratio**	2.51
P > F	<.0001	**P > F**	0.0101
Wilcoxon		**Anderson Darling test for Normality**	
χ2	39.30	**A2**	4.65
P > χ2	<.0001	**P > A2**	<.0001

Table II.104: Mean summary for total fresh weight (g) ± SE of arugula provided different supplementations of black soldier fly frass (Figure I.1) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	2.28	2.11
BSF 20%	3.20	1.54
BSF 10%	11.52	2.5
CP 100%	2.89	1.56
VC 100%	10.84	3.034
VC 80%	8.42	2.64
VC 60%	8.72	3.28
VC 40%	6.94	2.60
VC 20%	12.18	1.30
VC 10%	7.42	2.28

Table II.105: Summary statistics for average fresh weight (g) of 2020 greenhouse arugula (Figure I.2), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.93	**F ratio**	2.77
P > F	0.0002	**P > F**	0.0049
Wilcoxon		**Anderson Darling test for Normality**	
χ2	37.16	**A2**	3.76
P > χ2	0.0002	**P > A2**	<.0001

Table II.106: Mean summary for average fresh weight (g) of arugula (Figure I.2) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.85	0.69
BSF 20%	2.25	0.82
BSF 10%	4.28	0.77
CP 100%	1.76	0.96
VC 100%	3.97	0.94
VC 80%	5.17	1.92
VC 60%	3.29	0.98
VC 40%	3.51	1.25
VC 20%	6.32	1.29
VC 10%	4.1	1.218

Table II.107: Summary statistics for total dry weight (g) of 2020 greenhouse arugula (Figure I.3), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.49	**F ratio**	3.99
P > F	0.011	**P > F**	0.0002
Wilcoxon		**Anderson Darling test for Normality**	
χ2	38.80	**A2**	4.41
P > χ2	0.0001	**P > A2**	<.0001

Table II.108: Mean summary for total dry weight (g) of arugula (Figure I.3) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.074	0.15
BSF 20%	0.19	0.15
BSF 10%	0.35	0.15
CP 100%	0.16	0.11
VC 100%	0.4	0.15
VC 80%	0.66	0.15
VC 60%	0.31	0.15
VC 40%	0.49	0.15
VC 20%	0.62	0.15
VC 10%	0.47	0.15

Table II.109: Summary statistics for average dry weight (g) of 2020 greenhouse arugula (Figure I.4), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	1.38	**F ratio**	5.28
P > F	0.20	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	35.85	**A2**	8.59
P > χ2	0.0003	**P > A2**	<.0001

Table II.110: Mean summary for average dry weight (g) of arugula (Figure I.4) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.034	0.14
BSF 20%	0.15	0.14
BSF 10%	0.13	0.14
CP 100%	0.15	0.10
VC 100%	0.17	0.14
VC 80%	0.57	0.14
VC 60%	0.14	0.14
VC 40%	0.25	0.14
VC 20%	0.38	0.14
VC 10%	0.28	0.14

Table II.111: Summary statistics for leaf area (mm^2) of 2020 greenhouse arugula (Figure I.5), Df = 12

ANOVA		Levene's test for Equal Variance	
F ratio	1.074	**F ratio**	7.50
P > F	0.40	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	37.11	**A2**	24.14
P > χ2	0.0002	**P > A2**	<.0001

Table II.112: Mean summary for leaf area (mm^2) of arugula (Figure I.5) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	Mean	Standard Error
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	25.36	1604.4
BSF 20%	59.39	1604.4
BSF 10%	107.18	1604.4
CP 100%	46.29	1134.5
VC 100%	108.78	1604.4
VC 80%	111.07	1604.4
VC 60%	89.93	1604.4
VC 40%	1525.28	1604.4
VC 20%	152.55	1604.4
VC 10%	5970.15	1604.4

Table II.113: Summary statistics for total fresh weight (g) of 2020 greenhouse radishes (Figure I.6), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.72	**F ratio**	4.21
P > F	0.0056	**P > F**	0.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	33.50	**A2**	3.78
P > χ2	0.0008	**P > A2**	<.0001

Table II.114: Mean summary statistics for total fresh weight (g) of radishes (Figure I.6) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	3.66	3.66
BSF 20%	12.98	6.49
BSF 10%	17.64	4.92
CP 100%	13.87	5.35
VC 100%	4.46	1.82
VC 80%	20.34	4.93
VC 60%	16.51	1.14
VC 40%	11.36	3.89
VC 20%	21.36	4.90
VC 10%	20.64	9.51

Table II.115: Summary statistics for above ground fresh weight (g) of 2020 greenhouse radishes (Figure I.7), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	4.53	**F ratio**	2.75
P > F	<.0001	**P > F**	0.0051
Wilcoxon		**Anderson Darling test for Normality**	
χ2	37.69	**A2**	1.71
P > χ2	0.0002	**P > A2**	<.0001

Table II.116: Mean Summary statistics for above ground fresh weight (g) of radishes (Figure I.7) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	3.51	1.61
BSF 20%	8.09	1.61
BSF 10%	6.1	1.61
CP 100%	6.72	1.14
VC 100%	4.6	1.61
VC 80%	9.19	1.61
VC 60%	8.38	1.61
VC 40%	5.35	1.61
VC 20%	8.06	1.61
VC 10%	8.64	1.61

Table II.117: Summary statistics for below ground fresh weight (g) of 2020 greenhouse radishes (Figure I.8), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.99	**F ratio**	3.75
P > F	0.0027	**P > F**	0.0003
Wilcoxon		**Anderson Darling test for Normality**	
χ2	41.57	**A2**	5.14
P > χ2	<.0001	**P > A2**	<.0001

Table II.118: Mean Summary statistics for below ground fresh weight (g) of radishes (Figure I.8) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.43	2.65
BSF 20%	5.50	2.65
BSF 10%	11.11	2.65
CP 100%	3.14	1.89
VC 100%	3.5	2.65
VC 80%	6.79	2.65
VC 60%	10.87	2.65
VC 40%	8.55	2.65
VC 20%	12.02	2.65
VC 10%	8.51	2.65

Table II.119: Summary statistics for total dry weight (g) of 2020 greenhouse radishes (Figure I.9), Df = 12

ANOVA		Levene’s test for Equal Variance	
F ratio	5.30	**F ratio**	3.43
P > F	<.0001	**P > F**	.0008
Wilcoxon		**Anderson Darling test for Normality**	
χ2	43.34	**A2**	2.28
P > χ2	<0.0001	**P > A2**	<0.0001

Table II.120: Mean Summary statistics for total dry weight (g) of radishes (Figure I.9) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	Mean	Standard Error
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.34	0.31
BSF 20%	0.90	0.31
BSF 10%	1.34	0.31
CP 100%	0.89	0.22
VC 100%	0.83	0.31
VC 80%	1.41	0.31
VC 60%	1.73	0.31
VC 40%	1.29	0.31
VC 20%	2.24	0.31
VC 10%	1.59	0.31

Table II.121: Summary statistics for above ground dry weight (g) of 2020 greenhouse radishes (Figure I.10), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.07	**F ratio**	2.64
P > F	<.0001	**P > F**	0.0070
Wilcoxon		**Anderson Darling test for Normality**	
χ2	39.61	**A2**	1.67
P > χ2	<0.0001	**P > A2**	<0.0001

Table II.122: Mean Summary statistics for above ground dry weight (g) of radishes (Figure I.10) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.31	0.16
BSF 20%	0.63	0.16
BSF 10%	0.62	0.16
CP 100%	0.6	0.16
VC 100%	0.54	0.16
VC 80%	0.93	0.16
VC 60%	0.93	0.16
VC 40%	0.63	0.16
VC 20%	0.92	0.16
VC 10%	0.89	0.16

Table II.123: Summary statistics for below ground dry weight (g) of 2020 greenhouse radishes (Figure I.11), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	4.75	**F ratio**	4.29
P > F	<.0001	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
χ2	46.43	**A2**	5.46
P > χ2	<0.0001	**P > A2**	<.0001

Table II.124: Mean Summary statistics for below ground dry weight (g) of radishes (Figure I.11) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	0	0
BSF 80%	0	0
BSF 60%	0	0
BSF 40%	0.030	0.19
BSF 20%	0.27	0.19
BSF 10%	0.78	0.19
CP 100%	0.25	0.13
VC 100%	0.29	0.19
VC 80%	0.48	0.19
VC 60%	0.80	0.19
VC 40%	0.66	0.19
VC 20%	1.32	0.19
VC 10%	0.70	0.19

Table II.125: Summary statistics for total fresh fruit weight (g) of 2020 greenhouse tomatoes (Figure I.12), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	5.062	**F ratio**	1.77
P > F	<.0001	**P > F**	0.077
Wilcoxon		**Anderson Darling test for Normality**	
$\chi 2$	37.31	**A2**	0.82
P > $\chi 2$	0.0002	**P > A2**	0.033

Table II.126: Mean Summary statistics for total fresh fruit weight (g) of tomatoes (Figure I.12) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	155.7	155.7
BSF 80%	424.5	120.29
BSF 60%	471.5	195.50
BSF 40%	1189.1	177.79
BSF 20%	693.3	159.43
BSF 10%	595.4	250.80
CP 100%	363.75	101.38
VC 100%	1616	305.64
VC 80%	1151.8	130.35
VC 60%	1432.6	211.19
VC 40%	1012.8	402.84
VC 20%	1451.8	312.27
VC 10%	646.7	166.72

Table II.127: Summary statistics for average individual fresh fruit weight (g) of 2020 greenhouse tomatoes (Figure I.13), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	4.59	**F ratio**	2.35
P > F	<.0001	**P > F**	0.0155
Wilcoxon		**Anderson Darling test for Normality**	
$\chi 2$	34.048	**A2**	1.085
P > $\chi 2$	0.0007	**P > A2**	0.0030

Table II.128: Mean Summary statistics for average individual fresh fruit weight (g) of tomatoes (Figure I.13) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	15.57	14.27
BSF 80%	54.34	14.27
BSF 60%	51.32	14.27
BSF 40%	84.31	14.27
BSF 20%	82.72	14.27
BSF 10%	77.011	14.27
CP 100%	67.30	10.09
VC 100%	106.26	14.27
VC 80%	111.16	14.27
VC 60%	100.20	14.27
VC 40%	94.55	14.27
VC 20%	129.51	14.27
VC 10%	103.45	14.27

Table II.129: Summary statistics for total biomass (g) of 2020 greenhouse tomato plants (Figure I.14), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.26	**F ratio**	2.98
P > F	0.0016	**P > F**	0.0033
Wilcoxon		**Anderson Darling test for Normality**	
χ2	32.65	**A2**	0.94
P > χ2	0.0011	**P > A2**	0.013

Table II.130: Summary statistics for total biomass (g) of tomato plants (Figure I.14) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	669.5	97.99
BSF 80%	654.9	97.99
BSF 60%	998.67	89.45
BSF 40%	963.5	126.5
BSF 20%	769.13	109.55
BSF 10%	804.75	154.93
CP 100%	641.61	73.04
VC 100%	862.2	97.99
VC 80%	895.2	97.99
VC 60%	1149.13	109.55
VC 40%	1078.2	97.99
VC 20%	1101	97.99
VC 10%	894	97.99

Table II.131: Summary statistics for number of 2020 greenhouse tomato fruits per plant (Figure 10), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	3.52	**F ratio**	1.80
P > F	0.0006	**P > F**	0.070
Wilcoxon		**Anderson Darling test for Normality**	
χ2	28.98	**A2**	0.62
P > χ2	0.004	**P > A2**	0.11

Table II.132: Mean Summary statistics for number of tomato fruits per plant (Figure I.15) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	2	2.31
BSF 80%	8	2.31
BSF 60%	8	2.31
BSF 40%	14.8	2.31
BSF 20%	8.4	2.31
BSF 10%	7	2.31
CP 100%	4.4	1.63
VC 100%	16	2.31
VC 80%	10.8	2.31
VC 60%	14.2	2.31
VC 40%	9	2.31
VC 20%	11.6	2.31
VC 10%	6.2	2.31

Table II.133: Summary statistics for average individual weight (g) of BER 2020 greenhouse tomato fruits (Figure I.16), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.82	**F ratio**	2.84
P > F	0.001	**P > F**	0.0009
Wilcoxon		**Anderson Darling test for Normality**	
χ2	46.20	**A2**	26.44
P > χ2	<.0001	**P > A2**	<.0001

Table II.134: Mean Summary statistics for Average Individual weight of BER tomato fruits (Figure I.16) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	50.25	28.46
BSF 80%	46.78	11.31
BSF 60%	58.49	11.62
BSF 40%	63.92	10.63
BSF 20%	64.36	11.022
BSF 10%	71.52	13.17
CP 100%	63.79	12.52
VC 100%	98.7	15.59
VC 80%	109.36	10.51
VC 60%	92	11.46
VC 40%	100.21	15.21
VC 20%	103.038	13.67
VC 10%	78.87	11.02

Table II.135: Summary statistics for total weight (g) of BER 2020 greenhouse tomato fruits (Figure I.17), Df = 12

ANOVA		**Levene's test for Equal Variance**	
F ratio	2.14	**F ratio**	5.23
P > F	0.032	**P > F**	<.0001
Wilcoxon		**Anderson Darling test for Normality**	
$\chi 2$	15.83	**A2**	0.70
P > $\chi 2$	0.20	**P > A2**	0.068

Table II.136: Mean Summary statistics for total weight (g) of BER fruits (Figure I.17) grown in the greenhouse in 2020. Treatments include mixtures of control peat (CP) and black soldier fly larvae frass (BSF) or vermicompost (VC).

Treatment	**Mean**	**Standard Error**
BSF 100%	150.75	194.06
BSF 80%	355.54	122.74
BSF 60%	526.37	137.22
BSF 40%	549.7	122.74
BSF 20%	514.9	122.74
BSF 10%	400.5	122.74
CP 100%	346.1	122.74
VC 100%	394.8	122.74
VC 80%	962.34	122.74
VC 60%	680.8	122.74
VC 40%	420.9	122.74
VC 20%	535.8	122.74
VC 10%	631	122.74