EVALUATING THE POTENTIAL OF OYSTER MUSHROOM COMPOST WASTE FOR
NEMATODE MANAGEMENT

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Abstract

Several *Pleurotus* spp. have been documented to release allelopathic compounds against nematodes in laboratory trials. Introduction of this fungus to a crop production field for nematode management are challenged by various environmental factors. Two approaches were proposed to introduce spent oyster mushroom compost waste to a basil (*Ocimum basilicum*) agroecosystem: 1) amending transplant media mix with spent mushroom compost substrate, and or 2) delivering the mycotoxin through drenching plant roots with mushroom compost water extract (MCWE). Specific objectives of this thesis include 1) determining amendment rates of *Pleurotus* sp. compost into potting media to suppress root-knot nematodes (*Meloidogyne incognita*), 2) determine concentrations of mushroom compost water extract for nematode suppression, and 3) evaluate a spent oyster mushroom compost based technology for pre- and post-plant nematode management in field trials. A greenhouse pot trial demonstrated that amending spent *Pleurotus ostreatus* compost with coffee grounds as the substrate, did not suppress population densities of *Meloidogyne incognita* on basil planted in a sand: soil mix. Four laboratory trials were conducted using spent oyster mushroom compost amended into media with high organic matters in Cone-tainers: yard waste compost was used in Cone-tainer I, II, and III. And peat: moss: perlite was used in Cone-tainer IV. Numbers of *M. incognita* were suppressed at mushroom compost amendment rate ≥1% in a yard waste compost media. When amended into peat moss potting mix, mushroom compost amendment also suppressed *M. incognita* penetration at 1 and 33%. When preparing mushroom compost into MCWE, a minimum of 1% concentration is needed to suppress mobility of *M. incognita* if incubated for ≥2 days. However, the nematode suppressive effect
of MCWE was nematostatic instead of nematicidal. When *M. incognita* J2s were incubated for 7 days in MCWE Trial II, MCWE suppressed 22 and 41% of viability of *M. incognita* at as low as 10 and 25% concentration, respectively. Application of oyster mushroom compost was then evaluated in two field trials at Poamoho (Trial I) and Magoon (Trial II), respectively. Although mushroom compost amendment did not suppress plant-parasitic nematodes in both trials, its effects on soil health varied by trial. In Trial I, mushroom compost (amendment or drenching) plots were dominated by bacterial decomposition (lower CI, *P* < 0.01) initially, and resulted in a reduced soil food web structure (lower SI, *P* < 0.05). Lack of enhancement of fungivorous nematodes in this trial indicated a poor mushroom mycelia establishment in this field. However, in Trial II, mushroom compost amendment enhanced fungal decomposition as indicated by higher abundance of fungivorous nematodes than no mushroom compost treatments (*P* < 0.05) throughout the basil crop. The enhancement of fungivorous nematodes throughout Trial II coincided with enhancement of soil health conditions as indicated by an increase in abundance of bacterivorous (*P* < 0.05) and omnivorous (*P* < 0.01) nematodes compared to no mushroom treatments. When used as a seedling potting mix amendment, nematode richness in amendment plots was also increased (*P* < 0.05) compared to no amendment. However, spent oyster mushroom compost did not improve basil yield due to heavy infestation of *Peronospora belbahrii*, but amendment improved height in Poamoho. Spent oyster mushroom compost as amendment and sometimes as drench increased bacterivorous and fungivorous nematodes, and if practiced in area with higher humidity, it could also potentially increase omnivorous and predatory nematodes. In conclusion, although mushroom compost amendment and drench did not suppress plant-parasitic nematodes,
they had varying effects in improving soil health. Spent oyster mushroom compost as amendment and sometimes as drench increased bacterivorous and fungivorous nematodes, and if practiced in area with higher humidity, it could also potentially increase omnivorous and predatory nematodes. Overall, the benefits of oyster mushroom compost for suppression of plant-parasitic nematodes and improving soil health might take time. Continual practice of introducing mushroom compost waste in an agroecosystem might be needed to observe the benefits of this saprophytic fungus. Future research is needed to evaluate more frequent MCWE drenching for the management of plant-parasitic nematodes.
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Chapter 1

Literature Review

Damage of plant-parasitic nematodes on Basil

Basil (*Ocimum basilicum*) in Hawaii had a farm gate value of $2.08 million in 2012 (NASS, 2013). Unfortunately, basil is considered as a minor crop with only a few pesticides registered for use. However, basil is susceptible to root-knot nematodes, *Meloidogyne incognita* (Vovlas and Troccoli, 2008). The nematode damages the roots and impedes the ability of the plant to take up water and nutrients (Hamasaki *et al.*, 1994). We proposed to work with this specialty crop partly due to its important economic contribution to Hawaii, also partly because of its long-term crop production, making nematode management at post-plant rather challenging. Typically, a basil crop is grown for 9 to 12 months before a new crop planting. During this period, farmers would harvest the shoot tissue periodically for sale. Since root-knot nematode has a life cycle of approximately 30 days, this makes population densities of root-knot nematodes unbearable on basil crop.

*Root-knot nematodes*

Root-knot nematodes in the genus *Meloidogyne* are found worldwide as an important pest to many agricultural and ornamental plants. Root-knot nematodes feed and reproduce within the plant, inducing gall formation. This disfigures the roots and interferes with the plants ability to uptake water and nutrients. They are obligate plant parasites that are highly adaptive. The adult female has a pear shaped body without a posterior
protrudence. They have a stylet that is 15-16μm with rounded offset knobs. They produce eggs within a gelatinous matrix that prevents desiccation outside of the body. Depending on the host plant, the female can produce several hundred eggs. The first molt occurs within the egg, producing the second stage juvenile (J2). The J2 is considered the infectious stage and will infect nearby roots. The juvenile is around 350-450 μm long with a slender cone shape tail. Root-knot nematodes are known to have unbalanced sex ratios, males may be absent or abundant pending on the environmental conditions. The male is non-sedentary, vermiform shape, and ranges in length from 600-2500 μm. The tail is very short and bluntly rounded (Karssen and Moens, 2006).

Among the Meloidogyne species, *M. incognita*, *M. javanica*, and *M. arenaria* are the most economically damaging species in the tropic and subtropical regions (Sasser *et al*., 1983). *M. incognita* has a wide host range including tomatoes, cowpea, okra, banana, ornamentals, carrot, tobacco, cotton, potatoes, etc. (Sasser *et al*., 1983; Kaur and Attri, 2013).

*Meloidogyne incognita* populations reproduce through mitotic parthenogenesis and can have either a diploid or triploid chromosome. *M. incognita* chromosomes in the prolonged prophase period are bunched close to each other and cannot be counted.

**Damage of Meloidogyne incognita to basil**

Sweet basil planted in field conditions in low-density conditions produce fresh yields of 8kg/m² (Vovlas and Troccoli, 2008). Economic damage of *M. incognita* to basil shoot yield was estimated to be 47% over a 10-month grow period in Florida (average temperature ranging from 15-35°C) with initial inoculum of 5,000 eggs per plant (Rhoades, 1988). These
microscopic round worms damage the roots and impede the ability of basil to take up water and nutrients from the soil. As a result, affected plants may show symptoms of nutrient deficiency, wilting, and yield decline. Galling and root rot occur on plants that are heavily infected, and resulted in limited functional roots. Based on the study conducted by Rhoades (1988), nematode damage of *M. incognita* on basil is second to sting nematode (*Beloidolaimus longicaudatus*), but more damaging than lesion (*Pratylenchus scribneri*), lance (*Hoplolaimus geleatus*), and awl (*Dolichodorus heterocephalus*) nematodes. The level of damage could vary pending on climate weather. Environmental conditions have the potential to increase fungal disease presence in the leaf and root tissues. Resistant cultivars for nematodes in sweet basil are not available so management options are dedicated to the reduction of population (Vovlas and Troccoli, 2008).

**Challenge of Nematode Management**

Among many agriculture related pests, plant-parasitic nematodes are prevalent in nearly all agroecosystems in tropical climates (Schmitt and Sipes, 1998). However, few viable, reduced risk approaches are available to manage plant-parasitic nematodes. In general, crop losses due to plant-parasitic nematodes are estimated to be US $125 bil/yr worldwide, and U.S. $10 bil/yr in the United States (Chitwood, 2003). The most common approach for farmers to deal with nematode damage in intensive farming systems is to apply soil fumigants or other synthetic nematicides. Soil fumigants and post-plant nematicides are highly toxic, carcinogenic, can build up pesticide residues in food crops, and can infiltrate into the ground water (Abawi and Widmer, 2000). The remaining nematicides on the market are either continuing to be reviewed by EPA, voluntarily
removed by the manufacturers from the market, or simply not very effective (Abawi and Widmer, 2000; Barker and Koenning, 1998). Thus, the development of alternative control strategies to complement nematicides is urgently needed.

**Reduced Risk Nematode Management**

In 1983 (Sasser *et al*.), focus was on screening and selecting for crop resistance and recognized that nematicides usage was not feasible. Integrated crop protection systems include control measures such as resistant cultivars, crop rotation, nematicides, and cultural practices. Most of the traditional nematode management methods, though not always satisfactory for farmers that prefer a quick and easy solution, are being reinvestigated for sustainable agricultural production. Current traditional approaches include amending soil with animal and green manures, composted materials, nematicidal plants and proteinous wastes (Oka, 2010). Mechanisms on how these amendments resulted in longer-term nematode suppression are reviewed in detail by Oka (2010). Another traditional approach but with more advanced understanding on its mechanisms against plant-parasitic nematodes is planting of plants with allelopathic compounds against nematode pests. A thorough review on this subject was published by Kokalis-Burelle and Rodriguez-Kabana (2006). Some of the most well-known plants with allelopathic compounds against plant-parasitic nematodes include American joint vetch (*Aeschynomene sp.*), bahia grass (*Paspalum spp.*), castor bean (*Ricinus communis*), marigold (*Tagetes spp.*), hairy indigo (*Indigofera hirsuta*), horse bean (*Canavalia ensiformis*), partridge pea (*Cassia fasciculata*), sesame (*Sesamum indicum*), showy crotalaria (*Crotalaria spectabilis*), sorghum-sudan grass (*S. bicolor* × *S. vulgare* var. *sudanense*), sudan grass (*Sorghum vulgare* *vulgare*)...
var. *sudanense*), sunn hemp (*Crotalaria juncea*), velvet bean (*Mucuna deeringiana*), and vetch (*Vicia spp.*). In Hawaii, several cover crops examined to be effective against root-knot nematodes and that are easy to grow are sunn hemp (*Crotalaria juncea*) and marigold (*Tagetes spp.*) both have a detrimental effects on root-knot nematodes (Wang *et al.*, 2011). Other nematode suppressive cover crops suitable in tropical climate are sesame (*Sesamum indicum*) and sorghum × sudangrass DeKalb ST6E (*Sorghum bicolor × S. bicolor* var. *sudanense*) (Sipes and Arakaki, 1997).

Fallow is another cultural practice that can reduce population densities of plant-parasitic nematodes. It involves leaving the field without a crop in between cropping cycles for a period of time to deplete the food source for the parasitic nematodes (Cadet *et al.*, 2005). To be more efficient, some would combine fallow with soil solarization by mulching the fallow field with transparent polyethylene mulch for 4-6 weeks (Katan *et al.*, 1976). Soil solarization heated up the topsoil layer and was found to be effective to reduce *M. incognita* in sandy soil (Wang and McSorley, 2008). Unfortunately, in tropical areas that have high clay content like in Hawaii, soil solarization alone fails to suppress plant-parasitic nematodes satisfactorily. However, when integrating nematode allelopathic cover crops with soil solarization, population densities of reniform nematode (*Rotylenchulus reniformis*) were suppressed significantly towards the end of a cowpea (*Vigna unguiculata*) crop (Marahatta *et al.*, 2012).

Vermicompost tea prepared from leafy vegetable and fruit wastes decomposed by specific composting earthworms comprise compost commonly known as vermicompost. There are two methods to extract the vermicompost tea, aeration and non-aeration. Aerated compost teas include all of organisms in the original compost (Igham, 2005). The
process involved in making vermicompost tea is the ratio of 1:10 compost to water and aeration for 24 hours. The vermicompost tea is then strained and used as a drench or foliar spray. The beneficial microorganisms that were present in the original compost are in the tea when aerated, thus helping the plants. According to Igham, species diversity in good vermicompost can be as high as 25,000 species (2005). Vermicompost tea is a recently developed non-chemical approach found to be suppressive against plant-parasitic nematodes (Arancon et al., 2003; Arancon et al., 2007). However, field trials conducted in Hawaii using chicken manure based vermicompost tea to drench rhizosphere of zucchini (Cucurbita pepo) only suppressed reniform nematodes early in the season but not toward the end of the zucchini crop (Wang et al., 2014).

Beside cultural practices, introduction of biological control agents is another approach that had been investigated by many nematologists. Biological control consists of using natural enemies to manage a target pest. A detailed review on nematode biological control agents was published in a book by Stirling (1991) and more recently a thorough review by the same author was published on the progress on the applied use of various nematode biological control agents (Stirling, 2014). Among which, Paecilomyces, Pochonia chlamydosporia, and Pasteuria showed the most promising results and had been commercialized as biocontrol agents in certain regions in the world (Atkins, et al., 2003). Paecilomyces lilacinus was listed as a potential biological control as it is particularly pathogenic to root-knot nematodes, harmless to other animals and plants, and can be easily cultured in the laboratory (Sasser et al., 1983).

Another group of nematode biological control agents that have long been studied but are mostly limited to be used for conservation biological control (enhancement of
indigenous biological control agents in an agroecosystem through cultural practices) are
the nematode-trapping fungi (NTF). These fungi produce trapping structures such as
adhesive knobs, nets, branches, constricting or non-constricting rings (Duddington, 1954)
that could trap plant-parasitic nematodes as well as free-living nematodes. For example,
*Arthrobotrys oligospora* is a commonly found nematode-trapping fungus, which produces
adhesive nets (Zouhar et al., 2013). *Arthrobotrys dactyloides* produces constricting rings
that trap nematodes with well studied trapping behaviors (Farrell et al., 2006)

This thesis research will explore less studied nematode biocontrol agents, fungi that
naturally produce nematode allelopathic toxin. This type of biocontrol agent would require
less manufacturing for mass production, but rather recycling of farm waste from
commercial mushroom growers. The benefit of investigating mushroom compost for
nematode management include 1) avoiding the labor intensive or costly mass production
of biocontrol agents; 2) added value to mushroom production; 3) providing alternatives to
synthetic nematicides.

**Nematode management with mushroom compost**

Several edible mushrooms are well known for their nematode antagonistic capabilities
(Kwok, 1992; Luo et al., 2007; Palizi et al., 2009). Oyster mushrooms (*Pleurotus ostreatus*)
exude toxin droplets from the fungal hyphae, containing a toxin known as trans-2-
decenedioic acid (Kwok, 1992). This toxin paralyzes the nematode upon contact, which
allows the hyphae to colonize and digest the nematode (Thorn and Barron, 1984). Studies
of oyster mushroom effects on nematodes have been predominantly conducted *in vitro.*
The only literature available that tested oyster mushroom compost for nematode
management in greenhouse pot experiment was based on straw grown mushrooms against sugar beet cyst nematodes, *Heterodera schachtii* (Palizi *et al.*, 2009). Their result indicated that 3% of oyster mushroom compost would be sufficient to suppress 85% of sugar beet cyst nematodes in greenhouse soil. This is equivalent to 15 tons/acre (16.5 tons/ha) of mushroom compost amendment. This would be too cumbersome to gather and apply in a commercial field situation. Clearly, more research needs to be conducted to apply mushroom compost for nematode management in a feasible manner.

**Mechanisms of nematode suppression by mushroom**

Several mushroom species known to suppress plant-parasitic nematodes beside oyster mushroom included shaggy mushroom (*Coprinus comatus*), Shiitake (*Lentinula edodes*), *Nematoctonus concurrens*, and King Stropharia (*Stropharia rugosoannulata*). These fungi kill the nematodes using different mechanisms as reviewed below. In general, after killing the nematodes, these nematophagous fungi will decompose the nematodes and consume the nematodes. Fungi destroy nematodes in virtually all soils when present. Fungi convert nematode materials into plant available nutrients (Mankau, 1980).

**Oyster mushroom:** Several species of *Pleurotus* in the *Pleurotaceae* family are able to inhibit nematode population growth (Palizi *et al.*, 2009). Among which, gray oyster (*Pleurotus ostreatus*) is most potent. It produces a toxin to paralyze the nematodes when contact occurs and is capable of killing the nematodes within 24-48 hours. The nematodes gather around the fungal colony and become inactive. Directional hyphae are involved in colonizing the paralyzed nematodes because of the precision in which it enters the nematode (Mamiya *et al.*, 2005). *Pleurotus eryngii* also known as the king oyster mushroom
has been used as an antihelminthic drug. A water extract of *P. eryngii* have shown 95% effectiveness in controlling *Hymenolepis nana*, also known as dwarf tapeworm (Samsam-Shariat, *et al.*, 1994).

*Nematode murderer: Nematoctonus* is another genus in the family of *Pleurotaceae*. Literally, Nematoctonus means nematode murderer. As its name implies, this genus of fungus is an efficient nematode killer. Among this genus, *Nematoctonus concurrens* have conidia that produce toxins that immobilize nematodes within 24 hours. Inactivation can be seen before penetration of the fungal hyphae. Giuma *et al* (1973) had demonstrated that the toxin was thermodynamic and suggested that it is a polysaccharide (Barron, 1977). *Nematoctonus* also have distinct hourglass shaped adhesive knobs that exude a sticky substance at maturity. These knobs are strongly anchored to the hyphae. Many nematodes rip their own cuticle by trying to escape (Barron, 1977).

*Shaggy mushroom: Coprinus comatus* produces spiny ball-like structures, and damage the nematode mechanically once the nematodes come into contact with the spiny balls (Luo *et al*. 2007). In addition to producing mechanically damaging structures, *C. comatus* also produce seven toxins that aid in the immobilization of nematodes.

*Shiitaki mushroom: In an in vitro test, Lentinula edodes* penetrated the nematode body 5 days after nematodes were introduced to the fungal plate. The nematodes swarm to the edge of the hyphal mat but would then be paralyzed. This is a relatively weak toxin to the nematodes as compared to *Pleurotus ostreatus*, which is capable of killing the nematodes within 24-48 hours (Mamiya *et al*., 2005).

*King stropharia:* This fungus also known as garden giant, burgundy mushroom, or king stropharia (Japanese: *saketsubatake*) is an agaric of the family *Strophariaceae*. Although
many species in this family are considered non-edible, the species *Stropharia rugosoannulata* is edible and can immobilize nematodes by producing finger-like projections known as acanthocytes. These structures are spiny and damage the nematode mechanically (Luo *et al*., 2006).

Among these mushroom species known to be nematophagous or able to immobilize nematodes, *Pleurotus* shown to possess the greatest potential to be used as cultural practice against plant-parasitic nematodes in Hawaii. This is due to the relatively rapid kill of nematodes by *Pleurotus sp.* (Thorn and Barron, 1984), as well as its compost waste, which is commercially available in Hawaii. Thus, this thesis research will mainly focus on examining the potential of mushroom compost waste of *Pleurotus* against root-knot nematode, *Meloidogyne incognita*, one of the most commonly encountered plant-parasitic nematodes by basil farmers in Hawaii (Hamasaki *et al*., 1994).

**Use of mushroom compost for nematode suppression**

Previous studies on effect of mushroom for nematode suppression have been conducted *in vitro* on potato dextrose agar (PDA) plates. Fungi were grown on agar plates and then the nematodes were added to test for nematicidal, nematostatic, or nematophagous effects of the fungi (Barron, 1977; Luo *et al*., 2006). Common mushroom substrates used to produce oyster mushrooms include straw, sawdust, logs and stumps of broad leaf trees (Stamets, 1993). Most recently, some literatures suggest that coffee (*Coffea arabica*) grounds are an easy substrate to work with for oyster mushroom cultivation (Wall, 2004). Wall (2004), who is a researcher at the University of Guam, has shown that oyster mushrooms were produced more efficiently on coffee grounds and filter paper than
the other substrates such as shredded office paper and newspaper. Commercialized oyster mushroom kits use spent coffee grounds as oyster mushroom growing substrate and are available from Back to the Roots, Oakland, CA. While this thesis research is not about screening mushroom substrates for best mushroom growth, the outcome of this research would provide more incentive for farmers or home gardeners to recycle some kitchen or yard waste for mushroom cultivation and subsequently recycle the spent mushroom compost back in the gardens or farms for nematode suppression.  

There are several approaches to use mushroom compost for nematode suppression. The only published method to use mushroom compost in vivo is by direct incorporation of the mushroom compost into soil at 3% (w/w) and suppressed more than 85% of sugar beet cyst nematode (Heterodera Schachtii) (Palizi et al., 2009). Although direct incorporation of the mushroom substrate into the soil could ensure direct contact of the mushroom mycelia with the root system, the amendment rate needed for nematode suppression using this approach could be unfeasible in field- or even garden-scale.  

To ensure a more consistent performance of nematode suppression using mushroom compost, this thesis research explored mixing mushroom compost into organic based potting mix used for growing basil seedlings. It was anticipated that seedlings would be exposed to the fungi at an early stage allowing the fungi to establish prior to introduction to the field conditions. It was hypothesize that this would provide a layer of protection for the basil roots from nematode infection.  

The second approach examined in this thesis was to explore the potential of extracting toxic substances from mushroom compost against root-knot nematodes, here by referred
to as mushroom compost water extract (MCWE). The application of this water extract was applied by drenching the soil surface of the plants, adding a post-plant benefit.

**Mushroom compost water extract**

Making mushroom compost waste into mushroom compost water extract (MCWE) provides another approach to apply mushroom compost waste into agroecosystem. Preparing compost water extract is a relatively new organic farming practice by suspending compost in water. This extract might contain nutrients and diverse organisms, and is normally applied as a root drench or foliar spray (Xu et al., 2012). Drenching plants with water extract of food-waste based vermicompost has been demonstrated to promote plant and soil health (Pant et al., 2011). One hypothesis to be examined is that MCWE prepared from oyster mushroom compost could provide a means of extracting toxins, nutrients and fungal propagules associated with the oyster mushroom compost. Drenching MCWE to the root system of a crop would allow for periodic post-plant application, thus extending the application time for applying mushroom compost waste into a crop, especially for a relatively long-term crop like basil which can typically be grown and harvested for 9 -12 months.

**Use of mushroom compost waste for soil health enhancement**

Another benefit of introducing spent oyster mushroom compost into agroecosystem is its potential to enhance plant and soil health. Soil health can be defined as the capacity of the soil to sustain biological activity, promote environmental quality and focus on plant and animal health (Doran et al., 1996; Wang and McSorley, 2005). Common attributes that a
healthy soil should have include the ability to support life processes by retaining optimal water and soil properties. Soils play a major role in transforming sunlight, stored energy, and recycling matter to plants and animals (Doran et al. 1999). The healthy soils are also able to maintain microbial diversity, support the food web, remediate pollutants, and sequester heavy metals (Wang and McSorley, 2005).

The improvement of plant health can be achieved by maintaining the soil ecosystem. Organic matter has been a basic addition to the soil ecosystem as it can improve crop yield and possibly suppress soil pests (Wang and McSorley, 2005). Nematodes have been known to regulate the rates of decomposition and affecting growth and the metabolic activities of the microbes (Neher, 2001). Free-living nematodes are cosmopolitan organisms that have been proven to be good soil health indicators to reflect dominant decomposition pathways, and structure and function of soil food web (Bongers and Ferris, 1999; Ferris et al., 1997; Freckman, 1988; Ingham et al., 1985; Wang et al., 2004). Nematodes have diverse feeding behaviors, life strategies, and can survive in varying environments (Wang and McSorley, 2005). Free-living nematodes feed on microbes in the soil and affect soil nutrient mineralization and the decomposition pathways in the soil (Wang et al., 2004; Ingham et al., 1985). Based on their oral structure, nematodes are classified into trophic groups, such as bacterivores, fungivores, omnivores, predators, and herbivores (Yeates et al., 1993). Bongers and Bongers (1998) and Bongers and Ferris (1999) further assign the nematodes based on their life strategies into colonizer-persister (c-p) continuum in a scale of 1-5. Ferris et al. (2001) computed the sum of weighted abundance of nematodes in guilds (trophic groups × c-p value) to calculate enrichment, structure, and channel indices. These
indices have been widely used to evaluate soil health conditions affected by different soil treatments (Wang and McSorley, 2005).

When organic matters are added into the soil, the organic residues need to be broken down (mineralized) to release nutrients trapped in the residues in order for plants to uptake. There are two decomposition channels that are active in the soil food web, the faster bacterial channel and the slower fungal-based channel (Wang and McSorley, 2005). Bacterivorous nematodes feed on bacteria, thus mineralize nutrients tied up in bacteria. Similarly, fungivorous nematodes feed on fungi, thus mineralize nutrients stored in fungi. Calculations of fungivores/bacterivores ratios would indicate if the soil food web is dominated by bacteria decomposition or fungal decomposition (Freckman, 1988). Omnivorous and predatory nematodes prey on bacterivorous and fungivorous nematodes, thus allowing more nutrients to be released. The abundance of many free-living nematodes correlates with the concentrations of soil nutrients (Wang, et al., 2004). Free-living nematodes occupies key positions in the soil food web (Bongers and Ferris, 1999). Ingham et al. (1985) showed that additional presence of bacteria and fungi and their respective predators in the soils resulted in better soil health and plant growth compared to soils without the presence of microbial-grazing nematodes.

This project will evaluate the efficacy of adding spent oyster mushroom compost to enhance soil health conditions. It is anticipated that mycelia from the oyster mushroom compost would provide a food source for fungivorous nematodes, thus stimulating fungal decomposition pathways. Through the enhancement of the initial soil food web, a more structured soil food web can be established.
Objectives of thesis

The overall goal of this thesis research is to examine the potential of using spent oyster mushroom compost waste for managing nematodes. Specific objectives of this project were to:

1. determine the amendment rates of spent *Pleurotus* spp. compost in potting medium to suppress root-knot nematodes (*M. incognita*);
2. determine concentrations of mushroom compost water extract for nematode suppression; and
3. evaluate a spent oyster mushroom compost based technology for pre- and post-plant nematode management (plant-parasitic and free-living nematodes) in field trials.
Literature Cited


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Chapter 2

Effects of oyster mushroom compost waste against root-knot nematode *Meloidogyne incognita*

Abstract

Oyster mushroom (*Pleurotus* spp.) produces an allelopathic compound that suppresses plant-parasitic nematodes. Greenhouse and laboratory experiments were conducted to determine the effects of oyster mushroom compost against root-knot nematode, *Meloidogyne incognita*. Spent oyster mushroom compost was amended into sterile sand: soil 1:1 (v/v) mix at 0, 0.25, 0.5, and 1% in a greenhouse basil (*Ocimum basilicum*) pot trial. The oyster mushroom compost amendment did not suppress *M. incognita* reproduction and root penetration in this greenhouse trial. Oyster mushroom compost amendment was then amended into yard waste compost at 0, 33, and 50% (w/w) in Cone-tainer soil tubes. Oyster mushroom compost amendment suppressed *M. incognita* at 33 and 50% rates compared to unamended (*P* ≤ 0.05). The suppression was more effective if incubated for > 7 days than 1 or 4 days (*P* ≤ 0.05). Two additional trials showed that spent compost of *P. ostreatus* and *P. eryngii* can suppress *M. incognita* in the yard waste compost media at 1 or 2% amendment rates, but not the *M. incognita* root penetration. When *P. ostreatus* compost waste was tested in a peat moss: perlite (1:1 v/v) mix, root penetration of *M. incognita* into zucchini (*Cucurbita pepo*) was lower in 1 and 33% amendment rates than the unamended control (*P* ≤ 0.05), but it did not suppress the nematodes in the media. When oyster mushroom compost was prepared into mushroom compost water extract (MCWE), it reduced mobility of *M. incognita* compared to the water control. However, this effect was
nematostatic rather than nematicidal when the nematodes were incubated in the solution for 2-5 days, only achieving 20% suppression of the viability of *M. incognita* at 50% MCWE. A second trial where J2 of *M. incognita* was incubated for 7 days, MCWE suppressed 22 and 41% viability of *M. incognita* at as low as 10 and 25% concentration, respectively.

**Introduction**

Many nematophagous fungi have been recognized to be able to prey on nematodes and shown to have potential as biological control agents against plant-parasitic nematodes (Stirling, 2014). These fungi can be categorized into nematode egg parasites, female or cyst parasites, nematode-trapping fungi, or endoparasitic fungi. Thorough reviews of the fungi have been published by Chen and Liu (2005), Kerry (1984), Siddiqui and Mahmood (1996), Mamiya et al. (2005), Zouhar et al. (2013), Luo et al. (2006), and Stirling (2014). Reports indicate that mycorrhizal fungi can protect plant roots from root-knot nematode infection (Grandison and Cooper, 1986; Stirling, 2014). Another group of fungi with nematode suppressive capabilities belongs to the order Agaricales that are mostly edible mushrooms. Some of these mushrooms produce nematode-trapping structures like the *Nematoctonus* (teleomorph: *Hohenbuehelia*) that produces hourglass knobs (Barron and Dierkes, 1977), and *Pleurotus ostreatus* (oyster mushroom) that produces adhesive knobs (Saikawa and Wada, 1986) to capture their nematode prey. However majority of this group of fungi produce toxins or enzymes to paralyze or digest their nematode preys. For example, *P. ostreatus* produces a mycotoxin (trans-2-decenedioic acid) that can immobilize nematodes (Kwok *et al.*, 1992), and penetrate, colonize and digest the prey within 24 hours (Thorn and Barron, 1984). *Hohenbuehelia* and *Resupinatus* are fungi closely related to Pleurotaceae
that also have nematode antagonistic effects (Heydari et al., 2006). Shaggy mushroom 
(Coprinus comatus), Shiitake (Lentinula edodes), and King Stropharia (Stropharia 
rugosoannulata) are other agaric mushrooms known to suppress plant-parasitic 
nematodes (Luo et al., 2006; Mamiya et al., 2005; Luo et al., 2007). This project focused on 
examining the biological control potential of P. ostreatus and P. eryngii against M. incognita.

A common challenge of introducing nematode biological control agents into the field is the mass production of the biological control agents (Mankau, 1980). Fortunately, the mass production of oyster mushrooms, such as P. ostreatus and P. eryngii, has already been 
developed for commercial mushroom production. The allelopathic compounds of the 
oyster mushroom are excreted by the mycelium. Since oyster mushroom farmers harvest 
only the fruiting bodies, the spent oyster mushroom compost is readily available after 
mushroom harvest. This research examined two approaches to use spent oyster mushroom 
compost waste for nematode management: as soil an amendment or mushroom compost 
water extract.

Previously, Palizi et al. (2009) showed that straw grown oyster mushroom compost 
amended into soil at 3% suppressed 85% of sugar beet cyst nematodes (Heterodera 
schachtii) in a greenhouse pot experiment. The 3% amendment rate is equivalent to 16.5 
tons of mushroom compost/ha, a cumbersome amount to transport and accumulate. Thus, 
one approach of this project is to examine the effect of using spent oyster mushroom 
compost as transplant media amendment. Research is needed to determine optimal 
amendment rates for suppression of plant-parasitic nematodes.

Another approach to deliver the oyster mushroom compost for nematode suppression 
is through application of mushroom compost water extract (MCWE) as a soil drenching
solution. The compost water extract technique with an aerator used by organic farmers to prepare vermicompost tea (Pant et al., 2011) could provide a means to extract allelopathic compounds from the oyster mushroom compost.

Specific objectives of this research were to 1) determine amendment rates of spent Pleurotus spp. compost for transplant media against M. incognita, and 2) determine concentrations of MCWE that could suppress M. incognita.

Materials and Methods

Greenhouse Trial I: Spent mushroom compost of P. ostreatus was obtained from a commercial oyster mushroom growing kit (Back to the Roots. Oakland, CA) grown on brewed coffee grounds. Freshly brewed, cool coffee grounds (250 g) and 15 grams of the commercial oyster mushroom compost from the growing kit were bagged into plastic bags (12.7 cm x 25.4 cm clear plastic cellophane) with a 5-cm diameter hole covered with cotton filter for air exchange. Once the bags were colonized by P. ostreatus (approximately after 2 months), bags were poked with a sterilized needle to initiate fruiting bodies. After two mushroom flushes were harvested, the bag of spent oyster mushroom compost was used as soil amendment in a greenhouse experiment.

A total of 25 pots of 15-cm diameter plastic pots containing 1:1 sterile sand: soil mix (v/v) was prepared. Pots were amended with mushroom compost at 0.25, 0.5, or 1% (w/w) of soil weight. Two additional treatments received either no coffee grounds or 1 % coffee grounds without the oyster mushroom mycelia were included. The experiment was arranged in a completely randomized design (CRD) with 5 replications. Four-week old basil seedlings were transplanted into each pot and inoculated with 200-second stage juveniles
(J2) of *M. incognita* cultivated on coleus (*Plectranthus* spp.) and tomato (*Solanum lycopersicum*) in the greenhouse. Basil (*Ocimum basilicum*) was grown for 2.5 months after nematode inoculation with a slow released fertilizer (Osmocote Smart Release Plant Food Flower and Vegetable 14-14-14, Marysville, OH) and irrigation applied as needed.

At 2.5 months after nematode inoculation, shoot and root weights, and stem diameter at the base of each basil plant were measured. Potting media from each pot were composited and 250-cm³ soil subsamples were collected, and nematodes extracted using elutriation and centrifugal floatation (Byrd *et al.*, 1976; Jenkins, 1964). Nematode eggs from roots in each pot were extracted using NaOCl method (Hussey and Barker, 1973). Eggs were incubated in a Baermann tray (Whitehead and Hemming, 1964) for 1 week, and hatched J2 were counted. Numbers of *M. incognita* extracted were counted under an inverted microscope (Leica DM IL LED, Wetzlar, Germany).

**Greenhouse Trial II:** A 3×2 (compost amendment × nematode inoculum) factorial designed greenhouse pot experiment was established in Gilmore Greenhouse, University of Hawaii. The compost amendment treatments include *P. ostreatus* mushroom compost amended at 0, 1, and 2% (w/w) into the potting medium. The potting medium was sterile sand: soil 1:1 (v/v) plus 4% (w/w) yard waste compost (Hawaiian Earth Product, Ewa Beach, HI). Each pot was either inoculated or not inoculated with 600 J2 of *M. incognita*. The experiment was arranged in a CRD with 5 replications. Thus, a total of 30 pots of 15-cm diameter paper pots were used.

**Cone-tainer Experiment I (determine optimum incubation time):** The performance of oyster mushroom compost waste against *M. incognita* was examined again in a medium that contained higher organic matter, yard waste compost (Hawaiian Earth Product, Ewa
Beach, HI). Thirty Cone-tainers (Ray Leach Cone-tainer, Corvallis, OR) (4-cm in diameter and 13.5-cm tall) were filled with Hawaiian Earth Product compost amended with spent *P. ostreatus* mushroom compost at 0, 33, or 50% (w/w). Cone-tainer tubes were arranged in completely randomized design with 3 replications in a laboratory. Eighty *M. incognita* J2 were introduced into each tube and incubated for 1, 4, 7 and 30 days. Nematodes were extracted from each Cone-tainer at the end of the incubation period using Baermann trays (McSorley and Frederick, 1998). Nematodes were collected after 7 days and counted under an inverted microscope (Leica DM IL LED, Wetzlar, Germany). To ensure all nematodes were extracted from the compost, the compost was further processed using rolling, sieving and centrifugal flotation (Jenkins, 1964). Nematodes from the Baermann tray and Centrifugal flotation methods were combined as total nematodes recovered from the media.

**Cone-tainer Experiment II (Comparison of two Pleurotus species):** Nematode suppressive effects of spent oyster mushroom compost of *P. ostreatus* and *P. eryngii* were compared in Hawaiian Earth Products yard waste compost amended at 1, 2, 33, and 50% (w/w). These spent oyster mushroom compost were obtained from Hamakua Heritage Farm (Hamakua Heritage Farm, Laupahoehoe). The substrate mix includes eucalyptus sawdust, wheat bran, and corncobs. A control containing only the yard waste compost without spent oyster mushroom compost was included. This was a 2 ×5 (*Pleurotus* × amendment rate) factorial designed experiment arranged in CRD with 4 replications. A ‘Felix’ zucchini (*C. pepo*) (Harris Seeds, New York) seed was planted into each tube, 200 J2 of *M. incognita* were inoculated into each tube at 10 days after germination. The experiment was terminated one week after inoculation to examine *M. incognita* root penetration using acid fuchs in (Daykin
Nematodes were extracted from the compost media using rolling, sieving and centrifugal flotation (Jenkins, 1964).

**Cone-tainer III:** Cone-tainer III experiment was a repeat of Cone-tainer II except that the experiment was terminated 2 weeks after *M. incognita* inoculation, and only spent *P. eryngii* substrate from Hamakua Heritage Farm was tested.

**Cone-tainer IV:** Cone-tainer IV experiment was similar to the Cone-tainer III except peat moss: perlite mix (1:1 v/v) commonly used by growers as transplant mix was used as the growing medium. Spent *P. ostreatus* compost was used as an amendment. *C. pepo* shoots and roots were harvested 2 weeks after nematode inoculation and weight were recorded. Roots were stained using acid fuchsin (Daykin and Hussey, 1985) to examine for nematode penetration rate. Nematodes were extracted from the transplant media of each cone-tainer by elutriation (Byrd *et al.*, 1976) and centrifugal floatation (Jenkins, 1964).

**MCWE I:** Effects of mushroom compost water extract (MCWE) on *M. incognita* were examined *in vitro* by incubating the nematode in 25, 33, and 50% (w/w) MCWE in 6-cm diameter petri dishes. The spent oyster mushroom compost was obtained from laboratory grown *P. ostreatus* using coffee grown described in Greenhouse Trial I. MCWE was prepared by aerating the designated amount of oyster mushroom compost in water for 24 hours with an aquarium pump (Scheuerell and Mahaffee, 2002). Water was used as an untreated control. Fifty *M. incognita* J2 were incubated into 5 ml of the test solution per dish for 1, 2, 3, 4, or 5 days. The experiment was a 4×5 (MCWE concentration × incubation time) factorial design with 3 replications, a total of 60 petri dishes were examined. Active and inactive nematodes were determined by probing nematodes at the end of the incubation period. If a nematode moved it was considered active. After evaluating
nematode mobility, all nematodes were washed by transferring into water using 625-mesh screen, and incubated in water for another day and reexamined for mobility.

**MCWE II:** A second MCWE assay was conducted using a 0.25-ml microdish and viability of *M. incognita* was determined with the aid of Medola’s Blue stain (Pfaltz and Bauer Inc., Waterbury, CT). Approximately 50 J2s of *M. incognita* were incubated in 0, 10, 25 and 50% MCWE (w/w) with 1% Medola’s Blue for 7 days. Each treatment had four replications. Numbers of *M. incognita* in each micro-dish were counted initially (right after nematodes were added), and their viability was determined 7 days after incubation. Clear colored nematodes were viable, whereas blue stained nematodes were non-viable. MCWE or Medola’s Blue solution was then carefully removed from the top of each dish by pipetting as much as possible without removing the nematodes. Water was then added to each dish and nematodes were washed into a BPI dish (approximately 500μl of water per dish) to count stained and cleared nematodes. These nematodes were left in water in the BPI dishes for another 24 hours before recounting to determine % viability.

*Statistical analysis:* All data collected from each experiment were checked for normality using Proc Univariate (SAS Institute, Cary, NC). Parameters that were not normally distributed were transformed as suggested by Steel and Torrie (1980). Most of the nematode abundance were log-transformed (log10[x+1]) before statistical analysis. Data from the Greenhouse Trial I were subjected to one-way analysis of variance (ANOVA) followed by Waller-Duncan multiple range test using Proc GLM (SAS, Institute, Cary, NC). No statistical analysis was conducted for Greenhouse Trial II as no nematodes were recovered at the end of the experiment. Data from Cone-tainer I was analyzed by 3×4 (amendment rate × incubation time) factorial ANOVA whereas data from Cone-tainer II
were subjected to 5×2 (compost amendment rate × Pleurotus spp.) factorial ANOVA. If significant interaction occurred between the factors, data in each subplot were subjected to one-way ANOVA. Data from Cone-tainer III and IV were subjected to one-way ANOVA. Only untransformed means are presented. Means were separated using the Waller-Duncan k ratio ($k=100$) $t$-test when the treatment effects were significant.

Data from MCWE I experiment were subjected to 4 × 5 (MCWE concentration × days of incubation) factorial ANOVA using Proc GLM, whereas data from the MCWE II experiment were subjected to one way analysis of variance. Means were separated using the Waller-Duncan k ratio ($k=100$) $t$-test when the treatment effects were significant ($P \leq 0.05$).

Results

Greenhouse Trials: Spent P. ostreatus mushroom compost as a soil amendment in Greenhouse Trial I did not affect basil shoot and root weights ($P > 0.05$), but increased ($P \leq 0.05$) the reproduction of M. incognita (Fig. 2-1). The numbers of J2/g root were higher ($P \leq 0.05$) in the plants amended with the mushroom compost than in the unamended control. An amendment rate at 0.5% had more nematodes in the soil compared to the two controls (1% coffee grounds and unamended control). All oyster mushroom compost amendment rates increased the number of eggs/g root compared to the controls ($P \leq 0.05$). The highest number of nematodes was in the 1% mushroom amendment (Fig. 2-1). Shoot and root weights were not affected by oyster mushroom compost amendment.

Unfortunately, no nematodes were recovered from basil roots and soil at termination of Greenhouse Trial II. Thus, no data were reported from Trial II.
Fig. 2-1 Effects of different amendment rates of spent *Pleurotus ostreatus* compost (using coffee grounds as substrate) against *Meloidogyne incognita* A) J2 in soil, and B) eggs in roots of basil planted in sterile sand-soil mix in Greenhouse Trial I. Coffee grounds amended at 1% (w/w) and unamended potting mix are served as controls. Means are an average of 5 replications. Columns followed by same letters are not different according to Waller-Duncan k ratio (k=100) t-test.

*Cone-tainer I:* Based on the 3 × 4 ANOVA, no interaction between amendment rate and days of incubation were observed (*P* > 0.05). Spent *P. ostreatus* compost amendment at ≥ 33% suppressed *M. incognita* compared to the control (*P* ≤ 0.05). Regardless of the mushroom compost amendment rates, this mushroom compost amendment achieved 100% suppression of the mobility of *M. incognita* in the media if incubated for ≥ 7 days (Fig. 2-2).
Effects of mushroom compost of *Pleurotus ostreatus* A) amendment rate, and B) incubation time with *Meloidogyne incognita* using yard waste compost as potting medium in Cone-tainer Experiment I. Means are average of 5 replications. Columns followed by same letters are not different based on Waller-Duncan $k$ ratio ($k=100$) $t$-test.

*Cone-tainer II*: When zucchini seedlings were planted into the Cone-tainer with yard waste compost, mushroom compost amendment of both *P. eryngii* and *P. ostreatus* did not suppress root penetration of *M. incognita* ($P > 0.05$) but had fewer nematodes recovered in the media compared to the unamended control ($P \leq 0.05$; Fig 2-3A, B). Regardless of *Pleurotus* species, amending mushroom compost at 2, 33, and 50% resulted in higher zucchini root weight compared to the untreated control ($P \leq 0.05$; Fig 2-3C). Root weight of zucchini was higher in media amended with spent *P. eryngii* compost than in the unamended control ($P \leq 0.05$; Fig 2-3D).
Fig. 2-3. Effects of oyster mushroom compost amendment rates and species on A, B) *Meloidogyne incognita* extracted from roots (Root) or media by Elutriation, and C, D) zucchini root weight in Cone-tainer II Experiment. Two species of oyster mushroom examined are *Pleurotus eryngii* and *P. ostreatus*. Means are average of 4 replications. Columns followed by same letters are not significantly different based on Waller-Duncan k ratio ($k=100$) $t$-test.

*Cone-tainer III:* No *M. incognita* were recovered from the media amended with 2, 33, and 50% of *P. eryngii* compost, significantly lower than the number of nematodes recovered from the media amended with 1% mushroom compost or unamended ($P \leq 0.05$; Fig 2-4A). However, zucchini shoot weights were lower in media amended with 33 and
50% of *P. eryngii* compost than that amended with 2% or the unamended control (*P* ≤ 0.05; Fig 2-4B).

![Graph A](image)

**Fig. 2-4.** Effects of oyster mushroom compost amendment rates on A) numbers of *Meloidogyne incognita* recovered in yard waste compost medium, and B) zucchini shoot weight in Cone-tainer III Experiment. Means are an average of 8 replications. Columns followed by the same letter are not significantly different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.
Cone-tainer IV: Numbers of *M. incognita* penetrated into zucchini roots were only lower in media amended with 1 and 33% *P. ostreatus* compost than the unamended tubes (*P* ≤ 0.05, Fig 2-5). Oyster mushroom compost amendment did not affect nematodes in the media or zucchini shoot and root weights.

![Graph showing effects of compost amendment on root penetration of Meloidogyne incognita](image)

**Fig. 2-5.** Effects of oyster mushroom compost amendment rates on root penetration of *Meloidogyne incognita* in zucchini, Cone-tainer IV Experiment. Means are an average of 4 replications. Columns followed by the same letter(s) are not significantly different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

**MCWE assays:** No significant interaction (*P* > 0.05) occurred between MCWE concentrations and days of incubation on the final numbers of inactive *M. incognita* before washing (Fi) and after washing (Wi). All MCWE treatments (25, 33, and 50%) reduced % Fi (Fi/total initial number) and % Wi (Wi/total initial) compared to the water control (0% MCWE) (*P* < 0.01, Fig. 2-6A). Concentration of MCWE at 50, 33 and 25% had immobilized 55, 47, and 37% *M. incognita* before washing, respectively, significantly higher than 11% in
the water control ($P \leq 0.05$; Fig. 2-6A). However, most *M. incognita* became active again 1-day after removal from MCWE by washing in water. Only the 50% MCWE had higher % nematode inactive than the water control ($P < 0.05$; Fig. 2-6A). Incubation of *M. incognita* in MCWE for ≥ 2 days increased %Wi compared to 1 day of incubation ($P \leq 0.05$; Fig. 2-6B).

Fig. 2-6. Effects of oyster mushroom compost water extract (MCWE) on viability of *M. incognita* incubated A) in different concentrations of MCWE, or B) over different length of time in MCWE. Mobility and viability of *M. incognita* was examined by probing and observing under an inverted microscope before and after washing the nematodes in water. No difference among days after incubation before washing. Means are an average of 3 replications. Means of concentration (n=15) and time (n=12) followed by same letters are not significantly different based on Waller-Duncan k-ratio (k=100) t-test.
Fig. 2-7. Effect of concentration of *Pleurotus ostreatus* mushroom compost water extract (MCWE) on the viability of *Meloidogyne incognita* measured as the percent of active J2 over total J2 after transferring the nematodes from MCWE into water 7 days after incubation in MCWE Trial II. Means (n=4) with same letters are not significantly different based on Waller-Duncan k-ratio (k=100) t-test.

**MCWE II:** When J2s of *M. incognita* was incubated for 7 days in MCWE, 10% MCWE suppressed viability of *M. incognita* (numbers of colorless nematode/total numbers of nematodes dead or alive after washing) compared to the water control (*P* ≤ 0.05, Fig. 2-7).

**Discussion**

*Effects of oyster mushroom compost amendment on M. incognita*: The greenhouse experiment demonstrated that spent oyster mushroom compost amendment did not suppressed *M. incognita* when amended into sterile sand: soil mix. In fact, coffee grounds alone or spent oyster mushroom compost amendment in the coffee ground substrate
increased the abundance of *M. incognita* in the soil. Oyster mushrooms are normally cultivated in substrates with high organic matter (Aslam and Saifullah, 2013). Low organic matter in the sterile sand-soil mix might not be favorable for the *P. ostreatus* mycelia to establish.

Soil or potting media with higher organic matter could be more favorable for the mycelial growth of *Pleurotus* spp., and may support more mycotoxin production than media with low or no organic matter. Results from the four Cone-tainer experiments where oyster mushroom compost was examined in high organic matter media (yard waste compost or peat moss) supported this hypothesis. Oyster mushroom compost amended at 33 or 50% suppressed *M. incognita* in the yard waste compost media in Cone-tainer I, especially if the incubation time in the mushroom compost amendment was ≥ 7 days. Cone-tainer II and III experiments showed that amendment rates could be as low as 1 or 2% to suppress *M. incognita* when incubated for 7 or 14 days, respectively. Although Heydari *et al.* (2006) reported that *P. ostreatus* was the most suppressive among the five *Pleurotus* species tested (including *P. eryngii*). The suppressive effects of *P. eryngii* and *P. ostreatus* were equivalent in the Cone-tainer II experiment. Besides suppressing *M. incognita* in the soil, oyster mushroom compost amendment rates ≥ 2% could also increase zucchini root weight.

Difference in nematode suppressive effects by oyster mushroom compost amendment between the greenhouse and Cone-tainer experiments could also be due to differences in the mushroom growing substrates used. Spent coffee grounds were used in the greenhouse and Cone-tainer I experiments whereas a sawdust, wheat bran, and corncob mix were used in Cone-tainer II, III and IV experiments. The substrate could have affected the proliferation or establishment of the fungal mycelia. On the other hand, effects of oyster mushroom
compost amendment on root penetration by *M. incognita* varied from no suppressive effect in Cone-tainer II, increased penetration in Cone-tainer III (both using yard waste compost as media) to suppressive at 1 and 33% but not at 2 and 50% in Cone-tainer IV (using peat moss: perlite as media). More research is needed on the substrate source for *Pleurotus* spp. and the organic media that it is amended into, to understand the performance of spent oyster mushroom compost amendment against *M. incognita*

These results were deviated from *in vitro* and greenhouse experiments conducted by Palizi *et al.* (2009) where they found *P. ostreatus* grown on straw compost had suppressed populations of sugar beet cyst nematodes in soil as well as their root penetration into sugarbeet. Confined root systems in Cone-tainer soil tubes used in this study may have shortened the time for the fungi to paralyze and colonize the nematode prior to root penetration by the nematodes.

**Effects of MCWE on *M. incognita***: Heydari *et al.* (2006) tested culture filtrates from different *Pleurotus* species cultivated in a malt extract broth for their suppressiveness against *M. javanica* and found that compost extract from *P. ostreatus* culture was most suppressive to the nematodes compared to those from *P. eryngii*, *P. sajor-caju*, *P. florida*, and *P. cornucopiae*. Similar root-knot nematode suppressive effects were observed in the current research by using water to extract the mycotoxin from *P. ostreatus* (MCWE trials). However, the nematode suppressive effect is only nematostatic (paralyzing) rather than nematicidal (killing). Only the 50% MCWE suppressed 13% viability of *M. incognita* after washing the nematodes in water. However, if *M. incognita* J2 were continuously exposed to MCWE for more than 2 days, higher percentage (approximately 15%) of J2 would be suppressed as compared to the water control (~5%). There was a 26% increase in
percentage of inactive nematodes in 2 days of incubation compared to 1 day of incubation. When *M. incognita* J2s were incubated for 7 days in MCWE II, MCWE suppressed 22 and 41% of viability of *M. incognita* at as low as 10 and 25% concentration, respectively.

Although this nematicidal effect of MCWE was minimal, farmers could make use of MCWE as a soil drench by avoiding irrigation for at least 2 days after drenching. Heydari *et al.* (2006) incubated *M. javanica* in 20% *P. ostreatus* culture filtrates in malt extract broth and found 54% of nematodes paralyzed after 1 day. They cautioned that mycotoxin production in field soil or crop rhizosphere might be more sophisticated than incubating the target nematodes in MCWE in the lab. The current studies reveal that efficacy of MCWE in suppressing plant-parasitic nematodes could be affected by concentration and incubation time. It is most likely that extraction media (water vs malt agar broth), virulence of *P. ostreatus* mycelia, and frequency of MCWE drenching into the field could also affect the nematode antagonistic effect of oyster mushroom. Future research should investigate environmental factors affecting the delivery of MCWE for field nematode management.

**Conclusions**

Current greenhouse and Cone-tainer experiments suggested that use of spent oyster mushroom compost for suppression of *M. incognita* could provide certain levels of suppression against *M. incognita* in soil containing high organic matter. This nematode management strategy might be more effective when introduced to soil or growing media with higher organic matter. The implication of using spent oyster mushroom compost to amend potting or transplant mix is that farmers can provide protection for seedlings prior to planting into a plant-parasitic nematode infested fields. Amending the potting mix also
provides easier application of the spent oyster mushroom compost to the field especially when using peat moss: perlite mix as transplant media. Oyster mushroom compost amendment mainly suppressed nematodes moving in the soil or growing media rather than parasitizing *M. incognita* that penetrated the root system. Thus, cultural practices to enhance the establishment of the fungal mycelia in the soil would be critical in using oyster mushroom compost waste for field nematode management. Since MCWE is only nematostatic and not nematicidal. Thus, caution should be used when irrigating after the introduction of oyster mushroom compost water extract into the field.
Literature Cited


Chapter 3

Oyster mushroom compost waste for nematode management in a basil agroecosystem

Abstract

Two field trials were conducted to examine the effects of oyster mushroom (*Pleurotus ostreatus*) compost on plant-parasitic nematodes and soil health conditions on a basil (*Ocimum basilicum*) crop. A 2×2×2 (cover crop × amendment × drenching) split-split plot designed experiment was conducted. Basil seedlings with or without mushroom compost amendment (A) at 50% (v/v) were transplanted into field plots previously planted or not planted with buckwheat (*Fagopyrum esculentum*) as a cover crop (C). Half of the basil plants were either drenched or not drenched (D) with 25% mushroom compost water extract in Trial I. Trial II was similar to Trial I except that yard waste compost mulch (M) was used instead of planting cover crop. Oyster mushroom compost A and D did not suppress plant-parasitic nematodes in both trials. While cover crop increased abundance of bacterivorous and fungivorous nematodes throughout the cropping cycle and increased richness towards the end of the crop, oyster mushroom compost A and D only enhanced bacterial decomposition compared to the control early in the cropping cycle ($P < 0.05$), and D only increased nematode richness in the middle of the cropping cycle. However, A or D decreased structure index (SI) when no cover crop was planted in Trial I, indicating a more disturbed soil food web. On the other hand, A enhanced abundance of fungivores and omnivores and richness throughout the crop, and increased abundance of bacterivores towards the end of the crop in Trial II ($P < 0.05$) regardless of yard waste compost or drenching treatments. Mushroom A or D increased enrichment index (EI) and reduced
channel index (CI) towards the end of Trial II compared to no A and no D ($P < 0.05$). These indicated that the oyster mushroom compost improved soil nutrient enrichment, soil food web structure and overall soil health in Trial II. Difference in the performance of oyster mushroom compost between these two trials was discussed. Unfortunately effects of oyster mushroom compost on basil yield were complicated by the downy mildew disease, thus was inconclusive.

**Introduction**

An allelopathic compound in oyster mushroom has been known to suppress nematodes since the 1990s (Kwok, 1992). However most of these studies were conducted in laboratory settings (Satou et al., 2008; Thorn and Barron, 1984) or greenhouse pot conditions (Palizi et al., 2009; Okorie et al., 2011). Unlike most classical fungal biological control agents of plant-parasitic nematodes that require mass rearing of fungi, recycling of mushroom compost substrate from commercial mushroom producers could offer an alternative to labor intensive rearing technologies. This research project aimed to develop field application methods for nematode management using spent oyster mushroom compost waste.

Basil (*Ocimum basilicum*) is very susceptible to infection of root-knot nematodes (*Meloidogyne* spp.) (Vovlas and Troccoli, 2008). Basil is an economically important specialty crop in Hawaii with a farm gate value of $2.08$ million in 2012 (NASS, 2013). Typically, a basil crop is grown for 9 to 12 months before a new crop planting. The long-term crop production of basil makes post-plant nematode management very challenging. Root-knot nematodes have a life cycle of approximately 30 days. This allows the nematodes
to reproduce many generations on basil, and could reach economic threshold early in the crop cycle and shorten the production time.

Results from the laboratory assays (Chapter 2) indicated that a minimal of 1% oyster mushroom amendment is needed to reduce root-knot nematodes population in the media. An alternative approach was to introduce spent *P. ostreatus* substrate as an amendment in the transplant media. The idea was to allow the mycelium to colonize the rhizosphere prior to field planting, providing a first line of defense for the seedlings against plant-parasitic nematodes.

Increasing soil organic matter is a well-known method to enhance activities of fungal biological control agents (Hoffmann and Sikora, 1993; Perez- Rodriguez *et al.*, 2011; Khan *et al.*, 2012; Timper, 2014). Adding organic matter could increase the population densities of soil microbial-feeding nematodes, which are alternative hosts for nematode-trapping fungi (Linford, *et al.*, 1938, Oka, 2010; Timper, 2014). Therefore, the current research examined spent oyster mushroom in field plots with or without organic matter added. To further evaluate the potential of this mushroom compost in basil cropping systems, a root drenching solution was prepared by extracting the mycotoxin into water from oyster mushroom compost using compost tea brewer aerated overnight (Pant *et al.*, 2011). This would allow a post-plant delivery of oyster mushroom compost water extract (MCWE) to plant roots. Laboratory experiments indicated that 25% MCWE paralyzed *M. incognita* effectively (Chapter 2).

Another benefit of introducing spent oyster mushroom compost into agroecosystem is its potential to enhance plant and soil health. A healthy soil can be characterized by high biodiversity, stability to disturbance or stress, maintenance of soil nutrient cycling,
suppression of pests and pathogens, and improved plant health (Wang and McSorley, 2005). Free-living nematodes are cosmopolitan organisms that have been proven to be good soil health indicators that reflect dominant decomposition pathways, structure and function of soil food web (Bongers and Ferris, 1999; Ferris et al., 1997; Freckman, 1988; Ingham et al., 1985; Wang et al., 2004). Free-living nematodes feed on microbes or microfauna in the soil and affect soil nutrient mineralization (Wang et al., 2004; Ingham et al., 1985). Abundance of nematode trophic groups (bacterivores, fungivores, omnivores, predators, and herbivores) classified by Yeates et al. (1993), and life strategies of nematodes (colonizer-persister values) (Bongers and Bongers, 1998) are computed into enrichment, structure, and channel indices (Ferris et al., 2001). All of these indices have been demonstrated to be good soil health indicators (Ferris et al., 2012).

Specific objectives of this project were to examine if integration of mushroom-compost waste amendment and MCWE drenching can 1) suppress plant-parasitic nematodes, 2) enhance soil health, and 3) improve basil growth and yield.

**Materials and Methods**

**Trial I**: A field was conducted in a field infested with plant-parasitic nematodes at the Poamoho Experimental Station, Waialua, HI. The soil type at this location is a Wahiawa silt-clay Tropeptic Eutrustox, clayey, kaolinitic, isohyperthermic soil, containing 18.6% sand, 37.7% silt, and 43.7% clay in the top 25-cm soil (Wang, et al., 2011). Soil organic matter was approximately 2% with pH of 6.5. The experiment was a 2×2×2 (cover crop × amendment × drenching) split-split plot design arranged in randomized complete block with 6 replications. Each sub-plot was 2.4 × 0.6 m² in size. Spent mushroom compost was
obtained from Hamakua Heritage Farm (Laupahoehoe, HI) before each drenching treatment. The 25% (w/w) MCWE was aerated in water for 24 hours before use and drenched at 100 ml per plant. Thus, there were 8 treatments including cover crop + amendment + drench (CAD), cover crop + amendment (CA), cover crop + drench (CD), cover crop (C), amendment + drench (AD), mushroom compost amendment (A), drench (D), and untreated control (0). Half of these subplots were planted with buckwheat (Fagopyrum esculentum) cover crop (C) at 9.16 kg seeds/ha for 4 weeks and then soil incorporated as a source of added organic matter. Basil seedlings were transplanted at 30-cm spacing within a row, i.e. 8 plants per plot. The other half of the plots was not planted with buckwheat. Half of the basil seedlings received 50% (w/w) mushroom amendment (A) and the other half not. Beginning at 4 weeks after transplanting, half of the plants were drenched (D) with 25% (w/w) MCWE at a 2-month interval and the other half were not drenched.

The basil crop was grown for 6 months and plants were fertilized with a fertilizer derived from aerobically composted turkey litter, feather meal, and sulfate of potash (Sustane 5-2-4, Sustane, Inc., Cannon Falls, MN) equivalent to 7.84 kg N/ha, 1.37 kg P/ha, and 5.21 kg K/ha every 2 months. Due to infection of Peronospora belbahrii, basil plants were sprayed with a rotation of Fosphite (JH Biotech, Ventura, CA, Mono-and dipotassium salts of Phosphorous Acid), OxiDate (BioSafe Systems, East Hartford, CT, Hydrogen Dioxide and Peroxyacetic acid), Trilogy (Certis, Columbia, MD, clarified hydrophobic extract of neem oil), Quadris (Syngenta, Greensboro, NC, Azoxyastrobin), and Revus (Syngenta, Greensboro, NC, Mandipropamid) on a weekly interval.
**Trial II:** A similar field trial to Trial I was conducted at the Magoon Experimental Station, Manoa, HI with slight modifications. This area consists of a sandy loam soil of fine, mixed, active, and isohyothermal typic haplustepts. Half of the field was mulched with yard waste compost mulch (M) obtained from the University of Hawaii Buildings and Grounds Management whereas the other half was not M was used as organic inputs instead of planting a cover crop like in Trial I. Thus, this was a $2 \times 2 \times 2$ (MxAxD) split-split plot experiment with 4 replications. Each subplot was $0.3 \times 2.4$ m$^2$ in size. Treatments included mulch + amendment + drench (MAD), mulch + amendment (MA), mulch + drench (MD), yard waste compost mulch (M), amendment + drench (AD), mushroom compost amendment (A), drench (D) and untreated control (0).

**Plant growth and yield:** Plant health data were evaluated by measuring plant height and chlorophyll content using a chlorophyll meter (SPAD-502Plus, Konica Minolta, Inc, NJ, USA) every 3 weeks. One week after each plant measurement, basil shoots were harvested according to commercial practice over a 6-month period for Trial I or 4-month period for Trial II.

**Nematode assay:** Soil samples were taken before basil transplanting and at 2-month intervals thereafter. Four 20-cm deep soil cores were collected from each subplot and composited. Nematodes were extracted from a 250-cm$^3$ sample by elutriation and centrifugal floatation (Byrd et al., 1976; Jenkins, 1964). All nematodes extracted were identified to the genus level wherever possible except for Rhabditidae, which was identified only to family level. Nematodes were counted using an inverted microscope (Leica DM IL LED, Leica Microsystems, Wetzlar, Germany) and assigned to a trophic group.
(algivores, bacterivores, fungivores, herbivores, omnivores, or predators) based on Yeates et al. (1993). Several members of the Tylenchidae family, such as Filenchus and Tylenchus, were classified as fungivores. Nematode richness was calculated for each sample. Enrichment, structure, and channel indices of the soil food web were calculated based on the weighted system of the c-p value and feeding groups described by Ferris et al. (2001).

**Statistical analysis:** Prior to analysis, data was tested for normality using Proc Univariate in SAS 9.2 (SAS Institute. Cary, NC). Nematode abundance was \((\log_{10}[x+1])\) transformed to normalize the data prior to analysis of variance (ANOVA). Data from each trial were subjected to sampling date × cover crop × amendment × drench factorial ANOVA using Proc GLM in SAS. When interaction between treatment and sampling date was not significant, repeated measure analysis over sampling dates was performed using Proc Mixed in SAS and means were then subjected to contrast analysis: cover crop vs no cover crop (CAD, CA, CD, C vs AD, A, D, 0), amendment vs no amendment (CAD, CA, AD, A vs CD, C, D, 0), drench vs no drench (CAD, CD, AD, D vs CA, C, A, 0), mushroom (CAD, CA, CD, AD, A, D) vs no mushroom (C and 0), cover crop with mushroom vs without mushroom (CAD, CA, CD vs C), no cover crop with mushroom vs without mushroom (AD, A, D vs 0), and amendment with drench vs without drench (CAD, AD vs CA, A). When interaction between treatment and sampling date was significant, data were subjected to ANOVA using Proc GLM by sampling date.
## Results

Table 3-1. Analysis of variance of oyster mushroom compost treatments on nematodes and nematode community indices at Poamoho Experimental Station.

<table>
<thead>
<tr>
<th>Indices</th>
<th>C×A×D</th>
<th>C×A</th>
<th>C×D</th>
<th>C</th>
<th>A×D</th>
<th>A</th>
<th>D</th>
<th>Date</th>
<th>Date×C</th>
<th>Date×A</th>
<th>Date×D</th>
<th>Date×C×A</th>
<th>Date×C×D</th>
<th>Date×A×D</th>
<th>Date×C×A×D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterivore</td>
<td>NS(^b)</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
| Fungivore   | NS | NS | NS | **| NS | NS | NS | NS  | * | NS | NS | NS | NS | NS | NS | *
| Herbivore   | NS | NS | NS | NS | NS | NS | NS | NS  | ** | NS | NS | NS | NS | NS | NS | NS |
| Omnivore    | NS | NS | NS | NS | NS | NS | NS | NS  | ** | NS | NS | NS | NS | NS | NS | NS |
| Total       | NS | NS | NS | NS | NS | NS | NS | NS  | * | NS | NS | NS | NS | NS | NS | NS |
| Richness    | NS | NS | NS | * | NS | * | NS | ** | NS | NS | NS | NS | NS | NS | NS | * |
| Diversity   | NS | NS | NS | NS | NS | NS | NS | NS  | ** | NS | NS | NS | NS | NS | NS | NS |
| El\(^c\)    | * | NS | NS | * | NS | NS | NS | NS  | ** | NS | NS | NS | NS | NS | NS | NS |
| SI          | NS | NS | NS | NS | NS | NS | NS | NS  | ** | NS | NS | NS | NS | NS | NS | NS |
| CI          | NS | NS | NS | NS | NS | NS | NS | NS  | ** | * | ** | NS | NS | ** | NS | NS |

\(^a\) Treatment and treatment interactions: C×A×D= cover crop × amendment × drench, C×A=cover crop × amendment, C×D= cover crop × drench, C=cover crop, A×D=amendment × drench, A= mushroom compost amendment, D=drench, Date = sampling date, Date×C=date × cover crop, Date×A=date × amendment, Date×D=date × drench, Date×C×A=date × cover crop × amendment, Date×C×D=date × cover crop × drench, Date×A×D=date × amendment × drench, Date×C×A×D=date × cover crop × amendment × drench.

\(^b\) NS = not significant, * and ** indicate significant at P ≤ 0.05 and 0.01, respectively.

\(^c\) MI = Maturity index, EI = Enrichment index, SI = Structure index, CI = Channel index.
Effects on plant-parasitic nematodes: Root-knot (*Meloidogyne incognita* and *M. javanica*) and reniform (*Rotylenchulus reniformis*) nematodes were the main plant-parasitic nematodes found in both basil trials, with root-knot nematodes being the most dominant genera in Trial I and *R. reniformis* being the most dominant species in Trial II. Initial sampling after terminating buckwheat cover crop in Trial I, cover crop plots increased abundance of root-knot nematodes (382 nematodes/250 cm³ soil) compared to the no cover crop plots (65 nematodes/250 cm³ soil) (*P* ≤ 0.01, data not shown).

Fig. 3-1. Abundance of A) root-knot nematode and B) reniform nematodes in Trial I in Poamoho experiment in 250 cm³ soil. Treatments CAD= cover crop + amendment + drench, CA=cover crop + amendment, CD= cover crop + drench, C=cover crop, AD=amendment + drench, A= mushroom compost amendment, D=drench, 0= untreated control.

Although the abundance of plant-parasitic nematodes (herbivores) varied over time, none of the treatment factors had significant interactions with sampling date after basil
planting (Table 3-1). Thus, repeated measures of the abundance of plant-parasitic nematodes from three sampling dates were conducted. Abundance of root-knot nematode and reniform nematode were not affected by cover crop (C), oyster mushroom compost amendment (A) or MCWE drenching (D) ($P > 0.05$, Fig 3-1 A,B).

Fig. 3-2. Effects of mushroom amendment, compost water extract drench, and cover crop on a) bacterivores, b) fungivores, c) richness, and d) channel index (CI) over time in Trial I at Poamoho Experimental Station. C=cover crop, A= mushroom compost amendment, D=drench, 0=control. Means (n=48) on each date followed by treatments indicate significant difference based on Proc GLM by date.
Nematode community analysis: Interaction between treatment and sampling date only occurred in the abundance of bacterivores, fungivores, richness, and channel Index (CI) (Table 3-1). Thus, data of these four parameters were analyzed by sampling dates (Fig.3-2). Contrast analysis showed that planting of buckwheat cover crop (C) increased ($P \leq 0.05$) the abundance of bacterivores and fungivores throughout the trial (Fig.3-2A, B), and richness only at the last sampling date ($P \leq 0.05$, Fig.3-2C). Adding oyster mushroom compost (CAD, CA, CD, AD, A, D) decreased CI compared to no mushroom (C and 0) treatments on the first sampling date ($P \leq 0.01$, Fig.3-2D). Drenching further increased richness in the second date ($P \leq 0.05$), but decreased bacterivores in the final date ($P \leq 0.05$, Fig.3-2A, C, D).

However, under no cover crop conditions, mushroom treatments decreased richness on the first date ($P \leq 0.05$, Fig.3-2C). Although amendment in combination with drenching (CAD and AD) had lower CI than amendment without drenching (CA and A) on the first sampling date ($P \leq 0.01$, Fig.3-2D), amendment with drenching decreased bacterivores in the third sampling date compared to amendment without drenching ($P \leq 0.05$, Fig.3-2A).

Since no interaction ($P > 0.05$) occurred between date and treatment on abundance of omnivores, diversity, MI, EI, and SI, these parameters were analyzed using repeated measures over time prior to contrast analysis (Table 3-2). Cover cropping (CAD, CA, CD, C) alone had increased abundance of omnivorous nematodes ($P \leq 0.05$) and diversity ($P \leq 0.05$), but decreased MI ($P \leq 0.05$) compared to no cover crop (AD, A, D, 0). In no cover crop conditions, mushroom compost treatments (AD, A, D) had decreased SI ($P \leq 0.05$, Table 3-2) compared to the no mushroom treatment (0), indicating a disturbed soil food web.
Table 3-2. Contrast analysis of oyster mushroom compost effects on repeated measured of nematodes and nematode community indices at the Poamoho experimental station, Trial I.

<table>
<thead>
<tr>
<th>Indices</th>
<th>CADa</th>
<th>CA</th>
<th>CD</th>
<th>C</th>
<th>AD</th>
<th>A</th>
<th>D</th>
<th>0</th>
<th>C vs No C</th>
<th>A vs No A</th>
<th>D vs No D</th>
<th>PO vs no PO</th>
<th>C with PO vs without PO</th>
<th>No C with PO vs without PO</th>
<th>A with D vs no D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematodes / 250 cm$^3$ soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Herbivore</td>
<td>654</td>
<td>661</td>
<td>918</td>
<td>641</td>
<td>655</td>
<td>627</td>
<td>644</td>
<td>696</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Omnivore</td>
<td>32</td>
<td>32</td>
<td>24</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Diversity</td>
<td>5</td>
<td>5.33</td>
<td>4.73</td>
<td>5.34</td>
<td>4.2</td>
<td>4.72</td>
<td>4.1</td>
<td>4.59</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>F/F+Bd</td>
<td>31.1</td>
<td>30.7</td>
<td>37.8</td>
<td>33.5</td>
<td>28</td>
<td>27.3</td>
<td>31.4</td>
<td>31.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
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</tr>
<tr>
<td>MI (%)</td>
<td>2</td>
<td>2</td>
<td>2.06</td>
<td>2</td>
<td>2.06</td>
<td>2.17</td>
<td>2.06</td>
<td>2.17</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>EI (%)</td>
<td>36.36</td>
<td>36.75</td>
<td>38.98</td>
<td>33.58</td>
<td>37.7</td>
<td>34.17</td>
<td>31.6</td>
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<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SI (%)</td>
<td>33.71</td>
<td>35.94</td>
<td>37.41</td>
<td>30.55</td>
<td>35.87</td>
<td>39.61</td>
<td>31.17</td>
<td>47.15</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

a C = cover crop, A = mushroom compost amendment, D = drench, 0 = control.

b Contrast analysis: C = cover crop, No C= no cover crop, A= amendment, No A = no amendment, D = drench, and No D = no drench. NS = not significant ($P > 0.05$), * indicates significant contrast at $P \leq 0.05$.

c Values are least square means of repeated measures over three sampling dates using Proc Mixed analysis (n=36).

d F= abundance of fungivores, B= abundance of bacterivores, MI = Maturity index, EI = Enrichment index, SI = Structure index
Table 3-3. Analysis of variance of the effects of oyster mushroom compost amendment and mushroom compost water extract on nematode communities in Trial II at the Magoon Experiment Station.

<table>
<thead>
<tr>
<th>Indices</th>
<th>M×A×D</th>
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<th>A</th>
<th>D</th>
<th>Date</th>
<th>Date×M</th>
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<td>NS</td>
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<td>**</td>
<td>NS</td>
<td>**</td>
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<td>NS</td>
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<td>NS</td>
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</tr>
<tr>
<td>Fungivore</td>
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<td>NS</td>
<td>*</td>
<td>NS</td>
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<td>NS</td>
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<tr>
<td>Omnivore</td>
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<td>NS</td>
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<td>NS</td>
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<tr>
<td>Herbivore</td>
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<td>NS</td>
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<tr>
<td>Richness</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diversity</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MI(^c)</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EI</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SI</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CI</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>**</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\)Treatment and treatment interactions: M×A×D = mulch × amendment × drench, M×A = mulch × amendment, M×D = mulch × drench, M=Yard waste compost mulch, A×D = amendment × drench, A = mushroom compost amendment, D = drench, Date = sampling date, Date×M = date × mulch, Date×A = date × amendment, Date×D = date × drench, Date×M×A = date × mulch × amendment, Date×M×D = date × mulch × drench, Date×A×D = date × amendment × drench, Date×M×A×D = date × mulch × amendment × drench

\(^b\)NS = Not significant, * and ** indicate significant interaction at \(P \leq 0.05\) and \(0.01\), respectively.

\(^c\)MI = Maturity index, EI = Enrichment index, SI = Structure index, CI = Channel index.
Based on the ANOVA of the nematode community analysis from Trial II (Table 3-3), treatment only interacted with date on bacterivores, EI, and CI (P ≤ 0.05). Thus, a contrast analysis of these parameters was performed by the two sampling dates (Table 3-4). Mulching with yard waste compost (MAD, MA, MD, M) increased bacterivores (P ≤ 0.05), and decreased CI (P ≤ 0.01) in the first sampling date. In contrary, yard waste compost mulch decreased the abundance of bacterivores (P ≤ 0.05) and EI (P ≤ 0.05), but increased CI (P ≤ 0.05) in the second sampling date. On the other hand, amendment (MAD, MA, AD, A) increased bacterivores (P ≤ 0.01) and EI (P ≤ 0.01), but decreased CI (P ≤ 0.01) only in the second date (Table 3-4). Similarly, MCWE drenching increased EI (P ≤ 0.05) and decreased CI (P ≤ 0.05) on the second sampling date. Regardless of mulching, mushroom compost treatments increased bacterivores and EI in the first sampling date and subsequently decreased CI on the second date (Table 3-4).

Since no significant interaction between date and treatment for the abundance of fungivores, omnivores, and predators, richness, diversity, MI and SI based on ANOVA (Table 3-3), these parameters were subjected to repeated measures prior to contrast analysis (Table 3-5). Yard waste compost mulch increased the abundance of fungivores (P ≤ 0.05), and amendment (A) increased abundance of omnivores (P ≤ 0.05) and richness (P ≤ 0.01) compared to no A (Table 3-5).
Table 3-4. Contrast analysis of the effects of oyster mushroom compost amendment and mushroom compost water extract on nematodes and nematode community indices by sampling dates in Trial II at the Magoon Research station.

<table>
<thead>
<tr>
<th>Indices</th>
<th>MAD</th>
<th>MA</th>
<th>MD</th>
<th>M</th>
<th>AD</th>
<th>A</th>
<th>D</th>
<th>M vs no M</th>
<th>A vs no A</th>
<th>D vs no D</th>
<th>PO vs no PO</th>
<th>M+PO vs M-PO</th>
<th>No M+PO vs No M-PO</th>
<th>A+D vs A-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterivore</td>
<td>320b</td>
<td>550</td>
<td>243</td>
<td>395</td>
<td>225</td>
<td>333</td>
<td>260</td>
<td>130</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(250 cm³ soil)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El (%)</td>
<td>39</td>
<td>35</td>
<td>41</td>
<td>40</td>
<td>39</td>
<td>35</td>
<td>41</td>
<td>41</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CI (%)</td>
<td>89</td>
<td>77</td>
<td>78</td>
<td>75</td>
<td>100</td>
<td>90</td>
<td>92</td>
<td>100</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>16-weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterivore</td>
<td>200</td>
<td>130</td>
<td>130</td>
<td>55</td>
<td>400</td>
<td>558</td>
<td>198</td>
<td>75</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>(250 cm³ soil)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El (%)</td>
<td>57</td>
<td>44</td>
<td>62</td>
<td>32</td>
<td>74</td>
<td>84</td>
<td>55</td>
<td>42</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CI (%)</td>
<td>59</td>
<td>81</td>
<td>39</td>
<td>100</td>
<td>41</td>
<td>7</td>
<td>61</td>
<td>90</td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

\(^a\) Contrast analysis: M=yard waste compost mulch, No M= no yard waste compost mulch, A=amendment, No A= no amendment, D=drench, and No D= no drench. Mush = mushroom treatment (amendment and or drench), no Mush= no mushroom treatments, M+Mush= mulch and mushroom treatments, M-Mush= mulch without mushroom, No M+Mush= no mulch with mushroom, No M-Mush= no mulch without mushroom, A+D= amendment with drench, A-D= amendment without drench.

\(^b\) Values are least square means based on repeated measure over time using Proc Mixed analysis (n=18).

\(^c\) NS = Not significant, * and ** indicate significant contrast at \( P \leq 0.05 \) and 0.01, respectively.

\(^d\) EI = Enrichment index, CI = Channel index.
Table 3-5. Repeated measures of nematode community indices over time affected by oyster mushroom compost amendment and mushroom compost water extract drenching in Trial II at the Magoon Experimental Station.

<table>
<thead>
<tr>
<th>Indices</th>
<th>MAD</th>
<th>MA</th>
<th>MD</th>
<th>M</th>
<th>AD</th>
<th>A</th>
<th>D</th>
<th>0</th>
<th>M vs no M</th>
<th>A vs no A</th>
<th>D vs no D</th>
<th>PO vs no PO</th>
<th>PO vs M-PO</th>
<th>No M+PO vs No M-PO</th>
<th>M+PO vs A+D</th>
<th>A+D vs A-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungivore</td>
<td>319</td>
<td>250</td>
<td>170</td>
<td>176</td>
<td>280</td>
<td>223</td>
<td>189</td>
<td>189</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Omnivore</td>
<td>11</td>
<td>30</td>
<td>4</td>
<td>0</td>
<td>14</td>
<td>6</td>
<td>4</td>
<td>9</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Predator</td>
<td>28</td>
<td>48</td>
<td>5</td>
<td>15</td>
<td>31</td>
<td>11</td>
<td>16</td>
<td>21</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Herbivore</td>
<td>74</td>
<td>53</td>
<td>126</td>
<td>68</td>
<td>39</td>
<td>58</td>
<td>46</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Richness</td>
<td>11</td>
<td>13</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diversity</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MI (%)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SI (%)</td>
<td>29</td>
<td>31</td>
<td>15</td>
<td>13</td>
<td>41</td>
<td>15</td>
<td>34</td>
<td>32</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* M = Yard waste compost mulch, A = mushroom amendment, D = drench, 0 = control.

b Contrast analysis where M = yard waste compost mulch, No M = no yard waste compost mulch, A=amendment, No A= no amendment, D=drench, and No D= no drench. PO = amendment and or drench, no PO= no amendment or drench, M +PO= mulch and amendment and or drench, M-PO= mulch without amendment or drench, No M +PO= no mulch with amendment and or drench, No M-PO= no mulch without amendment or drench, A+D= amendment with drench, A-D= amendment without drench.

c NS = Not significant, * and ** indicate significant contrast at $P \leq 0.05$ and 0.01, respectively.
Fig. 3-3. Mushroom amendment and drenching effect on A) basil height, B) yield and C) chlorophyll readings. Combined chlorophyll content from basil was measured by Soil Plant Analysis Development (SPAD). Means in each column are repeated measures over time in field trial I at the Poamoho experimental station. Means are average of 36, 48, and 36 for height from 3 sampling dates, total yield from 6 sampling dates, and chlorophyll from 3 sampling dates, respectively in field Trial I at the Poamoho Experiment Station.

A=amendment, No A= no amendment, D=drench, No D= no drench, C=cover crop, No C= no cover crop. * and NS indicate significant difference based on the Proc mixed analysis at $P \leq 0.05$, and no significance respectively

*Basil growth and yield:* Based on ANOVA, no significant interaction between treatments and sampling date was detected for basil height, yield, and chlorophyll content in Trial I (Fig. 3-3). Thus repeated measure analysis over the harvesting or data taking dates were conducted for these parameters. Although both A and D increased basil height ($P \leq 0.05$,
Fig. 3-3A), A only slightly increased basil yield ($P < 0.10$) while D had no effect on yield in Trial I (Fig. 3-3B). Drenching of MCWE increased chlorophyll content of basil if cover crop was planted ($P < 0.05$) but not when cover crop was not planted (Fig. 3-3C).

Table 3-6. Analysis of variance of effects of oyster mushroom compost amendment and mushroom compost water extract drench on basil height, chlorophyll content and yield, in Trial II, Magoon Research Station.

<table>
<thead>
<tr>
<th>Variable</th>
<th>M×A×D</th>
<th>M×A</th>
<th>M×D</th>
<th>M</th>
<th>×AD</th>
<th>A</th>
<th>D</th>
<th>Date×M</th>
<th>Date×A</th>
<th>Date×D</th>
<th>DateM×A</th>
<th>DateM×D</th>
<th>DateM×A×D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Yield</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Treatment and treatment interactions: M×A×D= yard waste compost mulch × amendment × drench, M×A=mulch × amendment, M×D= mulch × drench, M=Yard waste compost mulch, A×D=amendment × drench, A= mushroom compost amendment, D=drench, Date= sampling date, Date×M=date × mulch, Date×A=date × amendment, Date×D=date × drench, Date×M×A=date × mulch × amendment, Date×M×D=date × mulch × drench, Date×A×D=date × amendment × drench, Date×M×A×D=date × mulch × amendment × drench.

b NS = Not significant, * and ** indicate significant difference for the contrast analysis at $P \leq 0.05$ and 0.01 respectively.

In Trial II, no interaction occurred between treatment and date for basil height, yield, and chlorophyll content (Table 3-6), thus these parameters were subjected to repeated
measure analysis, but both mushroom compost A and D did not affect basil chlorophyll content and height ($P > 0.05$, data not shown). Mushroom compost A and D did not increase basil yield, however, yard waste compost mulch increased basil yield compared to no mulch ($P \leq 0.05$, Fig. 3-4).

![Graph](image)

**Fig. 3-4.** Yard waste compost mulch and mushroom compost waste amendment effect on yield in Trial II at the Magoon Research Station. M= mulch, No M= no mulch, A= amendment, No A= no amendment, D=drench, and No D= no drench. * indicates significant difference between treatments at $P \leq 0.05$. NS = not significant. Value of each column (n=48) is least square means based on repeated measure over 6 sampling dates.

**Discussion**

*Effects on plant-parasitic nematodes:* Spent *P. ostreatus* compost used as amendment or drenching did not suppress *Meloidogyne* spp. (predominant in Trial I) or *R. reniformis* (predominant in Trial II). These field results were not consistent with the laboratory results that showed that spent *P. ostreatus* compost suppressed *M. incognita* at 1-2% amendment rate in Chapter 2. Palizi, *et al.* (2009) reported that *P. ostreatus* compost waste suppressed *H. schachtii* on sugarbeet, whereas Okorie *et al.* (2011) reported that compost
waste of *P. ostreatus* and *P. tuberregium* suppressed *M. incognita* on soybean in greenhouse pot studies. These field trials were based on natural infestation of plant-parasitic nematodes in the field. High abundance of *Meloidogyne* spp. (> 800 nematodes/250 cm³ soil at termination of the basil crop) in Trial I and too low infestation of *R. reniformis* in Trial II (< 120 nematodes/250 cm³ soil at termination of the basil crop) might be limiting factors to evaluate the effects of oyster mushroom compost waste on plant-parasitic nematodes for these field trials.

Several other factors could also play roles in the lack of nematode antagonistic effect of oyster mushroom compost in a field highly infested with *M. incognita*. These include 1) unfavorable environment for the mycelium to establish in the field, such as exposure to sunlight, 2) antagonism from soil organisms such as fungivorous fauna, 3) dilution of MCWE into soil after drenching, and 4) basil root masses that extend beyond the colonization of mycelia after a few months of growth. The attempt to use spent oyster mushroom compost in a heavily infested *Meloidogyne* spp. field was not successful despite the addition of organic amendment in the soil. This is similar to many other fungal nematode biological control approaches in the field (Mankau, 1980; Sikora, 1992). Mankau (1980) suggesting that fungal biological control is difficult because of the complex nature of the soil habitat. Sikora (1992) referred to the dynamics of the natural microbial community as not well understood and performance of nematode antagonistic fungi in the tropics has not been investigated thoroughly.

Buckwheat was used as a green manure and weed-smothering crop, which also provided a source of organic matter. Buckwheat dry matter generated approximately 3.36 tons/ha in 8 weeks. Soil incorporation of buckwheat residues has been documented to
improve the physical condition and the moisture-holding capacity in the soil (Oplinger et al., 1989). Unfortunately, buckwheat is susceptible to root-knot nematode, as indicated by higher abundance of root-knot nematodes in the cover crop plots vs bare ground plots at termination of cover crop in Trial I. Future research should examine more frequent MCWE drenching (weekly as opposed to once every two months in the current project) when plant-parasitic nematode abundance reaches a critical level.

**Effects on Soil Health:** In Trial I, oyster mushroom compost treatments (amendment or drenching) initially were dominated by bacterial decomposition as indicated by lower CI on the first sampling date, but eventually resulted in reduced soil food web structure (lower SI) compared to the no mushroom treatment especially in field plots without cover crop planting. This suggested that oyster mushroom compost only enhanced soil bacterial activities temporarily at the Poamoho site, and the effect was not long lasting. Although drenching MCWE increased richness ($P \leq 0.05$) in the second date, it decreased bacterivores in the final date. Overall, oyster mushroom compost treatments did not enhance soil health conditions in Trial I despite the enhancement of bacterivores, fungivores and omnivores by buckwheat cover crop throughout the basil crop. Lack of response of the fungivorous nematodes to the mushroom compost treatments in Trial I also suggesting that the mycelia were unsuccessfully introduced in this field. As suggested in a review of conservation biological control for nematode management, multiple seasons of application might be needed for biological control agents to establish in a field (Timper, 2014).

On the other hand, in Trial II adding oyster mushroom compost as an amendment increased abundance of fungivorous nematodes than the no amendment plots ($P < 0.05$)
throughout the basil crop at the Magoon site, suggesting that the fungivorous nematodes were responding to the mushroom compost amendment. This effect was not significant when oyster mushroom compost was introduced by MCWE drenching. Higher fungal microbial activities in amended plots regardless of the yard waste compost mulch treatment suggesting that introduction of *P. ostreatus* had led to establishment of the mycelia in the treated plots. Better performance of *P. ostreatus* in terms of improving soil health in Trial II could be due to higher humidity at Magoon (annual rainfall of 1137 mm) than at Poamoho (annual rainfall of 881 mm). In addition, oyster mushroom compost amendment increased abundance of bacterivorous nematodes, EI and decrease of CI at the end of basil crop, and increased omnivorous nematodes and richness throughout the basil crop in Trial II indicating an overall improvement of soil health conditions by introducing this mushroom compost.

Results of Trial II did not support the need of adding organic matter such as yard waste compost mulch to facilitate the establishment of oyster mushroom compost. Mulching with yard waste compost alone did not increase the abundance of omnivorous nematodes, but adding oyster mushroom amendment increased omnivorous nematodes within one cropping cycle. Wang *et al.* (2011) and Marahatta, *et al.* (2010) reported that at least two cropping cycles are needed to see improvement in soil food web structure through conservation cover cropping practices. It is encouraging to see improvement of soil health condition by the oyster mushroom compost amendment in Trial II within one cropping cycle. It is possible that over time, the soil food web structure would improve with oyster mushroom compost treatments at the Poamoho site.
As seen from the contrast analysis, an increase in omnivorous nematodes was mainly from the contribution of oyster mushroom amendment rather than drenching effect in Trial II. Since each plant was treated with 50 grams of mushroom amendment, this would equate to 2.69 mt/ha of mushroom compost. This rate is considerably lower than the recommended mulching with yard waste compost at 11mt/ha -110mt/ha recommended by Biernbaum (2012) or with spent mushroom compost at 100 mt/ha recommended by Courtney and Mullen (2007) for soil health improvement. Oyster mushrooms are good saprophytes that can help to break down organic matter in the soil for nutrient recycling. Enhancement of soil health by adding oyster mushroom compost would be profitable for farmers, as the agroecosystem becomes more efficient in nutrient cycling, fertilizer inputs can be reduced.

*Effects on basil yield:* Although oyster mushroom compost increased basil plant height in Trial I, it did not increase basil yield in both trials. Okorie *et al.*, (2011) reported higher soybean yield in *P. ostreatus* compost treatment in a greenhouse pot trial, so there is potential for this compost in increasing crop yield. However, basil yield evaluation in the current experiment was complicated by heavy infection of *Peronospora belbahrii* (Downy Mildew) in both field trials. This might be responsible for the lack of significant effect of mushroom compost on basil yield in the current field trials. However, adding yard waste compost mulch increased basil yield ($P \leq 0.05$). It is possible that mulch on the soil surface reduced splashing of spores, and thus reduced the dispersal of this pathogen.

In conclusion, although mushroom compost amendment and drench did not suppress plant-parasitic nematodes, they had varying effects in improving soil health. Spent oyster mushroom compost as amendment and sometimes as drench increased bacterivorous and
fungivorous nematodes, and if practiced in area with higher humidity, it could also potentially increase omnivorous and predatory nematodes. Overall, the benefits of oyster mushroom compost for suppression of plant-parasitic nematodes and improving soil health might take time. Continual practice of introducing mushroom compost waste in an agroecosystem might be needed to observe the benefits of this saprophytic fungus. Future research is needed to evaluate more frequent MCWE drenching for the management of plant-parasitic nematodes.
Literatures Cited


https://www.hort.purdue.edu/newcrop/afcm/buckwheat.html


