Short-term Effects of Organic Amendments on Raspberry Root Pathogens: *Phytophthora rubi* and *Pratylenchus penetrans*

by

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Abstract

Raspberry fields in the Lower Mainland of British Columbia are often prepared for replanting by fumigating and/or applying broiler manure in order to control soil borne pathogens such as those causing raspberry root rot. Broiler manure applications pose a threat of nitrate leaching and contamination of groundwater, while fumigation poses environmental risks because it is a broad-spectrum biocide. The aim of my research was to evaluate the effectiveness of readily available composts to reduce the impacts of plant parasitic nematodes and *Phytophthora rubi* and to improve plant growth. In 2013, two outdoor pot experiments using 'Malahat' cultivar red raspberry plants were conducted with raspberry field soils naturally infested with *Pratylenchus penetrans*. The first experiment held during the spring compared two concentrations of two composts, two concentrations of manure, and fumigation, in the presence or absence of *P. rubi* and arranged in a randomized complete block design. A 72-h flooding period after *P. rubi* inoculation was used to facilitate *P. rubi* infection. The second experiment during the summer compared the same amendment treatments without *P. rubi* inoculation to optimize conditions for *P. penetrans*. Soil samples taken from each pot were analyzed for nematode populations on the day of planting and at harvest (13 weeks later). The number of shoots and the biomass of shoots and roots were assessed at the end of both experiments, while root rot ratings were performed at the end of the first experiment.

P. rubi was not detected in experiment 1 due to suboptimal environmental conditions after inoculation. Broiler manure suppressed root lesion nematode populations and improved plant growth relative to the control nearly as well as fumigation in experiment 1. There was no significant treatment effect on plant growth in experiment 2. However, broiler manure and fumigation did suppress *P. penetrans* populations relative to the control as in the first experiment. In contrast with earlier field studies, compost treatments did not suppress nematode populations or improve plant growth relative to the control. Overall, plant vigor and nematode suppression in compost treatments were limited. Based on previous compost studies, I speculate that longer experiment periods may be needed in order to detect benefits from composting.

Preface

This study built on previous field experiments research conducted by Forge et al. (2015; 2016). I designed and put together both experiments. I collected plant biomass data and soil samples, conducted root rot ratings, and extracted and counted soil nematodes. Quantitative PCR and *Phytophthora rubi* inoculation procedures were based on previous work done at PARC in Agassiz. I performed all quantitative PCR work for experiment 1 with the guidance of Carol Koch from Agriculture Agri-Food Canada, Agassiz. I conducted the statistical analyses using SAS at PARC in Agassiz with the guidance of Dr. Tom Forge. All thesis chapters were written with the guidance of Drs. Tom Forge and Louise Nelson. Thesis chapters were reviewed by the members of my supervisory committee: Drs. Miranda Hart and Daniel Durall from the University of British Columbia Okanagan campus.

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List of Abbreviations

AAFC – Agriculture and Agri-Food Canada

Al – aluminum

ANOVA – Analysis of variance

BC – British Columbia

BD – bulk density

B – boron

bp – base pairs

C – carbon

Ca – calcium

CEC – cation exchange capacity

C/N – carbon to nitrogen ratio

Cq – quantification cycle in qPCR

ctrl – control

Cu – copper

dF/dT – derivative – change in fluorescence relative to change in temperature

dNTPs – deoxy nucleotide phosphates

Fe – iron

FLN – free living nematodes

fum – fumigation

HiMan – high rate of broiler manure amendment

HiMC – high rate of mushroom compost amendment

HiPARC – high rate of compost amendment

K – potassium

LoMan – low rate of broiler manure amendment

LoMC – low rate of mushroom compost amendment

LoPARC – low rate of compost amendment

Mg – magnesium

Mn – manganese

N - nitrogen

Na – sodium

ND – not detected

NTC – non-template control

OM – organic matter

P – phosphorus

*PARC – Pacific Agri-Food Research Centre

PCR – Polymerase Chain Reaction

Pos – positive

P. penetrans; P. p – *Pratylenchus penetrans*

P. rubi; PR – *Phytophthora rubi*

qPCR – Quantitative Polymerase Chain Reaction

∗ Name of the research station in Agassiz at the time of this study

std – standard

S – sulphur

TM – temperature moisture probes

wt – weight

Zn – zinc

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Chapter 1 Introduction

1.1 Raspberry production in the Lower Mainland

The Lower Mainland region of British Columbia, especially the central Fraser Valley, is an ideal area for red raspberry (*Rubus idaeous L.*) production with its mild climate and well-drained soils (Luttmerding, 1981; Raspberry Industry Development Council, 2011). Around 1700 ha of farmland provide more than 80% of Canada's red raspberry production with 12 million kilograms being shipped across Canada and worldwide each year (Raspberry Industry Development Council, 2011).

Red raspberry growers in the Lower Mainland of British Columbia have used the same practices to maintain high yields of their crops for many years. Generally, after about 4-7 years, berry yields and crop vigour begin to decline due to soil borne pathogens such as raspberry root rot caused by the pathogen *Phytophthora rubi* and root lesion nematodes (*Pratylenchus penetrans*) (Gigot et al., 2013; pers. comm. Tom Forge, Agriculture and Agri-Food Canada; Walters et al., 2009), and raspberry bushy-dwarf virus. Consequently, every 5 to 8 years, growers remove the established stand of raspberry plants, fumigate the soil, usually in the fall, to reduce *P. rubi* and *P. penetrans* populations, and replant the following spring (British Columbia Ministry of Agriculture and Lands, 2013). The fumigant most often used by BC growers is Vapam® (metam sodium, active ingredient – methyl isothiocyanate) (pers. comm. Tom Forge, Agriculture and Agri-Food Canada). Many growers also amend soil with poultry manure during this renovation period (British Columbia Ministry of Agriculture and Lands, 2013). Sometimes the manure is applied instead of fumigation, but often manure is applied in addition to fumigation (British Columbia Ministry of Agriculture and Lands, 2013). As well as providing organic matter and ample nutrients for the new crop, high rates of poultry manure can reduce nematode populations through "biocidal activity" (Forge et al., 2012), probably as a result of the high concentration of organic acids, ammonia or ammonium ions in the manure (Gamliel et al., 2000; Rodriguez-Kabana, 1986).

The nitrogen requirements of raspberry are relatively low and because the soil in the central Fraser Valley is composed of medium textured, well drained silt loam, it is important to not overfertilize for risk of nitrate leaching (Luttmerding, 1981). Generally, there is a loss of

about 50 kg N ha^{-1} per year through the removal of berries and plant foliage (Zebarth et al., 2015). As a result, British Columbia Ministry of Agriculture and Lands (2013) recommends an annual N fertilization application rate between 50 and100 kg N ha^{-1}. Historically, growers would fertilize established crops with poultry manure or fertilizer on an annual basis in addition to the poultry manure that may have been incorporated prior to replanting. Nitrate contamination of the Sumas-Abbotsford aquifer was linked to excessive nitrogen inputs to raspberry crops in the 1990s, and best management practices were developed to encourage growers to account for the nitrogen inputs from manure (Dean et al., 2000; Zebarth et al., 2015; Zebarth et al., 1998; British Columbia Ministry of Agriculture and Lands, 2013). In more recent years, the fertilization of established raspberry crops with poultry manure has declined (pers. comm. Tom Forge, Agriculture and Agri-Food Canada).

1.1.1 Pre-plant soil treatments for raspberry

1.1.1.1 Fumigation

Both pre-plant fumigation and high manure applications have environmental consequences. Soil fumigants are broad-spectrum biocides that destroy most of the organisms living in the soil, including beneficial soil organisms that could naturally contribute to pathogen regulation (Gamliel et al., 2000; James, 1989; Klose et al., 2006). As a result of the loss of these natural regulators, once populations of soil borne pathogens, such as plant parasitic nematodes, become re-established in fumigated soil, they can build up to greater levels than in non-fumigated soil (Forge et al., 2001; Gamliel et al., 2000; Gigot et al., 2013; Walters et al., 2009). Soils with low microbial populations are more vulnerable to reinvasion of pathogens as a result of regular fumigation (Gamliel et al., 2000).

In addition to fumigants being detrimental to soil health, fumigation can pose risks to human health and, consequently, regulations governing fumigation practices have become more restrictive to ensure the protection of people near fumigation sites (Health Canada, 2012; Rudolph and DeVetter, 2015; Walters et al., 2009). New restrictions on fumigation include requirements for the creation of large buffer zones, with the size of these prescribed buffer zones depending on the type of fumigant used, the rate and the application method (e.g. whether

fumigated soil is covered), and whether public daycare facilities, schools or hospitals are nearby (Health Canada, 2012; Rudolph and DeVetter, 2015).

1.1.1.2 Poultry manure

The majority of the province's poultry production is located in the Fraser Valley, particularly in the Abbotsford area where the raspberry industry is concentrated, and there is an oversupply of manure in the region (Chesnaux et al., 2012; Ference Weicker & Company, 2009; Timmenga & Associates Inc., 2003; Zebarth et al., 1999). Historically, many growers have used this readily available and inexpensive manure as a soil amendment prior to replanting, at rates of up to about 250 m^3/ha (2.5 cm thick layer). At a typical bulk density of 250 kg/m^3 and total N concentration of 5% (Forge et al., 2015), such applications can result in total nitrogen inputs of up to about 3000 kg/ha. The mineralization of N in soil amended with such high rates of poultry manure would undoubtedly exceed nitrogen requirements of the crop in the first few years of production. As a consequence, these manure applications would have high potential for nitrate to leach from the soil profile to groundwater with the fall rains (Forge et al., 2015; Dean et al., 2000; Zebarth et al., 1998). Nitrate contamination of the Sumas-Abbotsford aquifer has been associated with such heavy applications of manure to raspberry crops (Chesnaux et al., 2012; Dean et al., 2000; Jeffries et al., 2008; Zebarth et al., 1998). Growers are currently discouraged from applying poultry manure at these high rates prior to replanting. However, the guidelines (Berry Production Guide, 2012) are open to interpretation and some growers may still be applying manure in excess of crop requirements for N at the time of replanting (pers. comm. Tom Forge, Agriculture and Agri-Food Canada).

1.1.1.3 Compost as an alternative

New restrictions on fumigation make it more costly and inconvenient for growers to fumigate. There is growing recognition that longer-term impacts of fumigation on soil health may negate short-term benefits of pathogen population reduction. Additionally, the practice of applying manure amendments can lead to nitrate leaching. Therefore, there is a need to develop alternative soil amendments or practices that will improve early growth of raspberry without the environmental risks associated with fumigation and broiler manure application. Composts made from manures have lower nitrogen contents and less readily available forms of nitrogen than raw

manures. Forge et al. (2015; 2016) recently demonstrated that pre-plant incorporation of at least one compost made from broiler manure and yard and vegetable wastes could reduce *P. penetrans* populations and improve early establishment of raspberry, without increasing soil nitrate relative to non-amended treatments. Mushroom compost is made using poultry manure and is a readily available resource for raspberry growers to use as the majority of British Columbia's mushroom producers are also located in the Fraser Valley (Suess and Curtis, 2006; Timmenga & Associates Inc., 2003).

1.2 Soil borne pathogens of raspberries in the Lower Mainland

1.2.1 Raspberry root rot (*Phytophthora rubi*)

Phytophthora rubi (*P. rubi*) is a microscopic fungus-like organism from the class Oomycota also known as the water molds (Agriculture and Horticulture Development Board, 2015; EPPO, 2013). Some of the differences between Oomycota and true fungi include the cell wall of Oomycota being composed of beta glucans and cellulose instead of chitin, mitochondria having tubular cristae instead of flattened cristae, vegetative cells being typically diploid and the production of oospores, which are not produced by true fungi (Rossman and Palm, 2006). The primary host of *P. rubi* is cultivated raspberries (EPPO, 2013; Hoashi-Erhardt, 2008). As with most species of *Phytophthora*, *P. rubi* can survive for many years in soil as resistant oospores (EPPO, 2013; Hardham, 2001). These oospores can germinate to form one or several sporangia (EPPO, 2013; Hardham, 2001). The optimum temperature for germination can range from 10-15ºC; however, these spores can also germinate at a slower rate at both temperature extremes of 20ºC and 5ºC (EPPO, 2013). *Phytophthora rubi* sporangia then release motile double flagellated zoospores, which swim through water films between soil particles towards the root tips of the host plant (Agrios, 1978; EPPO, 2013; Hardham, 2001; Rudolph and DeVetter, 2015). This organism is able to locate host plants through the chemical exudates released from the plant roots (EPPO, 2013; Hardham, 2001). These motile zoospores will encyst and penetrate just behind the root tip, producing hyphae that will grow towards the phloem and cambium, which will collapse at the time of infection (Laun and Zinkernagel, 1997). Once the plant is infected, *P. rubi* mainly grows within the stele, with hyphae also growing out from the roots to form new sporangia, which release more zoospores to continue the cycle of infection onto new roots (EPPO, 2013; Hardham, 2001). *Phytophthora rubi* infection of raspberry generally occurs during the wet and

cool months of late autumn and early spring, perfect conditions for zoospores to move through the saturated soil (Agrios, 1978; EPPO, 2013; Hoashi-Erhardt, 2008; Rudolph and DeVetter, 2015). Below ground symptoms are apparent on the roots from late autumn onwards, but generally infection is undetected until the above ground parts of the plants begin to show stress (EPPO, 2013). Once the weather becomes drier during the late spring or summer there is dieback of plant roots, and stunted and chlorotic plants or even plant death become evident as a result of restricted uptake of water and nutrients through the affected root systems caused by the collapse of the cambium and phloem due to infection (EPPO, 2013; Hoashi-Erhardt, 2008; Laun and Zinkernagel, 1997; Rudolph and DeVetter, 2015).

1.2.2 Root lesion nematodes (*Pratylenchus penetrans*)

Root lesion nematodes (*Pratylenchus spp.*) are migratory endoparasites that damage a wide range of crops, causing chronic yield losses and long term declines of root systems and overall plant vigour (Agrios, 1978; Gigot et al., 2013; Vrain et al., 1997; Walters et al., 2009). Root lesion nematodes feed on root cortical cells, killing the tissues in the root cortex and causing the appearance of necrotic lesions or spots on the roots (Agrios, 1978). As a result, feeder roots are destroyed over time leaving only coarse woody roots (Agrios, 1978; Forge et al., 2008; Walters et al., 2009). The primary species affecting temperate fruit crops worldwide is *P. penetrans*. In addition to directly causing damage to roots, root lesion nematodes can predispose plants to other soil borne pathogens such as *P. rubi*, which can contribute to the decrease in plant vigour and productivity, or even potentially plant death (Agrios, 1978; Rudolph and DeVetter, 2015; Walters et al., 2009). Therefore, before planting red raspberries, growers often fumigate their soils to decrease the populations of plant parasitic nematodes (Vrain et al., 1996; Rudolph and DeVetter, 2015; Walters et al., 2009). However, even with soil fumigation, the populations of nematodes can build up to damaging levels higher than in non-fumigated soil within several years (Forge et al., 2001; Gamliel et al., 2000; Walters et al., 2009). This rise in population densities of plant parasitic nematodes in previously fumigated soil is due to a lack of natural enemies (Forge et al., 2001; Gamliel et al., 2000; Walters et al., 2009).

1.3 Compost amendments

As a result of prolonged and intensive agricultural cultivation, there can be a loss of soil organic matter, degradation of soil physical properties, decreased soil biological activity and overall reduced soil fertility (Bailey and Lazarovits, 2003; Zinati, 2005). Compost has been used in agriculture for many years as an organic slow release fertilizer and a material that improves soil properties (Bailey and Lazarovits, 2003; Bonilla et al., 2015; Hoitink and Boehm, 1999; Mehta et al., 2014; Saxena et al., 2015; Zinati, 2005). Composting is a long, thermophilic, aerobic stabilization process that transforms organic wastes to a more stable, humified form of organic matter that has properties more similar to native soil organic matter (Hoitink and Boehm, 1999; Mehta et al., 2014; Zinati, 2005). Additions of compost can aid in preventing or decreasing soil degradation by improving properties such as aggregate stability, water holding capacity, and cation exchange capacity. Not only is the use of compost practical, especially with an increase in accumulation of waste by humans and livestock, but compost applications have been shown to suppress pests and diseases of agricultural crops (Bonilla et al., 2015; Hoitink and Boehm, 1997; Mehta et al., 2014; Saxena et al., 2015).

1.3.1 How composts can suppress plant diseases

Compost appears to suppress plant diseases via any of four possible mechanisms: competition, production of antibiotic compounds, parasitism and predation, and activation of disease resistance genes in plants (Bonilla et al., 2015; Chen et al., 2015; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink and Grebus, 1994; Hoitink et al., 1997; Litterick and Wood, 2009; Lockwood, 1988; Mehta et al., 2014; Noble and Coventry, 2005; Postma and Schilder, 2015; Rahman et al., 2014; Saxena et al., 2015; Zinati, 2005).

In the first mechanism, competition, growth of beneficial microflora (including those naturally dwelling within the compost) is enhanced and they compete with plant pathogens in the rhizosphere for nutrients, such as sugars and amino acids exuded from roots, resulting in reduced pathogen activity and disease (Hoitink and Boehm, 1999; Lockwood, 1988; Mehta et al., 2014; Postma and Schilder, 2015; Saxena et al., 2015). An example of this can be seen under iron limited conditions, where fluorescent *Pseudomonas* spp. produce siderophores which can

compete for iron with pathogenic organisms causing their growth to be suppressed (Lockwood, 1988; Mehta et al., 2014).

The next form of biological control is antibiosis, the production of antibiotic compounds by beneficial microorganisms (Lockwood, 1988; Mehta et al., 2014; Saxena et al., 2015). This process involves antagonism mediated by the production of specific or non-specific metabolites including lytic agents, volatile compounds or other toxic substances as a result of microbial activity (Lazarovits, 2001).

Predation and parasitism of plant pathogens is another mechanism of biological control that can be enhanced by compost amendments. This third mechanism occurs since organic amendments stimulate the growth of populations of beneficial microorganisms such as fungivorous nematodes, which can search out and consume soil borne pathogens (Lazarovits, 2001; Mehta et al., 2014; Rahman et al., 2014). A well-known example of this is the beneficial fungus, *Trichoderma sp*. It has been found to destroy the sclerotia of the pathogenic fungus, *Rhizoctonia*, and also to parasitize other soil borne pathogens such as *Phytophthora cactorum* (Hoitink and Boehm, 1999; Mehta et al., 2014; Rudolph and DeVetter, 2015; Saxena et al., 2015).

The last known method of biocontrol by composts involves the induction of systemic resistance or a state of enhanced defensive capacity in plants elicited by specific environmental stimuli, which potentially can aid the plant to defend itself against subsequent biotic challenges (Chen et al., 2015; Mehta et al., 2014; Rahman et al., 2014; Vallad and Goodman, 2004). There are two ways in which this resistance can occur, induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Vallad and Goodman, 2004). The SAR mechanism can be triggered by exposing the plant to virulent, avirulent or non-pathogenic microbes or by various chemical agents such as salicylic acid (Chen et al., 2015; Vallad and Goodman, 2004; Zhang et al., 1996, 1998). On the other hand, ISR is activated by plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* spp. and it also has pathways regulated by different plant compounds such as ethylene and jasmonate rather than salicylic acid (Raaijmakers et al., 2009; Vallad and Goodman, 2004). Plants grown in compost-amended mixtures that induce systemic resistance also have higher concentrations of enzymes related to host defense mechanisms (Zhang et al., 1996).

Furthermore, some researchers believe that physical or chemical characteristics of composts work to reduce disease severity by either directly or indirectly affecting the pathogen or host capacity for growth by altering availability of key nutrients, organic matter, moisture, and pH. Organic amendments that have high nitrogen content, such as hog or poultry manure, or immature composts made from such materials, have the potential to suppress soil-borne diseases via the toxic effects of ammonia, nitrous acid or volatile fatty acids (Cao et al., 2014; Lazarovits, 2001). However, these same chemical compounds can also be toxic to plants and beneficial organisms, as previous research has found that immature composts applied with excessive nitrogen loading have been implicated in numerous diseases such as *Phytophthora* dieback, fireblight and *Fusarium* wilt (Hoitink and Grebus, 1994).

Composts and manures can also contain relatively large concentrations of certain elements such as sulphur, copper, zinc and iron, all of which have the potential to affect soil microbial activity which in turn can also affect both *Phytophthora* and root lesion nematodes. Although, elemental sulphur has long been known to be a foliar fungicide and is used commercially to control common foliar diseases such as apple scab, the same has not previously been seen with *Phytophthora* root diseases possibly due to the fact that *Phytophthora* is not a fungus (De Curtis et al., 2012; Williams and Cooper, 2004). Copper is another element that can affect microbes and is used as a foliar fungicide, especially for organically managed fruit crops (Mackie et al., 2013). Copper is essential for healthy cellular functioning, but can become toxic when supply exceeds demand and stresses macrofauna, microorganisms and their enzyme activities, and also potentially becomes toxic to plants (Mackie et al., 2013; Mayor et al., 2013). Zinc also has an important effect on soil microbes and has the ability to enhance as well as reduce activity depending on its concentration (Joshi and Jaiswal, 2013). Although accumulation of high levels of copper or zinc has been associated with reduced soil microbial activity, it seems unlikely that the quantities added to soil with a single application of manure or compost would be enough to significantly alter the activity of soil-borne plant pathogens (Table A.2 and Table A.4). In addition, especially with composts, most metallic elements such as copper are not readily available and released into the soil, as the availability of bio-active metal ions is strongly affected by organic matter, soil pH and cation exchange capacity (Mackie et al., 2013). Although, it is possible for sulphur, copper, zinc and iron within compost to be a mechanism of control for soil-borne pathogens, previous research points to biological factors as the most likely

causes of disease suppression in compost-amended soils (Bonilla et al., 2015; De Ceuster and Hoitink, 1999; Fichtner et al., 2004; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink and Grebus, 1994; Hoitink et al., 1997; Litterick and Wood, 2009; Lockwood, 1988; Mehta et al., 2014; Noble and Coventry, 2005; Postma and Schilder, 2015; Rahman et al., 2014; Saxena et al., 2015; Timper 2014; Zinati, 2005). Nonetheless, such factors should not be ruled out, and experiments demonstrating disease suppression from application of composts should consider relationships between inputs of S, Cu, Zn and Fe and disease suppression.

1.3.1.1 Compost-induced suppression of diseases caused by *Phytophthora*

Many studies of organic amendments on suppression of *Phytophthora* have shown efficacy in both container media and under field conditions (Aryantha et al., 2000; De Ceuster and Hoitink, 1999; Fichtner et al., 2004; Gilardi et al., 2013; Hoitink and Boehm, 1999; Litterick and Wood, 2009; Maloney et al., 2005; Mehta et al., 2014; Milner et al., 2004; Noble and Coventry, 2005). Negative environmental implications of using fumigants, and a lack of efficacy of fungicides against *Phytophthora* show that there is a market for an alternative such as compost (Kempler et al., 2012; Milner et al., 2004). Negative correlations between total microbial activity and *Phytophthora* root and crown rot, suggest that higher levels of microbial activity are responsible for the greater suppression of this disease in amended soils (Kim et al., 1997). Other studies found a relationship between low organic matter levels and increased activity of *Phytophthora* (Downer et al., 2001). With additions of compost, suppression of *Phytophthora cinnamomi* was achieved through biological control; first because the compost provided a substrate for growth of fungal antagonists, and second because composts also created an environment that promoted activity of enzymes associated with degradation of hyphae of oomycetes (Downer et al., 2001). Furthermore, additions of organic matter have been associated with improved soil structure (Ownley and Benson, 1991). *Phytophthora* thrives in wet soils because of elevated zoospore formation and dispersal; also oxygen deficiency as a result of waterlogging can cause roots to be leakier, inhibit root regeneration and affect the host plant's resistance mechanisms, predisposing the plant to infection (Duncan and Kennedy, 1989). Improving soil structure through the decomposition of organic matter results in better water retention during dry weather as well as improved drainage during periods of increased precipitation; therefore, reducing conduciveness to the *Phytophthora* infection process under wet

conditions and decreasing water stress under dry conditions (Duncan and Kennedy, 1989). Some composts can have relatively high calcium concentrations and augmenting soil with calcium has been found to lower the incidence of disease of *Phytophthora* (Maloney et al., 2005; Serrano et al., 2011; Sugimoto et al., 2010). Calcium restricts the liberation and movement of infective *Phytophthora* propagules, thus causing an interference with zoospore formation and reduction in disease (Maloney et al., 2005; Serrano et al., 2011; Sugimoto et al., 2010).

1.3.1.2 Compost-induced suppression of plant parasitic nematodes

With plant parasitic nematodes being major pests of red raspberry and the new restrictions on pre-plant soil fumigation, the need arises for more studies looking at alternative nematode management strategies (Walters et al., 2009). Forge and Kempler (2009) found a negative correlation between the population densities of omnivorous and predacious nematodes and the population densities of *P. penetrans* (root lesion nematode) under several organic mulch treatments applied to red raspberry. They also found a positive correlation between root biomass and populations of *P. penetrans* (Forge and Kempler, 2009). These larger populations of omnivorous and predacious nematodes are an example of how organic amendments promote populations of soil organisms that are antagonistic to plant parasitic species. A similar study found that shredded paper mulch increased these antagonistic nematodes and decreased populations of *P. penetrans* in roots of apple trees (Forge et al., 2008). Populations of fungal and bacterial antagonists of parasitic nematodes, such as *Trichoderma* or *Pseudomonas* spp., along with predacious invertebrates such as Collembola also can be stimulated by the addition of organic matter to soil (Oka and Yermiyahu, 2002; Rahman et al., 2014; Thoden et al., 2011). Depending on the parent material of the organic amendments, some amendments released nematotoxic compounds such as organic acids, plant secondary metabolites and nitrogenous compounds during decomposition (Oka and Yermiyahu, 2002; Rahman et al., 2014; Thoden et al., 2011). Organic amendments with low C/N ratios, such as poultry manure, often contain substantial amounts of ammonia that is liberated in soil and can reduce populations of parasitic nematodes, which are known to be very sensitive to ammonia (Oka and Yermiyahu, 2002; Thoden et al., 2011). However, the success of organic acids and ammonia is dependent on soil physico-chemical properties such as soil pH (Thoden et al., 2011). This was evident as swine

manure was discovered to be more effective in reducing the numbers of plant parasitic nematodes in acidic soils as opposed to neutral or alkaline soils (Lazarovits et al., 2001).

Increased knowledge of several types of composts and their influences on *Pratylenchus penetrans* and *Phytophthora rubi*, would help improve the understanding of the potential benefits of composts for use in raspberry production in the Fraser Valley.

1.4 Objective

The objective of my thesis was to determine the effects on inoculum densities of *Phytophthora rubi* and *Pratylenchus penetrans* of incorporating composts into soil prior to planting red raspberry. Two different compost amendments were compared to incorporation of raw broiler manure and fumigation, both of which have been standard practices of commercial raspberry growers but have negative implications for environmental quality. The two composts that I evaluated were made from readily available materials in the Lower Mainland and are described in more detail below. All of the organic amendments used in these experiments were applied in two different concentrations based on nitrogen contents as described below. Two outdoor greenhouse pot experiments at the Pacific Agri-Food Research Centre-Clearbrook research substation were set up to address this objective and to test the following hypotheses:

1.5 Hypotheses tested

1. Compost will improve plant growth relative to the non-amended control, but not compared to fumigation and manure treatments.
2. Compost will decrease population densities of *Pratylenchus penetrans* relative to the non-amended control, but not as well as fumigation and manure treatments.
3. Compost will decrease the incidence of disease by *Phytophthora rubi* relative to the non-amended control and fumigation, but not as well as the manure amendment.
4. Compost will increase population densities of free living nematodes relative to the non-amended control and fumigation, but but not as well as the manure amendment.
5. There will be no differences between PARC compost and spent mushroom compost with respect to plant growth, nematode populations and incidence of *Phytophthora* root rot.

Chapter 2: Site and Methods

2.1 Research site and soil

The study consisted of two outdoor pot experiments located at the Agriculture and Agri-Food Canada research substation at 510 Clearbrook Rd., Abbotsford, BC. The first experiment was conducted during spring of 2013 and the second experiment occurred during the summer of 2013. Both experiments used soil from an old raspberry field at the site that was naturally infested with *Pratylenchus penetrans*. Six composite samples were taken from the site and subjected to a complete suite of chemical analyses (Table 2.1). Kuchta (2012) reported results of basic soil properties such as dry bulk density (BD) and texture prior to establishing his research plots at the same Clearbrook substation field site (Table 2.2) (Figure 2.1).

The soil was collected from random locations in the field to a depth of 30 cm using a shovel. The soil was then passed through a 5 mm sieve, mixed thoroughly, and then 5 L aliquots were placed into each of 160 black polyethylene "2 gallon" greenhouse pots (80 pots for experiment 2) (Diameter 8.5" x Height 8.5"). A Decagon EM50 data logger with five 5TM probes was used to monitor soil moisture and temperature regimes in the pots for both experiments. The six probes were placed in six randomly chosen pots.

Table 2.1: Soil analyses of six separate 0-30cm composite samples from the field soil at Clearbrook substation. Each composite sample was composed of 20 cores taken from an 80 x 40 m area used to provide soil for the experiments, and was analyzed by A&L Canada Laboratories Inc., ON, Canada. Values in parentheses are standard deviations.

Macronutrients and properties									
OM (%)	N (%)	C/N Ratio	pH	CEC (meq/100g)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
5.35 ±.37	.19 ±.02	17.6 ±1.7	5.6 ±.15	16.1 ±1.2	52.5 ±13.2	91.3 ±8.7	621.7 ±144	45 ±6.3	17.3 ±3.9

Micronutrients (ppm)						
Zn	Cu	Fe	Al	Mn	B	Na
2.55±.47	.88±.21	58±3.29	2015±106	11.3±.82	.1±0.0	9.5±2.5

*Organic matter and nitrogen determined by combustion analysis
OM= organic matter
CEC= electrical conductivity of the soil

Table 2.2: Physical and textural properties of the field soil at Clearbrook substation (Kuchta, 2012).

Horizon	Depth (cm)	Dry BD[a] (g cm $^{-3}$)	Texture
A_p	0-26	1.18	Loam
B_{fj}	26-61	1.27	Loam
II_c	62-78+	1.80	Sand to gravelly sand

[a]using brass cores ~137cm^3

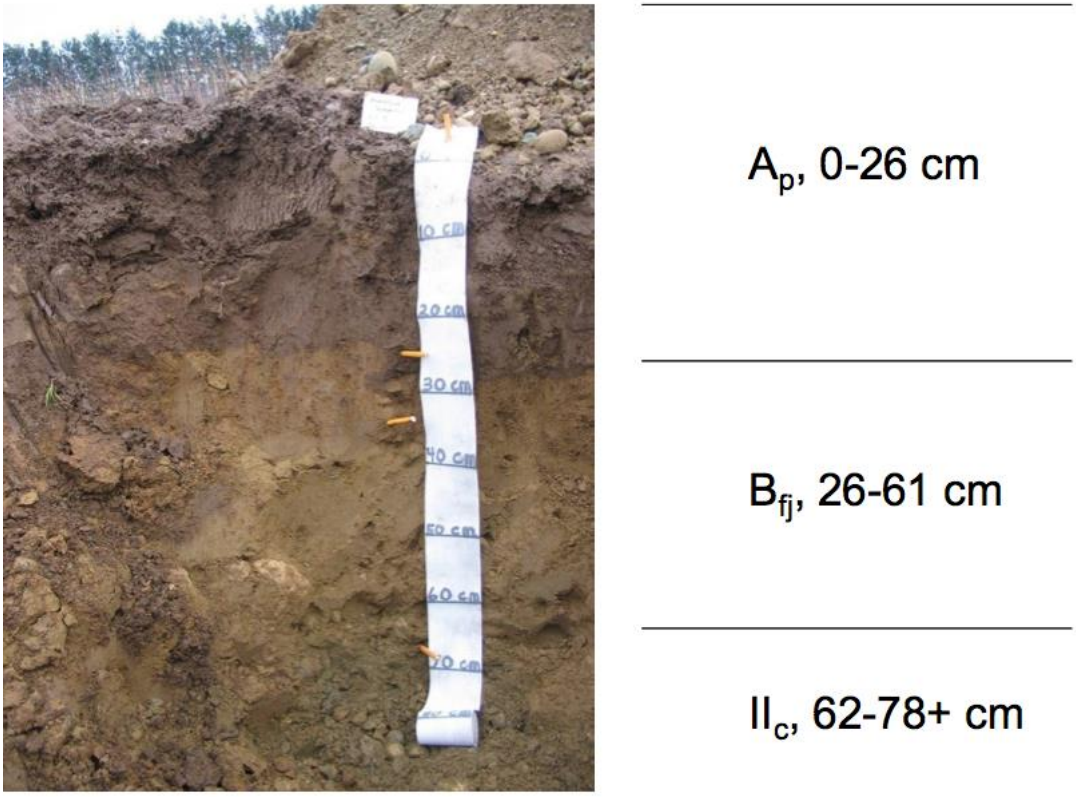

Figure 2.1: Soil profile with horizons identified, Clearbrook substation (Kuchta, 2012)

2.2 Raspberry plants

Red raspberry plants used for both experiments were cultivar 'Malahat', which is susceptible to *Phytophthora rubi* infection, and were propagated through rooted cuttings. Roots from greenhouse-grown Malahat plants from AAFC Pacific Agri-Food Research centre in Agassiz, British Columbia were spread out in trays with pasteurized potting medium. New shoots emerging from roots were cut, rinsed with deionized water, dipped into rooting hormone and transplanted into pots containing pasteurized potting medium. They were grown under standard greenhouse conditions (20°C, 16h photoperiod, 60-70% relative humidity) for several weeks before being used.

2.3 Soil treatments

Soil treatments for both experiments were arranged in a randomized complete block design consisting of 1) control (no amendment); 2) 2X PARC compost (high PARC); 3) 1X PARC compost (low PARC); 4) 2X mushroom compost (high mushroom compost); 5) 1X mushroom compost (low mushroom compost); 6) 2X broiler manure (high manure); 7) 1X broiler manure (low manure); 8) fumigation. The broiler manure was obtained from local commercial broiler chicken farms and also consisted of wood shavings. The PARC compost was produced at the AAFC Pacific Agri-Food Research Centre in Agassiz, British Columbia. The primary feedstocks for this compost are layer manure (from laying hens), greenhouse waste (discarded plants with potting media) and yard waste (grass clippings and prunings), and the compost was made using the turned windrow approach, with weekly turning for six weeks followed by approximately six weeks curing. The mushroom compost is by-product of *Agaricus* mushroom production and was obtained from Champ's Mushrooms farm located in Aldergrove, BC. Primary feedstocks of mushroom composts used in Fraser Valley mushroom production are wheat straw, bedded horse manure, poultry manure and gypsum. Properties of the composts and manure used in the experiments are presented in Table A.1 and Table A.3.

The fumigant, Basamid® (Mitsui & Co., Toronto, ON; active ingredient Dazomet), is a powder that was mixed into the soil at a rate of 1.5 g per pot which corresponded to the recommended label application rate of 200 g/m^3 applied to a depth of 30 cm. Dazomet hydrolyzes in soil to methyl isothiocyanate, which is the active ingredient in Vapam®, the commercial fumigant normally used on raspberry farms in the Fraser Valley. The pots were then hand watered to near saturation and covered with plastic. After four weeks, the plastic was removed and the pots were allowed to off-gas for two weeks.

The compost and manure amendments were analyzed for nitrogen content beforehand (Table A.1 and Table A.3) and application rates were based on a benchmark application rate of 500 kg total N/ha equivalent for 1X or "low" treatments. The rationale for using 500 kg total N/ha as a benchmark application rate was that raspberry growers typically fertilize at N application rates of between 50 and 100 kg N/ha each year, and annual losses of N from the system (primarily harvested berries) are expected to be between 30 and 50 kg N/ha. Using 50 kg N/ha per year as a crude estimate of losses from the agroecosystem, and making a conservative

assumption that replanting will occur within 10 years, it can be argued that the incorporation of an organic amendment at 500 kg total N ha^{-1} at the time of replanting would roughly balance losses from the cropping system over the following 10 years and would be a sensible base rate. The rate at which N is mineralized and made available to the crop will actually vary considerably among the composts and manures, so this one-time application would not be expected to offset fertilizer requirements for the life of the crop. A 2X or "high" treatment was also included for experimental reasons and corresponds approximately to the 250 m^3/ha rates used in the previous field experiments (Forge et al., 2015; 2016) and approximates rates of application that were used historically as pre-plant amendments (Zebarth et al., 2015) (Appendix A). Pre-plant incorporation of compost at such rates did not increase nitrate leaching relative to unamended soil (Forge et al., 2015; 2016), so such an application rate could be justified if it was necessary to suppress *P. penetrans* and/or *P. rubi*.

All of the organic amendments were thoroughly hand mixed with the field soils within their pots at the same time as fumigation. Pots not treated with an organic amendment were fertilized with urea at the equivalent of 100 kg N/ha (as within the recommended guidelines for growers by the British Columbia Ministry of Agriculture and Lands, 2013) to ensure that plant growth was not nitrogen limited.

2.4 Experimental design

Phytophthora rubi infects raspberry roots during periods when soil temperatures are cool and soil is often saturated, which typically occurs during the spring and fall seasons (Agrios, 1978; EPPO, 2013; Hoashi-Erhardt, 2008; Rudolph and DeVetter, 2015). Because of this, the first experiment of this project was set-up in spring and involved inoculating the soil with *P. rubi* followed by intentional flooding of the pots to create conditions conducive to *P. rubi* infection. Ideally, this experiment would have included flooding vs no flooding as a variable. However, if set up as a factorial experimental design, such an experiment would have had 32 treatment combinations or 320 pots. I did not have adequate resources to do an experiment of this size and, because assessing the effect of flooding was not an objective of my research, I decided to not include flooding as a variable and to subject all pots to flooded conditions conducive to *P. rubi* infection.

In contrast to *P. rubi*, root lesion nematodes do not prefer cool, moist conditions and flooding the pots after inoculation with *P. rubi* was likely to be suppressive to the native populations of root lesion nematodes in the soil. The conditions of the first experiment were not optimal for determining the effects of compost on root lesion nematodes. Therefore, a second experiment was set-up in summer of 2013 using soil naturally infested with high population densities of *P. penetrans* but did not involve *P. rubi* inoculation or flooding of the pots.

In Experiment 1 there were 20 pots of each of the eight soil treatments for a total of 160 pots. After planting, ten of the pots of each soil treatment were inoculated with *Phytophthora rubi* as described below. Because *P. rubi* inoculation was not included as a factor in Experiment 2, there were 10 pots of each of the 8 soil treatments for a total of 80 pots. For both experiments the pots were arranged in a randomized complete block design on a gravel pad at the experimental site. A blocked experimental design was chosen over a completely randomized design to facilitate harvesting the experiment and processing samples in batches corresponding to blocks. The pots were incubated for six weeks before planting. At the time of planting, a small core of soil was removed from each pot to estimate "time-zero" root lesion and free living nematode population densities.

2.5 Sampling procedure

For both experiments, the plants were removed from the pots thirteen weeks after the initial planting. At thirteen weeks, the root systems had largely filled the pots and I rationalized that because the root systems would become increasingly pot-bound, a longer growth period would not be beneficial to the experimental outcome. The shoots were collected and air dried in a greenhouse for determination of shoot dry weights. Each of the pots of soil was sieved through a coarse sieve (6-mm opening) and the whole root mass was removed and washed. For Experiment 1, the whole root mass was evaluated for root rot symptoms on a 1 to 8 root rating (1= healthy roots, 8= high decay) after they were washed. Soil samples were also collected from each pot during the sieving process. A portion of fine roots (~1 g) was collected from each treatment pot for nematode extraction using a mist chamber that was set up previously at PARC-Agassiz (Forge and Kimpinski, 2007). In Experiment 1, an additional sample of roots was taken and stored in a freezer at -20ºC for subsequent *P. rubi* analyses as described below. The remaining root system was air dried in the greenhouse and weighed. Soil samples were also

collected from each pot during the sieving process and stored in a refrigerator at 4ºC before nematode extractions occurred.

2.6 Nematode extraction and enumeration

Nematodes from both experiment 1 and 2 were extracted from the soil using the Baermann pan technique (Forge and Kimpinski, 2007), placed into 20-ml vials, and then stored in a refrigerator until the numbers of *Pratylenchus* nematodes were counted using an inverted microscope and a 10x10 gridded counting plate. The population present at the site was previously identified as *P. penetrans*. Characteristics that differentiate *Pratylenchus* species nematodes from other stylet-bearing nematodes are as follows: a distinctive short and thick stylet with large basal knobs, overlapping esophagus, flat head, an intestine packed with numerous dark granules as well as relatively slow and graceful movement (Mai and Mullin, 1996; Shurtleff and Averre, 2000).

In addition to *P. penetrans*, the numbers of free living nematodes were also counted for both experiments. The abundance of free living nematodes, which includes bacterivores, fungivores, omnivorous and predacious types, is indicative of inputs of food resources to the soil web in the absence of other stresses (Ferris and Bongers, 2006; Rahman et al., 2014; Thoden et al., 2010). The total number of free living nematodes, which are overall more numerous than *P. penetrans*, was estimated by counting a fraction of each plate and then multiplying the count by the fraction of the plate counted. No other species of plant-parasitic nematodes were present in the test soil, and free living nematodes were considered any nematodes that were not *P. penetrans*.

2.7 *Phytophthora rubi* inoculation and analyses (Experiment 1)

Inoculation with *Phytophthora rubi* was included as a factor in Experiment 1, followed by qPCR analyses of root tissues for the presence of *P. rubi*.

2.7.1 *Phytophthora* cultures

An isolate of *Phytophthora rubi* (JL11) from Pacific Agriculture Research Centre in Agassiz, BC, was grown in clarified V8 juice broth, [V8 juice, 340 ml; $CaCO_3$, 5 g; centrifuged at 3500 rpm for 30 min to clarify, the supernatant was diluted 1:9 with distilled water and

autoclaved for 20 min] (C. Koch, personal communication, December 13, 2012). Cultures were incubated at room temperature for 5 weeks. Mycelial mats were collected in a Buchner funnel, vacuum filtered, and rinsed with deionized water. The hyphae were added to deionized water to a concentration of 0.04 g/ml, and mixed in a blender with four 10-s pulses on low speed.

2.7.2 Inoculation procedure

The raspberry plants were inoculated 5 weeks after they were transplanted into the pots, thus allowing the root systems to recover from replant shock and begin to initiate new root growth. *P. rubi* inoculum was added to one-half of the pots of each soil amendment treatment (+PR treatment). A glass rod was used to create one 8-cm deep hole beside each plant and 5 ml of inoculum were delivered into each hole via a pipettor. This inoculation procedure is used routinely by the berry breeding program at PARC for standardized assessment of genetic resistance, so there is a high degree of confidence about its efficacy. Control plants (-PR) were inoculated with 5 ml of deionized water only. Following the inoculation, all of the plants were flooded for 72 h to create conditions that were favourable for infection. This experiment occurred during spring while the weather was cooler, which is also favorable for *Phytophthora rubi* growth and infection (Agriculture and Agri-Food Canada, 2007; Agrios, 1978; EPPO, 2013). The resulting experimental design consisted of 16 treatments (8 soil amendment treatments x 2 *Phytophthora* treatments (+/-), each replicated in 10 pots).

2.7.3 PCR procedure

Conventional PCR was also run in order to confirm that no *P. rubi* was already present in the soil (tested from time-zero soil samples) using primers DC1 and MP5 which target a portion of the ITS1 region of the ribosomal DNA specific to *P. rubi* (Bonants et al., 2004) obtained from Sigma-Aldrich Canada. DNA was extracted from the time-zero soil samples using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) as described by the manufacturer. Cycling parameters for conventional PCR were 94ºC for 5 min, followed by 39 cycles of 94ºC for 45 sec, 58ºC for 45 sec and 72ºC for 45 sec, and a final 10-min extension at 72ºC. Final concentrations of reagents were 1X Bioline buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.25 units Bioline Taq polymerase and 0.5 µM of each primer, plus 1 µl of template DNA to a final volume of 20 µl in sterile deionized water. Products were then analyzed by gel electrophoresis on 1.5%

agarose, followed by staining in ethidium bromide, and imaging using GelDoc apparatus (Bio-Rad Laboratories Inc., Carlsbad, CA).

2.7.4 Quantitative PCR procedure

The quantitative PCR assays were run using BioRad C1000 Thermal Cycler (Bio-Rad Laboratories Inc., Carlsbad, CA). Following nematode extraction, ~1g portion of fine roots from each treatment pot was dried, ground and passed through a #20 sieve (0.85 mm mesh size). DNA was extracted from about 10-mg samples of ground tissue using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA). Use of this kit eliminated the PCR inhibitors present in the root samples (pers. comm., Carol Koch, plant pathology technician, Agriculture and Agri-Food Canada). Primers DC1 and MP5 (Bonants et al., 2004) obtained from Sigma-Aldrich Canada were then used for quantitative PCR. These primers, which yield an amplicon of 127 bp, were developed and tested by Bonants et al. (2004). They were also previously tested in the plant pathology laboratory at PARC-Agassiz for specificity with respect to BC isolates of *P. rubi* and cross-reaction with other oomycetes that may occur in the root zone of raspberry. They are currently being used extensively in the plant pathology laboratory at PARC-Agassiz. DNA was extracted from pure *P. rubi* tissue (isolate JL11) using the FastDNA kit (MP Biomedical, Solon, OH) and its protocol for fungal tissue. This DNA isolated from a pure culture of *P. rubi* (diluted 600X) was used to make up a 2X dilution standard series for qPCR ranging in concentration from 6250 to 49 pg/ml. Cycling parameters for qPCR included an initial denaturation step of 3 min at 95ºC followed by 41 cycles of 95ºC for 10 sec and 63ºC for 30 sec. This was followed by a melt temperature determination ranging from 72ºC to 90ºC. Reagents were Evagreen Supermix (consists of buffer, dNTPs, polymerase and Evagreen dye) (Biorad, cat.#1725202) and primers with final concentrations of 1X Evagreen mix and 0.5 µM of each primer, plus 1 µl of template DNA to a final volume of 20 µl in sterile deionized water.

2.7.5 Positive controls and standard series for qPCR and PCR

DNA extracted from infected root tissue from a root rot experiment performed at PARC (Agassiz) that used *P. rubi* isolate JL11, was used as the positive control for qPCR of the root samples. The concentration of DNA in the positive controls and standard series was calculated in

the Life Sciences Centre lab at University of British Columbia Vancouver using the Qubit Broad Range kit (Invitrogen, Carlsbad, CA) as described by the manufacturer.

2.8 Statistical Analyses

Parameters subjected to statistical analyses in both experiments included: shoot dry weight, root dry weight, *P. penetrans* in soil per pot, *P. penetrans* per g root, total *P. penetrans* per pot (root and soil populations combined) and free living nematodes per pot. The data were first tested for homogeneity of variances with Bartlett's test. Population data of *P. penetrans* were log-transformed prior to final analyses to compensate for non-normal distribution. For Experiment 1, a blocked two-way analysis of variance (ANOVA) was performed on the response parameters to assess the simple and interactive effects of amendment and *P. rubi* inoculation factors. For Experiment 2, a blocked one-way analysis of variance was performed.

Planned linear contrasts were used to test the significance of differences between groups of treatments, and were designed to test the five main hypotheses. Contrasts are used for testing the significance of a difference between two groups of means, such as comparing all manure treatments to all compost treatments, or for testing the significance of difference between a group of means and a single mean, such as comparing the grand mean of all compost-amended treatments to the control. Analyzing contrasts involves assigning coefficients to the means in a way that they add up to zero. For instance, for comparing the control to all compost treatments, the set of coefficients would be (4 0 0 0 -1 -1 -1 -1) if the treatments were entered into the analyses in the order: control, fumigation, low manure, high manure, PARC low, PARC high, Mushroom low, Mushroom high. The test of significance is then a test of whether the weighted grand mean deviates significantly from zero, relative to a weighted variance (Snedecor and Cochran, 1980).

For each parameter, the first contrast compared composts as a group to the untreated control; the second contrast compared composts as a group to the fumigation treatment; the third contrast compared composts as a group to the manure treatments combined; the fourth contrast compared mushroom compost to PARC compost. For further exploratory analyses of relationships among treatments, supplemental contrasts were used to compare manure treatments combined to the control and to the fumigation treatment. Additional informal comparisons

between means were performed using Duncan's test. All of the data were analyzed using SAS (SAS Institute Inc. 2000). The significance of correlations between *P. penetrans* and plant weight, free living nematodes and plant weight, and free living nematodes and *P. penetrans* was determined using the Pearson's correlation coefficient computed using Microsoft Excel (Microsoft Corporation 2010).

Chapter 3: Results

3.1 Experiment 1

3.1.1 *Phytophthora rubi*:

Prior to planting experiment 1, the soil used in the pots was tested for the presence of *P. rubi*. No pathogen was detected prior to inoculation of the pots (Fig. 3.1).

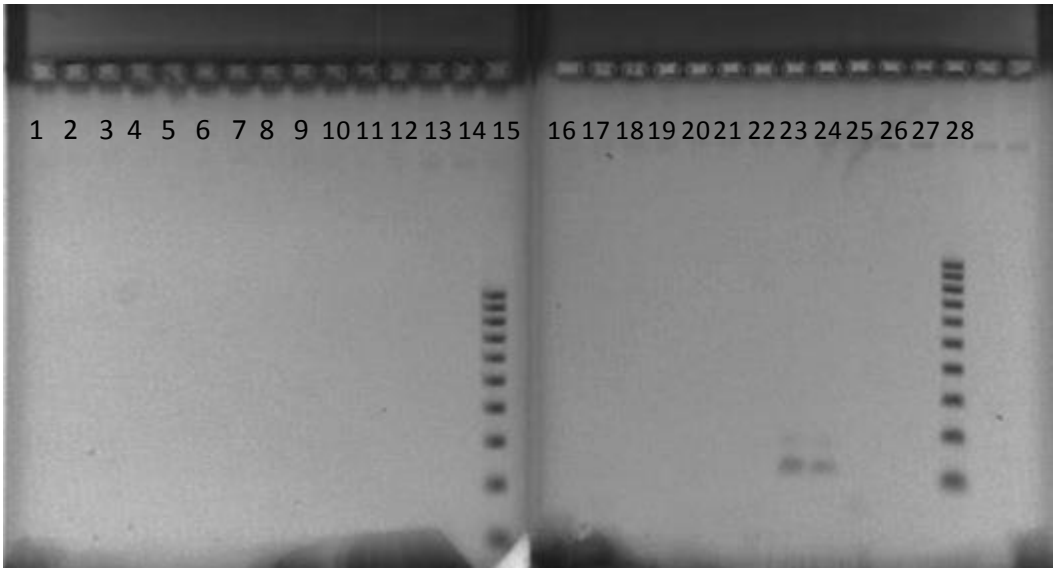

Figure 3.1: Products of PCR amplification testing for the presence of *P. rubi* from 19 different samples of soil before the start of the experiment 1 using *P. rubi* specific primers (DC1 and MP5). Samples were loaded on a 1.5% TBE agarose gel and stained using ethidium bromide 0.5 ug/ml in distilled water. Lanes 1-12 &16-22: experimental site soil samples; lanes 23 & 24: positive controls; lane 25: blank/NTC; lanes 15 & 28 GeneRuler100 bp DNA ladder (Carlsbad, CA); Lanes 13,14 & 26,27: empty lanes.

Roots harvested after 13 weeks from each treatment were assayed for the presence of *P. rubi* using qPCR. *Phytophthora rubi* was not detected in the non-inoculated controls. *Phytophthora rubi* was detected in one inoculated pot from block 1 and it was present in trace amounts in two samples from block 10 (Table A.7) (Figure A.7, A.10 and A.11), in one root sample each from blocks 7 and 8 (Table A.5) (Figure A.5, A.16 and A.17), but no detection of *P. rubi* occurred in blocks 6 and 9 (Table A.5)(Figure A.14 and A.15) and one sample from block 4 was positive for *P. rubi* (Table A.6) (Figure A.6 and A.9); however, there was none detected in blocks 3 and 5 and block 2 (Table A.6) (Figure A.12, A.13 and A.8). The positive samples from block 10 were treatments with low mushroom compost and fumigation. The positive sample from block 1 was a control treatment. The positive sample from block 7 was high mushroom

compost and the positive sample from block 8 was the low mushroom compost treatment. The positive treatment from block 4 was fumigation. These five blocks were the only blocks where some samples were positive for *P. rubi* via. qPCR (Appendix D)

3.1.2 Plant analyses

The amendment factor had a significant main-factor effect on the mean shoot and root dry weights in the overall analysis of variance. There was no significant effect of *P. rubi* inoculation nor was the amendment x *P. rubi* interaction significant (Table 3.1).

3.1.2.1 Shoot weight

Compost treatments did not result in greater shoot weights than the control (control vs compost contrast $p < 0.001$), fumigation (fumigation vs control contrast $p < 0.001$) and manure treatments (manure vs compost contrast $p < 0.001$), as shown in Figure 3.2. There were no significant differences in shoot weight between the mushroom and PARC compost amendments (mushroom vs PARC contrast $p = 0.79$), and the rate of compost application did not have a significant effect on shoot weight (Table A.11). The manure treatment resulted in greater shoot weights than the control (control vs manure contrast $p < 0.001$). There was no significant difference between the fumigation and manure treatments (fumigation vs manure contrast $p= 0.28$).

3.1.2.2 Root weight

Compost treatments did not result in greater root weights than the control (control vs compost contrast $p = 0.05$), manure (manure vs compost contrast $p < 0.001$) and fumigated treatments (fumigation vs control contrast $p < 0.001$), as shown in Figure 3.2. There was no significant difference in root weights between the mushroom and PARC compost (mushroom vs PARC contrast $p = 0.37$), and the rate of compost application did not have a significant effect on root weight (Table A.11). The manure treatment had significantly greater root weight than the control (control vs manure contrast $p = 0.0015$) and there were no significant differences in root biomass between the fumigation and manure treatments (fumigation vs manure contrast $p = 0.75$).

Table 3.1: Summary of p-values from analysis of variance of data from Experiment 1. Data were analyzed using a two-way ANOVA model with *P. rubi* inoculation and amendment treatments as the two factors. In all, there were 16 treatment combinations (8 amendment treatments x 2 *P. rubi* inoculations (+/-)) in each of ten replicate blocks. The dependent variables (going across the top of the table) were analyzed separately.

Plant Parameters			***Pratylenchus penetrans* (P.p.)**				**Free living nematodes (FLN)**	
			Day of planting	**End of experiment (13 weeks after planting)**			**Day of planting**	**End of experiment (13 weeks after**
	Shoot wt (g)	**Root wt (g)**	**P.p./pot**	**P.p./ g root**	**P.p./pot of soil**	**P.p. /pot (roots + soil)**	**FLN/pot**	**FLN/pot of soil**
ANOVA summary—P-values								
Block	.0013	<0.0001	.3686	.0206	<0.0001	.3136	.6666	<0.0001
Amendment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P. rubi	.1344	.3520	.6121	.9786	.3766	.7967	.7867	.0747
***P. rubi**Amendment**	.3336	.3675	.7933	.9565	.8367	.9334	.6774	.2086
Planned Contrasts								
control vs compost	0.0011	0.0495	0.3752	0.1386	0.0082	0.5071	0.0320	0.0137
fumigation vs compost	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
manure vs compost	<0.0001	<0.0001	<0.0001	<0.0001	0.0022	<0.0001	<0.0001	<0.0001
mushroom vs PARC	0.7886	0.3690	0.3154	0.1675	0.1064	0.1389	0.3828	0.0261
control vs manure	<0.0001	0.0015	<0.0001	<0.0001	0.7696	0.0095	<0.0001	<0.0001
fumigation vs manure	0.2815	0.7546	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

wt= weight
P.p.= *Pratylenchus penetrans*
FLN= Free living nematodes

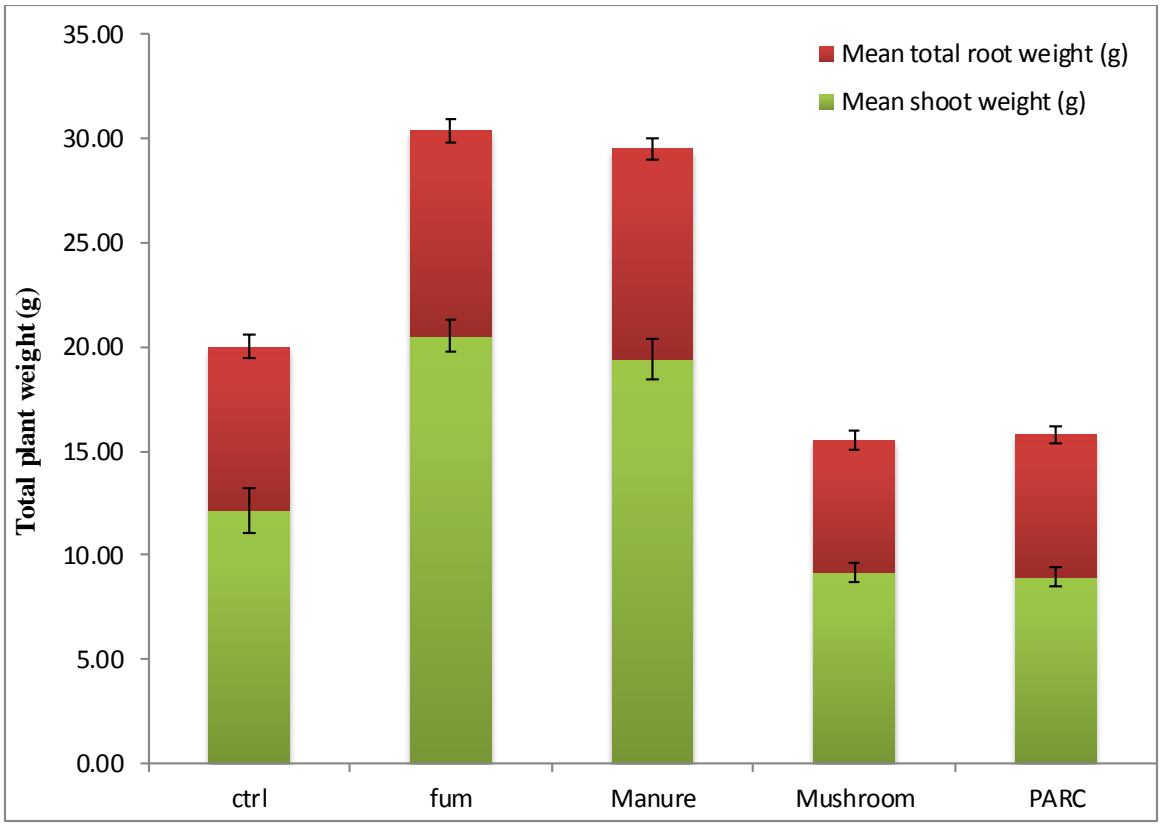

Figure 3.2: Effects of amendment treatments on mean shoot and root dry weights (g) of raspberry plants 13 weeks after planting, ±standard error of the mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

3.1.3 Nematode populations

3.1.3.1 Pre-plant nematode populations

The amendment factor had a significant effect on the number of *P. penetrans* at the time of planting in the overall analysis of variance (Table 3.1). However, there was no significant effect of *Phytophthora* inoculation nor was there an interaction effect of inoculation and amendment treatment on the number of *P. penetrans*. Compost amendments did not significantly reduce abundance of *P. penetrans* at the time of planting compared to the control (control vs compost contrast $p = 0.38$). The abundance of *P. penetrans* at planting was greater in compost-amended pots relative to manure (manure vs compost contrast $p < 0.001$) and fumigation (fumigation vs compost contrast $p < 0.001$), as shown in Figure 3.3. There was no significant difference at the time of planting in the abundance of *P. penetrans* between mushroom and PARC compost amendments (mushroom vs PARC contrast $p = 0.32$), and rate of compost

application did not have a significant effect on population densities of *P penetrans* (Table A.11). Manure treatments significantly decreased populations of *P. penetrans* compared to the control (control vs manure contrast $p < 0.001$). Fumigated pots had significantly smaller populations of *P. penetrans* at the time of planting compared to manure (fumigation vs manure contrast $p < 0.001$).

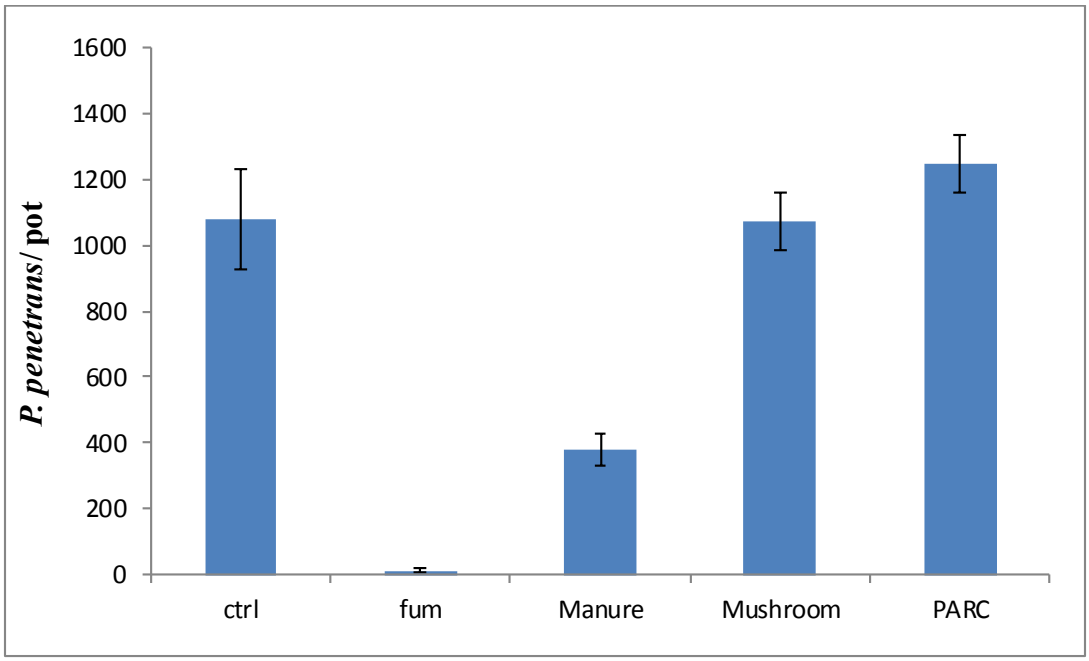

Figure 3.3: Effects of amendment treatments on mean abundance of *Pratylenchus penetrans* per pot, on the day the raspberry plants were planted into the pots, ±standard error of the mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

The only plant parasitic nematode in the test soil was *P. penetrans*. The free living soil nematode community was comprised primarily of bacterivorous and secondarily fungivorous nematodes. In the overall analysis of variance, the amendment factor had a significant effect on the total number of free living soil nematodes at the time of planting Experiment 1 (Table 3.1). There was no effect of *P. rubi* inoculation or the *P. rubi* x amendment interaction in the overall analysis of variance. Compost amendments significantly increased the total number of free living soil nematodes at the start of Experiment 1 relative to the non-amended control (control vs compost contrast $p = 0.032$) and fumigation (fumigation vs compost contrast $p < 0.001$), as shown in Figure 3.4. Compost did not increase population densities of free living soil nematodes compared to manure (manure vs compost contrast $p < 0.001$). There was no significant

difference between the two composts on populations of free living nematodes (mushroom vs PARC contrast $p = 0.38$), and the rate of compost application did not have a significant effect on population densities of free living nematodes (Table A.11). Poultry manure had significantly larger populations of free living soil nematodes than the non-amended control (control vs manure contrast $p < 0.001$) and fumigation treatment (fumigation vs manure contrast $p < 0.001$).

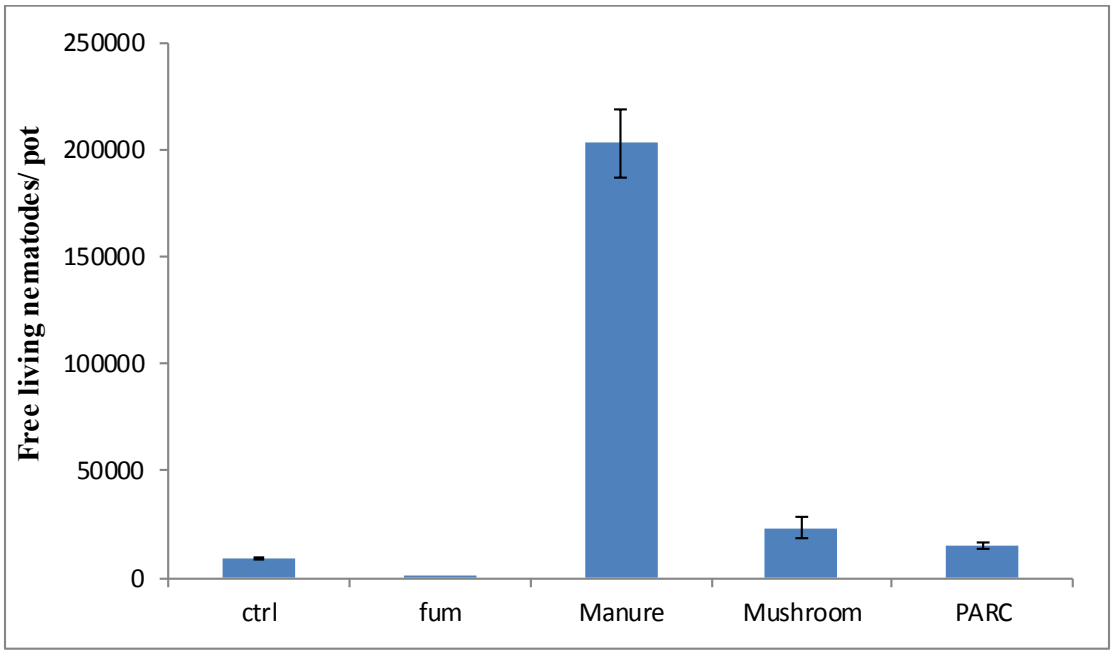

Figure 3.4: Effects of amendment treatments on mean abundance of free living nematodes per pot, on the day raspberry plants were planted into the pots, ±standard error of the mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

3.1.3.2 Final nematode populations

There was a significant amendment effect on the number of *P. penetrans* found in raspberry roots in the overall analysis of variance (Table 3.1). There was no significant effect of *P. rubi* inoculation or of an interaction between inoculation and pre-plant amendment. The compost amendments had significantly larger populations of *P. penetrans* per gram of root compared to manure (manure vs compost contrast $p < 0.001$) and fumigation (fumigation vs compost contrast $p < 0.001$), as shown in Figure 3.5. There was no significant difference between compost amendments and the non-amended control (control vs compost contrast $p = 0.14$) and no difference between the two compost treatments with respect to *P. penetrans* per gram of root (mushroom vs PARC contrast $p = 0.17$) The rate of compost application did not

have a significant effect on *P penetrans* per gram of root (Table A.11). Fumigation resulted in significantly smaller populations of *P. penetrans* per gram of root compared to manure (fumigation vs manure contrast $p < 0.001$). Broiler manure amendment resulted in significantly lower populations of *P. penetrans* per gram of root compared to the non-amended control (control vs manure contrast $p < 0.001$).

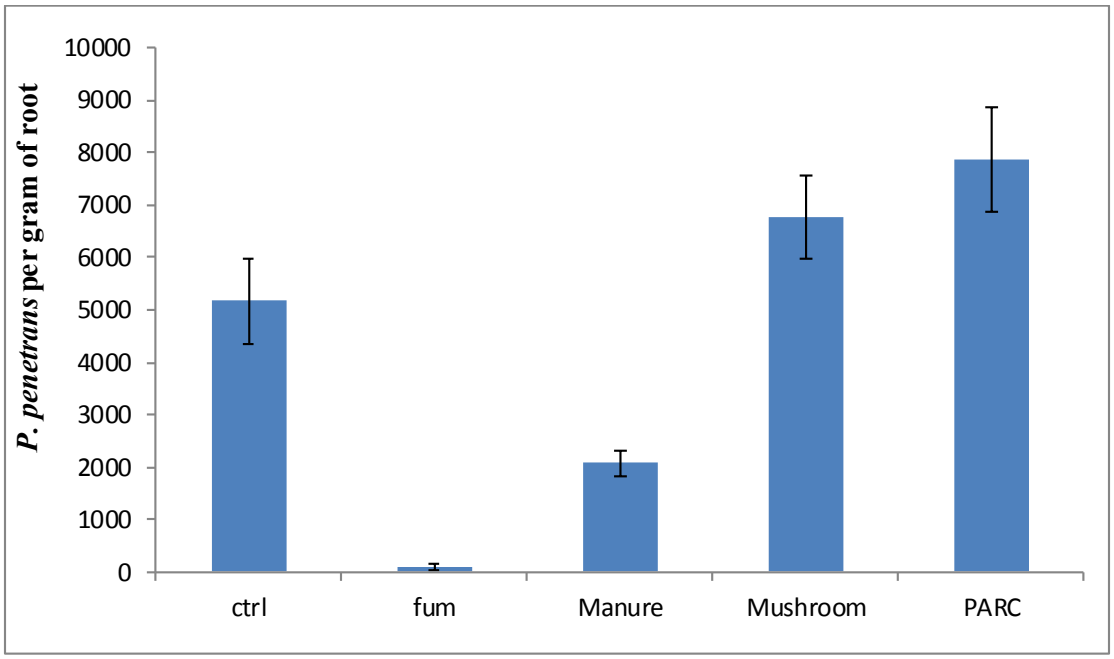

Figure 3.5: Effects of amendment treatments on mean *Pratylenchus penetrans* per gram of raspberry root, collected 13 weeks after planting ±standard error. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

At the end of the experiment the overall analysis of variance indicated that there was a significant effect of amendments on the number of *P. penetrans* per pot of soil (Table 3.1). There was no significant effect of *P. rubi* inoculation, nor was there an interaction effect between *P. rubi* and pre-plant amendment in the overall analysis of variance**.** Compost resulted in significantly larger populations of *P. penetrans* per pot of soil than the non-amended control (control vs compost contrast $p = 0.008$), fumigation (fumigation vs compost contrast $p < 0.001$) and manure (manure vs compost contrast $p = 0.002$), as shown in Figure 3.6. There was no significant difference between the two different compost amendments in their effect on mean populations of *P. penetrans* per pot of soil (mushroom vs PARC contrast $p = 0.11$), and rate of compost application did not have a significant effect on abundance of *P penetrans* per pot of soil

(Table A.11). There was no significant difference between the non-amended control and manure treatments on populations of *P. penetrans* per pot of soil (control vs manure contrast p = 0.77). Fumigated pots had significantly smaller soil populations of *P. penetrans* than manure-amended pots (fumigation vs manure contrast p < 0.001).

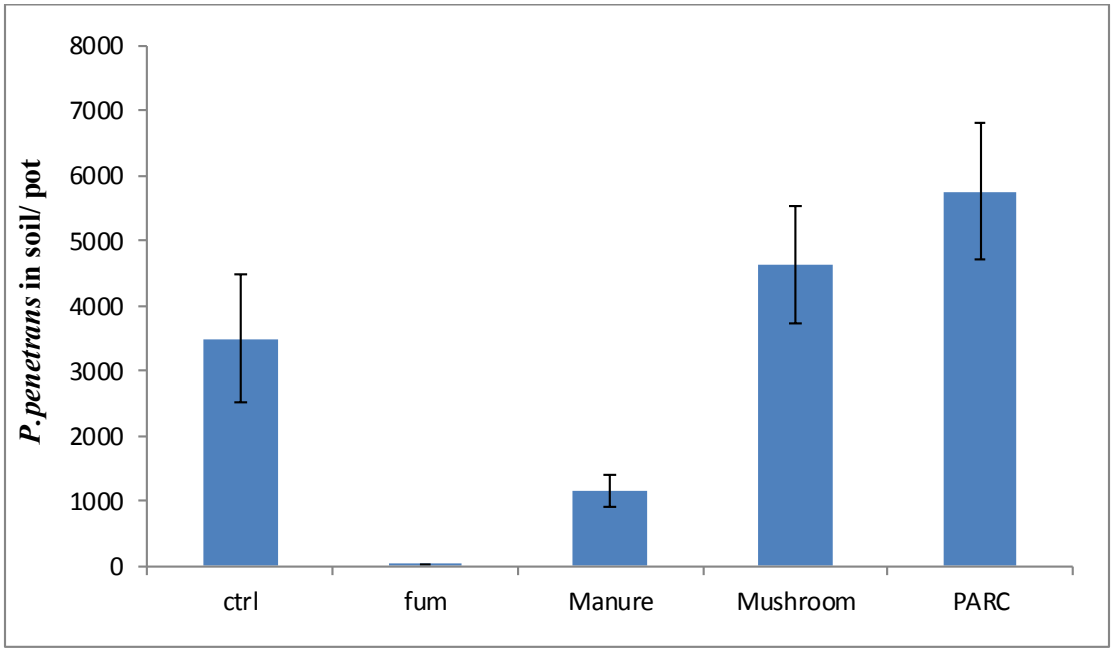

Figure 3.6: Effects of amendment treatments on mean population densities of *Pratylenchus penetrans* extracted from the soil per pot 13 weeks after planting ±standard error of the mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

There was a significant effect of amendment factor on the mean total number of *P. penetrans* per pot of soil (nematodes in roots and soil combined) in the overall analysis of variance (Table 3.1). There was no effect of *P. rubi* and of the interaction of inoculation x pre-plant amendment in the overall analysis of variance. Compost amendments had significantly larger total *P. penetrans* populations per pot than broiler manure (manure vs compost contrast p < 0.001) and fumigation (fumigation vs compost contrast p < 0.001), as shown in Figure 3.7. There was no significant difference between compost amendments and the non-amended control (control vs compost contrast p = 0.51) and no difference between the two different compost treatments (mushroom vs PARC contrast p = 0.14) on total numbers of *P. penetrans* per pot. The rate of compost application did not have a significant effect on total *P penetrans* per pot (Table A.11). Broiler manure resulted in significantly smaller numbers of *P. penetrans* per pot

compared to the non-amended control (control vs manure contrast p = 0.01). Fumigation resulted in significantly smaller numbers of total *P. penetrans* per pot than manure-amended pots (fumigation vs manure contrast p < 0.001).

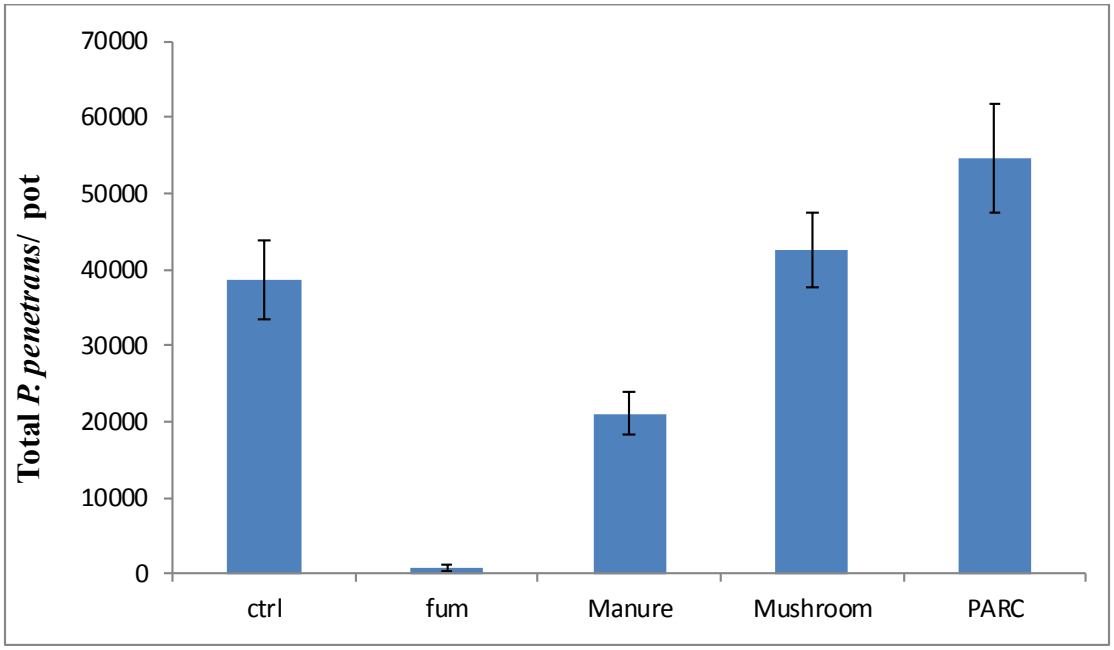

Figure 3.7: The mean total number of *Pratylenchus penetrans* per pot (sum of *P. penetrans* in soil and roots) after 13 weeks raspberry growth ±standard error of mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

There was a significant effect of amendment on the number of free living nematodes per pot of soil in the overall analysis of variance (Table 3.1). No effect of *P. rubi* and no interaction effect of inoculation x pre-plant amendment were found. Compost had significantly larger populations of free living soil nematodes than the non-amended control (control vs compost contrast p = 0.014) and fumigation (fumigation vs compost contrast p < 0.001). Free-living nematode populations in manure-treated pots were significantly greater than in compost-amended pots (manure vs compost contrast p < 0.001), as shown in Figure 3.8. PARC compost supported significantly greater numbers of free-living nematodes than mushroom compost (mushroom vs PARC contrast p = 0.03), but, the rate of application did not have a significant effect on mean population densities of free living nematodes per pot (Table A.11). Manure-treated pots had significantly larger populations of free living nematodes than non-amended control pots (control vs manure contrast p < 0.001) and fumigated pots (fumigation vs manure contrast p < 0.001).

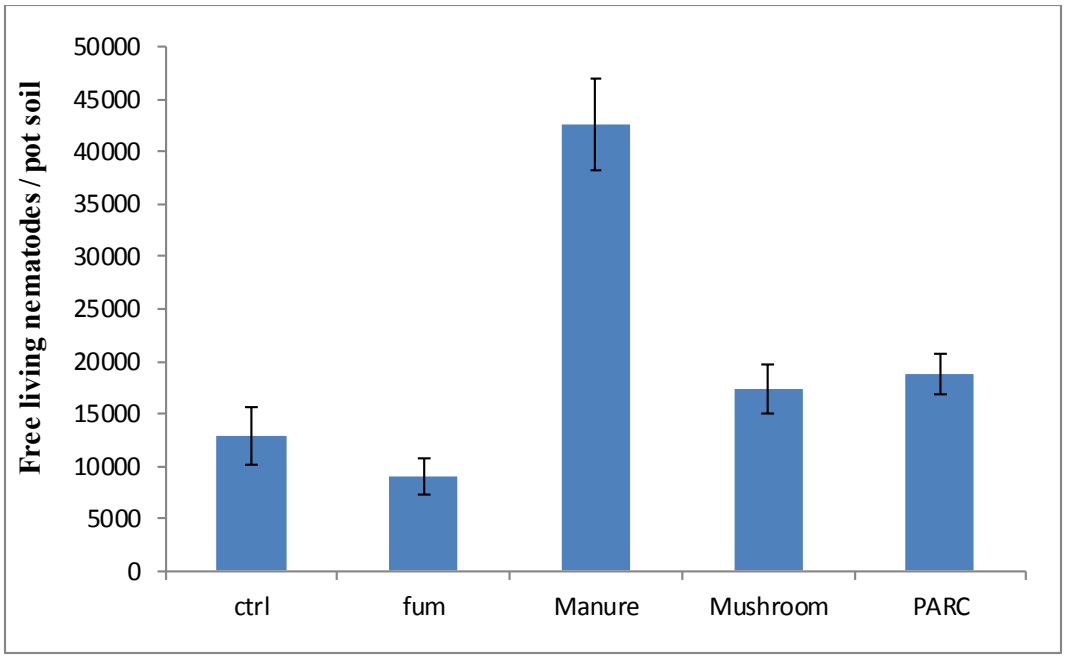

Figure 3.8: Effects of amendment treatments on mean abundance of soil-dwelling free living nematodes per pot after 13 weeks raspberry growth ±standard error of the mean. Ctrl= control (n=20), fum= fumigation (n=20), Manure= broiler manure (n=40), Mushroom= mushroom compost (n=40), PARC= PARC compost (n=40). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

3.2 Experiment 2 results

3.2.1 Plant analyses

The amendment factor did not have a significant main-factor effect on the mean shoot and root dry weights in the overall analysis of variance (Table 3.2). There was a significant block effect for mean shoot dry weight but not for root weight.

3.2.1.1 Shoot weight

There was no significant difference between compost and the non-amended control (control vs compost contrast p = 0.46) and fumigation (fumigation vs compost contrast p = 0.47), as shown in Figure 3.9. There was a significant difference in mean shoot weights between the mushroom and PARC compost (mushroom vs PARC contrast p = 0.03), but the rate of application did not have a significant effect (Table A.12). There was no significant difference in mean shoot weights between the manure and the non-amended control (control vs manure contrast p = 0.38) and fumigated pots (fumigation vs manure contrast p = 0.38). Manure had

significantly larger shoot weights than compost-amended pots (manure vs compost contrast p = 0.032).

3.2.1.2 Root weight

There was no significant difference amongst any of the treatments (Figure 3.9).

Table 3.2: Summary of p-values from analysis of variance of data from experiment 2. Data were analyzed using a blocked one-way ANOVA model with 8 amendment treatments in each of ten replicate blocks. The dependent variables (going across the top of the table) were analyzed separately.

	Plant parameters		***Pratylenchus penetrans* (P. p)**				**Free living nematodes (FLN)**	
			Day of planting	**End of experiment (13 weeks after planting)**			**Day of planting**	**End of experiment (13 weeks after planting)**
	Shoot wt (g)	**Root wt (g)**	**P. p/ pot**	**P. p/ g root**	**P. p/pot of soil**	**P. p/pot (roots + soil)**	**FLN/pot**	**FLN/pot of soil**
ANOVA summary— P-values								
Block	0.0002	0.0566	0.1146	0.1154	<0.0001	0.1621	0.1501	0.2612
Amendment	0.1585	0.8644	<0.0001	0.0004	0.003	0.0003	<0.0001	<0.0001
Planned Contrasts								
control vs compost	0.4644	0.7864	0.0219	0.7641	0.1257	0.5477	0.1009	0.093
fumigation vs compost	0.4688	0.7349	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001
manure vs compost	0.0316	0.3069	0.0028	0.0003	0.0048	0.0029	0.0886	0.0011
mushroom vs PARC	0.0302	0.9753	0.2177	0.2512	0.3198	0.2649	0.1181	0.3916
control vs manure	0.3809	0.3324	<0.0001	0.004	0.5179	0.0076	0.7675	0.0002
fumigation vs manure	0.3774	0.6775	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

wt=weight
P.p= *Pratylenchus penetrans*
FLN= Free living nematodes

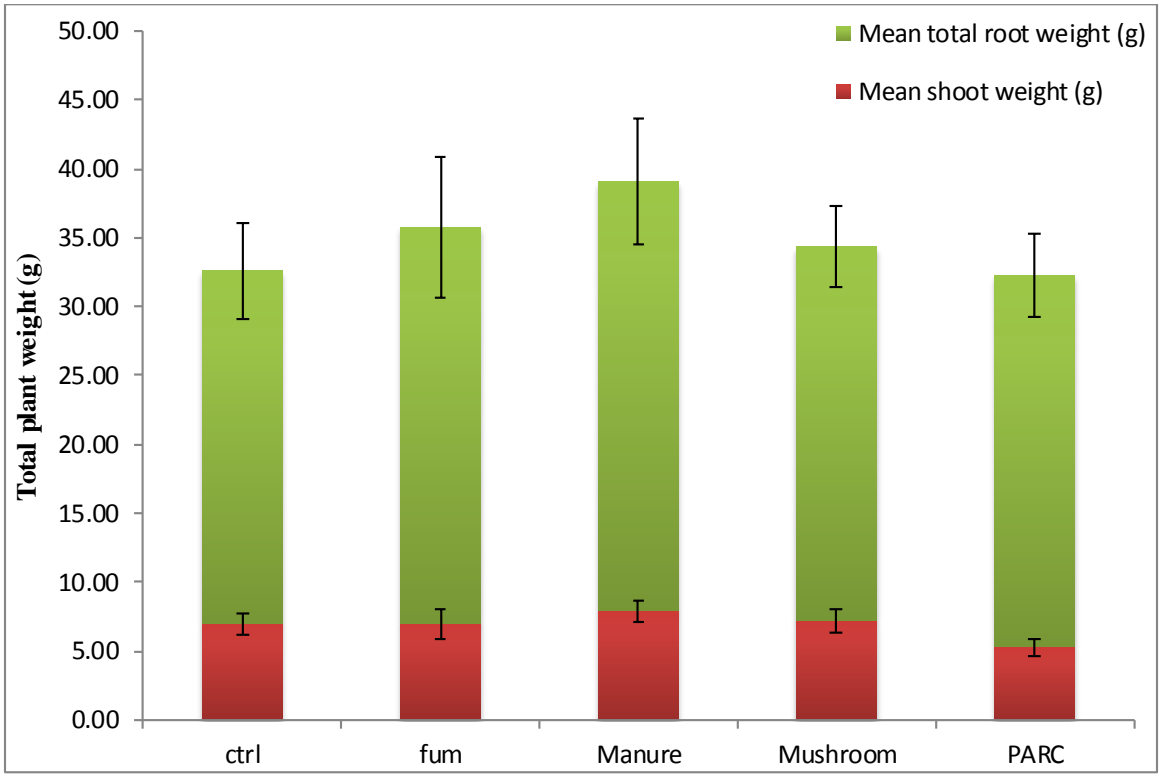

Figure 3.9: Effects of amendment treatments on mean shoot and root dry weights (g) of raspberry plants collected and dried 13 weeks after planting (end of experiment 2) ±standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

3.2.2 Nematode populations

3.2.2.1 Pre-plant nematode populations

The amendment factor had a significant effect on *P. penetrans* numbers at the time of planting in the overall analysis of variance (Table 3.2). Compost-amended pots had significantly lower populations of *P. penetrans* per pot than the non-amended control (control vs compost contrast $p = 0.022$), as shown in Figure 3.10. Compost-amended pots did not significantly reduce the abundance of *P. penetrans* per pot compared to fumigated (fumigation vs compost contrast $p < 0.001$) and manure-amended pots (manure vs compost contrast $p = 0.003$). There was no significant difference in treatment effect between mushroom and PARC compost-amended pots (mushroom vs PARC contrast $p = 0.22$), and rate of compost application did not have a significant effect on the mean abundance of *P penetrans* (Table A.12). Fumigated pots had significantly lower mean populations of *P. penetrans* per pot on day of planting than manure-amended pots (fumigation vs manure contrast $p < 0.001$).

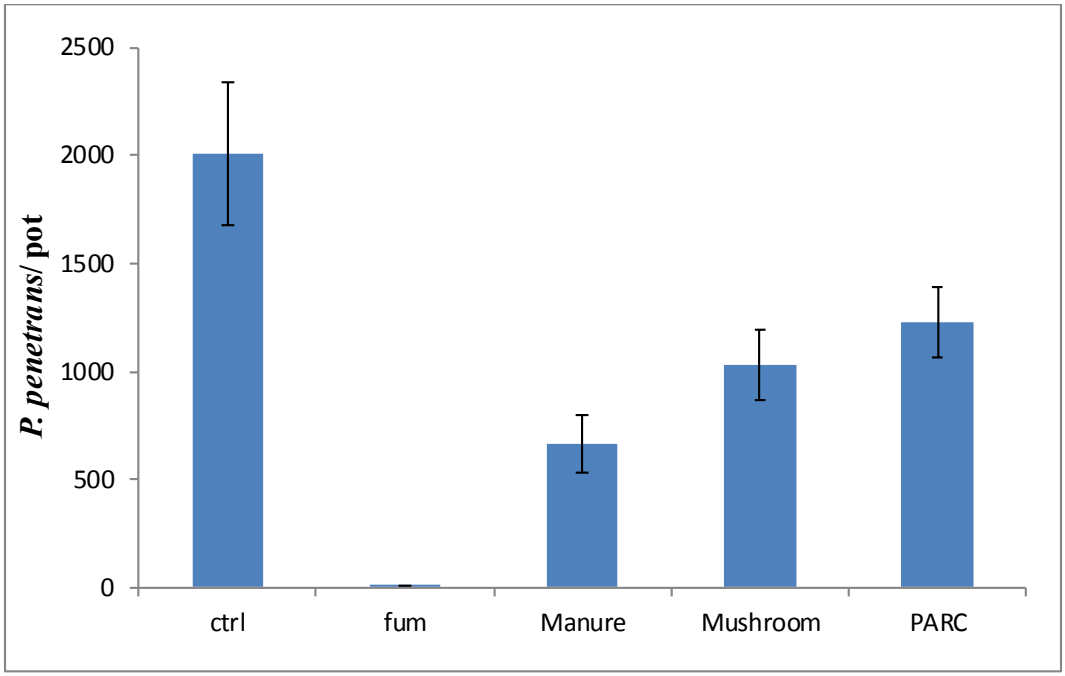

Figure 3.10: Effects of amendment treatments on mean population densities of *Pratylenchus penetrans* (*P. penetrans* per pot), on the day the raspberry plants were planted into the pots ± standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

As with experiment 1, the only plant parasitic nematode in the test soil was *P. penetrans*. The free living soil nematode community was comprised primarily of bacterivorous and secondarily of fungivorous nematodes, although I did not distinguish among trophic groups of free living nematodes when counting. In the overall analysis of variance, the amendment factor had a significant effect on the total number of free living soil nematodes at the time of planting Experiment 2 (Table 3.2). Compost-amended pots significantly increased the total number of free living soil nematodes compared to the non-amended control (control vs compost contrast $p = 0.022$) and fumigated pots (fumigation vs compost contrast $p < 0.001$), as shown in Figure 3.11. Compost amendments did not enhance populations of free living nematodes in soil compared to manure-treated pots (manure vs compost contrast $p = 0.003$). There was no significant difference between pots amended with mushroom and PARC compost (mushroom vs PARC contrast $p = 0.22$), and rate of application did not have a significant effect on the abundance of free living nematodes for PARC compost but, there was a difference in mushroom compost ($p = 0.05$; Table A.12). Manure significantly enhanced populations of free living soil nematodes compared to the

non-amended control (control vs manure contrast $p < 0.001$) and fumigated pots (fumigation vs manure contrast $p < 0.001$).

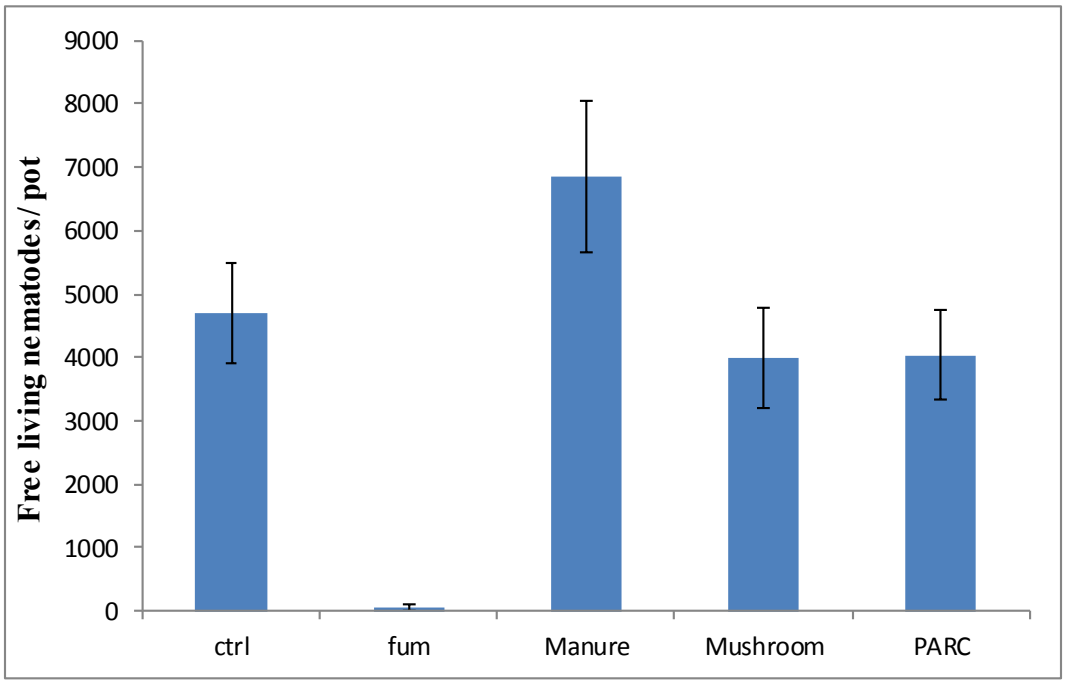

Figure 3.11: Effects of amendment treatments on mean abundance of free living nematodes per pot, on the day raspberry plants were planted into the pots, ±standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

3.2.2.2 Final nematode populations

There was a significant amendment effect on the number of *P. penetrans* found in raspberry roots in the overall analysis of variance (Table 3.2). There was no significant difference between compost-amended pots and the non-amended control (control vs compost contrast $p = 0.76$) and there was no difference between the two different compost amendments with respect to *P. penetrans* per gram of root (mushroom vs PARC contrast $p = 0.25$), as shown in Figure 3.12. The rate of compost application did not have a significant effect on *P. penetrans* per gram of root (Table A.12). Compost amendments did not significantly lower populations of *P. penetrans* per gram of root compared to fumigated pots (fumigation vs compost contrast $p < 0.001$) and -amended pots (manure vs compost contrast $p < 0.001$). Manure had significantly lower populations of *P. penetrans* per gram of root than non-amended control (control vs manure contrast $p = 0.004$). Fumigated pots had significantly less *P. penetrans* per gram of root than manure-amended pots (fumigation vs manure contrast $p < 0.001$).

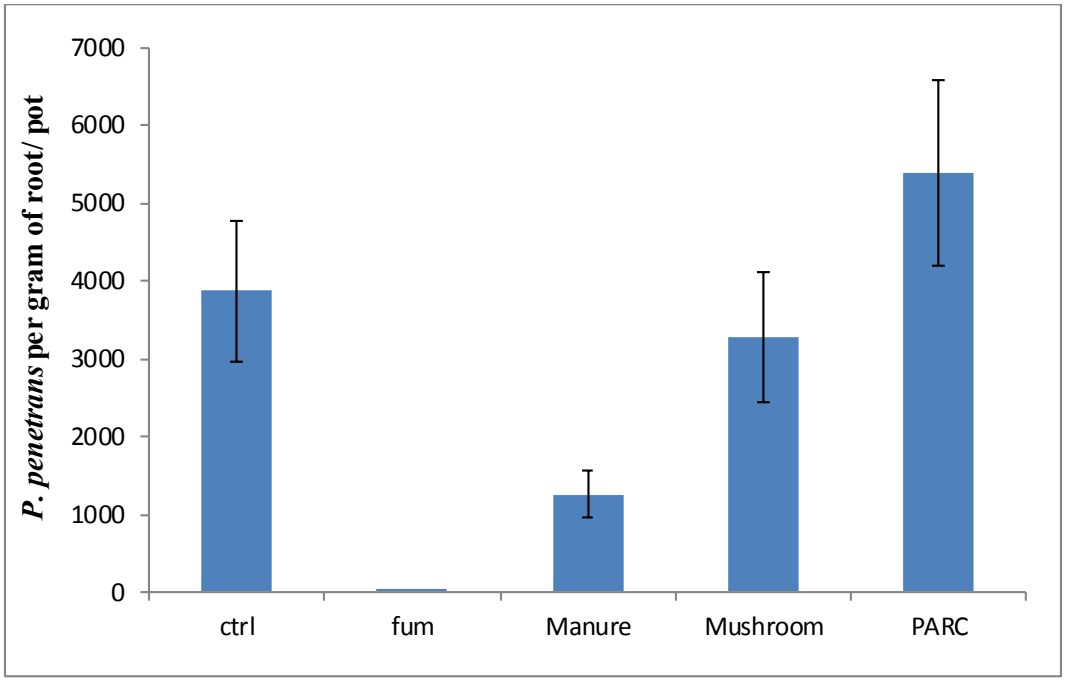

Figure 3.12: Effects of amendment treatments on mean population densities of *Pratylenchus penetrans* per gram raspberry root per pot, collected 13 weeks after day of planting (end of experiment 2) ± standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

At the end of the second experiment, the overall analysis of variance indicated that there was a significant effect of amendments on the number of *P. penetrans* per pot of soil (Table 3.2). There was no significant difference between compost amendments and the non-amended control (control vs compost contrast $p = 0.13$) and there was no difference between the two compost treatments (mushroom vs PARC contrast $p = 0.32$), as shown in Figure 3.13. There was also no significant effect of application rate of compost amendments on the numbers of *P. penetrans* per pot of soil (Table A.12). Compost amendments did not significantly lower populations of *P. penetrans* per pot of soil compared to manure-amended pots (manure vs compost contrast $p = 0.005$) and fumigated pots (fumigation vs compost contras $p < 0.001$). There was no significant difference between manure-amended pots and the non-amended control with respect to the abundance of *P. penetrans* per pot of soil (control vs manure contrast $p = 0.52$). Fumigated pots had significantly smaller soil populations of *P. penetrans* per pot than manure-amended pots (fumigation vs manure contrast $p < 0.001$).

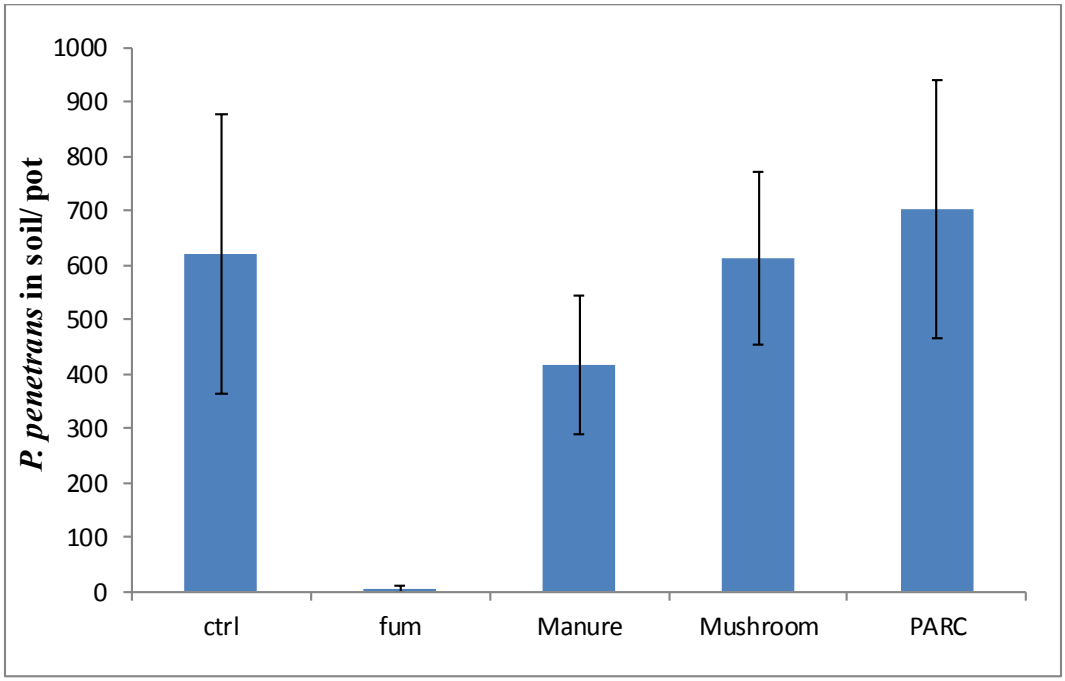

Figure 3.13: Effects of amendment treatments on mean population densities of *Pratylenchus penetrans* extracted from the soil per pot at the end of experiment 2 (13 weeks after day of planting) ± standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

There was a significant effect of amendment factor on the mean total number of *P. penetrans* per pot (nematodes in roots and soil combined) in the overall analysis of variance (Table 3.2). There was no significant difference between compost amendments and the non-amended control (control vs compost contrast $p = 0.55$) and there was no difference between the two compost treatments (mushroom vs PARC contrast $p = 0.26$), as shown in Figure 3.14. The rate of compost application also did not have a significant effect on the mean population densities of total *P. penetrans* per pot (Table A.12). Compost amendments did not significantly lower populations of *P. penetrans* per pot compared to fumigated pots (fumigation vs compost contrast $p < 0.001$) and manure-amended pots (manure vs compost contrast $p = 0.003$). Fumigation had significantly less *P. penetrans* per pot compared to manure-amended pots (fumigation vs manure contrast $p < 0.001$). Manure resulted in significantly smaller numbers of total *P. penetrans* per pot than the non-amended control (control vs manure contrast $p = 0.008$).

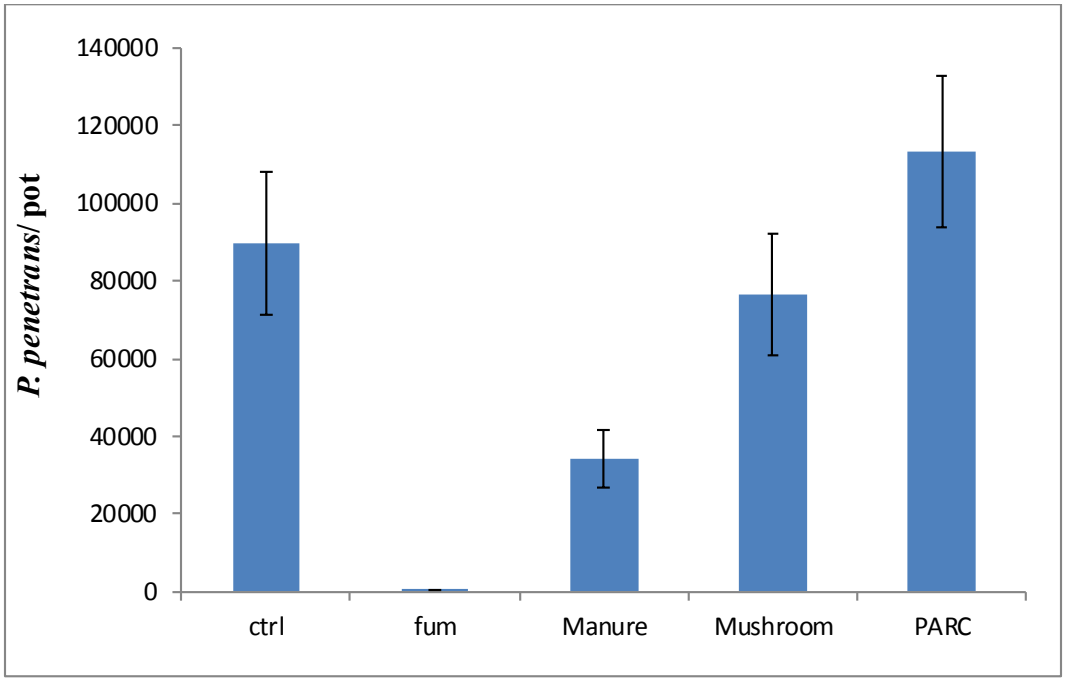

Figure 3.14: The mean total number of *Pratylenchus penetrans* per pot (sum of *P. penetrans* in soil and roots) after 13 weeks raspberry growth ±standard error of mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

There was a significant effect of amendment on the number of free living nematodes per pot of soil in the overall analysis of variance (Table 3.2). There was no significant difference between compost amendments and the non-amended control (control vs compost contrast p= 0.09) and there was no difference between the two compost treatments (mushroom vs PARC contrast p = 0.39), as shown in Figure 3.15. The rate of compost application also did not have a significant effect on the mean population densities of free living nematodes per pot (Table A.12). Compost amendments significantly enhanced numbers of free living nematodes compared to fumigation (fumigation vs compost contrast p = 0.001). Compost treatments did not have a significant effect on the abundance of free living nematodes per pot compared to manure-amended pots (manure vs compost contrast $p < 0.001$). Manure-amended pots had significantly larger populations of free living nematodes compared to the non-amended control (control vs manure contrast $p < 0.001$) and fumigated pots (fumigation vs manure contrast $p < 0.001$).

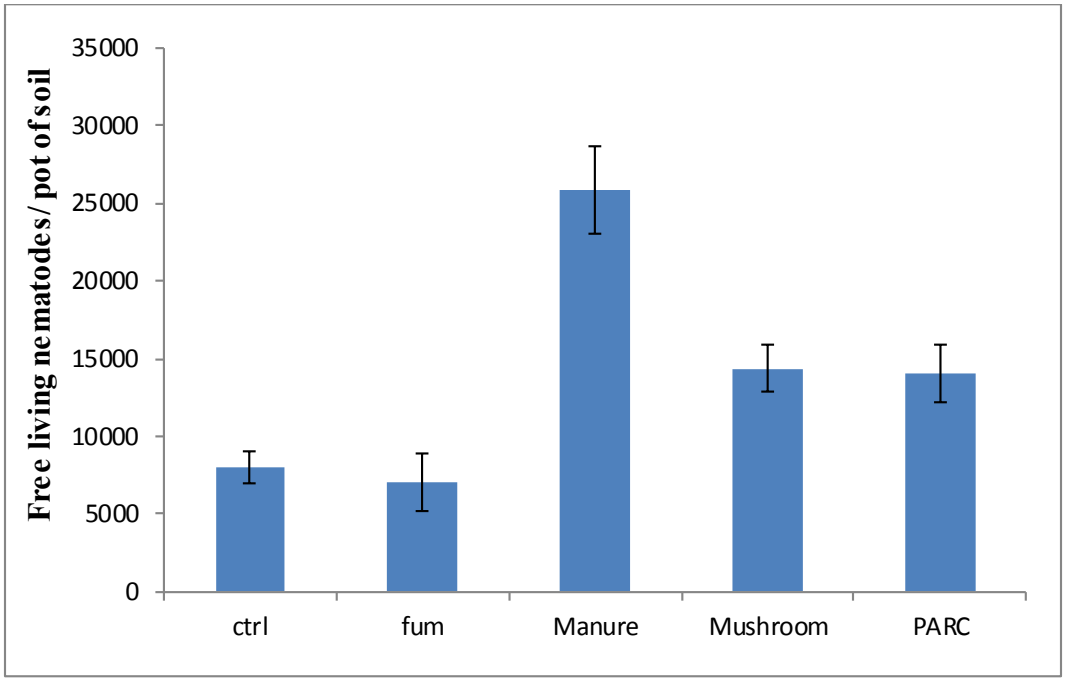

Figure 3.15: Effects of amendment treatments on mean abundance of soil-dwelling free living nematodes per pot after 13 weeks raspberry growth ±standard error of the mean. Ctrl= control (n=10), fum= fumigation (n=10), Manure= broiler manure (n=20), Mushroom= mushroom compost (n=20), PARC= PARC compost (n=20). Manure, Mushroom and PARC data are averaged over rates to visualize principal planned contrasts.

Chapter 4 Discussion

The objective of this study was to determine if either of two regionally-available composts could suppress the soil borne pathogens, *Pratylenchus penetrans* and *Phytophthora rubi*, and improve early growth of raspberry plants relative to non-amended soil and conventional grower practices of fumigation or the application of poultry manure. The latter two practices are known to suppress soil borne plant pathogens and improve early raspberry growth, but have undesirable effects on environmental quality that compost amendments would not have. My experiments were designed to address five primary hypotheses:

Hypothesis 1. Compost will improve raspberry plant growth relative to the non-amended control but not compared to fumigation and manure treatments: The compost treatments did not improve plant growth relative to the non-amended control, refuting this hypothesis. The manure and fumigation treatments resulted in greater plant growth than the compost and control treatments in one of the two experiments. Some differences between the results of the two experiments were that in experiment 1 there was approximately a 3-fold difference in shoot weight between the lowest and highest treatments while there was only a 2-fold difference in shoot weight for experiment 2. The experimental period for experiment 2 was from August to November; therefore, with the decrease in photoperiod, it is possible this had a negative effect on the shoot growth, especially since the dry weights for roots in the second experiment were much larger in comparison to experiment 1. Treatment differences for plant growth parameters may also have not been seen due to experiment 2 having fewer replicates than the first experiment.

Hypothesis 2. Compost will decrease population densities of Pratylenchus penetrans relative to the non-amended control but not as well as fumigation and manure treatments: Compost treatments suppressed *P. penetrans* populations at planting relative to the untreated control in one of the two experiments, and the compost treatments had larger at-plant *P. penetrans* populations than the fumigation and manure treatments, partially supporting this hypothesis.

Both poultry manure and fumigation are known to suppress plant parasitic nematodes (Forge et al., 2015; Forge et al., 2016). Poultry manure most likely reduces nematode populations via a "biocidal" effect perhaps due to the high concentrations of ammonia, ammonium ions and possibly volatile fatty acids and sulphides (Forge et al., 2012; Forge et al., 2016; Gamliel et al.,

2000; Rodriguez-Kabana, 1986), and suppressive effects on plant-parasitic nematode populations are observed relatively rapidly. Compost, by definition, does not contain significant concentrations of ammonia or volatile fatty acids, and therefore should not have a rapid biocidal effect. As discussed in more detail in the Introduction, an extensive body of research suggests that composts may enhance suppressiveness by promoting populations of antagonistic organisms such as predacious nematodes or fungi (Forge et al., 2008; Forge et al., 2015; Forge et al., 2016; Forge and Kempler, 2009; Rahman et al., 2014). This mechanism, which would depend on the development of populations of antagonists, may take longer to develop and may not develop to the same extent in soil that has been sieved and contained in greenhouse pots as in field conditions. I speculate that pre-setup sieving of the soil and inadequate time for the compost to foster development of antagonistic organism populations are the main reasons that I did not observe compost-induced suppression of *P. penetrans,* whereas compost-induced suppression was observed by Forge et al. (2015; 2016), which were field experiments that ran through two full growing seasons. The compost and manure treatments were applied at similar rates of total nitrogen, and all treatments received adequate nitrogen for growth. Therefore, the increase in plant growth parameters for both fumigation and manure amendments was not likely due to gross differences in nutrient availability. Given that *P. penetrans* was the dominant pathogen in the soil, I infer that the fumigation and manure treatments increased growth primarily via their suppression of *P. penetrans* populations. Across treatments, this relationship was illustrated by the significant negative correlations between *P. penetrans* populations and plant growth (Figure A.18a and A.19a).

Hypothesis 3. Compost will decrease the incidence of disease by Phytophthora rubi relative to the non-amended control and fumigation but not as well as the manure amendment: Inoculation with *Phytophthora rubi* in the first experiment did not result in measurable root rot. Analysis of variance of plant growth data did not indicate an effect of inoculation on plant growth, and visual assessment of the roots of the raspberry plants at the end of the first experiment did not reveal any disease. Quantitative PCR of root samples from *Phytophthora*-inoculated pots from experiment 1 showed either no detection of *P. rubi*, or only trace amounts when looking at the Cq values and melt curves (Appendix D). Therefore my third primary hypothesis could not be tested.

It seems unlikely that the lack of infection was due to a non-pathogenic isolate or improper inoculation procedures. The isolate of *P. rubi* and the procedures for culturing and preparation of inoculum are used on a routine basis in the plant pathology lab at PARC for screening raspberry genotypes for resistance to *P. rubi*, and no changes in pathogenicity of the *P. rubi* culture have been noticed (C. Koch, personal communication) It seems more likely that the lack of success of the *P. rubi* inoculation was due to suboptimal environmental conditions after inoculation. As a result of an unusually early heat wave in May of 2013, the pots were subjected to unusually high temperatures immediately after inoculation, with temperatures in the pots rising to about 22°C (Figure A.1). *P. rubi* is known to prefer cooler temperatures and generally infects roots at soil temperatures between 1 and 12 ^{o}C (Agriculture and Agri-Food Canada, 2007; EPPO, 2013). It seems likely therefore that the high temperatures in the pots may have been lethal to it or at least suppressed zoospore activity. In the future, an experiment like this should be held even earlier in the year or under controlled conditions to ensure cooler temperatures during the time of inoculation.

Hypothesis 4. Compost will increase population densities of free living nematodes relative to the non-amended control and fumigation but, not as well as the manure amendment: I found that compost and manure treatments both enhanced populations of free living nematodes in comparison to both control and fumigation treatments, supporting this hypothesis. Because manure contains more labile organic matter than compost, it is expected to stimulate larger pulses of microbial growth and resulting growth of bacterivorous nematode populations than compost. My data are consistent with this expectation and support the second part of this hypothesis. Populations of free living nematodes thus generally indicate enhanced soil fertility or nutrient turnover (Ferris and Bongers, 2006; Forge et al., 2008) and may also be indicative of more suppressive soil food webs, particularly when the increase in overall free-living nematode abundance is accompanied by increased abundance and diversity of omnivorous and predacious nematodes (Rahman et al., 2014; Timper 2014; Sanchez-Moreno and Ferris 2007). Since I did not categorize free living nematodes into feeding groups for example: bacterial feeders, predacious etc., it is difficult to relate the degree of antagonism these nematodes could have on root lesion nematodes.

Hypothesis 5. There will be no differences between PARC compost and spent mushroom compost with respect to plant growth, nematode populations and incidence of Phytophthora root rot: It is important to know if the disease suppressive qualities of compost are generally

associated with most composts of a general type, or they are very specific or unique to a particular compost. The process of composting can have a homogenizing effect on organic waste amendments, transforming very dissimilar feedstocks into finished composts that are more similar to each other than original feedstocks, which led me to hypothesize that there would not be substantial differences between the two composts used in my experiments. I did not find any significant differences, in any parameter, between the PARC compost and the mushroom compost used in these experiments, supporting this hypothesis

Merits of compost vis-à-vis fumigation and manure treatments:

Fumigation, although successful at controlling soil-borne pathogens, is detrimental to soil health because it destroys both pathogens and beneficial organisms and, consequently, it can drastically alter soil communities and create a "biological vacuum" (Gamliel et al., 2000; Griffiths et al., 2000; James, 1989; Klose et al., 2006; Yao et al., 2006). With a lack of natural regulators of plant parasitic nematodes, such as predacious nematodes and nematode-trapping fungi, these pathogens can become re-established in fumigated soils and eventually reach greater levels than in non-fumigated soil (Forge et al., 2001; Gamliel et al., 2000; Gigot et al., 2013; Walters et al., 2009). Both experiments were only active for a period of 13 weeks, so it was probably not enough time to be able to see an effect of *P. penetrans* possibly building to greater population densities within fumigated pots. That being said, it can be noted that *P. penetrans* populations did grow slightly in fumigated pots when comparing density levels from the day of planting to the end of both experiments. With longer experimental periods, it may have been possible to observe a greater population build-up of *P. penetrans* due to the biological vacuum created in fumigated pots. Lastly, because fumigation is a concern for human health, regulations governing the use of fumigants have in recent years become stricter (Health Canada, 2012). This limits growers in their ability to fumigate their soils; therefore, no matter its success, an alternative is still needed (Rudolph and DeVetter, 2015; Walters et al., 2009).

While manure appeared to be better than compost for nematode suppression and plant growth, it has some potential negative impacts on the environment that need to be considered. Up to 50% of the nitrogen in broiler manure may be plant available ammonium. In addition, with low C/N ratios, the organic fraction of manure N stimulates the growth of bacteria and bacterial feeding nematodes, leading to rapid mineralization of organic N in manure (Gale et al., 2006; Griffiths et al., 1998). Consequently, large applications can provide mineral N in excess of crop

needs which can be leached from the soil profile into the groundwater, (Chesnaux et al., 2012; Dean et al., 2000; Forge et al., 2013; Jeffries et al., 2008; Zebarth et al., 1998). Compost, on the other hand, has very low concentrations of nitrate and ammonium and more stable forms of organic N than raw manure (Forge et al., 2012; Forge et al., 2013; Forge et al., 2016). Forge et al. (2015; 2016) compared pre-plant amendments of compost and broiler manure, applied at similar rates, with respect to risk of nitrate leaching and found that broiler manure presents a greater risk of nitrate leaching than compost.

Chapter 5 Conclusions

With such a large abundance of raspberries coming from the Fraser Valley, management practices used by growers to control soil borne pathogens have the potential to create a large impact on soil health and can influence the quality of groundwater in the Abbotsford-Sumas Aquifer located below most of these farms. This thesis was built upon previous research done by Forge et al. (2015; 2016) and addressed the objective of determining the impact of pre-plant incorporation of two different composts on raspberry root health and inoculum densities of *Phytophthora rubi* and *Pratylenchus penetrans*, relative to incorporation of raw broiler manure and fumigation, both of which are standard practices of commercial raspberry growers.

Compost data were not consistent between both experiments but the results indicated that pre-plant amendment of soil with compost did not promote plant growth and control *P. penetrans* relative to the non-amended control. According to previous studies of the mechanisms of how composts control soil-borne pathogens, it seems likely that 13 weeks was not enough time to observe an effect of compost (Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink and Grebus, 1994; Hoitink et al., 1997; Lockwood, 1988; Postma and Schilder, 2015; Zinati, 2005).

Results from visual root rot rating, quantitative PCR and analysis of plant growth data indicated that the *P. rubi* inoculum did not effectively infect the raspberry roots, most likely as a result of unusually high temperatures that occurred immediately after inoculation, and which may have been detrimental to *Phytophthora rubi*. Future research should ensure that environmental conditions will remain conducive to the pathogen throughout the experimental period, Future research would also benefit from use of better techniques to quantify the pathogen in soil and root tissues, including using a plasmid to clone the PCR fragment in order to determine the copy number of the *P. rubi* DNA of interest.

Previous research suggested that composts possibly control *P. penetrans* via enhanced populations of soil food web organisms that are antagonistic to *P. penetrans* (Forge et al., 2008; Forge et al., 2015; Forge et al., 2016; Forge and Kempler, 2009; Rahman et al., 2014). Populations of free-living nematodes appear to be indicative of such enhanced soil food webs (Timper 2014; Sanchez-Moreno and Ferris 2007). The results confirmed that fumigation pots had the lowest numbers of free living nematodes, manure treatments had the highest, and

compost treatments enhanced free living nematode numbers more than control pots. These free living nematodes were not categorized by trophic group; however, I observed that they were primarily microbivores, which is consistent with previous observations that omnivorous and predacious nematodes, which are generally known to be sensitive to disturbance (e.g. Ferris and Bongers 2001; Rahman et al., 2014), generally do not proliferate in greenhouse pot studies over relatively short time periods (Forge, pers. comm.).

I conclude that compost should not be considered as a reliable short term pre-plant treatment for the specific purpose of controlling populations of *P. penetrans*. Despite the lack of effect of compost on *P. penetrans* populations and plant growth in my pot study; however, I propose that compost can still be considered a promising pre-plant soil amendment for raspberry growers. Compost clearly has the potential to suppress *P. penetrans* populations under field conditions (Forge et al., 2016), although my data suggest that this effect may not be consistent or evident in the short-term. Compost also provides a slow-release source of nutrients and has beneficial effects on soil chemical and physical properties (Forge et al., 2016). Much of the theory behind the mechanisms of how compost suppresses soil-borne pathogens suggests that it may require more time for the suppressive soil food web to develop relative to manure bio-fumigation and fumigation. (Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Hoitink and Grebus, 1994; Hoitink et al., 1997; Lockwood, 1988; Postma and Schilder, 2015; Zinati, 2005). In the future, a longer experimental period should be considered as a more realistic test of the effects of compost on controlling soil borne pathogens. Furthermore, a longer experiment would also have allowed for observations of the effects of fumigation on soil communities and natural soil regulators; thus allowing for a better comparison in the long term between these pre-plant amendments. Finally, additional research with a broader range of types of composts made from readily available manures in the Lower Mainland could indicate if the lack of pathogen-suppressive effects in my experiments was due to batch-to-batch variation in suppressive qualities. Manure was highly successful at suppressing *P. penetrans* populations and improving plant growth in this experiment, but concern around its use and the potential for nitrate leaching into the Abbotsford-Sumas Aquifer cannot be ignored.

Bibliography:

Agriculture and Agri-Food Canada. 2007. *Crop profile for raspberry*. Retrieved from Government of Canada publications: http://publications.gc.ca/collections/collection_2009/agr/A118-10-18-2007E.pdf.

Agriculture and Horticulture Development Board. 2015. *Report: A desk study to review global knowledge on best practice for oomycete root-rot detection and control*. University of Worcester, England. Retrieved from: http://www.hdc.org.uk/sites/default/files/research_papers/CP%20126_Report_Final_2014.pdf.

Agrios, G. N. 1978. *Plant pathology* (2nd edition). New York, NY: Academic Press Inc.

Aryantha, I. P., Cross, R., Guest, D. I. 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted manures. *Phytopathology* 90: 775-782.

Bailey, K.L. and Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil & Tillage* 72: 169-180.

Bonants, P.J.M., van Gent-Pelzer, M.P.E., Hooftman, R., Cooke, D.E.L. 2004. A combination of baiting and different PCR formats, including measurement of real-time quantitative fluorescence, for the detection of *Phytophthora fragariae* in strawberry plants. *European Journal of Plant Pathology* 110: 689-702.

Bonilla, N., Vida, C., Martinez-Alonso, M., Landa, B.B., Gaju, N., Cazorla, F.M., de Vicente, A. 2015. Organic amendments to avocado crops induce suppressiveness and influence the composition and activity of soil microbial communities. *Applied and Environmental Microbiology* 81 (10): 3405-3418.

British Columbia Ministry of Agriculture and Lands. 2013. Berry Production Guide 2012. Beneficial management practices for berry growers in British Columbia, Lower Mainland Horticulture Improvement Association, Victoria, BC.

Cao, Y., Chang, Z., Wang, J., Ma, Y., Yang, H., Fu, G. 2014. Potential use of anaerobically digested manure slurry to suppress *Phytophthora* root rot of chilli pepper. *Scientia Horticulturae* 168: 124-131.

Chen, J.T., Lin, M.J., Huang, J.W. 2015. Efficacy of spent blewit mushroom compost and *Bacillus aryabhattai* combination on control of Pythium damping-off in cucumber. *Journal of Agricultural Science* 153: 1257-1266.

Chesnaux, R., Allen, D.M., Simpson, M.W.M. 2012. Comparing isotopic groundwater age measurements with simulated groundwater ages: Example of the Abbotsford-Sumas aquifer (USA and Canada) and application. *Water and Environment Journal* 26: 30-37.

Dean, D.M., Zebarth, B.J., Kowalenko, C.G., Paul, J.W., Chipperfield, K. 2000. Poultry manure effects on soil nitrogen processes and nitrogen accumulation in red raspberry. *Canadian Journal of Plant Science*: 849-86.

De Ceuster, T.J.J. and Hoitink, H.A.J. 1999. Prospects for composts and biocontrol agents as substitutes for Methyl Bromide in biological control of plant diseases. *Compost Science & Utilization* 7(3): 6-15.

De Curtis, F., De Cicco, V., Lima, G. 2012. Efficacy of biocontrol yeasts combined with calcium silicate or sulphur for controlling durum wheat powdery mildew and increasing grain yield components. *Fields Crop Research* 134: 36-46.

Downer, A., Menge, J.A., Pond, E. 2001. Association of cellulytic enzyme activities in Eucalyptus mulches with biological control of *Phytophthora cinnamomi*. *Phytopathology* 91: 847-855.

Duncan, J.M. and Kennedy, D.M. 1989. The effect of waterlogging on *Phytophthora* root rot of red raspberry. *Plant Pathology*. 38: 161-168.

EPPO. 2013. Data Sheets on quarantine pests: *Phytophthora fragariae* [Data Sheet]. Retrieved from: http://www.eppo.int/QUARANTINE/data_sheets/fungi/PHYTFR_ds.pdf.

Ference Weicker & Company. 2009. *Fraser valley poultry manure movement study*. Vancouver, BC: British Columbia Agriculture Council.

Ferris, H. and Bongers, T. 2006. Nematode indicators of organic enrichment. *Journal of Nematology* 38(1): 3-12.

Fichtner, E.J., Benson, D.M., Diab, H.G., Shew, H.D. 2004. Abiotic and biological suppression of *Phytophthora parasitica* in a horticultural medium containing composted swine waste. *Phytopathology* 94: 780-788.

Forge, T., Muehlchen, A., Hackenberg, C., Neilsen, G., Vrain, T. 2001. Effects of preplant inoculation of apple (*Malus domestica* Borkh.) with arbuscular mycorrhizal fungi on population growth of the root-lesion nematode, *Pratylenchus penetrans*. *Plant and Soil* 236: 185-196.

Forge, T., Kimpinski, J., 2007. *Nematodes*. In: Gregorich, E.G., Carter, M.R. (Eds), Soil Sampling and Methods of Analysis, Second Edition. CRC Press, Boca Raton, FL, pp 415.

Forge, T.A., Hogue, E.J., Neilsen, G., Neilsen, D. 2008. Organic mulches alter nematode communities, root growth and fluxes of phosphorus in the root zone of apple. *Applied Soil Ecology* 39 (1): 15-22.

Forge, T.A. and Kempler, C. 2009. Organic mulches influence population densities of root-lesion nematodes, soil health indicators, and root growth of red raspberry. *Canadian Journal of Plant Pathology* 31: 241-249.

Forge, T.A. Personal communication. 2012.

Forge, T., Kenney, E., Zebarth, B. 2012. Alternatives to Fumigation. Pp. 30-32 in: Proceedings of the 2012 Horticulture Growers Short Course, Lower Mainland Horticultural Improvement Assoc. Abbotsford, BC, Jan. 26-28, 2012.

Forge, T., Hashimoto, N., Zebarth, B., Kenney, E., Neilsen, D. 2013. The use of compost as a preplant amendment to minimize impacts of parasitic nematodes and improve soil health and early establishment of red raspberry. Proceedings, 2nd International Symposium on Organic Matter Management And Compost Use In Horticulture. International Society for Horticultural Science, Santiago, Chile, 21-24 October, 2013.

Forge, T., Hashimoto, N., Neilsen, D., Kenney, E., Zebarth, B. 2015. The use of compost as a preplant amendment to minimize impacts of parasitic nematodes and improve soil health and early establishment of red raspberry. *Acta Horticulturae* 1076: 225-231.

Forge, T., Kenney, E., Hashimoto, N., Neilsen, D., Zebarth, B. 2016. Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. *Agriculture, Ecosystems and Environment* 223: 48-58.

Gale, E.S. Sullivan, D.M., Cogger, C.G., Bary, A.I., Hemphill, D.D., Myhre, E.A. 2006. Estimating plant-available nitrogen release from manures, composts and specialty products. *Journal of Environmental Quality* 35: 2321-2332.

Gamliel, A., Austerweil, M., Kritzman, G. 2000. Non-chemical approach to soilborne pest management- Organic amendments. *Crop Protection* 19: 847-853.

Gigot, J., Walters, T.W., Zasada, I.A. 2013. Impact and occurrence of *Phytophthora rubi* and *Pratylenchus penetrans* in commercial red raspberry (*Rubus ideaus*) fields in Northwestern Washington. *International Journal of Fruit Science* 13: 357-372.

Gilardi, G., Baudino, M., Moizio, M., Pugliese, M., Garibaldi, A., Gullino, M.L. 2013. Integrated management of *Phytophthora capsici* on bell pepper by combining grafting and compost treatment. *Crop Protection* 53: 13-19.

Griffiths, B.S. Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of biodiversity-ecosystem function relationship. *OIKOS* 90 (2): 279-294.

Griffiths, B.S., Wheatley, R.E., Olesen, T., Henriksen, K., Ekelund, F., Ronn, R. 1998. Dynamics of nematodes and protozoa following the experimental addition of cattle or pig slurry to soil. *Soil Biology & Biochemistry* 30: 1379-1378.

Hardham, A.R. 2001. The cell biology behind *Phytophthora* pathogenicity. *Australian Plant Pathology* 30: 91-98.

Health Canada. 2012. *New label requirements for soil fumigant products containing Chloropicrin, Dazomet, Metam Sodium or Metam Potassium.* Retrieved from: http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/soil-fumigant-fumigation-sol/index-eng.php

Hoashi-Erhardt, W.K. and Moore, P.P. 2008. Field and greenhouse response of red raspberry genotypes to root rot. *Horticulture Science* 43 (5): 1367-1370

Hoitink, H. A. J. and Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* 24: 93-114.

Hoitink, H. A. J. and Grebus, M. E. 1994. Status of biological control of plant diseases with composts. *Compost Science & Utilization*. 2(2): 6-12.

Hoitink, H.A.J., Stone, A.G., Han, D.Y. 1997. Suppression of plant diseases by composts. *HortScience* 32 (2): 184-187.

Hoitink, H. A. J. and Boehm, M. J. 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology* 37: 427-446.

James, R.L. 1989. *Effects of fumigation on soil pathogens and beneficial microorganisms*. Paper presented at the Intermountain Forest Nursery Association annual meeting, Bismark, North Dakota, August 14-18, 1989.

Jeffries, M., Hughes-Games, G., Sweeney, M. 2008. Sustainable nitrogen management in British Columbia raspberry crops. *Acta Horticulturae* 777: 435-438.

Joshi, A. and Jaiswal, P. 2013. Microorganisms living in zinc contaminated soil- a review. *Journal of Pharmacy and Biological Sciences* 6 (2): 67-72.

Kempler, C., Muehlchen, A.M., Forge, T.A. 2012. Screening for resistance to *Phytophthora* root rot in raspberries: Identifying new sources of resistance. *Acta Horticulturae* 926: 59-64.

Kim, K.D., Nemec, S., Musson, G. 1997. Effects of composts and soil amendments on soil microflora and *Phytophthora* root and crown rot of bell peppers. *Crop Protection* 16(2): 165-172.

Klose, S., Acosta-Martinez, V., Ajwa, H.A. 2006. Microbial community composition and enzyme activities in a sandy loam soil after fumigation with methyl bromide or alternative biocides. *Soil Biology and Biochemistry* 38 (6): 1243-1254.

Kuchta, S. 2012. The effect of nitrogen, water and alley management strategies on nitrate and water loss from the root zone in perennial red raspberry. M.Sc. Thesis. University of

British Columbia, Okanagan. In cIRcle [database on-line]; retrieved from: https://circle.ubc.ca/bitstream/handle/2429/43534/ubc_2013_spring_kuchta_shawn.pdf?sequence=1 (accessed April 2015)

Laun, N. and Zinkernagel, V. 1997. A comparison of the resistance against *Phytophthora fragariae var. rubi*, the causal agent of a raspberry root rot. *Journal of Phytopathology* 145: 197-204.

Lazarovits, G. 2001. Management of soilborne plant pathogens with organic soil amendments: A disease control strategy salvaged from the past. *Canadian Journal of Plant Pathology* 23: 1-7.

Lazarovits, G., Tenuta, M., Conn, K.L. 2001. Organic amendments as disease control strategy for soilborne diseases of high-value agriculture crops. *Australian Plant Pathology* 30: 11-117.

Litterick, A. and Wood, M. 2009. The use of composts and compost extracts in plant disease control. In Walters, D. (Ed.), *Disease Control in Crops: Biological and Environmentally Friendly Approaches* (pp. 93-121). Blackwell Publishing: ISBN: 978-1-405-16947-9.

Lockwood, J. L. 1988. Evolution of concepts associated with soilborne plant pathogens. *Annual Review of Phytopathology* 26: 93-121.

Luttmerding, H.A. 1981. *Soils of the Langley- Vancouver map area*. Report number 15. RAB Bulletin 18. Volume 3. Description of soils, Province of British Columbia. Ministry of Environment, Kelowna.

Mackie, K.A., Muller, T., Zikeli, S., Kandeler, E. 2013. Long-term copper application in an organic vineyard modifies spatial distribution of soil micro-organisms. *Soil Biology & Biochemistry* 65: 245-253.

Mai, W.F. and Mullin, P.G. 1996. *Plant-parasitic nematodes: A pictorial key to genera, fifth edition*. Cornell University, New York, NY, pp 94.

Maloney, K., Pritts, M., Wilcox, W., Kelly, M. 2005. Suppression of *Phytophthora* root rot in red raspberries with cultural practices and soil amendments. *HortScience* 40(6): 1790-1795.

Mayor, D.J., Gray, N.B., Elver-Evans, J., Midwood, A.J., Thornton, B. 2013. Metal-macrofauna interactions determine microbial community structure and function in copper contaminated sediments. *PLOS ONE* 8 (5): 1-9.

Mehta, C.M., Palni, U., Franke-Whittle, I.H., Sharma, A.K. 2014. Composts: Its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Management* 34: 607-622.

Milner, P.D., Ringer, C.E., Maas, J.L. 2004. Suppression of strawberry root disease with animal manure composts. *Compost Science & Utilization* 12 (4): 298-307.

Noble, R. and Coventry, E. 2005. Suppression of soil-borne plant diseases with composts: A review. *Biocontrol Science and Technology*, 15 (1): 3-20.

Oka, Y. and Yermiyahu, U. 2002. Suppressive effects of composts against the root-knot nematode *Meloidogyne javanica* on tomato. *Nematology* 4(8): 891-898.

Ownley, B.H. and Benson, D.M. 1991. Relationship of matric water potential and air-filled porosity of container media to development of *Phytophthora* root rot of Rhododendron. *Phytopathology* 81: 936-941.

Postma, J. and Schilder, M.T. 2015. Enhancement of soil suppressiveness against *Rhizoctonia solani* in sugar beet by organic amendments. *Applied Soil Ecology* 94: 72-79.

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., Moenne-Loccoz, Y. 2009. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321: 341-361.

Rahman, L., Whitelaw-Weckert, M.A., Orchard, B. 2014. Impact of organic soil amendments, including poultry-litter biochar, on nematodes in a Riverina, New South Wales, vineyard. *Soil Research*, 52: 604-619.

Raspberry Industry Development Council. 2011. *Welcome to the Raspberry Industry Development Council*. Retrieved from : http://bcraspberries.com/index.php?page_id=1

Rodriguez-Kabana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology* 18(2): 129-135.

Rossman, A.Y. and Palm, M.E. 2006. *Why are Phytophthora and other Oomycota not true Fungi?* Retrieved from: http://www.apsnet.org/edcenter/intropp/pathogengroups/pages/oomycetes.aspx (accessed June 2016)

Rudolph, R.E. and DeVetter, L.W. 2015. Management strategies for *Phyophthora rubi* and *Pratylenchus penetrans* in floricane red raspberry (*Rubus idaeus L.*) *Journal of the American Pomological Society* 69(3): 118-136.

Sanchez-Moreno, S., Ferris, H. 2007. Suppressive service of the soil food web : Effects of environmental management. *Agriculture, Ecosystems & Environment* 119: 75-87.

Saxena, J., Choudhary, S., Pareek, S., Choudhary, A.K., Iquebal, M.A. 2015. Recycling of organic waste through four different composts for disease suppression and growth enhancement in mung beans. *Clean- Soil, Air, Water* 43 (7): 1066-1071.

Serrano, M. S., De Vita, P., Fernandez-Rebollo, P., Hernandez, M.E.S. 2011. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* 132: 271-279.

Shurtleff, M.C. and Averre, C.W. 2000. *Diagnosing plant diseases caused by nematodes*. The American Phytopathological Society, Minnesota, USA, pp 118-119.

Snedecor, G.W. and Cochran, W.G. 1980. *Statistical Methods, Seventh Edition*. Iowa State University Press, Ames, IA, USA.

Suess, A. and Curtis, J. 2006. *Report: Value-added strategies for spent mushroom substrate in BC*. *British Columbia Ministry of Agriculture and Lands*. Retrieved from: http://www.al.gov.bc.ca/mushroom/guide/value_added_strategies.pdf

Sugimoto, T., Watanabe, K., Yoshida, S., Aino, M., Furiki, M., Shiono, M., Matoh, T., Biggs, A.R. 2010. Field application of calcium to reduce Phytophthora stem rot of soybean, and calcium distribution in plants. *Plant Disease* 94: 812-819.

Thoden, T.C., Korthals, G.W., Termorshuizen, A.J. 2011. Organic amendments and their influences on plant parasitic and free-living nematodes: A promising method for nematode management? *Nematology* 13(2): 133-153.

Timmenga & Associates Inc. 2003. *Report: Evaluation of options for Fraser Valley poultry manure utilization*. Vancouver, BC. Retrieved from: http://www.al.gov.bc.ca/poultry/publications/documents/evaluation_poultry_manure.pdf

Timper, P. 2014. Conserving and enhancing biological control of nematodes. *Journal of Nematology* 46(2): 75-89.

Vallad, G.E. and Goodman, R.M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* 44(6): 1920-1934.

Vrain, T., DeYoung, R., Hall, J. 1996. Cover crops resistant to root-lesion nematodes in raspberry. *Horticulture Science* 31 (7): 1195-1198.

Vrain, T. C., Forge, T.A., DeYoung, R. 1997. Population dynamics of *Pratylenchus penetrans* parasitizing raspberry. *Fundamental and Applied Nematology* 20 (1): 29-36.

Walters, T.W., Pinkerton, J.N., Riga, E., Zasada, I.A., Particka, M., Yoshida, H.A. 2009. Managing plant-parasitic nematodes in established red raspberry fields. *HortTechnology* 19(4): 726-768.

Williams, J.S. and Cooper, R.M. 2004. The oldest fungicide and newest phytoalexin- a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathology* 53: 263-279.

Yao, S., Merwin, I.A., Abawi, G.S., Thies, J.E. 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biology & Biochemistry* 38: 587-599

Zebarth, B.J., Hii, B., Liebscher, H., Chipperfield, K., Paul, J.W., Grove, G., Szeto, S.Y. 1998. Agricultural land use practices and nitrate contamination in the Abbotsford Aquifer, British Columbia, Canada. *Agriculture, Ecosystems & Environment* 69: 99-112.

Zebarth, B.J., Paul, J.W., Van Kleeck, R. 1999. The effect of nitrogen management in agricultural production on water and air quality: evaluation on a regional scale. *Agriculture, Ecosystems & Environment* 72: 35-52.

Zebarth, B.J., Ryan, C.M., Graham, G., Forge, T.A., Nielsen, D. 2015. Groundwater monitoring to support development of BMPs for groundwater protection: The Abbotsford-Sumas aquifer case study. *Groundwater Monitoring & Remediation* 35: 82-96.

Zhang, W., Han, D.Y., Dick, W.A., Davis, K.R., Hoitink, H.A.J. 1996. Compost-induced systemic acquired resistance in cucumber to Pythium root rot and anthracnose. *Phytopathology* 86: 1066-1070.

Zhang, W., Han, D.Y., Dick, W.A., Davis, K.R., Hoitink, H.A.J. 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. *Phytopathology* 88: 450-455.

Zinati, G.M. 2005. Compost in the 20th century: A tool to control plant diseases in nursery and vegetable crops. *HortTechnology* 15 (1): 61-66.

Appendices

Appendix A: Amendment application calculations

Before the experiment was set up, samples of each of the organic amendments was sent to a commercial analytical lab and analyzed for N content (% of dry weight) and numerous other parameters. Calculations of application rates (g fresh amendment/pot) that would be equivalent to field application of 500 kg N/ha incorporated to a depth of 30 cm are below:

1X poultry manure

Total nitrogen (N) = 4.7%

Dry matter= 25%

Want 500kg N/hectare:

500 kg N•ha^{-1} /.047= 10638 kg manure•ha^{-1}

10638.3 kg manure•ha^{-1}/ 10000= 1.0638 kg•m^{-2}

1.0638 kg manure•m^{-2}/0.3m^3 (incorporated depth) => 1.0638 kg manure/300L soil

= 3.5g/L and each pot had 5L of soil => 17.7 g dry amount of broiler manure

17.7g / .25 = 71 g fresh broiler manure per pot

2X poultry manure

71 g • 2 = 142 g broiler manure per pot

1X mushroom compost

Total N= 2.3%

Dry matter= 43%

500 kg N•ha^{-1}/ .023 N= 21739 kg compost•ha^{-1} => 2.1739 kg compost•m^{-2}

2.1739 kg compost•m^{-2} /0.3 m^3 => 2.1739 kg compost/ 300L soil

7.25 g/L • 5L= 36 g dry weight

36 g/ .43= 84 g fresh mushroom compost per pot

2X mushroom compost

84 g • 2= 169 g mushroom compost per pot

1X PARC compost

Total N= 1.7%

Dry matter= 46%

500 kg N•ha^{-1} / .017 N= 29411 kg compost•ha^{-1} => 2.9411 kg•m^{-2}

2.9411 kg compost•m^{-2}/ 0.3 m^3 => 2.9411 kg compost/ 300L soil

9.8 g/L • 5L= 49 g dry weight

49 g/ .46= 107 g fresh PARC compost per pot

2X PARC compost

107 g • 2= 214 g PARC compost per pot

Appendix B: Nutrient analyses of organic amendments

Table A.1: Preliminary analysis results for experiment 1. Analyses were performed by A&L labs Inc. (London, ON) on amendment samples prior to project setup.

Parameter	*PARC compost*	*Mushroom compost*	*Broiler manure*
Dry matter	46%	43%	25%
Nitrogen (total)	1.80%	2.12%	3.26%
Phosphorous (total)	1.86%	.64%	1.95%
Potassium (total)	2.09%	2.51%	2.53%
Organic matter	47.9%	53.1%	46.9%
pH	7.25	8.08	8.70
C/N	15:1	14:1	14:1
Sulphur	5670.0 ppm	33500.0 ppm	7550.0 ppm
Bulk density	706 kg/m^3	499 kg/m^3	228 kg/m^3
Conductivity (@ 25ºC)	7.06 ms/cm	8.59 ms/cm	8.74 ms/cm
Sodium	.28%	.25%	.44%
Aluminum	6210.0 ppm	2427.0 ppm	282.1 ppm
Boron	23.6 ppm	16.4 ppm	33.8 ppm
Calcium	8.84%	11.8%	3.30%
Copper	66.0 ppm	94.4 ppm	413.2 ppm
Iron	12655.0 ppm	3085.5 ppm	983.0 ppm
Magnesium	.89%	.68%	.71 %
Manganese	661.0 ppm	275.3 ppm	537.0 ppm
Zinc	421.2 ppm	179.0 ppm	460.8 ppm

**Calculated on dry weight basis*

Table A.2: Grams of amendment elements per kilogram of soil for experiment 1

	1X Broiler manure	2X Broiler manure	1X Mushroom compost	2X Mushroom compost	1X PARC compost	2X PARC compost
Cu	1.22	2.44	0.57	1.14	0.54	1.08
Fe	2.90	5.80	18.5	37.0	103	206
S	22.3	44.6	201	402	46.3	92.6
Zn	1.36	2.72	1.07	2.14	3.44	6.88

Table A.3: Experiment 2 preliminary analysis results. Analyses were performed by A&L labs Inc. (London, ON).

Parameter	*PARC compost*	*Mushroom compost*	*Broiler manure*
Dry matter	46%	43%	25%
Nitrogen (total)	1.85%	2.13%	4.21%
Phosphorous (total)	1.80%	.63%	1.66%
Potassium (total)	2.60%	2.02%	1.87%
Organic matter	45.7%	50.5%	87.5%
pH	7.08	7.14	8.47
C/N	14:1	13:1	12:1
Sulphur	6025.0 ppm	30875.0 ppm	5870.0 ppm
Bulk density			
Conductivity (@ 25ºC)	8.45 ms/cm	7.99 ms/cm	7.69 ms/cm
Sodium	.34%	.19%	.30%
Aluminum	5755.0 ppm	2799.5 ppm	260.7 ppm
Boron	25.0 ppm	15.4 ppm	26.9 ppm
Calcium	8.95%	13.2%	2.71%
Copper	61.7 ppm	94.1 ppm	338.8 ppm
Iron	12620.0 ppm	3324.5 ppm	711.0 ppm
Magnesium	.89%	.64%	.56%
Manganese	543.0 ppm	282.6 ppm	463.1 ppm
Zinc	406.3 ppm	171.7 ppm	372.6 ppm

**Calculated on dry weight basis*

Table A.4: Grams of amendment elements per kilogram of soil for experiment 2

	1X Broiler manure	2X Broiler manure	1X Mushroom compost	2X Mushroom compost	1X PARC compost	2X PARC compost
Cu	1.00	2.00	0.56	1.12	0.50	1.00
Fe	2.10	4.20	19.9	39.8	103	806
S	17.3	34.6	185	370	49.2	98.4
Zn	1.10	2.20	1.03	2.06	3.32	6.64

Appendix C: Decagon EM50 soil temperature and moisture data

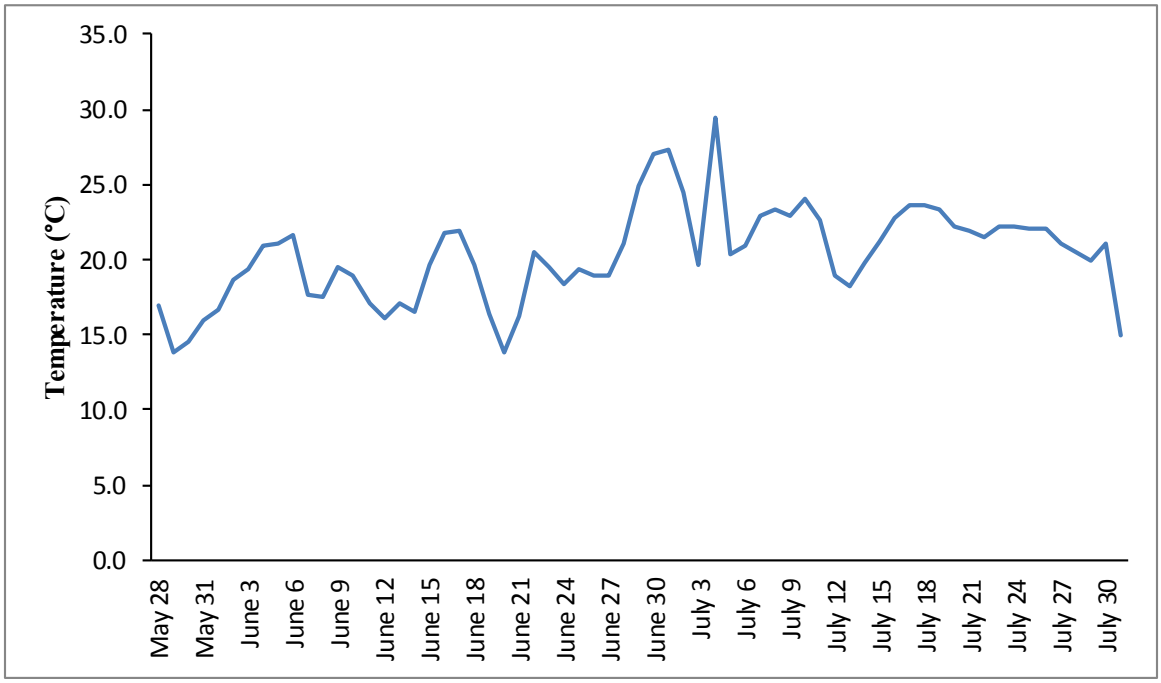

Figure A.1: Average daily temperatures during experiment 1 measured with Decagon EM50 datalogger 5TM probes (inoculation date of May 31).

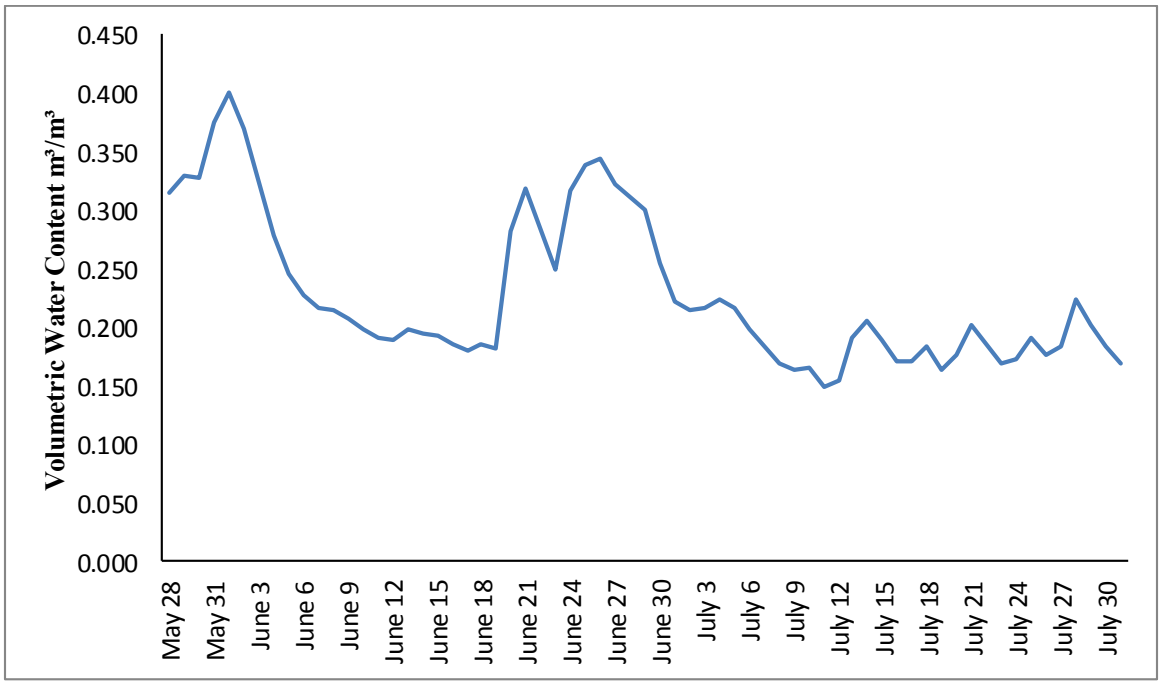

Figure A.2: Average daily soil moisture during experiment 1 measured with Decagon EM50 datalogger 5TM probes (inoculation date of May 31).

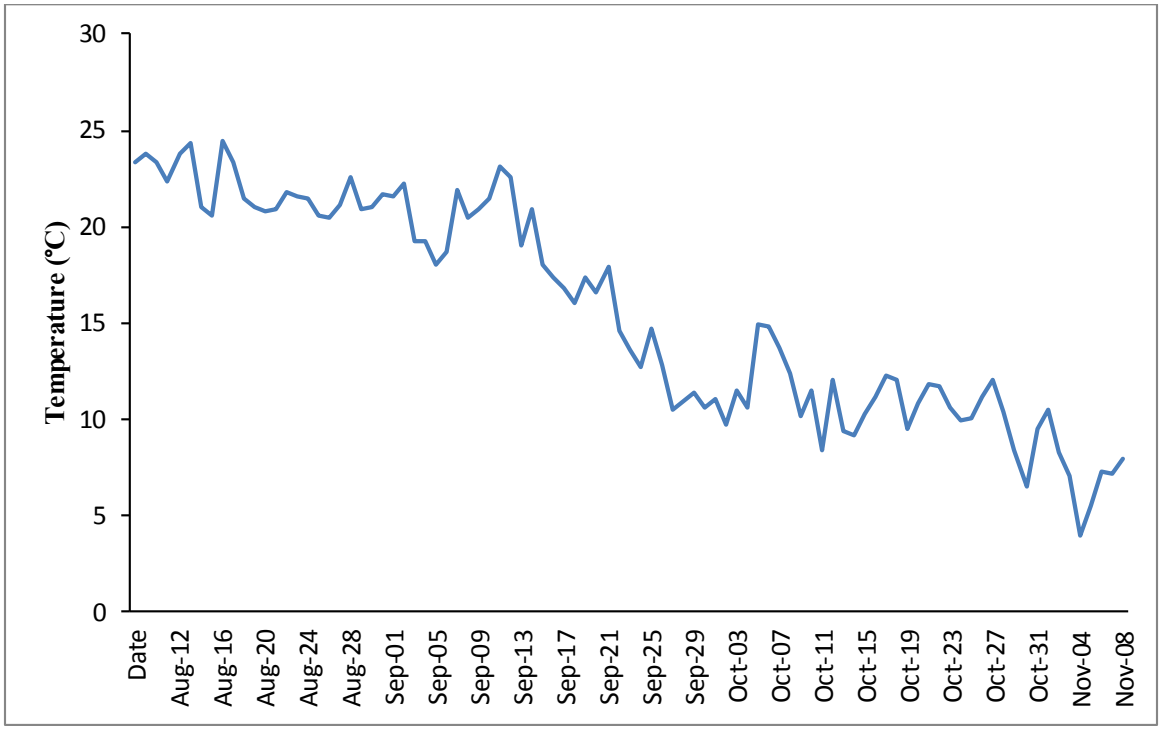

Figure A.3: Average daily temperatures during experiment 2 measured with Decagon EM50 datalogger with 5TM probes.

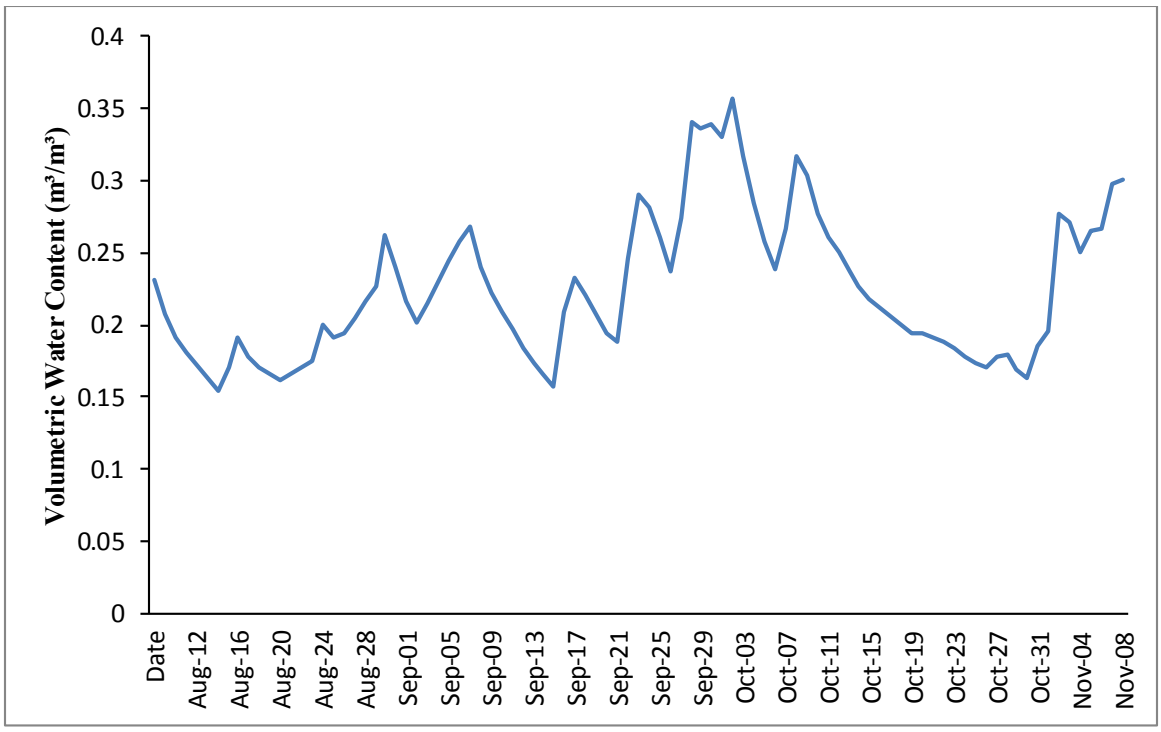

Figure A.4: Average daily soil moisture during experiment 2 measured with Decagon EM50 datalogger 5TM probes.

Appendix D: Quantitative PCR data

Table A.5: Results of quantitative PCR of raspberry roots from block 6+9 (left) and 7+8 (right) using primers specific to *P. rubi*.

Sample	Block	Treatment	[a]Cq mean	[d]*P. rubi* presence (pg/ml)	Mean melt temperature (° C)	Sample	Block	Treatment	[a]Cq mean	[d]*P. rubi* presence (pg/ml)	Mean melt temperature (°C)
[b]NTC			ND	ND	None	[b]NTC			ND	ND	None
Pos Ctrl			29.4	26275	86.5	Pos Ctrl			29.45	43900	86.5
Std-01			31.31	6250	86.0	Std-01			32.05	6250	86.5
Std-02			32.56	3130	86.0	Std-02			33.39	3130	86.5
Std-03			33.77	1560	86.0	Std-03			34.21	1560	86.5
Std-04			34.40	781	86.0	Std-04			35.51	781	86.5
Std-05			35.71	391	86.0	Std-05			36.13	391	86.5
Std-06			36.34	195	86.0	Std-06			37.34	ND	None
Std-07			37.23	98	86.0	Std-07			38.26	ND	None
Std-08			39.03	49	86.5	Std-08			39.17	ND	None
92	6	ctrl	40.10	ND	None	111	7	ctrl	39.20	ND	None
81	6	fum*	37.13	ND	89.5	97	7	LoMan*	39.69	ND	None
82	6	ctrl*	39.28	ND	None	98	7	ctrl*	38.84	ND	None
83	6	LoMC*	38.69	ND	None	99	7	HiPARC*	40.74	ND	None
84	6	LoPARC*	38.60	ND	88.0	100	7	LoMC*	40.17	ND	None
85	6	LoMan*	38.48	ND	89.5	101	7	HiMan*	40.77	ND	None
86	6	HiMC*	38.86	ND	None	102	7	fum*	39.58	ND	None
87	6	HiMan*	38.85	ND	89.5	103	7	HiMC*	35.73	566	86.5
88	6	HiPARC*	39.76	ND	None	104	7	LoPARC*	ND	ND	None
144	9	ctrl	40.30	ND	None	121	8	ctrl	40.41	ND	None
129	9	LoPARC*	40.73	ND	None	113	8	fum*	ND	ND	None
130	9	HiMan*	39.61	ND	None	114	8	LoPARC*	ND	ND	None
131	9	HiPARC*	39.62	ND	None	115	8	HiMan*	38.89	ND	89.5
132	9	LoMan*	0.00	ND	None	116	8	ctrl*	ND	ND	None
133	9	HiMC*	41.57	ND	None	117	8	HiMC*	40.93	ND	None
134	9	ctrl*	39.27	ND	None	118	8	LoMC*	32.62	494	86.5
135	9	LoMC*	39.35	ND	89.5	119	8	LoMan*	39.71	ND	None
136	9	fum*	40.34	ND	89.5	120	8	HiPARC*	39.02	ND	None

[a]Cq, the number of cycles before fluorescence threshold is reached.

[b]NTC, non-template control.

[c]Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
ND, not detected.

[d]*P. rubi* presence determined based on melt temperature (86-87°C)

*Inoculated treatment pots.

Table A.6: Results of quantitative PCR of raspberry roots from block 3+5 (left) and 2+4 (right) using primers specific to *P. rubi*.

Sample	Block	Treatment	[a]Cq mean	[d]*P. rubi* presence (pg/ml)	Mean melt temperature (°C)	Sample	Block	Treatment	[a]Cq mean	[d]*P. rubi* presence (pg/ml)	Mean melt temperature (°C)
[b]NTC			ND	ND	None	[b]NTC			ND	ND	None
Pos Ctrl			29.73	14250	86.5	Pos Ctrl			29.35	54150	86.5
Std-01			31.80	6250	86.5	Std-01			32.33	6250	86.0
Std-02			32.64	3130	86.0	Std-02			33.21	3130	86.0
Std-03			33.69	1560	86.0	Std-03			33.91	1560	86.0
Std-04			35.28	781	86.5	Std-04			35.26	781	86.0
Std-05			35.98	391	86.5	Std-05			35.86	391	86.5
Std-06			37.67	195	86.5	Std-06			36.25	195	86.0
Std-07			40.02	98	86.5	Std-07			38.41	ND	None
Std-08			40.76	ND	None	Std-08			38.67	ND	None
47	3	ctrl	36.74	ND	88.5	29	2	ctrl	41.08	ND	None
33	3	LoMC*	37.82	ND	None	17	2	ctrl*	39.17	ND	None
34	3	HiMan*	38.20	ND	89.5	18	2	fum*	37.31	ND	89.5
35	3	ctrl*	40.21	ND	None	19	2	HiMC*	38.47	ND	89.5
36	3	LoMan*	37.54	ND	89.5	20	2	HiMan*	37.33	ND	88.75
37	3	fum*	36.43	ND	89.5	21	2	LoMC*	38.16	ND	89.5
38	3	LoPARC*	37.16	ND	89.5	22	2	LoMan*	37.94	ND	89.5
39	3	HiPARC*	37.40	ND	89.5	23	2	LoPARC*	39.27	ND	None
40	3	HiMC*	36.89	ND	None	24	2	HiPARC*	38.14	ND	89.5
80	5	ctrl	38.04	ND	89.5	61	4	ctrl	39.32	ND	None
65	5	LoMan*	39.22	ND	None	49	4	HiPARC*	39.42	ND	None
66	5	fum*	40.68	ND	None	50	4	ctrl*	38.46	ND	89.5
67	5	HiPARC*	39.66	ND	None	51	4	HiMan*	37.83	ND	None
68	5	HiMC*	37.41	ND	89.5	52	4	LoMan*	39.15	ND	89.5
69	5	LoPARC*	37.55	ND	89.5	53	4	LoMC*	39.70	ND	None
70	5	ctrl*	37.65	ND	89.5	54	4	LoPARC*	39.19	ND	None
71	5	LoMC*	37.19	ND	89.5	55	4	HiMC*	38.28	ND	89.5
72	5	HiMan*	38.48	ND	None	56	4	fum*	37.30	145	86.5

[a]Cq, the number of cycles before fluorescence threshold is reached.
[b]NTC, non-template control.
[c]Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
ND, not detected.
[d]*P. rubi* presence determined based on melt temperature (86-87°C)
*Inoculated treatment pots.

Table A.7: Results of quantitative PCR of raspberry roots from block 1+10 using primers specific to *P. rubi*.

Sample	Block	Treatment	[a]Cq mean	[d]*P. rubi* presence (pg/ml)	Mean melt temperature (°C)
[b]NTC			ND	ND	None
Pos Ctrl			29.08	17050	86.5
Std-02			31.88	3130	86.5
Std-03			32.25	1560	86.5
Std-04			33.35	781	86.5
Std-05			34.46	391	86.5
Std-06			35.29	195	86.5
Std-07			37.17	98	86.0
Std-08			37.48	49	86.5
15	1	ctrl	35.12	ND	87.5
1	1	LoPARC*	37.60	ND	None
2	1	HiMan*	38.11	ND	89.5
3	1	HiPARC*	37.96	ND	89.5
4	1	LoMC*	38.65	ND	None
5	1	ctrl*	37.04	78	86.5
7	1	fum*	37.62	ND	89.5
8	1	LoMan*	38.10	ND	None
13	1	HiMC*	37.90	ND	88.0
157	10	ctrl	37.30	ND	89.5
145	10	HiMC*	37.88	ND	87.0
146	10	LoPARC*	36.48	ND	87.0
147	10	ctrl*	38.04	ND	88.0
148	10	HiMan*	37.40	ND	None
149	10	LoMC*	34.78	332	86.5
150	10	HiPARC*	35.60	ND	89.5
151	10	LoMan*	37.15	ND	89.5
152	10	fum*	36.88	78	87.0

[a]Cq, the number of cycles before fluorescence threshold is reached.
[b]NTC, non-template control.
[c]Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
ND, not detected.
[d]*P. rubi* presence determined based on melt temperature (86-87°C)
*Inoculated treatment pots.

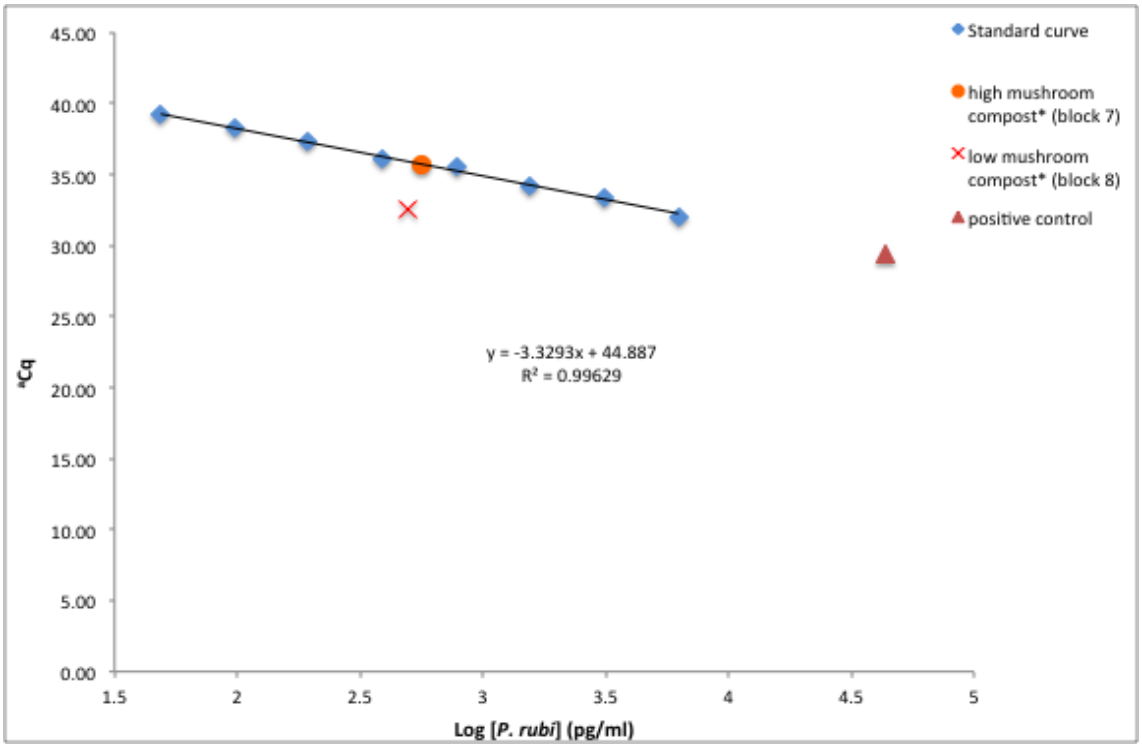

Figure A.5: Quantitative PCR standard curve with positive root samples for block 7+8 using primers specific to *P. rubi*. Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
[a]Cq, the number of cycles before fluorescence threshold is reached.
*Inoculated treatment pots.

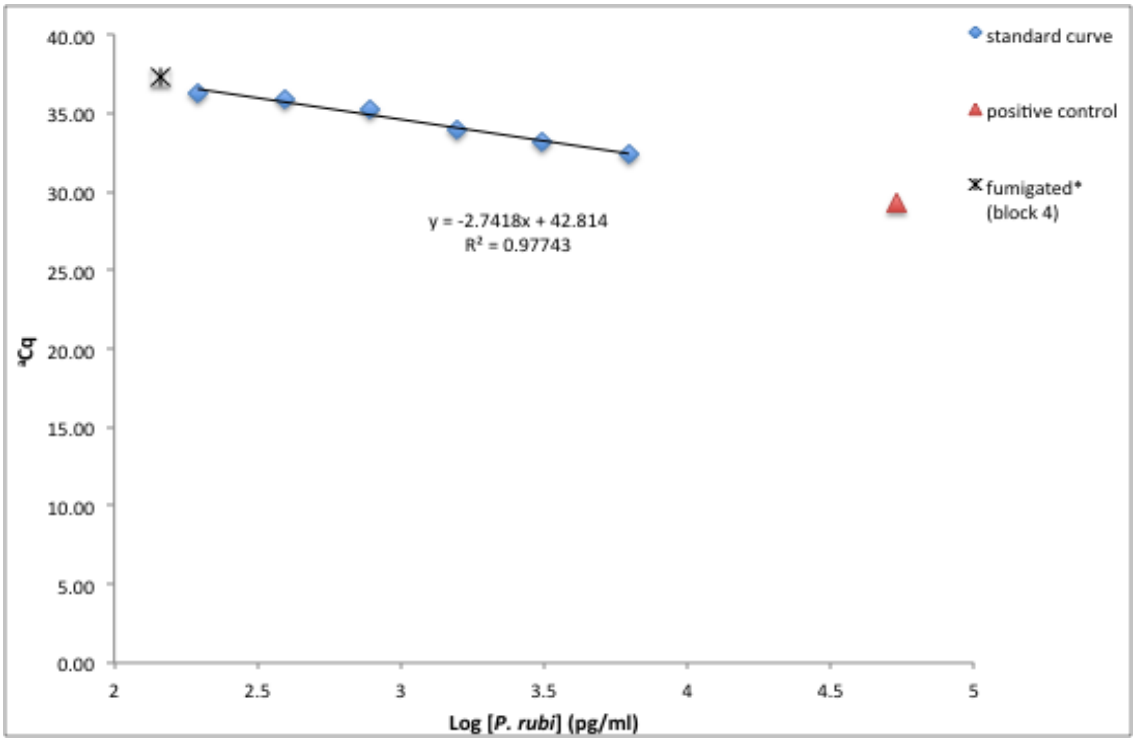

Figure A.6: Quantitative PCR standard curve with positive root samples for block 4 using primers specific to *P. rubi*. Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
[a]Cq, the number of cycles before fluorescence threshold is reached.
*Inoculated treatment pots.

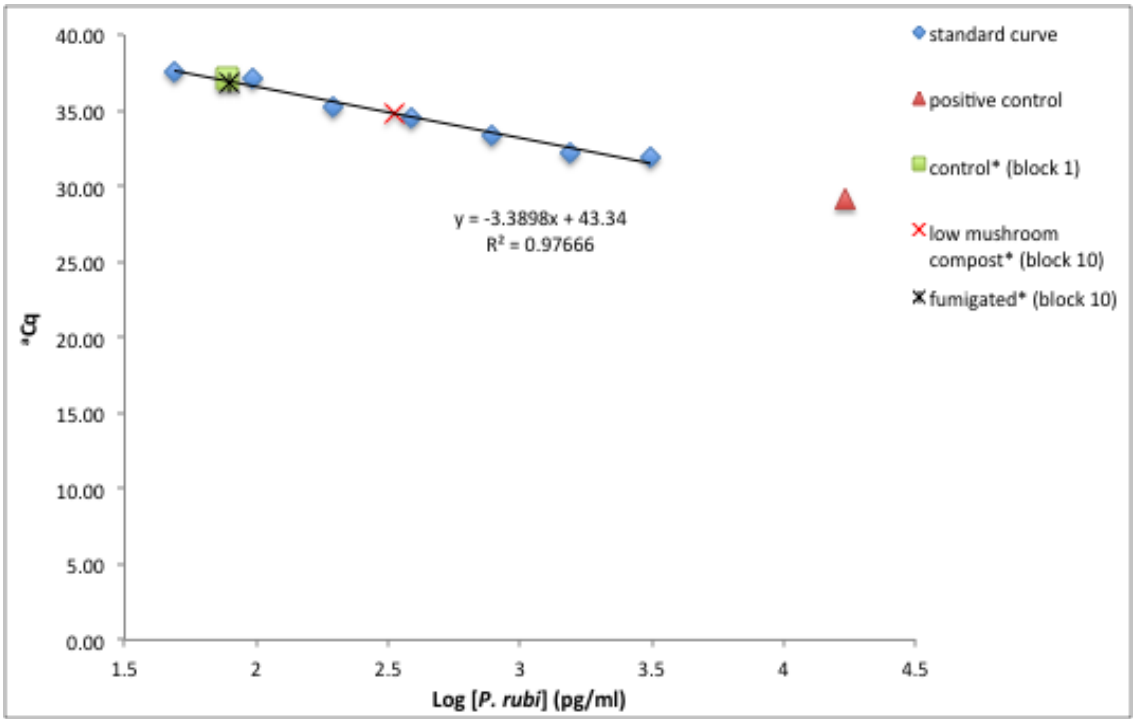

Figure A.7: Quantitative PCR standard curve with positive root samples for block 1+10 using primers specific to *P. rubi*. Standard DNA concentration values in pg/ml, concentrations determined using Qubit Broad Range kit (Invitrogen, Carlsbad, CA).
[a]Cq, the number of cycles before fluorescence threshold is reached.
*Inoculated treatment pots.

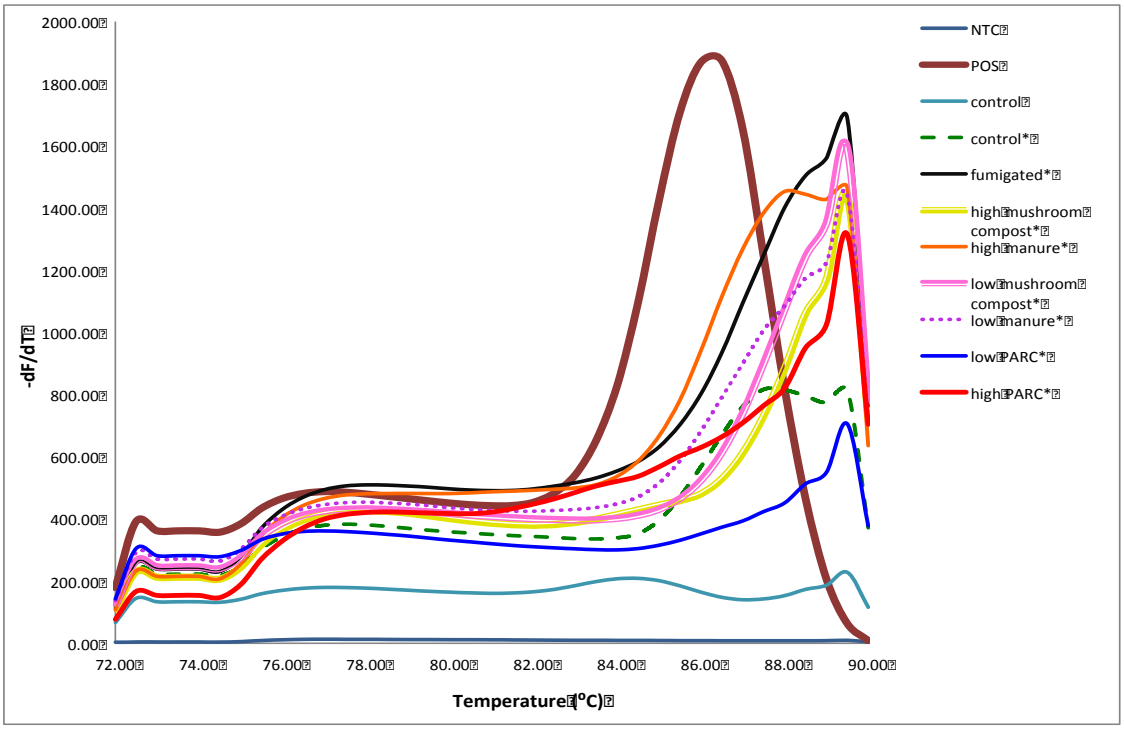

Figure A.8: Melting curve profile of block 2. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

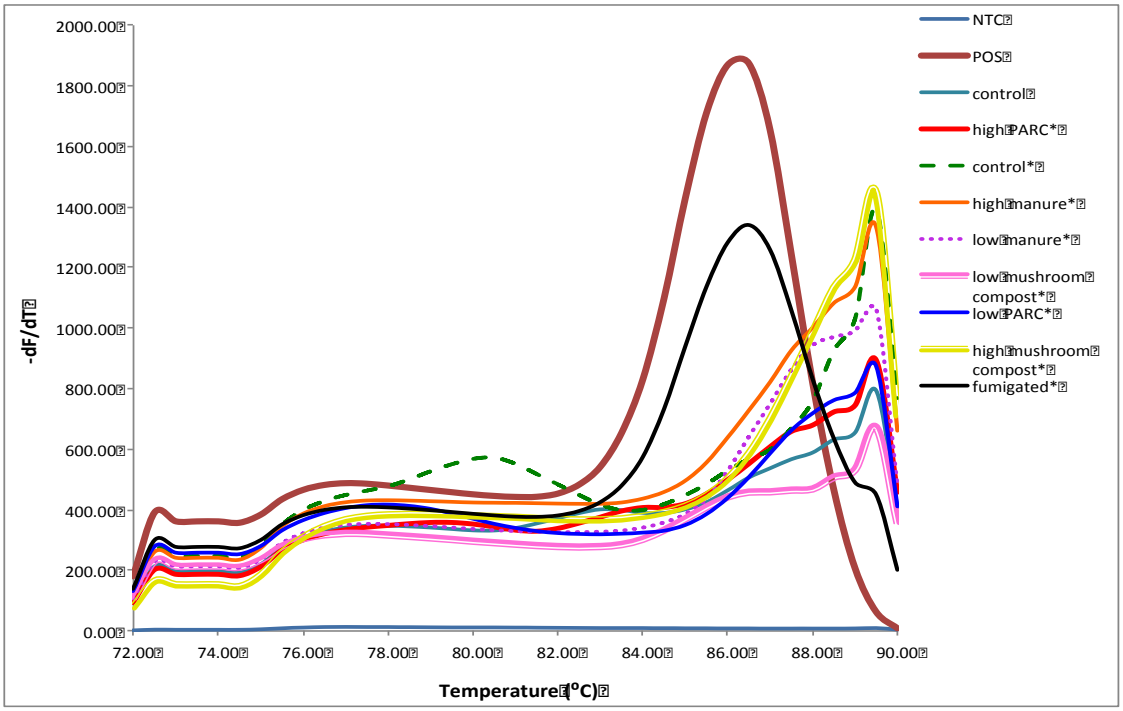

Figure A.9: Melting curve profile of block 4. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

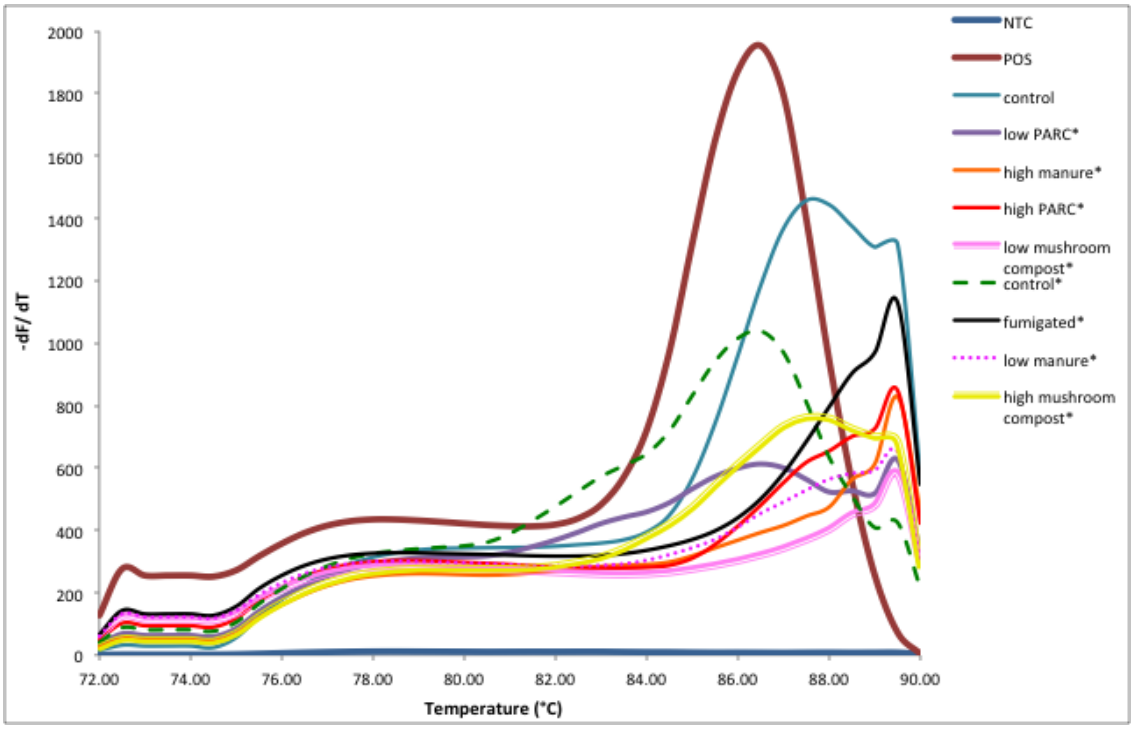

Figure A.10: Melting curve profile of block 1. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

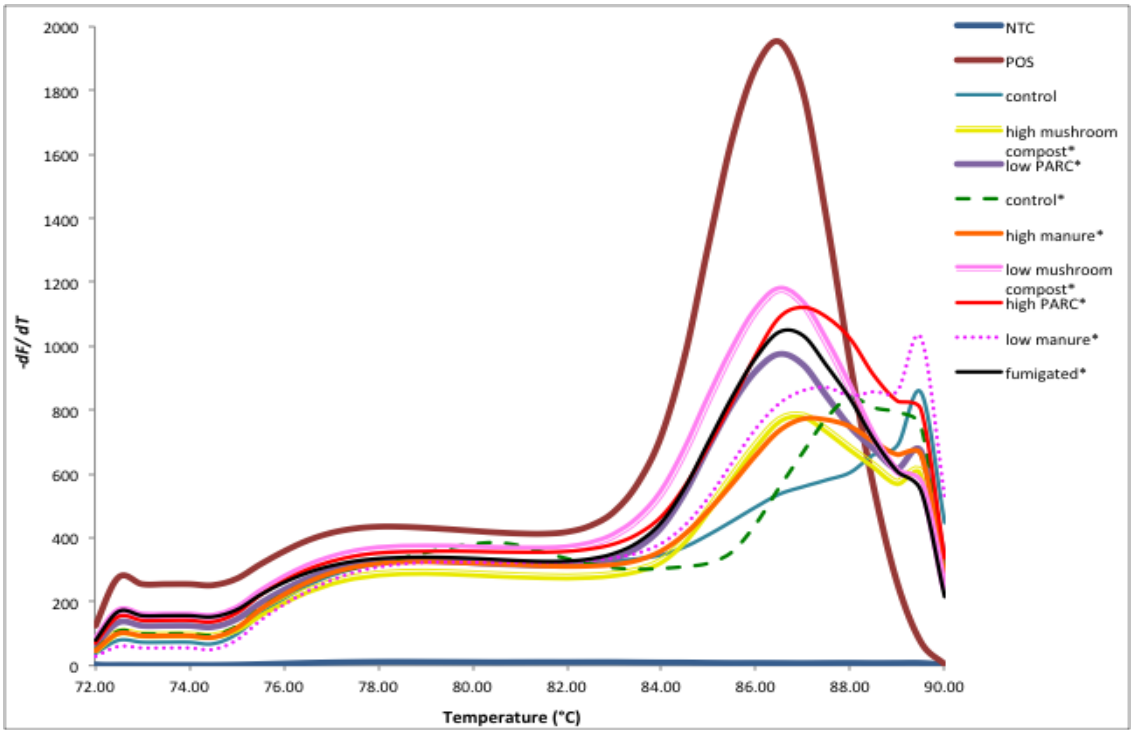

Figure A.11: Melting curve profile of block 10. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

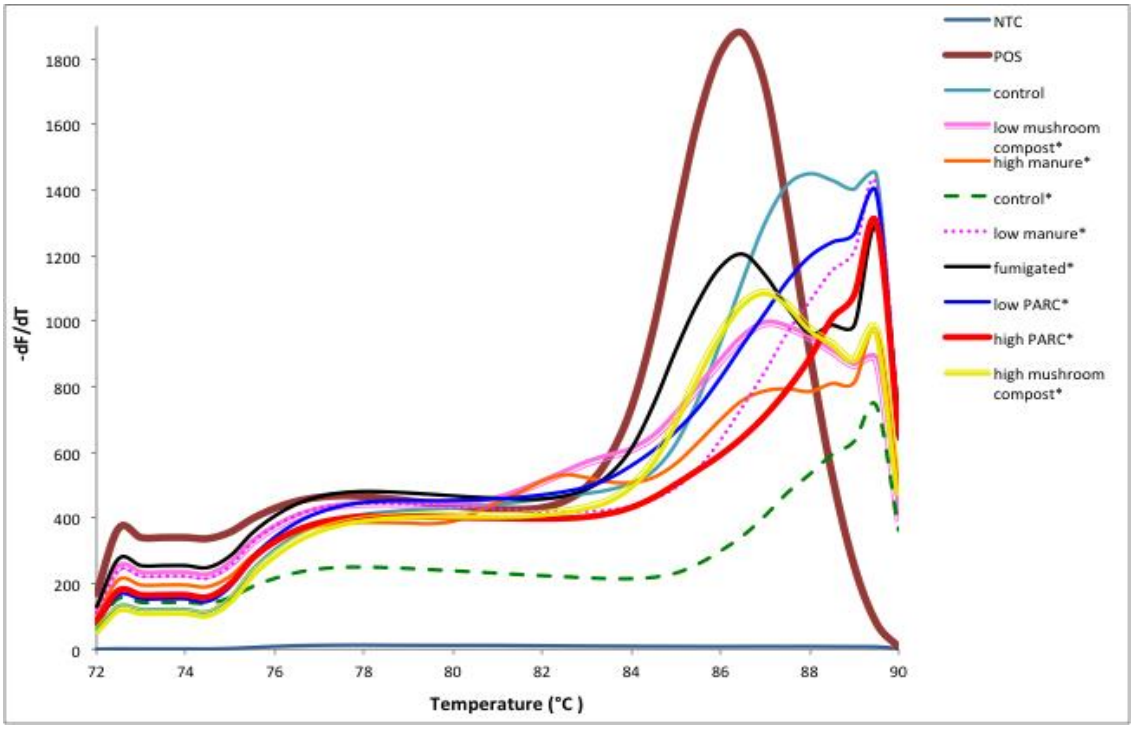

Figure A.12: Melting curve profile of block 3. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as – *dF*/*dT* versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

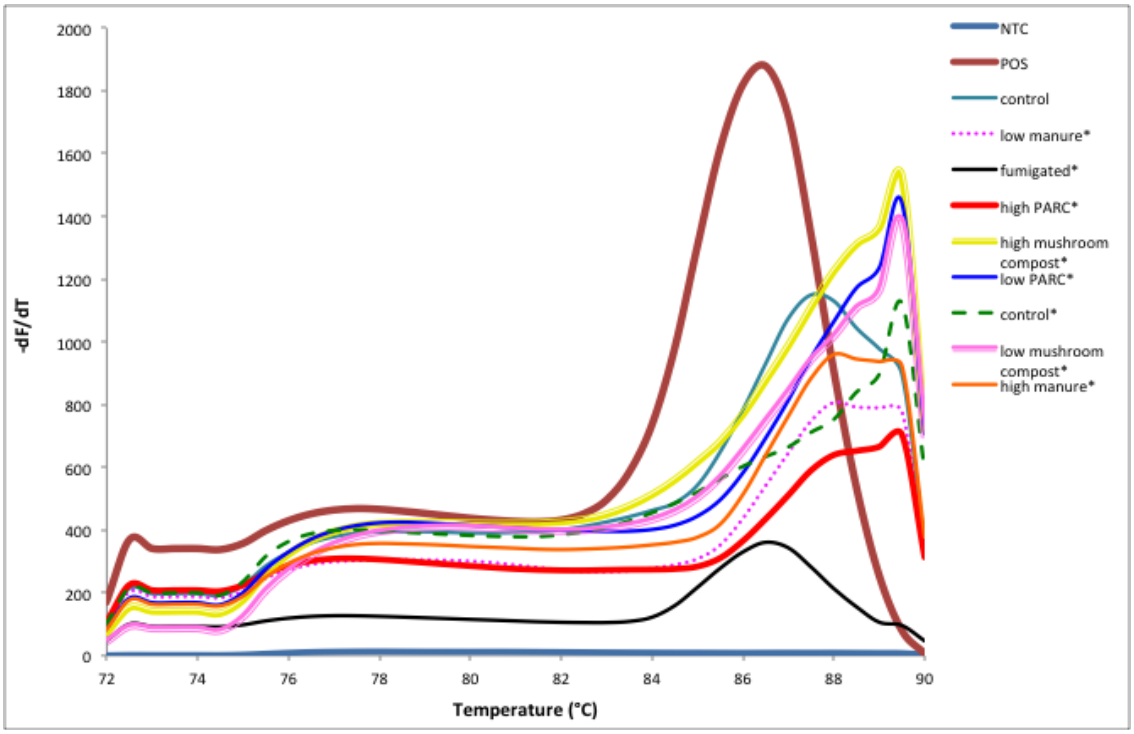

Figure A.13: Melting curve profile of block 5. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as – *dF*/*dT* versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

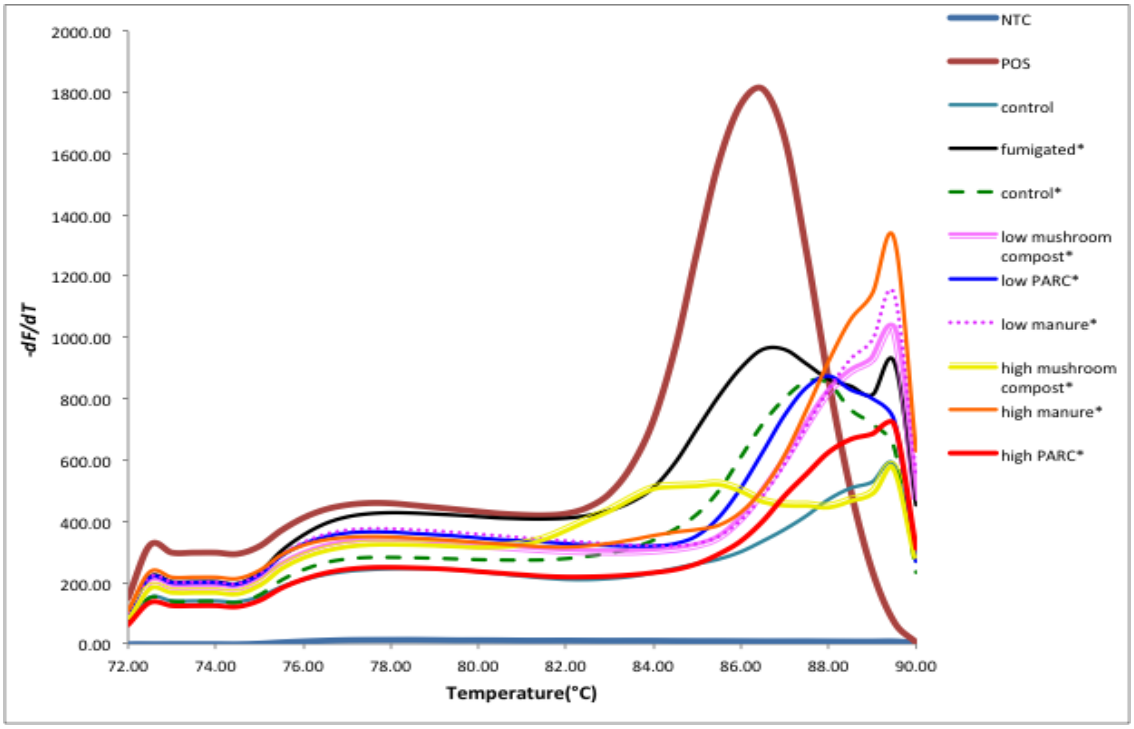

Figure A.14: Melting curve profile of block 6. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

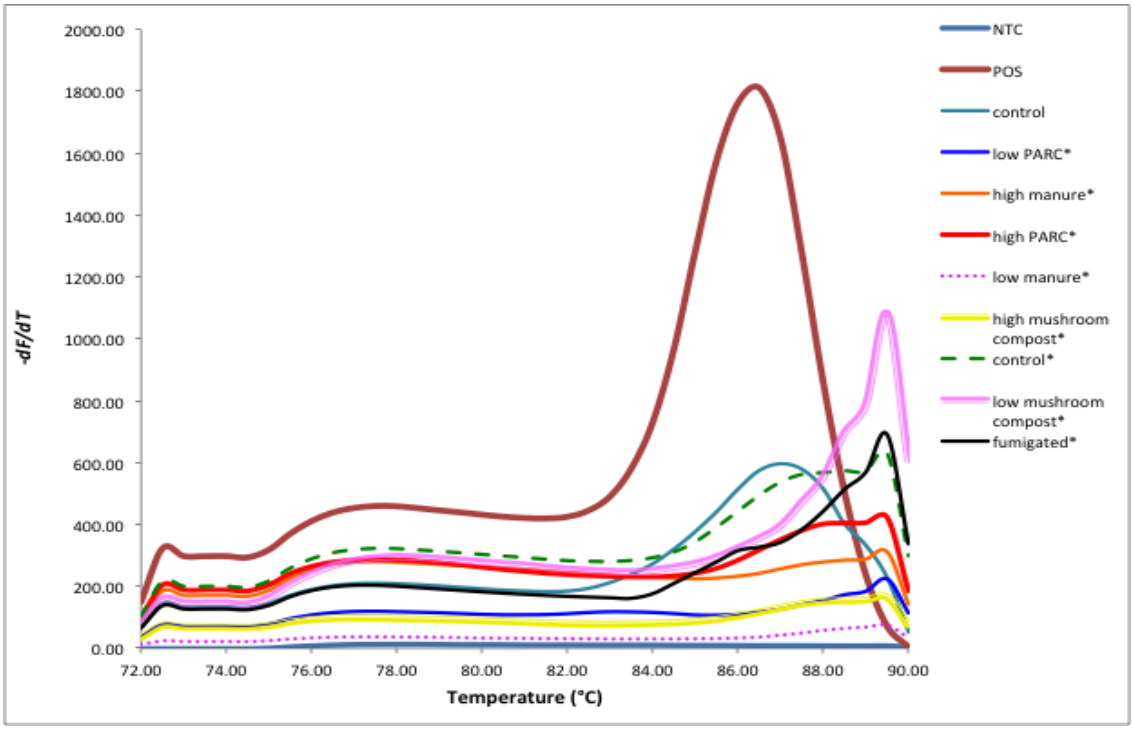

Figure A.15: Melting curve profile of block 9. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

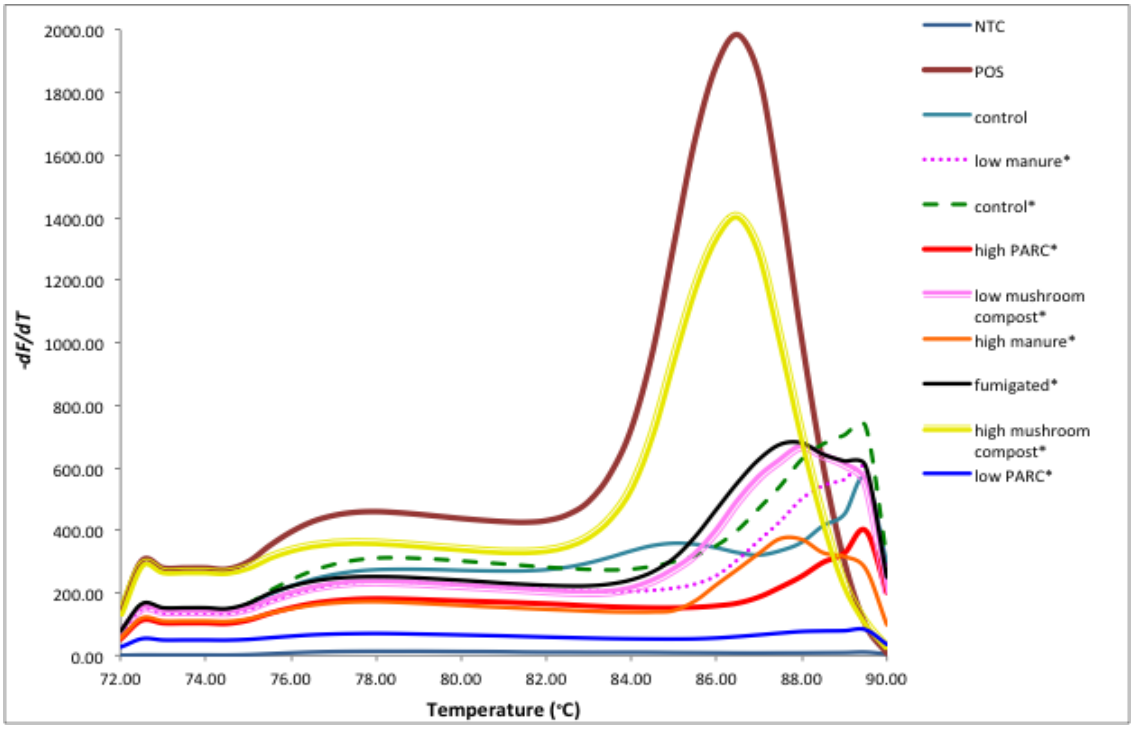

Figure A.16: Melting curve profile of block 7. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

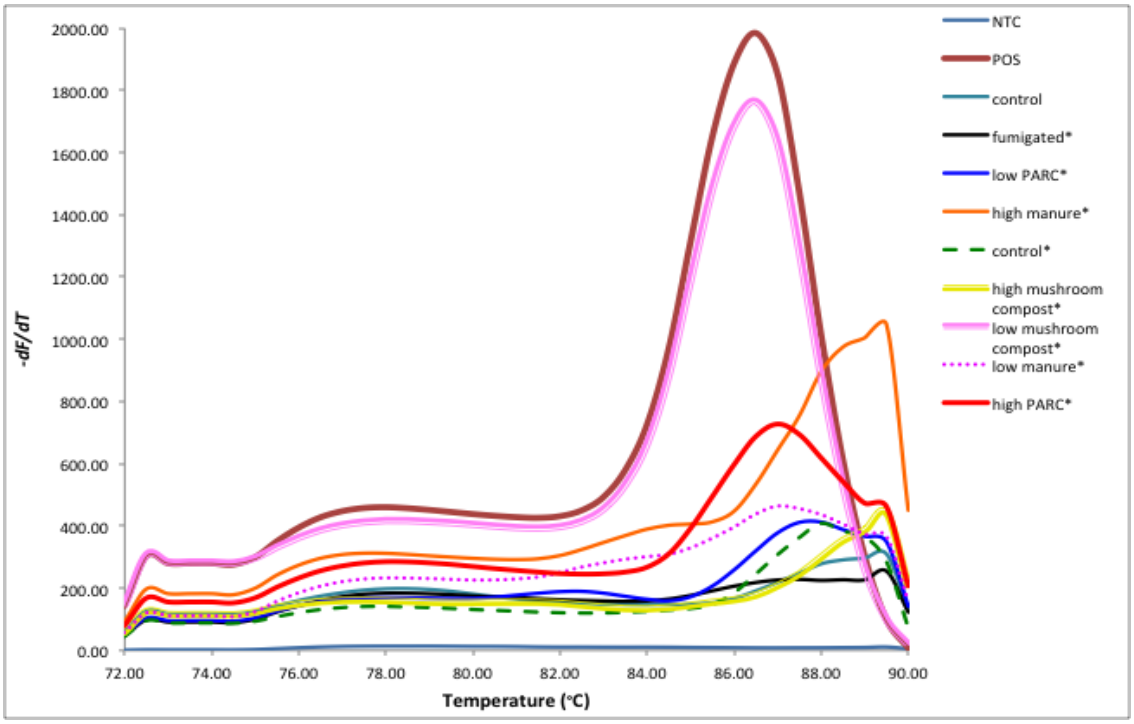

Figure A.17: Melting curve profile of block 8. The negative derivative of SYBR green fluorescence with respect to temperature is plotted as $-dF/dT$ versus temperature to obtain a graphical representation of melting peaks.
NTC, non-template control
POS, positive control
*Inoculated treatment pots

Appendix E: Statistical output data

Table A.8: Experiment 1 Analysis of Variance SAS output. L= data were log-transformed prior to analyses, T1= day of planting.

Shoot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	400.4	44.49	3.25	0.0013
Treatment	7	4555.7	650.8	47.56	<0.0001
Phyophthora	1	31.03	31.03	2.27	0.1344
Treatment* *Phytophthora*	7	110.5	15.79	1.15	0.3336
Model	24	5097.6	212.4	15.52	<0.0001
Error	135	1847.3	13.68		
Corrected total	159	6944.9			

Root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	276.4	30.71	4.73	<0.0001
Treatment	7	449.7	64.24	9.89	<0.0001
Phyophthora	1	5.66	5.66	0.87	0.352
Treatment* *Phytophthora*	7	49.94	7.13	1.10	0.3675
Model	24	781.7	32.57	5.02	<0.0001
Error	135	876.5	6.49		
Corrected total	159	1658.2			

Root rot rate

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	6.01	0.667	1.42	0.184
Treatment	7	74.14	10.59	22.59	<0.0001
Phyophthora	1	0.006	0.0063	0.01	0.9083
Treatment* *Phytophthora*	7	3.24	0.463	0.99	0.4424
Model	24	83.4	3.48	7.41	<0.0001
Error	135	63.29	0.469		
Corrected total	159	146.7			

L *P. penetrans*/g of root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	3.65×10^8	4.06×10^7	2.28	0.0206
Treatment	7	1.32×10^9	1.89×10^8	10.62	<0.0001
Phyophthora	1	12888	12888	0.00	0.9786
Treatment* *Phytophthora*	7	3.63×10^7	5.18×10^6	0.29	0.9565
Model	24	1.72×10^9	7.19×10^7	4.04	<0.0001
Error	135	2.40×10^9	1.78×10^7		
Corrected total	159	4.13×10^9			

L 13wks total *P. penetrans*/ pot (roots + soil)

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	9.89×10^{9}	1.10×10^{9}	1.18	0.3136
Treatment	7	5.12×10^{10}	7.32×10^{9}	7.85	<0.0001
Phyophthora	1	6.21×10^{7}	6.21×10^{7}	0.07	0.7967
Treatment**Phytophthora*	7	2.23×10^{9}	3.19×10^{8}	0.34	0.9334
Model	24	6.34×10^{10}	2.64×10^{9}	2.83	<0.0001
Error	134	1.25×10^{11}	9.33×10^{8}		
Corrected total	158	1.88×10^{11}			

L T1 *P. penetrans*/ pot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	2.25×10^{6}	250506	1.10	0.3686
Treatment	7	3.29×10^{7}	4.70×10^{7}	20.60	<0.0001
Phyophthora	1	58931	58931	0.26	0.6121
Treatment**Phytophthora*	7	881888	125984	0.55	0.7933
Model	24	3.61×10^{7}	1.50×10^{6}	6.59	<0.0001
Error	132	3.01×10^{7}	228129		
Corrected total	156	6.62×10^{7}			

L T1 FLN/ pot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	$2.06 \text{ X } 10^{10}$	$2.29 \text{ X } 10^{9}$	0.75	0.6666
Treatment	7	1.08×10^{12}	1.54×10^{11}	50.06	<0.0001
Phyophthora	1	2.26×10^{8}	2.26×10^{8}	0.07	0.7867
Treatment**Phytophthora*	7	1.49×10^{10}	2.13×10^{9}	0.69	0.6774
Model	24	1.11×10^{12}	4.64×10^{10}	15.09	<0.0001
Error	132	4.06×10^{11}	3.07×10^{9}		
Corrected total	156	1.52×10^{12}			

L 13wks *P. penetrans*/ pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	1.21×10^{9}	1.34×10^{8}	8.37	<0.0001
Treatment	7	7.41×10^{8}	1.06×10^{8}	6.60	<0.0001
Phyophthora	1	1.26×10^{7}	1.26×10^{7}	0.79	0.3766
Treatment**Phytophthora*	7	5.56×10^{7}	7.94×10^{6}	0.50	0.8367
Model	24	2.02×10^{9}	8.40×10^{7}	5.24	<0.0001
Error	134	2.15×10^{9}	1.60×10^{7}		
Corrected total	158	4.16×10^{9}			

L 13wks FLN/ pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	2.28×10^{10}	2.54×10^{9}	16.03	<0.0001
Treatment	7	2.41×10^{10}	3.45×10^{9}	21.78	<0.0001
Phyophthora	1	5.11×10^{8}	5.11×10^{8}	3.23	0.0747
Treatment* *Phytophthora*	7	1.56×10^{8}	2.22×10^{8}	1.40	0.2086
Model	24	4.90×10^{10}	2.04×10^{9}	12.91	<0.0001
Error	134	2.12×10^{10}	1.58×10^{8}		
Corrected total	158	7.02×10^{10}			

Table A.9: Experiment 1 contrast Analysis of Variance SAS output. Contrast analyses were conducted after removing *Phytophthora* inoculation factor from the model. L= data were log-transformed prior to analyses, T1= day of planting.

Shoot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	400.4	44.49	3.20	0.0015
Treatment	7	4556	650.8	46.79	<0.0001
control vs manure	1	702.0	702.0	50.47	<0.0001
control vs compost	1	153.2	153.2	11.01	0.0011
fumigation vs manure	1	16.25	26.25	1.17	0.2815
fumigation vs compost	1	2099	2099	150.9	<0.0001
manure vs compost	1	2857	2857	205.4	<0.0001
mushroom vs PARC	1	1.00	1.00	0.07	0.7886
Model	16	4956	309.8	22.27	<0.0001
Error	143	1989	13.91		
Corrected total	159	6945			

Root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	276.4	30.71	4.71	<0.0001
Treatment	7	449.7	64.24	9.86	<0.0001
control vs manure	1	67.98	67.98	10.43	0.0015
control vs compost	1	25.57	25.57	3.92	0.0495
fumigation vs manure	1	0.639	0.639	0.10	0.7546
fumigation vs compost	1	174.6	174.6	26.78	<0.0001
manure vs compost	1	330.8	330.8	50.76	<0.0001
mushroom vs PARC	1	5.29	5.29	0.81	0.3690
Model	16	726.1	45.38	6.96	<0.0001
Error	143	932.1	6.52		
Corrected total	159	1658			

L *P. penetrans*/g of root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	15.65	1.74	3.2	0.0015
Treatment	7	231.6	33.09	60.83	<0.0001
control vs manure	1	12.31	12.31	22.64	<0.0001
control vs compost	1	1.21	1.21	2.22	0.1386
fumigation vs manure	1	75.26	75.26	138.3	<0.0001
fumigation vs compost	1	208.7	208.7	383.6	<0.0001
manure vs compost	1	40.72	40.72	74.86	<0.0001
mushroom vs PARC	1	1.05	1.05	1.92	0.1675
Model	16	247.3	15.46	28.41	<0.0001
Error	143	77.8	0.544		
Corrected total	159	325.1			

L 13wks total *P. penetrans*/ pot (roots + soil)

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	23.21	2.58	2.69	0.0064
Treatment	7	469	67	68.86	<0.0001
control vs manure	1	6.63	6.63	6.91	0.0095
control vs compost	1	0.424	0.424	0.44	0.5071
fumigation vs manure	1	258.8	258.8	269.9	<0.0001
fumigation vs compost	1	447.8	447.8	466.9	<0.0001
manure vs compost	1	20.86	20.86	21.76	<0.0001
mushroom vs PARC	1	2.12	2.12	2.21	0.1389
Model	16	492.2	30.76	32.08	<0.0001
Error	142	136.2	0.959		
Corrected total	158	628.4			

L T1 *P. penetrans*/ pot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	3.43	0.382	0.88	0.5423
Treatment	7	312.5	44.64	103.3	<0.0001
control vs manure	1	16.89	16.89	39.09	<0.0001
control vs compost	1	0.342	0.342	0.79	0.3752
fumigation vs manure	1	116.2	116.2	268.9	<0.0001
fumigation vs compost	1	287.2	287.2	664.6	<0.0001
manure vs compost	1	44.39	44.39	102.7	<0.0001
mushroom vs PARC	1	0.439	0.439	1.02	0.3154
Model	16	315.9	19.74	45.69	<0.0001
Error	140	60.5	0.432		
Corrected total	156	376.4			

L T1 free living nematodes nematodes/ pot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	17.47	1.94	1.88	0.0594
Treatment	7	701.7	100.2	97.13	<0.0001
control vs manure	1	113	113	109.53	<0.0001
control vs compost	1	4.84	4.84	4.69	0.032
fumigation vs manure	1	692.2	692.2	670.8	<0.0001
fumigation vs compost	1	367.2	367.2	355.8	<0.0001
manure vs compost	1	152.4	152.4	147.7	<0.0001
mushroom vs PARC	1	0.791	0.791	0.77	0.3828
Model	16	719.2	44.95	43.55	<0.0001
Error	140	144.5	1.03		
Corrected total	156	863.6			

L 13wks *P. penetrans*/ pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	352.6	39.17	17.55	<0.0001
Treatment	7	299.3	42.75	19.15	<0.0001
control vs manure	1	0.192	0.192	0.09	0.7696
control vs compost	1	16.07	16.07	7.2	0.0082
fumigation vs manure	1	147.3	147.3	66	<0.0001
fumigation vs compost	1	285.7	285.7	128	<0.0001
manure vs compost	1	21.7	21.7	9.72	0.0022
mushroom vs PARC	1	5.89	5.89	2.64	0.1064
Model	16	651.8	40.74	18.25	<0.0001
Error	142	316.9	2.23		
Corrected total	158	969			

L 13wks free living nematodes/ pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	133.2	14.8	17.91	<0.0001
Treatment	7	82.33	11.76	14.23	<0.0001
control vs manure	1	31.13	31.13	37.68	0.7696
control vs compost	1	5.15	5.15	6.24	0.0082
fumigation vs manure	1	65.12	65.12	78.81	<0.0001
fumigation vs compost	1	24.35	24.35	29.47	<0.0001
manure vs compost	1	25.41	25.41	30.75	0.0022
mushroom vs PARC	1	4.17	4.17	5.05	0.1064
Model	16	215.5	13.47	16.3	<0.0001
Error	142	117.3	0.826		
Corrected total	158	332.9			

Table A.10: Experiment 2 contrast Analysis of Variance SAS output. L= data were log-transformed prior to analyses, T1= day of planting.

Shoot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	301.5	33.50	4.39	0.0002
Treatment	7	84.25	12.04	1.58	0.1585
control vs manure	1	5.94	5.94	0.78	0.3809
control vs compost	1	4.13	4.13	0.54	0.4644
fumigation vs manure	1	6.03	6.03	0.79	0.3774
fumigation vs compost	1	4.05	4.05	0.53	0.4688
manure vs compost	1	36.86	36.86	4.83	0.0316
mushroom vs PARC	1	37.50	37.50	4.92	0.0302
Model	16	385.7	24.11	3.16	0.0006
Error	63	480.6	7.63		
Corrected total	79	866.3			

Root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	3889	432.1	1.98	0.0566
Treatment	7	692.1	98.88	0.45	0.8644
control vs manure	1	208.2	208.2	0.95	0.3324
control vs compost	1	16.16	16.16	0.07	0.7864
fumigation vs manure	1	38.10	38.10	0.17	0.6775
fumigation vs compost	1	25.25	25.25	0.12	0.7349
manure vs compost	1	231.5	231.5	1.06	0.3069
mushroom vs PARC	1	0.212	0.212	0.00	0.9753
Model	16	4581	286.3	1.31	0.2185
Error	63	13750	218.3		
Corrected total	79	18331			

L T1 *P. penetrans*/pot

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	16.30	1.81	2.22	0.0322
Treatment	7	182.5	26.07	31.92	<0.0001
control vs manure	1	15.42		18.88	<0.0001
control vs compost	1	4.51		5.53	0.0219
fumigation vs manure	1	77.70		95.14	<0.0001
fumigation vs compost	1	140.0		171.5	<0.0001
manure vs compost	1	7.90		9.67	0.0028
mushroom vs PARC	1	1.27		1.55	0.2177
Model	16	198.8	12.42	15.21	<0.0001
Error	63	51.45	0.817		
Corrected total	79	250.2			

L T1 free living nematodes

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	19.87	2.21	1.63	0.1262
Treatment	7	281.9	40.27	29.72	<0.0001
control vs manure	1	0.119	0.119	0.09	0.7675
control vs compost	1	3.76	3.76	2.77	0.1009
fumigation vs manure	1	212.2	212.2	156.7	<0.0001
fumigation vs compost	1	207.3	207.3	153.0	<0.0001
manure vs compost	1	4.05	4.05	2.99	0.0886
mushroom vs PARC	1	3.40	3.40	2.51	0.1181
Model	16	301.7	18.86	13.92	<0.0001
Error	63	85.35	1.35		
Corrected total	79	387.1			

L *P. penetrans*/g of root

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	13.87	1.54	1.37	0.2202
Treatment	7	95.79	13.68	12.19	<0.0001
control vs manure	1	10.05	10.05	8.95	0.004
control vs compost	1	0.102	0.102	0.09	0.7641
fumigation vs manure	1	28.16	28.16	25.08	<0.0001
fumigation vs compost	1	79.87	79.87	71.13	<0.0001
manure vs compost	1	16.39	16.39	14.59	0.0003
mushroom vs PARC	1	1.51	1.51	1.34	0.2512
Model	16	109.7	6.85	6.10	<0.0001
Error	62	69.62	1.12		
Corrected total	78	179.3			

L 13wks *total P. penetrans*/pot (roots + soil)

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	22.51	2.50	2.01	0.0534
Treatment	7	363.5	51.93	41.67	<0.0001
control vs manure	1	9.50	9.50	7.63	0.0076
control vs compost	1	0.455	0.455	0.37	0.5477
fumigation vs manure	1	205.4	205.4	164.8	<0.0001
fumigation vs compost	1	336.4	336.4	270.0	<0.0001
manure vs compost	1	12.02	12.02	9.65	0.0029
mushroom vs PARC	1	1.57	1.57	1.27	0.2649
Model	16	386.0	24.13	19.36	<0.0001
Error	62	77.27	1.25		
Corrected total	78	463.3			

L 13wks *P. penetrans*/pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	40.74	4.53	5.70	<0.0001
Treatment	7	107.8	15.40	19.39	<0.0001
control vs manure	1	0.336	0.336	0.42	0.5179
control vs compost	1	1.91	1.91	2.41	0.1257
fumigation vs manure	1	57.24	57.24	72.07	<0.0001
fumigation vs compost	1	106.2	106.2	133.7	<0.0001
manure vs compost	1	6.79	6.79	8.55	0.0048
mushroom vs PARC	1	0.799	0.799	1.01	0.3`98
Model	16	148.5	9.28	11.69	<0.0001
Error	63	50.04	0.794		
Corrected total	79	198.6			

L 13wks free living nematodes/pot of soil

	DF	Sum of Squares	Mean Square	F Value	P Value
Block	9	5.80	0.645	1.42	0.1982
Treatment	7	19.20	2.74	6.05	<0.0001
control vs manure	1	7.16	7.16	15.79	0.0002
control vs compost	1	1.32	1.32	2.91	0.0930
fumigation vs manure	1	14.11	14.11	31.11	<0.0001
fumigation vs compost	1	5.44	5.44	12.0	0.0010
manure vs compost	1	5.30	5.30	11.68	0.0011
mushroom vs PARC	1	0.338	0.338	0.74	0.3916
Model	16	25.01	1.56	3.45	0.0002
Error	63	28.58	0.454		
Corrected total	79	53.59			

Table A.11: Experiment 1 mean *Pratylenchus penetrans* and free living nematode (FLN) population densities, and plant growth parameters, by individual treatment. Values within a column followed by the same letter are not significantly different according to Duncan's mean separation test ($p < 0.05$) after two-way ANOVA. Nematode data were log-transformed prior to analyses. Treatment names are as follows: ctrl= control, fum= fumigation, HiMan= high manure, HiPARC= high PARC compost, LoMan= low manure, LoMC= low mushroom compost, LoPARC= low PARC compost.

	Pratylenchus penetrans				**Free living nematodes**	
Treatment	**At-plant/ pot**	**/g root**	**/pot of soil**	**/pot**	**At-plant/pot**	**FLN/pot**
ctrl	1,079a	5,177b	3,503bc	38,676ab	9,132c	12,897cd
fum	13d	94e	21d	852d	665d	9,000d
HiMan	252c	1,299d	776c	13,909c	216,704a	45,938a
HiMC	1,048a	6,525ab	4,004abc	44,341ab	31,119b	18,084c
HiPARC	1,106a	7,082ab	5,312a	54,243ab	16,919bc	21,722ab
LoMan	504b	2,856c	1,550abc	28,295b	189,249a	39,270a
LoMC	1,101a	7,032ab	5,265ab	40,909ab	15,703bc	16,619bc
LoPARC	1,386a	8,630a	6,208a	55,136a	13,561bc	15,906bc

	Plant growth parameters	
	Root weight (g)	**Shoot weight (g)**
ctrl	7.84d	12.15d
fum	9.88b	20.51b
HiMan	10.82a	23.08a
HiMC	6.87de	9.89de
HiPARC	7.02e	8.77e
LoMan	9.37c	15.74c
LoMC	5.76e	8.45e
LoPARC	6.64e	9.13e

Table A.12: Experiment 2 mean *Pratylenchus penetrans* and free living nematodes (FLN) population densities, and plant growth parameters, by individual treatment. Values within a column followed by the same letter are not significantly different according to Duncan's mean separation test ($p < 0.05$) after one-way ANOVA. Nematode data were log-transformed prior to analyses. Treatment names are as follows: ctrl= control, fum= fumigation, HiMan= high manure, HiPARC= high PARC compost, LoMan= low manure, LoMC= low mushroom compost, LoPARC= low PARC compost.

	Pratylenchus penetrans				**Free living nematodes**	
Treatment	**At-plant/ pot**	**/g root**	**/pot of soil**	**/pot**	**At-plant/pot**	**FLN/pot**
ctrl	2,009a	3,872ab	620b	89,889ab	4,705ab	8,041bcd
fum	5c	5e	5c	142d	51d	7,016d
HiMan	3,95b	1,078d	393b	2,862c2	4,372abc	26,257a
HiMC	5,84b	2,354bcd	472ab	69,190abc	2,880c	13,520abc
HiPARC	1,363a	3,829abc	794ab	108,295abc	4,941ab	17,041ab
LoMan	943a	1,424cd	441b	39,796bc	9,307a	25,389a
LoMC	1,476a	4,204bc	753a	84,116abc	5,084ab	15,185abc
LoPARC	1,097a	6,970a	610ab	11,8427a	3,131bc	11,075cd

	Plant growth parameters	
	Root weight (g)	**Shoot weight (g)**
ctrl	25.61a	6.97ab
fum	28.81a	6.97ab
HiMan	31.36a	8.05a
HiMC	31.31a	6.59ab
HiPARC	28.27a	5.54ab
LoMan	31.05a	7.78ab
LoMC	22.91a	7.85ab
LoPARC	25.65a	5.03b

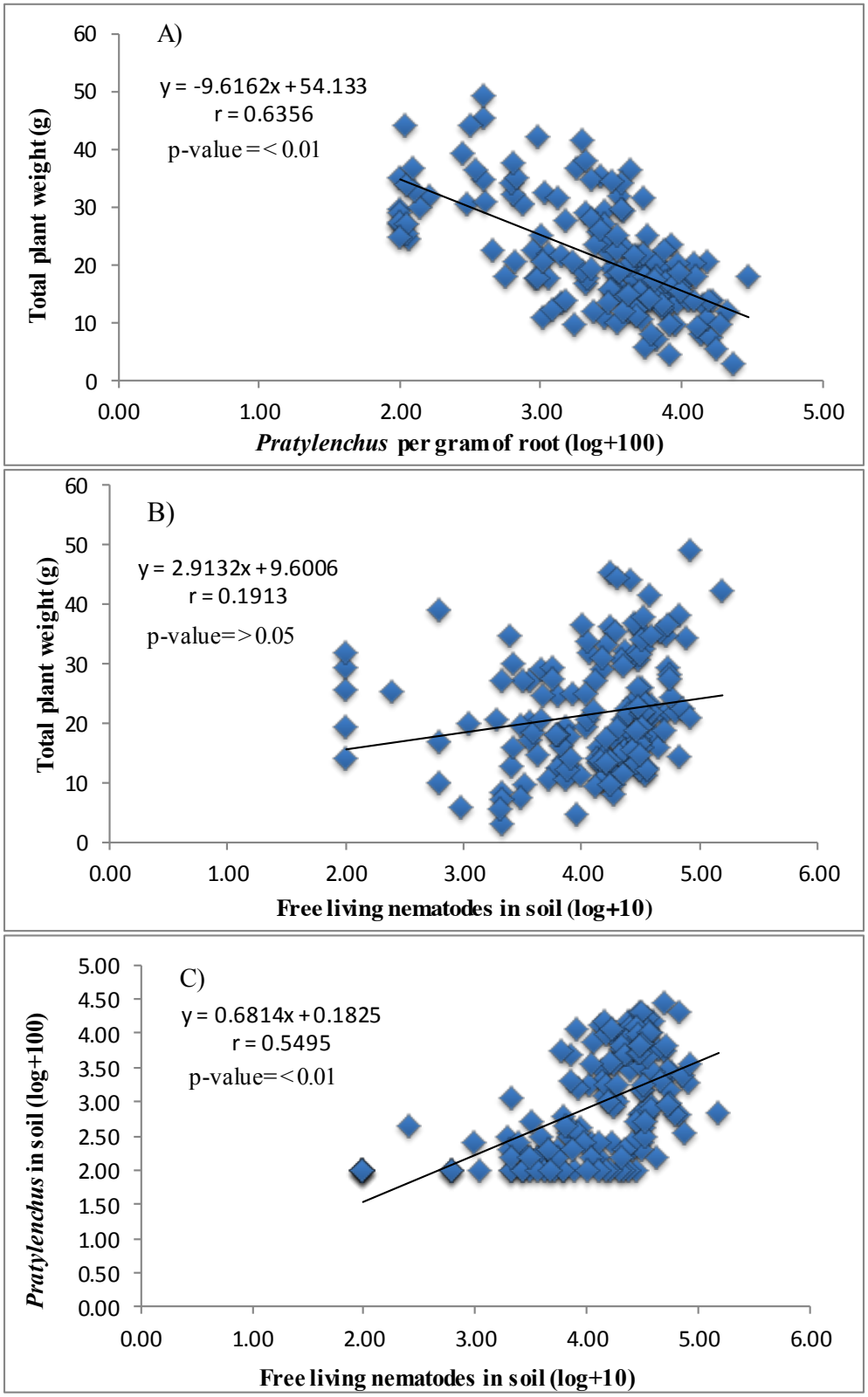

Figure A.18: Experiment 1 correlations between **A)** *P. penetrans*/gram of root and total plant weight (n=159). **B**) Free living nematode abundance and total plant weight (n=159). **C)** Free living nematode abundance and *P. penetrans* (n=159).

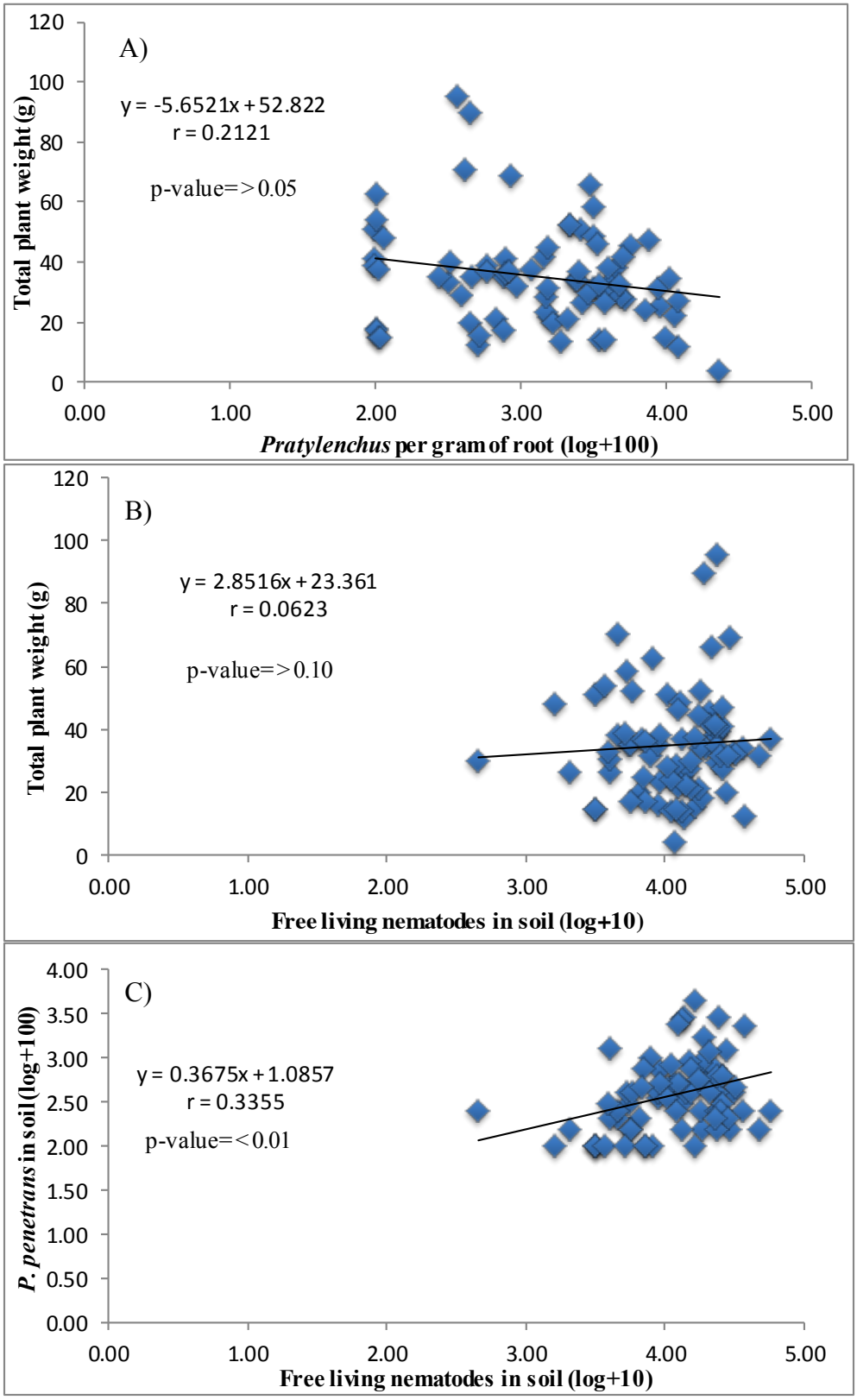

Figure A.19: Experiment 2 correlations between **A)** *P. penetrans*/gram of root and total plant weight (n=79). **B)** Free living nematode abundance and total plant weight (n=79).
C) Free living nematode abundance and *P. penetrans* in soil (n=79).