

THE EFFECTS OF AGRICULTURAL WASTE-BASED COMPOST AMENDMENTS IN
ORGANIC PEST MANAGEMENT

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ABSTRACT

The Effects of Agricultural Waste-Based Compost Amendments in Organic Pest Management

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Soil-borne pests and pathogens, such as *Verticillium dahliae*, can devastate a wide range of annual and perennial crops. Current management options for organic production are limited and sustainable management of pathogens, weeds, and arthropods is important for staying profitable and reducing the use of harmful chemicals. Organic soil amendments play an important role in supplying some of the nutritional needs of vegetable crops and improving soil structure, while also contributing to pest control. The objective of this research was to determine the effects of grape and olive-based composts on soil pathogen load, arthropod communities, and weed biomass and diversity. Field experiments were conducted in both organic and conventionally grown bell pepper (*Capsicum annuum* L.) systems during the summer growing season of 2018 on the Cal Poly campus in San Luis Obispo, California. Four different organic amendment treatments were tested including: olive based compost, grape based compost, dairy manure compost, and plant waste compost. Abundance of the fungal pathogen *Verticillium dahliae* was assessed from composite soil samples collected at several time points throughout the growing season. Olive, grape, and plant waste composts all displayed significant reduction of *V. dahliae* abundance between two to eight weeks post application of treatment when compared to the control. Insufficient evidence was found correlating farm management type with *V. dahliae* abundance after adjusting for treatment and time. Total dry weed biomass was assessed after one month of unhindered growth. The organic amendments tested appeared to alter weed species composition but not overall biomass though no significant differences were found. The soil

arthropods symphylans and collembolans were sampled throughout the growing season, significant trends in population were found over time but not across treatments. This research demonstrates how agricultural waste-based compost amendments have potential as tools in pest management.

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CHAPTER 1

Introduction

Pest and pathogen control are at the forefront of many vegetable grower's production challenges. The 2016 National Organic Research Agenda listed "Soil health, biology, quality, and nutrient management" as the highest priority research area for organic farmers while "weed management" and "insect management" also were in the top five (Jerkins and Ory, 2016).

Control of soil pathogens, insect pests, and weeds are top priorities due to their damaging effects on yield and limited management options in organic production. Careful management of soil microbial communities can reduce pathogen disease incidence and increase plant and soil health (Beneduzi et al., 2012; Santoyo et al., 2012). The use of organic soil amendments promotes soil microbial diversity, including specific taxa known to suppress soil-borne pathogens (Lupatini et al., 2017). Composted olive and grape pomace are two organic amendments shown to suppress soil-borne fungal pathogens (Ntougias et al., 2008). These pomaces are readily available in Mediterranean climates as byproducts of olive oil and wine production. Experimenting with the use of these locally available organic soil amendments may result in novel uses for sustainably protecting agricultural crops from disease and pests. Additionally, these amendments contain phytotoxic molecules that may have potential in the management of weeds and soil arthropod communities.

Decreasing disease incidence with an effectively managed soil microbial community is a sustainable alternative to harsher chemical controls. Methyl bromide was one such chemical control that was widely employed but was phased out of agriculture use starting in 2005 due to environmental concerns (EPA, 2018). Restrictions on methyl bromide prompted the exploration for alternative, sustainable methods. Disease suppression by soil microbial communities

functions through direct and indirect mechanisms. Direct suppression by antagonistic organisms occurs through the production of antibiotics and competition for resources. Indirect suppression functions through the introduction of systemic resistance and increased nutrient availability; leading to improved plant health and vigor (Hoitink and Bohem, 1999; Noble and Coventry, 2005).

Weeds can reduce yields as they compete with crops for water, nutrients, light, and space. Organic management options for weed management are often limited, costly, and labor intensive (Hartz et al., 2008; Smith et al., 2000). Organic amendments have shown potential for the suppression of weeds via the introduction of phytotoxic molecules to the soil (Cayuela et al., 2008; Ferrara et al., 2008; Ibrahim and Balah, 2018). Additionally, stimulation of the soil microbial community is likely to include taxa that feed on or otherwise limit the germination and growth of weeds (Kennedy, 1999).

Soil arthropod communities also are influenced by the applications of organic amendments (Chamberlain et al., 2006; Stirling et al., 2012; Tworowski, 2004). Arthropods have a wide variety of ecological roles in an agricultural setting from herbivorous pests to detritivores and fungivores that help with nutrient cycling and pest control (Anslan et al., 2018; Gao et al., 2017). The implications of compost application for soil arthropods are not well studied but may pose novel methods for managing pests such as root feeding symphylans. The addition of organic matter to the soil may prove to be an effective way to promote diversity of other soil arthropod communities such as omnivorous soil dwelling collembola, which contribute to soil nutrient cycling directly as detritivores and indirectly through microbial grazing (Ferlian et al., 2015; Ponge et al., 2003).

Grape and olive pomace are high in nutrients and contain phytotoxic polyphenols (Ntougias et al., 2008). Due to high salt content and the presence of phytotoxic compounds the direct application of grape and olive pomace can reduce soil quality and harm crops; however, composting produces a stabilized organic matter that is safe for application (Albuquerque et al., 2009; Aranda et al., 2015; Hachicha et al., 2008; Martinez et al., 2019). Thus, grape and olive-based composts maybe ideal candidates for soil amendment uses due to their disease suppressive qualities and abundance as waste products of agricultural processing. More research is needed regarding the potential uses of organic amendments as an alternative pest management strategy. Research is needed into the mechanisms and efficacy of organic amendment disease suppressive properties in different cropping systems to promote interest in their use. The effects of composted grape and olive pomace on disease suppression are yet to be fully understood, especially in vegetable systems where soil-borne pathogens are of major importance. Compost sources are also variable and often region specific, so a larger consensus of research is needed to better understand how agricultural waste-based amendments effect soil ecology. The objectives of this research are to determine the effects of dairy-based compost, plant waste-based compost, olive pomace-based compost, and grape pomace-based compost on soil-borne pathogen load, soil microbial communities, soil arthropod communities, weed abundance, health and yield of a bell pepper crop (*Capsicum annuum* L.).

We hypothesized that each organic amendment tested would have an effect on the soil microbial community and soil arthropod communities. In addition, we hypothesized that grape and olive-based amendments would have a suppressive effect on the soil-borne pathogen load and weed abundance. Finally, we hypothesized that organic amendment application would increase bell pepper health and yield.

CHAPTER 2

Literature Review

California agricultural production receipts totaled over \$47 billion in 2015 making it the largest producer in the nation (USDA, 2017). That production is hindered by a plethora of soil-borne diseases that can cause serious economic loss and have relatively few solutions in terms of suppression (Hartz et al., 2008). In recent years, soil management and disease suppression efforts have focused on sustainable practices that reduce the dependence on chemical fertilizers and pesticides (Curlango-Rivera et al., 2013). One promising sustainable method for suppressing these pathogens is by managing the soil microbial communities with the use of organic soil amendments (Lupatini et al., 2017). Existing olive oil and wine production in California creates an abundance of readily available organic waste products such as olive and grape pomaces. These waste products have been shown to have suppressive effects on certain soil-borne diseases (Noble and Coventry, 2004; Ntougias et al., 2007; Yangui et al., 2010). The objective of this literature review is to explore the current state of the research on organic soil amendments and how they affect soil microbial communities, soil-borne diseases, and specifically the effects of olive and grape pomace-based soil amendments.

Using organic soil amendments to manage the microbial community and suppress pathogens has been a topic of interest to researchers due to its potential applications as a sustainable alternative to pesticides and fumigation techniques. Organic amendments have been shown to increase the activity and diversity of the soil microbial community, which has beneficial effects on crop health and can help suppress soil-borne pathogens (Beneduzi et al., 2012; Hoitink and Boehm, 1999). Olive and grape-based composts have shown particular promise for disease suppression and may be ideal candidates for future application due to their

availability (Barbera et al., 2013; Vivas et al., 2009). A variety of studies and analysis have been performed but due to the complicated processes and ecology of each different cropping system and its soil the results are often inconsistent and hard to predict. More research is needed due to the potentially beneficial implications of having a sustainable method for soil-borne pathogen management.

Additionally, organic farmers are limited in their weed control methods; many of which are labor intensive and inviable on a large scale (Smith et al., 2000). Control techniques focus on whole systems management where the seed bank, water availability, and weed ecology are all considered (Smith et al., 2000). With further research, organic amendments could become a viable alternative to costlier and labor intensive weed control methods. The use of natural soil amendments also is attractive due to the lower environmental risks and impacts as opposed to traditional agrochemicals (Chaparro et al., 2012; Trillas et al., 2002).

2.1 Soil Amendments and Soil Microbial Communities

A well-researched benefit of organic soil amendments is their impact on the soil microbial community. Individual plant-microbe relationships have been the focus in many studies (Berg, 2009; Mendes et al., 2011). The symbiotic relationship between plants and rhizobia bacteria has been of particular interest with over 500 peer-reviewed journal articles, containing the word ‘rhizobia’ in the title, published in the last five years alone. These nitrogen fixing bacteria are likely the best described individual plant-microbe interactions; they are able to fix atmospheric nitrogen making it bioavailable for plants, in return, typically receiving carbonaceous nutrients. However, looking at individual relationships oversimplifies the complex nature of plant-microbiome interactions. Recent research has highlighted the benefit of a diverse

and balanced microbiome, which can offer a range of benefits in an agricultural system leading to increased crop health and productivity (Chaparro et al., 2012). In addition to this, many studies have also shown that soil microbial communities can play a role in suppression of soil-borne diseases (Santoyo et al., 2012). Different management methods have been tested to stimulate the soil microbial community to aid plant growth as well as suppress soil-borne diseases. These methods, if shown to be effective, could be a more environmentally friendly and sustainable alternative to other harsher methods such as methyl bromide fumigation (Hoitink and Bohem, 1999).

Different cropping methods can affect the soil microbial community (Degruene et al., 2017; Lupatini et al., 2017). However, it is challenging to trace the relationships between changes in the microbial community and the ecosystem function. Evidence has been found that organic farming systems harbor a more biodiverse soil microbial community than their conventional counterparts and that there are “Soil Health Treatments” that can stimulate minor increases in diversity (Lupatini et al., 2017). A field study on sweet corn, *Zea mays* L. (Poales: Poaceae), in Spain examined the connection between soil microbial community structure and function when treated with organic and conventional fertilizers. They found that integrating organic fertilizers stimulated microbial growth, increased enzyme activity, and changed the structure of the soil microbial community while maintaining crop yield and nutrient supply at similar levels to the inorganic fertilizer (Lazcano et al., 2013). This study was short term and took place over a single growing season in soils that already had high soil organic content. Their results show how small changes in cropping methods, like changing 25% of the fertilizer applied to organic, can have a large impact on the soil microbial community structure. They were unable

to see any large change in the ecosystem function, which they attributed to the short length of the study run time (Lazcano et al., 2013).

There are several known mechanisms by which certain soil-dwelling bacteria can achieve their plant growth promoting and disease suppressing properties. These effects typically occur through induction of a systemic resistance in the plant or a local antagonism to the soil-borne pathogen itself. Some soil bacteria have been seen to produce substances that directly suppress pathogens including antibiotics and siderophores (Beneduzi et al., 2012; Inderbitzen et al., 2018). Beyond these direct mechanisms there are also several ecological benefits of increased microbial diversity including the increase in plant nutrients making crops more resilient and successful competition for resources by beneficial microorganisms (Barbera et al., 2013; Chaparro et al., 2012). Changes in soil microbial communities directly impact crop health and yield, thus effectively managing the soil microbiome can have economic impacts by reducing reliance on chemical fertilizers and pesticides (Chaparro et al., 2012).

2.2 Organic Soil Amendments and Disease Suppression

The threat of soil-borne fungal pathogens to agricultural output is becoming increasingly severe for several reasons. Powerful conventional tools for the management of such pathogens are being phased out due to environmental concerns, such as methyl bromide (EPA, 2018; Trillas et al., 2002). Additionally, models predict an increase in the frequency and severity of soil-borne fungal pathogens due to climate change (Manici et al., 2014). Organic soil amendments have been reported to be an effective tool for suppressing soil-borne diseases from root rots to wilts. A number of studies focusing on various agricultural waste-based products have shown a consistent trend of disease suppression through the application of organic amendments with 15 articles

published on the topic in 2018 alone. In a review article on the use of composts to suppress soil-borne diseases, Noble and Coventry (2005), found that disease suppressive effects increased with the rate of application of organic amendments in the majority of the 92 studies reviewed. Disease suppression of soil-borne pathogens with organic amendments functions through a variety of mechanisms; many of which are context dependent and poorly understood. Known mechanisms can be divided into two major categories, direct suppression by the addition of phytotoxic molecules and indirect suppression via stimulation of a more robust microbial community including microbes that are antagonistic to pathogens or through increasing plant health and inducing systemic disease resistance (Hoitink and Boehm, 1999; Mendoza et al., 2012; Noble and Coventry, 2005; Weller et al., 2002).

Suppression of root disease through the application of compost water extract (CWE) has been demonstrated in several studies. The impact of CWE on the infection rate of pea roots by *Fusarium solani* Sacc. (Hypocreales: Nectraceae) and *Phoma pinodella* L. K. Jones (Pleosporales: Didymellaceae) was measured in a laboratory study by Curlango-Rivera, et al. (2013). They inoculated the diseases and compared the root infection rates by checking for symptoms in the peas grown in cellophane growth pouches. One variety, “Little Marvel” peas inoculated with *F. solani* achieved 100% suppression with the CWE treatment compared to a 93% infection rate without the CWE treatment (Curlango-Rivera, et al. 2013). The results were similar for the “Alaska” Pea trials run inoculated with both *F. solani* and *P. pinodella* where suppression was 90% or higher for both compared to over 98% infection rates following inoculation without treatment (Curlango-Rivera et al., 2013).

Verticillium dahliae Kleb. (Hypocreales: Plectosphaerellaceae) is a soil-borne fungal pathogen with a wide host range of over 150 economically important crops (McCain et al.,

1981). Inderbitzen et al. (2018) observed significant suppression of *V. dahliae* after the application of both broccoli residue and crab meal-based amendments. They attributed the observed suppression to a significant increase in the abundance of known antagonists of filamentous fungal plant pathogens within the soil microbial community after the application of organic amendments. This is consistent with the results observed in other studies tying the application of organic amendments to an increase in soil bacterial activity and diversity in turn suppressing fungal plant pathogens (Beneduzi et al., 2012; Hoitink and Boehm, 1999; Noble and Coventry, 2004). De Corato et al. (2016) reviewed the use of several waste-based organic amendments on suppression of seven pathogens, including *V. dahliae*, and found significant suppression of each of the pathogens with different amendments. Organic amendments have shown promise in the management of fungal diseases, but more research is required to thoroughly understand the context-specific mechanisms of suppression in order to make informed management decisions.

2.3 Grape and Olive Pomace-Based Organic Soil Amendments

Grape and olive pomaces are the products of post-harvest processing in wine and olive oil production. Grape pomace, also referred to as grape marc, is the skin, pulp, stalks, and seeds of grapes after wine production (Carmona et al., 2012; Mendoza et al., 2012). Olive pomace is the pulp, seeds, and skin of olives left after the production of olive oil (Albuquerque et al., 2004). Grape and olive pomace based organic amendments have shown potential for disease suppression (Noble and Coventry, 2004; Ntougias et al., 2008; Yangui et al., 2010). These products are an ideal candidate for research and future use because of their abundance and availability as waste products of the wine and olive oil industries in California. California is the

top grape and olive producing state in the USA with over 30,000 acres of olives and over 560,000 acres of wine grapes (Lazicki and Geisseler, 2016; USDA, 2018). Composted organic pomaces can also be rich in nutrients and antimicrobial compounds like polyphenols (Barbera et al., 2013). Solid grape waste before composting is particularly high in K and Fe among other nutrients and micronutrients crucial to plant growth and vigor (Alburquerque et al., 2004).

Ntougias, et al. (2007) examined infection rates of tomato plants, (*Solanum lycopersicum* L.), by *Phytophthora nicotianae* Breda de Haan (Peronosporales: Peronosporaceae) and *Fusarium oxysporum* Schlecht (Hypocreales: Nectriaceae) two major soil-borne pathogens, under olive and grape compost treatments. Tomatoes were inoculated with each of the pathogens and subsequently grown in different composts derived from olive leaves, olive mill wastewater, olive press cake, and grape marc (Ntougias et al., 2008). The effects of composting time were explored by treating inoculated tomatoes with amendments immediately after composting and after 9 months of storage. The authors observed that all of the organic composts tested suppressed the pathogens examined. *Phytophthora nicotianae* was particularly sensitive to the organic amendment applications and suppression was seen consistently in each of the compost mixes and with the two compost storage times compared to the control. *Fusarium oxysporum* disease incidence was suppressed by all compost mixes, but the effect was not significant with the 9 month storage time for olive leaves and olive press cake (Ntougias et al., 2007).

Compared to control of no compost, application of composted olive and grape waste products was observed to stimulate the soil microbial community and increase diversity as measured by a Shannon Index that analyzes 16S rRNA genes and using denaturing gradient gel electrophoresis (Ntougias et al., 2008; Vivas et al., 2009). The activity of enzymes was

monitored and in addition to distinctly different community makeup, increased overall microbial activity was observed through the process of composting olive waste compared to a manure waste (Vivas et al., 2009). Application of olive mill waste and wastewater has also been seen to benefit soil structure by increasing porosity and aggregate stability; similar to many other organic soil amendments (Barbera et al., 2013).

2.4 Organic Soil Amendments and Weed Suppression

Weeds exact a large toll on crop yield, and weed management strategies available to organic growers can be limited (Smith et al., 2000). Organic systems commonly employ a combination of techniques, including cover cropping, hand weeding, cultivation, flammers, sterilization and mulches, to maximize both weed control and crop yield while remaining economically sustainable without the use of synthetic chemicals (Smith et al., 2000). There is also a push for alternative weed management strategies with concerns over ever increasing incidence of herbicide resistance (Heap, 2014; Holt, 1990). Many organic amendments contain phytotoxic molecules that may inhibit weed seed germination or suppress young growth (Cayuela et al., 2008; Ferrara et al., 2008; Ibrahim and Balah, 2018).

An experiment on Cal Poly campus during the summer of 2018 tested the effects of several organic amendments for their suppressive effects on weed germination and biomass. In a pot study, soil from the Cal Poly Organic Farm was amended with composts derived from plant residue, dairy manure, and olive waste. Significant suppression of purslane, *Portulaca oleracea* L. (Caryophyllales: Portulacaceae) biomass was observed after the application of the dairy manure, plant residue, and the olive-dairy manure mix treatments compared to the control with

no compost application. Significant suppression of weed abundance and total weed biomass was observed after plant residue treatment compared to the control (Mondragon et al., 2018).

Olive mill waste water extracts have been shown to suppress weed biomass as well as fungal pathogens and nematodes in lab bioassays due to their high content of phytotoxic polyphenols (Boz et al., 2009; Cayuela et al., 2008). In the field, the use of olive waste as a mulch in vineyards was observed to suppress weed emergence, alter weed composition, as well as increase soil potassium (K) and magnesium (Mg) (Ferrara et al., 2015). Organic amendments were consistently observed to alter weed flora composition and suppress certain weed species germination (Cayuela et al., 2008; Ferrara et al., 2015; Mondragon et al., 2018). However, the use of olive waste in vegetable cropping systems requires more exploration as the same phytotoxic effects observed on some weed species may also cause damage and yield loss to the crop.

Animal waste-based compost sources have also shown potential for organic weed management. In an apple orchard, poultry manure-based compost significantly reduced total weed ground cover for up to a year after application (Brown et al., 2004). The most abundant weeds reported were *Sorghum halepense* L. (Poales: Poaceae), *Phytolacca americana* L. (Caryophyllales: Phytolaccaceae), *Ailanthus altissima* Swingle (Sapindales: Simaroubaceae), and *Toxicodendron radicans* L. (Sapindales: Anacardiaceae). The authors did not determine the mechanism of weed suppression but hypothesized the physical ground cover of the compost played a role. There also was a significantly reduced incidence of the brown rot fungus, *Monilinia fructicola* Honey (Helotiales: Sclerotiniaceae) and two insect pests, the spotted tentiform leafminer, *Phyllonorycter blancardella* Fabricius (Lepidoptera: Gracillariidae) and woolly apple aphid, *Eriosoma lanigerum* Hausmann (Hemiptera: Sternorrhyncha) in compost

treated plots (Brown et al., 2004). However, in another study using a dairy manure-based compost that was incorporated in the soil resulted in an increase in weed biomass (Wedryk et al., 2012). The compost application also significantly increased soil fertility, including available K, Ca, Mg, and organic matter, and increased the tomato harvest (Wedryk et al., 2012).

Compost tea, extract made from compost and water mixtures, was tested for its efficacy as a bio-pesticide. In a petri dish experiment, germination was suppressed in both purslane and maize seeds after the application of compost tea (Ibrahim et al., 2018). Compost tea has been observed in field application to reduce weed biomass compared to no treatment, hand hoeing, and mechanical cultivation in vegetable fields though the mechanism of suppression remains to be explored (Zinati, 2017).

2.5 Organic Soil Amendments and Soil Arthropod Communities

Soil arthropods have a wide variety of ecological roles in an agricultural system including fungivores and detritivores that help with nutrient cycling and herbivorous pest species that feed on roots and seeds (Bedano et al., 2006; Goncalves and Pereira, 2012; Singh et al., 2012). Soil arthropods may also function to help physically distribute soil microbes by carrying spores and hyphal fragments through the soil often into direct contact with roots; this may be especially important in the plant-microbe relations of mycorrhizal fungi and plant pathogens (Anslan et al., 2018).

Springtails (Entognatha: Collembola) are one of the most abundant soil dwelling arthropods (Anslan et al., 2018; Bedano et al., 2006). They are a diverse group mostly made up of opportunistic omnivores (Rusek, 1998). They play a fundamental role in soil nutrient cycling as microbial grazers and detritivores (Ferlian et al., 2015; Ponge et al., 2003). Springtails have

demonstrated a significant effect on fungal community structure through grazing and physical distribution of fungal hyphae through the soil (Anslan et al., 2018). Additionally, springtails have been associated with fluctuations in other meso and macrofauna communities including earthworms and centipedes (Gao et al., 2017).

Springtails are sensitive to a wide range of environmental changes; springtail diversity and abundance fluctuate with changes in soil temperature, nutrient levels, organic matter, site, and time (Abhilasha and Kumar, 2015). As with many taxa of soil fauna, temperature and moisture appear to be the most significant external influencers of springtail community structure (Machado et al., 2019); this leads to a significant amount of seasonal variation (Ford, 1937; Muturi et al., 2010).

Soil arthropod communities are highly sensitive to soil physical and chemical characteristics that may be altered by the application of organic soil amendments. The direct effects of organic soil amendments are not well understood, but certain amendments show potential for use to stimulate beneficial soil arthropods as well as potentially suppress pest species.

Chamberlain et al. (2006) observed a significant increase in springtail abundance following the application of organic alder waste. Springtail activity in turn resulted in an increase in litter derived carbon available to soil microbes. Manure application has demonstrated similar positive effects on springtail abundance while the application of inorganic fertilizers can have a negative effect by increasing crop health, in turn increasing water uptake. The resultant drying of the soil can negatively impact springtail abundance (Muturi et al., 2010). Due to the significant relationship between springtail morphotypes and soil physical and chemical characteristics, including soil carbon, bulk density, pH, porosity, and moisture, it was proposed to use springtail

diversity as an indicator of soil quality (Machado et al., 2019). However, not all results are consistent, soil arthropods can be difficult to quantify and there is limited consistency in community evaluation metrics (Umble and Fisher, 2003). Some studies have seen insufficient evidence to attribute differences in soil microbial biomass due to the activity of springtails (Wang et al., 2017).

Other soil arthropods have similarly complex relationship with soil chemical and physical attributes. Garden symphylans, *Scutigera immaculata* Newp. (Symphyla: Scutigeraellidae) can be a significant agricultural pest as they feed on seeds, seedlings, and roots of many important crops (Michelbacher, 1938). Symphylans affect production of root harvested crops such as tubers and carrots the most (Michelbacher, 1938; Stirling et al., 2012). The damage they cause to the roots may also provide points of entry for soil-borne pathogens (Bryant, 2004). Stirling et al. (2012) observed an increase in symphylans after the application of poultry manure. Though this increase was not significant, it is consistent with other studies where an impact of organic matter, fertilization, and crop choice influenced symphylan abundance (Bedano et al., 2006; Peachey et al., 2002). The application of composted poultry waste was shown to significantly suppress other arthropod pests including spotted tentiform leaf miner and woolly apple aphid (Brown and Tworowski, 2004).

CHAPTER 3

Materials and Methods

3.1 General Field Characteristics

Studies were conducted during the summer growing season of 2018 from May to November, on the California Polytechnic State University campus in San Luis Obispo, CA (35°18'15"N, 120°40'21"W). Two fields, 0.5 km apart, were divided into 25 plots each (7.5m x 3m), for a total of 50 plots (maps in Appendix A and B). Field 1 was located within the CCOF certified Cal Poly Organic Farm (county field identification number 090034) and field 2, located within the conventionally managed Cal Poly farm (county field identification number 090025). Previous crops in the organic field, field 1, include vegetable row crops such as kale (*Brassica oleracea sabellica* L.), broccoli (*Brassica oleracea italica* L.), and cauliflower (*Brassica oleracea botrytis* L.). Previous crops in the conventional field, field 2, include pumpkins (*Cucurbita pepo pepo* L.) and broccoli. Throughout the duration of the experiment both fields were managed organically. The soil is classified as clay loam with 45% sand, 23% silt and 32% clay. A summary of soil chemical properties from both fields is included in Table 1. A summary of weather data for the summer season of 2018 and 30-year averages are provided in Appendix G.

Table 1: Soil Chemical Properties of Each Field (n = 3).

	Organic Field	Conventional Field
Organic Matter (g kg ⁻¹)	37.0 ± 3.4	29.7 ± 2
Olsen P (mg kg ⁻¹)	75.3 ± 7.4	65.3 ± 3.8
K (mg kg ⁻¹)	509 ± 88.2	565 ± 56.6
Mg (mg kg ⁻¹)	960 ± 82.1	881 ± 33.8
Ca (mg kg ⁻¹)	2057 ± 199	2979 ± 103
Na (mg kg ⁻¹)	51.0 ± 8.5	49.6 ± 2.5
pH	7.23 ± 0.1	7.67 ± 0.1
C.E.C. (meq/100 g)	19.7 ± 1.9	23.7 ± 0.5
N (NO ₃ -N) (mg kg ⁻¹)	18.7 ± 2.5	4.7 ± 1.5
S (SO ₄ -S) (mg kg ⁻¹)	9.3 ± 4.4	12.0 ± 1.7
Zn (mg kg ⁻¹)	3.23 ± 0.21	1.60 ± 0.10
Mn (mg kg ⁻¹)	3.00 ± 1.00	6.33 ± 2.08
Fe (mg kg ⁻¹)	18.0 ± 1.0	8.67 ± 0.6
Cu (mg kg ⁻¹)	3.60 ± 0.10	1.50 ± 0.10
B (mg kg ⁻¹)	0.63 ± 0.06	0.70 ± 0.10
Soluble Salts (dS m ⁻¹)	0.53 ± 0.40	0.60 ± 0.10

Values represent means ± standard deviation.

3.2 Organic Amendment Sources

The organic amendment treatments applied were: dairy manure compost, olive pomace-based compost, grape pomace-based compost, plant waste compost, and a control with no compost treatment. The dairy manure compost consisted of mainly dairy manure mixed with horse manure and wood chips to achieve target C:N ratio, around 25, and pathogen reduction goals. Olive pomace was provided by La Panza Ranch in the San Juan Creek Valley, CA. The olive pomace was mixed (1:1 vol.) with dairy manure and windrow composted from December 2017 to application in June 2018 on the Cal Poly campus. Composts were processed at the California Polytechnic State University Composting Unit according to California Department of

Food and Agriculture and organic certification guidelines. The grape pomace-based compost was provided by the Cagliero Ranch in Paso Robles, California; they compost on site with windrow composting. The plant waste compost was produced from a mixture of plant waste materials including strawberry crowns, grape pomace, general green and vegetable waste, and it was provided by Engel and Gray, Inc. in Santa Maria, CA. Compost samples were analyzed by Control Laboratories (Watsonville, CA); a summary of characterization is given in Table 1, and complete results are included in Appendix F.

Table 2: Characterization of Organic Amendment Treatments

	Total N (%)	Total P (mg/kg)	Total K (mg/kg)	Electrical Conductivity (dS/m)	pH value	C/N ratio	Organic matter (%)
Dairy manure compost	1.4	5700	14000	3.6	8.40	10.7	30.7
Grape pomace compost	1.7	2500	14000	1.4	8.20	14.7	44.6
Olive pomace compost	1.7	4000	12000	2.6	8.71	16.5	47.6
Plant waste compost	1.4	3900	11000	4.4	8.10	12.1	35.9

3.3 Experimental Design and Field Setup

A completely randomized design with five replicates was laid out in each field (map in Appendix B). Each compost was applied at the rate of 25 tonnes dry matter per hectare, based upon recommendations in a review article of composts and fungal disease suppression (Bonanomi et al., 2007); and spread within its assigned plots on 12 June 2018. Then, each plot was fertilized with a pre-planting feather meal fertilizer (13-0-0) at the rate of 225 kg N per hectare. After compost and fertilizer application to the plots the fields were disked to a depth of 10 cm to incorporate the compost and prepare the fields for planting. Then beds were shaped 1 meter apart across the field, creating three beds within each plot. On 26 June 2018 the field was planted with bell peppers, *Capsicum annuum* L. 'Huntington' (Solanales: Solanaceae), at a

spacing of 20 cm provided as transplants from Plantel Nursery in Santa Maria, CA. Bell peppers were selected because they are known to be susceptible to *Verticillium dahliae*, as are many members of Solanaceae, and California is the top bell pepper producer in the USA with acreage in excess of 28,000 (Hartz et al., 2008). All plots were watered at the same rate as per UC Cooperative Extension guidelines for peppers so that plants were not subject to water stress (Hartz et al., 2008). Transplants were irrigated with sprinklers for the first two weeks after planting for establishment. Then 5/8" high flow drip tape was laid, one line per bed, and ran 4 hours at a time twice per week through July, three times per week through August and September, and twice per week through final harvest in November.

Treatment effects on soil chemical characteristics were evaluated from composite samples collected 30 June 2018; approximately two weeks after application of compost treatment. Composite soil samples were collected from three randomly chosen plots of each treatment from each field. Composite samples were made up of three subsamples collected from random locations within the plot at a depth of 1-15 cm with a soil auger then mixed thoroughly. Random plots were chosen with a random number generator and random locations within plots were chosen by breaking plots into a grid with spacing of 10 cm and using a random number generator to choose grid blocks for sampling. Samples were sent to A & L Western Agricultural Laboratories (Modesto, Ca, USA) for chemical analysis.

3.4 *Verticillium dahliae* Field Soil Load

The abundance of the pathogenic fungus *Verticillium dahliae* was assessed, following the methods reported in Kabir et al. (2004). A composite soil sample was collected from each plot at the time points given below. Each composite sample was made up of three subsamples collected

from random locations within the plot at a depth of 1-15 cm with a soil auger then mixed thoroughly. Random locations were chosen by breaking plots into a grid with spacing of 10 cm and using a random number generator to choose which grid blocks to sample. The first sample collection occurred on 18 May 2018 before treatments were applied and prior to tillage and plant establishment. *Verticillium dahliae* pathogen loads in the fields were high and uniform enough that inoculation was not required. The second sample collection occurred two weeks after the application of the treatments on 30 June 2018. Subsequent sample collections occurred every six weeks after the treatment application; 11 August, 21 September, and 1 November 2018. On each date composite soil samples were collected and then air dried for 14 days. After drying, samples were mixed again and ground with a mortar and pestle. Five separate 0.1 gram subsamples were removed, mixed with 0.9 ml deionized water, and plated individually onto Sorenson's NP-10 selective media in 100 mm x 15 mm polystyrene petri dishes (Fisherbrand, USA). Sorenson's NP-10 is a selective media including agar and antibiotics developed specifically to grow cultures of *V. dahliae* (see Appendix C for listing of ingredients).

Once plated, the samples were cultured for two weeks in the dark at room temperature; between 19°C and 22°C. Each plate was examined under a Luxeo 4D dissecting microscope (Labomed, Los Angeles, Ca, USA) and microsclerotia or colony forming units (CFU's) of the fungi were counted as per Goud et al. (2003). As each microsclerotia in the field could begin a new colony, the count from each dish is a sample number of *V. dahliae* CFU's per 0.1 gram of soil from the sample site.

3.5 *Verticillium dahliae* In Vitro Growth

Extracts of each compost were prepared from a compost to deionized water ratio of 1:5 by volume. In a sterile 1 liter beaker, 200 cm³ of compost was mixed with 800 ml of deionized water and left at 20°C for five days. Extractions were stirred once per day for 60 seconds throughout the 5-day extraction period. Extracts were then poured through Whatman 1 filter paper (Sigma-Aldrich, Darmstadt, Germany) and run through a 0.45 µm syringe filter (Corning Inc., New York, USA) to obtain the final compost extracts used in this experiment as per Curlango-Rivera et al. (2013).

To assess the effects of the compost extracts, also referred to as compost teas, on *V. dahliae* growth, two trials were conducted. The first trial was to assess compost tea effects in a general acidified potato dextrose agar (APDA) media (see Appendix D for list of ingredients). The second trial was to assess effects in a more selective media for *V. dahliae* growth. In trial one, 20 ml of each compost extract was added to 200 ml of APDA. Each mix was then stirred for 2 minutes and poured into five petri dishes. An additional five petri dishes were filled with 200 ml APDA and 20 ml deionized water to function as the control; making a total of 25 dishes. Media solidified after 48 hours and inoculations were made with a 3-week-old colony of *V. dahliae*. Each dish was inoculated from the same colony and left in the dark at room temperature, between 19°C and 22°C, to culture. At each 48-hour interval, for 13 days, the maximum linear growth within each plate was measured. Maximum linear growth was considered as the measurement from the center site of inoculation to the furthest growth within each plate, in other words, the maximum radius of the colony as per De Corato et al. (2016). This trial faced minor issues with contamination. Despite the sterilization filtration of all the compost extracts, roughly 10% of the plates displayed growth of microbes other than *V. dahliae*. In plates with obvious

contamination, measurements were taken of *V. dahliae* when contaminant growth was greater than 5 mm separated. Plates with contaminant growth within 5 mm of *V. dahliae* colonies were discarded.

Trial two had the same experimental design but replaced the APDA with Sorenson's NP-10 selective media. Twenty ml of each compost extract was added to 200 ml of NP-10. Each mix was then stirred for 2 minutes and poured into five petri dishes. An additional five dishes were filled with a 20 ml deionized water and 200 ml NP-10 mix to act as a control. After waiting 48 hours for the solidification of these media, inoculation was made with a 6-week-old colony of *V. dahliae*. Each dish was inoculated from the same colony and left in a dark contained space at 20°C to culture. At each 48-hour interval, for 15 days, the maximum linear growth within each plate was measured. Due to the use of the selective media, no contamination issues were observed in this trial.

3.6 Soil Microbial Community Assessment

Soil samples were collected from three randomly chosen plots of each treatment from both farm sites. Randomization procedure was consistent with soil sampling for *V. dahliae* assessment. Samples were first collected on 19 May 2018 before the application of treatments. A second round of sampling occurred in the same plots on 11 October 2018. Sampling protocol was the same as for *V. dahliae* culturing. A composite sample made of three subsamples gathered using a soil auger to take a core from 1-15 cm depth. Between each sample the auger was sterilized using a 70% ethanol wipe.

Samples were all stored in a minus 18°C freezer until 28 November 2018. Each sample was then mixed thoroughly and a subsample of 250 mg soil was weighed out. Extractions were

made from each sample using the DNeasy Powersoil Kit (QIAGEN, Venlo, Netherlands) per manufacturer's instructions.

Prokaryote (Bacterial and archaeal) communities from the soil samples were amplified and sequenced by Molecular Research, LP (MR DNA, Shallowater, TX, USA). PCR was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with the 16S rRNA gene V4 variable region primers 515F and 806R. The thermal cycling consisted of 94°C for 3 minutes, 30 cycles of 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 minute, then an elongation step at 72°C for 5 minutes. An Ion Torrent PGM was used for sequencing as per manufacturer's guidelines. Data was processed by Molecular Research, LP using a proprietary analysis pipeline (MR DNA, Shallowater, TX, USA). Sequences were depleted of barcodes, primers, sequences <150bp, and sequences with ambiguous base calls and with homopolymer runs exceeding 6bp. Then they were denoised, chimeras were removed, and Operational Taxonomic Units (OTUs) were generated by clustering at 97% similarity. Those final OTUs were then classified with BLASTn using the RDPII and NCBI databases.

3.7 Weed Biomass Assessment

Weed biomass was assessed for field 1, the organic field, on 28 August 2018. After planting, the field was hand weeded for the first month until 26 July and cultivated on 17 July 2018. On 26 July 2018 a final manual weed removal of the entire field took place to ensure a cleared field baseline. From 26 July to 28 August 2018, weeds were allowed to grow uninhibited. Collection of above ground weed biomass took place through the center bed of each plot with a width of 30 cm. This made up an area of 2.25 m² or 10% of the total plot area. Within this area

all weeds were cut at soil level, identified and bagged. Weeds were divided by species and placed in paper bags and dried in an oven (ThermoFisher Scientific, USA) at 82°C for five days.

3.8 Soil Arthropod Abundance

The abundances of soil dwelling garden symphylans and collembolans were monitored within each research plot May through August 2018. Symphylans were monitored using a soil surface potato bait station as per Umble and Fisher (2003b). ‘Russet’ potatoes (*Solanum tuberosum* L.) were sliced in half and left under an upside down 18.5 cm diameter white bucket in the center of each plot. After 48 hours the bucket was gently removed and baits were flipped and photographed. Later those photos were assessed for the number of Symphyla present per bait (see Appendix E for bait station set up and Appendix H for Symphyla photo).

Collembola were monitored using a similar baiting strategy. A square foot space in the center of the plot was cleared and dampened with 100 ml of water. A 21.5 x 27.9 cm piece of acetate (Canon, Lake Success, New York, USA) was then laid on the ground with a 3 x 3 cm hole cut in the center. A fresh broccoli (*Brassica oleracea* L. *sabellica* (Brassicales: Brassicaceae)), spinach (*Spinacia oleracea* L. (Caryophyllales: Amaranthaceae)), or lettuce (*Lactuca sativa* L. (Asterales: Asteraceae)) leaf was then placed over the open hole in the sheet and an 18.5 cm diameter white bucket was placed upside down over the sheet and leaf. After 48 hours the bucket was removed, the leaf flipped over and the ground and underside of the leaf were photographed. Again, these photographs were assessed for the number of Collembola present within the 3 x 3 cm baiting area (see Appendix I for Collembola photo).

From 15 May to 15 August 2018 five baits were set every other day in each field, one plot from each treatment per field. After 48 hours these baits were photographed and reset in a

new plot of the same treatment. Baits were assessed and reset between the hours of 9 and 11am each sampling day. One sample was taken within each plot for each of the following sampling periods: 17 May through 28 May, 29 June through 10 July, and 20 August through 31 August 2018.

3.9 Bell Pepper Health and Yield Measurement

Bell pepper ‘huntington’ (*Capsicum annuum* L.) health was measured through the height and the measure of relative chlorophyll content of the plants. Height was measured from five randomly chosen plants within each plot on 2 August and 2 September 2018. Height was recorded from ground level to the apical meristem. The relative chlorophyll content of the plant was measured using a SPAD 502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan). Five random plants were chosen from each plot on 2 August and 2 September 2018. Randomization was achieved by assigning each plant within a plot a number, then using a random number generator to select plants. Chlorophyll content was measured in a randomly chosen young and fully-expanded leaf on selected plants. Random leaves were chosen by assigning the 12 youngest fully-expanded open leaves a number and using a random number generator to select one of those leaves.

Peppers were harvested on three separate dates; 3 September, 4 October, and 5 November 2018. On each harvest date a crew worked through both fields clean harvesting all ripe bell peppers. Peppers were considered ripe based on maturity, this was determined in the field based on size and development of the shoulder ridge above the calyx (Lindgren and Hodges, 1990). At the final harvest in November 2018, all fruit were collected including immature fruits to be

included in the yield biomass assessment. For analysis all harvest fresh weights from each plot were pooled into a season total harvest weight per plot.

3.10 Statistical Analyses

Statistical analyses were carried out with JMP 14 software (SAS Institute, USA). All data, except microbial sequencing data, were analyzed treating sample, plot, and field as replicated random effects and treatment and time as main effects. When no significant interaction between treatment and field was observed, data between fields was pooled for analysis. Soil *V. dahliae* CFU data was square root transformed to maximize consistency in residuals of the model. Differences in least square means were tested by ANOVA then p-values from pairwise comparisons were generated by way of students t test. Benjamini-Hochberg procedure was utilized for assessment of significance from p-values to ensure maximum statistical power and maintain type one error rate at 0.05.

Statistical analysis of microbial sequencing data was conducted with PRIMER-e software (Massey University, Auckland, New Zealand) based on the OTU counts after a square root transformation. Similarity percentage (SIMPER) analysis was conducted as a one-way S17 Bray-Curtis similarity analysis and used to create non-metric Multidimensional Scaling Plots (MDS). Analysis of similarities (ANOSIM) was run with maximum 999 permutations. Significance levels below 5% were considered significant. Analysis of differences is based on Bray-Curtis dissimilarity / standard deviation (Diss/SD).

Additional analysis was performed exclusively on genera that are known filamentous fungal plant pathogen antagonists. OTU counts were filtered at the genus level to include only known antagonists of filamentous fungal plant pathogens as per Inderbitzin et al. (2018).

CHAPTER 4

Results and Discussion

4.1 Organic Amendment Effects on Soil Chemical Properties

In the organic farm the four compost treatments had no significant effect on soil organic matter percentage, magnesium (Mg), calcium (Ca), pH, cation exchange capacity, N (NO₃-N), manganese (Mn), iron (Fe), Copper (Cu), boron (B), or soluble salts compared to control. Dairy and plant waste-based composts increased the levels of soil phosphorus (P) ($p = 0.006$), potassium (K) ($p = 0.040$), sodium (Na) ($p = 0.008$), sulfur (S) (SO₄-S) ($p = 0.029$), and zinc (Zn) ($p = 0.015$) compared to control. Grape and olive pomace-based compost treatments had no significant effect on soil P, K, Na, S, or Zn compared to control. Dairy had the largest impact on soil Na with an average of 221 mg/kg, 400% higher than in control, while plant waste-based compost had 250% higher Na levels. Plant waste-based compost had the largest effect on soil S with an average of 83 mg/kg, over 300% higher than control plots; dairy based compost had 250% more S than control.

Table 3: Effect of Each Compost Treatment on Soil Chemical Properties in the Organic Field (n = 3).

	Control	Dairy	Grape	Olive	Plant	p-value
Organic Matter (g kg ⁻¹)	39.0 ± 1.73	49.0 ± 9.54	49.3 ± 10.1	51.7 ± 7.02	53.3 ± 11.9	0.368
Olsen P (mg kg ⁻¹)	82.3 ± 6.66 A	161 ± 36.4 C	94.0 ± 8.72 AB	99.7 ± 9.29 AB	129 ± 25.9 BC	0.006*
K (mg kg ⁻¹)	613 ± 65.2 A	1094 ± 224 B	816 ± 13.8 AB	916 ± 149 AB	1124 ± 313 B	0.040*
Mg (mg kg ⁻¹)	1059 ± 30.2	1004 ± 50.1	1037 ± 32.6	1047 ± 29.0	1038 ± 26.4	0.444
Ca (mg kg ⁻¹)	2272 ± 106	2275 ± 43.4	2280 ± 113	2223 ± 62.1	2373 ± 161	0.552
Na (mg kg ⁻¹)	54.0 ± 6.08 A	221 ± 78.2 C	75.7 ± 1.53 AB	124 ± 29.0 AB	150 ± 55.4 BC	0.008*
pH	6.93 ± 0.21	7.17 ± 0.15	7.07 ± 0.12	7.07 ± 0.06	7.10 ± 0.10	0.384
C.E.C. (meq/100g)	22.2 ± 1.23	23.4 ± 0.53	22.3 ± 0.84	22.6 ± 0.90	23.9 ± 1.91	0.384
N (NO ₃ -N) (mg kg ⁻¹)	89.3 ± 22.1	123 ± 39.9	89.0 ± 17.1	81.7 ± 29.5	112 ± 41.8	0.472
S (SO ₄ -S) (mg kg ⁻¹)	26.7 ± 4.62 A	70.0 ± 22.9 BC	30.3 ± 6.03 A	35.7 ± 7.64 AB	83.0 ± 40.8 C	0.029*
Zn (mg kg ⁻¹)	3.50 ± 0.26 A	5.70 ± 0.82 BC	4.10 ± 0.26 AB	4.30 ± 0.61 AB	6.43 ± 1.72 C	0.015*
Mn (mg kg ⁻¹)	7.33 ± 4.51	10.0 ± 0.00	7.00 ± 3.46	8.33 ± 3.51	9.67 ± 1.15	0.677
Fe (mg kg ⁻¹)	18.3 ± 1.53	18.7 ± 0.58	18.3 ± 0.58	18.3 ± 2.08	20.7 ± 1.53	0.248
Cu (mg kg ⁻¹)	4.40 ± 0.46	4.43 ± 0.46	4.77 ± 0.32	4.37 ± 0.21	4.63 ± 0.51	0.713
B (mg kg ⁻¹)	0.73 ± 0.06	1.37 ± 0.38	0.87 ± 0.06	1.03 ± 0.23	1.33 ± 0.42	0.066
Soluble Salts (dS m ⁻¹)	1.70 ± 0.36	2.63 ± 0.45	1.77 ± 0.29	2.07 ± 0.81	3.23 ± 1.54	0.200

Values represent means ± standard deviation. *Means connected by the same letter within a row are not significantly different at $p \leq 0.05$.

In the conventional field the four compost treatments had no significant effect on soil organic matter percentage, P, Mg, Ca, pH, cation exchange capacity, N (NO₃-N), S, Zn, Mn, Fe, Cu, B, or soluble salts compared to control. Olive pomace-based and plant waste-based compost treatments increased soil K ($p = 0.044$) and Na ($p = 0.043$) compared to control. Additionally,

dairy waste-based compost increased soil Na compared to control. Grape pomace-based compost had no significant effect on soil K or Na. In the conventional field, dairy manure only had a significant effect on Na, which was 150% higher than control. Plant waste and olive pomace-based compost had similar effects on soil K and Na; K being 125% of control and Na over 150% of control.

Table 4: Effect of Each Compost Treatment on Soil Chemical Properties in the Conventional Field (n = 3).

	Control	Dairy	Grape	Olive	Plant	p-value
Organic Matter (g kg ⁻¹)	31.0 ± 5.29	33.3 ± 2.08	37.0 ± 5.20	46.7 ± 11.6	38.0 ± 7.81	0.153
Olsen P (mg kg ⁻¹)	63.0 ± 5.20	82.7 ± 12.4	71.3 ± 4.16	80.3 ± 11.9	80.3 ± 7.77	0.106
K (mg kg ⁻¹)	520 ± 35.0 A	556 ± 22.8 A	619 ± 76.4 AB	668 ± 88.8 B	672 ± 57.6 B	0.044*
Mg (mg kg ⁻¹)	852 ± 143	874 ± 49.7	962 ± 63.5	882 ± 128	910 ± 62.9	0.692
Ca (mg kg ⁻¹)	3049 ± 136	2652 ± 333	2949 ± 96.8	2790 ± 41.2	2982 ± 116	0.107
Na (mg kg ⁻¹)	57.0 ± 6.56 A	96.3 ± 27.0 B	73.3 ± 7.57 AB	99.7 ± 13.3 B	87.3 ± 16.0 B	0.043*
pH	7.50 ± 0.15	7.53 ± 0.12	7.43 ± 0.12	7.47 ± 0.15	7.63 ± 0.15	0.493
C.E.C. (meq/100g)	23.8 ± 0.56	22.3 ± 1.86	24.5 ± 0.36	23.3 ± 0.61	24.5 ± 0.47	0.082
N (NO ₃ -N) (mg kg ⁻¹)	42.3 ± 24.5	28.7 ± 27.7	52.0 ± 21.0	44.3 ± 11.5	45.0 ± 23.6	0.779
S (SO ₄ -S) (mg kg ⁻¹)	31.0 ± 7.81	21.7 ± 11.6	27.3 ± 6.43	30.7 ± 2.52	32.0 ± 8.54	0.528
Zn (mg kg ⁻¹)	1.60 ± 0.10	1.97 ± 0.45	2.10 ± 0.20	2.33 ± 0.67	2.60 ± 0.61	0.170
Mn (mg kg ⁻¹)	3.00 ± 1.53	6.33 ± 1.15	6.33 ± 3.21	5.67 ± 1.53	4.00 ± 1.00	0.247
Fe (mg kg ⁻¹)	8.00 ± 1.00	9.67 ± 0.58	9.33 ± 1.15	8.00 ± 0.00	10.0 ± 1.73	0.124
Cu (mg kg ⁻¹)	1.60 ± 0.06	1.63 ± 0.25	1.73 ± 0.12	1.73 ± 0.12	1.70 ± 0.17	0.864
B (mg kg ⁻¹)	0.70 ± 0.12	0.77 ± 0.15	0.80 ± 0.00	0.97 ± 0.31	0.93 ± 0.15	0.428
Soluble Salts (dS m ⁻¹)	1.10 ± 0.30	0.90 ± 0.44	1.20 ± 0.26	1.20 ± 0.20	1.17 ± 0.35	0.761

Values represent means ± standard deviation. *Means connected by the same letter within a row are not significantly different at $p \leq 0.05$.

4.2 Organic Amendment Effects on Soil *Verticillium dahliae* Abundance

Soil *V. dahliae* abundances expressed in CFU's/g soil, demonstrated clear trends both across treatments and through time. The initial samplings for *V. dahliae* CFU's/g soil were taken in May before any field preparation or treatments. At this time, all of the treatment-assigned plots had significantly similar pathogen loads (treatment: $p = 0.063$) with a mean of 52.4 ± 29.6 CFU/g soil ($n = 250$; $0 - 220$). Thus, there was a similar pathogen load before treatment application throughout the treatment blocks, and CFUs well above the threshold for many plants to become infected (Bolda and Koike, 2013). This pathogen load is considered sufficient to cause disease in certain crops such as strawberries, which are particularly susceptible and will begin to show symptoms with as little as 3 CFU per gram of soil (Bolda and Koike, 2013). Bell peppers are less susceptible, thus requiring a higher field pathogen load than strawberries to become infected (Bhat et al., 2003; McCain et al., 1981). There was also no significant difference between the pathogen load of the two fields tested (field: $p = 0.141$). Additionally, there was no significant interaction between any of the amendment treatments and the field site (i.e., conventional vs. organic) (treatment * field: $p = 0.477$). These factors allowed analysis to continue with fields as a random effect and replicate in the design, all further analysis of *V. dahliae* was performed by pooling the data from both fields.

By treating date as a fixed effect there was a consistent pattern in *V. dahliae* pathogen load through the season with no two sequential sampling dates being equivalent (time: $p < 0.0001$). The first sampling, on 18 May 2018, had the highest mean pathogen load at 52.43 ± 29.61 CFU/g soil ($n = 250$; $0 - 22$) (Table 3). By the second sampling on 30 June 2018, pathogen loads had dropped to 21.08 ± 18.12 CFU/g soil ($n = 250$; $0 - 90$). This drop was expected due to several soil disturbance events in early June: the four organic amendment treatments were

applied and the fields were both disked, the beds were shaped and planted. All of these practices cause major disruption to soil microbes, particularly fungi, as hyphae are susceptible to such mechanical disturbances (Degruene et al., 2017). Thus, it is expected to see a significant drop in *V. dahliae* soil load between these times. Though there was a universal pathogen load decrease between May and June, it was also clear that all organic amendment treated plots had a greater reduction in pathogen load when compared to untreated control plots (Table 2). All four amendment treatments displayed a significant reductive effect in June, two weeks after the application of treatment (treatment: $p = 0.001$). Grape, olive, and plant-based amendments had the largest reduction in soil *V. dahliae* with insufficient evidence to differentiate between them. Mean observed *V. dahliae* CFU/g soil within grape, olive, and plant-based amendment treated plots were 14.6 ± 14.5 ($n = 50$; $0 - 70$), 16.8 ± 15.9 ($n = 50$; $0 - 50$), and 14 ± 12.7 ($n = 50$; $0 - 50$), respectively; compared to the control at 34.4 ± 17.5 ($n = 50$; $0 - 70$) CFU/g soil. The dairy manure-based amendment had less pathogen load reduction than the other three amendment treatments at 25.6 ± 20.4 CFU/g soil ($n = 50$; $0 - 90$) but *V. dahliae* CFU's were still significantly reduced compared to the control ($p = 0.028$).

Field pathogen load increased in the third sampling on 11 August 2018 compared to June, with mean *V. dahliae* at 34.88 ± 25.1 CFU/g soil ($n = 250$; $0 - 130$). This sampling date was two months after the application of treatment and well into the growing season only three weeks before first harvest. The significant effect of the grape, olive, and plant-based amendments on reducing soil pathogen load, compared to control plots at 53.6 ± 28.9 CFU/g soil ($n = 50$; $10 - 130$), remained through this sampling ($p < 0.01$). Mean observed *V. dahliae* CFU/g soil for grape, olive, and plant-based amendment treatments were 29.8 ± 18.5 ($n = 50$; $0 - 80$), 23.8 ± 24.2 ($n = 50$; $0 - 120$), and 24.6 ± 17.2 ($n = 50$; $0 - 70$), respectively. The effect of the dairy

manure-based amendment was no longer significant compared to control at 42.6 ± 21.8 CFU/g soil ($n = 50$; 10 – 130) (treatment: $p = 0.141$).

By the fourth sampling date, 21 September 2018, overall field pathogen loads were nearly returned to pretreatment levels from May with an overall mean of 45.44 ± 27.0 CFU/g soil ($n = 250$; 0 – 160). There were no significant differences in the *V. dahliae* soil pathogen load between any treatments ($p = 0.443$). This was the first sampling date with no reduction of soil pathogen load observed by any treatment. Thus, reduction in *V. dahliae* CFU's from applied compost treatments was measurable only up to eight weeks post-application. At the final sampling date, 1 November 2018, overall *V. dahliae* pathogen loads dropped down to 29.64 ± 18.6 CFU/g soil ($n = 250$; 0 – 120) with no significant differences between any treatments on this date ($p = 0.194$).

Table 5: Least Square Mean <i>Verticillium dahliae</i> Colony Forming Units/g Soil for Each Treatment over Time.					
	Control	Dairy	Grape	Olive	Plant
18 May (NS)	70.36	63.66	68.50	71.89	73.74
30 June	56.26 A	45.22 B	31.05 C	33.58 C	29.97 C
11 August	70.60 A	63.26 A	50.47 B	41.26 B	45.47 B
21 September (NS)	61.75	63.40	68.91	66.66	58.60
1 November (NS)	57.40	45.79	50.89	51.91	47.25
Connecting letters represent significant differences at each sampling date with $\alpha = 0.05$ (NS) at sampling date there were no significant differences with $\alpha = 0.05$					

The reduction in *V. dahliae* soil pathogen load observed after the application of these amendments could be due to a variety of mechanisms (Noble and Coventry, 2004). Reduction of

V. dahliae microsclerotia has been observed after the application of many organic amendments including plant residue, crab meal, cork, potato, spent mushroom, and olive waste-based composts (Barbera et al., 2013; Inderbitzen et al., 2017; Noble and Coventry, 2004). Additionally, suppression of other soil-borne fungal pathogens has been observed consistently after organic amendment application (Beneduzi et al., 2012; Curlango-Rivera et al., 2013; Ntougias et al., 2007). Thus, the observed reduction is consistent within the larger body of literature exploring organic amendments and disease suppression.

Olive and grape waste-based composts have been the focus of research due to their availability and known polyphenolic content. Significant suppression of a variety of soil-borne pathogens has been observed including *P. nicotianae*, *F. oxysporum*, *P. pinodella*, *F. solani*, and *V. dahliae* (Curlango-Rivera et al., 2013; Ntougias et al., 2007; Yangui et al., 2010). Dairy manure-based composts have also been shown to have suppressive qualities on soil-borne fungal pathogens (Hoitink and Boehm, 1999; St. Martin, 2014). The reduction in *V. dahliae* soil pathogen load observed in this study is consistent with the suppressive effects observed in other studies. The duration and extent of suppression depends on the specific amendment source, pathogen, and field management context.

Suppressive mechanisms in previous studies have often been difficult to identify. Most studies point to either chemical suppression via introduction of toxic compounds, induced systemic resistance, or biological suppression from stimulation of the microbial community including fungal antagonistic genera (Inderbitzen et al., 2017; Noble and Coventry, 2004).

4.3 Organic Amendment Extracts and *Verticillium dahliae* In Vitro Growth

An *in vitro* laboratory experiment was designed to replicate the field study design in a controlled setting away from field soil microbial communities. In trial 1, clear trends of organic amendments inhibiting *V. dahliae* growth mirroring field results were observed (Fig. 1). There was no significant difference between control *V. dahliae in vitro* growth and dairy extract-treated *V. dahliae in vitro* growth ($p = 0.902$) with mean maximum linear growth of 32.32 ± 4.09 mm ($n = 5$; 27.89 – 38.58) and 27.58 ± 3.07 mm ($n = 5$; 23.75 – 31.39) respectively after 11 days. The three other organic amendment extracts showed significant reduction ($p < 0.001$) of the *V. dahliae in vitro* growth from day 3 through day 11 with mean maximum linear growth of grape compost extract at 19.69 ± 2.47 mm ($n = 5$; 16.2 – 21.59), olive compost extract at 16.78 ± 4.24 mm ($n = 5$; 12.4 – 23.67), and plant compost extract at 16.16 ± 5.47 mm ($n = 5$; 9.04 – 23.23) at day 11. However, contamination with other microbes was observed in 12 of the 25 total plates in this trial. The effects of these contaminants were unclear so the experiment was replicated on a selective media.

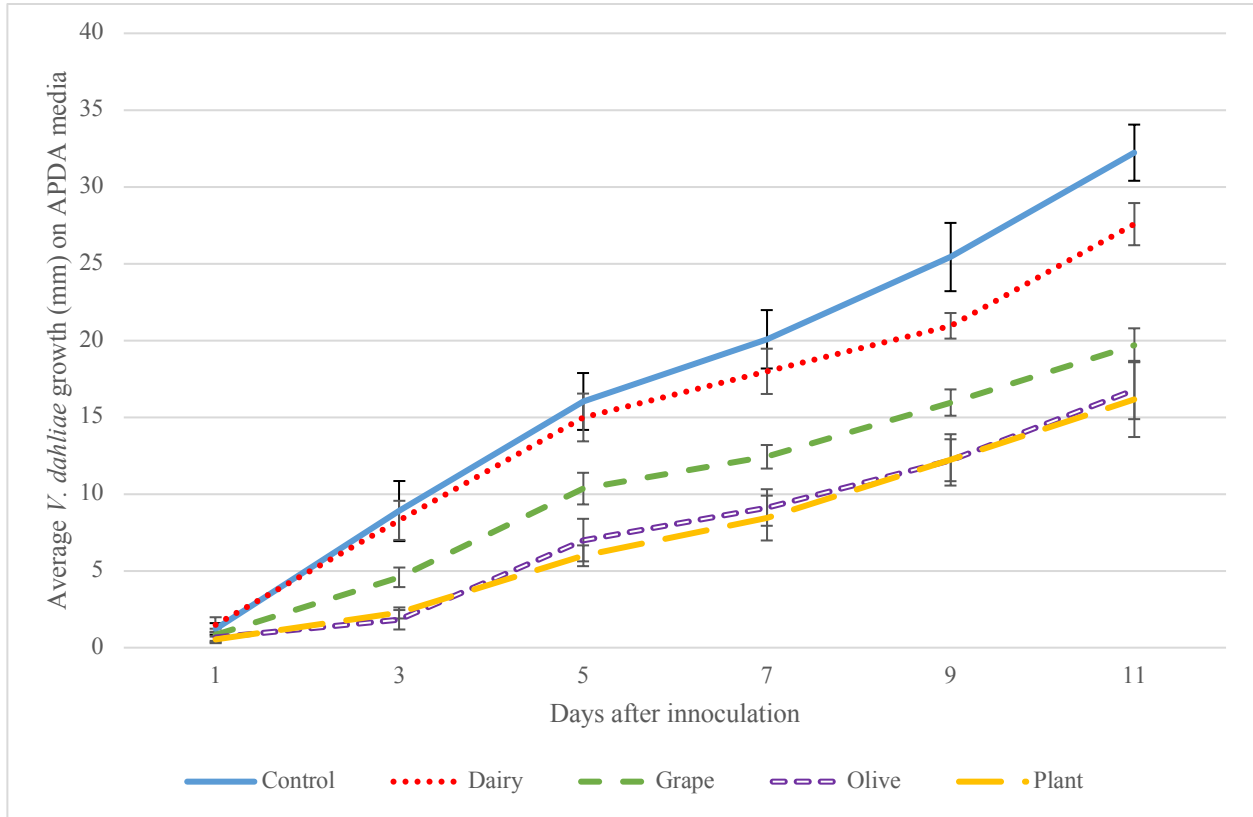


Fig. 1. Mean *V. dahliae* Linear Growth *In Vitro* on APDA Media as Affected by Compost Treatments over Time. Error Bars Represent One Standard Error of the Mean.

Trial 2 used the selective media Sorenson’s NP-10 and demonstrated similar results that supported the field data. After 15 days of incubation all *V. dahliae* colonies in control plates had grown to the edge of the petri dish with a 40 ± 0 mm radius ($n = 5$; $40 - 40$). The dairy extract-treated colonies had an average maximum linear growth of 36.18 ± 4.28 mm ($n = 5$; $29.75 - 40$), not significantly different from the control ($p = 0.288$). Grape, olive and plant-based extracts all demonstrated a significant reduction of *V. dahliae in vitro* growth compared to the control from day 3 through day 15 with average maximum linear growth of 29.71 ± 7.96 mm ($n = 5$; $21.37 - 40$), 30.29 ± 6.38 mm ($n = 5$; $23.25 - 40$), and 29.50 ± 5.55 mm ($n = 5$; $24.61 - 38.23$), respectively, at day 15 ($p = 0.022$). These results are consistent with the findings of our field trial and reduction of soil pathogen load.

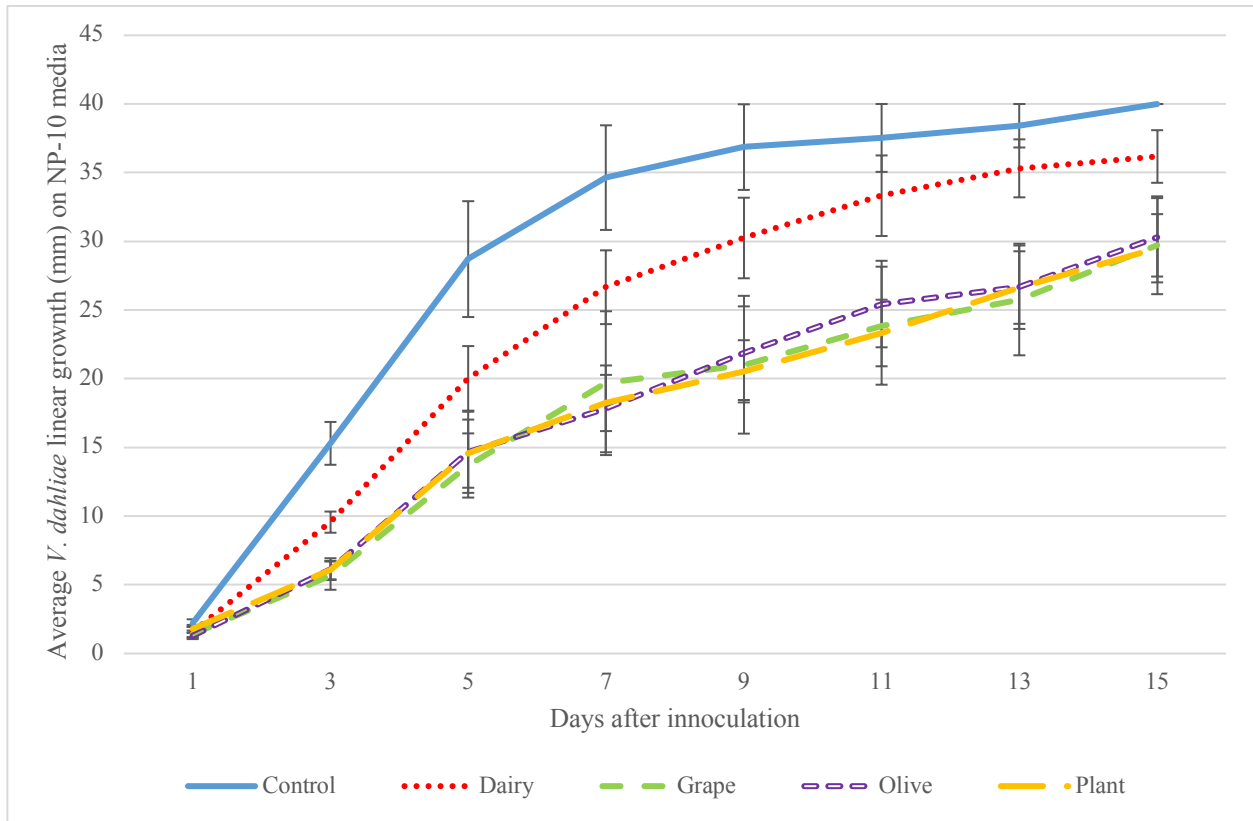


Fig. 2. Mean *V. dahliae* Linear Growth *In Vitro* on NP-10 Media by Treatment over Time. Error Bars Represent One Standard Error of the Mean.

The results of the laboratory study helped illuminate one mechanism of action by which the organic amendments were reducing the *V. dahliae* pathogen load. In a petri dish experiment where there were no other microorganisms present, the reduction demonstrated was likely due to direct chemical suppression from dissolved compost materials. As previously discussed, grape and olive wastes are known to be high in polyphenolic molecules that have already demonstrated toxic effects (Barbera et al., 2013). These results also indicated that some amount of the field reduction was due to chemicals within the amendments. In the field, other factors may have been at play such as stimulation of beneficial microbial communities helping to compete with or directly antagonize *V. dahliae* and keep their populations lower (Noble and Coventry, 2004).

4.4 Soil Microbial Community Assessment

Analysis of prokaryote communities was conducted to further explore the effects of treatments on the soil microbiome. The diversity and species composition of the soil microbial community has been linked to suppression of soil-borne pathogens (Benduzi et al., 2012; Chaparro et al., 2012; Hoitink and Boehm, 1999; Inderbitzen et al., 2018). The analysis included in this paper is preliminary; differences were assessed at the phyla level but exact nature of those differences is not evaluated. Further investigation of the data is necessary to help illuminate the makeup of compositional differences in the microbial communities between farms and treatment groups. Bray-Curtis analysis of dissimilarity was conducted to evaluate the structural diversity of the microbial community by comparing the number of species in common and those not shared between samples (Buttigieg and Ramette, 2014). Samples collected on 19 May 2018, before the application of treatments, displayed significant differences in the soil bacterial communities at the Phylum level between the organic and conventional field sites ($p = 0.019$). This is consistent with other research where the microbial community structure has been observed to be different between organic and conventionally managed soils (Lupatini et al., 2017). There were no significant differences in the bacterial communities at the Phyla level between any treatment assigned plots before treatment application ($p = 0.111$).

The next samples were collected on 11 October 2018, four months after the application of treatments. The bacterial community structure at the Phyla level between fields remained significantly different ($p = 0.006$). Based on ANOSIM, there was no significant effect of treatment in the conventional field on the species composition of microbial communities at the Phylum level ($p = 0.983$). However, in the organic field, the treatments did have a significant effect on the bacterial community structure at the Phylum level ($p = 0.026$). The microbial

community of dairy manure compost treated plots differed from the control strongly, with a Bray-Curtis dissimilarity of 10.63. The microbial community of plant waste compost treated plots varied strongly from control, with a Bray-Curtis dissimilarity of 12.36. The microbial communities of grape pomace compost treated plots varied from control with a Bray-Curtis dissimilarity of 10.68. Olive pomace compost treated plots microbial communities were dissimilar from control plots with a Bray-Curtis dissimilarity of 8.40. These differences demonstrate that within the organic farm each of the four treatments were having a significant effect on the microbial community structure when compared with the control plots. Further analysis of the data is required to explore in what ways the microbial communities are being affected. This preliminary analysis demonstrates that all four organic amendment treatments tested had a significant effect on the microbial community structure as is consistent with previous studies (Chaparro et al., 2012; De Corato et al., 2016; Vivas et al., 2008).

No significant impact was observed on the Shannon Diversity Index at the Phylum level in either field ($p = 0.831$) (Figure 3). The Shannon Diversity Index is based upon entropy models and can be used as a tool for quantifying ecological diversity by assessing both the species richness and relative abundances (Shannon, 1948). Despite treatments having a significant effect on the composition of bacterial communities, the overall diversity was not significantly affected.

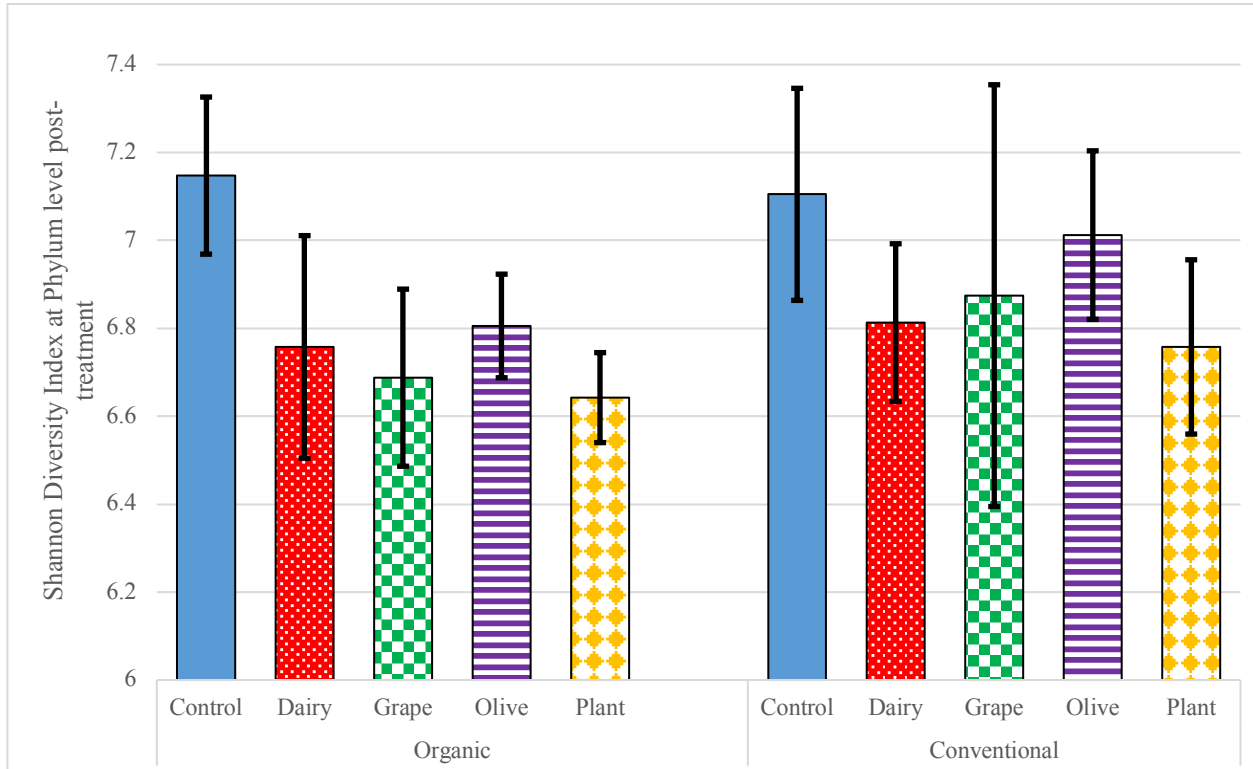


Fig. 3. Shannon Diversity Index at Phylum Level Post-Treatment by Treatment and Field. Error Bars Represent One Standard Error of the Mean.

After determining significant differences in microbial community structure with ANOSIM, SIMPER analysis was utilized to evaluate how closely related microbial communities were between treatment groups (Wakefield et al., 2013). Clustering of communities, or the relative similarity of the microbial communities between treated plots compared to overall field similarity, was seen with some treatments having a stronger effect than others (Figure 4). Within the organic farm, post-treatment similarities within plant, dairy, olive, and grape plots were 95.1%, 92.9%, 94.8%, and 90.3%, respectively. Compared to the control plots in the organic field with similarity of 92.96%. In the conventional field, similarities were less tightly clustered by treatment. Similarity between control plots was 88.4%. Similarities within plant, dairy, olive, and grape plots were 85.1%, 86.5%, 91.4%, and 83.6%, respectively. This is consistent with

observations by Lupatini et al. (2017) that organic systems and conventional systems have different soil microbial community structures. Again, further analysis is required to explore the differences observed between the farms in this study.

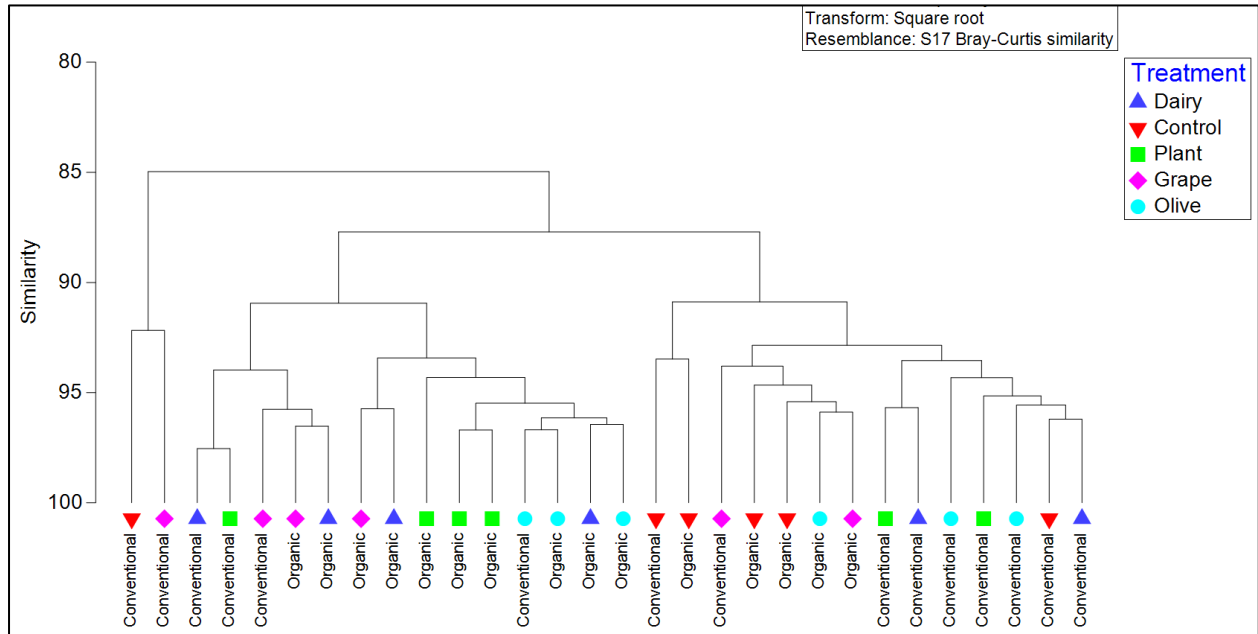


Fig. 4. Post-Treatment Bray-Curtis Similarity Clustering of Bacterial Communities by Treatment and Field.

4.5 Community Assembly of Known Fungal Antagonist Genera

Evaluating the species composition of known fungal antagonists helps to illuminate potential biological mechanisms of suppression (Hoitink and Boehm, 1999; Inderbitzen et al., 2018; Noble and Coventry, 2005). Known fungal antagonists were identified based upon the meta-analysis published by Inderbitzin et al. (2018). Known biological mechanisms of suppression of fungus are summarized for each genus discussed in table 3. Before the application of treatment on 19 May 2018, there was a significant difference in the composition of fungal antagonist communities between the organic and conventional fields ($p = 0.016$). Between fields, the largest differences in microbial species composition of known fungal antagonists were within

the genera *Paenibacillus*, *Chitinophaga*, *Burkholderia*, *Lysinibacillus*, *Cupriavidus*, and *Solibacillus*. However, at that time there were no significant differences between treatment assigned plots ($p = 0.071$). Further review of the data is required to evaluate the differences in these communities and their distributions.

Table 6: Known Fungal Antagonistic Bacterial Genera Discussed and Recorded Mechanisms of Suppression. Table Adapted from Inderbitzin et al. (2018).

Genus	Mechanism of suppression
<i>Achromobacter</i>	Siderophores
<i>Acidovorax</i>	Unknown
<i>Acinetobacter</i>	Antibiotics
<i>Actinomadura</i>	Antibiotics
<i>Arthrobacter</i>	Cell-wall-degrading enzymes
<i>Azospirillum</i>	Induced resistance, siderophores
<i>Cupriavidus</i>	Pathogenicity factor degradation
<i>Cytophaga</i>	Cell-wall-degrading enzymes
<i>Flavobacterium</i>	Unknown
<i>Mycobacterium</i>	Cell-wall-degrading enzymes
<i>Paenibacillus</i>	Cell-wall-degrading enzymes
<i>Sphingobacterium</i>	Cell-wall-degrading enzymes
<i>Streptosporangium</i>	Unknown

On 11 October 2018, four months after the application of compost treatments the composition of fungal antagonist communities remained significantly different between the organic and conventional fields ($p = 0.006$). The treatments also had a significant effect on the

composition of fungal antagonist communities ($p = 0.001$). Differences observed in the composition of fungal antagonistic bacteria communities between different treatments provided evidence that one mechanism of the observed field suppression of *V. dahliae*, after compost treatment, was biological suppression due to stimulation of these bacteria. Despite significant changes in structural diversity of these communities, relative abundance of fungal antagonists was not significantly affected by compost treatments ($p = 0.638$) (Figure 5).

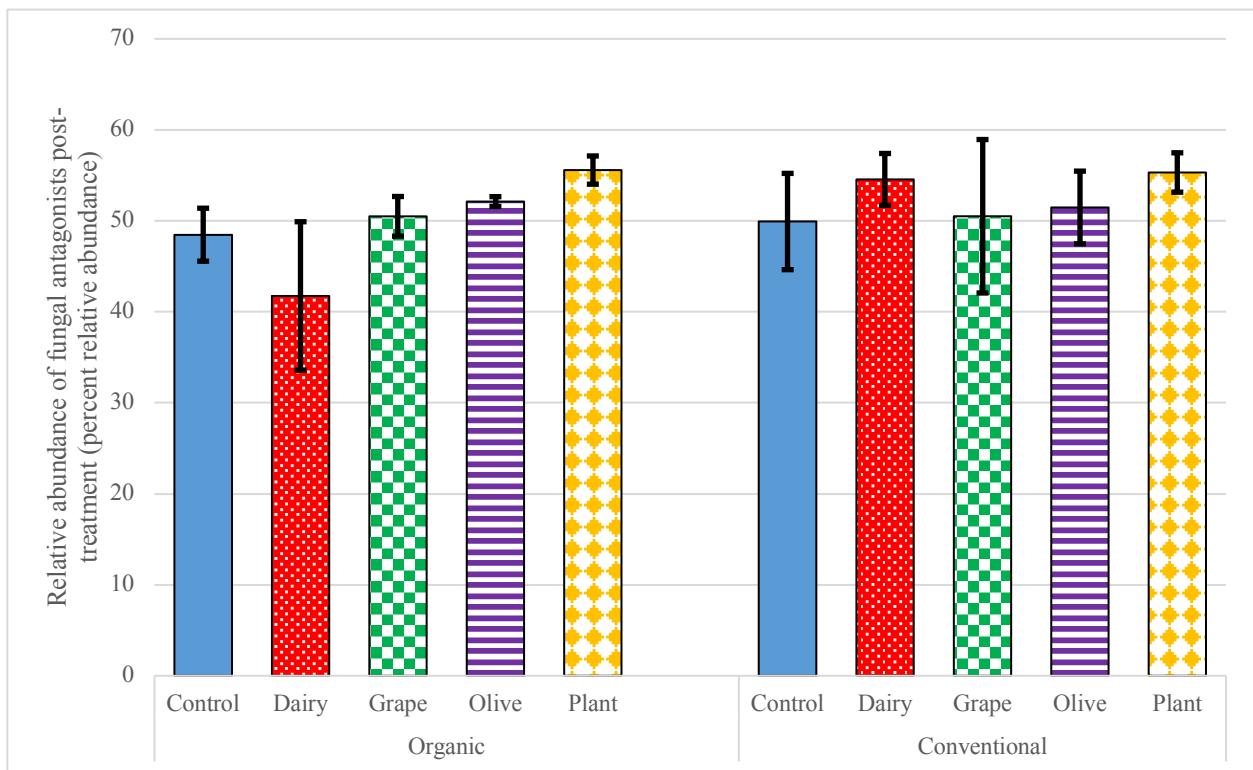


Fig. 5. Relative Abundance of Fungal Antagonists Post-Treatment by Compost Treatment and Field. Error Bars Represent One Standard Error of the Mean.

Again, the conventional farm was more variable with lower similarity observed between control plots or any treatment plots than in the organic farm. There were no significant differences observed between compost treated plots in the community of fungal antagonists ($p = 0.849$). On the organic farm, treatments did have a significant effect on the composition of fungal antagonistic communities ($p = 0.001$). The fungal antagonistic bacteria communities of plant

compost treated plots were most dissimilar from control within the genera *Sphingobacterium*, *Flavobacterium*, *Cupriavidus*, *Azospirillum*, and *Paenibacillus*; with Bray-Curtis Diss/SD values of 5.88, 4.44, 4.38, 3.69, and 2.2 respectively. Communities in dairy manure treated plots were most dissimilar from control within *Azospirillum*, *Achromobacter*, *Arthrobacter*, *Streptosporangium*, *Cytophaga*, and *Actinomadura*; with Bray-Curtis Diss/SD values of 4.93, 3.28, 2.32, 2.26, 2.23, and 2.07 respectively. Olive pomace compost treated plots were most dissimilar from control with Bray-Curtis Diss/SD values of 4.5 for *Achromobacter*, 2.18 for *Arthrobacter*, and 2.05 for *Acinetobacter*. Finally, grape pomace compost treated plots were most dissimilar from control within *Azospirillum*, *Actinomadura*, *Acidovorax*, *Mycobacterium*, and *Cytophaga*; with Bray-Curtis Diss/SD values of 4.56, 3.87, 2.48, 2.45, and 2.18 respectively.

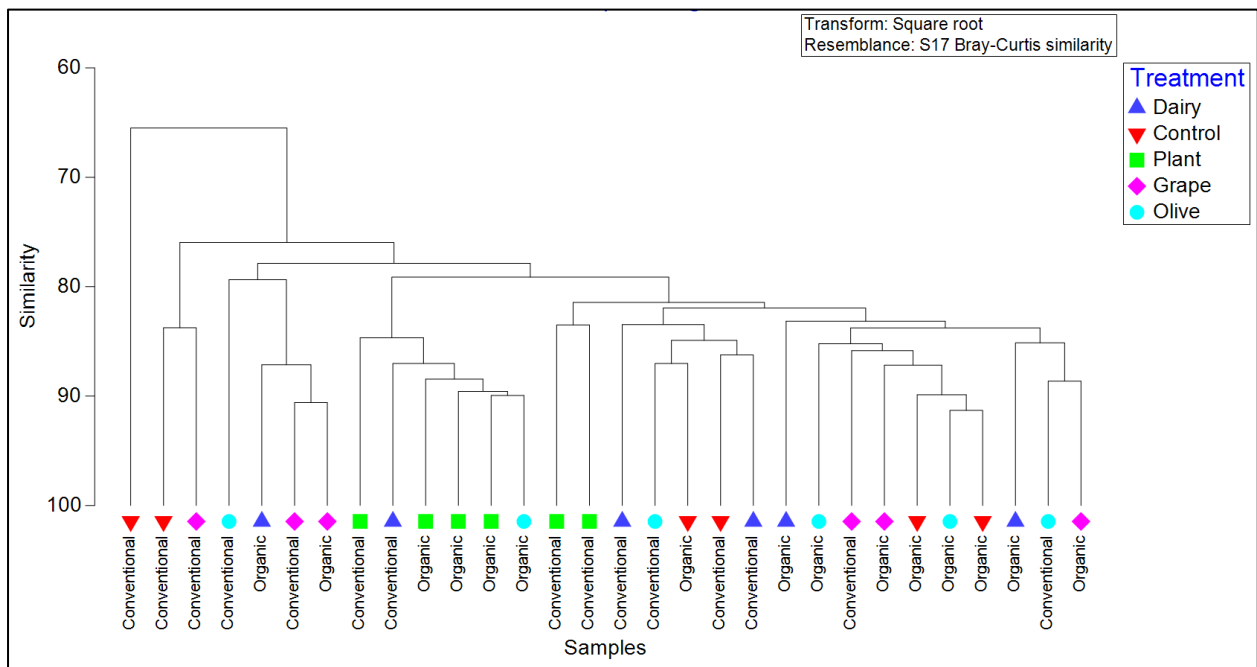


Fig. 6. Post-Treatment Bray-Curtis Similarity Clustering of Known Fungal Antagonistic Bacterial Communities by Treatment and Field.

Community structure of known fungal antagonists between treatment plots was again more similar within the organic farm. Control plots on the organic farm were an average of

86.0% similar at the genus level for known fungal antagonists. On the organic farm, plant compost treated plots were 88.7% similar, dairy manure treated plots were 80.0% similar, olive pomace treated plots were 86.4% similar, and grape pomace compost treated plots were 84.2% similar. The conventional farm showed more variation of the fungal antagonist communities within treatment types where none were significantly similar ($p = 0.849$) (Figure 6).

The preliminary analysis of prokaryote microbial communities included in this paper helps establish that the organic amendment treatments tested had a significant effect on microbial community structure. These structural differences require further analysis to determine more precisely the effects of each treatment and how that may be related to observed reduction in *V. dahliae* soil pathogen load. Biological suppression of fungal pathogens has been consistently reported (Hoitink and Boehm, 1999; Inderbitzen et al., 2018; Noble and Coventry, 2005; Trivedi et al., 2017; Weller et al., 2002). Based upon the significant treatment effects on bacterial community structures observed, including known fungal antagonists, it is likely that some of the *V. dahliae* reduction observed is due to changes in the microbial community. To establish which members of the microbial community were most influenced by treatment and potentially involved in pathogen suppression, further data review is required.

4.6 Weed Biomass and Diversity

The different compost treatments applied did not significantly affect total weed biomass on the organic farm ($p = 0.662$). Mean biomass assessment demonstrated no clear trends in part due to high variability (Fig. 7). The highest mean biomass was observed in the control plots averaging 214.5 ± 84.0 g/m² ($n = 5$; 82.1 – 307.6) while the lowest was observed in plant waste compost treated plots with an average of 134.5 ± 67.7 g/m² ($n = 5$; 26.4 – 198.9). Plots were

completely randomly assigned, but there did appear to be an edge effect on the weed biomass with an average of $211.5 \pm 74.1 \text{ g/m}^2$ ($n = 16$; $109.2 - 353.3$) in edge plots and an average of $130.7 \pm 108.5 \text{ g/m}^2$ ($n = 9$; $26.4 - 380.6$) in interior plots despite including 2 border rows on either side of the field. This may be due to inconsistency within the natural seed bank throughout the field though no measures of seed bank were taken so no conclusion can be made.

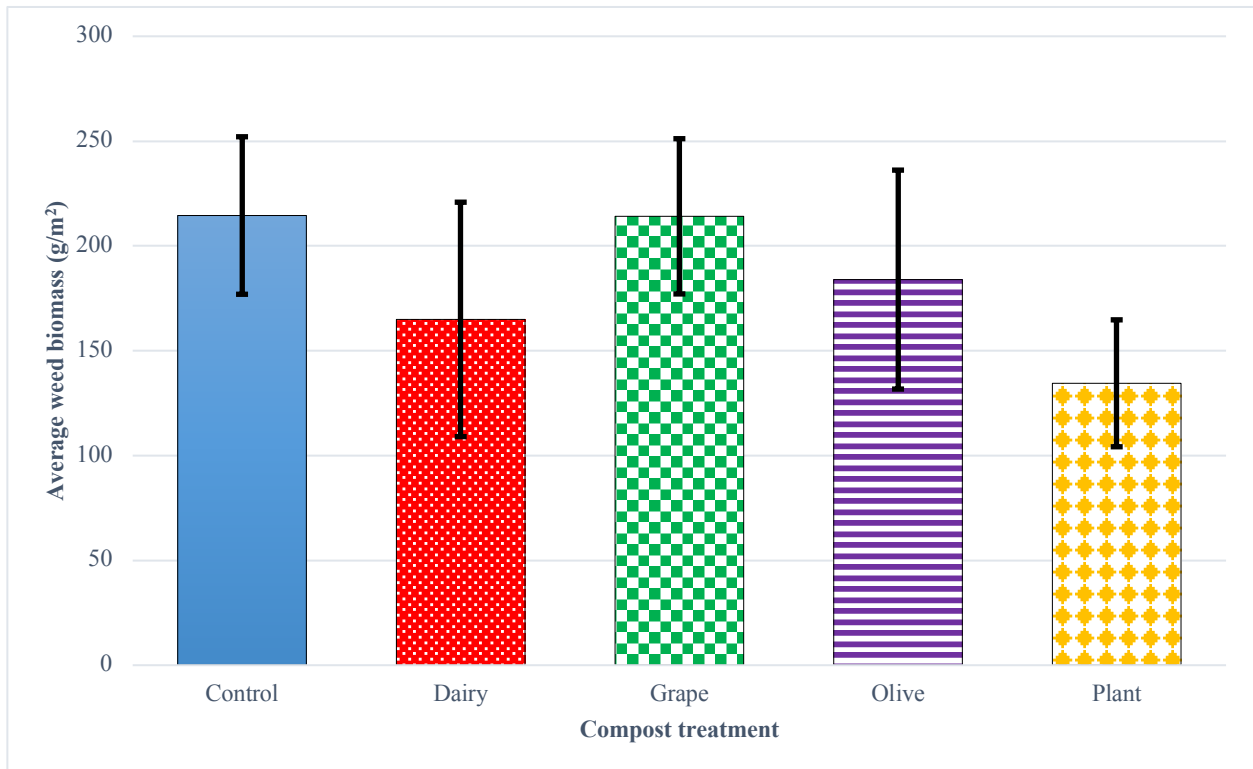


Fig. 7. Mean Total Weed Biomass in g/m^2 by Treatment. Error Bars Represent One Standard Error of the Mean.

The relationship between organic amendments and weed health and biomass is complicated and not well understood. There are likely phytotoxic effects of the amendments suppressing the germination of some weed seeds (Cayuela et al., 2008; Ferrara et al., 2014). Additionally, organic amendments stimulate the microbial community, which in turn can have detrimental effects on weed seeds including seed rot, shrunken seeds, and reduction or elimination of germination capacity (Kennedy, 1999). Some organic amendments may display a

positive effect on weeds by supplying them with nutrients and improving soil quality in the same way they do for crops.

The two weed species that were the most abundant both by number and biomass were little mallow, *Malva parviflora* L. (Malvales: Malvaceae) and annual sowthistle, *Sonchus oleraceus* L. (Asterales: Asteraceae). Both weeds were isolated and compared separately at collection. There was no significant effect of any compost treatment on the little mallow biomass ($p = 0.746$). There was again high variation between plots (Fig. 8). Although not statistically different from other treatments, the olive compost treated plots had the lowest average little mallow biomass with an average of only $42.5 \pm 59.4 \text{ g/m}^2$ ($n = 5$; 5.3 – 143.7). Little mallow is a very hardy weed with seeds that are resistant to environmental stresses because they are protected by a thick seed coat and have proven difficult to control with mulches and organic amendment treatments (Daugovish et al., 2007). Significant suppression, after olive waste compost application, of a variety of seeds has been observed including *Portulaca oleracea*, *Chrysanthemum segetum*, *Sonchus oleracea*, *Sinipis arvensis*, *Digitaria sanguinalis*, *Festuca arundinacea*, *Solanum nigrum*, *Trifolium incarnatum*, and *Amaranthus retroflexus* (Cayuela et al., 2008; Ferrara et al., 2015; Mondragon et al., 2018). Observed suppression varies depending on age of compost, application process, and application rate but suppression is consistently attributed to phytotoxic effects of polyphenols (Cayuela et al., 2008; Ferrara et al., 2015).

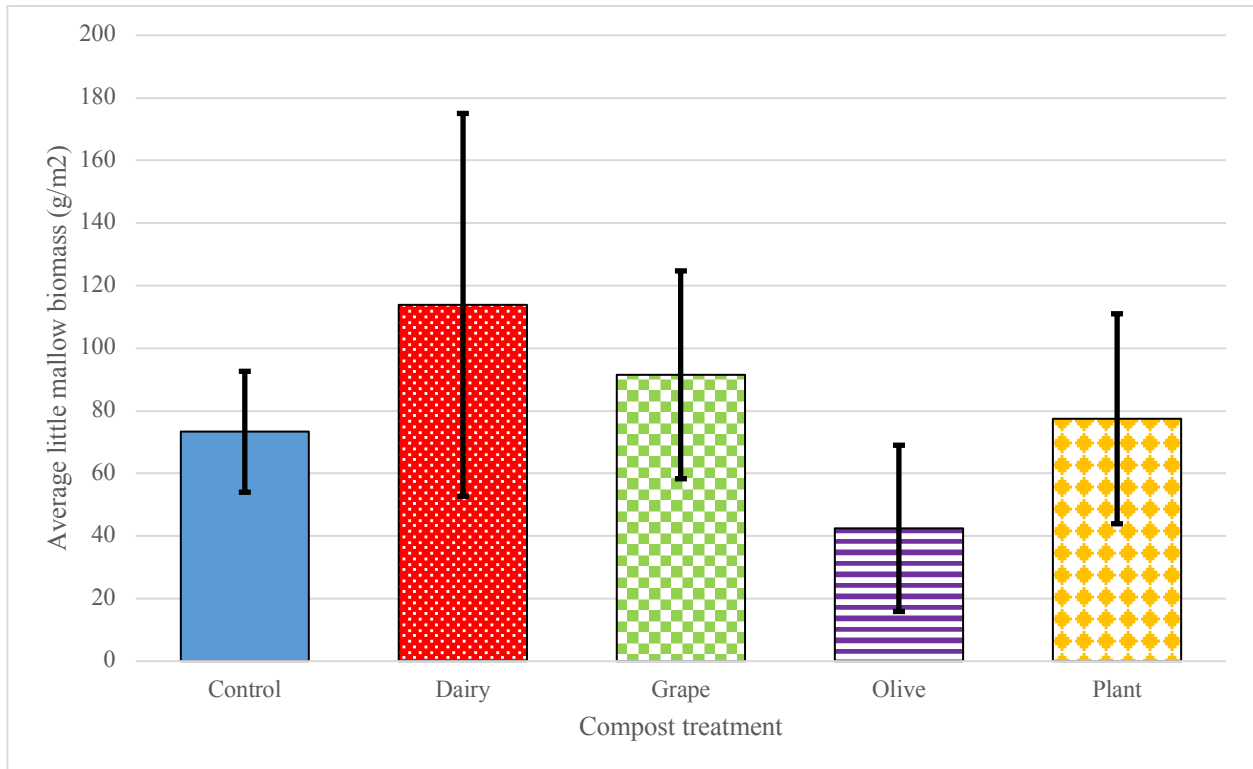


Fig. 8. Mean Little Mallow Biomass in g/m^2 by Treatment. Error Bars Represent One Standard Error of the Mean.

There was no significant effect ($p = 0.265$) of any treatment on sowthistle biomass although the control treatment tended to have a higher biomass (Fig. 9). Plant waste compost treated plots had the lowest average biomass at only $34.9 \pm 28.1 \text{ g/m}^2$ ($n = 5$; $6.3 - 79.6$) while control plots had the highest at $113.4 \pm 77.9 \text{ g/m}^2$ ($n = 5$; $14.8 - 192.5$). This was the largest mean difference between any treatment and control weed biomass observed ($p = 0.066$).

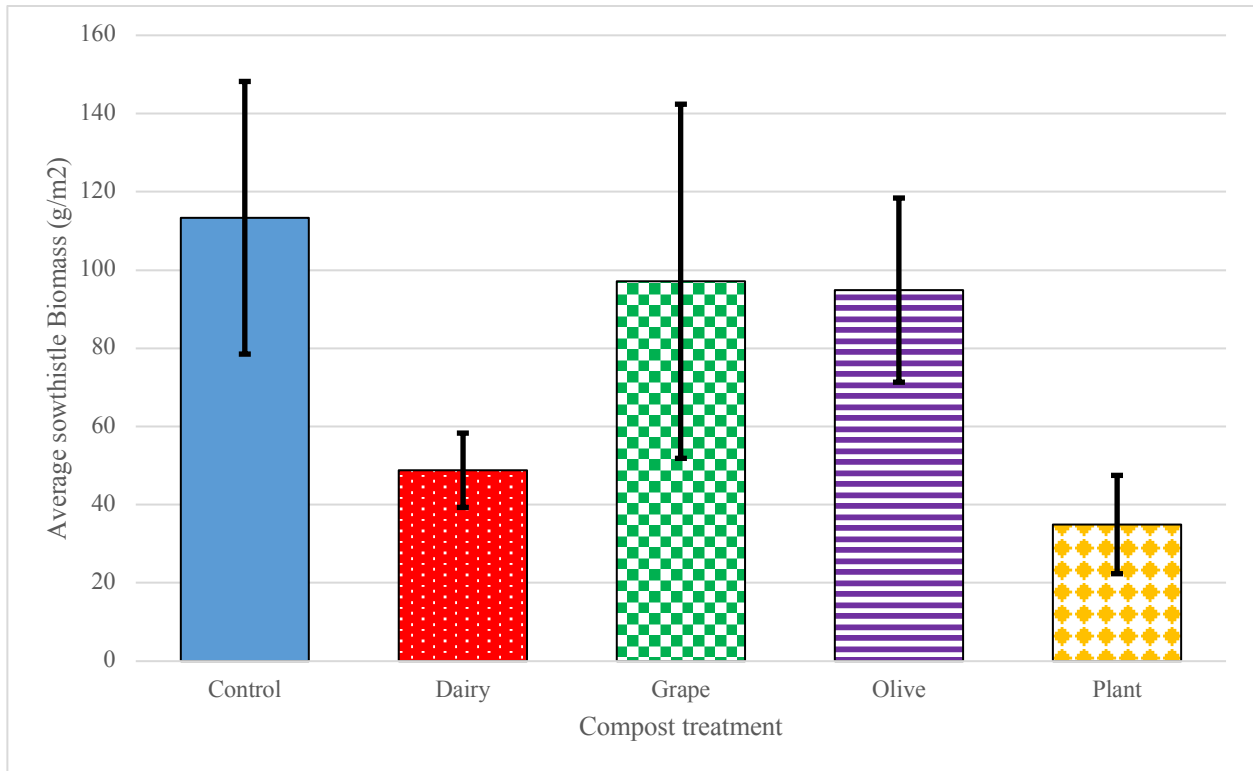


Fig. 9. Sowthistle Biomass in g/m² by Treatment. Error Bars Represent One Standard Error of the Mean.

4.7 Soil Arthropod Abundance

There was no significant treatment effect on either of the soil arthropod samples tested in this study in either field and any time. Collembola levels were higher pre-application, with a mean of 12.2 ± 12.1 ($n = 50$; $0 - 72$) per bait, than at any point post-application of compost treatments (Fig. 10). Insufficient evidence was collected to observe a treatment effect on Collembola abundance ($p = 0.551$). However, a significant effect of time was observed with a decrease from pre-treatment to all post-treatment samplings ($p < 0.0001$). This may be due to seasonal fluctuation in populations as the temperature increased, soils dried, and populations naturally declined; though due to the one-year duration of this study seasonality cannot be differentiated from natural temporal fluctuations (Ford, 1937; Singh et al., 2012). Another

explanation could be that the bait stations became less attractive to Collembola after the application of organic amendments and planting the field with peppers. The herbivorous soil arthropods had a readily available organic matter food source and were no longer as attracted to the bait stations.

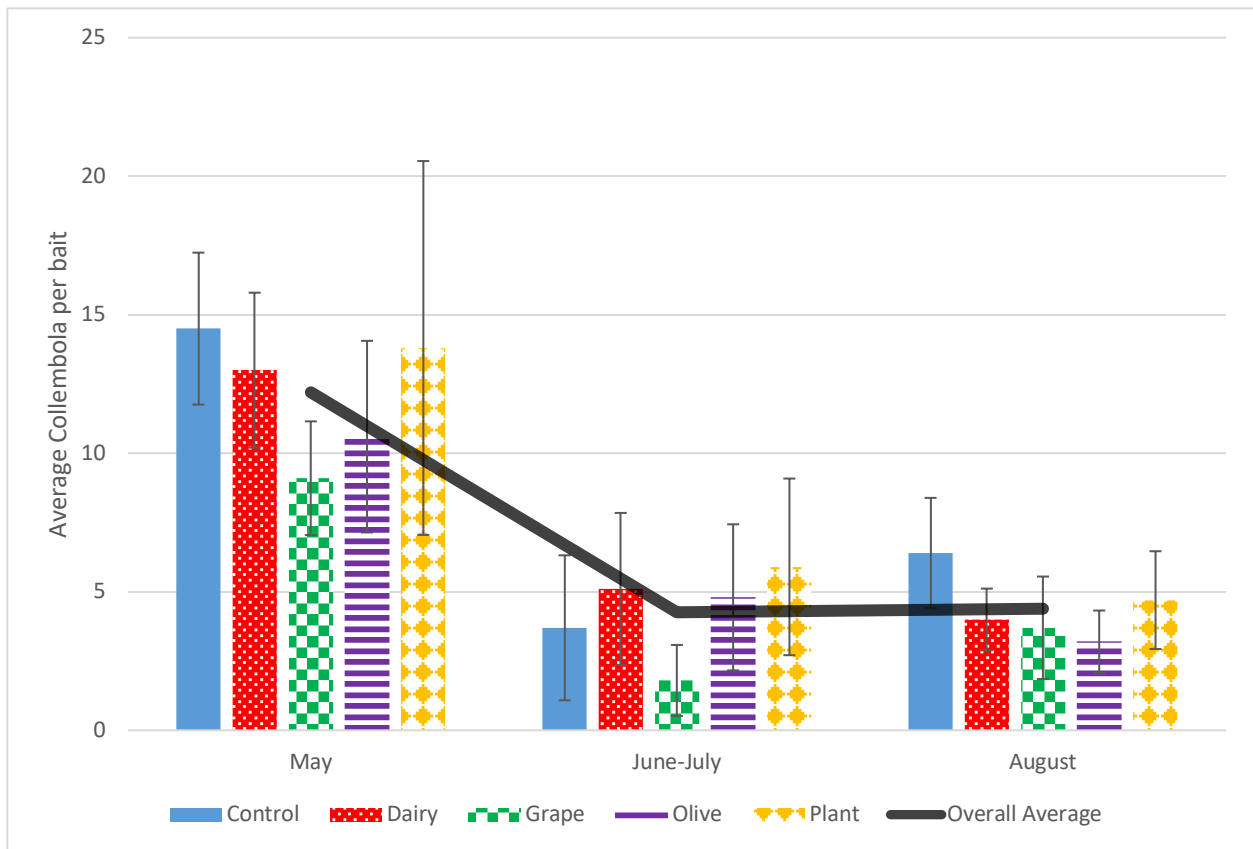


Fig. 10. Average Number of Collembola per Trap by Treatment over Time. Error Bars Represent One Standard Error of the Mean.

Symphyla populations showed a similar trend as seen with Collembola, but with much lower overall abundances (Fig. 11). There was no significant treatment effect on Symphyla abundance ($p = 0.870$). Time was again the most significant factor in bait station observations with a significant decrease observed during all sampling after the application of treatment when compared with pre-treatment bait averages ($p < 0.0001$). Again, this is likely due to the effect of adding an abundance of organic matter to the field not present during pre-treatment baiting.

Baiting Symphyla proved difficult and highly variable despite more consistent results observed in other studies (Umble and Fisher, 2003a). In a future study there are several design changes that may allow better observations of soil arthropods. Increasing sample size and utilizing a combination of several different sampling methods may allow for a broader assessment of arthropod abundances through time. The use of several baiting stations in combination with trapping tools such as Berlese funnels could help reduce the variability observed with a single sampling protocol.

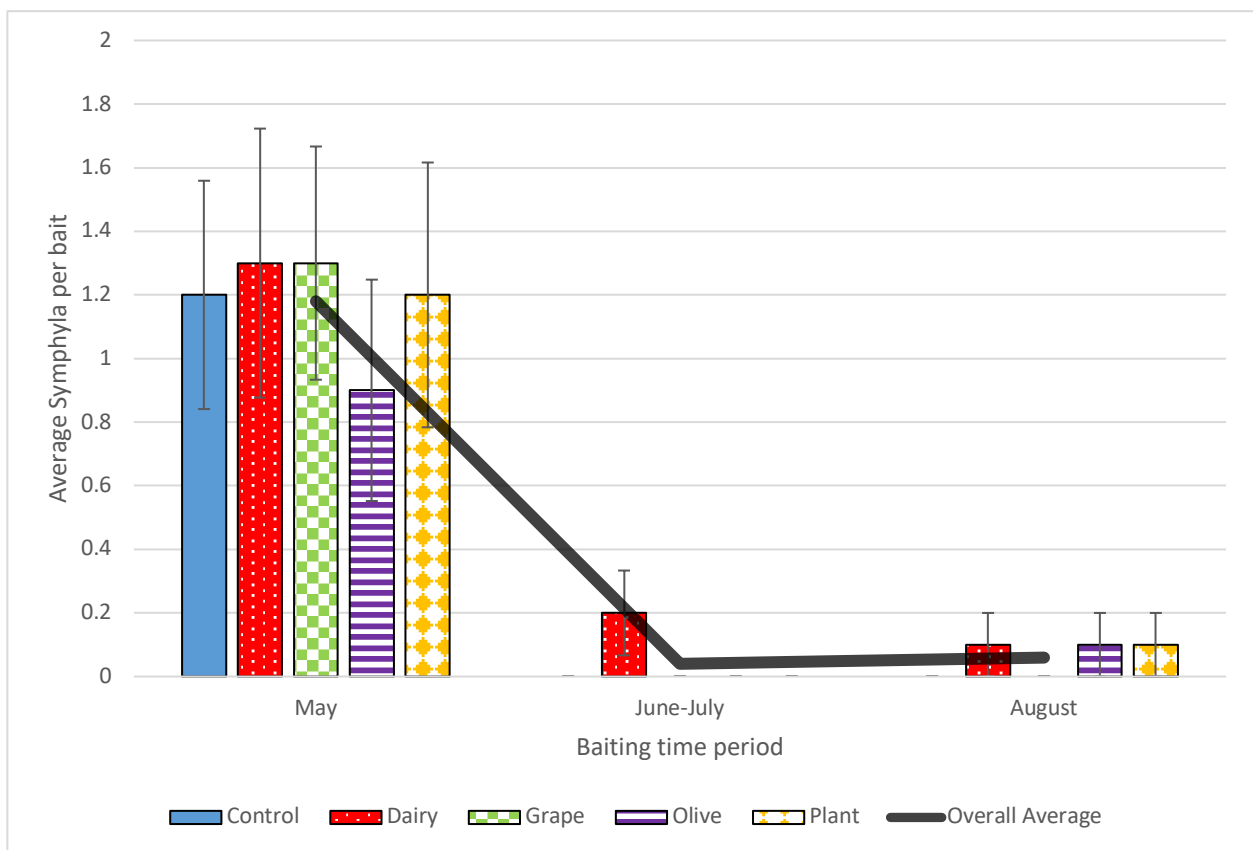


Fig. 11. Average Number of Symphyla per Trap by Treatment over Time. Error Bars Represent One Standard Error of the Mean.

4.8 Bell Pepper Health and Yield

None of the compost treatments tested had a significant effect on the growth of bell peppers, as measured by height, with an overall average growth of 27.7 ± 5.2 cm ($n = 250$; 15.5 – 41.4) on 2 September 2018 ($p = 0.833$) (Fig. 12). Similarly, relative leaf chlorophyll readings were not significantly affected by any treatment ($p = 0.554$) (Fig. 13). The effect of sampling date was significant in both instances with average height increasing from 26.06 ± 4.3 cm ($n = 250$; 15.2 – 37.8) to 27.67 ± 5.2 cm ($n = 250$; 15.5 – 41.4) ($p = 0.0002$). Average SPAD absorbance reading decreased significantly from 66.70 ± 9.3 ($n = 250$; 45.8 – 129.4) on 2 August down to 62.35 ± 9.2 ($n = 250$; 38 – 124.5) on 2 September ($p < 0.0001$). This decrease in SPAD absorbance is consistent with normal leaf aging (Yang et al., 2014).

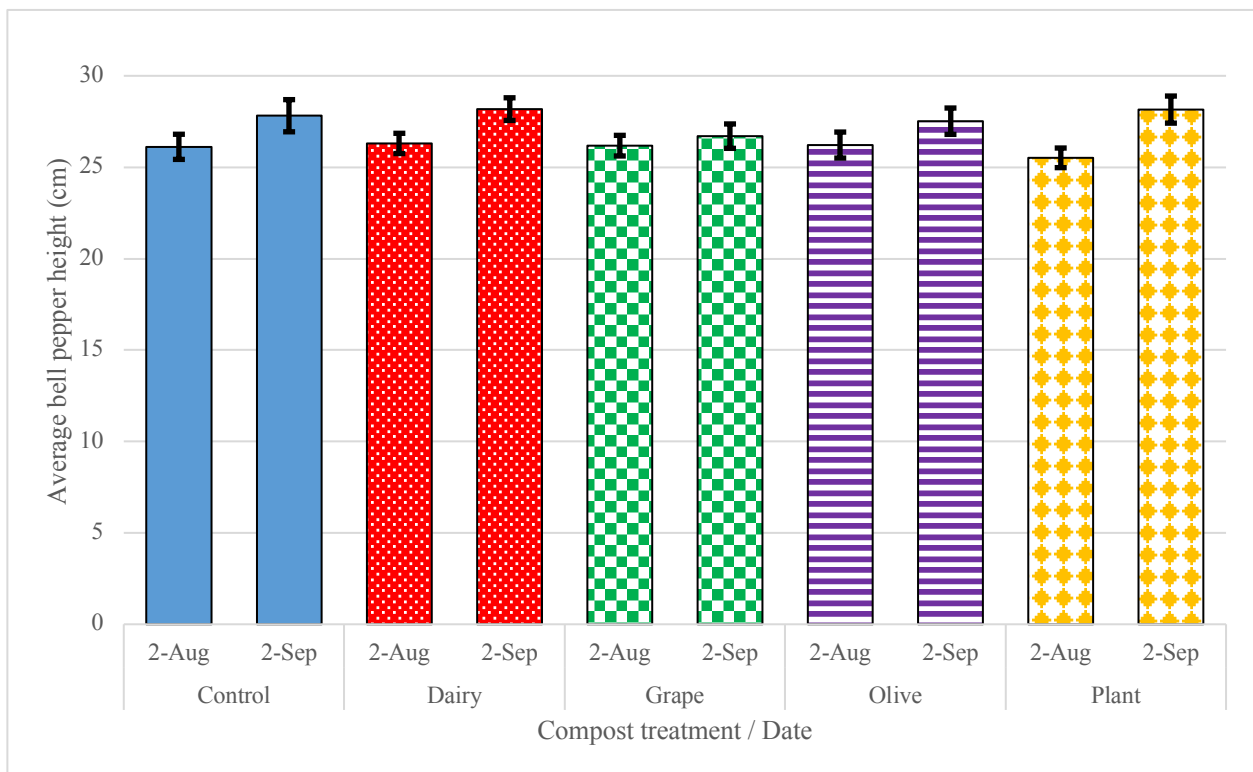


Fig. 12. Average *C. annuum* Height (cm) by Treatment over Time. Error Bars Represent One Standard Error of the Mean.

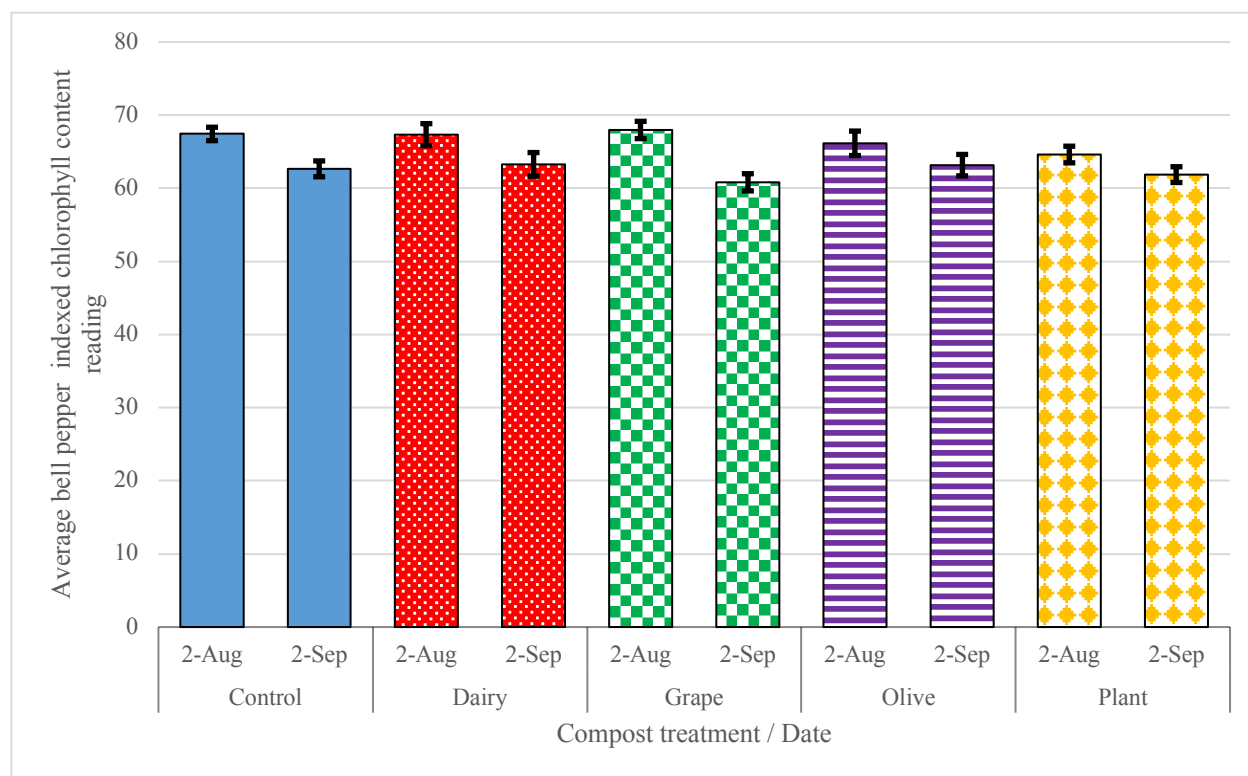


Fig. 13. Average *C. annuum* Indexed Chlorophyll Content Reading by Treatment over Time. Error Bars Represent One Standard Error of the Mean.

There was no significant effect on pepper yield between the fields ($p = 0.761$) (Fig. 14) or by treatment on yield ($p = 0.869$). Dairy treated plots had the highest yield with an average of 27.78 ± 10.67 kg per plot ($n = 10$; 6.03 – 52.21) and plant waste treated plots had the lowest yield with an average of 21.79 ± 10.26 kg per plot ($n = 10$; 11.14 – 40.83). Despite a significant effect of treatment on pathogen load there was no significant effect on the yield of peppers. Although the pathogen load of *V. dahliae* was above the threshold for disease development, the pepper plants had no observable disease symptoms throughout the trial. The chosen bell pepper variety is known to be susceptible to verticillium wilt, but it is not well researched how sensitive it is to the disease. Thus, it is plausible that the observed pathogen load was not enough for visible disease outbreak. A possible explanation for the absence of disease is the fact that this

piece of land has not had any pepper crops at least within the last five years. Bhat et al. (2003) have shown that in order for *V. dahliae* to develop on bell peppers, it requires pathogen isolates from another bell pepper crop (or eggplant), confirming earlier reports on the high specificity of *V. dahliae* in peppers (Tsrer et al., 1998). This is not the case with other nightshade family plants such as tomato, potato and eggplant, which can accept isolates from other nightshade crops (Bhat and Subbarao, 1999). Yield was highly variable from plot to plot and there appeared to be confounding variables in the field conditions. Transplanting was inconsistent in several areas of the field and some plants had to be replaced due to improper establishment. As previously discussed, an edge effect appeared to alter the biomass and composition of weeds observed throughout the study. It is likely that if there was an edge effect it also influenced yield and health of peppers. In the conventional field, yield in plots on the west side of the field averaged 95.67 kg while the rest of the field averaged 45.81 kg. These outside variables, among others, made yield readings highly variable and results difficult to interpret. All four compost treatments contained less than 2% total N, less than 0.6% P, less than 1.5% K, and over 30% organic matter. Nutrients found in composts are often slow to become available to plants; N specifically exists within composts mostly in the organic form, which is not immediately available to plants. Thus, composts do not often function as effective fertilizers rather as soil amendments; improving soil structure, increasing organic matter content and, through mineralization and other biological processes providing nutrients over time (Anonymous, 2016). Given this and the fact that the soils in both fields tested are routinely subject to fertilization, crop rotations, and soil amendments it is plausible that the nutrients from the composts applied did not influence plant growth and yield.

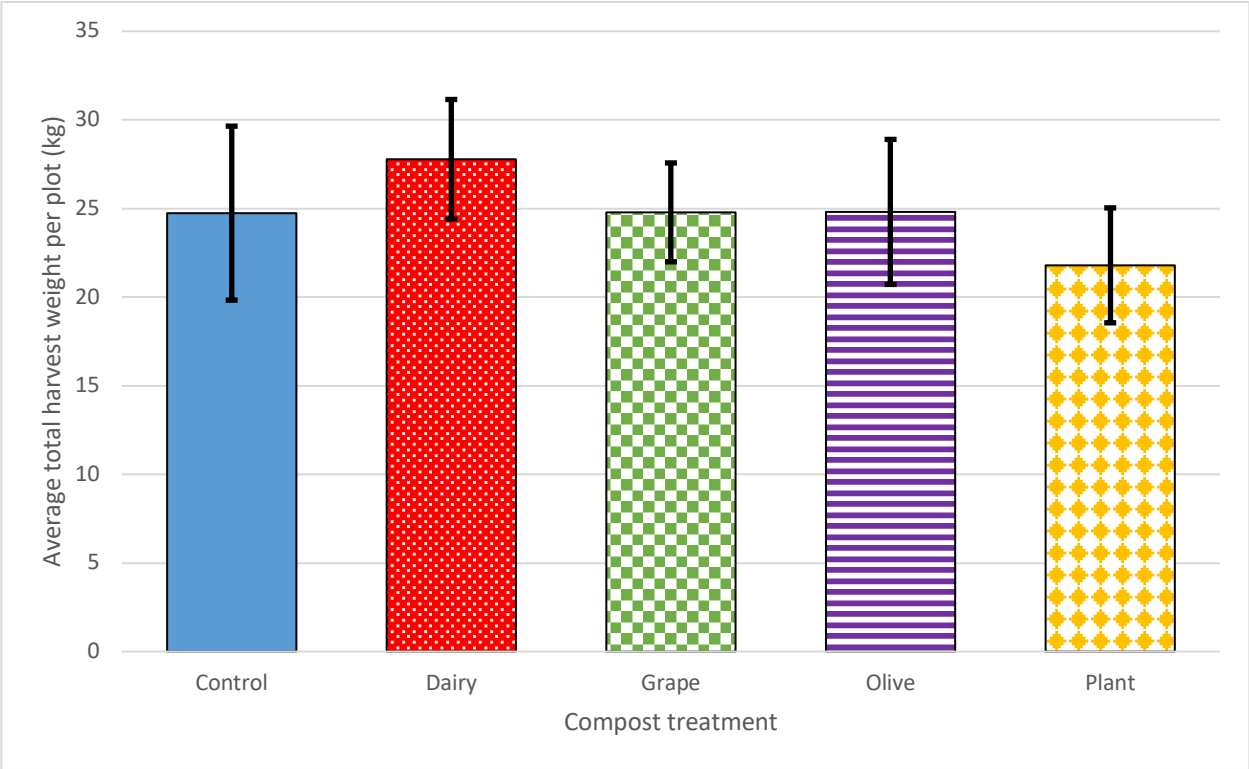


Fig. 14. Average Bell Pepper Yield (kg) per Plot (250 ft²) by Treatment. Error Bars Represent One Standard Error of the Mean.

CHAPTER 5

Conclusion

This study sought to explore some of the ecological effects of the application of several organic soil amendments. Four organic amendments were chosen due to their ease of access as abundantly available agricultural waste products in California as well as other Mediterranean regions. The effects of dairy manure compost, grape pomace-based compost, olive pomace-based compost, and a plant waste compost were all tested on the soil-borne pathogen *Verticillium dahliae*, soil arthropods Symphyla and Collembola populations, weed abundance, and bell pepper crop health and yield.

Significant reduction of the soil-borne fungal pathogen *V. dahliae* was observed up to two months after the application of olive, grape, and plant based organic amendments with an average decrease between 29% - 42% of the CFU/g soil for those three composts compared to control. Additionally, significant inhibition of *V. dahliae in vitro* growth was observed after treatment with grape, olive, and plant compost extracts; presenting average growth 25% - 50% less than the control. This supports the growing body of literature that organic amendments have potential as tools for sustainable soil disease management, though no disease outbreak was observed in this study. With particularly sensitive crop varieties, organic amendments may function as part of a broader control plan utilizing a variety of soil management tools to protect crops. This is supported by the findings of our laboratory experiment, which establishes that part of the mechanism of suppression is chemical. A significant effect of compost treatments on the microbial community structure and the structure of known fungal antagonistic bacterial communities was also observed within the organic farm. This is evidence that a biological mechanism of suppression of fungal pathogens after the application of organic amendments also

was occurring. In the field there are a variety of potential mechanisms by which organic amendments can suppress pathogens. These mechanisms include changes to the microbial community that hinder pathogen growth and the introduction of systemic resistance to the crop. In the laboratory study, these factors were not present, therefore the *V. dahliae* growth suppression seen must be in part due to chemicals introduced with the organic amendments having a deleterious effect on the pathogen. Between our observed laboratory suppression and changes to the microbial communities it is likely the significant suppression of *V. dahliae* by grape pomace, olive pomace, plant waste and dairy manure-based composts was multifaceted and worked through more than one mechanism.

We were unable to observe any significant effects of the compost on either of the arthropod communities sampled. Arthropods are highly mobile and difficult to accurately track or trap. This makes estimating their abundance and diversity distributions extremely challenging. Observations of all soil arthropods dropped significantly after the application of treatment which may be due to the attractiveness of the bait station decreasing after additional organic material was introduced as an alternative food source. These trends also may be due to natural seasonal variation but further research is required to illuminate the ecology of Collembola and their response to organic soil amendments.

Contrary to what we expected, total weed biomass was not significantly impacted by any of the treatments. Field weed populations are inherently highly variable in part due to an inconsistent seed bank. Our results were variable and appeared to be affected by several confounding variables masking any potential trends from the treatment application. Results were likely confounded by the inconsistent seed bank and the edge effect observed in both our fields. In a future study exploring the effect these amendments have on weeds, a controlled setting

could be utilized to avoid the pitfalls experienced here. A laboratory study could be designed to have equal seed numbers unbiased by field and seed bank conditions.

No negative effects of the treatments were observed on our crop. This has been a concern with the application of organic amendments with potentially phytotoxic content. Our compost analysis did show relatively high levels of soluble salts and olive and grape based amendments are known to be high in polyphenols, which can be phytotoxic. Despite these contents, and observed suppression trends of certain weed species, our compost treatments had no significant effect on the health or yield of bell peppers. We were not able to observe any significant impact on peppers from the treatments tested despite their measurable suppression on soil-borne pathogen loads. The cultivar of pepper used is known to be susceptible to verticillium wilt but is not highly sensitive and can be resistant to some *V. dahliae* strains. In a future study, a more sensitive crop, such as strawberries, could be utilized to observe effects from minor differences in the soil pathogen load. The lack of observable impact of compost treatment on bell pepper health or yield is likely due to high variability and confounding factors existing in field conditions. The relationships between soil physical and chemical characteristics, soil microbial communities, and plant health is complex, making it difficult to assess the impacts of the compost treatments on the bell peppers.

Overall, we were able to observe similar effects of suppression on soil-borne pathogen load as is consistent in the literature. This suppression was observed in both fields and both lab *in vitro* growth trials run. We were unable to determine the effects of our organic amendments on weed biomass or species composition, Symphyla or Collembola populations, or the health of our bell pepper crop. Organic amendments have potential as a pest suppression tool in organic management where other management options are limited and costly.

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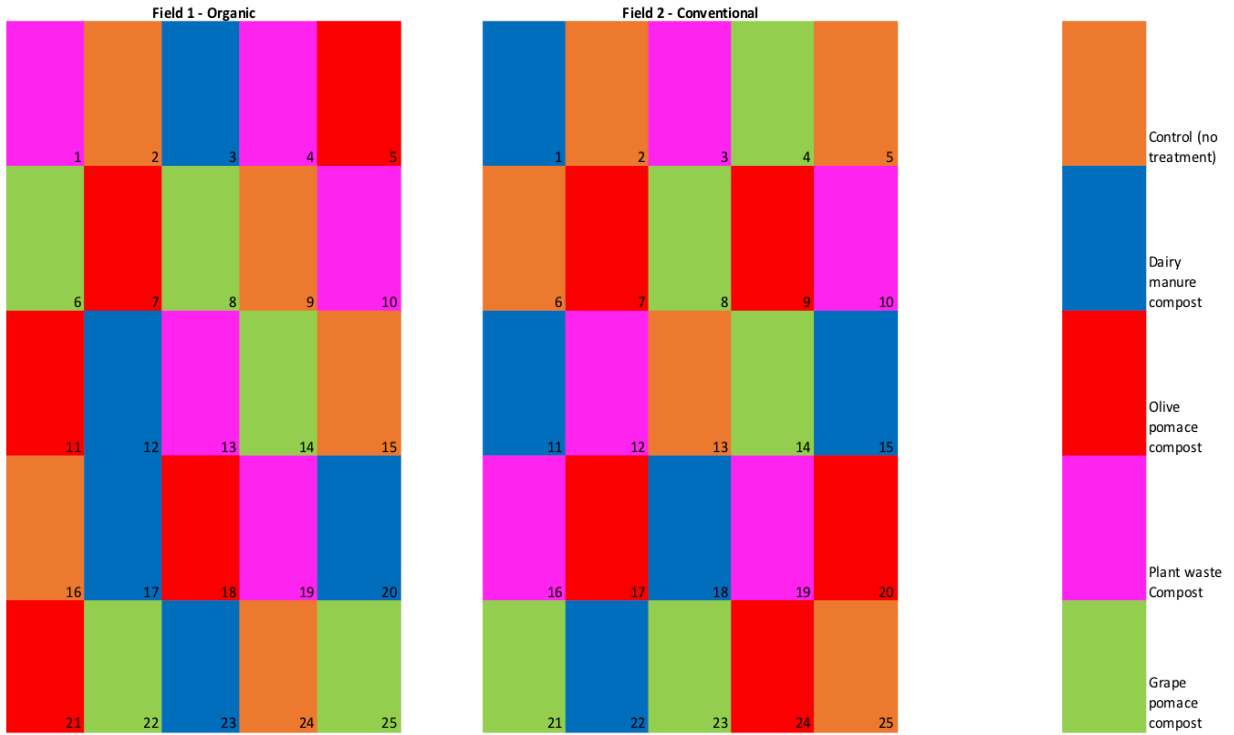
Appendix A

Field Maps (©2019 Google)



Appendix B

Plot Arrangement within Fields.



Appendix C

Sorenson's NP-10 Medium

Selective Medium for *Verticillium dahliae*

(This protocol was originally obtained from Mary Powelson's group)

I. Add these ingredients to 2 different flasks

FLASK 1:

	500 ml Medium	1 liter Medium
Distilled water	250 ml	500 ml
NaOH (1 N solution)	12.5 ml	25 ml
Polygalacturonic acid*	2.5 g	5.0 g

(*Na salt, **Sigma P-3889**)

FLASK 2:

	500 ml Medium	1 liter Medium
Distilled water	250 ml	500 ml
Agar	7.5 g	15.0 g
KNO ₃ (potassium nitrate)**	0.5 g	1.0 g
KH ₂ PO ₄ (potassium phosphate monoba)	0.5 g	1.0 g
KCl (potassium chloride)	0.25 g	0.5 g
MgSO ₄ -7H ₂ O (Magnesium sulfate heptahydrate)	0.25 g	0.5 g

ADD A STIR BAR TO FLASK 2.

II. Autoclave FLASKS 1 and 2 for 20 minutes.

III. Cool in waterbath to 55°C.

IV. To FLASK 2, add stock solutions (see below). You don't have to use stock solutions of each item, they can be added directly.

	500 ml Medium	1 liter Medium
Streptomycin SO ₄	0.025 g (i.e. 25 mg)	0.05 g (i.e. 50 mg)
Chlortetracycline HCl	0.025 g (i.e. 25 mg)	0.05 g (i.e. 50 mg)
Chloramphenicol	0.025 g (i.e. 25 mg)	0.05 g (i.e. 50 mg)
Tergitol NP-10 (filter sterilized)	0.25 ml (i.e. 250 ul)	0.5 ml (i.e. 500 ul)

In hood, pour FLASK 2 into FLASK 1; then stir, and pour immediately.

Appendix D

APDA (Acidified Potato Dextrose Agar)

For 1 liter:

- I. Add 1000 ml of diH₂O to a 2 L Erlenmeyer flask.
- II. Add stir bar and turn on hear, staying below ~ 60°C.
- III. Weigh out 39 grams of powdered potato dextrose agar and add to flask.
- IV. After solution has mixed thoroughly, autoclave for 20 minutes.
- V. Place flask in water bath set at 55°C for at least 30 minutes.
- VI. Move the flask onto a hot plate inside the laminar flow hood.
- VII. Add 1 ml of 85% lactic acid.
- VIII. Mix well without creating any bubbles, and then pour into Petri plates.

Appendix E

Bait Setup for Soil Arthropod Sampling.

For Collembola a leaf was placed over the plastic sheet over the hole and the bucket would be placed on top. For garden symphylans the plastic sheet was removed and a potato cutting was placed flat on the soil. The bucket was then left on the potato cutting.



Appendix F

Soil Control Lab Results.



TEL: 831-724-5422
 FAX: 831-724-3188
www.compostlab.com

Account #: 9020076-2/5-7772
 Group: Feb19B #61
 Reporting Date: February 20, 2019

Cal Poly State University
 1 Grand Avenue (Building 9)
 San Luis Obispo, CA 93407
 Attn: Ashraf Tubeileh

Date Received: 05 Feb. 19
 Sample Identification: Grape Waste Compost
 Sample ID #: 9020076 - 2/5

	Units	Wet wt. Basis	Dry wt. Basis	TMECC Method
Nutrients-Primary + Secondary				
Total Nitrogen:	%	1.5	1.7	4.02-D
Ammonia (NH ₄ -N):	mg/kg	10	11	4.02-C
Nitrate (NO ₃ -N):	mg/kg	62	70	4.02-B
Organic Nitrogen (Org.-N):	%	1.5	1.7	Calc.
Phosphorus (as P ₂ O ₅):	%	0.50	0.57	Calc.
Phosphorus (P):	mg/kg	2200	2500	4.03-A
Potassium (as K ₂ O):	%	1.4	1.7	Calc.
Potassium (K):	mg/kg	12000	14000	4.04-A
Calcium (Ca):	%	1.6	1.8	4.05
Magnesium (Mg):	%	0.99	1.1	4.05
Sulfate (SO ₄):	mg/kg	53	60	4.12-D/IC
Nutrients - Trace elements				
Copper (Cu):	mg/kg	26	29	4.05-Cu
Zinc (Zn):	mg/kg	76	85	4.05-Zn
Iron (Fe):	mg/kg	11000	13000	4.05-Fe
Manganese (Mn):	mg/kg	260	290	4.05-Mn
Boron (B):	mg/kg	19	22	4.05-B
Salts, pH, Bulk Density, Carbonates				
Sodium (Na):	%	0.064	0.071	4.05-Na
Chloride (Cl):	%	0.055	0.061	04.05/IC
pH Value:	units	8.20	NA	04.11-A
Electrical Conductivity (EC5 dw):	mmhos/cm	NA	1.4	04.10-A
Bulk Density :	lb/cu ft	38	34	SCL
Carbonates (as CaCO ₃) :	lb/ton	11	12	04.08-A
Organic Matter:	%	39.8	44.6	05.07-A
Organic Carbon:	%	23	25	4.01
Ash:	%	49.5	55.4	3.02
C/N Ratio	ratio	14.7	14.7	calc.
Moisture:	%	10.6	0	3.09
AgIndex	ratio	> 10	> 10	SCL

To Calculate lbs/ton: (%Nutrient) x (20)
 To Calculate lbs/ton: (mg/kg Nutrient/10,000) x (20)
 To Calculate lbs/cu yd: (%Nutrient/100) x B.D. x 27
 To Calculate lbs/cu yd: (mg/kgNutrient/1,000,000) x B.D. x 27

Analyst: Assaf Sadeh

ANALYTICAL CHEMISTS
and
BACTERIOLOGISTS
Approved by State of California

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FAX: 831-724-3188
www.compostlab.com

SOIL CONTROL LAB

42 HANGAR WAY
WATSONVILLE
CALIFORNIA
95076
USA

Account #: 9020076-1/5-7772
Group: Feb19B #60
Reporting Date: February 20, 2019

Cal Poly State University
1 Grand Avenue (Building 9)
San Luis Obispo, CA 93407
Attn: Ashraf Tubeileh

Date Received: 05 Feb. 19
Sample Identification: Dairy Waste Compost
Sample ID #: 9020076 - 1/5

Nutrients-Primary + Secondary	Units	Wet wt. Basis	Dry wt. Basis	TMECC Method
Total Nitrogen:	%	1.2	1.4	4.02-D
Ammonia (NH ₄ -N):	mg/kg	11	13	4.02-C
Nitrate (NO ₃ -N):	mg/kg	370	430	4.02-B
Organic Nitrogen (Org.-N):	%	1.2	1.4	Calc.
Phosphorus (as P ₂ O ₅):	%	1.1	1.3	Calc.
Phosphorus (P):	mg/kg	4800	5700	4.03-A
Potassium (as K ₂ O):	%	1.4	1.7	Calc.
Potassium (K):	mg/kg	12000	14000	4.04-A
Calcium (Ca):	%	2.1	2.5	4.05
Magnesium (Mg):	%	1.4	1.6	4.05
Sulfate (SO ₄):	mg/kg	1100	1200	4.12-D/IC
Nutrients - Trace elements				
Copper (Cu):	mg/kg	45	53	4.05-Cu
Zinc (Zn):	mg/kg	180	210	4.05-Zn
Iron (Fe):	mg/kg	16000	18000	4.05-Fe
Manganese (Mn):	mg/kg	420	500	4.05-Mn
Boron (B):	mg/kg	18	21	4.05-B
Salts, pH, Bulk Density, Carbonates				
Sodium (Na):	%	0.28	0.33	4.05-Na
Chloride (Cl):	%	0.24	0.28	04.05/IC
pH Value:	units	8.40	NA	04.11-A
Electrical Conductivity (EC5 dw):	mmhos/cm	NA	3.6	04.10-A
Bulk Density :	lb/cu ft	52	44	SCL
Carbonates (as CaCO ₃) :	lb/ton	27	32	04.08-A
Organic Matter:	%	25.9	30.7	05.07-A
Organic Carbon:	%	13	15	4.01
Ash:	%	58.6	69.3	3.02
C/N Ratio	ratio	10.7	10.7	calc.
Moisture:	%	15.6	0	3.09
AgIndex	ratio	7.1	7.1	SCL

To Calculate lbs/ton: (%Nutrient) x (20)
To Calculate lbs/ton: (mg/kg Nutrient/10,000) x (20)
To Calculate lbs/cu yd: (%Nutrient/100) x B.D. x 27
To Calculate lbs/cu yd: (mg/kgNutrient/1,000,000) x B.D. x 27

Analyst: Assaf Sadeh



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SOIL CONTROL LAB

42 HANGAR WAY
WATSONVILLE
CALIFORNIA
95076
USA

Account #: 9020076-3/5-7772
Group: Feb19B #62
Reporting Date: February 20, 2019

Cal Poly State University
1 Grand Avenue (Building 9)
San Luis Obispo, CA 93407
Attn: Ashraf Tubeileh

Date Received: 05 Feb. 19
Sample Identification: Olive Waste Compost
Sample ID #: 9020076 - 3/5

Nutrients-Primary + Secondary	Units	Wet wt. Basis	Dry wt. Basis	TMECC Method
Total Nitrogen:	%	1.3	1.7	4.02-D
Ammonia (NH ₄ -N):	mg/kg	16	21	4.02-C
Nitrate (NO ₃ -N):	mg/kg	11	15	4.02-B
Organic Nitrogen (Org.-N):	%	1.3	1.7	Calc.
Phosphorus (as P ₂ O ₅):	%	0.68	0.91	Calc.
Phosphorus (P):	mg/kg	3000	4000	4.03-A
Potassium (as K ₂ O):	%	1.1	1.4	Calc.
Potassium (K):	mg/kg	8900	12000	4.04-A
Calcium (Ca):	%	1.4	1.8	4.05
Magnesium (Mg):	%	0.82	1.1	4.05
Sulfate (SO ₄):	mg/kg	390	530	4.12-D/IC
Nutrients - Trace elements				
Copper (Cu):	mg/kg	31	42	4.05-Cu
Zinc (Zn):	mg/kg	98	130	4.05-Zn
Iron (Fe):	mg/kg	8200	11000	4.05-Fe
Manganese (Mn):	mg/kg	220	300	4.05-Mn
Boron (B):	mg/kg	20	27	4.05-B
Salts, pH, Bulk Density, Carbonates				
Sodium (Na):	%	0.14	0.19	4.05-Na
Chloride (Cl):	%	0.17	0.22	04.05/IC
pH Value:	units	8.71	NA	04.11-A
Electrical Conductivity (EC5 dw):	mmhos/cm	NA	2.6	04.10-A
Bulk Density :	lb/cu ft	47	35	SCL
Carbonates (as CaCO ₃) :	lb/ton	11	15	04.08-A
Organic Matter:	%	35.0	47.6	05.07-A
Organic Carbon:	%	20	28	4.01
Ash:	%	38.6	52.4	3.02
C/N Ratio	ratio	16.5	16.5	calc.
Moisture:	%	26.5	0	3.09
AgIndex	ratio	9.9	9.9	SCL

To Calculate lbs/ton: (%Nutrient) x (20)
To Calculate lbs/ton: (mg/kg Nutrient/10,000) x (20)
To Calculate lbs/cu yd: (%Nutrient/100) x B.D. x 27
To Calculate lbs/cu yd: (mg/kgNutrient/1,000,000) x B.D. x 27

Analyst: Assaf Sadeh



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SOIL CONTROL LAB

42 HANGAR WAY
WATSONVILLE
CALIFORNIA
95076
USA

Account #: 9020076-4/5-7772
Group: Feb19B #63
Reporting Date: February 20, 2019

Cal Poly State University
1 Grand Avenue (Building 9)
San Luis Obispo, CA 93407
Attn: Ashraf Tubeileh

Date Received: 05 Feb. 19
Sample Identification: Plant Waste Compost
Sample ID #: 9020076 - 4/5

Nutrients-Primary + Secondary	Units	Wet wt. Basis	Dry wt. Basis	TMECC Method
Total Nitrogen:	%	1.1	1.4	4.02-D
Ammonia (NH ₄ -N):	mg/kg	13	17	4.02-C
Nitrate (NO ₃ -N):	mg/kg	190	240	4.02-B
Organic Nitrogen (Org.-N):	%	1.1	1.4	Calc.
Phosphorus (as P ₂ O ₅):	%	0.68	0.89	Calc.
Phosphorus (P):	mg/kg	3000	3900	4.03-A
Potassium (as K ₂ O):	%	1.0	1.3	Calc.
Potassium (K):	mg/kg	8400	11000	4.04-A
Calcium (Ca):	%	1.9	2.4	4.05
Magnesium (Mg):	%	0.46	0.60	4.05
Sulfate (SO ₄):	mg/kg	2000	2500	4.12-D/IC
Nutrients - Trace elements				
Copper (Cu):	mg/kg	55	71	4.05-Cu
Zinc (Zn):	mg/kg	150	190	4.05-Zn
Iron (Fe):	mg/kg	8200	11000	4.05-Fe
Manganese (Mn):	mg/kg	230	300	4.05-Mn
Boron (B):	mg/kg	17	22	4.05-B
Salts, pH, Bulk Density, Carbonates				
Sodium (Na):	%	0.12	0.16	4.05-Na
Chloride (Cl):	%	0.29	0.37	04.05/IC
pH Value:	units	8.10	NA	04.11-A
Electrical Conductivity (EC5 dw):	mmhos/cm	NA	4.4	04.10-A
Bulk Density :	lb/cu ft	50	39	SCL
Carbonates (as CaCO ₃) :	lb/ton	19	25	04.08-A
Organic Matter:	%	27.7	35.9	05.07-A
Organic Carbon:	%	13	17	4.01
Ash:	%	49.4	64.1	3.02
C/N Ratio	ratio	12.1	12.1	calc.
Moisture:	%	22.9	0	3.09
AgIndex	ratio	6.8	6.8	SCL

To Calculate lbs/ton: (%Nutrient) x (20)
To Calculate lbs/ton: (mg/kg Nutrient/10,000) x (20)
To Calculate lbs/cu yd: (%Nutrient/100) x B.D. x 27
To Calculate lbs/cu yd: (mg/kgNutrient/1,000,000) x B.D. x 27

Analyst: Assaf Sadeh



Appendix G

Summary of Weather Data.

Summary of weather data for 2018 and 30 year averages for San Luis Obispo, CA
(station 52) as provided by California Irrigation Management Information System (CIMIS).

Monthly average climate data from 1986 - 2018 for San Luis Obispo, CA					
Month	Total ETo (mm)	Total Precip (mm)	Avg Sol Rad (W/m²)	Avg Air Temp (C)	Avg Rel Hum (%)
January	57.99	107.63	112.81	11.99	62.53
February	64.62	103.48	140.31	12.44	64.81
March	97.82	83.01	197.78	13.15	66.31
April	125.22	26.39	255.00	13.71	66.50
May	144.07	13.50	283.97	14.83	67.91
June	155.71	5.69	307.19	16.47	68.03
July	158.62	3.03	295.91	17.59	70.66
August	147.13	103.09	271.59	17.81	70.38
September	122.13	158.47	228.97	17.98	66.88
October	99.81	29.52	172.53	17.10	63.03
November	69.50	37.95	128.53	14.53	59.91
December	55.53	86.78	103.19	11.72	62.34

Monthly average climate data from 2018 for San Luis Obispo, CA					
Month	Total ETo (mm)	Total Precip (mm)	Avg Sol Rad (W/m²)	Avg Air Temp (C)	Avg Rel Hum (%)
January	61.80	81.0	104	15.2	68
February	86.59	3.4	165	14.3	55
March	82.20	199.2	165	13.9	76
April	130.00	12.8	251	16.7	75
May	125.68	0.2	250	16.2	79
June	151.99	0.1	287	19.1	76
July	161.38	0.0	272	20.8	79
August	148.71	0.0	259	21.2	75
September	119.25	0.0	224	19.7	78
October	101.50	16.3	178	17.3	63
November	75.12	115.5	135	15.6	56
December	59.58	27.3	110	12.5	65

Appendix H

Photo of Symphyla on Potato Bait. (9 May 2018)



Expanded portion of same photo to clearly show Symphyla.



Appendix I

Photo of Collembola from Soil Baiting Station. (23 May 2018)

