NON-FUMIGANT ALTERNATIVE SOIL MANAGEMENT PRACTICES FOR MITIGATING REPLANT DISEASE OF FRUIT TREES: MECHANISMS CONTRIBUTING TO *PRATYLENCHUS PENETRANS* SUPPRESSION

by

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Abstract

Replant disease presents a significant barrier to the reestablishment of orchards. In the Okanagan Valley, Canada, the root-lesion nematode, *Pratylenchus penetrans*, is widely distributed and implicated in poor growth of newly planted fruit trees. Restrictions on soil fumigants have generated interest in alternative management strategies for disease control. Using a combination of greenhouse and field experiments, this dissertation evaluated the effects of composts, bark chip mulch, biocontrol inoculation, and two different irrigation systems (drip emitter and microsprinkler) on the establishment of apple and sweet cherry trees in old orchard soil, *P. penetrans* population dynamics, as well as biotic and abiotic factors that may have contributed to enhanced plant growth and nematode suppression. In the first greenhouse experiment, compost amendments, but not biocontrol inoculation, improved growth of apple and sweet cherry seedlings in old orchard soil, suppressed *P. penetrans*, enhanced soil populations of total bacteria, 2,4-diacetylphloroglucinol-producing (DAPG+) bacteria, pyrrolnitrin-producing (PRN+) bacteria, and altered the composition of the soil microbial community. In the field experiment, compost amendment and surface application of bark chip mulch increased trunk diameters of sweet cherry trees planted at an old orchard site as well as suppressed *P. penetrans* populations. Compost enhanced rhizosphere populations of total bacteria, DAPG+ bacteria, and PRN+ bacteria, soil microbial activity, soil biological suppressiveness, and root colonization by arbuscular mycorrhizal fungi (AMF). Bark chip mulch enhanced rhizosphere populations of total fungi, soil microbial activity, and soil biological suppressiveness. Drip irrigation promoted greater trunk diameters and fruit yield, suppressed *P. penetrans* populations, and trees had greater root colonization by

AMF relative to microsprinkler irrigation. In the second greenhouse experiment, compost amendments increased plant growth relative to that of untreated soil in six out of twelve of the orchard soil x compost type combinations evaluated, and suppressed *P. penetrans* in four out of twelve treatment combinations. Inoculation with antagonistic *Pseudomonas* isolates provided minor plant growth promotion and *P. penetrans* suppression in orchard soil. Overall, preplant incorporation of composts and surface application of bark chip mulch, alongside the use of drip irrigation resulted in the best establishment of sweet cherry trees in old orchard soil.

Lay Summary

Replant disease is responsible for the poor growth of fruit trees planted into soil previously used for tree-fruit production, and has been linked to elevated populations of plant-parasitic nematodes in soil, among other possible causes including fungal pathogens. Recent restrictions on use of soil fumigants are increasing the interest in alternative soil management strategies that can provide similar levels of disease control. Using a combination of greenhouse and field experiments, the goal of this research was to evaluate the effects of compost amendments, bark chip mulch, inoculation with potential biocontrol microbes, and choice of low-volume irrigation system (drip emitter versus microsprinkler) on the establishment of fruit trees planted into old orchard soil. This research demonstrated that preplant incorporation of composts and surface application of bark chip mulch, alongside the use of a drip irrigation system provided the best establishment of fruit trees replanted into old orchard soil.

Preface

Chapter 2 is work that was conducted by myself under the guidance of Dr. Tom Forge and Dr. Louise Nelson. Segmented flow-injection analysis of soil mineral N content and combustion analysis of soil organic C and N contents were conducted by Dr. Denise Neilsen's research technician, Shawn Kuchta, at the Summerland Research and Development Centre. Optimization of the real-time polymerase chain reaction (PCR) assay for quantification of total fungi was performed in collaboration with Dr. Louise Nelson's Master of Science student, Paige Munro, at the University of British Columbia - Okanagan Campus. Optimization of the real-time PCR assay for quantification of total bacteria was performed by Dr. Tanja Vogel, at the University of British Columbia - Okanagan Campus. I designed and conducted the greenhouse experiment, collected the data, performed all other analyses, and conducted data analysis and interpretation.

Chapter 3 is work that was conducted by myself, Dr. Tom Forge, Dr. Denise Neilsen, and Dr. Gerry Neilsen. Segmented flow-injection analysis of soil mineral N content, combustion analysis of soil organic C and N contents, and inductively coupled plasma-optical emission spectroscopy of leaf nutrient concentrations were performed by Dr. Gerry Neilsen's research technician, Lana Fukumoto. Set-up of time-domain reflectometry (TDR) equipment and subsequent monitoring of soil volumetric water contents in sub-plots were performed by Dr. Denise Neilsen's research technicians, Shawn Kutcha and Istvan Losso. From 2014 through 2016, nematodes were extracted from soil and root samples by Dr. Tom Forge's research technician, Paul Randall. Experimental design was decided upon by Dr. Tom Forge, Dr. Denise Neilsen, and Dr. Gerry Neilsen prior to my involvement in the project. I collected the samples, performed

all other analyses, as well as conducted data analysis and interpretation.

A portion of Chapters 2 and 3 has been published: Watson, T.T., Nelson, L.M., Neilsen, D., Neilsen, G.H., Forge, T.A. (2017) Organic soil amendments influence *P. penetrans* populations, beneficial rhizosphere microorganisms, and growth of newly planted sweet cherry. *Applied Soil Ecology* 117-118: 212-220. I wrote the manuscript with guidance and input from Dr. Tom Forge, Dr. Louise Nelson, Dr. Denise Neilsen, and Dr. Gerry Neilsen. I independently designed and conducted the greenhouse experiment. For the field experiment, I collected the soil samples, quantified nematodes from samples, quantified rhizosphere/soil microorganisms, and performed plant growth measurements.

A portion of Chapter 3 has been published: Forge, T.A., Neilsen, D., Neilsen, G., Watson, T. (2016) Using compost amendments to enhance soil health and replant establishment of tree-fruit crops. *Acta Horticulturae* 1146: 103-108. I collected the soil samples, quantified nematodes from samples, and performed plant growth measurements.

Chapter 4 is work that was conducted by myself under the guidance of Dr. Tom Forge and Dr. Louise Nelson. I designed and conducted the experiment, collected the data, performed all other analyses, and conducted data analysis and interpretation.

A portion of Chapter 4 has been accepted for publication: Watson, T.T., Nelson, L.M., Neilsen, D, Neilsen, G.H., Forge, T.A. (XXXX) Evaluation of composts as a non-fumigant alternative to promote the establishment of fruit trees at old orchard sites. *Acta Horticulturae* (in press). I wrote the manuscript with guidance and input from Dr. Tom Forge and Dr. Louise Nelson. I independently designed and conducted the greenhouse experiments, as well as performed data analysis and interpretation.

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List of Abbreviations

AG-A	anastomosis group A
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
AWA	alkaline water agar
AWC-2014	agricultural waste compost from 2014
AWC-2014+BCA	agricultural waste compost from 2014 and biocontrol agent
AWC-2017	agricultural waste compost from 2017
BCA	biocontrol agent (*Serratia plymuthica* 6-5)
BCM	bark chip mulch
BLAST	basic local alignment search tool
CEC	cation-exchange capacity
CFU	colony forming units
CI	channel index
CLPP	community-level physiological profile
Comp	compost
Comp+BCM	compost and bark chip mulch
DAPG+	2,4-diacetylphloroglucinol-producing
EI	enrichment index
FAME	fatty acid methyl ester
Fum	fumigation
Fum+BCA	fumigation and biocontrol agent
GmS1	Gould's modified S1

ITS	internal transcribed spacer
MS	microsprinkler
MWC-2017	municipal waste compost from 2017
NH_4-N	ammonium nitrogen
NO_2-N+NO_3-N	nitrite and nitrate nitrogen
NO_3-N	nitrate nitrogen
NPMANOVA	non-parametric multivariate analysis of variance
OM	organic matter
PBS	phosphate buffered saline
PCA	principal components analysis
PCR	polymerase chain reaction
PDA	potato dextrose agar
Pf/Pi	nematode reproductive factor
pi	post-inoculation
PRN+	pyrrolnitrin-producing
PVK	Pikovskaya
SI	structure index
TCSA	trunk cross-sectional area
TDR	time domain reflectometry
TOC	total organic carbon
TSA+PDA	tryptic soy and potato dextrose agar
TSB	tryptic soy broth
TSB+PDB	tryptic soy and potato dextrose broth

YTC-2014	yard trimmings compost from 2014
YTC-2014+BCA	yard trimmings compost from 2014 and biocontrol agent
YTC-2016	yard trimmings compost from 2016
YTC-2017	yard trimmings compost from 2017

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First and foremost, I would like to express my sincere gratitude to my co-supervisors Dr. Tom Forge and Dr. Louise Nelson for their scientific insight, support, and guidance throughout my studies. I would also like to extend my thanks to my supervisory committee members Dr. José Úrbez-Torres and Dr. Melanie Jones for their guidance and constructive feedback on my project.

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1 Chapter 1: Introduction

1.1 Poor Growth of Replanted Fruit Trees

In annual production systems, it is well documented that prolonged monoculture can reduce crop yields through increased activity and accumulation of soil-borne plant pathogens and parasites (Weller et al., 2002). A similar problem exists in perennial production systems, including pome fruit (Ark and Thomas, 1936), and stone fruit (Hoestra and Oostenbrink, 1962), where subsequent planting on an old orchard site often results in poor tree establishment. A variety of terms have been used to refer to this phenomenon, including soil sickness (Cohen and Gur, 1988), soil exhaustion (Kviklys et al., 2008), double stubble (Guo et al., 2014), replant disorder (Browne et al., 2006), and more commonly replant disease (Mai and Abawi, 1981). Varying reports on the capacity of orchard soil previously planted with one particular fruit tree species to cause poor growth of a different species led earlier authors to divide replant disease into two types (Mai and Abawi, 1978). Non-specific replant disease refers to instances where subsequent planting with a different fruit tree species results in poor tree establishment, whereas specific replant disease is restricted to poor growth of the same species (Traquair, 1984). Replant disease presents a significant barrier to establishing productive orchards worldwide, and has been documented in every major fruit-growing region, including regions in North America (Braun, 1991; Jaffee et al., 1982; Mazzola, 1998; Neilsen et al., 1991), Europe (Hoestra, 1968; Manici et al., 2003; Savory, 1966), Israel (Gur et al., 1998), South Africa (Tewoldemedhin et al., 2011b), China (Sun et al., 2014), New Zealand (Fullerton et al., 1999), and Australia (Dullahide et al., 1994; Stirling et al., 1995). As a result of intensification of production in areas specialized in fruit growing,

the occurrence of replant disease is expected to increase (Mazzola and Manici, 2012).

Symptoms of replant disease include stunting of aboveground growth, root discolouration, root tip necrosis, reductions in root biomass, and occasionally death of the tree (Traquair, 1984), and are usually observed a few months after planting (Mazzola and Manici, 2012). Left untreated replant disease can delay fruit production, decrease fruit quality, and reduce fruit yield, preventing an orchard from reaching an acceptable level of productivity (Mazzola, 1998). In an economic analysis conducted on apple (*Malus domestica* (Borkh.)) in South Africa, Rabie et al., (2001) reported that delayed fruit production as a result of replant disease can decrease profitability by as much as 50% throughout the life of an orchard. The majority of research conducted on replant disease has primarily been focused on apple production systems, likely because apple is the most widely grown temperate perennial fruit tree. Stone fruit trees are also susceptible to replant disease (Browne et al., 2006; Yang et al., 2012), and possess a remarkably similar disease complex to that described for apple (Larsen, 1995).

1.2 Disease Etiology

Both biotic and abiotic factors have been associated with replant disease (Covey et al., 1979; Oh and Carlson, 1976). Early studies demonstrated a link between soil biology and replant disease, with numerous reports of significant plant growth enhancement after fumigating (Covey et al., 1979; Mai and Abawi, 1981; Ross et al., 1984) or steam pasteurizing (Hoestra, 1968) old orchard soil. Further support for biological agents was provided using soil re-inoculation experiments, where inoculating steam pasteurized orchard soil with as little as 10% untreated old orchard soil resulted in significant reductions in plant growth relative to plants grown in 100% steam pasteurized

orchard soil (Hoestra, 1968). Additional studies in New York using semi-selective biocides showed that replant disease appears to be linked to a disease complex primarily composed of nematodes and fungi (Jaffee et al., 1982; Mai and Abawi, 1978). The exact consortium of biological agents contributing to replant disease has shown considerable variability among geographic regions, among orchards within the same region (Manici et al., 2013; Mazzola, 1998; Yang et al., 2012), and likely also displays spatial variability within an individual orchard. In Central Europe, Manici et al. (2013) reported that nematodes did not contribute significantly to replant disease on apple, and that fungi were the predominant cause of replant disease in the orchards surveyed. Other growing regions have reported that plant-parasitic nematodes as well as plant-pathogenic fungi both contribute significantly to replant disease complex (Dullahide et al., 1994; Hoestra and Oostenbrink, 1962; Mai and Abawi, 1981; Traquair, 1984; Utkhede et al., 1992).

1.2.1 Plant-Parasitic Nematodes

In most tree-fruit growing regions, plant-parasitic nematodes have a widespread distribution and contribute significantly to replant disease complex of pome and stone fruit trees (Dullahide et al., 1994; Hoestra and Oostenbrink, 1962; Mai and Abawi, 1981; Traquair, 1984; Vrain and Yorston, 1987). Species from several nematode genera can cause root damage and yield reductions on fruit trees when present at high populations, including root-lesion nematodes (*Pratylenchus* (Filipjev)), dagger nematodes (*Xiphinema* (Cobb)), ring nematodes (*Mesocriconema* (Andrassy)), and root-knot nematodes (*Meloidogyne* (Göldi)) (Mai and Abawi, 1981). The plant-parasitic nematode most frequently associated with replant disease complex is *P. penetrans* (Chitwood & Oleifa); however, other *Pratylenchus* spp., such as *P. vulnus* (Allen & Jensen), *P. brachyurus*

(Filipjev & Schuurmans Stekhoven), *P. jordanensis* (Hashim, Zeid), and *P. coffeae* (Goodey) can be important parasites of fruit trees in other growing regions (Dullahide et al., 1994; Jones and Aldwinckle, 1991; Ogawa et al., 1995; Pinochet et al., 1993b; Stirling et al., 1995). In tree fruit growing regions in the Pacific Northwest of North America, *P. neglectus* (Filipjev & Schuurmans Stekhoven) and *P. crenatus* (Loof) are also widespread (Nyczepir, 1991; Sheedy et al., 2008), but are not thought to be problems on fruit trees. Without adequate species-level resolution during identification of nematodes from samples the presence of *P. neglectus* and *P. crenatus* in orchard soil can obscure understanding the relationships between plant-parasitic nematode population densities and plant growth, highlighting why it is critical to determine the species of *Pratylenchus* when quantifying populations in orchards. High populations of *X. americanum* (Cobb) have been associated with poor root growth of pome and stone fruit trees; however, the major importance of this nematode is as vector of plant viruses, including tomato ringspot virus (Jones and Aldwinckle, 1991; Ogawa et al., 1995). On *Prunus* spp., root feeding by *M. xenoplax* (Loof & De Grisse) causes destruction of feeder roots, and plays a role as a predisposing agent to bacterial canker (Lownsbery et al., 1973; Melakeberhan et al., 1994; Ogawa et al., 1995). In warmer growing regions, *M. incognita* (Chitwood), *M. javanica* (Chitwood), and *M. arenaria* (Chitwood) have been associated with reduced growth and yield of *Prunus* spp. (L.) (Ogawa et al., 1995).

Populations of *P. penetrans* are influenced by a variety of abiotic soil characteristics, including soil texture, moisture content, and temperature. One of the most important soil properties influencing the distribution of *Pratylenchus* spp. is soil texture, with *P. penetrans* reported more frequently in sandy soils (Florini et al., 1987; Jordaan et

al., 1989; Wallace, 1973). Another important factor governing *P. penetrans* populations in soil is moisture content (Kable and Mai, 1968). Population densities of *P. penetrans* have been reported to increase in drier soil conditions relative to overly wet soil (Kable and Mai, 1968; Norton and Burns, 1971). Increased metabolic stress on the nematode as a result of more drastic differences in osmotic potential between the nematode and wet soil (Townshend, 1973; Wallace, 1973), as well as variability in oxygen availability (van den Bergh et al., 2006), have been proposed to contribute to differential root-lesion nematode population development in varying soil moisture conditions. Soil temperature also has a strong influence on *P. penetrans* population abundance in soil, and contributes significantly to seasonal fluctuations in nematode populations in temperate growing regions (Kimpinski and Dunn, 1985; MacGuidwin and Forge, 1991).

Pratylenchus spp. are obligate migratory plant endoparasites that feed on a wide range of plant hosts, which may explain why these nematodes have been associated with non-specific replant disease (Mai and Abawi, 1981). Invasion of susceptible plant roots by *Pratylenchus* involves mechanical forces from thrusting of the needle-like stylet; the secretion of cell-wall degrading enzymes through the stylet has also been proposed (Morgan and McAllan, 1962). Once inside the roots, the nematode feeds on the cytoplasmic contents of infected plant cells as it travels within the root cortex, resulting in extensive destruction of cortical tissues (Sijmons et al., 1994; Townshend et al., 1989). At any given time, a portion of the *P. penetrans* population may be in the roots and in the soil, highlighting why it is crucial to sample both root tissue and soil when quantifying populations of migratory endoparasitic nematodes. At high enough populations, *P. penetrans* can result in significant growth reduction of fruit trees (Dullahide et al., 1994);

however, during an orchard renovation event young plants are rarely ever subject to the influence of only one soil-borne pathogen or pest.

1.2.2 Plant-Pathogenic Fungi and Oomycetes

A diverse array of fungi and oomycetes has been associated with replant disease of fruit trees across growing regions worldwide. The genera most commonly associated with replant disease include "*Cylindrocarpon*" (Wollenweber), *Rhizoctonia* (DeCandolle), *Phytophthora* (de Bary), and *Pythium* (Pringsh) (Braun, 1991; Jaffee et al., 1982; Manici et al., 2013; Mazzola, 1998; Tewoldemedhin et al., 2011a, 2011c; Traquair, 1984). *Fusarium* species have also been frequently recovered from roots of fruit trees exhibiting replant disease symptoms; however, many subsequent pathogenicity trials using such isolates have failed to demonstrate significant plant growth reduction on apple (Kelderer et al., 2012; Manici et al., 2003; Mazzola, 1998; Tewoldemedhin et al., 2011a). Nevertheless, *F. oxysporum* (Schltdl.) was recently reported to have a role in root rot of sweet cherry (*P. avium* (L.)) (Úrbez-Torres et al., 2016), and *F. tricinctum* (Corda) has been reported to decrease apple seedling biomass (Dullahide et al., 1994). Discrepancies among studies on the effects of *Fusarium* species on fruit tree growth may be attributed to fungi from this genus having highly variable effects on plant growth (Manici et al., 2017), ranging from beneficial to detrimental depending on genotypic differences among species and isolates, and the prevailing environmental conditions (Lewis, 1985).

The majority of fungi implicated in replant disease of fruit trees are non-obligate plant pathogens that can live in soil as saprophytes (Sun et al., 2017), potentially explaining why replant disease can persist in orchards through extended fallow periods (Mazzola and Mullinix, 2005). Soil fungi that facultatively parasitize plant roots

generally secrete enzymes and/or toxins that kill host cells, and then utilize the cellular contents of the deceased plant cells (Agrios, 1997). Various *Fusarium* species have been demonstrated to produce broad-spectrum mycotoxins capable of inducing root necrosis and reducing root growth, including equisetin, enniatin B, enniatin, D, and fusaric acid, and these compounds have been suggested to contribute to non-specific replant disease of fruit trees (Manici et al., 2017). Similarly, "*Cylindrocarpon*" have been documented to release lytic enzymes into the rhizosphere, which aid in nutrient acquisition from the plant host (Henriksson et al., 1997) as opposed to nutrient acquisition through extensive root penetration (Evans et al., 1967); this may also contribute to the non-specificity of many replant disease problems. In a comprehensive study of gamma ray-sterilized fungal culture filtrates from various species of fungi associated with the rhizosphere of apple trees, filtrates from *Fusarium* isolates had variable effects on plant growth, ranging from severe growth inhibition to modest growth promotion (Manici et al., 2017). Furthermore, reduced plant growth was observed regardless of root infection by *Fusarium* isolates, and the authors attributed this phenomenon to the production of various mycotoxins in the rhizosphere (Manici et al., 2017). Conversely, the modest phytotoxic effect of *Rhizoctonia* anastomosis group A (AG-A) culture filtrates suggests that this group of replant disease-associated fungi may primarily interact with plant roots through tissue colonization (Manici et al., 2017, 2015a; Tewoldemedhin et al., 2011a; Weinhold and Sinclair, 1996). Although oomycetes do not appear to contribute to poor growth of sweet cherry in old orchard soil in the Okanagan Valley, Canada (O'Gorman et al., 2016), *Pythium* and *Phytophthora* may contribute to replant disease in other growing regions (Dullahide et al., 1994; Manici et al., 2013; Mazzola, 1998; Tewoldemedhin et al.,

2011b).

1.2.3 Interaction Between Parasitic Nematodes and Pathogenic Fungi/Oomycetes

Synergistic interactions between plant-parasitic nematodes and fungal/oomycete pathogens are thought to contribute to the full impact of replant disease complexes. In the context of replant disease, synergistic interactions refer to instances where the co-presence of the nematode and fungus result in plant damage greater than the sum of the damage caused by each organism alone (Back et al., 2002). *Pratylenchus* spp. have commonly been reported to form disease complexes with fungal and oomycete pathogens, particularly *Rhizoctonia*, *Fusarium*, *Pythium*, and *Phytophthora* (Back et al., 2002). A variety of mechanisms have been proposed to account for why synergistic interactions occur between *P. penetrans* and plant-pathogenic fungi and oomycetes. A common mechanism proposed for the production of disease complexes is that nematode feeding sites predispose roots to infection by producing wounds that are easily colonized by fungal pathogens (Inagaki and Powell, 1969). Nematode feeding has also been suggested to increase the release of root exudates into the soil as well as alter their chemical profile, thereby making the exudates more favourable to fungal pathogens (Bergeson, 1972). Conversely, fungal pathogens have also been shown to increase root infestation by plant-parasitic nematodes. On alfalfa, infection with *F. oxysporum* was demonstrated to elevate CO_2 levels in roots, making them more attractive to *P. penetrans* (Edmunds and Mai, 1966). The most well documented example of synergism between soil-borne fungi and *P. penetrans* is on potato with the fungal pathogen *Verticillium dahliae* (Kleb.) (Rowe and Powelson, 2002), where in the absence of *V. dahliae*, *P. penetrans* does not reduce yield or growth of potato. Compelling evidence for synergistic

interactions between *P. penetrans* and pathogenic fungi on fruit trees is currently lacking. Dullahide et al., (1994) reported that *P. penetrans*, *P. jordanesis*, *F. tricinctum* and *C. destructans* (Zinssm.) could each, individually, reduce weight of apple in a greenhouse pathogenicity bioassay, but there was no evidence of interaction effects. Considering the worldwide co-occurrence of *P. penetrans* with fungi/oomycete pathogens on fruit trees, synergistic interactions seem plausible.

1.2.4 Bacteria

Mazzola (1998) demonstrated that bacteria did not contribute to poor growth of apple in Washington state, USA, through experiments utilizing soil application of chloramphenicol, which reduced bacterial populations but did not subsequently improve plant growth. Similarly, Dullahide et al., (1994) demonstrated that bacteria isolated from the roots of apple grown in old orchard soil were not pathogenic to apple when re-inoculated onto roots in a greenhouse pathogenicity assay. Nevertheless, recent studies have correlated changes in bacterial community structure with replant disease (Caputo et al., 2015; Franke-Whittle et al., 2015; Manici et al., 2015b; Nicola et al., 2017; Peruzzi et al., 2017; Sun et al., 2014). In one study, increases in the proportion of *Pseudomonas* spp. in the rhizosphere were associated with enhanced tree growth after gamma irradiation, and the authors suggested that these bacteria might contribute to pathogen suppression in old apple orchards (Caputo et al., 2015). At a newly planted orchard site that had not previously been used for tree fruit production, young apple trees displayed a neutral/negative growth response to soil pasteurization, increased rhizosphere colonization by *Burkholderia cepacia*, as well as enhanced overall fungistatic activity in the rhizosphere pseudomonad community (Gu and Mazzola, 2003; Mazzola, 1999).

Taken together, these studies suggest bacterial communities may contribute indirectly to replant disease complexes, potentially through their regulatory role on populations of soil-borne pathogens and pests (Nicola et al., 2017).

1.2.5 Abiotic Factors

Although replant disease is thought to be primarily a biological phenomenon, abiotic factors, such as unbalanced soil nutrition, low or high soil pH, poor soil structure and drainage, cold or drought stress, accumulation of plant chemical residues, and accumulation of pesticide residues likely contribute to variation in disease severity (Braun et al., 2010; Redman et al., 2001; Traquair, 1984). The abiotic factor most often implicated in poor establishment of fruit trees in old orchard soil is phosphorus (P) (Neilsen et al., 1994; Neilsen and Yorston, 1991; Slykhuis and Li, 1985; Wilson et al., 2004). Fertilization with P, particularly monoammonium phosphate, has been shown to improve the growth of newly planted apple (Slykhuis and Li, 1985; Wilson et al., 2004), potentially as a result of enhanced plant nutrition (Neilsen et al., 1994; Slykhuis and Li, 1985) or beneficial alterations in soil biology (Wilson et al., 2004). Similarly, increased root colonization by AMF has also been associated with improved establishment of fruit trees (Caruso et al., 1989; Čatská, 1994; Forge et al., 2001; Pinochet et al., 1993a; Ridgway et al., 2008; Utkhede et al., 1992). Forge et al., (2001) demonstrated that preplant inoculation of apple rootstock with *Rhizophagus irregularis* (Walker & Schubler) or *Glomus mosseae* (Gerd & Trappe) improved plant growth and leaf concentrations of P, Mg, Zn, and Cu, suggesting enhanced AMF colonization may have contributed to improved growth of replanted apple by enhancing plant nutrition. In addition to suppressing soil-borne pathogens and pests, enhancing soil P nutrition should

be one of the principal aims of an integrated replant management approach for fruit trees.

Accumulation of toxic compounds in the root zone of mature fruit trees has also been suggested to inhibit early growth of subsequently planted fruit trees (Nicola et al., 2016; Traquair, 1984); however, given that replant disease can persist in fallowed soil for a number of years (Savory, 1966), any such toxin would have to be very stable and resistant to degradation (Mazzola and Manici, 2012). Nicola et al. (2016) recently demonstrated that autotoxic phenolic compounds released into the soil by apple roots (particularly phlorizin and phloretin) can reduce root growth of newly planted apple seedlings; however, the deleterious effects of these compounds on plant growth were transient (<3 months), likely as a result of rapid degradation in the soil environment. Similarly, on *Prunus*, wounding of roots by nematode feeding can lead to hydrolysis of amygdalin, thereby releasing phytotoxic by-products into the soil (Tagliavini and Marangoni, 1992); this has also been proposed to contribute to poor tree establishment. Moreover, build-up of pesticide residues from application of lead arsenate insecticides has been implicated in poor establishment of fruit trees, albeit in the background of biological factors (Benson et al., 1978).

1.2.6 The Underlying Soil Health Problem

Although biological agents, and the abiotic factors that exacerbate their effect, are the direct cause of replant disease, the underlying problem orchardists face is poor soil ecosystem health (Forge et al., 2016b). Soil fumigants can provide temporary relief of the array of soil-borne pathogens and pests associated with replant disease; however, they do not address the intrinsic soil properties that contribute to the establishment and proliferation of these pathogens in orchard soil in the first place. These properties include

diminished soil OM content and depleted C pools (Kennedy, 1999; Mazzola and Manici, 2012). Future management approaches should be directed towards establishing production practices that manage replant disease through the establishment and maintenance of healthy soil ecosystem functioning (Brown and Tworkoski, 2006), a component of which includes natural suppression of soil-borne pathogens and pests.

Conventional agricultural production systems are often associated with depleted soil C pools (Bailey and Lazarovits, 2003; Wang et al., 2011) as well as significant plant disease and other pest problems (Hoitink and Boehm, 1999; Pimentel et al., 1991). Increases in soil C content have been documented to enhance soil fertility, reduce erosion and nutrient runoff, improve water quality, as well as reduce root disease incidence and severity (Davey, 1996; Drinkwater et al., 1995; Kurkalova et al., 2003; Lubowski et al., 2005). In natural ecosystems, increased soil OM content has been linked with natural suppression of soil-borne pathogens, including *Phytophthora* root rot of eucalyptus in Australia (Nesbitt et al., 1979), and *Pythium* root rot in forested soil in the Brazilian Amazon (Lourd and Bouhot, 1987). Organic agricultural production systems are often associated with increases in stable C pools in soil (Lal, 2004; Paustian et al., 2000; Wang et al., 2011), and it is thought that this drives biological suppression of root diseases through increases in microbial abundance, diversity, and activity (Drinkwater et al., 1995). For example, at an avocado orchard in Australia several years of OM applications suppressed *Phytophthora* root rot, and disease suppression was associated with increased soil OM content and enhanced microbial activity (Malajczuk, 1983, 1979). Similarly, reductions in the recovery of pathogenic *Pythium* and *Rhizoctonia* spp. have been reported in organic apple production systems relative to that of conventional

production systems (Manici et al., 2003); however, soil C and OM contents were not monitored.

1.3 Managing Poor Growth of Replanted Fruit Trees

1.3.1 Chemical Approaches

Historically, conventional growers have used preplant soil fumigants to reduce the effect of replant disease complexes on fruit tree establishment in old orchard sites. Many of the most effective soil fumigants have a broad spectrum of activity on soil organisms, including nematodes, fungi, bacteria, insects, and even plants. For example, the active chemical in the granular soil fumigant Basamid® (Dazomet) rapidly decomposes in soil into the volatile compound methylisothiocyanate, which acts as a non-selective inhibitor of enzymes (Wright, 1981). Although highly effective at controlling the diverse range of organisms that have been linked to replant disease, use of broad-spectrum soil fumigants has many inherent drawbacks. In some studies, application of soil fumigants has been associated with increased subsequent infestation with pests, including *P. penetrans* (Mazzola and Manici, 2012), likely as a result of elimination of natural antagonists of parasitic nematodes (Munnecke, 1984). More targeted non-fumigant chemical approaches have been attempted, such as application of nematicides (e.g. fenamiphos) and fungicides (e.g. metalaxyl) (Dullahide et al., 1994; Mazzola et al., 2002; Santo and Wilson, 1990); however, success has often been limited, likely due to failure to control the entire range of organisms contributing to the replant disease complex. The nematicide Vydate® (Oxamyl) is registered for use on non-bearing apple, and until recently, fenamiphos was registered for use on fruit trees in USA. Broad-spectrum soil fumigants as well as non-fumigant pesticides are difficult to apply, have a

high economic cost, and are detrimental to environmental and human health. Recent restrictions limiting access to soil fumigants are increasing interest in non-chemical strategies to manage replant disease of fruit trees.

1.3.2 Land Management

A simplistic yet unsustainable alternative to the use of chemical pesticides for control of replant disease is to replace soil at old orchard sites with soil that has not previously been cultivated with fruit trees (Mai and Abawi, 1981). Wilson et al., (2004) demonstrated that replacing soil in the planting hole with fresh soil resulted in a near two-fold increase in shoot extension and trunk-cross sectional area of apple after two growing seasons. In contrast to soil replacement, use of an extended fallow period before planting a subsequent orchard has been explored as a means to reduce disease pressure in soil. Manici et al. (2013) demonstrated that fallow periods decreased "*Cylindrocarpon*" recovery from roots of subsequently planted apple; however, replant disease severity was not decreased by fallow to the same extent as soil sterilization. Conversely, Mazzola and Mullinix, (2005) reported that a three-year fallow period did not reduce replant disease severity on apple in Washington state, USA. Fallow periods may have a positive effect on subsequent plant growth, but this agricultural practice is not economically viable for fruit growers as a result of the land being out of production for a number of years. A more promising land management approach that has been explored is replanting new trees into the previous orchard drive-row, in an attempt to take advantage of the lower pathogen density in this soil relative to the adjacent soil in the previous tree row (Rumberger et al., 2004; St. Laurent et al., 2008). Kelderer et al. (2012) reported that planting apple trees in the drive-row significantly increased tree growth relative to planting in the previous tree

row; however, the fungal pathogens linked to replant disease were still endemic to soil in the drive-row, suggesting the potential for rapid colonization of tree roots by the pathogen populations. Another major limitation to planting in the drive-row lies in the significant capital invested in irrigation and trellis systems required to support high-density dwarfing fruit tree varieties, requiring most new trees being replanted into the exact same position as the previous orchard, in order to accommodate these structures.

1.3.3 Nutrient Management

Inadequate soil P availability is the abiotic factor most often linked with poor growth of fruit trees at old orchard sites. Numerous studies from various growing regions have demonstrated the benefits of P fertilization on apple establishment in old orchard sites (Moran and Schupp, 2003; Neilsen et al., 1994, 1990; Slykhuis and Li, 1985; Wilson et al., 2004); however, effects on root pathogens were not monitored in these studies. Soil P availability is important to fruit tree establishment, but fertilizer application and plant nutrient uptake must be synchronized in order to prevent environmental contamination from P leaching (Djodjic et al., 2004). Alternatively, Sewell et al., (1988) showed that the effects of poor soil P availability could be offset by increased root colonization by AMF, which presumably enabled sufficient plant access to soil P even when little was directly accessible to apple roots.

1.3.4 Disease-Tolerant Rootstocks

Growth of fruit trees in old orchard soil is significantly influenced by rootstock genotype (Isutsa and Merwin, 2000; Mazzola et al., 2009), presumably as a result of differential host genetic resistance to parasitism (Alcañiz et al., 1996; Sewell and Wilson, 1959; Westcott and Zehr, 1991). Recent studies indicate that host influences on microbial

communities in the rhizosphere, specifically the capacity to select for microflora that suppress root pathogens (Mazzola et al., 2009; St. Laurent et al., 2010), may also be related to differential susceptibility of rootstocks. Most of the commonly used dwarfing apple rootstocks in orchards in British Columbia, Canada (Malling-9 and Malling-26) are susceptible to replant disease (Auvil et al., 2011). Apple rootstocks with tolerance to replant disease do exist (Geneva-16, Geneva-41, Geneva-210) (Robinson et al., 2011); however, rootstocks with such tolerance are not yet widely available, or have not been demonstrated for many other fruit trees species, including sweet cherry.

1.3.5 Cover Crops

Some plants have been documented to suppress replant-associated fungi and nematodes when grown as preplant cover crops at orchard sites. Cover cropping with marigolds (*Tagetes* spp. (L.)) has previously been shown to reduce *P. penetrans* populations and improve subsequent growth of apple seedlings in old orchard (Merwin and Stiles, 1989), likely as a result of the production in roots, and exudation, of the nematicidal compound α-terthienyl (Bakker et al., 1979), which can be fatal to root feeding nematodes. Moreover, preplant cover cropping with certain wheat cultivars has been shown to improve subsequent growth of apple seedlings planted into old orchard soil, with reduced infection of roots by fungi and *P. penetrans* linked with shifts in the genetic and functional composition of the pseudomonad community in the apple rhizosphere (Gu and Mazzola, 2003; Mazzola, 2002). Cover crops have not received widespread acceptance as alternatives to soil fumigants as a result of the high cost of marigold seeds, and because the orchard site often must be out of production for a growing season prior to replanting.

1.3.6 Biofumigation

Many plants in the Brassicaceae contain high levels of glucosinolates, which rapidly hydrolyze into volatile isothiocyanates when incorporated into soil (Hanschen et al., 2015). Additionally, some *Sorghum* spp. (Moench) contain high levels of dhurrin, which hydrolyze into hydrogen cyanide when incorporated into soil (Widmer and Abawi, 2000). Use of such plants as green manures and seed meal amendments are two ways in which the allelopathic properties of these plants can be exploited to suppress the deleterious organisms associated with replant disease, a practice known as biofumigation. At a replant site in Washington state, USA, green manure incorporation of *Brassica napus* (L.) enhanced apple seedling growth and reduced root infection by *Rhizoctonia* relative to untreated soil (Mazzola and Mullinix, 2005). Similarly, fall incorporation of *S. vulgare* (Pers.) green manure reduced *M. xenoplax* populations at the time of planting in a peach (*P. persica* (L.) Batsch) replant site in Georgia, USA (Nyczepir and Rodriguez-Kabana, 2007). Drawbacks to the use of green manures are similar to those experienced with fallow periods and cover crops, often requiring orchards to be out of production for a growing season prior to subsequent establishment. Additionally, *Brassica* spp. and *Sorghum* spp. are hosts for many *Pratylenchus* spp., and if they are not used effectively as biofumigants they may subsequently result in increases in parasitic nematode populations in soil.

In an attempt to circumvent some of the limitations of green manures, some studies have explored amending soil with *Brassica* seed meal, a by-product of biofuel production which also contains high levels of glucosinolates. In Washington state, USA, amending soil with *Brassica* seed meal suppressed *P. penetrans* populations and

increased apple growth over three growing seasons relative to untreated and fumigated soil (Mazzola et al., 2015). Many other similar studies in the region have also demonstrated significant *P. penetrans* suppression and plant growth promotion through the use of *Brassica* seed meals (Mazzola et al., 2009, 2007; Mazzola and Mullinix, 2005). Interestingly, the suppressive effects of *Brassica* seed meals were not found to correlate with glucosinolate content levels; rather, increases in microbial antagonists have been proposed as a concurrent mechanism in disease control (Mazzola et al., 2006). Amending soil with *Brassica* seed meal has been associated with increases in populations of nematophagous fungi (Yulianti et al., 2007) and *Streptomyces* spp. (Mazzola et al., 2006), both of which have antagonistic activity to soil-borne pathogens and pests. Despite numerous reports of successful replant disease suppression as a result of the use of *Brassica* seed meals, studies have demonstrated that these amendments are not as effective as soil fumigants in enhancing fruit tree establishment (Mazzola and Brown, 2010). The high cost of *Brassica* seed meal amendments is another major limitation to widespread adoption of biofumigation as a replant disease management strategy (Forge et al., 2016b).

1.3.7 Biocontrol

Biocontrol refers to reductions in pathogen or parasite populations through the actions of antagonistic organisms (Stirling, 2014). Antagonists can be parasites, predators, pathogens, competitors, or any other organism that repels, inhibits, or kills another organism (Sikora, 1992). A diverse array of organisms has been documented to be antagonistic to plant-parasitic nematodes and pathogenic fungi, with numerous literature reviews dedicated to this specific topic (Dong and Zhang, 2006; Haas and

Défago, 2005; Heydari and Pessarakli, 2010; Jatala, 1986; Kerry, 2000; Khan and Kim, 2007; Siddiqui and Mahmood, 1997; Tian et al., 2007; Weller, 1988). With such a diverse range of organisms associated with antagonism, it comes as no surprise that a variety of mechanisms associated with pathogen suppression has been discovered, ranging from direct parasitism of nematodes and fungi, to indirect suppression as a result of increased competition for nutrients and space. It may be possible to harness the suppressive activities of antagonists to control replant disease-associated fungi and nematodes, either through direct inoculation or enhancement of indigenous populations in soil.

1.3.7.1 Antagonists of Parasitic Nematodes and Pathogenic Fungi/Oomycetes

Suppressive soils are characterized by minimal disease development despite the presence of a susceptible host and virulent pathogen, and are primarily attributed to the activity of soil antagonists (Mazzola, 2002). Some groups of bacteria can directly parasitize plant-parasitic nematodes, including the root-lesion nematode (Bird et al., 2003). Some members of the genus *Pasteuria* are endospore-forming bacteria that live as obligate endoparasites in the bodies of nematodes (Starr and Sayre, 1988). Spores of *Pasteuria* germinate upon attaching to the nematode cuticle, and then penetrate into the body and proliferate, eventually resulting in degradation of the reproductive capacity of female nematodes (Sayre and Wergin, 1977). The high tolerance of *Pasteuria* endospores to environmental stresses has made these bacteria intriguing candidates for microbial inoculant formulation.

Most other bacteria that have been reported to be detrimental to plant parasitic nematodes and pathogenic fungi act by producing biocidal metabolic by-products,

enzymes, toxins, or antibiotics (Haas and Défago, 2005; Tian et al., 2007; Weller, 1988); competing for limiting nutrients and space (Oostendorp and Sikora, 1990); or by inducing plant host systemic resistance (Duijff et al., 1997). Bacteria that colonize the rhizosphere (rhizobacteria) are prime candidates for biocontrol because they colonize the interface between roots and soil through which invading pathogens and pests must pass. Rhizobacteria constitute one of the most taxonomically diverse and functionally active groups of microorganisms in the soil, with a significant proportion having antagonistic activity to nematodes and fungi (Oostendorp and Sikora, 1989; Spiegel et al., 1991). The genera of rhizobacteria most consistently associated with biocontrol activity to fungi and nematodes include *Enterobacter* (Hadar et al., 1983; Kwok et al., 1987; Nair and Fahy, 1972), *Serratia* (Sneh et al., 1984), *Pseudomonas* (Burr et al., 1978; Kloepper et al., 1980; Kwok et al., 1987; Stutz et al., 1986), and *Bacillus* (Campbell and Capper, 1986; Kwok et al., 1987; Utkhede et al., 2001). *Pseudomonas* species have received particular research focus for their role in soil suppressiveness (Siddiqui et al., 2005; Spiegel et al., 1991). *Pseudomonas* species have been documented to produce a variety of antagonistic secondary metabolites that are detrimental to fungi and nematodes, including phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and hydrogen cyanide (Haas and Défago, 2005). Increases in populations of antibiotic-producing pseudomonad populations have been linked with soil suppressiveness in a number of pathosystems (de Souza et al., 2003; Garbeva et al., 2004a; Latz et al., 2012) including replant disease of fruit trees (Mazzola, 1999). Many of these bacteria are relatively easy to culture, making them ideal candidates for microbial inoculant development.

A diverse range of fungi has been shown to parasitize plant-parasitic nematodes and nematode eggs, and they may play a significant role in naturally regulating nematode populations in soil. The most extensively studied group of microbial antagonists of plant-parasitic nematodes are nematode-trapping fungi (Zopf, 1888). Nematode-trapping fungi use a variety of structures to trap nematodes, ranging from simple adhesives on the surface of fungal hyphae to elaborate constricting rings and snares (Barron, 1977). Nematode-trapping fungi are ubiquitous in soil (Gray, 1987) and tend to be associated with decomposition of high C organic material (Cooke, 1962). Common genera of nematode-trapping fungi include species from the genera *Arthrobotrys* (Corda, Pracht) and *Monacrosporium* (Oudem.) (Eren and Pramer, 1965; Gaspard and Mankau, 1986). Conversely, some groups of fungi persist within nematodes as obligate parasites, completing the majority of their lifecycle within the body of the nematode host, including fungi from the genera *Catenaria* (Sorokin) (Sayre and Keeley, 1969), *Lagenidium* (Schenk), and *Aphanomyces* (de Bary) (Jaffee and Schaffer, 1987). Opportunistic saprophytic fungi have also been documented to deleteriously colonize nematode eggs, including *Paecilomyces* (Bainier) (Jatala, 1986), *Verticillium* (Nees) (Morgan-Jones et al., 1983), and even "*Cylindrocarpon*" (Rodriguez-Kabana and Morgan-Jones, 1988).

Increased root colonization by AMF is associated with numerous benefits to plant hosts, including increased plant nutrition (Miller, 2000), drought tolerance (Ruiz-Lozano et al., 1995), and even pathogen tolerance and/or suppression (Azcón-Aguilar and Barea, 1997; Hussey and Roncadori, 1982). The root systems of fruit trees often are colonized by mycorrhizal fungi (Bâ et al., 2000; Purin et al., 2006; Wu et al., 2011), and these fungi likely interact, to some degree, with *P. penetrans* and pathogenic fungi during

root invasion. Mechanisms of disease suppression by AMF are still largely speculative; however, a variety have been proposed, including increased plant host nutrient status, microbial competition for nutrients and root penetration sites, activation of plant host defense mechanisms, as well as alterations to root exudates making them less favourable to invading pathogens (Azcón-Aguilar et al., 2002; Hussey and Roncadori, 1982). Several studies have reported that AMF can increase host tolerance to *Pratylenchus* root infestation on a variety of fruit tree species, including pear (Lopez et al., 1997), peach (Pinochet et al., 1996), and apple (Pinochet et al., 1993b). Increased root colonization by AMF has also been reported to directly reduce *Pratylenchus* populations on apple (Forge et al., 2001) and cherry (Pinochet et al., 1995).

Other extensively studied groups of antagonists of nematodes and fungi are predacious and fungivorous free-living nematodes, respectively (Jairajpuri and Bilgrami, 1990; Jones, 1974; Small, 1988). Interest in the biocontrol capacity of these groups of nematodes has existed since their initial discovery (Cobb, 1920; Thorne, 1924). Predacious nematodes are found in four major taxonomic groups including Monochids, Dorylaimids, Aphelenchids, and Diplogasterids (Stirling, 2014). These nematodes are believed to play an important role in the natural control of nematode populations in soil as a result of non-specific feeding on prey (Bae and Knudsen, 2001; Ishibashi and Choi, 1991; Khan and Kim, 2007). A number of other soil invertebrates have been reported to prey on nematodes and fungi, and they may also display some degree of biocontrol capacity in soil, including mites, Collembola, protozoa, turbellarians, tardigrades, and oligochaetes (Doncaster and Hooper, 1961; Hutchinson and Streu, 1960; Inserra and Davis, 1983; Murphy and Doncaster, 1957; Sayre and Powers, 1966). As a result of lack

of comprehensive knowledge of the efficacy of these organisms as biocontrol agents, as well as difficulties in mass-production, commercialization, and release, their potential for disease control still largely remains to be demonstrated.

1.3.7.2 Development of Microbial Inoculants

The use of microbial inoculants in an attempt to introduce specific beneficial microorganisms into the soil to increase crop growth has occurred in agricultural practices for well over 100 years (Nobbe et al., 1891; Nobbe and Hiltner, 1893). Biopesticides are introduced microorganisms used for pest control that reduce the impact of pathogens/pests by harnessing the suppressive activities of natural antagonists (Chandler et al., 2008). Biofertilizers are microorganisms that, when applied to plants and/or soil, increase plant access and uptake of nutrients, including but not limited to N and P (Sheraz Mahdi et al., 2010). Although there are many advantages to using microbial inoculants over soil fumigants and fertilizers, such as a high efficacy at low quantities, the ability to multiply in the environment, and improved environmental and personal safety (Berg, 2009), drawbacks to their use include short-shelf life as well as variable success in field trials (Weller, 1988). Improved efficacy of microbial inoculants can potentially be achieved through simultaneous incorporation of OM into soil (Bonkowski et al., 2009; Boulter et al., 2002), which presumably acts as a nutrient source to promote the establishment and activity of introduced biocontrol microorganisms (Kwok et al., 1987).

Both bacterial and fungal biopesticides have been explored as a means to reduce the effects of replant disease complex on fruit tree establishment. In the Okanagan Valley, Canada, Utkhede and Smith (1992) reported that preplant root inoculation of

apple with *B. subitilis* strain EBW-4 increased trunk cross-section area for 5 years relative to untreated orchard soil. Increased yield and growth of inoculated apple trees planted in soil from an old sweet cherry orchard have also been reported using this same biocontrol strain (Utkhede and Smith, 1993a). In China, inoculating apple seedlings with a different strain of *B. subtilis* reduced *Fusarium* root infection (Ju et al., 2014). In New Zealand, a *Trichoderma* sp. (Pers.) was evaluated for commercial development as a microbial inoculant for replant disease suppression; however, growth enhancement of apple was later found to be associated with nutrient additions found in the formulation as opposed to actual biocontrol activity of the *Trichoderma* sp. (Kandula et al., 2010).

Significant research has been devoted to the development of AMF inoculants as biofertilizers to improve the establishment of fruit trees. In the Okanagan Valley, Canada, Utkhede et al. (1992) demonstrated that inoculating apple with *G. mosseae* significantly improved growth in an old apple orchard site; however, effects on pathogen populations were not monitored. Inoculation of apple with various AMF species has also been reported to improve plant growth in old orchard soil in New Zealand (Ridgway et al., 2008), but similarly, effects on root pathogens were not monitored. In a different study, inoculation of sweet cherry rootstock with *R. irregularis* was reported to significantly increase plant growth but did not decrease *P. vulnus* populations (Pinochet et al., 1995). Conversely, a trial performed in the Okanagan Valley, Canada demonstrated that preplant inoculation of apple rootstock with AMF not only improved growth and nutrient uptake, but also suppressed root infestation by *P. penetrans* (Forge et al., 2001).

1.3.8 Composts

Interest in the use of compost as a soil amendment for growth promotion and disease control in horticultural systems is increasing. The use of compost amendments has many benefits to soil health including increased soil nutrition (Braun et al., 2010; Gagnon et al., 2012), enhanced phosphatase activity in the rhizosphere (Bastida et al., 2008), increased soil biological activity and diversity (Hargreaves et al., 2008; Kennedy, 1999), suppression of weed seeds (Jakobsen, 1995), erosion resistance (Bazzoffi et al., 1998), reductions in soil bulk density (Tester, 1989), and increased water and nutrient holding capacity (Brown and Cotton, 2011). Use of stable, mature compost as opposed to raw manure soil amendments also has the added benefit of reduced risk of ammonia phytotoxicity, less dramatic shifts in nutrient availability, and reduced potential for environmental contamination by fecal bacteria (Forge et al., 2016a). Additionally, many studies have also reported that amending soil with compost can induce suppressiveness to soil-borne pathogens and pests, suggesting that these amendments may be effective disease management tools (Hoitink et al., 1997; Hoitink and Boehm, 1999; Noble and Coventry, 2005). Both sterilized and unsterilized compost extracts have been associated with enhanced growth of apple in replant soil (van Schoor et al., 2009), suggesting induced soil suppressiveness through inputs of compost are likely a result of enhancing the activities of indigenous antagonistic soil microflora, as opposed to the action of introduced antagonists in the amendment (Lockwood, 1990; Stone et al., 2004).

In a recent literature review on the use of compost amendments to suppress plant-parasitic nematodes, Thoden et al. (2011) concluded that composts have potential to suppress as well as enhance plant-parasitic nematode populations. Studies on the use of

compost amendments for suppression of plant pathogenic fungi have reported similar trends (Bonanomi et al., 2007; Erhart et al., 1999; Pérez-Piqueres et al., 2006; Termorshuizen and Jeger, 2008). Nevertheless, there have been numerous reports of successful enhancement of growth through the utilization of compost soil amendments in a replant scenario. Growth benefits of applying compost to a replant site have been demonstrated in a number of different growing regions including Maine, USA (Moran and Schupp, 2003, 2005), Israel (Gur et al., 1998), Washington state, USA (Peryea and Covey, 1989), and South Africa (van Schoor et al., 2009); however, in all these studies the effects on soil-borne pathogens were not monitored. In Nova Scotia, Canada, Braun et al. (2010) demonstrated that preplant application of hog manure-wood mill waste compost not only increased trunk diameter and fine root abundance of apple, but also reduced *P. penetrans* populations relative to untreated soil. In New York, USA, manure-yard waste-vegetable waste compost also suppressed *P. penetrans* populations; however, growth was not improved by the compost amendment or even soil fumigation at this replant site (Rumberger et al., 2007, 2004; Yao et al., 2006).

1.3.8.1 OM-Mediated Soil Suppressiveness

Certain organic soil amendments contain pre-existing compounds, or precursor molecules to compounds, that are directly toxic to nematodes and fungi. As previously described, incorporation of *Brassica* and *Sorghum* plant material into soil can inhibit soil-borne pathogens and pests through the release of biocidal compounds. Similarly, incorporation of organic material rich in chitin or protein has been linked to suppression of fungi and nematodes as a result of increased enzymatic activity, specifically chitinase and protease, respectively (Galper et al., 1990; Mian et al., 1982; Rodriguez-Kabana et

al., 1983). Decomposition of OM rich in nitrogenous material, such as low C/N ratio composts (<10), manures, and raw sewage, have been linked with nematode and fungal suppression through the release of ammonia as the OM decomposes (Huber and Watson, 1970; Rodriguez-Kabana, 1986; Spiegel et al., 1986). Although decomposition of OM into ammonia may contribute to nematode and fungal suppression, such suppressive effects would be short lived, and would not account for the reports of long-term pathogen suppression as a result of OM inputs.

Other abiotic factors have also been associated with OM-mediated suppression of root diseases. The decomposition level of organic soil amendments has been shown to play an important role in the suppressiveness of the amendment, with mature, non-decomposed composts reported more suppressive than partially decomposed material (Tuitert et al., 1998). Similar trends in suppressiveness have also been observed with peat-based soil amendments (Boehm et al., 1997). Increases in extractable C content in soil (Tilston et al., 2002), the production of volatile fatty acids (Tenuta et al., 2002), as well as changes in soil pH, salinity and electrical conductivity, CO_2 and O_2 concentrations, redox potential, and soil structure have also been linked with suppressiveness of organic soil amendments (Oka, 2010). In a comprehensive review of the characteristics of organic soil amendments associated with suppression of soil-borne diseases, Bonanomi et al. (2010) concluded that biological indicators of suppressiveness, including microbial activity, microbial biomass, as well as the abundance of total bacteria, fluorescent *Pseudomonas*, and *Trichoderma* spp. were more predictive than physical or chemical indicators of suppressiveness.

The majority of organic soil amendments, including composts, are primarily thought to suppress plant-parasitic nematodes and pathogenic fungi by promoting the activity and abundance of indigenous soil antagonists. Linford et al. (1938) suggested that suppression of plant-parasitic nematodes through OM inputs into soil was associated with increases in populations of antagonistic organisms, including nematode-trapping fungi and predacious nematodes and mites. This hypothesis remained largely untested for a number of years; however, subsequent experiments by Cooke (1968) demonstrated that nematode-trapping fungi could be stimulated by organic amendments, and that they exhibited predatory activity towards nematodes. OM also provides a favourable environment for predatory invertebrates; however, little direct evidence indicates that they actually prey on nematodes and fungi on a scale that could account for the level of disease suppression often observed through OM inputs. In the absence of compelling evidence for a dominant role of nematode-trapping fungi and predatory invertebrates in OM-induced soil suppressiveness, there has been speculation about the possible role of other members of the soil microflora, including egg parasitizing fungi (Stirling, 2014), parasitic bacteria (Bird et al., 2003), and antibiotic-producing fungi and bacteria (Hoitink and Boehm, 1999; Stirling, 2014). Addition of OM to soil has been shown to stimulate populations of fast growing gram-negative bacteria with the capacity to produce antibiotics (Shanahan et al., 1992; You and Sivasithamparam, 1994), including *Pseudomonas* spp. (Boulter et al., 2002; Hoitink et al., 1997; Hoitink and Fahy, 1986), and it has been hypothesized that these bacteria colonize roots as they come into contact with OM aggregates, thereby promoting a rhizosphere microbiome that is rich in bacterial populations with high antagonistic activity (Stirling, 2014).

1.3.9 Mulches

The use of mulch has also gained significant interest as an alternative management approach for replant disease on fruit trees. Many different kinds of materials have been used as mulches, including straw, hay, alfalfa, wood chips, shredded paper, black plastic tarps, and stones (Granatstein and Sanchez, 2009). Mulching has many benefits to soil health, including weed suppression, soil moisture conservation, enhanced aggregate stability, temperature insulation, and reduced soil erosion (Granatstein and Mullinix, 2008; Mulumba and Lal, 2008; Ramakrishna et al., 2006; Smith et al., 2000). Additionally, the use of organic mulches has the added benefit of increasing soil nutrition and OM content in soil, as well as the potential for inducing soil suppressiveness to pathogens and pests (Brown and Tworkoski, 2004; Forge et al., 2008, 2003; Stirling et al., 1995). Nevertheless, use of mulch also has some drawbacks, including the potential requirement for frequent reapplication, risk of N immobilisation in soil (Huang et al., 2008), significant economic cost, as well as the potential for increases in other pests such as voles (Granatstein and Mullinix, 2008; Granatstein and Sanchez, 2009).

With regard to suppression of replant disease on fruit trees, a variety of mulches have shown significant management potential in a number of different growing regions. In the Okanagan Valley, Canada, use of black plastic tarps was associated with a 50% yield increase on apple (Neilsen et al., 2003). In the same region, application of shredded paper mulch and alfalfa mulch increased fruit yield, the abundance of fine roots in soil, and suppressed *P. penetrans* populations on apple (Forge et al., 2013b, 2008, 2003; Neilsen et al., 2003). Application of straw mulch has been associated with increased growth and yield of apple in various growing regions (Merwin et al., 1994; Shribbs and

Skroch, 1986; van Schoor et al., 2009). In the Okanagan Valley, Canada and Washington state, USA, use of wood chip mulches has resulted in enhanced growth of apple, elevated soil OM content, and even reduced irrigation water use (Granatstein and Mullinix, 2008; Neilsen et al., 2014). Similarly, in a study conducted in the Okanagan Valley, Canada on apple, mulching with compost improved root biomass, suppressed *P. penetrans* populations, as well as increased populations of omnivorous and predacious nematodes (Forge and Kempler, 2009).

1.3.10 Irrigation Type

Water management practices implemented after planting may also have a strong effect on tree establishment on replant sites (Forge et al., 2016b). Irrigation practices are known to influence soil water dynamics (Bryla et al., 2011, 2003; Hannam et al., 2016), soil nutrient availability (Neilsen et al., 2008), and even populations of soil-borne pathogens (Bryla and Linderman, 2007; Utkhede and Smith, 1996). Low-volume irrigation systems, such as drip emitters and microsprinklers, are rapidly becoming the industry standard in tree-fruit production systems due to improved water-use efficiency relative to overhead sprinklers (van der Gulik, 1999), and the capacity to deliver chemical fertilizers directly to the root zone via fertigation (Neilsen et al., 1998). Very little is known regarding the influence of different low-volume irrigation systems on fruit tree establishment, and almost nothing is known about how plant-parasitic nematode populations are affected by different types of irrigation systems. On a peach replant site in California, USA, irrigation with drip emitters improved growth, yield, and water use efficiency of young trees relative to microsprinklers (Bryla et al., 2003). Layne et al. (1996) reported that drip emitters increased soil water content relative to microsprinklers

in a high-density peach orchard in Ontario, Canada, but yield was not affected. A better understanding of the influence of low-water irrigation systems on the establishment of other fruit tree species, such as sweet cherry and apple, as well as effects on pathogen populations contributing to replant disease complex, will be essential to developing an integrated management strategy aimed at improving fruit tree establishment on old orchard sites.

1.4 Dissertation Objectives and Hypotheses

The aim of the research outlined in the following chapters of this dissertation is to expand knowledge of the effects of non-fumigant alternative soil management strategies, particularly composts, organic mulch, microbial inoculants, and low-volume irrigation systems, on the establishment of fruit trees planted in old orchard soil. Such soil management strategies have shown considerable potential to suppress replant disease in previous studies on apple; however, there is a lack in understanding of the effects of these management strategies on tree establishment and associated soil-borne pathogens on sweet cherry, as well as a knowledge gap with respect to the mechanisms that contribute to plant growth promotion and *P. penetrans* suppression.

1.4.1 Outline of Chapter 2

In Chapter 2, I describe using a greenhouse replant experiment to evaluate the effects of preplant soil incorporation of composts and root-dip inoculation with rhizobacteria that exhibited *in vitro* antagonism to fungal pathogens on (1) growth of apple and sweet cherry seedlings planted into orchard soil previously used for apple production, (2) *P. penetrans* populations and recovery of replant disease-associated fungal genera, and (3) the abundance of microbial populations associated with soil

suppressiveness (total bacteria, total fungi, *Pseudomonas* sp., DAPG+ bacteria, and PRN+ bacteria) as well as changes in soil microbial community composition. Using a subsequent greenhouse bioassay, the pathogenicity of fungi isolated from surface-sterilized apple and sweet cherry roots, as well as synergistic interactions with *P. penetrans*, were evaluated.

First, I predicted that compost amendments and root-dip inoculation with rhizobacteria that exhibited antagonism to fungal pathogens under lab conditions would improve the growth of apple and sweet cherry seedlings planted into orchard soil previously used for apple production relative to that of untreated soil because numerous studies had demonstrated benefits of these management strategies on young tree establishment (Moran and Schupp, 2003, 2005; Utkhede et al., 2001; Utkhede and Smith, 1993b, 1992; van Schoor et al., 2009). Second, I predicted that improved plant growth would be associated with lower recovery of *P. penetrans* and replant disease-associated fungal genera from roots, because plant-parasitic nematodes and pathogenic fungi are associated with poor growth of newly planted fruit trees (Braun, 1991; Dullahide et al., 1994; Hoestra and Oostenbrink, 1962; Jaffee et al., 1982; Mai and Abawi, 1981; Manici et al., 2013; Mazzola, 1998; Tewoldemedhin et al., 2011a, 2011b, 2011c; Traquair, 1984; Vrain and Yorston, 1987), and their populations have been shown to be influenced by composts (Bonanomi et al., 2007; Erhart et al., 1999; Pérez-Piqueres et al., 2006; Termorshuizen and Jeger, 2008; Thoden et al., 2011) and biocontrol organisms (Dong and Zhang, 2006; Haas and Défago, 2005; Heydari and Pessarakli, 2010; Jatala, 1986; Kerry, 2000; Khan and Kim, 2007; Siddiqui and Mahmood, 1997; Tian et al., 2007; Weller, 1988). Third, I predicted that composts would increase the abundance of

microbial populations associated with soil suppressiveness as well as alter the soil microbial community composition because numerous studies have demonstrated stimulation and alteration in the composition of these populations after soil management practices are implemented (Caputo et al., 2015; Franke-Whittle et al., 2015; Hoitink and Boehm, 1999; Manici et al., 2015b; Nicola et al., 2017; Peruzzi et al., 2017; Stirling, 2014; Sun et al., 2014).

In the subsequent pathogenicity bioassay, I predicted that inoculation with fungi isolated from surface-sterilized roots of apple and sweet cherry seedlings and *P. penetrans* would reduce the growth of apple seedlings because these groups of organisms have been associated with poor tree establishment in a number of different growing regions (Braun, 1991; Caruso et al., 1989; Dullahide et al., 1994; Jaffee et al., 1982; Mai and Abawi, 1981; Manici et al., 2013; Mazzola, 1998; Tewoldemedhin et al., 2011a, 2011b, 2011c; Traquair, 1984). I also predicted that synergistic interactions would be observed upon co-inoculation with *P. penetrans* and fungi, based on the worldwide co-occurrence of these two groups of organisms (Back et al., 2002; Dullahide et al., 1994).

1.4.2 Outline of Chapter 3

In Chapter 3 I used a field replant experiment to evaluate the effects of preplant incorporation of compost, surface application of bark chip mulch, and irrigation type (drip emitter versus microsprinkler) on (1) early growth and fruit yield of sweet cherry trees planted into orchard soil previously used for apple production, (2) *P. penetrans* population dynamics in soil and root tissue, (3) microbial indicators of soil suppressiveness and enhanced P nutrition, and (4) abiotic factors that may have also contributed to improved plant growth and *P. penetrans* suppression, specifically,

alterations in plant/soil nutrition and water status.

First, I predicted that organic soil amendments (compost and bark chip mulch) would improve early growth and fruit yield of sweet cherry trees planted into an old apple orchard site relative to trees planted into untreated soil because numerous field studies have previously demonstrated the benefits of organic soil amendments on the establishment of fruit trees (Forge et al., 2013b, 2003; Granatstein and Mullinix, 2008; Merwin et al., 1994; Moran and Schupp, 2003, 2005, Neilsen et al., 2014, 2003; Shribbs and Skroch, 1986; van Schoor et al., 2009). I also predicted, based on the limited literature available, that the drip irrigation system would promote greater growth of newly planted sweet cherry relative to the microsprinkler irrigation system. Studies in California, USA on peach and high-bush blueberry (*Vaccinium corymbosum* (L.)) have demonstrated greater plant growth under drip irrigation systems (Bryla et al., 2011, 2005, 2003), potentially as a result of reduced exposure to water stress under drip emitters than microsprinklers. Second, I predicted that compost and bark chip mulch would suppress *P. penetrans* populations in soil and roots because numerous studies have demonstrated the suppressive effects of organic soil amendments on *P. penetrans* during the establishment of fruit trees in old orchard soil (Braun et al., 2010; Forge et al., 2013b, 2008, 2003; Forge and Kempler, 2009; Rumberger et al., 2007, 2004; Yao et al., 2006). Third, I predicted that compost and bark chip mulch would enhance microbial indicators of soil suppressiveness (microbial populations, soil microbial activity, and suppression of inoculated nematodes) and enhanced P nutrition (AMF root colonization and populations of phosphate-solubilizing bacteria) because previous studies demonstrated stimulation of these variables through inputs of organic soil amendments (Bastida et al., 2008; Hoitink

and Boehm, 1999; Stirling, 2014; Zayed and Abdel-Motaal, 2005). Fourth, I predicted that abiotic factors that can also influence tree establishment, such as plant/soil nutrition and water status, would be positively altered by soil management practices based on studies that have demonstrated such changes (Braun et al., 2010; Redman et al., 2001; Traquair, 1984).

1.4.3 Outline of Chapter 4

In Chapter 4 I describe extending previous research findings reported in Chapters 2 and 3 by evaluating the capacity of three different compost amendments to enhance plant growth, suppress *P. penetrans* populations, and stimulate microbial indicators of soil suppressiveness (microbial populations and soil microbial activity) in orchard soil collected from four different orchard sites. The secondary aim of this experiment was to isolate *Pseudomonas* spp. from a suppressive sweet cherry rhizosphere, screen the isolates for desirable biocontrol traits, and evaluate the capacity of two isolates that exhibited antagonism to pathogenic fungi and *P. penetrans* under lab and controlled greenhouse conditions, respectively, to enhance plant growth and suppress *P. penetrans* populations in orchard soil collected from four orchard sites.

First, I predicted that the three different compost amendments would enhance plant growth, suppress *P. penetrans* root populations, and stimulate microbial indicators of soil suppressiveness in orchard soil collected from four different orchard sites because such benefits were previously observed in experiments described in Chapters 2 and 3, as well as in other studies (Hoitink and Boehm, 1999; Moran and Schupp, 2003, 2005, Rumberger et al., 2007, 2004; Stirling, 2014; van Schoor et al., 2009; Yao et al., 2006). Second, I predicted that a compost-amended orchard soil would be a good source for

rhizosphere-colonizing *Pseudomonas* spp. with antagonistic activity to *P. penetrans* and replant-associated fungi because this group of bacteria has previously been shown to have antagonistic activity to replant-disease associated pathogens (Costa et al., 2009; Mazzola, 1999; Mazzola and Gu, 2002, 2000; Nandi et al., 2015; Siddiqui and Shaukat, 2003), and be stimulated by the addition of organic soil amendments (Boulter et al., 2002; Hoitink et al., 1997; Hoitink and Fahy, 1986; Shanahan et al., 1992; You and Sivasithamparam, 1994). Third, I predicted that antagonistic *Pseudomonas* isolates from the rhizosphere of sweet cherry would enhance plant growth and suppress *P. penetrans* populations when applied to the roots of apple seedlings planted into orchard soil collected from four different orchard sites because other studies in the Okanagan Valley, Canada have demonstrated the effectiveness of biocontrol inoculants for replant disease control (Utkhede et al., 2001; Utkhede and Smith, 1993a, 1993b, 1993c, 1992).

2 Chapter 2: Greenhouse Replant Experiment

2.1 Background

Poor growth of fruit trees planted into soil previously used for tree-fruit production presents a significant barrier to the establishment of productive orchards at old orchard sites (Mai and Abawi, 1978). Specific replant disease has often been used to refer to instances where poor growth of newly planted young trees is restricted to when the same fruit tree species is replanted, whereas non-specific replant disease refers to instances where planting with a different fruit tree species also results in poor growth (Traquair, 1984). Non-specific replant disease occurs in the background of specific replant disease, and has been attributed, in part, to increases in populations of plant-parasitic nematodes in recognition of their wide host range (Mai and Abawi, 1978). The root-lesion nematode, *Pratylenchus penetrans,* and fungi/oomycetes from the genera "*Cylindrocarpon*", *Fusarium, Rhizoctonia*, *Phytophthora*, and *Pythium* have been implicated in replant disease worldwide (Braun, 1991; Caruso et al., 1989; Dullahide et al., 1994; Jaffee et al., 1982; Mai and Abawi, 1981; Manici et al., 2013; Mazzola, 1998; Tewoldemedhin et al., 2011a, 2011b, 2011c; Traquair, 1984). Given the common co-occurrence of *P. penetrans* with those fungal/oomycete genera, as well as previous reports of disease complexes forming in other pathosystems (Back et al., 2002), synergistic interactions between these organisms seem likely. In the Okanagan Valley, Canada, *P. penetrans* has a widespread distribution and contributes significantly to poor establishment of newly planted fruit trees in old orchard sites (Forge et al., 2013a; Utkhede et al., 1992; Vrain and Yorston, 1987). Less is known regarding which fungal/oomycete species contribute to replant disease in this region; however, *Fusarium*,

"*Cylindrocarpon*", and occasionally *Rhizoctonia* have been associated with poor growth of sweet cherry (O'Gorman et al., 2016).

A variety of naturally occurring antagonists of plant-parasitic nematodes and pathogenic fungi have been documented, including nematode-trapping fungi (Stirling et al., 1998), parasitic bacteria (Starr and Sayre, 1988), parasitic fungi (Kerry et al., 1984), predacious nematodes (Yeates and Wardle, 1996), microarthropods (McSorley and Wang, 2009), and antibiotic-producing rhizobacteria (Siddiqui and Shaukat, 2003; Siddiqui and Sayeed Akhtar, 2009). It may be possible to exploit the biocontrol capacity of these organisms, either through direct inoculation, or by enhancing the activity and/or abundance of indigenous populations of antagonists. Inoculating roots with antagonistic strains of rhizobacteria has previously been demonstrated to improve growth of apple trees in old orchard soil (Utkhede and Smith, 1993a). In the Okanagan Valley, Canada, inoculation with *Bacillus subtilis* strain EBW4 improved trunk diameter, shoot growth, and yield of apple planted into old orchard soil, as well as suppress root infection by fungal pathogens (Utkhede et al., 2001; Utkhede and Smith, 1993a, 1992). Many other rhizobacteria, such as members of Enterobacteriaceae and Pseudomonadaceae, also display antagonistic activity to soil-borne pathogens (Hynes et al., 2008), and may have potential to improve growth of newly planted fruit trees when inoculated onto roots as biocontrol agents.

Amending soil with compost has many potential benefits to soil health, including increases in soil nutrition (Gagnon et al., 2012), water holding capacity (Brown and Cotton, 2011), biological activity and diversity (Hargreaves et al., 2008; Kennedy, 1999), as well as, in some instances, suppression of soil-borne pathogens (Hoitink and

Boehm, 1999; Noble and Coventry, 2005; Thoden et al., 2011). The mechanisms associated with pathogen suppression through OM inputs have primarily been attributed to increases in populations of indigenous soil antagonists (Cooke, 1968; Linford et al., 1938; Stirling, 2014), such as *Pseudomonas* spp., and other groups of antibiotic-producing rhizobacteria (Hoitink and Boehm, 1999; Stirling, 2014). Preplant soil incorporation of compost has previously been shown to improve apple growth (Braun et al., 2010; Gur et al., 1998; Moran and Schupp, 2003, 2005; Peryea and Covey, 1989; van Schoor et al., 2009) and reduce root infestation by *P. penetrans* (Braun et al., 2010; Rumberger et al., 2007, 2004). Currently, there is interest in whether preplant soil incorporation of compost will also show the same capacity to improve plant growth in other growing regions as well as on other fruit tree species, such as sweet cherry.

The nutritional benefit of OM inputs to soil is largely dependent on the composition of the material, the C/N ratio, and the time course of decomposition (Ferris and Matute, 2003). Fungal decomposition channels tend to predominate if OM is recalcitrant, rich in cellulose or lignin, and possesses a high C/N ratio, and this typically results in slower decomposition and nutrient release, whereas bacterial decomposition channels predominate when OM is labile, N-rich, and has a low C/N ratio, thereby favouring more rapid decomposition and nutrient mineralization (Ferris and Matute, 2003).

There are several nematode fauna-based indices that have been developed to provide information about the structure of soil food webs and nutrient cycling. Bongers (1990) proposed that free-living nematodes could be categorized, at the family level of resolution, along a continuum of live-history strategies from "colonizers' to "persisters",

and that weightings could be applied to taxa along the colonizer-persister scale such that weighted abundances of nematode families could be used to construct a ratio indicative of the ecological maturity of the nematode community, called the Maturity Index. The colonizer-persister continuum proposed by Bongers (1990) is similar to the r/K selection continuum in classical ecological theory (MacArthur and Wilson, 1967). It was also recognized that families of bacterial and fungal feeding nematodes at the colonizer end of the life-history spectrum could be conceptualized as "enrichment opportunists" associated with increased availability of resources to the soil food web (Ferris et al., 2001). This led to the development of the Enrichment Index (EI), which is in essence a measure of the abundance of enrichment opportunists relative to basal taxa and reflects the availability of resources to the soil food web. The Channel Index (CI) is a measure of the abundance of fungal feeding enrichment opportunists relative to bacterial feeding enrichment opportunists and therefore provides information about the predominant decomposition pathway occurring in the soil food web. The Structure Index (SI) is essentially a measure of the abundance of nematodes with high rankings along the colonizer-persister scale relative to basal taxa; because most taxa with high c-p rankings are also omnivores and predators, the SI is considered to be indicative of the trophic complexity of the soil food web (Ferris et al., 2001). The CI and EI provide a basis for assessing soil fertility levels and nutrient availability, with higher EI values typically being associated with greater N mineralization in soil (Ferris et al., 2004), unless significant N immobilisation by microbial biomass occurs (Chen and Ferris, 2000). Nematode fauna analysis is a useful means of assessing the state of a soil food web, including potential decomposition and nutrient mineralization from OM inputs to soil.

The objective of the experiments described in this chapter was to use a greenhouse to evaluate the effects of preplant soil incorporation of composts and root-dip biocontrol inoculation with potentially nematode-antagonistic rhizobacteria on (1) growth of apple and sweet cherry seedlings planted into orchard soil previously used for apple production, (2) *P. penetrans* populations and recovery of replant disease-associated fungi from root tissue, (3) the abundance of microbial populations associated with soil suppressiveness (total bacteria, total fungi, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria), and (4) changes in soil microbial community composition assessed via the use of Biolog Ecoplate analyses of CLPPs. Using a subsequent greenhouse bioassay, the pathogenicity of fungi isolated from surface-sterilized apple and sweet cherry roots, and synergistic interactions with *P. penetrans*, were evaluated. As described in Chapter 1, I predicted that composts and biocontrol inoculation with antagonistic rhizobacteria would improve plant growth as well as suppress *P. penetrans* populations and the recovery of replant disease-associated fungal genera from roots. I also predicted that composts would increase the abundance of microbial populations associated with soil suppressiveness as well as alter the soil microbial community composition. In the subsequent greenhouse experiment I predicted that fungi isolated from fruit tree roots would reduce the growth of apple seedlings, and that synergistic interactions would be observed upon co-inoculation with *P. penetrans*.

2.2 Methods

2.2.1 Site Description

The soil selected for the greenhouse replant experiment was from a 13-year-old block of 'Braeburn' apple on M.26 rootstock located at the Summerland Research and

Development Centre (Summerland, British Columbia, Canada). Soil at the site is characterized as a Skaha loamy sand (Aridic Haploxeroll); (Wittneben, 1986). The original orchard block consisted of twelve 26-m long rows of trees with 3 m between row and 1.25 m tree spacing, with a 2-m wide tree row that was kept free of competing vegetation, primarily via the use of glyphosate approximately twice per year.

2.2.1.1 Survey of Indigenous Soil Fungi

The presence of indigenous replant disease-associated fungi from the genera "*Cylindrocarpon*", *Fusarium*, and *Rhizoctonia* was confirmed by direct isolation from soil in October 2013, after the previous apple trees were removed from the orchard site. A composite soil sample was collected from the first planting row by removing soil cores (30 cm length, 2.5 cm diameter) directly from the previous tree root zone (one soil core approximately every 2.6 m of tree row, for a total of ten cores). A dilution series using a 10-g subsample of the soil suspended in 90 mL of phosphate buffered saline (PBS) (Appendix A) was performed, and 100 µL of a 10^5 dilution of the soil suspension was spread onto potato dextrose agar (PDA) + 100 µg mL^{-1} streptomycin plates (Appendix A) as well as alkaline water agar (AWA) + 100 µg mL^{-1} streptomycin plates (Appendix A), in triplicate. Plates were incubated in the dark for 72 h at 20 °C. Individual colonies were isolated by hyphal tip transfer to PDA, followed by incubation in the dark for 14 days at 20 °C, prior to morphological and molecular identification.

Preliminary identification of fungal isolates was performed using macroscopic and microscopic characteristics of colony growth (colour, texture, and growing margin), and conidial morphology (size, shape, and presence/absence of septa). Slide cultures were prepared for each fungal isolate and examined microscopically (Harris, 1986). Molecular

identification of replant disease-associated genera ("*Cylindrocarpon*", *Fusarium*, and *Rhizoctonia)* was performed by sequencing the internal transcribed spacer (ITS) region. Prior to DNA extraction, fungal isolates were cultured on PDA and grown to a colony diameter of approximately 4 cm. Total genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). Amplification reactions contained 2.0 µL of 10X ThermoPol® Buffer (New England Biolabs, Beverly, MA, USA), 1.6 µL of 25 mM $MgCl_2$, 1.0 µL of 10 mM dNTPs, 2.5 µL of 0.1 mM forward and reverse primer, 1.0 µL of template DNA, 0.1 µL of *Taq* DNA Polymerase (New England Biolabs, Beverly, MA, USA), brought to a volume of 20 µL. The ITS region was amplified with the primer set ITS1/ITS4 (Table 2.1). Amplification reactions were carried out on a Veriti® 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA) using previously defined temperature profiles (White et al., 1990). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, USA) and were sent for sequencing by the Fragment Analysis and DNA Sequencing Services at the University of British Columbia Okanagan (Kelowna, Canada). Sequences were edited and assembled using Geneious 6.1.3 (Biomatters, Ltd., Auckland, NZ). Consensus sequences were compared for similarities within the National Center for Biotechnology Information GenBank database using the Basic Local Alignment Search Tool (BLAST).

Table 2.1 - Primers used for DNA-based identification of fungi, bacteria, and nematodes.

Gene region	Target organism	Primer name	Sequence (5' to 3')	Reference
ITS	Fungi	ITS1	TCCGTAGGTGAACCTGCGG	White et al., (1990)
		ITS4	TCCTCCGCTTATTGATATGC	
16S	Bacteria	8F	AGAGTTTGATCCTGGCTCAG	Hynes et al., (2008)
		531R	ACGCTTGCACCCTCCGTATT	
		515F	TGCCAGCAGCCGCGTAA	
		1542R	GGCTACCTTGTTACGACTT	
28S	Nematodes	D2A	ACAAGTACCGTGAGGGAAAGTTG	De Ley et al., (1999)
		D3B	TCGGAAGGAACCAGCTACTA	

2.2.2 Prospective Biocontrol Isolates

Prospective biocontrol bacteria evaluated for antagonistic potential to replant disease-associated fungal pathogens consisted of *P. fluorescens* isolates 4-2 and 4-6, *Commamonas acidovorans* 6-5, and *Pantoea agglomerans* 6-20, previously isolated from the rhizosphere of healthy pea, lentil and chickpea plants in Saskatchewan, Canada (Hynes et al., 2008). These isolates were chosen from a subset of 26 bacteria that had been previously screened for antagonistic potential to similar fungal pathogens (Watson and Nelson, unpublished). The prospective biocontrol isolates had been previously identified using fatty acid methyl ester (FAME) analysis as well as 16S rRNA sequencing for *P. fluorescens* isolates 4-2 and 4-6 (Hynes et al., 2008).

Full-length 16S rRNA sequencing was performed for each bacterial isolate to confirm previous FAME and 16S rRNA-based identifications. Total genomic DNA was prepared using the E.Z.N.A Bacterial DNA Isolation Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). Amplification reactions were performed in 30-µL volumes containing 3.0 µL of 10X Thermopol® Buffer, 3.0 µL of dNTPs, 0.6 µL of 0.1 mM forward and reverse primer, 1.0 µL of template DNA, 0.2 µL of *Taq* DNA Polymerase, and 21.6 µL of water. Previously defined temperature profiles were used to amplify the 16S rRNA gene, using two different primer sets (Hynes et al., 2008). The first portion of the gene was amplified with the primer set 8F/531R, and the remainder of the gene was amplified with the primer set 515F/1542R (Table 2.1). PCR products were purified, sequenced, and analyzed as described previously.

2.2.2.1 *In Vitro* Fungal Inhibition Assays

A dual culture inhibition assay was performed to screen prospective biocontrol bacteria for antagonistic activity to fungi associated with replant disease. *Fusarium* sp. FUS2, *Fusarium* sp. FUS10, *Rhizoctonia* sp. RHZ1, *Rhizoctonia* sp. RHZ2, and "*Cylindrocarpon*" sp. CYL3 associated with replant disease of apple in the Okanagan Valley, Canada, were provided by BC Tree Fruits Cooperative (Lake Country, Canada). *Dactylonectria macrodidyma* 2007b (Halleen, Schroers & Crous) was provided by Dr. José Úrbez-Torres (Summerland Research and Development Centre, Summerland, Canada). This particular isolate has been associated with black foot disease of grape in the Okanagan Valley, Canada (Úrbez-Torres et al., 2014), and the species is likely associated with replant disease of fruit trees in this region as well (O'Gorman et al., 2016). Fungal cultures were replicated by placing a 7-mm plug removed from the edge of an actively growing fungal colony into the center of a PDA plate, followed by incubating in the dark at 20 °C for 14 days.

The antagonistic potential of prospective biocontrol isolates to each of the fungal isolates was evaluated using an adaptation of the *in vitro* dual culture inhibition assay described by Etebarian et al. (2005). Bacteria were grown at room temperature in 10 mL of tryptic soy broth (TSB) (Appendix A) on a rotary shaker set at 200 rpm for 48 h. A 7-mm fungal plug removed from the edge of an actively growing fungal colony was placed into the center of a of tryptic soy agar/potato dextrose agar (TSA+PDA) plate (Appendix A). An inoculating loop full of bacteria from the liquid culture was then streaked 25-mm away from the center of the fungal plug on each side, forming two parallel lines. A total of 10 replicates was performed for each combination of fungi and

bacteria. Plates were incubated in the dark at 20 °C until the fungal colony diameter of control plates that did not receive bacterial inoculation reached approximately 50 mm in diameter. Fungal colony diameter was measured using a digital caliper, and taken as the average of three independent measurements. Percent growth inhibition was calculated as 100 x (1-(biocontrol inoculated colony diameter/control colony diameter)).

The effect of prospective biocontrol isolates on conidial germination of "*Cylindrocarpon*" and *Fusarium* was assessed using an *in vitro* antagonism assay (Spadaro et al., 2013). A conidial suspension (100 µL; 10^5 conidia mL^{-1}), as well as a bacterial suspension (100 µL; 10^9 colony forming units (CFU) mL^{-1}) were added to a test tube containing 5 mL of tryptic soy/potato dextrose broth (TSB+PDB) (Appendix A) and incubated at room temperature at a 45° angle on a rotary shaker set at 150 rpm. A control treatment consisting of conidial suspension mixed with 100 µL of PBS instead of the bacterial inoculant was also included. Five replicates were performed for each treatment combination. After 12 h incubation, a subsample was observed microscopically and 20 conidia were evaluated for germination. Percent conidial germination was calculated as the number of conidia showing a visually distinguishable germ tube divided by 20.

2.2.2.2 Root Colonization Assay

A greenhouse experiment was performed to evaluate the capacity of *Serratia plymuthica* 6-5 to colonize apple roots in old orchard soil. A rifampicin-resistant mutant of the biocontrol isolate *S. plymuthica* 6-5 was induced. A 100-µL volume of a 10^9 CFU mL^{-1} cell density of bacterial culture was spread on TSA+100 µg mL^{-1} rifampicin (Appendix A). Following a 72-h incubation at room temperature, colonies resistant to rifampicin that had a similar colony size to the wild type strain were selected. The genetic

marking procedure was repeated by subsequent plating on TSA+100 µg mL^{-1} rifampicin three more times. Prior to use in the root colonization bioassay, the rifampicin-resistant isolate was screened for retention of antagonistic activity to replant-associated fungi using the dual culture inhibition assay described previously.

S. plymuthica 6-5RifR^{+} was grown to a cell density of 10^{9} CFU mL^{-1} in TSB and inoculated onto apple seedlings. Inoculation consisted of dipping the root system of seedlings in the suspension of *S. plymuthica* 6-5RifR^{+}, as well as applying 5 mL of the inoculant to the planting hole immediately prior to transplanting the seedling. Additionally, a control group was also included, consisting of apple seedlings inoculated with sterile TSB. Seedlings were planted into 1-L pots filled with 800 mL of untreated soil collected in October 2013 from the previously described apple orchard site, with four replicate pots per treatment for each sampling date. Seedlings were grown in a temperature-controlled greenhouse and were fertilized biweekly with all-purpose fertilizer (20:8:20). Seedlings were evaluated for root colonization by *S. plymuthica* 6-5RifR^{+} at the time of inoculation as well as 14, 28, and 56 days post inoculation (*pi*). At harvest, entire root systems were washed under running water to remove adhering soil and were subsequently ground in a mortar and pestle in 10 mL of sterile PBS solution. The root suspension was serially diluted (10^{2} - 10^{4} dilution range) and 100 µL of suspension were spread onto TSA+100 µg mL^{-1} rifampicin plates, in triplicate. Plates were incubated for 48 h at room temperature prior to counting CFU (30 - 300 CFU $plate^{-1}$ range).

2.2.3 Greenhouse Experimental Design

The greenhouse experimental design was a randomized complete block with five blocks, two plant types (apple and sweet cherry), and eight soil treatments. Sweet cherry seeds were collected from an experimental planting of 'Sweetheart' located at the Summerland Research and Development Centre. Seeds were placed into polyethylene bags containing sterile coarse sand, and were lightly coated with Vitavax® (Chemtura Corp., Middlebury, CT) to prevent seed rot during stratification. Seeds were stored at 4 °C for six months to break dormancy. Germinated seeds were placed into plastic pots containing sterile potting mix (3 parts potting mix: 1 part vermiculite: 1 part perlite), and grown to the two-leaf seedling stage in a greenhouse, prior to transplanting into treatment pots. Apple seeds were extracted from 'Ambrosia' apples harvested from an experimental planting located at the Summerland Research and Development Centre. Apple seeds were thoroughly washed with water, and placed into plastic flats containing a 1:1 mixture of sterile perlite and vermiculite. Seedlings were grown in these flats to the two-leaf seedling stage prior to transplanting into treatment pots.

Old apple trees from the previously planted orchard were removed in October 2013 using an excavator. After tree removal, a composite soil sample (3.5 L of soil from six different locations within each of the 12 previous tree rows) was collected at a depth of 5 – 30 cm directly from the previous tree-rooting zone using a shovel, and thoroughly mixed and passed through a 6-mm mechanical sieve to remove rocks and organic debris. Two-leaf stage apple and sweet cherry seedlings (one per pot) were planted into five replicate 5-L pots of each of eight soil treatments for each fruit tree species (1) 100% fumigated orchard soil (Fum), (2) a control comprised of 80% untreated soil and 20%

fumigated soil (3) inoculation with *S. plymuthica* 6-5 into 80% untreated soil and 20% fumigated soil (BCA), (4) inoculation with *S. plymuthica* 6-5 into 100% fumigated soil (Fum+BCA), (5) yard trimmings compost at a 20% v v^{-1} application rate (YTC-2014), (6) inoculation with *S. plymuthica* 6-5 into 80% v v^{-1} untreated soil and 20% v v^{-1} yard trimmings compost (YTC-2014+BCA), (7) agricultural waste compost at a 20% v v^{-1} application rate (AWC-2014), and (8) inoculation with *S. plymuthica* 6-5 into 80% v v^{-1} untreated soil and 20% v v^{-1} agricultural waste compost (AWC-2014+BCA). The pots were placed in a temperature-controlled (24 °C) greenhouse (located at 49°56'52.5"N 119°63'79.8"W) in a randomized complete block design. Seedlings were grown using a 14-hour photoperiod supplied with supplemental high-pressure sodium lighting for approximately 19 weeks prior to analysis (planted January 5, 2014).

2.2.3.1 Soil Treatment Application

Soil was fumigated with Basamid® (Engage Agro Corp., Guelph, Ontario, Canada) at a rate of 0.33 g L^{-1} of soil, with a two-week off-gas period prior to planting. Biocontrol inoculation consisted of dipping the root system of seedlings in a 10^9 CFU mL^{-1} suspension of *S. plymuthica* 6-5, as well as applying 5 mL of the inoculant to the planting hole immediately prior to transplanting the seedling. Composts were mixed with soil at a 20% v/v application rate, and incorporated into the soil one week prior to planting. AWC-2014 is a commercially available Organic Materials Review Institute-certified compost produced from feedlot waste (mixture of bedding and manure), wood sawmill waste, straw, and grape pomace (BigHorn Natural Compost, Oliver, British Columbia, Canada). Analyses of compost chemical and physical properties were performed by A & L Canada Laboratories Inc. (London, Ontario, Canada) (Table 2.2).

YTC-2014 was produced locally by the City of Kelowna from lawn trimmings and tree pruning waste (trade name GlenGrow, Kelowna, British Columbia, Canada) (Table 2.2). Insect and mite pests were controlled by monthly foliar application of Beleaf 50 SG® (ISK Biosciences Corporation, Concord, Ohio, USA) at a rate of 0.3 g L^{-1}. Pots were fertilized biweekly with all-purpose fertilizer (20:8:20), with a cumulative application of 0.55 g of mineral N supplied to each seedling.

Table 2.2 - Chemical and physical properties of compost amendments used in the greenhouse replant experiment. OM refers to organic matter, AWC-2014 refers to agricultural waste compost, and YTC-2014 refers to yard trimmings compost.

	Compost amendment	
Parameter	AWC-2014	YTC-2014
pH	8.09	7.71
Conductivity (ms cm^{-1})	1.49	2.90
OM (%)	34.0	40.6
Ammonium N (NH_4-N) (mg kg^{-1})	32.1	33.5
Nitrate N (NO_3-N) (mg kg^{-1})	282	449
N (%)	1.22	1.25
P (%)	0.30	0.30
K (%)	0.84	0.98
Mg (%)	0.55	0.65
Ca (%)	2.40	2.40
Total Organic C (TOC) (%)	18.9	22.5
C/N ratio	15.0	18.0

2.2.3.2 Analyses of Plant Growth

In order to test the hypothesis that compost and biocontrol would increase the growth of seedlings, I measured various aspects of plant growth. At time of harvest, shoots were cut at soil level and shoot length recorded prior to drying the plant material in an oven at 70 °C for 48 h for determination of dry shoot weight. Entire root systems were carefully removed from each pot and washed thoroughly with tap water. Root systems were then scanned on an Epson Perfection V700 scanner (Epson, Long Beach, CA, USA) and analyzed using WinRHIZO Regular software (Regent instruments, Montreal, QC, Canada). When root systems were too large to fit on the root scanner, they

were cut in half and analyzed by combining data from two independent scans. After removing subsamples of root tissue for nematode and fungal analyses, the remaining root tissue was dried in an oven at 70 °C for 48 h and dry root weight recorded.

2.2.3.3 Nematode Analyses

In order to test the hypothesis that compost and biocontrol would suppress *P. penetrans*, I quantified nematode populations in roots and soil. A 500-mL subsample of soil was collected from each pot at harvest and stored at 4 °C for a maximum of 1 week prior to subsequent processing. Nematodes were then extracted from 50 mL of the soil using the Baermann pan technique, with a 7-day incubation period (Forge and Kimpinski, 2007). After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Additionally, migratory endoparasitic nematodes were also extracted from a subsample of root tissue collected from each seedling using the shaker agitation technique (Shurtleff and Averre, 2005). Approximately 2 g of fresh fine root tissue were placed into a 250-mL Erlenmeyer flask containing 100 mL of water. Flasks were incubated at room temperature on a rotary shaker set at 120 rpm for 4 days. Nematodes were collected over a 25-µm sieve, and were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Extracted root tissue was dried at 70 °C for 48 h and weighed prior to computing *P. penetrans* per gram of dry root.

In order to test the hypothesis that differences in plant growth promotion between the compost amendments were associated with differential nutrient mineralization, I performed nematode faunal analysis of the EI. Free-living soil nematode

community analyses were performed, which comprised of determining the relative abundance of four major nematode feeding groups. A total of 100 nematodes was observed microscopically from each soil nematode extraction, and classified as bacterivorous, fungivorous/phytophagus, predacious, or omnivorous on the basis of oral structure (Ferris et al., 2001). Data are represented as the number of nematodes from each feeding group per 100 mL of soil. Nematode faunal analysis was done to evaluate potential differences in soil food webs between the compost-amended soils. Analysis was based on the relative weighted abundance of nematode functional guilds along the colonizer-persister scale ($Ba_{1\text{-}5}$, $Fu_{1\text{-}5}$, $Ca_{2\text{-}5}$, $Om_{3\text{-}5}$) (Bongers and Bongers, 1998). EI was calculated as 100 x (e/(e+b)), and SI was calculated as 100 x (s/(s+b)), where e, b, and s, represent enrichment, basal, and structure food web components, respectively (Ferris et al., 2001). The basal component (b) was calculated as $\sum k_b n_b$, where k_b are the weightings assigned to guilds that indicate basal characteristics of the food web (Ba_2, Fu_2), and n_b are the abundances of nematodes in those guilds (Ferris et al., 2001). The enrichment (e) and structure (s) components were calculated similarly, using guilds indicative of enrichment (Ba_1, Fu_2) and structure (Ba_3-Ba_3, Fu_3-Fu_3, Om_3-Om_5, and Ca_2-Ca_5), respectively (Ferris et al., 2001). CI was calculated as 100 x ($0.8Fu_2$/($3.2Ba_1$+$0.8Fu_2$)), where the coefficients are the k_e enrichment weightings for the respective guilds (Ferris et al., 2001).

2.2.3.4 Root Colonization by Necrotrophic Fungi

In order to test the hypothesis that compost and biocontrol would suppress replant disease-associated fungi, I quantified the recovery of fungi from surface sterilized roots. Three necrotic roots (5 cm in length) were randomly selected from each root system for analysis of fungi. Roots were surface sterilized in a 5% hypochlorite solution

for 5 min on a rotary shaker set at 150 rpm. Roots were then washed with sterile water twice and air-dried in a laminar flow hood for 15 min. Each 5-cm piece of root was aseptically cut into five segments of equal length, which were subsequently placed on a culture plate containing AWA+100 μL mL^{-1} streptomycin. Plates were incubated in the dark at 20 °C for 4 days, after which a single hyphal tip from each fungal colony growing outward from the root fragment was individually transferred to a PDA growth plate. PDA plates used for isolate identification were incubated in the dark at 20 °C for 10 days prior to grouping into fungal morphotypes (fungi sharing the same morphology) on the basis of morphological characteristics (colour, texture, growing margin, soluble pigment production, and conidia morphology) (Lacap et al., 2003). Three representative isolates were randomly selected from within each fungal morphotype and were subsequently identified using sequence analysis of the ITS region, as described previously. Data are presented as percent isolation of a particular fungal morphotype.

2.2.3.5 Soil Microbiology

In order to test the hypothesis that compost and biocontrol would increase the abundance of microorganisms with potential biocontrol activity, I measured the number of gene copies of total bacteria, total fungi, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in soil. DNA was extracted from 0.25 g of frozen soil (-20 °C) from each pot using the PowerSoil DNA Isolation Kit. The abundance of select groups of rhizosphere microorganisms was monitored using a SYBR Green-based real-time PCR assay utilizing previously developed primer sets (Table 2.3). The abundance of total bacteria was quantified with the universal bacterial primers BACT1369F and PROK1492R (Suzuki et al., 2000) using a standard curve consisting of a serial dilution

(10^6 - 10^0 gene copies reaction^{-1}) of the 16S rRNA gene (GenBank accession number LN57450) cloned into the plasmid pJ201:9907. The abundance of total fungi was quantified with the universal fungal primers FF390 and FR1 (Prévost-Bouré et al., 2011) using a standard curve consisting of a serial dilution of a portion of the 18S rRNA gene (GenBank accession number KX011854) of *F. oxysporum* F1-1 cloned into the plasmid pJ201:236602. The abundance of *Pseudomonas* spp. was quantified with the genus specific primers Pse435F and Pse686R (Bergmark et al., 2012) using a standard curve consisting of a serial dilution of genomic DNA isolated from the type isolate *P. fluorescens* Pf-5 (NRRL B-23932). Similarly, the abundance of DAPG+ bacteria was quantified with the primers BPF2 and BPR4 (McSpadden Gardener et al., 2001) targeting the *phlD* gene (polyketide synthase) (Bangera and Thomashow, 1999), and the abundance of PRN+ bacteria with the primers PrnD-F and PrnD-R (Garbeva et al., 2004b) targeting the *prnD* gene (aminopyrrolnitrin oxygenase) (van Pée and Ligon, 2000), using a standard curve consisting of a serial dilution of genomic DNA isolated from *P. fluorescens* Pf-5.

Table 2.3 - Primers used for real-time PCR-based quantification of soil microorganisms. DAPG+ bacteria refers to 2,4-diacetylphloroglucinol-producing bacteria and PRN+ bacteria refers to pyrrolnitrin-producing bacteria.

Target group	Gene region	Primer name	Sequence (5' to 3')
Total Bacteria	16S	BACT1369F	CGGTGAATACGTTCYCGG
		PROK1492R	GGWTACCTTGTTACGACTT
Total Fungi	18S	FF390	CGATAACGAACGAGACCT
		FR1	AICCATTCAATCGGTAIT
Pseudomonas spp.	16S	Pse435F	ACTTTAAGTTGGGAGGAAGGG
		Pse686R	ACACAGGAAATTCCACCACCC
DAPG+ bacteria	*phlD*	BPF2	ACATCGTGCACCGGTTTCATGATG
		BPR4	CCGCCGGTATGGAAGATGAAAAAGTC
PRN+ bacteria	*prnD*	PrnD-F	TGCACTTCGCGTTCGAGAC
		PrnD-R	GTTGCGCGTCGTAGAAGTTCT

For the standard curve, each reaction contained 10.0 µL of SsoFast™ Evagreen® Supermix (Bio-Rad Laboratories Inc., Hercules, California, USA), 400 nM of appropriate forward and reverse primer, and 5.0 µL of template DNA, brought up to a reaction volume of 20.0 µL using PCR-grade water. For the environmental samples, each reaction contained 10.0 µL of SsoFast™ Evagreen® Supermix (Bio-Rad Laboratories Inc., Hercules, California, USA), 400 nM of the appropriate forward and reverse primer, 150 ng of T4 gene 32 protein (New England Biolabs, Pickering, Ontario, Canada), and 2.0 µL of template DNA, brought up to a reaction volume of 20.0 µL. Template DNA from environmental samples was diluted 1:1000 fold for quantification of total bacteria and fungi, 1:100 fold for *Pseudomonas* spp., and 1:10 fold for DAPG+ bacteria and PRN+ bacteria.

For quantification of total bacteria, the PCR temperature profile consisted of 2 min at 95 °C, 40 cycles of 30 s at 95 °C and 30 s at 56 °C. For quantification of total fungi, the PCR temperature profile consisted of 5 min at 95 °C, 40 cycles of 30 s at 95 °C and 60 s at 59 °C. For quantification of *Pseudomonas* spp., DAPG+ bacteria and PRN+ bacteria, the PCR temperature profile consisted of 2 min at 98 °C, 40 cycles of 30 s at 98 °C and 30 s at 60 °C. All PCR reactions were performed in triplicate for each DNA extraction on a Bio-Rad CFX-96 real-time PCR system (Bio-Rad, Hercules, CA). Reactions were checked for amplification specificity by analysis of melting curves for a single peak, as well as confirmation of a single band of appropriate size when analyzed on a 1% agarose gel.

2.2.3.6 Community Level Physiological Profiles (CLPPs)

In order to test the hypothesis that composts would alter the soil microbial community, CLPPs were determined. CLPPs were determined using Biolog Ecoplates (Biolog Inc., Hayward, CA, USA). Plates consisted of 31 different C substrates (Table 2.4) plated in triplicate on a microtiter plate, with a redox dye (tetrazolium violet) added to quantify oxidative catabolism. A gram of soil from each pot was mixed with 99 mL of sterile water on a rotary shaker set at 150 rpm for 30 min at room temperature. The suspension was allowed to settle for 30 min at 4 °C, after which 150 µL of supernatant were transferred into each well of the microtitre plate. Plates were incubated at room temperature for 72 h, measuring absorbance of each well at 590 nm every 24 h on a Spectramax M2 plate reader (Molecular Devices, Sunnyvale, CA, USA).

Table 2.4 - C substrates used for community level physiological profiling of soil microbial communities.

Biolog Ecoplate C substrate	
β-Methyl-D-Glucoside	N-Acetyl-D-Glucosamine
D-Galactonic Acid Ɣ-Lactone	Ɣ-Hydroxybutyric Acid
L-Arginine	L-Threonine
Pyruvic Acid Methyl Ester	Glycogen
D-Xylose	D-Glucosaminic Acid
D-Galacturonic Acid	Itaconic Acid
L-Asparagine	Glycyl-L-Glutamic Acid
Tween 40	D-Cellobiose
i-Eruthritol	Glucose-1-Phosphate
2-Hydroxy Benzoic Acid	α-Ketobutyric Acid
L-Phenylalanine	Phenylethylamine
Tween 80	α-D-Lactose
D-Mannitol	D,L-α-Glycerol
4-Hydroxy Benzoic Acid	D-Malic Acid
L-Serine	Putrescine
α-Cyclodextrin	*Water*

Analysis of CLPP data was performed as outlined by Weber and Legge (2010). Data analysis was performed at 48 h post inoculation, corresponding to the measurement interval that provided the highest resolution between treatments without reaching saturation point in any wells (OD_{590}>2.0). Data were standardized by correcting each

absorbance value by the blank, followed by dividing by the average well colour development for the plate at 48 h post inoculation. The formula is written as:

$$\overline{A_k} = \frac{A_k - A_o}{\frac{1}{31}\sum_{i=1}^{31}(A_i - A_o)}$$

where A_k represents the standardized absorbance for well k, A_i represents the absorbance of well i, and A_o represents the absorbance reading of the blank. In the case of negative values obtained from wells with minimal colour response, the values were coded as zeros.

2.2.3.7 Soil Nutrient Analyses

In order to assess the potential influences of differential nutrient abundance and availabilities between the two compost amendments, a 0.25-g subsample of dry soil from each pot was analyzed for total C and N content using a Leco CHN Analyzer (Leco Corp., St. Joseph, MI, USA). Soil OM content was calculated as 1.72 times the total organic C content of the soil (Pribyl, 2010). Similarly, a 20-g subsample of soil from each pot was extracted with 2 *M* KCl to assess the residual mineral N content (Bremner and Keeney, 1966). The NO_2-N+NO_3-N (nitrite and nitrate N) and NH_4-N content of filtered supernatant was determined by segmented-flow injection analysis (Astoria Pacific International, Clackamas, Oregon, USA).

2.2.4 Pathogenicity Experiments

Greenhouse experiments were performed to evaluate the capacity of newly isolated fungi to elicit plant growth reductions on apple, as well as to evaluate potential synergistic interactions with *P. penetrans*. In the first experiment, 2-year old Ottawa 3 apple rootstock was planted into steam-pasteurized orchard soil (3.5 gal pots) that received one of eleven different fungal treatments, with each treatment replicated four

times ("*Cylindrocarpon*" sp. C1-1, "*Cylindrocarpon*" sp. C1-2, "*Cylindrocarpon*" sp. C2-1, "*Cylindrocarpon*" sp. C2-2, *Fusarium* sp. F1-1, *Fusarium* sp. F1-2, *Fusarium* sp. F2-1, *Fusarium* sp. F2-2, *Rhizoctonia* sp. R1-1, *Rhizoctonia* sp. R1-2, and an untreated control). Fungi were cultured in 250 mL of sterile oat bran (Mazzola, 1998) for 21 days, and then incorporated into the steam pasteurized soil at a 0.5% w w^{-1} rate. Pots were incubated in a greenhouse for 10 days prior to planting. The untreated control received 0.5% w w^{-1} of sterile oat bran. Total shoot extension was measured every 4 weeks for a total of 12 weeks.

The second experiment was a factorial design (± *P. penetrans* x 11 fungal treatments) with four replicates, giving a total of 88 seedlings. Nematode treatments included inoculation with *P. penetrans* or no *P. penetrans*. Six-leaf stage apple seedlings (Ambrosia) were planted into steam-pasteurized orchard soil that received one of the 22 different treatment combinations. Fungi were cultured in sterile oat bran and inoculated into 10-cm square pots filled with 375 mL of steam-sterilized field soil, as described previously. Nematodes were from an indigenous population of *P. penetrans* from this study's old apple orchard site that had been maintained on mint plants (*Mentha* x *piperita* 'Chocolate' (L.)) in a greenhouse. Pots were inoculated with *P. penetrans* at a density of 375 nematodes per pot (approximately 100 *P. penetrans* 100 mL^{-1} soil). All pots were incubated in a greenhouse for 10 days prior to planting. The pots that did not receive fungal inoculation received amendment with sterile oat bran, as described previously. At harvest (12 weeks), total shoot extension, shoot weight, root weight, and total biomass were determined. Additionally, migratory endoparasitic nematodes were also extracted from fine roots using the shaker agitation technique, as described above.

Morphology-based species identification of the indigenous *P. penetrans* population at the apple orchard site was confirmed by DNA-based sequencing of the D2 and D3 segments of the 28S rRNA gene using the D2A/D3B primer set (Table 2.1). Total genomic DNA was extracted using an adaptation of the NaOH nematode digestion protocol (Floyd et al., 2002). A single nematode, extracted from a population maintained on mint plant roots, was placed in a microcentrifuge tube with 20 µL of 0.25M NaOH, spun down briefly, and incubated overnight at room temperature. The next day, the digestion was heated to 99 °C for 3 min, cooled to room temperature, and spun down briefly once more. To the digestion, 4 µL of 1*M* HCl, 5 µL of 2% Triton X-100, and 10 µL of 0.5*M* Tris-HCl were added, vortexed, and spun down briefly. The digestion was then heated to 99 °C for 3 min, cooled to room temperature, then used immediately in the PCR. Amplification reactions contained 2.5 µL of 10X ThermoPol® Buffer (New England Biolabs, Beverly, MA, USA), 0.5 µL of 10 mM dNTPs, 0.25 µL of 20 µM forward and reverse primer, 1.0 µL of nematode digestion solution, and 0.125 µL of *Taq* DNA Polymerase (New England Biolabs, Beverly, MA, USA), brought to a volume of 25 µL. Amplification reactions were carried out on a T100 Bio-Rad Thermal Cycler (Bio-Rad, Ca, USA) using the following temperature profile: 4 min at 95 °C, 35 cycles of 1 min at 95 °C, 1.5 min at 55 °C, and 2 min at 68 °C, followed by a final extension of 10 min at 68 °C. PCR products were purified, sequenced, and analyzed as described previously.

2.2.5 Statistical Analysis

Data from the greenhouse experiment were subjected to a blocked two-way ANOVA using a general linear model in SPSS 20.0. Terms in the model were block,

plant type, soil treatment, and plant type x soil treatment interaction. Treatment means were compared using the Bonferroni t-test (P-value < 0.05). *P. penetrans* abundance data were analyzed after a log(x+10) transformation. Data from percent recovery of replant-associated fungal genera, percent conidial germination, and proportion of free-living nematode feeding-groups were analyzed after an arcsine transformation. The relationship between plant growth and pathogen/parasite recovery was evaluated using Pearson's correlation coefficient. Normalized absorbance values from Biolog Ecoplates were analyzed by PCA based on a correlation matrix (Weber and Legge, 2010). The CLPP data set was tested for significant differences calculated at P-value < 0.05 with a non-parametric multivariate analysis of variance (NPMANOVA) (Anderson, 2001) using PAST 3.X software package (Hammer et al., 2009). Significance was computed by permutation with 10,000 replicates based on Bray-Curtis distance measure.

Data from the multifactorial pathogenicity assay were subjected to a blocked two-way ANOVA using a general linear model in SPSS 20.0. Terms in the model were block, fungal treatment, nematode treatment, and fungal x nematode treatment interaction. Treatment means were compared using the Bonferroni t-test (P-value < 0.05). *P. penetrans* abundance data were analyzed after a log(x+10) transformation, and as a one-way ANOVA of pots inoculated with *P. penetrans*.

2.3 Results

2.3.1 Survey of Indigenous Soil Fungi

Preliminary work at this apple orchard site revealed a relatively large indigenous population of *P. penetrans* in the soil; however, the presence of necrotrophic fungi associated with replant disease had not been evaluated previously. Fungal species

that have been linked to replant disease that were present in soil at the orchard site included "*Cylindrocarpon*", *Fusarium*, and *Rhizoctonia* (Table 2.5). Soil dilution series plated on PDA+streptomycin yielded seven replant disease-associated fungi out of 32 isolates; however, fungi from "*Cylindrocarpon*" were not recovered using this isolation medium. Soil dilution series plated onto AWA+streptomycin yielded seven replant disease-associated fungi out of 26 total fungi isolated, including "*Cylindrocarpon*". The nematode biocontrol fungus *Paecilomyces* was also present in soil from this orchard site.

Table 2.5 - Survey of indigenous soil fungi in the old apple orchard soil. PDA refers to potato dextrose agar and AWA refers to alkaline water agar.

Isolation medium	Genus	Number of isolates
PDA+streptomycin	*Aspergillus*	3
	Bionectria	6
	Fusarium	6
	Mortierella	7
	Paecilomyces	1
	Penicillium	6
	Rhizoctonia	1
	Trichoderma	1
	Volutella	1
AWA+streptomycin	*Bionectria*	6
	Fusarium	2
	"*Cylindrocarpon*"	2
	Mortierella	5
	Paecilomyces	3
	Penicillium	3
	Rhizoctonia	3
	Ulocladium	1
	Volutella	1

2.3.2 Biocontrol Screening

2.3.2.1 Sequencing Results

Full-length 16S rRNA sequencing revealed different identities for *C. acidovorans* 6-5 and *P. agglomerans* 6-20 than previous FAME-based identification (Hynes et al., 2008). Sequence results indicated that both isolates were *S. plymuthica* (>99% identity with *S. plymuthica* strain 265XY5; Genbank accession number KF818650) (Appendix B).

2.3.2.2 Growth Inhibition Assay

Prospective biocontrol isolates *S. plymuthica* 6-5 and *S. plymuthica* 6-20 inhibited growth of all six replant disease-associated fungal pathogens evaluated (Table 2.6), whereas *P. fluorescens* 4-2 and *P. fluorescens* 4-6 failed to inhibit *Fusarium* sp. FUS2. Isolate *S. plymuthica* 6-5 was more antagonistic to all six replant disease-associated fungal pathogens than both *P. fluorescens* isolates, whereas *S. plymuthica* 6-20 did not inhibit *Rhizoctonia* sp. RHZ1 more than either *P. fluorescens* biocontrol isolate. Antagonistic activity by *S. plymuthica* 6-5RifR$^+$ to all six replant disease-associated fungi did not differ from *S. plymuthica* 6-5.

Table 2.6 - Dual culture fungal colony growth inhibition assay. Data were analyzed with a one-way ANOVA. Values sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment. Values represent the mean ± standard error. Values followed by NS (not significant) indicate no difference in percent growth inhibition between *S. plymuthica* 6-5RifR$^+$ and *S. plymuthica* 6-5.

	Percent growth inhibition (%)					
Biocontrol isolate	'*Cylindro-carpon*' sp. CYL3	*Dactylo-nectria macro-didyma* 2007b	*Fusariu m* sp. FUS2	*Fusarium* sp. FUS10	*Rhizoc-tonia* sp. RHZ1	*Rhizoc-tonia* sp. RHZ2
Psuedomonas fluorescens 4-2 (N=10)	34.3±3.2 b	22.1±4.2 b	0±0 b	36.4±3.1 b	29.6±2.0 b	34.7±3.4 c
P. fluorescens 4-6 (N=10)	41.4±3.3 b	23.8±5.4 b	0±0 b	30.1±5.2 b	31.8±1.3 b	47.1±2.9 b
Serratia plymuthica 6-5 (N=10)	53.2±3.0 a	67.8±6.5 a	58.3±2.0 a	53.9±4.7 a	46.0±2.3 a	66.7±3.3 a
S. plymuthica 6-20 (N=10)	55.4±2.7 a	69.93.9 a	50.7±1.8 a	50.6±4.9 a	38.5±2.0 ab	64.7±2.7 a
S. plymuthica 6-5RifR$^+$ (N=10)	52.5±3.1 NS	69.4±4.5 NS	54.7±1.2 NS	51.1±3.3 NS	44.6±3.4 NS	63.2±3.0 NS

2.3.2.3 Conidial Germination Assay

Co-incubation of fungal conidia with prospective biocontrol bacteria did not affect conidial germination of "*Cylindrocarpon*" sp. CYL3 or *Fusarium* sp. FUS10 (Table 2.7). Inoculation with *S. plymuthica* 6-5 inhibited conidial germination of *D. macrodidyma* 2007b and *Fusarium* sp. FUS2, whereas inoculation with *S. plymuthica* 6-

20 only suppressed *D. macrodidyma* 2007b conidial germination.

Table 2.7 - Dual culture conidial germination inhibition assay. Data were analyzed with a one-way ANOVA. Values sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment. Values represent the mean ± standard error.

	Percent conidial germination (%)			
Biocontrol isolate	"*Cylindrocarpon*" sp.CYL3	*Dactylnectria macrodidyma* 2007b	*Fusarium* sp. FUS2	*Fusarium* sp. FUS10
Control	8±3	57±4	28±2	35±4
(N=5)	a	a	a	a
Psuedomonas fluorescens 4-2	5±2	54±5	30±3	31±3
(N=5)	a	a	a	a
P. fluorescens 4-6	2±3	56±4	29±2	30±3
N=5)	a	a	a	a
Serratia plymuthica 6-5	7±4	37±7	17±2	29±4
(N=5)	a	b	b	a
S. plymuthica 6-20	8±2	36±5	23±4	28±3
(N=5)	a	b	ab	a

2.3.2.4 Root Colonization Assay

Indigenous background populations of rifampicin resistant bacteria at this site were below 4 log CFU g^{-1} of root at all sampling dates. The biocontrol isolate was recovered from roots at densities greater than 7 log CFU g^{-1} of root from 14 days post-inoculation (*pi)* through to 56 days *pi* (Table 2.8).

Table 2.8 - Root colonization of apple seedlings by *S. plymuthica* 6-5RifR+. Data were analyzed with a one-way ANOVA. Values represent the mean ± standard error. CFU refers to colony forming units and *pi* refers to post inoculation.

	Log CFU g^{-1} root			
Biocontrol inoculation	0 days *pi*	14 days *pi*	28 days *pi*	56 days *pi*
Serratia plymuthica 6-5RifR+ (N=4)	4.86±0.12	7.84±0.15	8.21±0.21	7.04±0.34

2.3.3 Greenhouse Replant Experiment

2.3.3.1 Plant Growth

The effect of soil treatment on plant growth was not dependent on plant type (soil treatment x plant type interaction *P*-value >0.05). Seedling shoot length was greater than the control in the Fum, Fum+BCA, AWC-2014, AWC-2014+BCA, and YTC-2014

treatments (Table 2.9). Apple shoots were longer than those of sweet cherry. Shoot weight was greater in the Fum, Fum+BCA, AWC-2014, and AWC-2014+BCA treatments than in the control. Apple shoot weight was greater than sweet cherry shoot weight. Root weight was greater in the Fum, Fum+BCA, AWC-2014, AWC-2014+BCA, and YTC-2014 treatments than in the control; however, root weight promotion by the Fum and Fum+BCA treatments was even greater than the compost treatments. Sweet cherry root weight was greater than that of apple. Total biomass was greater than that of the control in the Fum, Fum+BCA, AWC-2014, and AWC-2014+BCA treatments.

Table 2.9 - Effect of soil treatment and plant type on growth of seedlings in old apple orchard soil. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Plant growth analysis			
Factor	Level	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Biomass (g)
Soil treatment	Control (N=9)	44.1±7.3 c	7.0±0.8 d	2.69±0.37 c	9.7±1.0 c
	BCA (N=10)	63.1±11.5 bc	9.9±1.5 cd	3.40±0.38 c	13.3±1.7 c
	Fum (N=10)	83.6±8.7 ab	14.7±0.9 ab	7.57±0.76 a	22.3±1.6 a
	Fum+BCA (N=10)	91.3±7.6 a	16.9±0.8 a	7.51±0.82 a	24.4±1.2 a
	YTC-2014 (N=9)	74.2±10.9 ab	11.6±1.1 bcd	4.04±0.34 b	15.6±2.0 bc
	YTC-2014+BCA (N=8)	59.7±9.7 bc	8.4±1.3 d	3.00±0.35 c	11.4±1.6 c
	AWC-2014 (N=10)	80.8±11.4 ab	14.5±0.9 abc	5.60±0.45 b	20.1±1.2 a
	AWC-2014+BCA (N=9)	84.5±7.2 ab	13.5±0.8 abc	5.48±0.35 b	19.0±1.1 ab
Plant type	Apple (N=38)	94.7±3.6 a	13.0±0.65 a	4.00±0.28 b	17.0±0.8 a
	Sweet cherry (N=37)	50.3±3.5 b	11.2±0.85 b	5.87±0.51 a	16.8±1.3 a
P-value	Soil trt	<0.001	<0.001	<0.001	<0.001
	Plant type	<0.001	0.016	<0.001	0.822
	Soil trt x plant type	0.375	0.266	0.075	0.096

Soil treatment did not significantly affect root length (Table 2.10), but apple seedlings had longer root systems than sweet cherry. Root surface area was greater than the control in the Fum, Fum+BCA, AWC-2014, and AWC-2014+BCA treatments. Apple root surface area was greater than sweet cherry. Root volume in the Fum and Fum+BCA treatments was greater than the control. Sweet cherry root volume was greater than apple root volume.

Table 2.10 - Effect of soil treatments and plant type on root growth. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Root system analysis		
Factor	Level	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)
Soil treatment	Control	966±211	451±47	14.8±6.1
	(N=9)	a	b	b
	BCA	960±199	453±59	17.6±6.1
	(N=10)	a	b	b
	Fum	1443±175	862±68	54.9±8.6
	(N=10)	a	a	a
	Fum+BCA	1612±194	848±53	61.0±10.5
	(N=10)	a	a	a
	YTC-2014	1265±222	517±50	19.6±6.2
	(N=9)	a	b	b
	YTC-2014+BCA	1309±204	481±61	15.6±6.7
	(N=8)	a	b	b
	AWC-2014	1647±186	709±92	41.8±9.9
	(N=10)	a	a	ab
	AWC-2014+BCA	1450±124	752±47	35.7±5.8
	(N=9)	a	a	ab
Plant type	Apple	1669±108	689±47	24.5±4.3
	(N=38)	a	a	b
	Sweet cherry	1001±176	596±34	41.3±5.9
	(N=37)	b	b	a
P-value	Soil trt	0.059	<0.001	<0.001
	Plant type	<0.001	0.04	0.001
	Soil trt x plant type	0.357	0.455	0.153

2.3.3.2 Nematode Analyses

The effect of soil treatment on nematode populations was not dependent on plant type (soil treatment x plant type interaction *P*-value >0.05). Fum, AWC-2014, and

YTC-2014 soil treatments, with or without BCA, decreased the abundance of *P. penetrans* in soil and roots, as well as the total abundance of *P. penetrans* pot^{-1}, relative to those of the control or BCA (Table 2.11). Fumigation decreased *P. penetrans* in roots and the total abundance of *P. penetrans* pot^{-1} to a greater extent than did the AWC-2014 or YTC-2014 treatments. The abundance of *P. penetrans* was greater in pots planted with sweet cherry than in those with apple. The abundance of *P. penetrans* in soil and roots, as well as the total abundance in each pot, showed a significant negative correlation with all growth parameters (Appendix C).

Table 2.11 - Effect of soil treatments and plant type on *P. penetrans* populations. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		P. penetrans populations		
Factor	Level	*P. penetrans* 100 mL^{-1} soil	*P. penetrans* g^{-1} root	*P. penetrans* pot^{-1}
Soil treatment	Control	37±3	3048±213	4873±550
	(N=9)	a	a	a
	BCA	33±4	2148±153	4259±584
	(N=10)	a	a	a
	Fum	1±1	5±4	54±40
	(N=10)	b	c	c
	Fum+BCA	1±1	7±5	78±25
	(N=10)	b	c	c
	YTC-2014	8±1	282±58	895±126
	(N=9)	b	b	b
	YTC-2014+BCA	6±1	184±56	534±143
	(N=8)	b	b	b
	AWC-2014	8±2	304±112	1065±294
	(N=10)	b	b	b
	AWC-2014+BCA	6±1	352±81	1130±222
	(N=9)	b	b	b
Plant type	Apple	6±1	640±164	1110±309
	(N=38)	b	b	b
	Sweet cherry	18±2	915±254	2030±478
	(N=37)	a	a	a
P-value	Soil trt	<0.001	0.032	<0.001
	Plant type	<0.001	<0.001	<0.001
	Soil trt x plant type	0.061	0.972	0.206

Fum, Fum+BCA, AWC-2014, and AWC-2014+BCA treatments increased the proportion of bacterial feeders in the soil relative to those in BCA and the control, but to a significantly greater extent in the Fum treatment (Table 2.12). Fum, Fum+BCA, or AWC-2014+BCA treatments had smaller proportions of fungal+plant feeders in the soil than the BCA treatment and the control. Sweet cherry supported a greater proportion of fungal+plant feeders in soil than apple. The relative abundance of predatory and omnivorous nematodes was lower in the Fum and Fum+BCA treatments than all other soil treatments. Fum and Fum+BCA had significantly greater total abundance of nematodes in soil relative to that in YTC-2014+BCA. Plant type did not have a significant effect on the relative abundance of nematode feeding groups or total abundance of free-living nematodes in soil.

Table 2.12 - Effect of soil treatments and plant type on free-living nematode abundance and relative abundance of feeding groups. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

			Free-living nematode feeding groups (%)			
Factor	Level	Nematodes 100 mL^{-1} soil	Bacterial feeders	Fungal+ plant feeders	Predatory	Omnivores
Soil treatment	Control (N=9)	818±87 ab	80.7±1.5 c	10.3±1.8 a	3.8±0.3 a	5.2±1.1 a
	BCA (N=10)	860±100 ab	77.5±1.4 c	12.0±1.4 a	4.7±0.4 a	5.8±1.2 a
	Fum (N=10)	1616±122 a	98.3±1.2 a	1.1±1.0 c	0±0 b	0.6±0.8 b
	Fum+BCA (N=10)	1502±133 a	99.5±1.4 a	0.4±0.8 c	0±0 b	0.1±0.6 b
	YTC-2014 (N=9)	466±57 ab	84.1±1.3 bc	7.1±1.5 ab	5.4±0.9 a	3.4±1.0 a
	YTC-2014+BCA (N=8)	320±71 b	84.3±1.5 bc	9.4±0.9 a	2.8±0.5 a	3.5±1.0 a
	AWC-2014 (N=10)	566±60 ab	92.0±0.8 b	4.6±1.3 ab	0.8±0.3 a	2.6±1.1 a
	AWC-2014+BCA (N=9)	640±82 ab	91.1±1.1 b	4.3±1.2 b	2.00.4 a	2.6±1.2 a
Plant type	Apple (N=38)	840±82 a	89.4±1.3 a	4.7±1.1 b	2.4±0.6 a	3.5±0.9 a
	Sweet cherry (N=37)	857±100 a	87.9±1.2 a	7.4±1.4 a	2.3±0.7 a	2.4±1.2 a
P-value	Soil trt	0.002	<0.001	<0.001	<0.001	<0.001
	Plant type	0.691	0.161	0.002	0.970	0.100
	Soil trt x plant type	0.469	0.462	0.526	0.752	0.060

The effect of soil treatment on EI, Si, and CI was not dependent on plant type (soil treatment x plant type interaction *P*-value >0.05). Soil receiving AWC-2014 and AWC-2014+BCA had greater EI values relative to Fum, Fum+BCA, YTC-2014, and the control (Table 2.13). The EI was greater in pots planted with sweet cherry seedlings than with apple. Fum and Fum+BCA reduced the SI relative to all other soil treatments. Fum+BCA had a lower CI relative to YTC-2014.

Table 2.13 - Effect of soil treatments and plant type on soil food web indices. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, AWC-2014 refers to agricultural waste compost, EI refers to enrichment index, SI refers to structure index, and CI refers to channel index. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Nematode faunal analysis		
Factor	Level	EI	SI	CI
Soil treatment	Control	13.3±1.5	35.7±5.2	6.9±1.8
	(N=9)	bc	a	ab
	BCA	16.1±1.5	42.4±3.8	9.0±1.7
	(N=10)	ab	a	ab
	Fum	8.7±1.2	6.6±3.2	1.4±1.1
	(N=10)	c	b	ab
	Fum+BCA	12.6±1.9	4.4±3.2	0.3±0.5
	(N=10)	bc	b	b
	YTC-2014	10.0±1.3	36.9±3.2	15.0±1.4
	(N=9)	c	a	a
	YTC-2014+BCA	13.5±1.5	44.1±2.6	13.0±1.3
	(N=8)	bc	a	ab
	AWC-2014	21.3±2.4	33.1±4.2	1.8±1.3
	(N=10)	a	a	ab
	AWC-2014+BCA	18.0±1.2	29.9±3.6	3.4±0.9
	(N=9)	ab	a	ab
Plant type	Apple	12.4±0.9	27.8±3.1	6.1±1.8
	(N=38)	b	a	a
	Sweet cherry	15.9±1.2	30.4±2.7	6.3±1.4
	(N=37)	a	a	a
P-value	Soil trt	<0.001	<0.001	0.006
	Plant type	0.003	0.337	0.919
	Soil trt x plant type	0.381	0.912	0.804

2.3.3.3 Isolation Frequency of Necrotrophic Fungi

Fum and Fum+BCA treatments decreased the recovery of *Fusarium* sp. F1 from roots relative to the control (Table 2.14). *Fusarium* sp. F1 was recovered more often from sweet cherry roots than from apple. Recovery of *Fusarium* sp. F2 from roots of apple and sweet cherry was reduced in the Fum+BCA and YTC-2014+BCA treatments relative to the control. Recovery of "*Cylindrocarpon*" sp. C1 from sweet cherry was greatest under the control and BCA treatments, and reduced by Fum, AWC-2014, and YTC-2014, regardless of biocontrol inoculation. Interestingly, "*Cylindrocarpon*" sp. C1 was not recovered from apple roots. Fum reduced the recovery of "*Cylindrocarpon*" sp. C2

relative to all other soil treatments. Soil treatment and plant type did not influence the recovery of *Rhizoctonia* sp. R1. Recovery of "*Cylindrocarpon*" sp. C1 was negatively correlated with shoot length, shoot weight, root length, root surface area, and total biomass (Appendix C). "*Cylindrocarpon"* sp. C2 was negatively correlated with root weight and total biomass. *Fusarium* sp. F1 was negatively correlated with shoot length, root length, and root surface area.

Table 2.14 - Effect of soil treatments and plant type on percent recovery of replant disease-associated fungi. Data were analyzed with a two-way ANOVA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Percent recovery (%)				
Factor	Level	*Fusarium* sp. F1	*Fusarium* sp. F2	"*Cylindro-carpon*" sp. C1	"*Cylindro-carpon*" sp. C2	*Rhizoctonia* sp. R1
Soil treatment	Control (N=9)	70.3±10.4 a	22.1±7.9 a	29.6±7.7 a	29.5±8.7 a	7.3±4.9 a
	BCA (N=10)	50.0±11.4 a	3.3±3.3 ab	26.7±8.0 a	16.6±7.5 a	13.3±7.4 a
	Fum (N=10)	13.3±8.4 b	3.3±3.3 ab	0±0 b	0±0 b	6.7±6.7 a
	Fum+BCA (N=10)	13.3±8.2 b	0±0 b	3.3±2.3 b	3.7±3.3 a	6.6±4.4 a
	YTC-2014 (N=9)	40.8±14.5 a	3.7±3.7 ab	11.0±5.5 b	11.1±7.9 a	3.7±3.7 a
	YTC-2014+BCA (N=8)	33.4±16.1 a	3.3±3.3 ab	12.4±6.0 b	8.3±5.4 a	0±0 a
	AWC-2014 (N=10)	29.9±10.5 ab	16.6±7.5 a	10.0±7.1 b	9.9±5.0 a	3.3±3.3 a
	AWC-2014+BCA (N=9)	48.1±16.8 a	11.1±7.9 a	7.3±4.9 b	11.0±5.5 a	3.7±3.7 a
Plant type	Apple (N=38)	21.0±5.3 b	5.6±2.0 a	0±0 b	12.9±3.4 a	2.4±1.6 a
	Sweet cherry (N=37)	55.4±6.4 a	9.7±3.4 a	24.8±4.7 a	9.8±2.8 a	8.6±3.1 a
P-value	Soil trt	0.003	0.052	0.006	0.022	0.552
	Plant type	<0.001	0.379	<0.001	0.545	0.053
	Soil trt x plant type	0.067	0.582	0.004	0.309	0.261

2.3.3.4 Soil Microbiology

The effect of soil treatment on soil microbiology was not dependent on plant type (soil treatment x plant type interaction *P*-value >0.05). An effect of soil treatment

was observed on each group of rhizosphere microorganism quantified (Table 2.15). For quantification of total bacteria, the BACT1369F/PROK1492R primer set provided reaction efficiencies of 98.9 to 100.6%, with R^2-values >0.999. AWC-2014 had a greater abundance of total bacteria than the Fum or control treatments. Total bacteria were more abundant in YTC-2014 than in the Fum treatment. For quantification of total fungi, the FF390/FR1 primer set provided reaction efficiencies of 91.3 to 93.7%, with R^2-values >0.999. Fum resulted in less fungal DNA in soil than the control, AWC-2014, and YTC-2014 treatments.

For quantification of *Pseudomonas* spp., the Pse435F/Pse686R primer set provided reaction efficiencies of 99.3 to 100.7%, with R^2-values >0.999. AWC-2014 had a greater abundance of *Pseudomonas* spp. than the Fum treatment. For quantification of DAPG+ bacteria, the BPF2/BPR4 primer set provided reaction efficiencies of 94.6 to 98.6%, with R^2-values >0.999. DAPG+ bacteria were more abundant in AWC-2014 than in the control or Fum treatments. YTC-2014 had a greater abundance of DAPG+ bacteria in than the Fum treatment. For quantification of PRN+ bacteria, the PrnD-F/PrnD-R primer set provided reaction efficiencies of 99.4 to 101.7%, with R^2-values >0.999. AWC-2014 had a greater abundance of PRN+ bacteria in soil than the control or Fum treatments. PRN+ bacteria were more abundant in YTC-2014 than in the Fum treatment. Plant type had a significant main-factor effect on the abundance of PRN+ bacteria. Populations of PRN+ bacteria were greater in pots planted with apple than those with sweet cherry.

Table 2.15 - Effect of soil treatments and plant type on the abundance of beneficial soil microorganisms. Data were analyzed with a two-way ANOA. BCA refers to biocontrol inoculation with *Serratia plymuthica* 6-5, Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, AWC-2014 refers to agricultural waste compost, DAPG+ bacteria refers to 2,4-diacetylphloroglucinol-producing bacteria, and PRN+ bacteria refers to pyrrolnitrin-producing bacteria. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Total bacteria (log 16S gene copies g^{-1} soil)	Total fungi (log 18S gene copies g^{-1} soil	*Pseudomonas* spp. (log 16S gene copies g^{-1} soil)	DAPG+ bacteria (log *phlD* gene copies g^{-1} soil)	PRN+ bacteria (log *prnD* gene copies g^{-1} soil)
Soil treatment	Control (N=9)	8.68±0.03 bc	7.20±0.17 a	5.92±0.28 ab	4.21±0.07 bc	4.94±0.12 bc
	Fum (N=10)	8.51±0.05 c	6.15±0.13 b	5.74±0.21 b	4.14±0.06 c	4.85±0.07 c
	YTC-2014 (N=9)	8.80±0.06 ab	7.21±0.09 a	6.51±0.15 ab	4.42±0.08 ab	5.26±0.09 ab
	AWC-2014 (N=10)	8.92±0.06 a	7.25±0.09 a	6.68±0.22 a	4.52±0.07 a	5.40±0.09 a
Plant type	Apple (N=19)	8.73±0.04 a	6.98±0.14 a	6.25±0.13 a	4.30±0.05 a	5.26±0.07 a
	Sweet cherry (N=19)	8.72±0.07 a	6.93±0.14 a	6.16±0.21 a	4.30±0.06 a	4.98±0.11 b
P- value	Soil trt	<0.001	<0.001	0.014	<0.001	0.004
	Plant type	0.847	0.688	0.692	0.604	0.014
	Soil trt x plant type	0.215	0.407	0.846	0.593	0.375

When normalized absorbance values from the Biolog Ecoplates were subjected to PCA (Figure 2.1); the first principal component accounted for 26.4% of the variance in the data set, and the second principal component accounted for 20.6% of the variance. According to the NPMANOVA, soil treatment (*P*-value = 0.007) had a significant effect on the correlation matrix; however, plant type (*P*-value = 0.137) and plant type x soil treatment interaction (*P*-value = 0.986) did not. CLPPs from different soil treatments appeared to separate along the second principal component, with Fum, compost amended, and the control pots each forming significantly distinct clusters from one another.

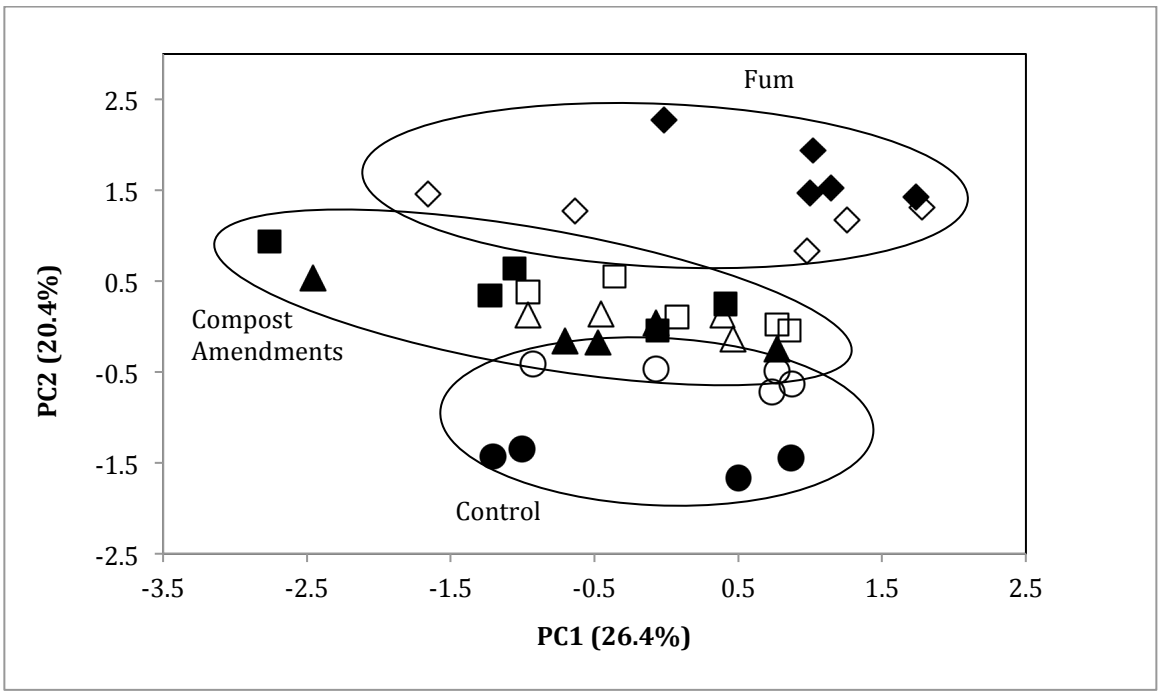

Figure 2.1 - Principal components analysis (PCA) of community level physiological profile (CLPP) data from Biolog Ecoplates, grouped according to soil treatment. Plant types include apple (shaded) and sweet cherry (unshaded). Soil treatments include a control (circle), Fum (diamond) = diamond, YTC-2014 (triangle) = triangle, and AWC-2014 (square). Fum refers to fumigation. Ellipses show significant differences (*P*-value < 0.05) in soil treatment, according to non-parametric multivariate analysis of variance (NPMANOVA).

2.3.3.5 Soil Nutrient Analyses

The effect of soil treatment on soil nutrition was not dependent on plant type (soil treatment x plant type interaction *P*-value >0.05). Total C and N contents were greater, and C/N ratios lower in YTC-2014 and AWC-2014-amended pots than the Fum and control treatments (Table 2.16). Soil OM content averaged 3.38% in compost-amended pots, compared with 2.05% in non-amended pots. Plant type did not have an effect on soil C or N. Pots amended with YTC-2014 possessed lower residual nitrite and nitrate N (NO_2-N+NO_3-N) than the control treatment or pots that received AWC-2014. Pots planted with apple possessed greater residual NO_2-N+NO_3-N than pots planted with sweet cherry. Soil treatment and plant type did not significantly affect residual NH_4-N content in pots.

Table 2.16 - Effect of soil treatments and plant type on soil nutrition. Data were analyzed with a two-way ANOVA. Fum refers to fumigation, YTC-2014 refers to yard trimmings compost, and AWC-2014 refers to agricultural waste compost, NO_2-N+NO_3-N refers to nitrite and nitrate N, NH_4-N refers to ammonium N, and OM refers to organic matter. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil mineral N contents		Soil C and N contents			
Factor	Level	NO_2-N+NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	C (%)	N (%)	C/N (%)	OM (%)
Soil treatment	Control (N=9)	50.7±5.49 a	1.83±0.50 a	1.18±0.08 b	0.07±0.01 b	15.8±0.28 a	2.03
	Fum (N=10)	36.6±5.20 ab	1.52±0.36 a	1.19±0.07 b	0.08±0.01 b	15.5±0.23 a	2.06
	YTC-2014 (N=9)	23.4±4.96 b	1.46±0.44 a	1.96±0.08 a	0.13±0.01 a	14.1±0.30 b	3.37
	AWC-2014 (N=10)	44.3±3.70 a	1.91±0.63 a	1.97±0.13 a	0.14±0.01 a	14.8±0.28 b	3.39
Plant type	Apple (N=19)	46.0±4.00 a	1.56±0.28 a	1.61±0.12 a	0.11±0.01 a	15.0±0.25 a	2.77
	Sweet cherry (N=19)	35.1±3.61 b	1.78±0.40 a	1.54±0.08 a	0.10±0.01 a	15.1±0.21 a	2.64
P- value	Soil trt	0.001	0.819	<0.001	<0.001	0.002	-
	Plant type	0.004	0.641	0.353	0.248	0.808	-
	Soil trt x plant type	0.537	0.808	0.185	0.124	0.364	-

2.3.4 Pathogenicity Trials

Sequence results from the 28S rRNA region of the indigenous root-lesion nematode population present in soil at the apple orchard site confirmed previous morphologically-based identification, showing 99.2% sequence identity with *P. penetrans* isolate MU2 (GenBank accession number KP161612) (Appendix B). In the first pathogenicity experiment, fungal inoculation did not affect total shoot extension of 2-year old Ottawa 3 rootstock at any measurement date (Appendix D).

In the second, multifactorial pathogenicity experiment, fungal inoculation had a significant effect on total shoot extension, shoot weight, root weight, and biomass of apple seedlings; however, inoculation with *P. penetrans* did not have a significant effect on these parameters (Table 2.17). A significant interaction effect was not observed between nematodes and fungi for any parameter. Total shoot extension was suppressed

by "*Cylindrocarpon*" sp. C1-1, and "*Cylindrocarpon*" sp. C2-1 relative to the control. Shoot weight and total biomass were suppressed by "*Cylindrocarpon*" sp. C1-1, "*Cylindrocarpon*" sp. C2-1, and "*Cylindrocarpon*" sp. C2-2, relative to the control. Root weight was supressed by inoculation with "*Cylindrocarpon*" sp. C1-1 and "*Cylindrocarpon*" sp. C2-2. Overall, *P. penetrans* populations in roots were very low (<15 *P. penetrans* g^{-1} root), and fungal inoculation did not significantly affect their population abundance.

Table 2.17 - Effect of fungal isolates and *P. penetrans* on growth of apple seedlings and root infestation by *P. penetrans*. Data were analyzed with a two-way ANOVA. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Shoot extension (cm)	Shoot weight (g)	Root weight (g)	Total biomass	*P. penetrans* g^{-1} root
Fungi	Control (N=8)	29.2±2.2 ab	6.15±0.44 a	2.60±0.19 a	8.75±0.49 a	14±2 a
	Fusarium sp. F1-1 (N=8)	25.5±3.3 abc	4.62±0.38 ab	1.62±0.31 ab	6.52±0.57 ab	7±1 a
	Fusarium sp. F1-2 (N=8)	26.4±3.2 abc	4.71±0.39 ab	2.24±0.28 ab	6.95±0.45 ab	8±1 a
	Fusarium sp. F2-1 (N=8)	37.0±4.1 a	5.93±0.49 ab	1.96±0.27 ab	7.89±0.61 ab	7±2 a
	Fusarium sp. F2-2 (N=8)	22.1±2.7 abc	4.90±0.34 ab	2.49±0.27 ab	7.39±0.46 ab	14±2 a
	"*Cylindrocarpon*" sp. C1-1 (N=8)	14.9±2.6 c	3.91±0.42 b	1.59±0.17 b	5.49±0.56 b	10±2 a
	"*Cylindrocarpon*" sp. C1-2 (N=8)	17.4±2.2 bc	4.81±0.27 ab	2.09±0.23 ab	6.90±0.45 ab	8±1 a
	"*Cylindrocarpon*" sp. C2-1 (N=8)	12.7±2.4 c	4.30±0.50 b	1.83±0.19 ab	6.12±0.63 b	13±2 a
	"*Cylindrocarpon*" sp. C2-2 (N=8)	17.1±2.9 bc	4.24±0.37 b	1.43±0.17 b	5.67±0.40 b	9±2 a
	Rhizoctonia sp. R1-1 (N=8)	23.8±3.0 abc	5.41±0.30 ab	2.72±0.30 a	8.13±0.40 a	7±1 a
	Rhizoctonia sp. R1-2 (N=8)	26.0±3.9 abc	5.69±0.42 ab	1.99±0.23 ab	7.67±0.60 ab	10±2 a
Nematode	Control (N=40)	22.2±1.59 a	5.03±0.22 a	1.97±0.11 a	7.00±0.30 a	-
	P. penetrans (N=40)	23.2±1.79 a	4.96±0.19 a	2.10±0.13 a	7.06±0.26 a	10±2
P-value	Fungi	<0.001	0.001	0.005	<0.001	0.652
	Nematode	0.513	0.757	0.348	0.858	-
	Fungi x nematode	0.120	0.453	0.323	0.407	-

2.4 Discussion

2.4.1 Influence of Compost Amendments

Previous studies have demonstrated that compost amendments can promote the growth of fruit trees planted into old orchard soil (Braun et al., 2010; Gur et al., 1998; Moran and Schupp, 2003; Peryea and Covey, 1989; van Schoor et al., 2009). Additionally, Braun et al. (2010) showed that improved plant growth in old orchard soil through the use of compost was also associated with reductions in *P. penetrans* populations. In this study, composts increased shoot and root growth of apple and sweet cherry seedlings planted in potted apple orchard soil. Composts also reduced *P. penetrans* populations and recovery of "*Cylindrocarpon*" sp. C1 from roots, and this pest/pathogen suppression likely contributed significantly to the improved plant growth associated with the compost amendments. Similar results have also been observed with red raspberry (*Rubus idaeus* (L.)) (Forge et al., 2016a), where preplant incorporation of compost reduced *P. penetrans* populations in soil and improved early plant growth. By providing significant control of parasitic nematode and pathogenic fungal populations in the early stages of plant growth, preplant soil incorporation of compost may lead to increased orchard productivity throughout the life of an orchard as a result of establishment of an extensive network of healthy roots (Braun et al., 2010). Overall, these data suggest composts have potential to improve growth of apple and sweet cherry seedlings planted into potted old apple orchard soil in a controlled greenhouse environment.

A number of organisms have been shown to suppress populations of plant-parasitic nematodes and pathogenic fungi, including antibiotic-producing rhizobacteria (Haas and Défago, 2005; Oostendorp and Sikora, 1989; Siddiqui et al., 2005; Spiegel et

al., 1991; Stirling et al., 1990) and predatory nematodes (Jairajpuri and Bilgrami, 1990; Jones, 1974; Small, 1988). In this study, increased plant growth and pathogen suppression as a result of agricultural waste compost application coincided with an increase in the abundance of total bacteria, DAPG+ bacteria, and PRN+ bacteria in soil, but not with increases in predacious nematodes. Increases in these groups of rhizobacteria have been linked with soil suppressiveness in a number of different pathosystems (de Souza et al., 2003; Garbeva et al., 2004b; Latz et al., 2012; Mazzola, 1999; Mazzola and Gu, 2002, 2000), and they are known to display strong antagonistic activity to nematodes and fungi (Costa et al., 2009; Nandi et al., 2015). Changes in soil bacterial community composition also have been linked with improved establishment of fruit trees in old orchard soil (Caputo et al., 2015; Franke-Whittle et al., 2015; Manici et al., 2015b; Nicola et al., 2017; Peruzzi et al., 2017; Sun et al., 2014). In this study, compost-amended, fumigated, and control pots each possessed a functionally distinct soil microbial community, based on their C-substrate utilization profiles. Manici et al. (2015b) demonstrated that improved growth in gamma-irradiated old orchard soil was associated with shifts in the rhizospheric bacterial community to increases in the proportion of *Pseudomonas* and *Novosphingobium* species. In this study, changes in soil microbial community composition, as evaluated by Biolog Ecoplates, could not be directly linked to the increases in populations of total bacteria, *Pseudomonas* spp., or antibiotic-producing bacteria; however, our data suggest that compost amendments promote the development of a functionally distinct soil microbial community that is also associated with increased abundance of total bacteria and antibiotic-producing bacteria, which may have contributed directly to *P. penetrans* and "*Cylindrocarpon*" suppression as well as

subsequent plant growth promotion.

Of the two composts evaluated, only agricultural waste compost consistently increased root surface area and total biomass of seedlings relative to that of the control. Similarly, agricultural waste compost resulted in greater residual NO_2-N+NO_3-N in soil, greater enhancement of bacterial populations in soil, greater promotion of bacterial feeding nematode populations, and a greater nematode EI than did yard trimmings compost. Although we did not observe a significant effect of compost type on the decomposition pathway, as indicated by CI value, agricultural waste compost appears to have increased populations of bacteria and bacterial grazers in soil, and this may have contributed to enhanced N mineralization by this amendment relative to yard trimmings compost (Ferris et al., 2004). Data on EI were consistent with other indicators of greater nutrient availability (mineral N content) with agricultural waste compost. Overall, this study suggests that, in addition to increasing the abundance of beneficial soil microorganisms, compost from agricultural waste appears to have increased net mineralisation of nutrients in amended soil relative to yard trimmings compost, which may have contributed to enhanced root growth and biomass promotion by this amendment.

2.4.2 Influence of Biocontrol Agent

Disease control through the introduction of natural antagonists, such as antibiotic-producing rhizobacteria, is an environmentally friendly and desirable alternative to the use of chemical fumigants. In this study, 16S rRNA sequence-based identification of prospective biocontrol isolates 6-5 and 6-20 suggested that their identity was *S. plymuthica*; however, previous FAME-based identification performed by Hynes et

al. (2008) suggested that the isolates were *C. acidovorans* and *P. agglomerans*, respectively. As has previously been documented, FAME-based identification does not always correspond to 16S rRNA sequence-based identification (Hynes et al., 2008). In this study, *S. plymuthica* 6-5 showed significant antagonistic potential to replant-associated fungal pathogens on growth plates. Similar levels of *in vitro* growth reduction to fungal pathogens of lentil (*F. avenaceum* (Fr.)) and chickpea (*R. solani* (Kuhn)) have previously been reported for this particular isolate (Hynes et al., 2008).

This study found that *in vitro* growth suppression of fungal pathogens did not correspond with plant growth promotion or pathogen suppression when the biocontrol agent was applied as a root-dip inoculant on apple and sweet cherry seedlings planted in potted orchard soil, with or without compost amendment. Failure of biocontrol isolates to suppress pathogens *in vivo*, despite considerable *in vitro* antagonism, has been a common obstacle during the development of microbial inoculants for control of plant diseases. Many studies have reported a lack of relationship between the ability of prospective biocontrol bacteria to inhibit pathogens *in vitro* and suppress disease caused by the pathogen *in vivo* (Schroth and Hancock, 1981; Wong and Baker, 1984). A number of factors have been proposed to contribute to inconsistencies in biocontrol performance in greenhouse and field trials (Weller, 1988), including loss of ecological competence through prolonged culturing in the lab, non-target pathogen interference, and failure to effectively colonize inoculated plant roots. In this study, biocontrol failure was not associated with inadequate root colonization, suggesting that this isolate may have lacked the required nutrients to produce secondary metabolites in sufficient quantities in the rhizosphere to effectively protect roots from invasion, or that any such antagonistic

metabolites that were produced were degraded/inhibited by the activity of other rhizosphere microorganisms. Future research on the development of rhizobacterial biocontrol inoculants should aim to isolate new strains from a naturally suppressive fruit tree rhizosphere, potentially increasing retention of ecological competence and presumably, biocontrol capacity.

2.4.3 Replant-Associated Fungi and Pathogenicity Trials

Fungi that were associated with replant disease and were indigenous to the soil from this apple orchard site included "*Cylindrocarpon*" sp. C1, *Fusarium* sp. F1, *Fusarium* sp. F2, and *Rhizoctonia* sp. R1, with AWA+streptomycin isolation medium showing a slightly greater breadth of coverage of relevant soil fungi relative to that of PDA+streptomycin isolation medium. Using AWA+streptomycin isolation medium, "*Cylindrocarpon*" sp. C1, "*Cylindrocarpon*" sp. C2, *Fusarium* sp. F1, *Fusarium* sp. F2, and *Rhizoctonia* sp. R1 were the predominant fungal morphotypes recovered from surface sterilized apple and sweet cherry roots. Seedling growth parameters showed a negative correlation with the recovery of "*Cylindrocarpon*" sp. C1, "*Cylindrocarpon*" sp. C2, and *Fusarium* sp. F1, suggesting that these fungi may have contributed to poor seedling growth in this orchard soil. These results are in agreement with a recent survey of rhizosphere fungal communities in replanted orchards in Central Europe using next-generation sequencing technology, which also showed a negative correlation between plant growth and "*Cylindrocarpon*" and *Fusarium* (Franke-Whittle et al., 2015). Similarly, the recovery of *Ilyonectria* spp. from apple roots has also bee reported to be strongly negatively correlated with plant growth in old orchard soil (Manici et al., 2013).

In the first pathogenicity bioassay, inoculation with fungal isolates did not significantly affect shoot extension of 2-year-old Ottawa 3 apple rootstock throughout the 12-week trial period. Such a result could potentially be due to the mature root system being able to tolerate the introduced pathogen (Jaffee and Mai, 1979) rather than host genetic resistance, as reports of genetic resistance to replant disease-associated pathogens have not been previously documented for this rootstock. Differences in root architecture and composition between the rootstocks used in the pathogenicity bioassay and the seedlings that the fungi were originally isolated from may have also contributed to differences in pathogen tolerance. In the multi-factorial experiment, planting apple seedlings into pasteurized soil that had been inoculated with "*Cylindrocarpon*" sp. C1 or "*Cylindrocarpon*" sp. C2 resulted in reduced plant biomass relative to non-inoculated pots; however, inoculation with *Fusarium* sp. F1, *Fusarium* sp. F2, or *Rhizoctonia* sp. R1 did not reduce seedling growth. In the greenhouse replant experiment, reduced plant growth of apple and of sweet cherry seedlings was correlated with increased recovery of *Fusarium* spp.; however, the isolates did not display negative effects on apple seedling growth in the pathogenicity bioassay. These discrepancies can potentially be attributed to fungi from this genus having highly variable effects on plant growth (Manici et al., 2017). In this study, the *Fusarium* isolates selected for this pathogenicity bioassay may not have been pathogenic; however, other members of the same species might still exhibit some degree of pathogenicity, or an environment conducive to disease development may not have been provided (Lewis, 1985). Interestingly, "*Cylindrocarpon*" sp. C1 was only isolated from sweet cherry seedlings in the initial greenhouse replant experiment, but isolates were later demonstrated to reduce apple seedling growth in the pathogenicity

trial. This finding suggests that reductions in apple growth may have been associated with production of deleterious extracellular metabolites and/or mycotoxins that could affect a range of fruit tree species and thereby contribute to non-specific replant disease. This is in agreement with a recent study which showed that gamma ray-sterilized fungal culture filtrates of *Ilyonectria europaea* (Cabral, Rego & Crous) and *I. robusta* (Cabral & Crous) isolated from the rhizosphere of apple trees reduced seedling growth (Manici et al., 2017). This may have been due to the high lytic activity of the filtrates, causing significant softening of the root tissue. Overall, these data support the hypothesis that fungal metabolites released into the soil may represent a significant component of the non-specific replant disease complex (Manici et al., 2017).

In the multi-factorial pathogenicity experiment, inoculation with *P. penetrans* was not associated with reductions in plant growth, likely due to the very low density of nematodes actively parasitizing the roots at the time of analysis. Jaffee et al. (1982) reported that addition of 140 *P. penetrans* per 100 mL of soil resulted in significant damage and necrosis to apple seedlings. Similarly, Dullahide et al. (1994) reported that inoculation with as few as 77 *P. penetrans* per 100 mL of soil could result in reductions in apple seedling biomass. In this experiment, *P. penetrans* were inoculated at a density of 100 *P. penetrans* per 100 mL of soil; however, very few *P. penetrans* were extracted from roots 12 weeks later. This may be due to suboptimal greenhouse environmental conditions at the start of this experiment. When the experiment was initiated (August 2016), the ambient greenhouse temperature regularly exceeded 40 °C during the day, which is beyond the optimal temperature for nematode survival (Acosta and Malek, 1979), and this may have adversely affected the survival of inoculated nematodes.

Similarly, nematodes may have been washed out of the soil as a result of excessive watering of the pots in order to maintain adequate moisture content during this period of thermal stress. Excessive ambient temperature in the greenhouse may have also had an effect on the pathogenicity of fungal pathogens and plant host tolerance in this study, suggesting that data from the pathogenicity trials be interpreted with caution.

3 Chapter 3: Field Replant Experiment

3.1 Background

Planting fruit trees in old orchard soil presents a significant barrier to the establishment of productive tree fruit orchards (Mai and Abawi, 1978). If disease management practices are not implemented, or are not successful, replant disease can delay fruit production, decrease fruit quality, and reduce fruit yield, preventing an orchard from reaching an acceptable level of productivity (Mazzola, 1998). The root lesion nematode, *Pratylenchus penetrans*, has a widespread occurrence throughout orchards in the Okanagan Valley, Canada (Forge et al., 2013a; Vrain and Yorston, 1987), and contributes significantly to poor establishment of fruit trees in old orchard soil (Utkhede et al., 1992). Similarly, inadequate soil P nutrition is the abiotic factor most commonly associated with replant disease (Neilsen and Yorston, 1991; Slykhuis and Li, 1985; Wilson et al., 2004), and likely contributes to poor tree growth alongside biological agents. The biological components of replant have commonly been controlled through application of chemical fumigants; however, recent restrictions limiting their use are increasing interest in non-fumigant control methods, particularly those that can be utilized in an integrated pest management approach.

Application of OM to soil, such as in the form of soil incorporated compost amendments or surface applied organic mulches, has many benefits to soil health (Gagnon et al., 2012; Granatstein and Mullinix, 2008; Hargreaves et al., 2008; Jakobsen, 1995), including suppression of soil-borne pathogens (Braun et al., 2010; Brown and Tworkoski, 2004; Forge et al., 2008, 2003; Stirling et al., 1995). Preplant incorporation of compost has previously been demonstrated to increase growth of apple trees in a field

replant setting (Moran and Schupp, 2003, 2005; van Schoor et al., 2009) as well as suppress *P. penetrans* populations (Braun et al., 2010). Surface application of high C organic mulch has also shown potential to suppress *P. penetrans* in perennial cropping systems (Forge et al., 2013b, 2008, 2003; Forge and Kempler, 2009) and has been associated with improved growth of apple trees (Forge et al., 2013b, 2003; Granatstein and Mullinix, 2008; Merwin et al., 1994; Neilsen et al., 2014, 2003; Shribbs and Skroch, 1986; van Schoor et al., 2009). Plant-parasitic nematode suppression through the use of organic soil amendments is thought to be a result of promoting the activity and abundance of natural antagonists in soil (Cooke, 1968; Linford et al., 1938; Stirling, 2014).

Organic soil amendments have previously been shown to increase populations of antagonistic rhizobacteria (Stirling, 2014; Chapter 2), antagonistic fungi (Cooke, 1968), predacious nematodes (Yeates and Wardle, 1996), and AMF (Franca et al., 2007; Gryndler et al., 2006), all of which may contribute to enhanced growth in old orchard soil. Increases in populations of antagonistic bacteria in the rhizosphere are believed to contribute to establishing suppressiveness to soil-borne pathogens/parasites of roots, including *P. penetrans* (Siddiqui and Shaukat, 2003; Chapter 2). Antagonistic fungi and predacious nematodes can exhibit suppressive capacity by actively preying on root-lesion nematodes (Dong and Zhang, 2006). Likewise, increases in AMF may contribute to improved P nutrition during early plant establishment (Forge et al., 2008). Currently, there is interest in whether these groups of organisms contribute to improving plant growth of fruit trees receiving organic soil amendments.

As a result of improved water-use efficiency relative to over-head sprinklers (van der Gulik, 1999) and the capacity to deliver chemical fertilizers directly to the root zone (Neilsen et al., 1998), low-volume irrigation systems, including drip emitters and microsprinklers, are rapidly becoming the industry standard. Water management practices implemented after planting may have strong effects on tree establishment at replant sites; however, very little is known regarding what influence different low-volume irrigation systems have on fruit tree establishment, and almost nothing is known about how plant-parasitic nematode and microbial antagonist populations are affected. To date, there have been a few reports of improved peach tree growth and yield through the use of drip emitters relative to microsprinklers (Bryla et al., 2003; Layne et al., 1996).

The objective of research described in this chapter was to evaluate the effects of preplant incorporation of compost, surface application of bark chip mulch, and irrigation type (drip emitter or microsprinkler) on (1) early growth and fruit yield of sweet cherry trees planted in orchard soil previously used for apple production, (2) *P. penetrans* population dynamics in soil and root tissue, (3) microbial indicators of soil suppressiveness (microbial populations, soil microbial activity, and biological suppressiveness) and enhanced P nutrition (AMF root colonization and populations of phosphate-solubilizing bacteria), and (4) plant/soil nutrition and water status. As described in Chapter 1, I predicted that organic soil amendments and drip irrigation would improve the establishment of sweet cherry trees planted into an old apple orchard site. I predicted that organic soil amendments would suppress *P. penetrans* populations in roots and soil as well as enhance microbial indicators of soil suppressiveness and enhanced P nutrition. I also predicted that soil management practices and irrigation

emitter type would influence plant/soil nutrition and water status.

3.2 Methods

3.2.1 Site Description

The site selected for renovation in this field replant experiment was the same apple orchard site sampled in the greenhouse replant experiment described in Chapter 2.

3.2.2 Experimental Design

After removing the old apple trees from the orchard site in fall 2013, sixty experimental sub-plots (5 m x 2 m) were overlaid onto the previous tree rows, with one of each of five of the following soil treatments randomly allocated to each of the twelve rows (1) untreated soil (Control), (2) fumigation (Fum), (3) compost (Comp) (4) surface application of bark chip mulch (BCM), and (5) Comp+BCM. The twelve rows were divided into six pairs, and within each pair one randomly selected row was irrigated with microsprinklers (MS) placed between trees, which delivered water over a 1.5-m wide tree root zone (Figure 3.1). The other row was irrigated with drip emitters (2 L h^{-1} rate) located at 30-cm intervals down the row, 15-cm outward from both sides of the tree row. Irrigation was applied daily to supply 100% of the estimated water lost to evapotranspiration the previous day in both treatments (Parchomchuk et al., 1996). In May 2014, sub-plots were planted with four sweet cherry trees ('Skeena' variety on Gi.6 rootstock), at a 1.25-m spacing between trees and 3-m spacing between rows, with all experimental measurements occurring on the two interior trees of each sub-plot. Foliar pest control and nutrient management measures were implemented according to standard production practices (www.bctfpg.ca; accessed January 1, 2014).

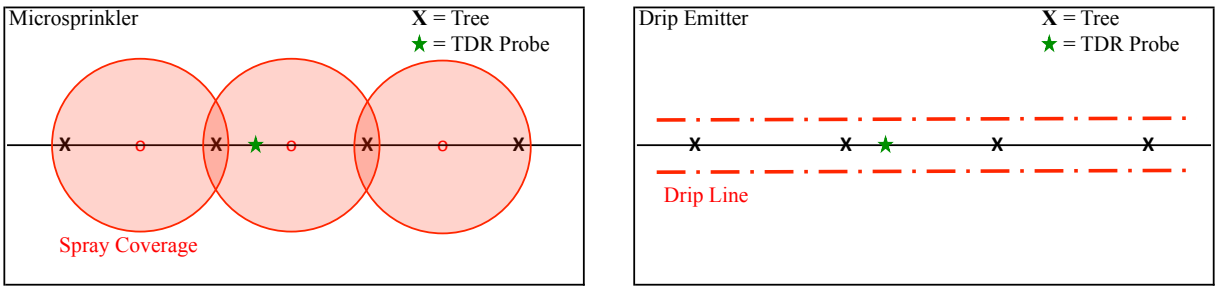

Figure 3.1 - Diagram of irrigation system and time domain reflectometry (TDR) probe layout.

3.2.3 Soil Treatment Application Rates

Sub-plots that were fumigated received 500 g sub-plot^{-1} of Basamid® (Dazomet) (Engage Agro Corp., Guelph, Ontario, Canada) evenly distributed on the soil surface and subsequently rototilled into the soil to a depth of 30 cm in October 2013, after the previous apple trees were removed. Compost (AWC-2014) (Table 2.2) was applied at a rate of 0.225 m^3 sub-plot^{-1} to a 1-m wide strip and rototilled into the soil to a depth of 30 cm on April 7, 2014. BCM (C:N ratio of 122; chip diameter of 5.0 - 7.5 cm) was applied to the soil surface to a height of 5 cm as a 1.5-m wide strip (0.375 m^3 sub-plot^{-1}) after the trees were planted on April 10, 2014. In May 2016, YTC-2016 (Table 3.1) and BCM (C:N ratio of 167) were reapplied to appropriate sub-plots at the same rate as described previously; however, compost was applied to the soil surface of sub-plots without being subsequently rototilled into the soil profile.

Table 3.1 - Chemical and physical properties of compost amendment. YTC-2016 refers to yard trimmings compost, OM refers to organic matter, NO_3-N refers to nitrate N, and TOC refers to total organic C.

Parameter	YTC-2016
pH	8.0
Conductivity (ms cm^{-1})	3.5
OM (%)	28.9
NO_3-N (mg kg^{-1})	75.0
N (%)	1.77
P (%)	0.09
K (%)	0.39
Mg (%)	0.13
Ca (%)	0.44
TOC (%)	17.9
C:N ratio	10.1

3.2.4 Plant Growth and Fruit Yield Measurements

In order to test the hypothesis that organic soil amendments and drip irrigation would improve the establishment of replanted sweet cherry trees, plant growth and fruit yield was measured. Total primary shoot extension was recorded at the end of the first growing season (November 2014) by measuring the distance between the primary shoot apical meristem and the base of the trunk. Trunk diameters were measured 15-cm above the bud union at the end of each growing season (November 2014 through 2017), and trunk cross-sectional areas (TCSA) were computed. In 2016 and 2017, total fruit yield was determined for each sub-plot. Average individual fruit weight was also determined using a random subsample of 100 fruit from each sub-plot.

3.2.5 *P. penetrans* Populations

In order to test the hypothesis that organic soil amendments and drip irrigation would suppress *P. penetrans*, nematode populations in roots and soil were quantified. Soil populations of *P. penetrans* were monitored throughout the first four years of establishment of the newly planted orchard. During each sampling date, a total of six soil cores (30 cm in length, 2.5 cm in diameter) were obtained from each sub-plot, at a

distance of 30 cm from the trunk (or proposed planting hole in the case of preplant sampling). Soil samples were obtained before compost incorporation, as well as in May and September from 2014 through 2017. Soil samples were placed into plastic bags and stored at 4 °C for a maximum of 48 h prior to subsequent processing. Nematodes were extracted from a 100-mL subsample of soil from each sub-plot using the centrifugal-floatation technique (Jenkins, 1964). After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. The abundance of *P. penetrans* was determined using an inverted compound microscope.

Sampling of root tissue for quantification of endoparasitic nematodes commenced in September 2014, for a total of eight sampling dates (September 2014, May 2015, September 2015, May 2016, July 2016, September 2016, May 2017, and September 2017). A hand trowel was used to remove root tissue from a 5 - 30-cm depth and a distance of 30-cm outward from the trunk of each measurement tree. Endoparasitic nematodes were extracted from 2-g subsamples of fine root tissue (<2 mm diameter) using the shaker agitation technique, with a 7-day incubation period (Shurtleff and Averre, 2005). After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting.

3.2.5.1 Other Nematode Populations

Analyses of the total abundance of nematodes in soil were performed on May and September sampling dates from 2014 through 2017. Analyses of other plant-parasitic nematode populations (*Paratrichodorus teres* (Khan & Singh), *Hemicycliophora similis*

(Thorne), *Mesocriconema xenoplax*) and the abundance of predatory nematodes (*Mononchus* spp. (Bastian)) were performed on the May and September sampling dates from 2015 through 2017. *M. xenoplax* was identified to the species level by sequencing a portion of the 28S rRNA gene region, as described previously in Chapter 2 (Appendix B). *P. teres* and *H. similis* were identified to the species level using morphological diagnostic features (Bongers, 1988).

3.2.5.2 Fine Root Length Density in Soil Cores

In order to test the hypothesis that organic soil amendments and drip irrigation would improve the establishment of replanted sweet cherry trees, root growth was measured. On the May and September 2016 and 2017 soil sampling dates, root tissue was collected from soil cores using a 5-mm sieve, and total fine root length (<2mm diameter) was quantified using WinRhizo Regular software (Regent instruments, Montreal, QC, Canada).

3.2.5.3 *P. penetrans* Populations and Fine Root Length Density in Mulch/Topsoil

On July 14, 2017, a supplementary soil sampling was performed to test the hypothesis that fine root density was enhanced in the mulch layer of sub-plots treated with BCM relative to the mineral soil layer below, potentially as a result of reduced *P. penetrans* parasitism. A mulch/soil core was obtained approximately 40 cm from the trunk of each measurement tree from each sub-plot (2 cores sub-plot^{-1}). Metal soil corers (5.25 cm diameter; 30 cm length) were driven through the mulch layer using a rubber hammer and removed from the soil using a pair of pliers. For sub-plots that received surface application of BCM and/or Comp, the height of the mulch layer was carefully measured and subsequently placed into a plastic bag. The remaining soil layer was

divided into 0 - 7.5 cm and 7.5 - 15.0 cm soil depth fractions, and placed into separate plastic bags. Samples from both measurement trees were compiled from each sub-plot, and stored at 4 °C for a maximum of 72 h prior to subsequent processing.

Roots were collected from the soil/mulch samples by sieving on a 5-mm sieve. Analysis of fine root (<2mm diameter) length was performed using WinRHIZO Regular software (Regent Instruments, Quebec City, QC, Canada). Endoparasitic nematodes were extracted from fine root tissue (<2 mm diameter) using the shaker agitation technique, with a 5-day incubation period (Shurtleff and Averre, 2005). Nematodes were also extracted from a 50-mL subsample of soil/mulch using the Baermann pan technique, with a 5-day incubation period (Forge and Kimpinski, 2007). After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting.

3.2.6 Soil Microbiology

3.2.6.1 Molecular Quantification of Rhizosphere Microorganisms

In order to test the hypothesis that organic soil amendments and drip irrigation would increase the abundance of rhizosphere microorganisms with potential biocontrol activity, I measured the number of gene copies of total bacteria, total fungi, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria. DNA was isolated from rhizosphere soil collected in May and September of 2015 and 2016. Approximately 6 g of fine root tissue with adhering rhizosphere soil were placed into a 50-mL centrifuge tube filled with 34 mL of sterile PBS. Tubes were vortexed at maximum speed for 10 min to remove adhering rhizosphere soil, and subsequently centrifuged at 5,000 rpm for 2 min. Clean root tissue was carefully removed from the tubes and the tubes were then centrifuged at

5,000 rpm for an additional 2 min, after which the supernatant was carefully poured off. DNA was isolated from 0.5 g of rhizosphere soil pellet according to the manufacturer's protocols using a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA). A subsample of rhizosphere soil pellet was dried in an oven at 65 °C and weight loss recorded to correct for variability in soil moisture content between samples. Isolated rhizosphere DNA was stored at -85 °C until subsequent analyses. Real-time PCRs for quantification of beneficial microorganisms in the sweet cherry rhizosphere were performed as described previously (Chapter 2).

3.2.6.2 Cultural Quantification of Rhizosphere Microorganisms

In order to test the hypothesis that organic soil amendments and drip irrigation would increase the abundance of rhizosphere microorganisms with potential biocontrol activity and enhanced P nutrition, I measured the abundance of *Pseudomonas* spp. and phosphate-solubilizing bacteria in the rhizosphere. In May and September of 2015 and 2016, approximately 2 g of fine root tissue with adhering rhizosphere soil were placed into a 50-mL centrifuge tube filled with 18 mL of sterile PBS. The tube was vortexed at maximum speed for 10 min to remove adhering rhizosphere soil, and subsequently centrifuged at 5,000 rpm for 2 min, followed by carefully removing the clean root tissue from the tube. The remaining soil pellet was suspended by vortexing for 5 s, and a serial dilution (10^2 - 10^5) was performed after letting the soil suspension settle for 1 min. The soil suspension was then centrifuged at 5,000 rpm for an additional 2 min, after which the supernatant was carefully poured off. The tube and wet rhizosphere pellet were dried in an oven and dry rhizosphere soil pellet weight was recorded. Using the prepared dilution series, 100 µL of solution from each dilution step were spread onto Gould's modified S1

(GmS1) and Pikovskaya's (PVK) growth medium for determination of *Pseudomonas* spp. and phosphate-solubilizing bacteria population abundances, respectively (Pikovskaya, 1948; Tarnawski et al., 2003). GmS1 plates were incubated at room temperature for 36 h prior to counting colonies. Colonies on GmS1 plates that fluoresced when placed under an ultraviolet light were counted as fluorescent *Pseudomonas*. PVK plates were incubated at room temperature for 5 days prior to counting colonies that formed clear halos in the growth medium.

3.2.6.3 Root Colonization by AMF

In order to test the hypothesis that organic soil amendments and drip irrigation would increase the abundance of microorganisms associated with enhanced P nutrition, I measured root colonization by AMF. Root colonization by AMF was determined using the magnified intersections method (McGonigle et al., 1990). Fine roots (20 cm total length) were collected from each sub-plot in May and September from 2015 through 2016, from a separate population of roots than those used for extraction of *P. penetrans*. Roots were washed free of adhering soil using distilled water and stored in 70% ethanol until further analyses. Roots were cut into twenty 1-cm fragments and soaked in 10% KOH for 12 h. Roots were then heated at 95 °C for 1 h in a fresh solution of 10% KOH. They were then rinsed twice with distilled water, and placed in 3% H_2O_2 for 15 min at room temperature. Roots were rinsed in distilled water and placed in 5% HCl for 5 min at room temperature, followed by soaking in trypan blue solution (0.2% w/v) (Vierheilig et al., 2005) for 24 h at room temperature. The roots were then placed in distilled water for 12 h and mounted on a microscope slide using glycerol (aligned parallel to the long axis of the slide). Slides were observed at x200 magnification and the presence of arbuscules

at 100 points of intersection was determined for each root fragment. For each root subsample, the abundance of arbuscules was calculated as the number of intersections with arbuscules present divided by the total number of intersections examined.

3.2.6.4 Soil Microbial Activity

In order to test the hypothesis that organic soil amendments and drip irrigation would increase soil parameters indicative of pathogen suppression, I measured soil microbial activity. In May 2017 and September 2017, hydrolysis of fluorescein diacetate was measured as described by Green et al. (2006). Approximately 5.0 g of soil obtained during routine soil sampling were placed in a 50-mL centrifuge tube with 22.5 mL of 60 mM sodium phosphate buffer and mixed thoroughly (approximate total volume of 25.0 mL). From this suspension, 5.0 mL were transferred to a 50-mL centrifuge tube with 42.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of 4.9 mM fluorescein diacetate (20 mg fluorescein diacetate in 10 mL of acetone). The centrifuge tube was capped, vortexed for 5 s, and placed in an incubator at 37 °C for 3 h. After incubation, 2 mL of acetone were added to the tube, vortexed for 5 s, and then centrifuged for 7.5 min at 5,000 rpm. Approximately 200 µL of supernatant were transferred, in triplicate, into wells of a 96-well microtitre plate and absorbance measured on a spectrophotometer at 490 nm (xMark^{TM} Microplate Absorbance Spectrophotometer, Bio-Rad, Hercules, California, USA). The concentration of fluorescein released was calculated by reference to a standard curve consisting of 0.03, 0.1, 0.3, and 0.5 mg solutions of fluorescein prepared in the same solution matrix described above. Controls were performed for each soil treatment x irrigation type combination to correct for variation in background fluorescence between treatments. A composite soil sample was obtained for each soil

treatment x irrigation type combination by combining 5 g of soil from each of the six replicate sub-plots, and mixing thoroughly. Approximately 5.0 g of soil were placed in a 50-mL centrifuge tube with 22.5 mL of 60 mM sodium phosphate buffer and mixed thoroughly (approximate total volume of 25 mL). From this suspension, 5.0 mL were transferred to a 50-mL centrifuge tube with 42.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of acetone (in place of fluorescein diacetate substrate). The centrifuge tube was incubated and analyzed as described above. An additional control was performed to correct for spontaneous fluorescence development by the fluorescein diacetate substrate at 37 °C, which consisted of 47.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of 4.9 mM fluorescein diacetate (no soil).

3.2.7 Soil Biological Suppressiveness Bioassay

In order to test the hypothesis that *P. penetrans* suppression in organic amended and drip irrigated soil was associated with soil biology I measured soil biological suppressiveness. In September 2016 and October 2016, three soil cores (30 cm depth, 2.5 cm diameter) were collected from each sub-plot independent of the routine soil sampling for nematode populations. In May 2017 and September 2017, a subsample of soil was obtained during routine soil sampling for nematode populations. Duplicate 100-g subsamples of field-moist soil were placed into plastic bags and one of each pair was heated at 70 °C for 1 h on two consecutive days. *Meloidogyne hapla* (Chitwood) eggs were extracted from a population maintained on tomato plants by vigorously shaking 1-cm root fragments in 1% NaOCl for 3 min. Extracted *M. hapla* eggs were then hatched on a Baermann funnel (Viglierchio and Schmitt, 1983) over a duration of 10 days. A total of 2,000 J2 stage *M. hapla* were added to the heated and untreated soil. After 14 days at

22 °C under a humidity dome, nematodes were extracted using the Baermann pan technique, using a two-day incubation period. The number of *M. hapla* retrieved from heated and untreated samples of each soil (N_H and N_U, respectively) were used to calculate the percentage suppressiveness (100 X (N_H - N_U)/N_H) (Jaffee et al., 1998). *M. hapla* were identified to the species level by sequencing a portion of the 28S rRNA gene region (Appendix B), as described previously in Chapter 2.

3.2.8 Soil Water Content

In order to test the hypothesis that organic soil amendments and irrigation type would influence soil water dynamics I measured soil water content. Soil volumetric water content was monitored using reflectometer probes (CS616 Water Content Reflectometer Probe; Campbell Scientific, Logan, USA) connected to a data logger (CR1000 Series Data Logger; Campbell Scientific, Logan, USA) using a relay multiplexer (AM16/32 Relay Multiplexer; Campbell Scientific, Logan, USA), which collected hourly measurements over the duration of the four-year study starting on June 24, 2014 (system initialization date). The reflectometer probes were positioned within the tree row (one probe per sub-plot) approximately 15 cm from either of the two drip emitter laterals that ran along both sides of the tree row, and approximately 15 cm from microsprinklers located within the tree row between each tree (Figure 3.1). Measurements were performed on the first six orchard rows (Block 1, 2, and 3) in the Fum, Comp, BCM, and Comp+BCM sub-plots. On September 12, 2016 a system failure occurred, and soil volumetric water content measurements were not collected for the remainder of the third growing season (2016) or the start of the fourth growing season (2017).

3.2.9 Photosynthetic Rate, Stomatal Conductance, Transpiration Rate, and Stem Water Potential

In order to test the hypothesis that organic soil amendments and irrigation type would influence plant water dynamics I measured photosynthetic rate, stomatal conductance, transpiration rate, and stem water potential. Photosynthetic rate, stomatal conductance, and transpiration rate were measured weekly during the growing season in 2017 (9 measurement dates; June through August) using an LCi-SD Ultra Compact Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, England). Measurements were taken between 10:00 am and 12:00 pm under clear skies on two south facing, fully expanded leaves on new extension growth, for each measurement tree. Measurements were performed on Blocks 1, 2, and 3 at approximately 7-day intervals in the Fum, Comp, and BCM sub-plots.

Stem water potentials were also measured weekly during the growing season in 2017 (9 measurement dates; June through August) using a PMS Model 610 pressure chamber (PMS Instrument Company, Albany, Oregon, USA). For each measurement tree, water potentials were measured on two north facing, fully expanded leaves located on new extension growth. While still attached to the tree, leaves were wrapped in black polyethylene and aluminum foil for 30-min prior to leaf removal for measurements, and measurements were taken between 10:00 am and 12:00 pm. Measurements were performed on Blocks 1, 2, and 3 at approximately 7-day intervals in the Fum, Comp, and BCM sub-plots.

3.2.10 Soil OM and Extractable Nutrients

In order to test the hypothesis that organic soil amendments and irrigation type will influence soil nutrition I measured soil OM and extractable nutrients. Prior to planting, as well as at the end of each growing season (2014 through 2015), total soil C and N were assessed on a 0.25 g subsample of air-dried soil from each sub-plot using a combustion analyzer (Leco CHN 628 Series, Leco Corp., St. Joseph, Michigan, USA). Soil OM content was calculated as 1.72 times the total C content (Nelson and Sommers, 1982). For the 2015 soil sampling date, air-dried soil samples from each sub-plot were also sent to A & L Canada Laboratories Inc. (London, Ontario, Canada) for full physiochemical analyses.

3.2.11 Tree Leaf Nutrition

In order to test the hypothesis that organic soil amendments and irrigation type would influence plant nutrition I measured leaf macro- and micronutrients. Leaf nutrient concentrations were determined in July from 2014 through 2016. Twenty leaves were sampled from each sub-plot, oven-dried at 65 $^{\circ}$C, and ground in a stainless steel mill. Leaf P, K, Ca, Mg, Zn, Fe, Mn, Cu, and B concentrations were determined by inductively coupled plasma-optical emission spectroscopy on a Spectroblue ICP-OES (Spectro; Kleve, Germany). Leaf N concentration was determined by combustion analysis on a Leco CHN Analyzer, (Leco CHN 628 Series; St. Joseph, MI).

3.2.12 Statistical Analyses

Data from the field experiment were subjected to a split-plot repeated measures ANOVA in SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). The experimental model was a randomized complete block with six blocks, two irrigation treatments (whole plots),

five soil treatments (split-plots), and a varying number of sampling dates depending on the parameter analyzed. When a significant soil treatment x date or irrigation type x date interaction effect was detected, analyses were performed individually for each sampling date as a split-plot two-way ANOVA; otherwise data were pooled across all sampling dates. When a significant soil treatment x irrigation type interaction effect was detected, analyses were performed across all treatment combinations as a one-way ANOVA. Differences between treatment means were examined using the Bonferroni t-test (*P*-value < 0.05). *P. penetrans* root abundance data were analyzed after a log(x+10) transformation to correct for heteroscedasticity. Soil volumetric water content data were analyzed as the average volumetric water content in each sub-plot for each month that the irrigation system was turned on (May through October). Percent AMF root colonization, percent soil biological suppressiveness, and soil volumetric water content data were analyzed after an arcsine(squareroot(x)) transformation. The relationship between plant growth and molecular and culture-based quantification of *Pseudomonas* spp. was evaluated using Pearson's correlation coefficient. The number of *Pseudomonas* CFU g^{-1} of rhizosphere soil was adjusted to reflect the average 16S rRNA gene copy number of 5.04 for *Pseudomonas* spp. (Klappenbach et al., 2001), prior to correlation analysis.

3.3 Results

3.3.1 Plant Growth and Fruit Yield

Neither soil treatment nor irrigation type significantly affected shoot length in 2014 (Table 3.2). The effect of soil treatment on TCSA was dependent on measurement year (soil treatment x date interaction *P*-value < 0.001); therefore, analyses were performed separately for each measurement date. There was an effect of soil treatment on

TCSA in 2014, when TCSA was found to be greater in the Fum treatment than in the Control, BCM, and Comp+BCM treatments. In 2015, TCSA was greater in the Fum and Comp+BCM treatments than in the Control. In 2016, all soil treatments increased TCSA relative to that of the Control. In 2017, soil treatment did not affect TCSA. Irrigation type affected TCSA throughout the first four years of tree establishment (2014 - 2017). TCSA was consistently greater under the drip irrigation system than trees grown using MS.

Table 3.2 - Effect of soil treatments and irrigation type on growth of newly planted sweet cherry trees. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, MS refers to microsprinkler, and TCSA refers to trunk cross-sectional area. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Shoot length (cm)	TCSA (cm^2)			
Factor	Level	2014	2014	2015	2016	2017
Soil treatment	Control (N=12)	124.9±15.3 a	3.13±0.18 b	6.25±0.48 b	15.1±1.5 b	21.0±1.9 a
	Fum (N=12)	223.3±26.5 a	4.75±0.29 a	8.85±0.53 a	20.5±1.4 a	26.6±1.7 a
	Comp (N=12)	198.5±31.5 a	3.45±0.22 b	7.50±0.49 ab	20.8±1.2 a	29.8±1.7 a
	BCM (N=12)	138.8±22.5 a	2.79±0.19 b	7.80±0.36 ab	20.8±1.1 a	28.2±1.7 a
	Comp+BCM (N=12)	175.1±29.9 a	3.20±0.22 b	8.76±0.45 a	23.5±1.3 a	30.4±2.0 a
Irrigation	Drip (N=30)	187.7±17.5 a	3.78±0.18 a	8.49±0.34 a	21.5±0.9 a	29.6±1.3 a
	MS (N=30)	156.6±16.4 a	3.15±0.18 b	7.18±0.29 b	18.8±0.9 b	24.8±1.1 b
P-value	SoilTrt	0.051	<0.001	<0.001	0.047	0.219
	Irrig	0.174	0.001	0.001	0.047	0.003
	SoilTrt x Irrig	0.183	0.543	0.769	0.093	0.282

In 2016, fruit yield in the Control and Fum sub-plots was greater than in the Comp+BCM treatment (Table 3.3). Individual fruit weight was not affected by soil treatment. In 2017, neither fruit yield nor individual fruit weight was affected by soil treatment. In 2016, irrigation type did not have a significant effect on fruit yield or individual fruit weight. In 2017, trees irrigated with drip emitters had larger fruit yield than trees irrigated using MS, but individual fruit weight was not affected.

Table 3.3 - Effect of soil treatments and irrigation type on yield and individual fruit weight. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Harvest 2016		Harvest 2017	
Factor	Level	Yield (kg tree^{-1})	Fruit weight (g)	Yield (kg tree^{-1})	Fruit weight (g)
Soil treatment	Control (N=12)	2.43±0.15 a	11.7±0.5 a	5.68±0.31 a	9.25±0.31 a
	Fum (N=12)	2.18±0.22 a	11.6±0.4 a	6.51±0.62 a	9.07±0.39 a
	Comp (N=12)	1.72±0.19 ab	11.4±0.8 a	6.59±0.59 a	9.66±0.24 a
	BCM (N=12)	1.55±0.21 ab	12.0±0.4 a	6.57±0.37 a	9.54±0.38 a
	Comp+BCM (N=12)	1.34±0.16 b	11.4±0.6 a	7.19±0.42 a	9.25±0.42 a
Irrigation	Drip (N=30)	1.96±0.11 a	11.6±0.2 a	7.19±0.29 a	9.11±0.20 a
	MS (N=30)	1.73±0.15 a	11.7±0.4 a	5.82±0.27 b	9.60±0.24 a
P-value	SoilTrt	0.004	0.763	0.352	0.408
	Irrig	0.334	0.853	0.018	0.139
	SoilTrt x Irrig	0.372	0.144	0.688	0.443

Soil treatment affected the abundance of fine root tissue at a soil depth of 0-30 cm (Table 3.4). The effect of soil treatment did not depend on sampling date; therefore data were pooled across all four sampling dates prior to analysis. Fine root length was smaller in the BCM treatment than in the Control, Fum, and Comp treatments. Irrigation type also affected the abundance of fine root tissue, irrespective of the sampling date. Fine root length was greater in the drip irrigation treatment than the MS irrigation treatment.

Table 3.4 - Effect of soil treatments and irrigation type on fine root length in soil (0-30 cm depth). Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across four sampling dates (May 2016, September 2016, May 2017, September 2017). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Fine root length (cm 100 ml^{-1} soil)
Soil treatment	Control (N=48)	23.2±2.5 a
	Fum (N=48)	22.1±2.9 a
	Comp (N=48)	24.6±3.2 a
	BCM (N=48)	10.3±3.3 b
	Comp+BCM (N=48)	14.0±3.0 ab
Irrigation	Drip (N=120)	23.7±1.9 a
	MS (N=120)	14.0±2.3 b
P-value	SoilTrt	<0.001
	Irrig	0.001
	Date	0.001
	SoilTrt x Irrig	0.397
	SoilTrt x Date	0.294
	Irrig x Date	0.243
	SoilTrt x Irrig x Date	0.941

3.3.2 *P. penetrans* Population Dynamics

3.3.2.1 Soil *P. penetrans* Populations

The effect of soil treatment on soil *P. penetrans* populations depended on sampling date (soil treatment x date interaction *P*-value < 0.001); therefore data from each sampling date were analyzed separately. Soil treatment affected *P. penetrans* population densities in soil at most sampling dates (*P*-value < 0.05) (Figure 3.2). Prior to planting in 2014, *P. penetrans* population densities in the Fum treatment averaged 20 *P. penetrans* 100 mL^{-1} of soil. In non-fumigated sub-plots, population densities ranged from 88 to 98 *P. penetrans* 100 mL^{-1} of soil prior to planting. In May 2014, population densities in soil were smaller in the Fum treatment than in the other treatments; however,

by September 2014 they did not differ from the Control. In September 2014, Comp, BCM, and Comp+BCM had smaller population densities of *P. penetrans* in soil than the Control. BCM had a lower abundance of *P. penetrans* in soil than the control in May 2015. In September 2015, BCM, and Comp+BCM had smaller population densities of *P. penetrans* in soil than the Control and Fum treatments. In May 2016, Comp, BCM, and Comp+BCM had smaller populations densities of *P. penetrans* than the Control. In September 2016, May 2017, and September 2017, soil treatment did not significantly affect soil *P. penetrans* populations.

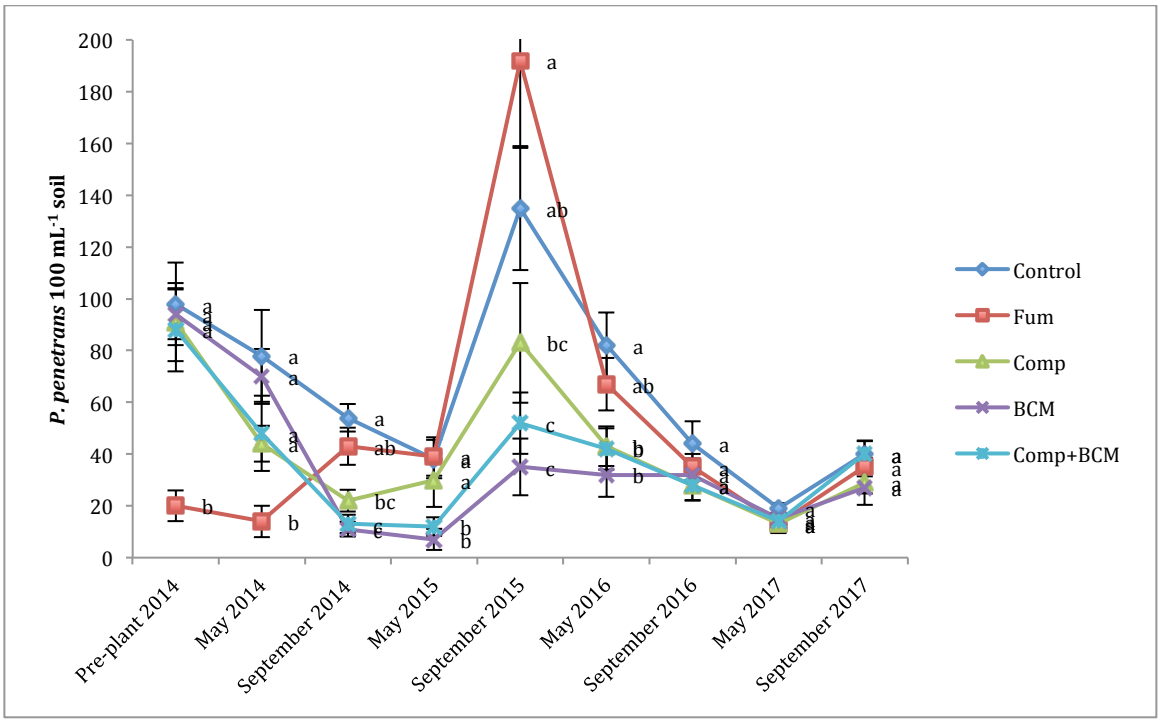

Figure 3.2 - Effect of soil treatments on *P. penetrans* populations in soil. Data were analyzed with a split-plot two-way ANOVA (N=12). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, and Comp+BCM refers to compost and bark chip mulch. Bars represent the standard error. Data points sharing the same letter within a sampling date do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

The effect of irrigation type on soil *P. penetrans* populations depended on sampling date (irrigation type x date interaction *P*-value < 0.001); therefore, data from each sampling date were analyzed separately. Throughout the first growing season

(Figure 3.3), irrigation type did not have a significant effect on soil *P. penetrans* populations. Irrigation type affected *P. penetrans* populations in 2015 and 2016. Drip emitters had fewer *P. penetrans* in soil than MS in both sampling dates in 2015, as well as September 2016. In May 2017 and September 2017, irrigation type did not significantly affect soil *P. penetrans* populations.

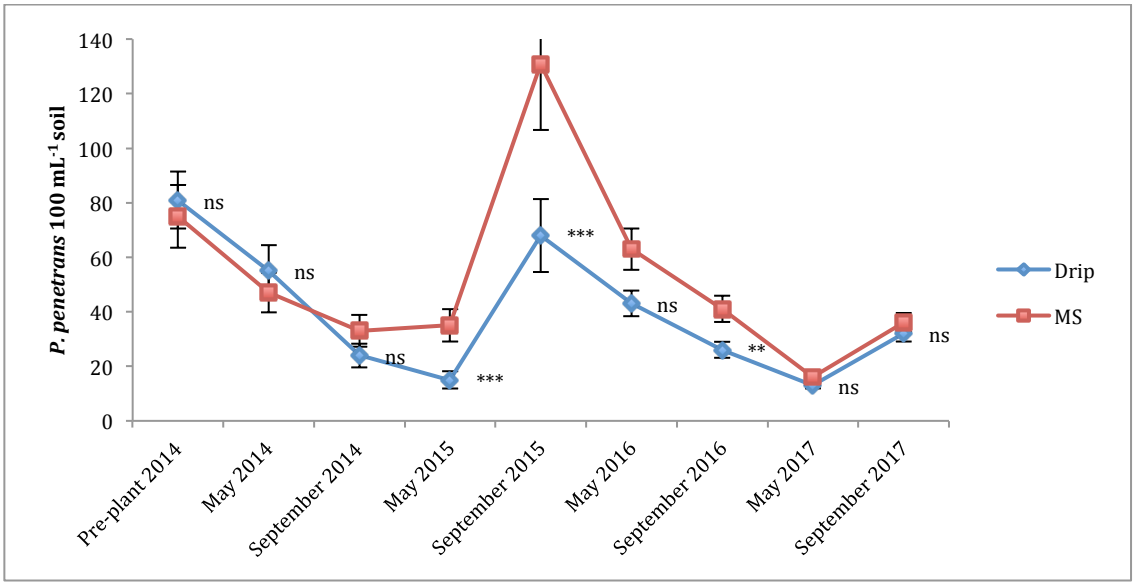

Figure 3.3 - Effect of irrigation type on *P. penetrans* populations in soil. Data were analyzed with a split-plot two-way ANOVA (N=30). MS refers to microsprinkler, NS refers to not significant (*P*-value > 0.05), * refers to significant at *P*-value < 0.05, ** refers to significant at *P*-value < 0.01, and * refers to significant at *P*-value < 0.001). Bars represent the standard error.**

3.3.2.2 Root *P. penetrans* Populations

The effect of soil treatment on *P. penetrans* populations in roots depended on sampling date (soil treatment x date interaction *P*-value < 0.001); therefore, data from each sampling date were analyzed separately. Soil treatment affected *P. penetrans* population densities in root tissue at most sampling dates (Figure 3.4). In September 2014, root population densities in the Comp, BCM, and Comp+BCM treatments were smaller than in the Control. In May 2015, only Comp+BCM had smaller *P. penetrans* population densities in root tissue than the Control. In September 2015, root population

densities in Comp, BCM, and Comp+BCM treatments were all smaller than in the Control. In May 2016, root population densities in BCM and Comp+BCM treatments were smaller than the Control and Fum treatments. In July 2016 soil treatment did not affect *P. penetrans* populations in roots. In September 2016, *P. penetrans* root populations were lower in the Comp+BCM treatment than the Control. In May 2017 and September 2017, soil treatment did not affect *P. penetrans* populations in roots. Root populations in the Fum treatment did not differ from the Control at any sampling date.

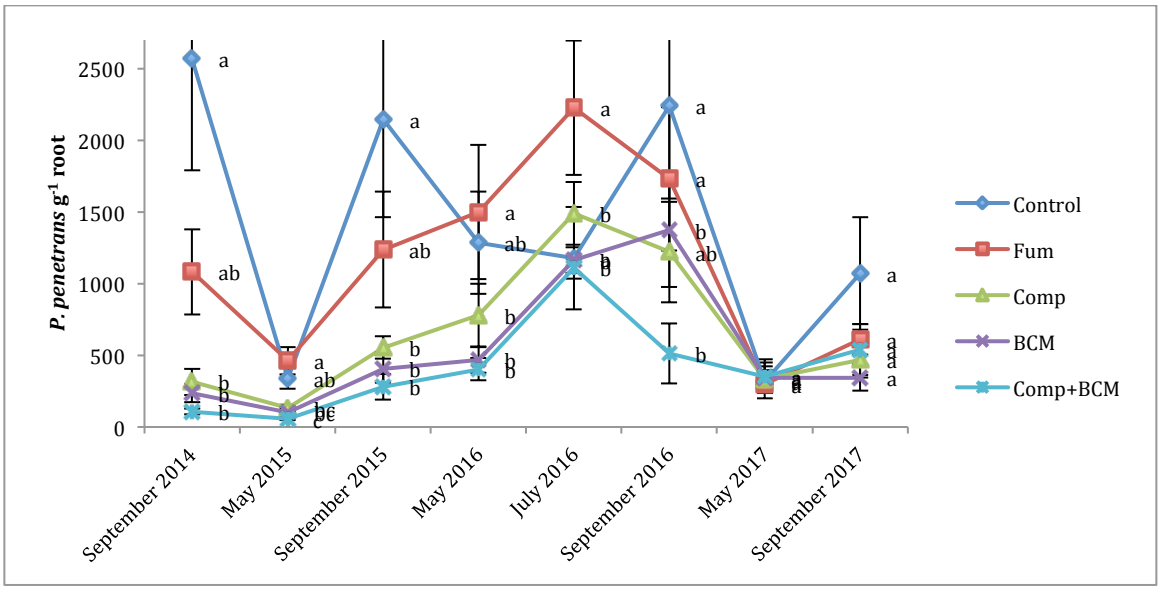

Figure 3.4 - Effect of soil treatments on *P. penetrans* populations in roots. Data were analyzed with a split-plot two-way ANOVA (N=12). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, and Comp+BCM refers to compost and bark chip mulch. Bars represent the standard error. Data points sharing the same letter within a sampling date do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

The effect of irrigation type on *P. penetrans* populations in roots depended on sampling date (irrigation type x date interaction *P*-value<0.001); therefore, data from each sampling date were analyzed separately. Irrigation type did not have a significant effect on *P. penetrans* populations in roots in the first growing season (Figure 3.5). In September of 2015 and 2016, *P. penetrans* populations in roots were smaller in the sub-

plots irrigated with drip emitters than sub-plots irrigated using MS. In May 2017 and September 2017, irrigation type did not have a significant effect on *P. penetrans* populations in the roots.

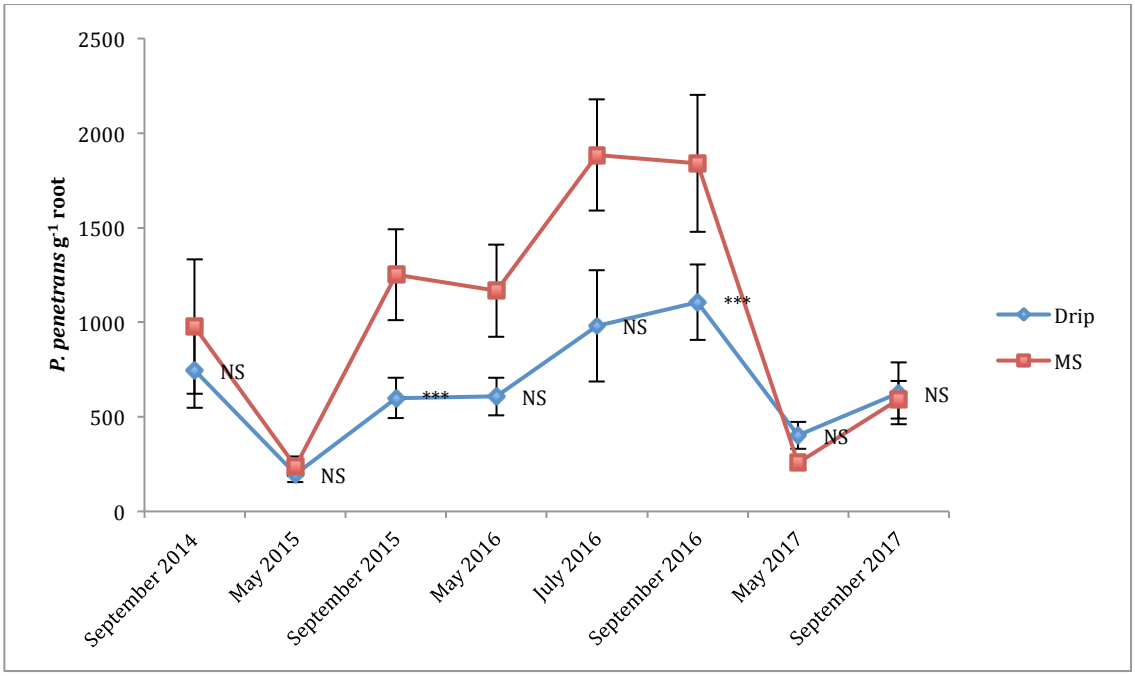

Figure 3.5 - Effect of irrigation type on *P. penetrans* populations in roots. Data were analyzed with a split-plot two-way ANOVA (N=30). MS refers to microsprinkler, NS refers to not significant (*P*-value > 0.05), * refers to significant at *P*-value < 0.05, ** refers to significant at *P*-value < 0.01, and * refers to significant at *P*-value < 0.001). Bars represent the standard error.**

3.3.2.3 *P. penetrans* Populations and Fine Root Density in Mulch/Topsoil

In July 2017, soil treatment affected the total length of fine roots in the mulch layer above the soil, as well as in the 0 - 7.5 cm depth mineral soil layer (Table 3.5). Comp+BCM increased the total length of fine roots in the mulch layer above the soil than sub-plots that received BCM alone. No roots were observed in the mulch layer in the sub-plots that received surface application of Comp alone. At a depth of 0 - 7.5 cm, total fine root length was greater in the Comp+BCM treatment than plots that received BCM alone. No significant differences in fine root length were observed between soil treatments at a

depth of 7.5 - 15 cm. Irrigation type did not significantly affect fine root length in the mulch layer or at any soil depth examined. Sampling depth had a main factor effect on the total length of fine root tissue in the mineral soil layer (0 - 30 cm), with greater root density in the 7.5 - 15 cm depth than the 0 - 7.5 cm depth (Appendix E). When plots that received application of mulch (BCM and BCM+Comp) were analyzed independent of non-mulch treatments (Control, Fum, Comp), sampling depth did not significantly affect root length.

Table 3.5 - Effect of soil treatments and irrigation type on fine root distribution in mulch/topsoil. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

			Fine root length (cm)		
Factor	Level	Mulch height (cm)	Mulch layer	Mineral soil (0 - 7.5 cm depth)	Mineral soil (7.5 - 15 cm depth)
Soil treatment	Control (N=12)	-	-	62.3±23.2 ab	126.3±31.7 a
	Fum (N=12)	-	-	87.2±26.1 ab	146.8±31.6 a
	Comp (N=12)	1.05±0.15	0±0 c	105.6±17.1 ab	121.7±38.9 a
	BCM (N=12)	6.05±0.25	83.6±18.4 b	49.7±18.9 b	123.7±40.3 a
	Comp+BCM (N=12)	7.07±0.24	188.1±24.5 a	147.4±24.8 a	194.5±57.7 a
Irrigation	Drip (N=30)	-	50.3±13.8 a	103.7±19.8 a	158.9±26.1 a
	MS (N=30)	-	58.4±18.5 a	77.1±12.2 a	126.3±25.2 a
P-value	SoilTrt	-	<0.001	0.040	0.899
	Irrig	-	0.559	0.181	0.220
	SoilTrt x Irrig	-	0.791	0.063	0.439

Soil treatment affected the abundance of *P. penetrans* in soil at a depth of 7.5 - 15 cm (Table 3.6), where *P. penetrans* populations were smaller in the Comp than the Control. Sampling depth did not significantly affect *P. penetrans* populations in soil in the mineral soil layer (Appendix E), irrespective of soil treatment or irrigation type.

When sub-plots that received application of mulch (BCM and BCM+Comp) were analyzed independent of the non-mulch treatments (Control, Fum, Comp), sampling depth affected soil *P. penetrans* populations (*P*-value = 0.013), with *P. penetrans* populations smaller in the mulch layer than either the 0 - 7.5 cm or 7.5 - 15 cm mineral soil layers.

Table 3.6 - Effect of soil treatments and irrigation type on *P. penetrans* distribution in mulch/topsoil. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		P. penetrans 50 mL^{-1} soil			*P. penetrans* g^{-1} root		
Factor	Level	Mulch Layer	0 - 7.5 cm	7.5 - 15 cm	Mulch Layer	0 - 7.5 cm	7.5 - 15 cm
Soil treatment	Control (N=12)	-	26±7 a	59±19 a	-	916±321 a	1936±711 a
	Fum (N=12)	-	38±10 a	51±9 ab	-	1074±371 a	1481±449 a
	Comp (N=12)	-	22±5 a	24±9 b	-	475±273 a	1083±490 a
	BCM (N=12)	16±5 a	36±10 a	31±8 ab	2522±747 a	431±168 a	1248±467 a
	Comp+BCM (N=12)	18±3 a	42±7 a	48±11 ab	953±238 b	493±123 a	1167±514 a
Irrigation	Drip (N=30)	12±4 a	33±7 a	36±6 a	1543±704 a	386±163 a	305±168 b
	MS (N=30)	22±4 a	32±4 a	49±9 a	1932±635 a	970±269 a	2461±328 a
P-value	SoilTrt	0.838	0.105	0.045	0.048	0.566	0.659
	Irrig	0.099	0.932	0.282	0.102	0.152	0.050
	SoilTrt x Irrig	0.561	0.170	0.559	0.211	0.557	0.381

Soil treatment significantly affected *P. penetrans* populations in roots in the mulch layer, where populations were found to be smaller in the Comp+BCM treatment than the BCM treatment (Table 3.6). Significant differences in root *P. penetrans* populations between soil treatments were not observed at a depth of 0 - 7.5 cm or 7.5 - 15 cm; however, at a depth of 7.5 - 15 cm, drip emitters had smaller *P. penetrans* populations in roots than MS. Sampling depth did not significantly affect *P. penetrans* populations in roots in the mineral soil layer (Appendix E). When plots that received

application of mulch (BCM and BCM+Comp) were analyzed independent of the non-mulch treatments (Control, Fum, Comp), sampling depth did not significantly affect root *P. penetrans* populations.

3.3.3 Other Nematode Populations in Soil

Other plant-parasitic nematodes recovered from this orchard from 2015 through 2017 included *P. teres*, *H. similis*, and *M. xenoplax.* Populations of *P. teres* and *H. similis* were relatively low and neither soil treatment nor irrigation type had a significant effect on their abundances (Appendix F). Soil treatment and irrigation type affected the abundance of *M. xenoplax* in soil; however, a soil treatment x irrigation type interaction effect was observed. Application of Comp in combination with drip irrigation increased *M. xenoplax* in soil relative to that of most other treatment combinations, with the exception of drip irrigation combined with BCM (Table 3.7).

Table 3.7 - Effect of soil treatments and irrigation type on *M. xenoplax* populations in soil from 2015 through 2016 (6 sampling dates). Data were analyzed with a split-plot repeated measures ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Irrigation	Soil treatment	*M. xenoplax* 100 mL^{-1} soil
Drip	Control	2±1
	(N=36)	b
	Fum	3±1
	(N=36)	b
	Comp	79±15
	(N=36)	a
	BCM	12±7
	(N=36)	ab
	Comp+BCM	1±1
	(N=36)	b
MS	Control	1±1
	(N=36)	b
	Fum	6±2
	(N=36)	b
	Comp	7±2
	(N=36)	b
	BCM	0±0
	(N=36)	b
	Comp+BCM	2±1
	(N=36)	b
P-value	Trt	0.004
	Date	0.034
	Trt x Date	0.350

Populations of *Mononchus* spp. were quantified in 2016 and 2017. Populations were relatively low and neither soil treatment nor irrigation type had a significant effect on their abundance (Appendix F). The total abundance of free-living soil nematodes was quantified from 2014 to 2017, and neither soil treatment nor irrigation type had a significant effect on their abundance (Appendix F).

3.3.4 Soil Microbiology

3.3.4.1 Molecular Quantification of Beneficial Microorganisms

Soil treatment affected the abundance of bacteria and fungi in the rhizosphere (Table 3.8). The effect of soil treatment was not dependent on sampling date; therefore

data were pooled across all four sampling dates prior to analysis. For quantification of total bacteria, the BACT1369F/PROK1492R primer set provided amplification efficiencies of 95.0 to 102.4%, with R^2-values that ranged from 0.995 to 0.999. Application of Comp+BCM increased the abundance of bacteria in the rhizosphere relative to that of the Control, Fum, and BCM treatments. Application of Comp alone increased the abundance of bacteria relative to that of the Control and Fum treatments. For quantification of total fungi, the FF390/FR1 primer set provided amplification efficiencies of 82.5 to 96.9%, with R^2-values that ranged from 0.991 to 0.999. BCM and Comp+BCM increased the abundance of fungi in the rhizosphere relative to that of the Control and Fum treatments. Irrigation type did not significantly affect the abundance of bacteria or fungi in the rhizosphere.

Table 3.8 - Effect of soil treatments and irrigation type on total bacteria and total fungi in the rhizosphere. Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across four sampling dates (May 2015, September 2015, May 2016, September 2016). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Total bacteria (log 16S gene copies g^{-1} rhizosphere soil)	Total fungi (log 18S gene copies g^{-1} rhizosphere soil)
Soil treatment	Control (N=48)	9.61±0.05 c	8.35±0.10 b
	Fum (N=48)	9.62±0.05 c	8.16±0.10 b
	Comp (N=48)	9.91±0.06 ab	8.54±0.08 ab
	BCM (N=48)	9.76±0.06 bc	8.56±0.09 a
	Comp+BCM (N=48)	9.97±0.05 a	8.56±0.09 a
Irrigation	Drip (N=120)	9.75±0.04 a	8.43±0.06 a
	MS (N=120	9.81±0.04 a	9.44±0.06 a
P-value	SoilTrt	<0.001	<0.001
	Irrig	0.244	0.873
	Date	<0.001	<0.001
	SoilTrt x Irrig	0.875	0.641
	SoilTrt x Date	0.056	0.539
	Irrig x Date	0.942	0.442
	SoilTrt x Irrig x Date	0.509	0.243

Soil treatment affected the abundance of *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in the rhizosphere (Table 3.9). The effect of soil treatment was not dependent on sampling date; therefore, data were pooled across all four sampling dates prior to analysis. For quantification of *Pseudomonas* spp., the Pse435F/Pse686R primer set provided amplification efficiencies of 92.4 to 101.7%, with R^2-values that ranged from 0.998 to 0.999. Application of Comp and Comp+BCM increased the abundance of *Pseudomonas* spp. in the rhizosphere relative to that of the Control and Fum treatments. Application of BCM increased the abundance of *Pseudomonas* spp. in the rhizosphere relative to that of the Control. For quantification of DAPG+ bacteria, the BPF2/BPR4

primer set provided amplification efficiencies of 90.5 to 99.6%, with R^2-values that ranged from 0.995 to 0.997. Application of Comp increased the abundance of DAPG+ bacteria relative to that of all other soil treatments. Application of Comp+BCM increased the abundance of DAPG+ bacteria relative to the Control and BCM treatments. For quantification of PRN+ bacteria, the PrnD-F/PrnD-R primer set provided amplification efficiencies of 95.4 to 104.7%, with R2-values that ranged from 0.998 to 0.999. Application of Comp+BCM increased the abundance of PRN+ bacteria relative to that of the Control, Fum, and BCM treatments. Application of Comp increased the abundance of PRN+ bacteria relative to that of the Control and Fum treatments. Irrigation type did not significantly affect the abundance of *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in the rhizosphere.

Table 3.9 - Effect of soil treatments and irrigation type on *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in the rhizosphere. Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across four sampling dates (May 2015, September 2015, May 2016, September 2016). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, MS refers to microsprinkler, DAPG+ bacteria refers to 2,4-diacetylphloroglucinol-producing bacteria, and PRN+ bacteria refers to pyrrolnitrin-producing bacteria. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	*Pseudomonas* spp. (log 16S gene copies g^{-1} rhizosphere soil)	DAPG+ bacteria (log *phlD* gene copies g^{-1} rhizosphere soil)	PRN+ bacteria (log *prnD* gene copies g^{-1} rhizosphere soil)
Soil treatment	Control (N=48)	8.22±0.09 c	5.00±0.13 c	6.56±0.06 c
	Fum (N=48)	8.42±0.09 bc	5.47±0.15 bc	6.46±0.08 c
	Comp (N=48)	8.61±0.09 a	6.10±0.14 a	6.81±0.07 ab
	BCM (N=48)	8.49±0.11 ab	5.30±0.13 c	6.60±0.08 bc
	Comp+BCM (N=48)	8.67±0.11 a	5.68±0.12 b	6.93±0.06 a
Irrigation	Drip (N=120)	8.46±0.07 a	5.45±0.10 a	6.61±0.05 a
	MS (N=120)	8.51±0.06 a	5.57±0.09 a	6.73±0.05 a
P-value	SoilTrt	<0.001	<0.001	<0.001
	Irrig	0.359	0.367	0.151
	Date	<0.001	<0.001	0.003
	SoilTrt x Irrig	0.441	0.272	0.098
	SoilTrt x Date	0.730	0.300	0.200
	Irrig x Date	0.336	0.202	0.878
	SoilTrt x Irrig x Date	0.323	0.100	0.661

3.3.4.2 Cultural Quantification of Beneficial Microorganisms

Soil treatment had an effect on the abundance of total *Pseudomonas* spp., fluorescent *Pseudomonas* spp., and phosphate-solubilizing bacteria cultured from the rhizosphere (Table 3.10). The effect of soil treatment did not depend on sampling date; therefore, data were pooled across all four sampling dates prior to analysis. *Pseudomonas* spp. populations were more abundant in the Comp, BCM, and Comp+BCM treatments than in the Control. Application of Comp increased fluorescent *Pseudomonas* spp. populations in the rhizosphere relative to that of the Control and Fum treatments.

Application of Comp+BCM increased the abundance of fluorescent *Pseudomonas* spp. populations relative to that of the Control treatment. Populations of phosphate-solubilizing bacteria were more abundant in the Fum, Comp, and Comp+BCM treatments than the Control. Irrigation type did not significantly affect the abundance of *Pseudomonas* spp., fluorescent *Pseudomonas* spp., or phosphate-solubilizing bacteria in the rhizosphere.

Table 3.10 - Effect of soil treatments and irrigation type on microorganisms cultured from the rhizosphere. Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across four sampling dates (May 2015, September 2015, May 2016, September 2016). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, MS refers to microsprinkler, and CFU refers to colony forming units. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	*Pseudomonas* spp. (log CFU g^{-1} rhizosphere soil)	Fluorescent *Pseudomonas* spp. (log CFU g^{-1} rhizosphere soil)	Phosphate-solubilizing bacteria (log CFU g^{-1} rhizosphere soil)
Soil treatment	Control (N=48)	6.86±0.08 b	5.19±0.12 c	6.78±0.06 b
	Fum (N=48)	7.05±0.09 ab	5.69±0.08 bc	7.13±0.09 a
	Comp (N=48)	7.13±0.10 a	6.08±0.10 a	7.14±0.11 a
	BCM (N=48)	7.15±0.07 a	5.71±0.11 abc	6.98±0.08 ab
	Comp+BCM (N=48)	7.23±0.09 a	6.01±0.12 ab	7.21±0.09 a
Irrigation	Drip (N=120)	7.09±0.08 a	5.68±0.10 a	7.04±0.04 a
	MS (N=120)	7.07±0.06 a	5.79±0.10 a	7.06±0.05 a
P-value	SoilTrt	<0.001	<0.001	<0.001
	Irrig	0.777	0.459	0.809
	Date	<0.001	0.020	<0.001
	SoilTrt x Irrig	0.488	0.225	0.125
	SoilTrt x Date	0.200	0.051	0.060
	Irrig x Date	0.573	0.225	0.210
	SoilTrt x Irrig x Date	0.900	0.710	0.521

3.3.4.3 Correlation Between *Pseudomonas* spp. Quantification Techniques

Data from DNA- and culture-based quantification of *Pseudomonas* spp. in the rhizosphere were pooled from all sampling dates and Pearson's correlation coefficient

was determined. A positive linear correlation (r = 0.363, n = 240, *P*-value = 0.002) was observed between DNA- and culture-based quantification techniques, with the culture-based technique enumerating, on average, only 3.72% of the total *Pseudomonas* spp. enumerated by the DNA-based technique.

3.3.4.4 Root Colonization by AMF

Soil treatment had an effect on root colonization by AMF (Table 3.11). The effect of soil treatment did not depend on sampling date; therefore, data were pooled across all four sampling dates prior to analysis. Application of Comp increased AMF root colonization relative to that of the Control treatment. Irrigation type also had an effect on root colonization by AMF. Drip irrigation resulted in greater root colonization by AMF than MS irrigation.

Table 3.11 - Effect of soil treatments and irrigation type on root colonization by AMF (arbuscular mycorrhizal fungi). Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across four sampling dates (May 2015, September 2015, May 2016, September 2016). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Arbuscular colonization (%)
Soil treatment	Control (N=48)	9.61±1.32 b
	Fum (N=48)	16.51±1.65 ab
	Comp (N=48)	17.78±1.52 a
	BCM (N=48)	15.78±1.21 ab
	Comp+BCM (N=48)	16.42±1.51 ab
Irrigation	Drip (N=120)	18.35±0.94 a
	MS (N=120)	12.09±0.87 b
P-value	SoilTrt	0.010
	Irrig	<0.001
	Date	0.009
	SoilTrt x Irrig	0.063
	SoilTrt x Date	0.656
	Irrig x Date	0.926
	SoilTrt x Irrig x Date	0.497

3.3.4.5 Soil Microbial Activity

The affect of soil treatment on soil microbial activity depended on sampling date; therefore, each sampling date was analyzed separately (Table 3.12). Soil treatment as well as irrigation type did not significantly affect soil microbial activity in May 2017. In September 2017, soil treatment affected soil microbial activity, but irrigation type did not have a significant effect. Comp and Comp+BCM had greater soil microbial activity than the Control and Fum treatments. Application of BCM resulted in greater soil microbial activity than the Control, but not the Fum treatment.

Table 3.12 - Effect of soil treatments and irrigation type on soil microbial activity. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil microbial activity (mg fluorescein released kg^{-1} soil $3h^{-1}$)	
Factor	Level	May 2017	September 2017
Soil treatment	Control	63.3±8.9	27.8±5.7
	(N=12)	a	c
	Fum	70.5±8.5	32.4±4.7
	(N=12)	a	bc
	Comp	58.6±6.1	50.8±7.2
	(N=12)	a	a
	BCM	68.5±4.2	43.6±5.6
	(N=12)	a	ab
	Comp+BCM	82.9±9.5	48.1±4.1
	(N=12)	a	a
Irrigation	Drip	73.5±5.2	37.1±3.2
	(N=30)	a	a
	MS	64.0±4.6	44.0±4.3
	(N=30)	a	a
P-value	SoilTrt	0.105	0.013
	Irrig	0.425	0.448
	SoilTrt x Irrig	0.241	0.144

3.3.5 Soil Biological Suppressiveness Bioassay

Soil treatment had an effect on soil biological suppressiveness; however, the effect depended on sampling date (soil treatment x date interaction *P*-value = 0.025), so data from each sampling data were analyzed separately (Table 3.13). In September 2016, the Comp, BCM, and Comp+BCM treatments had greater soil suppressiveness than the Control. In October 2016, the Comp and BCM treatments had greater soil suppressiveness than the Control. In May 2017, the Comp, BCM, and Comp+BCM treatments had greater soil suppressiveness than the Control. In September 2017, soil biological suppressiveness was greater in the Comp and BCM sub-plots than the Control and Fum sub-plots. Irrigation type did not have a significant effect on soil biological suppressiveness.

Table 3.13 - Effect of soil treatments and irrigation type on soil biological suppressiveness. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil biological suppressiveness (%)			
Factor	Level	September 2016	October 2016	May 2017	September 2017
Soil treatment	Control (N=12)	22.6±4.1 c	21.8±3.6 b	22.9±4.4 b	30.2±5.0 b
	Fum (N=12)	29.2±5.2 bc	33.0±5.2 ab	34.8±4.8 ab	20.7±5.2 b
	Comp (N=12)	56.5±6.8 a	47.8±6.3 a	42.3±4.5 a	57.9±7.0 a
	BCM (N=12)	48.3±6.9 ab	56.4±3.0 a	46.0±5.7 a	62.3±4.1 a
	Comp+BCM (N=12)	55.3±4.3 a	35.0±3.6 ab	48.0±5.1 a	42.8±6.8 ab
Irrigation	Drip (N=30)	37.0±4.1 a	37.6±3.9 a	42.9±3.1 a	39.1±3.9 a
	MS (N=30)	47.7±4.3 a	40.0±3.0 a	34.7±3.7 a	46.4±5.1 a
P-value	SoilTrt	0.002	0.001	0.008	<0.001
	Irrig	0.175	0.520	0.153	0.366
	SoilTrt x Irrig	0.510	0.403	0.877	0.482

3.3.6 Soil Water Content

Soil treatment had an effect on average monthly soil volumetric water content in September and October of 2014 and 2015 (Appendix G). In September 2014 (Appendix G, Table G.1), average soil volumetric water content was greater in the Comp+BCM treatment than the Control. In October 2014, average soil volumetric water content was greater in the Comp+BCM treatment than the BCM treatment. In September and October 2015 (Appendix G, Table G.3), a soil treatment x irrigation type interaction was observed. In September 2015, average soil volumetric water content was lower in the Comp treatment under microsprinkler irrigation than the Fum, Comp, and BCM treatments under drip irrigation. In October 2015, average soil volumetric water content was lower in the Comp treatment under microsprinkler irrigation than the Comp treatment under drip irrigation. On average, drip emitters maintained numerically greater

soil volumetric water content than MS throughout the first four years of tree establishment.

3.3.7 Photosynthetic Rate, Stomatal Conductance, Transpiration Rate, and Stem Water Potential

Soil treatment did not significantly affect photosynthetic rate, stomatal conductance, transpiration rate, or stem water potential when it was measured throughout the 2017 growing season (Table 3.14). The effect of irrigation type did not depend on sampling date; therefore, data were pooled across all nine sampling dates prior to analysis. Irrigation type affected photosynthetic rate, stomatal conductance, transpiration rate, and stem water potential, with all four parameters lower for the drip irrigated trees than trees irrigated with MS.

Table 3.14 - Effect of soil treatments and irrigation type on photosynthetic rate, stomatal conductance, transpiration rate, and stem water potential in 2017. Data were analyzed with a split-plot repeated measures ANOVA. Means represent pooled data across nine sampling dates (June - August 2017). Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	Photosynthetic rate (umol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stem water potential (MPa)
Soil treatment	Fum (N=54)	13.7±0.3 a	0.169±0.008 a	5.11±0.15 a	-0.95±0.31 a
	Comp (N=54)	13.0±0.4 a	0.158±0.007 a	4.78±0.19 a	-0.93±0.33 a
	BCM (N=54)	13.5±0.4 a	0.172±0.009 a	4.97±0.18 a	-0.93±0.33 a
Irrigation	Drip (N=81)	12.7±0.3 b	0.149±0.007 b	4.42±0.11 b	-0.99±0.22 b
	MS (N=81)	14.1±0.3 a	0.184±0.006 a	5.49±0.15 a	-0.89±0.29 a
P-value	SoilTrt	0.055	0.064	0.184	0.600
	Irrig	<0.001	<0.001	<0.001	<0.001
	Date	<0.001	<0.001	<0.001	<0.001
	SoilTrt x Irrig	0.285	0.526	0.446	0.052
	SoilTrt x Date	0.249	0.357	0.977	0.416
	Irrig x Date	0.945	0.582	0.873	0.301
	SoilTrt x Irrig x Date	0.788	0.609	0.796	0.382

3.3.8 Soil OM and Extractable Nutrients

Prior to planting or organic amendment application, soil C, N, and C/N ratio did not differ among sub-plots (soil treatments) or whole plots (irrigation treatments) (Appendix H, Table H.1). In September 2014, soil N content was greater in the Comp amendment sub-plots than Fum sub-plots. Similarly, soil N content was greater under MS irrigated trees than trees irrigated with drip emitters. In September 2015, soil C and N content was greater in the Comp and Comp+BCM treatment than Fum sub-plots. In September 2015, C/N ratio was greater in the BCM and Comp+BCM treatment than the Control, Fum, and Comp treatments.

In September 2015, soil treatment affected most soil nutrition parameters evaluated (Appendix H, Table H.2). Soil OM content was greater in the Comp and Comp+BCM amended sub-plots than Fum sub-plots. Soil P content (Bray-extractable P) was greater in the untreated Control sub-plots than the Fum and BCM sub-plots. Soil K content was greater in the Comp and Comp+BCM treatment than the untreated Control, Fum, and BCM treatments. Soil Mg content was greatest in the Comp-amended sub-plots, and was significantly greater than the untreated Control, Fum and BCM treatments. Soil Mg content was reduced relative to that of the untreated Control in the BCM treatment. Soil Ca content was greater in the Comp-amended sub-plots relative to that of the untreated Control. Soil Na content in the untreated Control and Comp amended sub-plots was greater than the BCM treatment. Soil pH did not differ between soil treatments. Soil cation exchange capacity was greater in the Comp amended sub-plots than the untreated Control, Fum, and BCM treatments. Soil S content was greater in the Fum and Comp sub-plots than the BCM and Comp+BCM treatments. Soil Al content was reduced

by all soil treatments relative to that of the untreated Control. Soil NO_3-N content was reduced in the BCM and Comp+BCM treatments relative to that of the untreated Control, Fum, and Comp treatments.

3.3.9 Tree Leaf Nutrition

Soil treatment had an effect on many leaf nutrient concentrations in 2014 (Appendix I, Table I.1). The effect of soil treatment on leaf N content depended on irrigation type, with N content lower in the Comp+BCM treatment under MS irrigation than the Comp+BCM treatment under drip irrigation (Appendix I, Table I.2). The effect of soil treatment on leaf P content was also dependent on irrigation type, with leaf P content greater in the Fum treatment under MS irrigation than the Comp+BCM treatment under MS irrigation (Appendix I, Table I.2). Leaf K content was enhanced by the Fum, Comp, and Comp+BCM treatments relative to that of the Control and BCM treatments (Appendix I, Table I.1). The Control and BCM treatments had greater leaf Ca and Mg content than the Comp treatment. Leaf B content was greater in the Control treatment than the BCM. Leaf Zn content was greater in the Comp and Comp+BCM treatments than the Control and Fum treatments. The effect of soil treatment on leaf Fe content depended on irrigation type, with greater leaf Fe in the Comp treatment under MS irrigation than the BCM treatment under drip irrigation (Appendix I, Table I.2). Leaf Mn content was greater under Fum treatment than the Comp and Comp+BCM treatments.

Soil treatment had an effect on some leaf nutrient contents in 2015 (Appendix I, Table I.3). Leaf P content was greater in the Comp+BCM treatment than the Control, Fum, and Comp treatments, whereas application of BCM alone increased leaf P content relative to that of the Control and Fum treatments. Leaf K content was enhanced by all

three organic soil treatments relative to that of the Control and Fum treatments. Leaf Ca content was greatest under the Control treatment. Leaf Zn content was greater in the BCM and Comp+BCM treatments than the Control and Fum treatments.

Soil treatment had a significant effect on some leaf nutrient contents in 2016 (Appendix I, Table I.4). Leaf N content was greater in the Comp treatment than the Control, BCM, and Comp+BCM treatments. Leaf K and Mg content was greater in the Comp, BCM and Comp+BCM treatments than the Control and Fum treatments. Leaf B content was greater in the Comp, BCM, and Comp+BCM treatments than the untreated Control. Leaf Zn content was greater in the Control, BCM, and Comp+BCM treatment than the Fum treatment.

Irrigation type had an effect on some leaf nutrient contents in 2014, and 2016 but not in 2015 (Appendix I). In 2014, the effect of irrigation type on N, P, and Fe leaf contents depended on soil treatment, as discussed above. Trees irrigated with MS also had greater leaf concentrations of Ca, Mg, and Mn, whereas trees irrigated with drip emitters had greater leaf K concentrations. In 2016, leaf K content was greater in the MS treatment than drip emitters.

3.4 Discussion

3.4.1 Influence of Soil Fumigant

Historically, conventional tree-fruit growers have used broad-spectrum chemical fumigants to control replant disease at orchard sites. Soil fumigants are highly effective at controlling the diverse array of organisms contributing to replant disease complex, and often result in significant enhancement of plant growth and fruit yield when applied to orchard soil (Arneson and Mai, 1976; Browne et al., 2006; Covey et al., 1979;

Mai and Abawi, 1981; Mai and Parker, 1972; Pitcher et al., 1966; Utkhede and Li, 1989); however, increasing restrictions on their use now limit availability in most growing regions. In this field experiment, soil fumigation resulted in significant increases in trunk diameters of newly planted sweet cherry trees at an apple orchard site over the first three growing seasons; however, enhanced root growth and fruit yield was not observed relative to the untreated soil when the data were collected in the third and fourth growing seasons.

Seedling greenhouse bioassays have previously been used to predict expected plant growth responses to soil management practices at orchard sites (Hoestra, 1968; Neilsen and Yorston, 1991; Sitepu and Wallace, 1974). The field and greenhouse replant experiments (Chapter 2) both showed similar trends in plant growth responses to the soil treatments; however, in the field experiment, tree trunk diameter increase relative to the untreated control in response to soil fumigation ranged from 26.7 - 51.8%, depending on growing season, whereas in the previous greenhouse experiment, growth responses to soil fumigation ranged from 89.6 - 129.9%, depending on which plant growth parameter was analyzed (shoot length, shoot weight, or plant biomass). Greater plant growth enhancement in response to soil fumigants in the greenhouse versus the field experiment could potentially be a result of the artificial growing environment provided by potted-container experiments, which presumably are less susceptible to reestablishment of pathogen/pest populations from adjacent, non-fumigated soil. Differences in abiotic environmental factors, such as precipitation and temperature, may have also contributed to differential plant growth promotion in response to soil fumigants in the greenhouse and field experiments. Overall, this suggests that seedling bioassays may overestimate the

expected plant growth response to soil fumigants in field settings.

At the time of planting, previous soil fumigation resulted in fewer *P. penetrans* in soil relative to that of the untreated control; however, by September of the first growing season (2014), soil populations had increased to levels that no longer differed from the control. Similarly, at no sampling date over the duration of this four-year study did root populations of *P. penetrans* in the fumigated sub-plots significantly differ from root populations in the untreated control sub-plots. Mazzola and Manici (2012) reported a similar trend on apple, where soil fumigation initially decreased *P. penetrans* populations in the first growing season; however, populations were found to increase in the second growing season to levels that exceeded the untreated control soil. Such severe reinfestation with *P. penetrans* after soil fumigation has typically been attributed to elimination of microbial antagonists of plant-parasitic nematodes (Munnecke, 1984; Rodriguez-Kabana and Curl, 1980). Because we fumigated 2-m wide strip plots rather than an entire field, the reinfestation by *P. penetrans* observed in fumigated sub-plots in this particular field experiment was likely more rapid than in a commercial-scale fumigation; however, it would be comparable to that expected in a bed fumigation, which is a practice under increasing consideration by growers because it facilitates a reduction in required buffer zones (Zasada et al., 2010). Despite rapid soil reinfestation with *P. penetrans*, newly planted sweet cherry trees had significantly greater trunk diameters relative to the control throughout the first three years of orchard establishment. Using a greenhouse experiment, Wilson et al. (2004) demonstrated that an initial growth period without exposure to replant disease-associated pathogens/pests had a marked effect on fruit tree growth, even after subsequent exposure to pathogens, which could potentially

explain the long-term positive growth response to soil fumigation observed in this field experiment, despite subsequent reinfestation with *P. penetrans*. Additionally, populations of fungal pathogens that may have been affecting roots along with *P. penetrans* may not have re-established in fumigated orchard soil at the same rate and/or intensity as was observed for *P. penetrans*.

Populations of *P. penetrans* and beneficial rhizosphere microorganisms were often affected by sampling date, with populations larger in fall than spring sample dates. Soil temperature can have a strong influence on *P. penetrans* population abundance, and likely contributed to seasonal fluctuations in nematode populations in this study (Kimpinski and Dunn, 1985; MacGuidwin and Forge, 1991). Additionally, accumulation of root exudates in the rhizosphere during the growing season may have contributed to larger populations of beneficial rhizosphere microorganisms in fall sampling dates as well.

Shifts in the soil bacterial community composition to a proportional increase in *Pseudomonas* spp. have been observed after gamma-irradiating apple orchard soil (Caputo et al., 2015), and the authors suggested that such changes in bacterial community composition might contribute to establishing suppressive microbial populations in orchard soils. In this field experiment, sub-plots that were fumigated had an increased abundance of total *Pseudomonas* spp. and fluorescent *Pseudomonas* spp. in the rhizosphere relative to the control, according to culture-based quantification techniques; however, such differences in bacterial abundance were not detected using the real-time PCR assay. Discrepancies between DNA- and culture-based quantification of *Pseudomonas* spp. can potentially be attributed to the growth plate assay quantifying a

smaller subset of the Pseudomonad population in the rhizosphere (3.72%), relative to the real-time PCR assay. Increases in the abundance of cultureable pseudomonads in the rhizosphere did not appear to contribute to establishing soil suppressiveness to plant-parasitic nematodes because fumigated soil was rapidly reinfested with *P. penetrans* and displayed minimal suppressive capacity to *M. hapla* in the soil suppressiveness bioassay; however, increases in the abundance of cultureable *Pseudomonas* spp. may have had other positive benefits on plant growth, perhaps by increasing plant nutrient availability (Selvakumar et al., 2009), by producing plant growth promoting hormones (Marasco et al., 2013) or by suppressing fungal root pathogens (Marchi et al., 2013).

In addition to increases in cultureable *Pseudomonas* spp., soil fumigants were also associated with increases in the abundance of phosphate solubilizing bacteria in the rhizosphere relative to that of the untreated control, suggesting a potential role of increased P nutrition in enhanced plant growth in the fumigated soil. Conversely, analyses of soil P concentrations showed lower nutrient concentrations in the fumigation soil relative to the untreated soil; however, all sub-plots were fertigated with sufficient P, and at no sampling date were trees under any soil treatment deficient in P, as indicated by leaf P concentrations. Microbial populations that enhance plant access to P can also have many other different functions in the rhizosphere (Sharma et al., 2013; Vassilev et al., 2006), including suppression of root diseases (Ahmad et al., 2008) and direct plant growth promotion (Brown, 1974), all of which could help explain the increases in bacterial populations associated with P nutrition that were observed in this particular study. Overall, responses of bacterial populations associated with enhanced plant P availability to soil fumigation require further research focus in the future.

3.4.2 Influence of Compost Amendment

Amending orchard soil with compost has previously been demonstrated to promote the growth of potted tree-fruit seedlings in greenhouse experiments (van Schoor et al., 2009) (Chapter 2), as well as newly planted fruit trees in field experiments (Braun et al., 2010; Gur et al., 1998; Moran and Schupp, 2003, 2005; Peryea and Covey, 1989; van Schoor et al., 2009). Additionally, using compost amendments has also been associated with reductions in *P. penetrans* populations on fruit trees planted into old orchard soil (Braun et al., 2010; Rumberger et al., 2007, 2004; Yao et al., 2006). In this field experiment, composts increased trunk diameters and fine root density of sweet cherry trees planted into an old apple orchard site, as well as sustained reductions in *P. penetrans* population densities in roots and soil over the first three years of orchard establishment, and this nematode suppression likely contributed significantly to improved growth in replant soil relative to the untreated control treatment. Data from this experiment suggest composts can promote the growth of fruit trees planted at old orchard sites, as well as provide prolonged suppression of *P. penetrans* populations relative to soil fumigants. In this experiment, compost was applied to soil at rates of 150 m^3 ha^{-1} orchard area. Assuming an estimated cost of \$20 m^{-3} (CAD) for compost, such an application would cost approximately \$3000 (CAD), in contrast to soil fumigation, which costs approximately \$5000 (CAD) ha^{-1} orchard area, assuming an estimated cost of \$10 kg^{-1} (CAD) for Basamid®. Alternatively, soil fumigation with Vapam® (metam sodium) would cost approximately \$3,500 (CAD) ha^{-1} orchard area, assuming an estimated cost of \$11 kg^{-1}.

In the first year that fruit was harvested (2016), sub-plots that received combined application of compost and bark chip mulch had a smaller fruit yield relative to the control and fumigation treatments. Reductions in initial fruit yield in the combined organic amendment sub-plots can potentially be explained by the fact that this treatment had the largest yearly trunk diameter increase, suggesting that the trees were allocating more resources to vegetative growth, whereas the trees planted in the untreated control treatment appear to have allocated more resources to reproductive growth. This could potentially represent a life history strategy in response to increased initial root parasitism by *P. penetrans* in the control treatment, as enhanced fecundity has previously been observed in response to host-parasitism in other biological systems (Minchella and LoVerde, 1981). In the three organic soil amendment treatments (Comp, BCM, Comp+BCM), resource allocation to vegetative growth in the early years of orchard establishment could potentially result in greater fruit yield in subsequent growing seasons as a result of an increase in the number of lateral branches available to support fruiting spurs (Lauri et al., 2004). This hypothesis is supported by the current field experiment, which displayed a trend towards numerically greater fruit yield in the combined organic amendment treatment (Comp+BCM) in the subsequent harvest year (2017) relative to that of the untreated control treatment. On-going monitoring of the current field experiment will be essential to evaluate the long-term effects of compost applications on orchard productivity.

Increases in the abundance of microbial populations that are antagonistic to plant-parasitic nematodes are thought to contribute to establishing nematode suppressive soils, including increases in total microbial abundance and activity in soil (Bonanomi et

al., 2010; Oka, 2010), as well as increases in the abundance of more specific groups of microbial antagonists, such as *Pseudomonas* spp. (Bonanomi et al., 2010; Hoitink et al., 1997; Hoitink and Fahy, 1986; Siddiqui et al., 2005) and other populations of antibiotic-producing bacteria (Oka, 2010; Siddiqui and Shaukat, 2003). In this study, increased plant growth and nematode suppression as a result of compost application coincided with an increase in the abundance of total bacteria, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in the rhizosphere, as evaluated using a real-time PCR assay. These groups of bacteria are known to have strong antagonistic activity to nematodes and pathogenic fungi (Costa et al., 2009; Nandi et al., 2015), and have been linked with soil suppressiveness in a number of pathosystems worldwide (de Souza et al., 2003; Garbeva et al., 2004b; Latz et al., 2012; Mazzola, 1999; Mazzola and Gu, 2002, 2000). In this study, compost may have promoted the development of a suppressive rhizosphere by increasing the overall abundance of bacteria in the rhizosphere, including *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria, which in turn, may have contributed to *P. penetrans* suppression and improved plant growth. Additionally, these groups of bacteria may also have contributed to suppression of opportunistic fungal pathogens, and perhaps had other positive influences on root growth (de Souza et al., 2003; Garbeva et al., 2004b; Latz et al., 2012; Mazzola, 1999; Mazzola and Gu, 2002, 2000).

In addition to increases in the abundance of antagonistic bacteria in the rhizosphere, compost amendments resulted in enhanced soil biological suppressiveness to plant-parasitic nematodes in bulk soil as well as enhanced soil microbial activity. A number of organisms inhabiting bulk soil have been shown to contribute to suppression of plant-parasitic nematode populations, including antibiotic-producing bacteria (Siddiqui

and Shaukat, 2003), predatory nematodes and mites (Jones, 1974), nematode-trapping fungi (Stirling et al., 1998), and other nematode-parasitizing bacteria and fungi (Sayre and Keeley, 1969; Starr and Sayre, 1988). In this study, increases in the abundance of predacious nematodes in response to compost application were not observed; however, increases in other groups of nematode antagonists could have potentially contributed to enhanced soil biological suppressiveness and reductions in *P. penetrans* populations. Comparisons were not made between differential colonization of rhizosphere and bulk soil by microbial populations associated with soil suppressiveness; therefore, increases in antagonistic bacteria in the bulk soil, in addition to increases in antagonistic bacteria populations in the rhizosphere, may have contributed significantly to *P. penetrans* suppression. Many studies on the use of compost amendments for root disease control have found a significant positive correlation between soil microbial activity and suppression of fungal root disease (Boehm and Hoitink, 1992; Hoitink et al., 1997). In this particular study, compost amendments stimulated soil microbial activity relative to the control, suggesting this may have been a contributing mechanism in *P. penetrans* suppression and/or soil biological suppressiveness in compost-amended orchard soil as well.

Other populations of plant-parasitic nematodes that were recovered from this orchard site included *P. teres*, *M. xenoplax*, and *H. similis*. Populations of *P. teres* and *H. similis* were relatively low, and neither soil treatment nor irrigation type significantly affected their abundances. Although these two plant-parasitic nematodes can negatively affect the growth of fruit trees at high enough population densities (Mai and Abawi, 1981), populations did not reach such levels over the first four years of tree

establishment. Interestingly, application of compost in combination with a drip emitter irrigation system resulted in significant stimulation of *M. xenoplax* populations in soil relative to most other treatment combinations. A similar phenomenon has been observed on wine grape grown in the Okanagan Valley, Canada using overhead sprinklers, where compost amendments were found to stimulate *M. xenoplax* populations relative to a fertilizer control (Smit, 2009). Mechanisms behind compost-induced stimulation of *M. xenoplax* under drip irrigation requires further research focus; however, this phenomenon appears to be easily mitigated on sweet cherry through co-amendment with bark chips, or the use of a microsprinkler irrigation system. In general, this study is in agreement with prior literature reporting *P. penetrans* as the primary plant-parasitic nematode associated with replant disease at old orchard sites (Hoestra and Oostenbrink, 1962; Jaffee et al., 1982; Mai and Abawi, 1981; Traquair, 1984; Vrain and Yorston, 1987). In a recent literature review, Thoden et al. (2011) concluded that increases in the abundance of free-living nematode populations after the addition of organic soil amendment were often associated with enhanced plant growth. In this study, compost amendments did not increase the abundance of free-living nematodes in soil.

In addition to increasing bacterial populations in the rhizosphere directly linked with soil suppressiveness, compost amendments were also associated with enhanced rhizosphere colonization by phosphate-solubilizing bacteria, as well as increased root colonization by AMF relative to that of the untreated control. Enhancing plant access to P has previously been shown to improve the establishment of apple at old orchard sites (Neilsen and Yorston, 1991; Slykhuis and Li, 1985; Wilson et al., 2004), and this has now become a central principle in developing an integrated replant disease management

strategy. In other cropping systems, composts have been associated with enhanced phosphatase activity in the rhizosphere (Bastida et al., 2008), as well as increases in populations of phosphate solubilizing bacteria and fungi in the rhizosphere (Zayed and Abdel-Motaal, 2005). Although all sub-plots were fertigated with sufficient P, increases in phosphate-solubilizing bacteria in the rhizosphere may have contributed significantly to enhanced plant uptake of phosphate in the compost-amended sub-plots. Similarly, root colonization by AMF was enhanced in the compost-amended soil, and this may have also contributed to the greater leaf P concentrations observed in the combined organic soil amendment treatment relative to that of the untreated control, despite sufficient P fertilization and/or any signs of P deficiency in the control treatment. Enhanced root colonization by AMF in the compost-amended soil may also have had other beneficial effects on plant growth at this orchard site, such as suppression of root pathogens (Azcón-Aguilar and Barea, 1997; Hussey and Roncadori, 1982) and perhaps even tolerance to water stress (Ruiz-Lozano et al., 1995).

Soil treatments significantly affected plant and soil nutrition when it was evaluated over the first three years of orchard establishment. Application of compost increased soil K, Mg, Ca, and CEC relative to that of the control treatment, as well as reduced Al concentration in soil. Similarly, application of compost increased leaf K and B concentrations relative to that of the control; however, concentrations for these nutrients, as well as most of the other leaf nutrients analyzed, were within the recommended range in production guidelines across all soil treatments (www.bctfpg.ca; accessed January 1, 2014), suggesting that differences in plant nutrition among soil treatments likely did not contribute significantly to the differences in plant growth

observed between soil treatments in this experiment. Despite greater soil Ca and Mg concentrations, trees that received compost amendment had reduced leaf Ca and Mg concentrations relative to that of the control, and the Mg concentration was below a concentration considered deficient; however, this did not appear to have severely affected plant growth in the compost-amended sub-plots. Sub-plots that received compost amendments had the greatest soil C content, supporting the hypothesis that increases in soil C content are associated with improved soil health (Lehman et al., 2015).

The effect of compost amendment on average monthly soil volumetric water content depended on the irrigation system used and sampling date, with application of compost under a microsprinkler irrigation system resulting in significantly lower average monthly soil volumetric water content relative to application of compost under a drip irrigation system in September and October of the second growing season (2015). Despite differences in soil water contents, significant differences in photosynthetic rate, transpiration rate, stomatal conductance, and stem water potential were not observed among soil treatments when they were analyzed in 2017, suggesting that differences in plant water status likely did not contribute strongly to the differences in plant growth response to soil treatments observed in this study.

3.4.3 Influence of Bark Chip Mulch

Surface application of high C organic mulch has previously been demonstrated to promote the growth of newly planted apple trees at old orchard sites (Forge et al., 2013b, 2008, 2003; Forge and Kempler, 2009; Granatstein and Mullinix, 2008; Merwin et al., 1994; Neilsen et al., 2014, 2003; Shribbs and Skroch, 1986; van Schoor et al., 2009), as well as reduce *P. penetrans* populations in orchards (Forge et al., 2013b, 2008,

2003; Forge and Kempler, 2009; Neilsen et al., 2003). In this field experiment, application of bark chip mulch improved trunk diameters of sweet cherry trees planted into an old apple orchard site, as well as sustained smaller *P. penetrans* population densities in roots and soil over the first three years of orchard establishment relative to that of the control, particularly when applied in combination with compost. Similar to what was also observed using compost amendments in the current field experiment, surface application of bark chips resulted in a trend towards greater resource allocation to vegetative growth in the early years of orchard establishment and a trend towards enhanced fruit yield in subsequent years relative to that of untreated soil. Overall, these data suggest bark chip mulch can promote the establishment of fruit trees planted at old orchard sites, as well as provide prolonged suppression of *P. penetrans* populations relative to that of soil fumigants, thereby showing significant potential as a component of an integrated replant disease management approach alongside compost amendments. In this experiment, bark chip mulch was applied to soil at rates of 250 m^3 ha^{-1} orchard area. Assuming an estimated cost of \$20 m^{-3} (CAD) for bark chips, such an application would cost approximately \$5000 (CAD), equivalent to that of soil fumigation with Basamid®.

When fine root density was quantified in soil cores taken from the mineral soil layer (0-30 cm soil depth) from 2016 through 2017, surface application of bark chips reduced fine root density in soil relative to that of the compost treatment, and showed a strong trend towards reduced fine root density relative to that of the control and fumigation treatments. While soil sampling, a large proportion of roots were observed to be growing in the mulch layer above the mineral soil; therefore, an additional soil coring procedure was performed in July 2017 to test the supplementary hypothesis that fine

roots were preferentially growing in the mulch layer above the soil, potentially to escape parasitism by *P. penetrans*. In the sub-plots that only received surface application of bark chips, fine root density was not significantly greater in the mulch layer relative to that of the mineral soil collected from a depth of 0 - 7.5 cm, suggesting that roots were not preferentially growing in the mulch layer, but rather, were growing in this layer at the same density as in the two mineral soil depths examined. When compost was applied to the soil surface in combination with bark chips, fine root density was increased in the mulch layer relative to that of application of bark chips alone, indicating a significant stimulatory effect of composts on root growth in the bark chip mulch layer. Interestingly, fine roots were not present in the thin layer of compost located directly above the soil surface when the amendment was applied without bark chips, possibly due to the excessively high concentration of nutrients present in the non-incorporated compost layer (Hunt et al., 1972). Contrary to the original hypothesis, roots growing in the bark chip mulch layer were equally parasitized by *P. penetrans* as roots growing in the bulk soil below, suggesting that the root density in the mulch layer above soil was not associated with parasitism-escape, but rather, other beneficial factors attributed to the bark chips, such as the availability of certain plant nutrients (Pickering and Shepherd, 2000), or root access to irrigation water (Pickering et al., 1998). These results are in contrast to studies in sugarcane production systems, which have consistently demonstrated nematode-suppression directly in the mulch/litter layer above the soil surface (Stirling et al., 2011, 2005), a discrepancy that might possibly be attributed to differences in the composition of the mulch material used and differences in the production systems. When compost was applied to the soil surface along with bark chip mulch, significant reductions in root

parasitism by *P. penetrans* were observed in the mulch layer, suggesting that compost amendments can help promote a mulch environment that is suppressive to *P. penetrans*.

Surface application of bark chips did not have the same stimulatory effect on bacterial populations in the rhizosphere of sweet cherry trees as was observed with preplant soil incorporated composts. Bark chip mulch was associated with increased soil microbial activity, enhanced rhizosphere populations of total fungi, as well as an increased abundance of *Pseudomonas* spp. (culture- and DNA-based quantification) and phosphate-solubilizing bacteria in the rhizosphere. Despite only minor stimulation of bacterial populations in the rhizosphere associated with soil suppressiveness relative to compost, surface application of bark chips enhanced soil biological suppressiveness to plant-parasitic nematodes in the soil below the mulch layer relative to non-amended soil, as well as reduced *P. penetrans* populations in soil and roots, suggesting that other types of antagonists were likely contributing to nematode suppression. Surface application of bark chips stimulated greater rhizosphere colonization by fungi and enhanced soil microbial activity, indicating a possible role of increased activity and abundance of fungal antagonists. Populations of nematode-trapping fungi have previously been associated with decomposition of high C organic material (Cooke, 1962), such as the bark chips applied in this field experiment, and selective enrichment of such fungi may have contributed significantly to nematode suppression. Similarly, many other groups of antagonistic fungi (Jatala, 1986; Morgan-Jones et al., 1983; Rodriguez-Kabana and Morgan-Jones, 1988), antagonistic bacteria (Starr and Sayre, 1988), and other soil invertebrates (Doncaster and Hooper, 1961; Hutchinson and Streu, 1960; Inserra and Davis, 1983; Murphy and Doncaster, 1957; Sayre and Powers, 1966), which were not

specifically quantified in this particular experiment, also may have contributed to nematode suppression in the bark chip mulch treatment. The abundant root growth observed in the bark chip mulch layer above the soil warrants examination of the rhizosphere community in roots growing in this zone; however, it does not appear that these microorganisms played a significant role in nematode suppression, at least without the co-addition of a compost amendment.

Mineral soil under the bark chip mulch treatment had a significantly larger C/N ratio relative to that of the control treatment. In contrast to the compost amendments, surface application of bark chips reduced soil concentrations of P, Mg, Na, and NO_3-N relative to the control, suggesting that significant nutrient immobilization may have occurred in this treatment. This was further supported by low leaf N concentrations in the bark chip mulch amended sub-plots irrigated by microsprinklers, demonstrating that combined application of bark chip mulch under a microsprinkler irrigation system can result in significant N immobilization, and thus may require supplemental fertilization. Bark chip mulch did increase leaf concentrations of B, Zn, and Mg, and the Zn concentrations were increased relative to levels that were considered deficient in the control treatment in 2015, suggesting some beneficial nutrient contribution did occur as a result of this soil amendment. Similar to compost, bark chip mulch reduced the concentration of Al in soil relative to that of the control. Overall, the majority of the leaf nutrient concentrations were within the production guidelines across all soil treatments, likely as a result of sufficient fertigation; it is therefore unlikely that differences in plant nutrition played a strong role in contributing to the differences in plant growth observed under soil treatments in this experiment.

Bark chip mulch did not significantly affect average monthly soil volumetric water content in sub-plots. Similarly, no differences in photosynthetic rate, transpiration rate, stomatal conductance, or stem water potential were observed among soil treatments when they were measured in 2017, suggesting that differences in plant water status likely did not contribute strongly to the differences in plant growth response to soil treatments in this experiment. Based on the results from this experiment, I would recommend the use of preplant soil incorporation of compost combined with surface application of bark chips as a non-fumigant alternative to enhance the establishment of sweet cherry in old orchard soil. Although such an application would be more costly than soil fumigation, an economic comparison of organic soil amendments and fumigation should note that the use of such amendments also brought additional benefits to soil that fumigants did not provide, including greater soil C sequestration, enhanced nutrient availability, and the establishment of biologically suppressive soil.

3.4.4 Influence of Irrigation Type

Choice of low-volume irrigation type has previously been demonstrated to influence the establishment of perennial crops, including peach (Bryla et al., 2005, 2003) and highbush blueberry (Bryla et al., 2011). On peach planted in a sandy loam soil, drip irrigation was demonstrated to increase TCSA, water use efficiency, soil water content, and marketable fruit yield relative to that of microsprinklers (Bryla et al., 2005, 2003). On highbush blueberry planted in a silty clay loam soil, drip emitters promoted greater dry cane weight, water use efficiency, and soil water content relative to that of microsprinklers (Bryla et al., 2011). This study is therefore in agreement with prior literature on other perennial crops; sweet cherry trees irrigated using drip emitters

displayed larger trunk diameters relative to that of microsprinklers throughout the first four years of orchard establishment. Additionally, drip emitters were associated with enhanced root growth as well as larger fruit yields relative to that of sweet cherry trees irrigated with microsprinklers.

While soil moisture regimes can have significant effects on the activity of plant-parasitic nematodes (Prot and Matias, 1995; Smitley et al., 1992), little is known of how different types of low volume irrigation systems affect plant-parasitic nematode populations. In this field experiment, there were smaller *P. penetrans* populations under drip irrigation relative to that of microsprinklers during the critical first four years of growth, and this likely contributed significantly to enhanced plant growth and fruit yield in this treatment. In contrast to the smaller *P. penetrans* population observed in this study, drip irrigation has, on occasion, been associated with increased susceptibility to root rot fungal pathogens, including *Pythium* and *Phytophthora* (Feld et al., 1990), particularly on sites with poor soil drainage (Bryla and Linderman, 2007). Populations of oomycete pathogens were not assessed, but the improved tree growth and fruit yield under the treatment resulting in greatest water contents (drip irrigation) suggests that such pathogens did not play a significant role in this study. *M. xenoplax* populations were significantly stimulated by the drip irrigation system, particularly when it was used in combination with compost; however, population densities were very small and were not likely to have as strong of an influence on tree health as *P. penetrans*.

In this study, soil biological suppressiveness to plant-parasitic nematodes, soil microbial activity, as well as rhizosphere colonization by antagonistic bacteria did not differ between irrigation treatments, suggesting that abiotic factors were likely

responsible for the differences in *P. penetrans* abundance. In general, when soil is saturated with water, *P. penetrans* population growth is poor, but population growth tends to improve as soil water content decreases, until reaching a critical point (Kable and Mai, 1968). This is believed to be attributed to reduced metabolic stress on the nematode as the soil and nematode approach osmotic equilibrium (Wallace, 1973), as well as increased oxygen availability for the nematode with decreasing soil water content (van den Bergh et al., 2006). It is possible that, by increasing the amount of water directly in the root zone, drip irrigation has promoted an environment that is sub-optimal for *P. penetrans* activity and/or population development.

Root colonization by AMF was reduced under microsprinkler relative to that of drip irrigation. Direct relationships between AMF and *P. penetrans* are complex, with AMF potentially suppressing *P. penetrans* in many studies (De La Peña et al., 2006; Forge et al., 2001; Pinochet et al., 1993a), while other studies have conversely demonstrated that significant nematode damage can suppress arbuscule formation (O'Bannon and Nemec, 1979; Pinochet et al., 1995). With regard to fruit trees, some studies have demonstrated that AMF can reduce *P. penetrans* populations (Forge et al., 2001; Pinochet et al., 1993a). Most of these studies compared AMF-inoculated and non-inoculated plants rather than differential colonization by indigenous AMF. Alternatively, greater root colonization by AMF under drip irrigation may have been a result of greater plant growth in this treatment, as opposed to direct microbe-nematode interactions, with drip-irrigated trees having more C to support mycorrhizal symbiosis. The contrasting responses of AMF and *P. penetrans* in this study are consistent with either model of interaction (1) AMF suppress *P. penetrans*, (2) *P. penetrans* suppress AMF, or (3)

irrigation type had more direct differential effects on *P. penetrans* and AMF, and the inverse relationship between the two root-colonizing organisms was coincidental.

Differences in root colonization by AMF in this particular study might be a direct result of differential root infestation by *P. penetrans* between the two irrigation treatments. Some nematodes, such as the ectoparasitic ring nematode (*M. xenoplax*), have been demonstrated to reduce AMF colonization on perennial crops through competition for plant photosynthate (Schreiner and Pinkerton, 2008). Migratory endoparasitic nematodes, including *P. penetrans*, have also been observed to reduce rates of root colonization by AMF on perennial plants (Forge et al., 2001; O'Bannon and Nemec, 1979; Pinochet et al., 1995); however, with this nematode, reductions in arbuscular colonization have generally been linked to the formation of necrotic lesions and destruction of root cortical tissue as a consequence of root feeding (Hussey and Roncadori, 1978). It seems reasonable to speculate that the reduced abundance of arbuscules in sweet cherry roots in the microsprinkler irrigation treatment may have been attributed to greater root infestation by *P. penetrans* in this study as well.

Microsprinkler irrigation was found to induce moderate N immobilization, particularly when applied over bark chip mulch, suggesting additional N fertilization may be required in the first year if microsprinklers are to be used in combination with bark chip mulch. Conversely, leaf concentrations of Mg were deficient in 2014 under the drip emitter irrigation system relative to that of microsprinkler, suggesting additional Mg fertilization may be required in the first year if drip emitters are to be used, particularly in combination with composts. Although other differences in leaf nutrient concentrations were observed between irrigation treatments, nutrient concentrations were within

recommended levels in the production guidelines for both irrigation treatments (www.bctfpg.ca; accessed January 1, 2014), and therefore, these differences likely played a minimal role in the differences in plant growth observed between irrigation treatments in this study.

On average, soil water contents were greater under trees irrigated with drip emitters relative to those of microsprinklers throughout the first four years of orchard establishment. Other studies exploring the effects of low-volume irrigation systems on perennial crops have also reported greater soil water content under drip emitters relative to those of microsprinklers (Bryla et al., 2005; Bryla and Linderman, 2007; Hannam et al., 2016; Layne et al., 1996), and this has generally been attributed to greater surface evaporation of water under microsprinklers relative to that of drip emitters. Despite greater soil water contents under the drip emitters, photosynthetic rate, transpiration rate, stomatal conductance, and stem water potentials were lower for drip emitter irrigated trees than microsprinkler irrigated trees when they were measured in 2017. Nevertheless, stem water potentials were not critically low and trees did not display any symptoms of water stress, with stem water potentials greater than -1.0 MPa across both irrigation treatments during the growing season. These results support the hypothesis that other factors, such as suppression of *P. penetrans* populations through alterations in the soil water content, are likely to have played a more prominent role than differential tree water availability in contributing to the differences in tree growth observed between these two irrigation types.

Very few interaction effects were observed between soil treatment and irrigation type, with the exception of populations of *M. xenoplax*, leaf N, P and Fe contents, and

soil water content. Soil treatment and irrigation type had very strong main factor effects on the majority of the variables evaluated, most importantly the growth of newly planted sweet cherry trees. This suggests that composts and bark chip mulch can be utilized irrespective of the low-volume irrigation system chosen. Overall, this implies that orchardists will not need to adapt their choice of soil management practices to the irrigation type that they have chosen.

4 Chapter 4: Multisoil Replant Experiment

4.1 Background

Variation in disease etiology (Manici et al., 2013; Mazzola, 1998; Yang et al., 2012), soil texture (Florini et al., 1987; Jordaan et al., 1989; Wallace, 1973), climatic conditions (Chakraborty and Newton, 2011), and various other environmental conditions (Neilsen and Yorston, 1991; Redman et al., 2001; Traquair, 1984), make it difficult to expand positive results from one experimental orchard to other orchard sites and growing regions. Most of the research conducted on replant disease has been focused on apple, and as a result, little is known about replant disease of other fruit tree species. Utkhede and Li (1988) demonstrated that apple seedling biomass was significantly improved by pasteurizing soil collected from apple, peach, sweet cherry, and pear (*Pyrus* (L.)) orchards, demonstrating the non-specific nature of replant disease. Whether non-fumigant management strategies, such as compost amendments and biocontrol agents, will similarly enhance the growth of apple planted into soil that was cropped with other fruit tree species remains to be demonstrated.

In addition to variability among sites in the effectiveness of compost amendments (Whipps, 1997), variation in the composition of compost amendments can also influence the level of plant growth promotion and nematode suppression observed (Atiyeh et al., 2000; Hoitink and Fahy, 1986; Thoden et al., 2011). Variable root growth promotion was observed between yard trimmings compost and agricultural waste compost amendments (Chapter 2), further supporting previous literature reporting differential plant growth enhancement among different compost amendments (Courtney and Mullen, 2008). Discrepancies in plant growth enhancement and nematode

suppression have been associated with variation in a number of compost physiochemical and biological parameters. For example, physiochemical parameters, such as particle size, N content, C/N ratio, feedstock composition, EC, pH, bulk density, porosity, decomposition level, and water-holding capacity (Hoitink and Fahy, 1986; Lozano et al., 2009; Renco et al., 2009, 2007), and biological parameters, including the capacity to promote an antagonistic soil/rhizosphere microflora and enhance soil microbial activity (Boehm and Hoitink, 1992; Hoitink et al., 1997), can contribute to differential plant growth promotion and pathogen suppression among different compost amendments. Most of the studies on mitigating replant disease with composts have been performed using a limited number of orchard sites and a limited number of soil amendments (Braun et al., 2010; Moran and Schupp, 2003; van Schoor et al., 2009). As a result, there is a need to demonstrate the reproducibility of replant disease control through the use of composts using a greater number of orchard sites and compost amendments.

Biocontrol of replant disease through the use of microbial inoculants remains an intriguing possible non-fumigant disease management strategy. In the Okanagan Valley, Canada, inoculating apple roots with *Bacillus subtilis* strain EBW4 was observed to improve plant growth and suppress fungal pathogens in apple orchard soil (Utkhede et al., 2001; Utkhede and Smith, 1993a, 1992). *Pseudomonas* spp. are able to produce broad-spectrum antibiotics, extracellular enzymes, and siderophores (Haas and Défago, 2005), and have been associated with improved establishment of fruit trees (Mazzola, 1999), as well as suppression of fungi and nematodes (Haas and Défago, 2005; Nandi et al., 2015; Siddiqui and Shaukat, 2003). Secondary metabolites produced by *Pseudomonas* spp. that have been linked to pathogen suppression include 2,4-diacetylphloroglucinol,

phenazine-3-carboxylic acid, pyrrolnitrin, pyoluteorin, and hydrogen cyanide (de Souza and Raaijmakers, 2003; Raaijmakers et al., 1997; Ramette et al., 2003). Increases in rhizosphere populations of *Pseudomonas* spp. were associated with improved plant growth and nematode suppression in compost-amended orchard soil (Chapter 3). Biocontrol with antagonistic *Pseudomonas* spp. isolated from a suppressive fruit tree rhizosphere might show potential as an alternative to fumigation for management of replant disease.

In Chapters 2 and 3 of this dissertation, I described how preplant soil incorporation of compost improved plant growth and suppressed *Pratylenchus penetrans* populations infecting apple and sweet cherry seedlings in apple orchard soil in a greenhouse experiment, as well as sweet cherry trees planted into an old apple orchard site. Moreover, compost-induced plant growth promotion and nematode suppression were correlated with increases in rhizosphere microbial populations that have been associated with soil suppressiveness. The primary aim of this chapter was to extend previous research findings by evaluating the capacity of three different compost amendments to enhance plant growth, suppress *P. penetrans* populations, and stimulate microbial populations associated with soil suppressiveness in orchard soil collected from four different orchard sites. In Chapter 2, root-dip inoculation of apple seedlings with *Serratia plymuthica* 6-5 failed to improve the growth of apple or sweet cherry seedlings planted into potted apple orchard soil. The secondary aim of this chapter was to isolate *Pseudomonas* spp. from a suppressive sweet cherry rhizosphere, screen isolates for desirable biocontrol traits, and evaluate the capacity of two antagonistic isolates to enhance plant growth and suppress *P. penetrans* populations in orchard soil collected

from four orchard sites. As described in Chapter 1, I predicted that composts would enhance plant growth, suppress *P. penetrans*, and stimulate microbial indicators of soil suppressiveness in soil collected from four orchard sites. I also predicted that compost-amended orchard soil would be a good source for antagonistic *Pseudomonas* isolates, and that inoculation of apple seedlings with such isolates would enhance plant growth and suppress *P. penetrans* populations in orchard soils.

4.2 Methods

4.2.1 Site Description and Initial Soil Analyses

Orchard sites MUPP, P20, and NENT are located at the Summerland Research and Development Centre (Summerland, British Columbia, Canada). MUPP is a conventionally managed experimental block of apple ('Gala' on Malling 26 rootstock) planted in an Osoyoos loamy sand soil for 10+ years. P20 is a conventionally managed experimental block of sweet cherry ('Lapins' on Krymsk 5 rootstock) planted in an Osoyoos loamy sand soil for 12+ years. NENT is a conventionally managed experimental block of pear ('Barlett') planted in a silty loam soil for 20+ years. Located at 49°51'44.3"N 119°23'51.8"W (Kelowna, British Columbia, Canada), DD is a conventionally managed commercial block of sweet cherry ('Sentennial' on Mazzard rootstock) planted in a Skaha sandy loam soil for 10+ years.

Soil was collected from MUPP, P20, and NENT on November 8, 2016, and from DD on November 9, 2016. Using a shovel, a composite soil sample (from >5 orchard rows and >20 total tree root zones) was collected from each orchard site directly from the root zone of established trees at a depth of 5 - 30 cm. For experimental orchard sites (MUPP, P20, and DD), orchard soil was collected from sub-plots that did not

receive soil treatment (no application of fumigants, nematicides, or soil amendments). Soil from within each orchard site was thoroughly mixed and passed through a 6-mm mechanical sieve to remove rocks and organic debris, prior to subsequent use. Analyses of soil physiochemical properties were performed by A & L Canada Laboratories Inc. (London, Ontario, Canada) (Table 4.1). Initial populations of *P. penetrans* were examined by extracting nematodes from 100-mL subsamples of soil from each site, using the centrifugal-floatation technique (Jenkins, 1964). After collecting the nematodes over a 25-µM sieve, nematodes samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. The abundance of plant-parasitic nematodes was determined using an inverted compound microscope.

Table 4.1 - Site descriptions and soil physiochemical properties. CEC refers to cation-exchange capacity, OM refers to organic matter, and NO_3-N refers to nitrate N.

	Orchard Site			
Parameter	MUPP	P20	NENT	DD
Species	*Malus domestica* Borkh.	*Prunus avium* L.	*Pyrus communis*	*P. avium* L.
Cultivar	'Gala' on Malling 26	'Lapins' on Krymsk 5	'Barlett'	'Sentenial' on Mazzard
Soil texture	Loamy sand	Loamy sand	Silty Loam	Sandy Loam
Pratylenchus penetrans 100 mL^{-1}	150	143	92	63
pH	7.2	6.9	7.2	6.3
Cation-exchange capacity (CEC) (meq 100 g^{-1})	8.8	10.3	11.9	11.7
OM (%)	1.5	2.3	2.4	3.5
C/N ratio	5.1	8.5	5.9	7.3
P (ppm)	68	66	66	110
K (ppm)	169	360	318	337
Mg (ppm)	210	170	270	200
Ca (ppm)	1220	1330	1640	1590
Na (ppm)	19	25	25	13
Al (ppm)	413	13	604	652
S (ppm)	8	9	8	11
NO_3-N (ppm)	16	23	25	39

4.2.2 Compost Greenhouse Replant Experiment

The greenhouse experimental design was a randomized complete block with eight blocks of twenty treatment combinations representing a complete factorial combination of four orchard soils (MUPP, P20, NENT, DD) and five soil treatments. Two-leaf stage apple seedlings (Ambrosia; one per pot) were planted into eight replicate 15-cm pots of each of the five soil treatments for each orchard site (1) steam pasteurized orchard soil, (2) a control compromised of 80% untreated orchard soil and 20% steam pasteurized orchard soil, (3) AWC-2017 at a 20% v v^{-1} application rate, (4) YTC-2017 at 20% v v^{-1}, and (5) municipal waste compost (MWC-2017) at 20% v v^{-1}. The pots were placed in a temperature-controlled (24 °C) greenhouse (located at 49°56'52.5"N 119°63'79.8"W) in a randomized complete block design. Seedlings were grown using a 14-hour photoperiod supplied with supplemental high-pressure sodium lighting for 16 weeks prior to analysis (planted January 13th, 2017).

Orchard soil was steam pasteurized at 70 °C for 1 h on two consecutive days in a Pro-Grow SS-30 Electric Soil Sterilizer (U.S. Global Resources Inc., Fair Oaks. Ranch, USA). Composts were incorporated into soil one week prior to planting. MWC-2017 was produced locally by the District of Summerland from lawn trimmings, tree prunings, and treated biosolids. Analyses of compost physiochemical properties were performed by A & L Canada Laboratories Inc. (London, Ontario, Canada) (Table 4.2). Insect and mite pests were controlled by monthly foliar application of Beleaf 50 SG® (ISK Biosciences Corporation, Concord, Ohio, USA) at a rate of 0.3 g L^{-1}. Pots were fertilized biweekly with all-purpose fertilizer (20:8:20), with a cumulative application of 0.75 g of mineral N supplied to each seedling.

Table 4.2 - Physiochemical properties of compost amendments. AWC-2017 refers to agricultural waste compost, YTC-2017 refers to yard trimmings compost, MWC-2017 refers to municipal waste compost, OM refers to organic matter, NO_3-N refers to nitrate N, and TOC refers to total organic C.

	Compost amendment		
Parameter	AWC-2017	YTC-2017	MWC-2017
pH	7.1	7.8	7.2
Conductivity (ms cm^{-1})	7.1	4.0	2.2
OM (%)	22.2	35.1	32.3
NO_3-N (mg kg^{-1})	1021	15	35
N (%)	1.64	2.31	1.9
P (%)	0.14	0.19	0.12
K (%)	0.52	0.83	0.23
Mg (%)	0.13	0.30	0.09
Ca (%)	0.66	0.78	0.29
TOC (%)	13.6	21.7	20.0
C:N ratio	8.3	9.4	10.5

4.2.2.1 Analyses of Plant Growth

In order to test the hypothesis that composts would increase plant growth, various aspects of plant growth were measured. At time of harvest, shoots were cut at soil level and shoot length recorded prior to drying in an oven at 65 °C for 72 h for determination of shoot dry weight. Entire root systems were carefully removed from each pot and washed thoroughly under running water. After removing a 2-g subsample of fine root tissue for extraction of endoparasitic nematodes, the remaining root tissue was dried in an oven at 65 °C for 72 h and root dry weight recorded.

4.2.2.2 *P. penetrans* Populations

In order to test the hypothesis that compost would suppress *P. penetrans,* populations in roots and soil were quantified. Nematodes were extracted from 100-mL subsamples of soil from each pot using the centrifugal-floatation technique (Jenkins, 1964) prior to planting, as well as at the time of harvest. After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Migratory endoparasitic nematodes were extracted at harvest from a subsample of root tissue

collected from each seedling using the shaker agitation technique (Shurtleff and Averre, 2005). Approximately 2 g of fine root tissue were placed into a 250-mL Erlenmeyer flask containing 100 mL of water. Flasks were incubated at room temperature on a rotary shaker set at 125 rpm for a duration of 5 days. Nematodes were collected over a 25-µm sieve, and were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Extracted root tissue was dried at 65 °C for 48 h prior to computing *P. penetrans* per gram of dry root.

4.2.2.3 Soil Microbiology

In order to test the hypothesis that composts would increase the abundance of microorganisms with potential biocontrol activity, I quantified the abundance of total bacteria, total fungi, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria. DNA was extracted from 0.25 g of soil from each pot using the E.Z.N.A Soil DNA Isolate Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). Real-time PCRs for quantification of beneficial microorganisms in soil were performed as described previously (Chapter 2); however, reactions were performed using Luna Universal qPCR Master Mix (New England Biolabs, Pickering, Ontario, Canada) in place of SsoFast™ Evagreen® Supermix (Bio-Rad Laboratories Inc., Hercules, California, USA)

4.2.2.4 Soil Microbial Activity

In order to test the hypothesis that composts would increase soil parameters indicative of pathogen control, soil microbial activity was measured. Hydrolysis of fluorescein diacetate was measured on a subsample of soil from each pot, as described by Green et al., (2006). Approximately 1.0 g of soil was placed in a 50-mL centrifuge tube with 47.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of 4.9 mM fluorescein

diacetate (20 mg fluorescein diacetate in 10 mL of acetone). The centrifuge tube was capped, vortexed for 5 sec, and placed in an incubator at 37 °C for 3 h. After incubation, 2 mL of acetone were added to the tube, vortexed for 5 sec, and then centrifuged for 7.5 min at 5,000 rpm. Approximately 200 µL of supernatant were transferred to three triplicate wells of a 96-well microtitre plate and absorbance measured on a spectrophotometer at 490 nm (xMark^{TM} Microplate Absorbance Spectrophotometer, Bio-Rad, Hercules, California, USA). The concentration of fluorescein released was calculated by reference to a standard curve consisting of 0.03, 0.1, 0.3, and 0.5-mg solutions of fluorescein prepared in the same solution matrix described above. Controls were performed for each soil treatment x orchard site combination to correct for variation in background fluorescence between treatments. A composite soil sample was obtained for each soil treatment x orchard site combination by combining 1.0 g of soil from each of the eight replicate pots, and mixing thoroughly. Approximately 1.0 g of the composite soil sample were placed in a 50-mL centrifuge tube with 47.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of acetone (in place of fluorescein diacetate substrate). The centrifuge tube was incubated and analyzed as described above. An additional control was performed to correct for spontaneous fluorescence development by the fluorescein diacetate substrate at 37 °C, which consisted of 47.5 mL of 60 mM sodium phosphate buffer and 0.5 mL of 4.9 mM fluorescein diacetate (no soil).

4.2.3 Biocontrol Greenhouse Replant Experiment

Prospective biocontrol isolates were obtained from the rhizosphere of the newly planted sweet cherry trees described in Chapter 3, and were evaluated for biocontrol of replant disease on apple seedlings planted into four different potted orchard soils (MUPP,

P20, NENT, DD).

4.2.3.1 Biocontrol Library Creation

Prospective biocontrol bacteria were isolated in May 2015 from the first planting row of the sweet cherry orchard described in Chapter 3 (five different soil treatment sub-plots; microsprinkler irrigation system). Single colonies were plucked from the GmS1 plates that were previously used to quantify *Pseudomonas* spp. in the sweet cherry rhizosphere (Chapter 3). A total of 20 isolates was obtained from each of the five soil treatments (10^2 dilution ratio), giving a total of 100 isolates in the biocontrol culture library (Appendix J). Each isolate was purified on TSA using the streak plate technique, and subsequently stored at -85 °C in 20% glycerol until subsequent use.

4.2.3.2 Cultural Based Biocontrol Screening

All 100 *Pseudomonas* isolates were evaluated for antagonistic activity to necrotrophic fungi associated with replant disease using a dual culture inhibition assay, as described previously (Chapter 2). Bacteria were screened for antagonistic activity to *Fusarium* sp. F1-1, *Fusarium* sp. F2-1, "*Cylindrocarpon*" sp. C1-1, "*Cylindrocarpon*" sp. C2-1, and *Rhizoctonia* sp. R1-1, all of which were isolated from surface-sterilized necrotic roots of apple and sweet cherry seedlings grown in old orchard soil (Chapter 2). *Pseudomonas* isolates that showed antagonistic potential to at least one necrotrophic fungus were further characterized for other desirable biocontrol traits on differential growth media. For each assay, an inoculating loop full of each *Pseudomonas* isolate was inoculated into the centre of a well on a 24-well microtitre plate containing 1 mL of growth medium in each well. Isolates were screened for phosphate solubilizing activity using PVK medium, as described previously (Chapter 3). Isolates were screened for

fluorescent pigment production on King's B agar (Johnsen and Nielsen, 1999). After 4-days of incubation at room temperature on King's B agar, colonies were evaluated for the production of a fluorescent pigment when placed under an ultraviolet light. Isolates were screened for siderophore production on chrome azurol S agar (Alexander and Zuberer, 1991). After 4 days of incubation at room temperature on chrome azurol S agar, colonies were evaluated for the production of an orange halo around colonies. Isolates were screened for protease activity on skimmed milk agar (Pailin et al., 2001). After a 7-day incubation period at room temperature on skimmed milk agar, colonies were evaluated for the production of a zone of clearing around colonies.

4.2.3.3 Molecular Characterization of Antagonistic Biocontrol Isolates

Pseudomonas isolates that showed antagonistic potential to at least one necrotrophic fungus were further characterized for desirable biocontrol traits using previously developed PCR probes (Table 2.3). Total genomic DNA was extracted from each *Pseudomonas* isolate using the freeze-thaw technique (Millar et al., 2000). Each isolate was grown in TSB at room temperature for 24 h, and then 0.5 mL of culture was centrifuged at 13,000 rpm for 5 min. The cells were suspended in 0.1 mL of TE Buffer (10 mM Tris-HCl pH 8.0; 1 mM EDTA), incubated at 95 °C for 45 min, and then placed in a freezer at -85 °C for 1 h. The frozen cell suspension was thawed at room temperature, centrifuged at 13,000 rpm for 15 min and then the supernatant was removed and stored at -20 °C until further analysis.

Isolates were screened for the presence of the *phlD* gene responsible for 2,4-diacteylphloroglucinol biosynthesis (Raaijmakers et al., 1997), the *phzCD* gene responsible for phenazine-3-carboxylic acid biosynthesis (Raaijmakers et al., 1997), the

prnD gene responsible for pyrrolnitrin biosynthesis (de Souza and Raaijmakers, 2003), the *pltC* gene responsible for pyoluteorin biosynthesis (de Souza and Raaijmakers, 2003), and the *hcnBC* gene responsible for hydrogen cyanide biosynthesis (Ramette et al., 2003) (Table 4.3). As a positive control for successful DNA extraction as well as confirmation of the absence of PCR inhibitors, a portion of the 16S region was amplified using the 8F/531R primer set, as described previously (Chapter 2). The type isolate *P. fluorescens* Pf-5 (NRRL B-23932) was included as a positive control for amplification of the *phlD*, *prnD*, *pltC* and *hcnBC* genes. The type isolate *P. fluorescens* 2-79 (NRRL B-15132) was included as a positive control for amplification of the *phzCD* gene.

Table 4.3 - Primers used for DNA-based detection of desirable biocontrol genes. The 16S gene region was included as a positive control.

Gene region	Primer name	Sequence (5' to 3')	Reference
phlD	Phl2a	GAGGACGTCGAAGACCACCA	Raaijmakers et al., (1997)
	Phl2b	ACCGCAGCATCGTGTATGAG	
phzCD	PCA2a	TTGCCAAGCCTCGCTCCAAC	Raaijmakers et al., (1997)
	PCA3b	CCGCGTTGTTCCTCGTTCAT	
prnD	PRND1	GGGGCGGGCCGTGGTGATGGA	de Souza and Raaijmakers, (2003)
	PRND2	YCCCGCSGCCTGYCTGGTCTG	
pltC	PLTC1	AACAGATCGCCCCGGTACAGAACG	de Souza and Raaijmakers, (2003)
	PLTC2	AGGCCCGGACACTCAAGAAACTCG	
hcnBG	ACa	ACTGCCAGGGGCGGATGTGC	Ramette et al., (2003)
	ACb	ACGATGTGCTCGGCGTAC	
16S	8F	AGAGTTTGATCCTGGCTCAG	Hynes et al., (2008)
	531R	ACGCTTGCACCCTCCGTATT	

For each gene region of interest, amplification reactions contained 2.5 µL of 10X ThermoPol® Buffer (New England Biolabs, Beverly, MA, USA), 1.25 µL of 100% dimethyl sulfide, 0.5 µL of 10 mM dNTPs, 0.1 µL of 0.1 mM forward and reverse primer, 1.0 µL of template DNA, and 0.1 µL of *Taq* DNA Polymerase (New England Biolabs, Beverly, MA, USA), brought to a volume of 25 µL. For amplification of the *phlD* gene, the PCR temperature profile consisted of 1.5 min at 94°C, and 35 cycles of 35 s at 94 °C, 30 s at 65 °C, 45 s at 68 °C. For amplification of the *phzCD* gene, the PCR

temperature profile consisted of 2 min at 94 °C, 30 cycles of 1 min at 94 °C, 45 s at 67 °C, 1 min at 68 °C, and 5 min at 68 °C. For amplification of the *prnD* and *pltC* genes, the PCR temperature profile consisted of 2 min at 94 °C, 30 cycles of 1 min at 94 °C, 2 min at 68 °C, and 5 min at 68 °C. For amplification of the *hcnBC* gene, the PCR temperature profile consisted of 2.5 min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 63 °C, 1 min at 68 °C, and 10 min at 68 °C. For the 16S gene region, the PCR temperature profile was performed as described previously (Chapter 2). Amplification reactions were carried out on a Veriti® 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA).

4.2.3.4 *P. penetrans* Antagonism Bioassay

Prospective biocontrol bacteria were screened for suppression of *P. penetrans* root infestation on 'Crimson Passion' sour cherry explants planted into steam pasteurized soil that was re-inoculated with *P. penetrans*. Soil was collected from a grassland at Summerland Research and Development Centre that had not been previously planted with any crop. Soil was steam pasteurized at 70 °C twice and placed into pots. *P. penetrans* were obtained from a population maintained in a greenhouse on mint plants (Chapter 2). Nematodes were extracted from mint roots using the shaker agitation technique (Shurtleff and Averre, 2005), and inoculated into the pasteurized soil to a density of 200 *P. penetrans* per 100 mL of soil by pipetting a known density of *P. penetrans* into three 1-cm deep holes in the top soil of each pot. Pots were incubated for 7 days prior to biocontrol inoculation and planting.

Pseudomonas isolates that showed antagonistic activity to necrotrophic fungi were grown in TSB for 2 days, and then diluted by 1:10 to prepare a liquid inoculant for the greenhouse trials. Isolates were inoculated into the planting hole with sour cherry

explant roots, as described previously (Chapter 2). Control treatments included pots that did not receive inoculation with a *Pseudomonas* isolate (sterile TSB), as well as pots that were not inoculated with *P. penetrans* or a *Pseudomonas* isolate. Each soil treatment was replicated four times. Pots were placed in a temperature-controlled greenhouse (24 °C) in a randomized block design and watered as required. Pots were fertilized biweekly with all-purpose fertilizer (20:8:20).

Plants were analyzed for total biomass and *P. penetrans* population density in fine roots at 12 weeks post inoculation. Plants were removed from pots and root systems thoroughly washed in water to remove adhering soil. A 2-g subsample of fine root tissue was used to extract migratory endoparasitic nematodes using the shaker agitation technique (Shurtleff and Averre, 2005). The remaining plant material was dried in an oven at 70 °C for 48 h and total dry biomass computed.

4.2.3.5 Multisoil Replant Experiment

The greenhouse experimental design was a randomized complete block with eight blocks of sixteen treatment combinations representing a complete factorial combination of four orchard soils (MUPP, P20, NENT, DD) and four soil treatments. Two-leaf stage apple seedlings (one per pot) were planted into eight replicate 15-cm pots of each of the four soil treatments for each orchard site (1) steam pasteurized orchard soil, (2) a control compromised of untreated orchard soil, (3) root-dip inoculation with *Pseudomonas* sp. P10-32 into untreated orchard soil, and (4) root-dip inoculation with *Pseudomonas* sp. P10-42 into untreated orchard soil. The pots were placed in a temperature-controlled (24 °C) greenhouse (located at 49°56'52.5"N 119°63'79.8"W) in a randomized complete block design. Seedlings were grown using a 14-hour photoperiod

supplied with supplemental high-pressure sodium lighting for 16 weeks prior to analysis (planted January 13th, 2017).

Orchard soil was steam pasteurized at 70 °C for 1 h on two consecutive days. Biocontrol inoculation consisted of dipping the root system of seedlings in a suspension of 10^9 CFU mL^{-1} of the appropriate *Pseudomonas* isolate, as well as applying 5 mL of the inoculant to the planting hole immediately prior to transplanting the seedling. Insect and mite pests were controlled by monthly foliar application of Beleaf 50 SG® (ISK Biosciences Corporation, Concord, Ohio, USA) at a rate of 0.3 g L^{-1}. Pots were fertilized biweekly with all-purpose fertilizer (20:8:20), with a cumulative application of 0.75 g of mineral N supplied to each seedling.

4.2.3.5.1 Analyses of Plant Growth

In order to test the hypothesis that antagonistic *Pseudomonas* isolates would increase plant growth on orchard soil, various aspects of plant growth were measured. At time of harvest, shoots were cut at soil level and shoot length recorded, prior to drying in an oven at 65 °C for 48 h for determination of dry shoot weight. Entire root systems were carefully removed from each pot and washed thoroughly with water. After removing subsamples of root tissue for extraction of migratory endoparasitic nematodes, the remaining root tissue was dried in an oven at 65 °C for 48 h and dry root weight recorded.

4.2.3.5.2 *P. penetrans* Populations

In order to test the hypothesis that antagonistic *Pseudomonas* isolates would suppress *P. penetrans,* nematode populations in roots and soil were quantified. Nematodes were extracted from a 50-mL subsample of soil from each pot before planting

and at harvest, using the Baermann pan technique with a 7-day incubation period (Forge and Kimpinski, 2007). After collecting the nematodes over a 25-µm sieve, nematode samples were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Additionally, migratory endoparasitic nematodes were also extracted from a subsample of root tissue collected from each seedling using the shaker agitation technique (Shurtleff and Averre, 2005). Approximately 2 g of fresh fine root tissue were placed into a 250-mL Erlenmeyer flask containing 100 mL of water. Flasks were incubated at room temperature on a rotary shaker set at 100 rpm for 4 days. Nematodes were collected over a 25-µm sieve, and were transferred in water into glass scintillation vials and stored at 4 °C for a maximum of four weeks prior to counting. Extracted root tissue was dried at 70 °C for 24 h prior to computing *P. penetrans* per gram of dry root.

4.2.3.5.3 Root Colonization Assay

In order to test the hypothesis that antagonistic *Pseudomonas* isolates would colonize the root system of apple in different orchard soils, root colonization by the *Pseudomonas* isolates was determined. A concurrent greenhouse bioassay was performed to evaluate the capacity of *Pseudomonas* P10-32 and P10-42 to colonize apple roots in each orchard soil. Rifampicin-resistant mutants of *Pseudomonas* P10-32 and P10-42 were induced on TSA+100 µg mL^{-1}, ad described previously (Chapter 2). Inoculation consisted of dipping the root system of seedlings in a 10^9 CFU mL^{-1} suspension of the rifampicin-resistant biocontrol isolate, as well as applying 5 mL of the inoculant to the planting hole immediately prior to transplanting the seedling. Treatments included a non-inoculated control, *Pseudomonas* P10-32RifR^+, and *Pseudomonas* P10-42RifR^+. Seedlings were

planted into 1-L pots filled with 800 mL of untreated soil collected from each orchard site, with four replicate pots for each sampling date. Seedlings were evaluated for root colonization by each biocontrol isolate at 14 and 56 days post inoculation (*pi*).

4.2.4 Statistical Analysis

4.2.4.1 Compost Experiment

Data were subjected to a univariate two-way ANOVA in SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA); differences between treatment means were examined using the Bonferroni *t*-test (*P*-value < 0.05). *P. penetrans* abundance data were analyzed after a log (x + 10) transformation. A step-wise multiple linear regression was performed to determine which variable(s) best accounted for variance in plant biomass in the data set.

4.2.4.2 Biocontrol Experiment

Differences in treatment means during the dual-culture fungal inhibition assay and preliminary *P. penetrans* suppression bioassay were evaluated using a one-way ANOVA in SPSS 20.0 and differences between treatment means were examined using the Bonferroni *t*-test (*P*-value < 0.05). The multisoil greenhouse data were subjected to a univariate two-way ANOVA in SPSS 20.0 and differences between treatment means were examined using the Bonferroni *t*-test (*P*-value < 0.05). *P. penetrans* abundance data were analyzed after a log (x + 10) transformation.

4.3 Results

4.3.1 Compost Greenhouse Experiment

A soil treatment x orchard soil interaction effect was observed for most of the parameters analyzed (Appendix K), with the exception of soil microbial activity, and the abundance of *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in soil; therefore,

data from each orchard site were analyzed separately.

4.3.1.1 Plant Growth

Soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings planted in soil collected from the MUPP orchard site (Table 4.4). Shoot length, shoot weight, root weight, and plant biomass were greater in the steam pasteurization treatment than the control, AWC-2017, YTC-2017, and MWC-2017 treatments. Shoot length, shoot weight, and plant biomass were greater in the AWC-2017 treatment than the control.

Table 4.4 - Effect of soil treatments (composts and steam pasteurization) on plant growth parameters of apple seedlings grown in MUPP, P20, NENT, and DD orchard soil. Data were analyzed with a one-way ANOVA (N=8). AWC-2017 refers to agricultural waste compost, YTC-2017 refers to yard trimmings compost, and MWC-2017 refers to municipal waste compost. Values represent the mean ± standard error. Values sharing the same letter within a column and within an orchard soil do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Soil treatment	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Biomass (g)
	MUPP Orchard Soil			
Control	41.8±1.1 c	4.87±0.31 c	0.88±0.07 b	5.75±0.44 c
Pasteurized	66.9±2.7 a	9.58±0.64 a	2.65±0.15 a	12.20±0.83 a
AWC-2017	53.6±1.2 b	7.05±0.51 b	1.13±0.10 b	8.18±0.53 b
YTC-2017	44.9±2.7 bc	5.69±0.71 bc	0.87±0.16 b	6.50±0.85 bc
MWC-2017	47.2±2.4 bc	5.65±0.50 bc	1.18±0.19 b	6.87±0.52 bc
P-value	<0.001	<0.001	<0.001	<0.001
	P20 Orchard Soil			
Control	43.0±3.9 b	6.16±0.75 b	1.74±0.14 a	7.89±0.73 b
Pasteurized	64.7±4.8 a	9.55±0.53 a	2.56±0.22 a	12.10±1.01 a
AWC-2017	66.3±2.9 a	10.08±0.48 a	2.45±0.18 a	12.53±0.58 a
YTC-2017	59.7±2.6 ab	9.34±0.44 a	1.92±0.15 a	11.25±0.47 a
MWC-2017	56.7±5.3 ab	7.84±0.63 ab	1.92±0.18 a	9.77±0.77 ab
P-value	0.021	0.036	0.164	0.044
	NENT Orchard Soil			
Control	31.5±3.9 b	3.09±0.27 b	1.22±0.17 ab	4.31±0.41 b
Pasteurized	36.4±2.6 ab	3.54±0.35 b	0.96±0.12 b	4.50±0.53 b
AWC-2017	38.8±3.1 ab	4.36±0.41 ab	1.58±0.15 a	5.94±0.51 ab
YTC-2017	49.7±3.5 a	5.28±0.58 a	1.61±0.19 a	6.90±0.75 a
MWC-2017	48.1±3.2 a	5.61±0.45 a	1.41±0.19 ab	7.02±0.46 a
P-value	0.001	0.001	0.024	0.001
	DD Orchard Soil			
Control	42.0±1.9 c	5.27±0.20 c	1.02±0.08 b	6.29±0.25 c
Pasteurized	67.1±3.3 a	11.78±0.44 a	3.35±0.14 a	15.14±0.44 a
AWC-2017	40.4±1.8 c	4.33±0.27 c	0.62±0.05 b	4.95±0.29 c
YTC-2017	44.5±2.2 bc	4.85±0.29 c	0.82±0.06 b	5.67±0.32 c
MWC-2017	54.2±3.3 b	7.22±0.43 b	1.20±0.11 b	8.42±0.52 b
P-value	<0.001	<0.001	<0.001	<0.001

In soil collected from the P20 orchard site, soil treatment affected shoot length, shoot weight, and biomass of apple seedlings. Shoot length was greater in the steam pasteurization and AWC-2017 treatments than in the control treatment. Plants grown in the steam pasteurization, AWC-2017, and YTC-2017 treatments had greater shoot weight and plant biomass than in the control treatment.

Soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings planted in soil collected from the NENT orchard site. Steam pasteurization did not increase any plant growth parameter relative to the control. Plants grown in YTC-2017 and MWC-2017 treatments had greater shoot length, shoot weight, and plant biomass than in the control.

In soil collected from the DD orchard site, soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings planted. Shoot length, shoot weight, root weight, and plant biomass were greater in the steam pasteurization treatment than in the control, AWC-2017, YTC-2017 and MWC-2017 treatments. Plants grown in the MWC-2017 treatment had greater shoot length, shoot weight, and plant biomass than the control and AWC-2017 treatment.

4.3.1.2 *P. penetrans* Populations

Soil treatment affected all *P. penetrans* variables in soil from the MUPP orchard site (Table 4.5). At the time of planting, *P. penetrans* were absent from steam pasteurized soil, whereas the control and compost-amended pots had larger populations, which ranged from 111 to 122 *P. penetrans* 100 mL^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil of steam pasteurized soil. Compost amendments did not significantly affect *P. penetrans* populations in soil relative to those in the control at harvest; however, populations in the MWC-2017-amended pots did not differ from the steam pasteurization treatment. Root populations of *P. penetrans* were smaller in the MWC-2017 treatment than in the control, whereas populations were larger in the AWC-2017 treatment than in all other soil treatments. The abundance of *P. penetrans* pot^{-1} was smaller in the MWC-2017 treatment than in the AWC-2017 or YTC-2017 treatments.

The reproductive factor (Pf/Pi) of *P. penetrans* was enhanced in the AWC-2017 treatment relative to those of all other soil treatments.

Table 4.5 - Effect of soil treatments (composts and steam pasteurization) on *P. penetrans* parameters of apple seedlings grown in MUPP, P20, NENT, and DD orchard soil. Data were analyzed with a one-way ANOVA (N=8). AWC-2017 refers to agricultural waste compost, YTC-2017 refers to yard trimmings compost, MWC-2017 refers to municipal waste compost, and Pf/Pi refers to nematode reproductive factor. Values represent the mean ± standard error. Values sharing the same letter within a column and within an orchard soil do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Soil treatment	Preplant *Pratylenchus penetrans* 100 mL^{-1} soil	Harvest *P. penetrans* 100 mL^{-1} soil	Harvest *P. penetrans* g^{-1} fine root	Harvest *P. penetrans* pot^{-1}	Pf/Pi
	MUPP Orchard Soil				
Control	111±3 a	13±2 a	2961±799 b	1931±627 ab	2.58±0.78 b
Pasteurized	0±0 b	0±0 b	0±0 d	0±0 c	-
AWC-2017	122±6 a	15±4 a	6936±813 a	4929±657 a	6.37±0.97 a
YTC-2017	118±4 a	20±4 a	3338±498 b	2378±761 a	3.13±1.06 b
MWC-2017	114±4 a	9±3 ab	1549±649 c	923±360 b	1.36±0.51 b
P-value	<0.001	0.001	<0.001	<0.001	<0.001
	P20 Orchard Soil				
Control	116±3 a	3±1 a	970±296 a	843±232 a	1.11±0.30 a
Pasteurized	0±0 b	0±0 a	0±0 c	0±0 c	-
AWC-2017	128±8 a	1±1 a	399±190 ab	454±165 ab	0.58±0.20 a
YTC-2017	120±5 a	1±1 a	233±94 b	333±159 b	0.46±0.22 a
MWC-2017	121±5 a	1±1 a	312±72 ab	373±106 ab	0.48±0.19 a
P-value	<0.001	0.213	<0.001	<0.001	0.165
	NENT Orchard Soil				
Control	72±2 a	1±1 a	0±0 a	4±2 a	0.01±0.01 a
Pasteurized	0±0 b	0±0 a	0±0 a	0±0 a	-
AWC-2017	79±3 a	1±1 a	15±14 a	22±12 a	0.04±0.02 a
YTC-2017	74±4 a	2±1 a	0±0 a	13±5 a	0.03±0.01 a
MWC-2017	73±4 a	2±1 a	2±2 a	15±5 a	0.03±0.01 a
P-value	<0.001	0.221	0.214	0.096	0.552
	DD Orchard Soil				
Control	57±2 a	15±3 a	1975±298 a	1669±283 a	4.60±0.91 a
Pasteurized	0±0 b	0±0 b	0±0 b	0±0 c	-
AWC-2017	55±3 a	12±2 a	2480±580 a	983±193 ab	2.91±0.65 ab
YTC-2017	55±1 a	13±2 a	863±153 b	590±101 b	1.62±0.44 b
MWC-2017	55±2 a	9±2 a	1169±229 b	935±182 ab	2.59±0.49 b
P-value	<0.001	<0.001	<0.001	<0.001	0.016

In soil collected from the P20 orchard site, soil treatment affected *P. penetrans* populations in soil prior to planting, with *P. penetrans* absent from steam pasteurized soil, and larger populations in the control and compost-amended pots, which ranged from

116 to 128 *P. penetrans* 100 ml^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil in the steam pasteurization treatment. At harvest, soil treatment did not significantly affect *P. penetrans* population abundance in soil, or reproductive factor; however, *P. penetrans* populations in roots and the abundance of *P. penetrans* pot^{-1} were smaller in the YTC-2017 treatment than in the control.

Soil treatment affected *P. penetrans* populations in soil from the NENT orchard site prior to planting, with *P. penetrans* absent from steam pasteurized soil, and larger populations in the control and compost-amended pots, which ranged from 72 to 79 *P. penetrans* 100 mL^{-1} of soil. At harvest *P. penetrans* were not recovered from roots or soil in the steam pasteurization treatment. At harvest, soil treatment did not significantly affect *P. penetrans* population abundance in soil and roots, the total population abundance pot^{-1}, or the reproductive factor of *P. penetrans*.

Soil treatment affected all *P. penetrans* variables in soil from the DD orchard site. At time of planting, *P. penetrans* were absent from steam pasteurized soil, whereas the control and compost-amended pots had larger populations, which ranged from 55 to 57 *P. penetrans* 100 mL^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil in the steam pasteurization treatment. Compost amendments did not significantly affect *P. penetrans* populations in soil relative to those of the control treatment. Root populations of *P. penetrans* were smaller in the YTC-2017 and MWC-2017 treatments than in the control and AWC-2017 treatments. The total abundance of *P. penetrans* pot^{-1} was smaller in the YTC-2017 treatment than in the untreated control. The reproductive factor of *P. penetrans* was reduced in the YTC-2017 and MWC-2017 treatments relative to that of the untreated control.

4.3.1.3 Soil Microbiology

Soil treatment affected microbial activity, as well as the total abundance of bacteria, fungi, and *Pseudomonas* spp. in soil from the MUPP orchard site (Table 4.6). Microbial activity was greater in the YTC-2017 treatment than in the steam pasteurization treatment. The total abundance of bacteria in soil was greater in the AWC-2017 and YTC-2017 treatments than in the control and steam pasteurization treatments. All compost treatments had a greater abundance of total fungi in soil relative to that in the steam pasteurization treatment. AWC-2017 and MWC-2017 treatments also had a greater abundance of total fungi in soil than the control treatment. The steam pasteurization treatment, as well as all compost treatments had a greater abundance of *Pseudomonas* spp. in soil than the control.

Table 4.6 - Effect of soil treatments (composts and steam pasteurization) on soil microbiology parameters of apple seedlings grown in MUPP, P20, NENT, and DD orchard soil. Data were analyzed with a one-way ANOVA (N=8). AWC-2017 refers to agricultural waste compost, YTC-2017 refers to yard trimmings compost, MWC-2017 refers to municipal waste compost, DAPG+ bacteria refers to 2,4-diacetylphloroglucinol-producing bacteria, and PRN+ bacteria refers to pyrrolnitrin-producing bacteria. Values represent the mean ± standard error. Values sharing the same letter within a column and within an orchard soil do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Soil treatment	Soil microbial activity (mg fluore-scein released kg^{-1} soil $3h^{-1}$)	Total bacteria (log 16S gene copies g^{-1} rhizosphere soil)	Total fungi (log 18S gene copies g^{-1} rhizosphere soil)	*Pseudomonas* spp. (log 16S gene copies g^{-1} rhizosphere soil)	DAPG+ bacteria (log *phlD* gene copies g^{-1} rhizosphere soil)	PRN+ bacteria (log *prnD* gene copies g^{-1} rhizosphere soil)
	MUPP Orchard Soil					
Control	54±6 ab	8.58±0.11 b	8.23±0.08 bc	6.12±0.12 b	5.28±0.14 a	4.53±0.10 a
Pasteurized	40±8 b	8.50±0.14 b	7.80±0.12 c	6.88±0.10 a	5.28±0.09 a	4.47±0.15 a
AWC-2017	48±6 ab	9.01±0.06 a	8.73±0.09 a	6.84±0.07 a	5.10±0.18 a	4.92±0.14 a
YTC-2017	75±11 a	8.96±0.08 a	8.48±0.05 ab	6.68±0.08 a	5.38±0.18 a	4.83±0.07 a
MWC-2017	71±8 ab	8.83±0.13 ab	8.79±0.11 a	6.70±0.06 a	5.12±0.15 a	4.95±0.07 a
P-value	0.009	0.001	<0.001	<0.001	0.746	0.069
	P20 Orchard Soil					
Control	49±6 b	8.40±0.16 a	7.90±0.15 a	5.71±0.16 b	5.24±0.08 a	4.86±0.07 a
Pasteurized	38±7 b	8.59±0.13 a	8.03±0.13 a	6.55±0.14 a	5.14±0.15 a	4.30±0.15 b
AWC-2017	49±5 b	8.69±0.08 a	8.01±0.11 a	6.42±0.14 a	5.27±0.13 a	4.76±0.14 ab
YTC-2017	64±5 ab	8.69±0.11 a	7.86±010 a	6.21±0.10 ab	5.33±0.15 a	4.61±0.09 ab
MWC-2017	70±6 a	8.45±0.12 a	8.19±0.11 a	6.32±0.09 a	5.36±0.16 a	4.64±0.10 ab
P-value	<0.001	0.072	0.315	0.002	0.764	0.029
	NENT Orchard Soil					
Control	66±5 b	8.98±0.09 b	8.83±0.10 a	6.65±0.10 a	5.00±0.16 ab	5.15±0.09 a
Pasteurized	63±7 b	8.71±0.07 c	8.16±0.07 b	6.87±0.06 a	5.08±0.16 ab	4.57±0.17 b
AWC-2017	66±6 b	9.20±0.09 a	8.91±0.08 a	7.07±0.09 a	4.97±0.16 b	4.98±0.10 ab
YTC-2017	62±7 b	9.23±0.05 a	8.84±0.09 a	6.96±0.08 a	5.26±0.12 ab	5.18±0.09 a
MWC-2017	96±9 a	9.12±0.07 ab	9.12±0.07 a	6.89±0.10 a	5.53±0.20 a	5.20±0.09 a
P-value	0.007	<0.001	<0.001	0.063	0.037	0.020
	DD Orchard Soil					
Control	59±4 b	8.96±0.06 bc	9.07±0.05 a	5.96±0.12 b	5.32±0.08 a	4.83±0.10 a
Pasteurized	57±9 b	8.90±0.10 c	8.64±0.08 b	6.89±0.14 a	4.99±0.16 a	4.33±0.14 b
AWC-2017	64±9 ab	9.23±0.06 ab	9.24±0.07 a	6.62±0.06 a	5.32±0.13 a	4.89±0.07 a
YTC-2017	69±7 ab	9.29±0.04 a	9.11±0.07 a	6.68±0.09 a	5.32±0.10 a	5.20±0.13 a
MWC-2017	81±8 a	9.13±0.08 abc	9.34±0.04 a	6.46±0.06 ab	5.30±0.15 a	5.08±0.10 a
P-value	0.014	<0.001	<0.001	<0.001	0.198	<0.001

In soil collected from the P20 orchard site, soil treatment affected microbial activity, and the abundance of *Pseudomonas* spp. and PRN+ bacteria in soil. Microbial

activity was greater in the MWC-2017 treatment than in the control, steam pasteurization, and AWC-2017 treatments. The abundance of *Pseudomonas* spp. was greater in the steam pasteurization, AWC-2017, and MWC-2017 treatments than in the control. The abundance of PRN+ bacteria was greater in the control treatment than in the steam pasteurization treatment.

Soil treatment affected microbial activity, and the abundance of total bacteria, total fungi, DAPG+ bacteria, and PRN+ bacteria in soil from the NENT orchard site. Microbial activity was greater in the MWC-2017 treatment than in all other soil treatments. The abundance of total bacteria in soil was greater in the AWC-2017 and YTC-2017 treatments than in the control. Steam pasteurized soil had fewer total bacteria and total fungi than all other soil treatments. The abundance of DAPG+ bacteria was greater in the MWC-2017 treatment than in the AWC-2017 treatment. The abundance of PRN+ bacteria was greater in the control, YTC-2017, and MWC-2017 treatments than in the steam pasteurization treatment.

In soil from the DD orchard site, soil treatment affected microbial activity, and the abundance of total bacteria, total fungi, *Pseudomonas* spp., and PRN+ bacteria in soil. Microbial activity was greater in the MWC-2017 treatment than in the control and steam pasteurization treatments. The AWC-2017 and YTC-2017 treatments increased the abundance of total bacteria in soil relative to that in the steam pasteurization treatment; however, YTC-2017 also increased the abundance of total bacteria compared to the control. All composts, as well as the control, had a greater abundance of total fungi in soil than the steam pasteurization treatment. The steam pasteurization treatment, as well as the AWC-2017 and YTC-2017 treatments had a greater abundance of *Pseudomonas* spp.

in soil than the control. All compost treatments, as well as the control, had a greater abundance of PRN+ bacteria in soil than the steam pasteurization treatment.

4.3.1.4 Step-Wise Multiple Linear Regression Analysis

A step-wise multiple linear regression was performed to determine which variable(s) best accounted for variance in plant biomass in the data set. The regression equation for plant biomass was: plant biomass (g) = - 0.1328 (log *P. penetrans* g^{-1} root) - 0.859 (log 18S gene copies g^{-1} soil) + 19.073. The multiple correlation coefficient was r=0.555 (*P*-value = 0.001), indicating that approximately 30.8% of the variance in plant biomass could be accounted for by *P. penetrans* root infestation and the abundance of total fungi in soil, to which both variables contributed negatively.

4.3.2 Biocontrol Isolate Screening

Of the 100 *Pseudomonas* isolates in the biocontrol library, only 23 showed antagonistic activity to at least one replant disease-associated fungal pathogen (Table 4.7), as evaluated by *in vitro* suppression of fungal mycelium on growth plates. Differences in the level of fungal suppression were not observed among antagonistic isolates. Most *Pseudomonas* isolates displayed some capacity to reduce root infestation by *P. penetrans*; however, this was only associated with enhanced plant growth with *Pseudomonas* sp. P10-32, P10-38, P10-42, P10-51, P10-53, P10-66, P10-86 and P10-88. Differences in the level of *P. penetrans* suppression and plant growth enhancement among antagonistic isolates were not observed.

Table 4.7 - Fungal growth inhibition, *P. penetrans* suppression, and promotion of plant biomass by antagonistic *Pseudomonas* isolates. For the *in vitro* fungal growth suppression assay, values are indicative of significant reductions in fungal colony diameter relative to the non-inoculated control according to one-way ANOVA (N=5). Values represent the mean ± standard error. Significant differences in *P. penetrans* suppression and plant growth promotion were not observed relative to the control in the preliminary greenhouse bioassay according to one-way ANOVA (N=4)

	In vitro fungal growth suppression (%)					Greenhouse bioassay	
Isolate	*Fusarium* sp. F1-1	*Fusarium* sp. F2-1	'*Cylindro-carpon*' sp. C1-1	'*Cylindro-carpon*' sp. C2-1	*Rhizoc-tonia* sp. R1-1	*Pratylenchus penetrans* suppression (%)	Plant growth promotion (%)
P10-18	45.3±1.2	15.0±0.5	39.5±2.5	62.3±3.4	46.8±2.5	12.4±6.7	-43.4±11.0
P10-32	38.8±0.9	-	55.3±2.2	23.5±2.0	35.2±3.4	83.4±4.5	36.1±12.3
P10-38	-	-	44.6±1.7	-	6.9±1.2	39.3±9.9	26.8±9.6
P10-42	-	-	16.8±1.0	-	-	76.8±5.5	39.3±10.0
P10-51	21.1±2.4	9.4±1.1	21.7±3.4	12.3±2.1	40.6±2.2	0.4±4.9	19.0±8.7
P10-53	24.9±0.8	7.7±0.7	23.1±2.6	14.0±2.2	37.7±2.8	65.3±3.8	7.4±12.2
P10-55	41.1±1.1	16.6±1.3	34.4±1.9	25.4±3.0	45.6±2.6	-27.3±7.7	-56. 0±13.2
P10-56	42.1±2.1	16.4±1.0	40.9±2.3	66.7±3.1	43.8±3.3	23.7±8.0	-47.2±14.0
P10-62	-	-	23.2±2.9	-	-	19.9±6.3	-29.6±7.7
P10-63	19.5±0.8	6.9±1.0	22.8±1.1	24.3±2.7	14.2±2.5	9.0±5.5	-4.1±9.9
P10-65	-	6.8±0.6	16.1±0.9	16.3±2.9	46.4±2.3	14.0±9.8	-18.3±11.1
P10-66	20.9±1.9	15.3±0.8	-	13.7±2.2	42.0±1.9	16.1±7.4	11.2±13.0
P10-67	28.7±1.4	20.7±1.1	-	20.0±2.5	41.7±3.0	17.9±6.8	-27.7±8.7
P10-69	13.1±2.0	17.8±0.8	-	20.0±1.6	42.6±2.6	92.8±10.0	-49.8±9.0
P10-72	22.6±1.2	17.2±1.1	-	24.1±2.1	43.4±2.8	90.2±9.9	-47.3±9.2
P10-75	19.7±1.5	2.8±1.5	-	14.5±1.3	18.2±3.1	59.7±8.3	-35.6±8.3
P10-76	23.0±1.5	4.4±0.9	14.9±1.4	12.8±1.9	8.7±2.1	69.1±7.2	-66.9±7.9
P10-77	-	13.6±1.3	23.3±2.0	17.5±1.7	43.0±2.4	46.3±5.4	-52.8±8.4
P10-78	15.8±2.1	14.1±1.0	-	-	40.9±2.7	26.3±6.7	-41.0±11.1
P10-81	23.4±1.9	10.0±1.0	-	-	-	87.8±7.2	-4.4±9.2
P10-84	-	5.6±0.9	18.5±1.8	-	-	66.8±6.3	-20.5±8.8
P10-86	-	4.6±0.7	14.9±1.9	-	-	52.5±12.0	14.8±7.7
P10-88	14.3±1.7	4.6±1.3	20.2±2.3	-	-	49.5±7.8	26.1±11.2

Of the 23 *Pseudomonas* isolates that showed antagonistic activity to at least one replant disease-associated fungal pathogen, only *Pseudomonas* sp. P10-51 had the capacity to solubilize phosphate (Table 4.8). All 23 antagonistic isolates were positive for siderophore production. Similarly, most of the 23 antagonistic isolates were also positive for protease activity, with the exception of *Pseudomonas* sp. P10-38, P10-75, P10-76, P10-81, and P10-86. *Pseudomonas* sp. P10-51 and P10-53 were positive for the *phlD* gene required for biosynthesis of 2,4-diacetylphloroglucinol. *Pseudomonas* sp. P10-55, P10-56, P10-63, P10-65, P10-66, P10-69, P10-72, P10-76, and P10-77 were positive for

the *prnD* gene required for biosynthesis of pyrrolnitrin. *Pseudomonas* sp. P10-51, P10-53, P10-63, P10-66, P10-69, P10-72, P10-75, P10-76, P10-77, and P10-78 were positive for the *hcnBC* gene required for biosynthesis of hydrogen cyanide.

Table 4.8 - Culture characteristics and presence of antibiotic biosynthesis genes for the antagonistic *Pseudomonas* isolates.

	Culture characteristics (+/-)			Biosynthesis gene (+/-)				
Isolate	Phosphate solubilisation	Siderophore production	Protease activity	*phlD*	*phzCD*	*prnD*	*pltC*	*hcnBC*
P10-18	-	+	+	-	-	-	-	-
P10-32	-	+	+	-	-	+	-	-
P10-38	-	+	-	-	-	-	-	-
P10-42	-	+	+	-	-	-	-	-
P10-51	+	+	+	+	-	-	-	+
P10-53	-	+	+	+	-	-	-	+
P10-55	-	+	+	-	-	+	-	-
P10-56	-	+	+	-	-	+	-	-
P10-62	-	+	+	-	-	-	-	-
P10-63	-	+	+	-	-	+	-	+
P10-65	-	+	+	-	-	+	-	-
P10-66	-	+	+	-	-	+	-	+
P10-67	-	+	+	-	-	-	-	-
P10-69	-	+	+	-	-	+	-	+
P10-72	-	+	+	-	-	+	-	+
P10-75	-	+	-	-	-	-	-	+
P10-76	-	+	-	-	-	-	-	+
P10-77	-	+	+	-	-	+	-	+
P10-78	-	+	+	-	-	+	-	+
P10-81	-	+	-	-	-	-	-	-
P10-84	-	+	+	-	-	-	-	-
P10-86	-	+	-	-	-	-	-	-
P10-88	-	+	+	-	-	-	-	-

4.3.3 Biocontrol Greenhouse Experiment

A soil treatment x orchard soil interaction effect was observed for most of the parameters analysed (Appendix L), with the exception of the abundance of *P. penetrans* g^{-1} root; therefore, data from each orchard soil were analyzed separately

4.3.3.1 Plant Growth

Soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings planted in soil collected from the MUPP orchard site (Table 4.9). Shoot length, shoot weight, root weight, and plant biomass were greater in the steam

pasteurization treatment than in the untreated control and both biocontrol treatments.

Table 4.9 - Effect of soil treatments (*Pseudomonas* isolates and steam pasteurization) on plant growth parameters of apple seedlings grown in MUPP, P20, NENT, and DD orchard soil. Data were analyzed with a one-way ANOVA (N=8). Values represent the mean ± standard error. Values sharing the same letter within a column and within an orchard soil do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Soil treatment	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Biomass (g)
	MUPP Orchard Soil			
Control	25.0±2.4 b	1.81±0.26 b	0.163±0.029 b	1.98±0.28 b
Pasteurized	48.8±3.2 a	4.66±0.36 a	0.947±0.054 a	5.61±0.50 a
Isolate P10-32	30.0±2.5 b	2.29±0.26 b	0.193±0.060 b	2.48±0.28 b
Isolate P10-42	30.2±3.0 b	2.34±0.24 b	0.163±0.032 b	2.50±0.32 b
P-value	<0.001	<0.001	<0.001	<0.001
	P20 Orchard Soil			
Control	42.1±4.4 b	3.60±0.53 b	0.328±0.058 c	3.93±0.53 b
Pasteurized	61.1±2.8 a	7.11±0.61 a	1.676±0.090 a	8.79±0.75 a
Isolate P10-32	31.4±4.6 b	2.75±0.38 b	0.508±0.069 bc	3.26±0.40 b
Isolate P10-42	42.3±4.7 b	3.99±0.44 b	0.789±0.082 b	4.78±0.49 b
P-value	<0.001	<0.001	<0.001	<0.001
	NENT Orchard Soil			
Control	28.7±3.9 a	2.09±0.43 a	0.314±0.082 a	2.40±0.50 a
Pasteurized	29.8±2.5 a	2.16±0.36 a	0.327±0.062 a	2.14±0.30 a
Isolate P10-32	25.8±2.2 a	1.81±0.23 a	0.327±0.039 a	1.64±0.23 a
Isolate P10-42	24.4±2.0 a	1.49±0.21 a	0.194±0.040 a	2.47±0.25 a
P-value	0.631	0.338	0.164	0.284
	DD Orchard Soil			
Control	30.5±1.6 b	2.28±0.25 c	0.246±0.035 b	2.52±0.27 b
Pasteurized	69.0±3.6 a	7.81±0.39 a	1.502±0.041 a	9.32±0.45 a
Isolate P10-32	36.7±3.1 b	2.88±0.34 bc	0.300±0.063 b	3.18±0.37 b
Isolate P10-42	44.4±3.3 ab	3.60±0.32 b	0.317±0.039 b	3.92±0.34 b
P-value	<0.001	<0.001	<0.001	<0.001

In soil collected from the P20 orchard site, soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings. Shoot length, shoot weight, root weight, and plant biomass were greater in the steam pasteurization treatment than in the untreated control and biocontrol treatments. Root-dip inoculation with *Pseudomonas* sp. P10-42 increased root weight relative to that of the untreated control.

Soil treatment did not significantly affect shoot length, shoot weight, root weight, or biomass of apple seedlings planted into soil collected from the NENT orchard

site.

In soil collected from the DD orchard site, soil treatment affected shoot length, shoot weight, root weight, and biomass of apple seedlings. Shoot length, shoot weight, root weight, and plant biomass were greater in the steam pasteurization treatment than in untreated control. Root-dip inoculation with *Pseudomonas* sp. P10-42 increased shoot weight relative to that of the untreated control.

4.3.3.2 *P. penetrans* Populations and Root Colonization by *Pseudomonas* Isolates

Soil treatment affected most of the *P. penetrans* variables in soil from the MUPP orchard site, with the exception of nematode reproductive factor (Pf/Pi) (Table 4.10). At the time of planting, *P. penetrans* were absent from steam pasteurized soil, whereas the control and biocontrol-inoculated pots had larger populations, which ranged from 58 to 62 *P. penetrans* 50 mL^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil of steam pasteurized soil. Biocontrol inoculation did not affect the abundance of *P. penetrans* in roots or soil relative to that of the untreated control. At 14 days *pi*, *Pseudomonas* sp. P10-42 was present on the root system of apple seedlings; however, populations were reduced to background population levels of indigenous rifampicin-resistant bacteria by 56 days *pi*.

Table 4.10 - Effect of soil treatments (*Pseudomonas* isolates and steam pasteurization) on *P. penetrans* parameters and root colonization by *Pseudomonas* isolates on apple seedlings grown in MUPP, P20, NENT, and DD orchard soil. Data were analyzed with a one-way ANOVA (N=8). Pf/Pi refers to nematode reproductive factor, and CFU refers to colony forming units. Values represent the mean ± standard error. Values sharing the same letter within a column and within an orchard soil do not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Soil treatment	Preplant *Pratylenchus penetrans* 50 mL^{-1} soil	Harvest *P. penetrans* 50 mL^{-1} soil	Harvest *P. penetrans* g^{-1} fine root	Harvest *P. penetrans* pot^{-1}	Pf/Pi	Log CFU g^{-1} root 14 days *pi*	Log CFU g^{-1} root 56 days *pi*
	MUPP Orchard Soil						
Control	60±2 a	43±9 a	2988±1274 a	784±168 a	1.99±0.40 a	5.0±0.1 b	4.9±0.1 a
Pasteurized	0±0 b	0±0 b	2±2 b	0±0 b	-	-	-
Isolate P10-32	62±2 a	34±5 a	1342±505 a	549±84 a	1.31±0.21 a	5.3±0.1 ab	4.9±0.1 a
Isolate P10-42	58±2 a	45±6 a	1996±652 a	781±94 a	1.79±0.25 a	5.4±0.1 a	5.1±0.1 a
P-value	<0.001	<0.001	<0.001	<0.001	0.491	0.025	0.195
	P20 Orchard Soil						
Control	57±2 a	14±3 a	1013±515 a	353±62 a	0.96±0.18 a	5.3±0.1 b	5.3±0.1 a
Pasteurized	0±0 b	0±0 b	0±0 b	0±0 b	-	-	-
Isolate P10-32	55±2 a	11±2 a	711±366 a	296±127 a	0.85±0.29 a	6.4±0.1 a	5.6±0.2 a
Isolate P10-42	52±2 a	10±2 a	1079±261 a	460±77 a	1.40±0.14 a	6.3±0.1 a	5.6±0.1 a
P-value	<0.001	<0.001	<0.001	<0.001	0.317	<0.001	0.151
	NENT Orchard Soil						
Control	34±3 a	1±1 a	2±2 a	13±5 a	0.06±0.03 a	3.9±0.1 a	3.8±0.1 a
Pasteurized	0±0 b	0±0 a	0±0 a	0±0 a	-	-	-
Isolate P10-32	36±1 a	2±1 a	5±3 a	124± a	0.05±0.02 a	4.2±0.1 a	4.0±0.1 a
Isolate P10-42	37±2 a	1±1 a	2±1 a	8±3 a	0.04±0.02 a	4.1±0.1 a	4.0±0.1 a
P-value	<0.001	0.257	0.637	0.274	0.863	0.056	0.346
	DD Orchard Soil						
Control	25±2 a	34±4 a	2097±603 a	733±87 a	4.68±0.69 a	5.0±0.1 b	5.3±0.1 b
Pasteurized	0±0 b	0±0 c	0±0 c	0±0 c	-	-	-
Isolate P10-32	25±1 a	13±3 b	1404±646 ab	361±60 b	2.24±0.38 b	7.0±0.1 a	5.5±0.1 b
Isolate P10-42	26±2 a	13±3 b	637±263 b	282±46 b	1.68±0.32 b	7.4±0.1 a	6.8±0.1 a
P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

In soil collected from the P20 orchard site, soil treatment affected most of the *P. penetrans* variables, with the exception of nematode reproductive factor (Pf/Pi). At the time of planting, *P. penetrans* were absent from steam pasteurized soil, whereas the control and biocontrol-inoculated pots had larger populations, which ranged from 52 to 57 *P. penetrans* 50 mL^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil of steam pasteurized orchard soil. Biocontrol inoculation did not affect the

abundance of *P. penetrans* in roots or soil relative to the untreated control. At 14 days *pi*, *Pseudomonas* sp. P10-32 and *Pseudomonas* sp. P10-42 were present on the root system of apple seedlings; however, populations were reduced to background population levels of indigenous rifampicin-resistant bacteria by 56 days *pi*.

Soil treatment affected *P. penetrans* populations at the time of planting in soil collected from the NENT orchard site; however, at the time of harvest, populations were low and did not differ among any soil treatment. Inoculated biocontrol isolates were not detected at 14 days or 56 days *pi*.

In soil collected from the DD orchard site, soil treatment affected all of the *P. penetrans* variables. *P. penetrans* were absent from steam pasteurized soil, whereas the control and biocontrol-inoculated pots had larger populations, which ranged from 25 to 26 *P. penetrans* 50 mL^{-1} of soil. At harvest, *P. penetrans* were not recovered from roots or soil in the pasteurization treatment. Root-dip inoculation with *Pseudomonas* sp. P10-32 and *Pseudomonas* sp. P10-42 decreased the abundance of *P. penetrans* in soil, the total abundance of *P. penetrans* pot^{-1}, and the reproductive factor of *P. penetrans* relative to those of the untreated control. Similarly, root-dip inoculation with *Pseudomonas* sp. P10-42 also decreased root infestation by *P. penetrans*. At 14 days *pi*, *Pseudomonas* sp. P10-32 and *Pseudomonas* sp. P10-42 were present on the root system of apple seedlings; however, only *Pseudomonas* sp. P10-42 was found on the root system of apple seedlings at 56 days *pi*.

4.4 Discussion

4.4.1 Influence of Compost Amendments

Preplant soil incorporation of compost improved the growth of apple seedlings planted into orchard soil in six out of twelve of the orchard soil x compost type combinations evaluated. Results from the six treatment combinations that resulted in a positive plant growth response to compost amendment are consistent with other studies, including the greenhouse and field experiments performed in Chapters 2 and 3 of this dissertation, which have demonstrated significant plant growth promotion through the utilization of composts during the establishment of fruit trees in old orchard soil (Braun et al., 2010; Moran and Schupp, 2003; van Schoor et al., 2009). In this study, plant growth promotion was dependent on the compost type used and the orchard soil selected, and no particular compost improved plant growth in all four orchard soils. Similarly, none of the orchard soils displayed a positive plant growth response to all three compost amendments. Inconsistencies in compost amendment effects on plant growth enhancement and disease suppression, as well as natural variability in the materials used as compost feedstocks often hinder widespread acceptance of such amendments in horticultural production systems (Bonanomi et al., 2010; Termorshuizen and Jeger, 2008). Examination of orchard site and compost amendment physiochemical parameters failed to reveal clear trends between plant growth promotion and any of the abiotic factors examined. In a recent literature review, Bonanomi et al. (2010) concluded that biological soil parameters, rather than physiochemical parameters were more useful predictors of OM-mediated soil suppressiveness. Future research should be directed to elucidating variability in compost-induced plant growth promotion among different

orchard soils and compost types.

In four out of twelve of the orchard soil x compost type combinations evaluated, preplant soil incorporation of compost suppressed *P. penetrans* root infestation, and in one orchard soil x compost type combination, enhanced *P. penetrans* root infestation was observed relative to that of the control. In a recent literature review by Thoden et al. (2011), compost amendments were concluded to have the potential to suppress as well as stimulate *P. penetrans* populations; however, positive plant growth responses were often observed, irrespective of the effect on nematodes. Studies on the use of compost amendments for suppression of plant pathogenic fungi have reported similar, variable trends in the level of disease suppression (Bonanomi et al., 2007; Erhart et al., 1999; Pérez-Piqueres et al., 2006; Termorshuizen and Jeger, 2008). Results from the current study are in agreement with prior literature; compost amendments had variable effects on *P. penetrans* populations, and even when populations were increased by compost amendment, positive plant growth responses were still observed. In the previous greenhouse (Chapter 2) and field (Chapter 3) experiments conducted at a nearby apple orchard site, amending soil with agricultural waste compost suppressed *P. penetrans* populations; however, in this greenhouse experiment, agricultural waste compost stimulated *P. penetrans* populations in apple orchard soil. Increases in plant-parasitic nematode populations alongside enhanced plant biomass can potentially be explained by (1) increased nutritional content of plant host roots stimulating nematode feeding (Yeates, 1987, 1976), (2) reductions in plant secondary metabolites as a result of improved host nutritional status (Herms, 2002), and/or (3) enhanced root development offering more nematode feeding sites (Thoden et al., 2011). Overall, this study demonstrates the

variability in nematode suppression that compost amendments can have when utilized as a non-fumigant alternative for replant disease control.

Preplant soil incorporation of compost enhanced microbial populations associated with soil suppressiveness relative to the control in 23.6% of the cases, across all orchard soil x compost type combinations evaluated, and in no event did compost amendment reduce soil microbial populations. In Chapters 2 and 3, composts were demonstrated to promote rhizosphere populations of total bacteria, *Pseudomonas* spp., PRN+ bacteria, and DAPG+ bacteria; with stimulation of such microbial populations associated with *P. penetrans* and "*Cylindrocarpon*" suppression as well as improved growth of apple and sweet cherry. Although a clear trend was not observed between promotion of the microbial populations enumerated and plant growth and/or *P. penetrans* suppression in this experiment, promotion of such populations likely had other benefits on plant health, such as suppression of fungal/oomycete pathogens (de Souza et al., 2003; Garbeva et al., 2004a; Latz et al., 2012), and enhanced plant nutrient availability (Mantelin and Touraine, 2004). Overall, this study suggests that compost amendments promote soil microbial populations associated with soil suppressiveness and enhanced plant growth; however, whether these microbial populations are directly responsible for *P. penetrans* suppression in orchard soil still remains to be demonstrated.

In the PCA of orchard soils that displayed a positive plant growth response to soil pasteurization (MUPP, P20, and DD), orchard soil had the greatest effect on the variables examined, separating along the first principal component, while soil treatment had a secondary effect on the data set, with yard trimmings and municipal waste compost separating from the control treatment along the second principal component. In the step-

wise multiple regression analysis, the biological factors that accounted for the most variance in plant biomass were *P. penetrans* root infestation and the total abundance of fungi in soil. It was not surprising to find *P. penetrans* associated with reductions in plant biomass, as this nematode is a well-established parasite of fruit trees in the region (Forge et al., 2013a; Vrain and Yorston, 1987). The total abundance of fungi in soil was evaluated as an indicator of soil suppressiveness, as many different types of soil fungi can have antagonistic potential to nematodes (Hallmann and Sikora, 2011; Stirling et al., 1998), and increases in overall microbial abundance in soil, to which fungi contribute significantly (Frey et al., 1999), are often associated with pathogen suppression (Stirling, 2014). Finding a negative correlation between plant biomass and the total abundance of fungi in soil suggests that increased fungal abundance was not associated with compost-induced soil suppressiveness in the orchard soils sampled. The real-time PCR assay that was used to quantify soil fungi did not distinguish between beneficial and pathogenic fungi; therefore, increased fungal abundance may have been associated with a greater density of fungal pathogens in soil, as opposed to beneficial fungi. Alternatively, the negative correlation between plant biomass and fungal abundance in soil could also potentially be an artifact of the limited number of replant disease-conducive orchard soils analyzed (N=3). Consequently, future research should be directed towards identifying the diversity and abundance of fungal pathogens present in soil that may be negatively affecting fruit tree growth, along with *P. penetrans*, using soil collected from a greater number of orchard sites.

Orchard soil collected from NENT did not display a positive plant growth response to soil pasteurization in the greenhouse experiment; however, plant growth was

relatively low compared to apple seedlings grown in soil from the other three orchard sites, suggesting that abiotic factors may have been responsible for the poor growth of seedlings in soil from this orchard site. Amending NENT orchard soil with yard trimmings compost and municipal waste compost enhanced plant growth relative to the control and steam pasteurization treatment; however, analyses of orchard site and compost type physiochemical parameters did not reveal a clear link between plant growth promotion in these soil treatments and any physiochemical parameters examined. Analysis of leaf micronutrient concentrations could help to delineate potential host nutrient deficiencies between the soil treatments and possibly help explain the compost-induced growth promotion that was observed by the application of yard trimmings compost and municipal waste compost in soil from this orchard site; however, all pots were fertilized sufficiently with an all-purpose fertilizer, suggesting other factors may have contributed to differences in plant growth. Despite moderate initial population densities of *P. penetrans* in soil from NENT at the time of planting, populations of *P. penetrans* were very low in all soil treatments by the time of harvest. This may have been related to the silty loam soil at this orchard site, which may not have provided an environment conducive to nematode survival in a potted greenhouse environment (Florini et al., 1987; Jordaan et al., 1989; Wallace, 1973).

Based on the findings from this study, compost amendments show potential to be an effective tool in an integrated management strategy for promoting plant growth of fruit trees at old orchard sites. Although the level and consistency of plant growth promotion was often inferior to soil pasteurization (and presumably soil fumigation), compost amendments offer many other potential benefits to soil health that soil fumigants

do not provide, including enhanced nutrient availability, improved water retention, reductions in soil bulk density (Bulluck et al., 2002; Debosz et al., 2002), as well as more prolonged *P. penetrans* suppression (Chapter 3). Similarly, soil fumigants have often been associated with severe subsequent reinfestation with *P. penetrans* (Mazzola and Manici, 2012), potentially as a result of elimination of microbial antagonists of plant-parasitic nematodes (Munnecke, 1984).

4.4.2 Influence of Biocontrol Agents

In vitro fungistatic activity was a time and cost effective approach for preliminary screening of the *Pseudomonas* library for antagonistic activity to *P. penetrans*, as many of the isolates that displayed antagonistic activity to fungi on growth plates also displayed antagonistic activity to *P. penetrans* in the preliminary greenhouse bioassay. This finding suggests that suppression of nematodes and fungi potentially occurs through common mechanisms, such as the production of broad-spectrum antibiotics or extracellular lytic enzymes. DNA-based screening for the presence of genes coding for biosynthesis of antagonistic secondary metabolites did not show a correlation between these genes and *in vitro* or *in vivo* antagonistic activity to fungi or nematodes, respectively. Similarly, a number of the isolates that displayed strong capacity for control of nematodes and fungi, including *Pseudomonas* sp. P10-42, failed to show the presence of any of the key antibiotic biosynthesis genes screened for in this study. This suggests that alternate mechanisms may be responsible for the suppression of nematodes and fungi observed in this study. This may include the production of a diverse range of other antibiotics that were not screened for (Beneduzi et al., 2012; Haas and Défago, 2005), or the production of volatile organic compounds (Giorgio et al., 2015). Many of the

antagonistic biocontrol isolates were positive for production of siderophores and protease, which may have also contributed to suppression of nematodes and fungi in this study. Induction of plant systemic resistance also may have contributed to suppression of nematode root infestation in the preliminary greenhouse bioassay.

Inoculating apple seedlings via root dip with *Pseudomonas* sp. P10-42 improved plant growth in the MUPP and DD orchard soils, whereas inoculation with *Pseudomonas* sp. P10-32 did not affect plant growth in any orchard soil evaluated. In DD soil, inoculation with *Pseudomonas* sp. P10-42 and *Pseudomonas* sp. P10-32 reduced *P. penetrans* populations on apple seedlings, and for *Pseudomonas* sp. P10-42, nematode suppression and plant growth promotion were associated with sustained root colonization. Effective colonization of the host root system is one of the factors most commonly associated with successful biocontrol (Weller, 1988), and in this study, DD was the only orchard soil where an introduced biocontrol agent was present at detectable levels at 56 days *pi*. Examination of soil physiochemical parameters from the DD orchard soil revealed a relatively high OM content, which may have contributed to sustained root colonization by the biocontrol isolate in this soil (Bonkowski et al., 2009; Boulter et al., 2002). Kwok et al. (1987) proposed that high concentrations of soil OM might help support populations of introduced biocontrol agents. Enhanced soil OM might also stimulate greater host root exudation in the rhizosphere (Grayston et al., 1997), which could also help sustain inoculated biocontrol rhizobacteria. Although these hypotheses were not directly tested, data from this study suggest that *Pseudomonas* biocontrol agents may prove useful in an integrated pest management strategy employing the use of organic soil amendments, such as composts and organic mulches, or in organic tree-fruit

production systems.

When compared with soil pasteurization and compost amendments, root-dip inoculation with antagonistic *Pseudomonas* isolates provided minimal levels of plant growth enhancement of apple seedlings in orchard soil. Similarly, inconsistencies in plant growth promotion among different orchard soils present another significant barrier to widespread adoption of these particular biocontrol isolates as non-fumigant alternatives for replant disease control. Moreover, potential downstream biopesticide developmental hurdles, including inoculant formulation, product registration, product application, and shelf-life issues, all present significant barriers that most microbial inoculants must overcome before they can be used commercially for disease control (Bailey et al., 2010; Montesinos, 2003). Overall, based on the level of and inconsistencies in plant growth promotion, further development of *Pseudomonas* sp. P10-42 and *Pseudomonas* sp. P10-32, as microbial inoculants for replant disease control, is not recommended. Promoting indigenous populations of antagonistic *Pseudomonas* spp., as well as other microbial antagonists, through the incorporation of OM into soil shows greater potential for controlling replant disease.

5 Chapter 5: Summary and Future Directions

5.1 Fumigation

Soil fumigation improved the growth of fruit trees planted into old orchard soil, emphasizing the strong role that biological factors play in replant disease. In the first greenhouse experiment (Chapter 2), fumigation resulted in suppression of *Pratylenchus penetrans* throughout the duration of the experiment, but in the field experiment, fumigated soil was rapidly reinfested with *P. penetrans*. In addition to rapid reinfestation with *P. penetrans*, root populations of *P. penetrans* in fumigated sub-plots never differed from the control, demonstrating that fumigation only provided relatively short-term control of *P. penetrans*. We fumigated 2-m wide strip plots rather than an entire field, so the reinfestation by *P. penetrans* observed in fumigated sub-plots in this particular field experiment was likely more rapid than in a commercial scale fumigation; however, it would be comparable to that expected in a bed fumigation, which is a practice under increasing consideration. Despite short-term nematode control, long-term growth promotion was observed in fumigated soil in the field experiment, emphasizing why increasing restrictions on, or complete loss of, this effective soil-borne disease management strategy will have such a severe detrimental effect on the productivity of the tree-fruit industry. There is considerable need for alternative soil management strategies that can provide similar levels of replant disease control to those observed with chemical fumigants.

5.2 Compost Amendments

In the first greenhouse experiment (Chapter 2), compost amendments improved plant growth, suppressed *P. penetrans* and "*Cylindrocarpon*" populations in roots, altered

the composition of the soil microbial community, and enhanced the abundance of total bacteria, DAPG+ bacteria, and PRN+ bacteria in soil. In the field experiment (Chapter 3), composts improved plant growth, suppressed *P. penetrans*, enhanced soil microbial activity, soil biological suppressiveness, root colonization by AMF, and the abundance of total bacteria, *Pseudomonas* spp., DAPG+ bacteria, and PRN+ bacteria in the rhizosphere. In the second greenhouse experiment (Chapter 4), composts increased plant growth in six out of twelve of the orchard site x compost type combinations evaluated, and suppressed *P. penetrans* in four out of twelve of the combinations. Significant variability in plant growth promotion and nematode suppression was observed among the various orchard soils and compost types evaluated, and the differences could not be directly linked to differential promotion of soil microbial antagonists, suggesting that other factors were responsible for the variation in nematode suppression and plant growth promotion observed among the different orchard sites and compost types evaluated.

I originally predicted that compost amendments would improve the growth and fruit yield of trees planted in old orchard soil based upon the abundance of literature demonstrating the beneficial effects of composts on tree establishment in old orchard soil. In this dissertation, compost amendments consistently improved plant growth of fruit trees and seedlings planted in soil from an old apple orchard site, as described in Chapters 2 and 3; however, variability in plant growth promotion was observed among the various orchard soils and compost types evaluated in the multisoil greenhouse experiment in Chapter 4. I also predicted that compost amendments would suppress *P. penetrans* populations, and similarly, compost amendments consistently suppressed *P. penetrans* in Chapters 2 and 3; however, significant variability in nematode suppression was observed

in Chapter 4. This is in agreement with other studies that have demonstrated variability in plant growth promotion and nematode suppression through the use of compost amendments, as reviewed by Thoden et al. (2011). I also predicted that composts would increase microbial indicators of soil suppressiveness and enhanced P nutrition. In general, composts enhanced microbial indicators of soil suppressiveness and enhanced P nutrition; however, variability in the enhancement of microbial indicators of soil suppressiveness was observed in the multisoil greenhouse experiment. Lastly, in Chapter 3, I predicted that composts would have a beneficial effect on abiotic factors that can also contribute to differential plant growth promotion and *P. penetrans* suppression. Although composts did result in some enhancement of soil nutrient availability and plant nutrition relative to that of the untreated control, such changes likely did not play a strong role in the differences observed in plant growth promotion and nematode suppression by composts because most of the leaf nutrient concentrations were within the recommended levels in production guidelines across all soil treatments, likely as a result of adequate fertigation. The effect of compost on soil volumetric water content was dependent on the irrigation system used; however, alterations in plant water status were not subsequently observed, suggesting that improvements in plant water status did not play a strong role in improved plant growth by compost amendments either.

The strengths of the research conducted in this dissertation on composts include the number of orchard soils sampled and the variety of compost amendments evaluated, thereby demonstrating the reproducibility of replant disease control with this management strategy. This study employed DNA and culture-based quantification techniques to monitor *Pseudomonas* populations in the rhizosphere, allowing for a

comparison of these two quantification methods. This study also contributed to expanding knowledge of the effect of compost amendments under different low-volume irrigation systems (drip emitters and microsprinklers). Interaction effects between compost and irrigation systems were only evaluated at one orchard site, primarily due to the significant resources required for field-scale research trials, which therefore limits the possibility of extrapolation of the results obtained in this study to other orchard sites with different soil textures and/or management histories. Throughout this dissertation, quantification of antibiotic-producing bacteria was performed using a real-time PCR assay that probed for the presence of key genes required for the biosynthesis of 2,4-diacetylphlorglucinol or pyrrolnitrin. Such an assay does not assess whether the genes are actively being expressed, or whether the antibiotic being produced is even present in the rhizosphere/soil in sufficient quantities to account for disease control, a limitation that could be overcome through the use of a reverse transcriptase PCR assay and/or the use of high performance liquid chromatography to directly quantify the antibiotics *in situ*.

Understanding variability in plant-parasitic nematode suppression among different orchard sites and compost types will be vital to widespread acceptance of composts for replant disease control. Future research directed towards understanding the intrinsic soil and compost properties associated with successful disease control will be essential. Although promotion of indigenous bacterial antagonists was observed through application of compost amendments, and the role of antagonistic *Pseudomonas* spp. in the control of *P. penetrans* confirmed using a subsequent greenhouse experiment (Chapter 4), many other groups of antagonists also could have contributed to nematode control in the compost-amended soil, alongside bacterial antagonists. Whether promotion

of bacterial antagonists in the rhizosphere was a direct result of stimulation by the compost as roots came into contact with the OM, or rather, from composts promoting greater host root exudation into the rhizosphere, which subsequently stimulated bacterial populations, is an area of research interest that was not addressed in this study. Moreover, populations of fungal pathogens that may have been affecting roots alongside *P. penetrans* were not monitored in Chapters 3 and 4, and would be an area of useful research focus.

5.3 Biocontrol

In the first greenhouse experiment (Chapter 2), root-dip inoculating apple and sweet cherry seedlings with *Serratia plymuthica* 6-5 prior to planting into old orchard soil did not suppress *P. penetrans* or improve plant growth. In the second greenhouse experiment (Chapter 4), both of the *Pseudomonas* isolates that were evaluated displayed significant antagonistic activity to *P. penetrans* during preliminary screening; however, only minor effects on plant growth and *P. penetrans* populations were observed when these isolates were evaluated in old orchard soil, and the degree of biocontrol was orchard soil-specific. In the soil where the *Pseudomonas* isolates did display biocontrol activity to *P. penetrans* (DD orchard soil), nematode suppression appeared to be linked with successful colonization of the apple root system. Soil OM has previously been shown to support inoculated biocontrol agents (Bonkowski et al., 2009), and this may explain why biocontrol was only successful in soil from the orchard site with the greatest soil OM. This suggests that biocontrol with *Pseudomonas* at sites rich in OM, such as in organic production systems (Mazzola and Manici, 2012), or at orchard sites utilizing organic soil amendments, might potentially provide situations where biocontrol could be

successfully utilized. Soil OM provides the essential nutrients required to support actively growing bacterial populations, and likely provides the essential nutrients required for the production of the secondary metabolites responsible for biocontrol (Kwok et al., 1987). Enhanced soil OM can also stimulate greater host root exudation in the rhizosphere as well (Grayston et al., 1997), which could help sustain inoculated biocontrol rhizobacteria as well. Overall, this study has successfully demonstrated the role of antagonistic *Pseudomonas* in suppression of *P. penetrans*, affirming the role of this genus of bacteria in suppression of root diseases in the rhizosphere of fruit trees.

I originally predicted that root-dip inoculation with antagonistic rhizobacteria would improve plant growth and suppress *P. penetrans* populations in old orchard soil based upon published research demonstrating successful biocontrol of replant disease in the Okanagan Valley, Canada. Successful biocontrol after root-dip inoculation with *Pseudomonas* isolates was only observed in one of the four soils considered in the mutlisoil experiment (Chapter 4), suggesting that this management strategy was not as reliable as previous literature may have suggested. Discrepancies between this dissertation and prior literature (see Utkhede et al. (2001)) could potentially be a result of differences in the genera of bacteria evaluated for biocontrol, or perhaps differences in the orchard soils evaluated.

A major strength of the research conducted on biocontrol in this dissertation includes the use of root colonization assays to assess whether biocontrol failure was associated with inadequate colonization of the plant host root system, a problem that has previously impeded the development of many other biocontrol agents (see Weller, (1988)). This dissertation evaluated biocontrol capacity at five different orchard sites with

varying cropping histories, allowing for evaluation of the role of orchard-level variation on biocontrol success. The main limitation of this research is the limited number of prospective biocontrol agents evaluated, primarily due to the resources required for adequate replication of each isolate in greenhouse trials. Numerous *Pseudomonas* isolates displayed only minor, or even no, antagonistic activity or desirable biocontrol traits in the preliminary *in vitro* screening, but these isolates may have still displayed significant biocontrol activity if evaluated in orchard soil. Although resource extensive, this limitation could be overcome by screening entire isolate libraries for successful disease suppression directly in orchard soil.

Greenhouse-based microbial inoculant studies allow for an experimentally controlled demonstration of the organisms responsible for suppression of plant pathogens and parasites, and therefore they have considerable value in research on microbial interactions. From an applied prospective, biocontrol with rhizobacteria has shown considerable variability in the level of disease control, possibly a result of the diverse array of pathogens, parasites, and abiotic factors contributing to this particular disease complex. Nevertheless, biocontrol of replant disease with rhizobacteria has many benefits over many other biocontrol organisms that have been explored previously, such as the ability to multiply and persist in the rhizosphere, a broad-spectrum of activity to fungi and nematodes, and relative ease in mass production and commercialization. Future research should be directed towards evaluating the role of OM in supporting inoculated biocontrol microorganisms in the rhizosphere. Additionally, understanding the factors associated with successful establishment of biocontrol agents, such as sufficient soil OM, could potentially help ameliorate variability in biocontrol success.

5.4 Organic Mulch

In the field experiment (Chapter 3), surface application of bark chip mulch improved plant growth and suppressed *P. penetrans* populations in roots and soil of sweet cherry trees planted into an old apple orchard site. This organic soil amendment also enhanced soil biological suppressiveness, soil microbial activity, and the abundance of fungi in the rhizosphere. Some nutrient immobilization did occur in the bark chip mulch treatment, particularly when applied under the microsprinkler treatment; however, this did not seem to have adversely affected plant growth in these sub-plots.

I hypothesized that surface application of bark chip mulch would improve early growth and fruit yield of sweet cherry trees planted into an old apple orchard based upon the abundance of literature that has demonstrated positive plant growth response through the use of organic mulches during tree establishment. Bark chip mulch successfully increased trunk diameters of sweet cherry trees relative to the control; however, enhanced fruit yield was not observed. I also predicted bark chip mulch would suppress *P. penetrans* populations, and such suppression was observed in the mineral soil layer by fall of the first growing season. I predicted that bark chip mulch would enhance microbial indicators of soil suppressiveness and enhanced P nutrition based on previous reports of such changes. In this dissertation, bark chip mulch enhanced microbial indicators of soil suppressiveness; however, rather than promotion of soil bacterial antagonists, promotion of the total abundance of fungi was observed. Bark chip mulch did interact with irrigation to result in some differences in plant and soil nutrition; however, such differences likely did not play a strong role in contributing to the differences observed in plant growth promotion and nematode suppression by mulch because most leaf nutrient concentrations

were within recommended levels in production guidelines across all soil treatments.

The strengths of this research on the utility of organic mulch for replant disease control include the length of this study, evaluation of the interaction with different irrigation systems, as well as the supplementary experiment that was conducted on *P. penetrans* populations and fine root length density as influenced by sampling depth in the mulch-amended sub-plots. This study was able to demonstrate remarkably fast suppression of *P. penetrans* populations relative to most other studies conducted on organic mulches. The supplementary experiment conducted on *P. penetrans* and fine root density in mulch demonstrated the importance of sampling the organic layer above the mineral soil layer when bark chip mulch has been applied to the soil surface, as this environment is actively exploited by plant roots as well as plant-parasitic nematodes. This study is limited to one orchard site, primarily due to the requirement of field-scale research to effectively evaluate mulch treatments; therefore, results from this study cannot be extrapolated with confidence to other orchard sites with different soil and/or cropping histories.

Further research is required to demonstrate the beneficial effects of bark chip mulch on soil suppressiveness, microbial indicators of suppressiveness, and *P. penetrans* populations at a greater number of orchard sites. Bark chip mulch increased the abundance of fungi in the rhizosphere; however, due to the nature of the real-time PCR assay that was used to assess fungal abundance, it is not possible to determine if any of the well known nematode-trapping or -parasitizing groups of fungi increased under the mulch and could have been responsible for the enhanced nematode suppression. Previous research has documented increased activity of nematode-trapping fungi in response to

inputs of OM with a high C/N ratio, such as bark chip mulch used in my study. Researchers speculate that inputs of such high C/N ratio materials to soil can stimulate antagonistic fungi to prey upon nematodes as a N source (Cooke, 1962). A multitude of other nematode antagonistic organisms, including predacious invertebrates, could also have been enhanced under bark chip mulch, but were not specifically assessed in my research. Significant root growth was observed in the mulch layer located directly above the soil, and, although roots were equally parasitized by *P. penetrans* as they were in the mineral soil, more detailed analysis of the chemical, physical, or biological factors associated with the extensive root growth in this region is justified. Populations of fungal pathogens that may have been affecting roots along with *P. penetrans* were not monitored, and would be an area of useful research focus as well.

5.5 Low-Volume Irrigation Systems

In the field experiment (Chapter 3), drip irrigation improved trunk diameters, the density of fine roots in soil, and fruit yield. Trees under drip irrigation also had fewer *P. penetrans* in roots and soil, and this likely contributed to greater plant growth and fruit yield. Inverse correlations between AMF and *P. penetrans* root parasitism were observed in the context of irrigation effects, and could have been the result of competition for the root cortex between these two organisms, with enhanced *P. penetrans* root-feeding excluding arbuscule formation. Alternatively, irrigation may have had more direct differential effects on *P. penetrans* and AMF, and the inverse relationship between the two root-colonizing organisms could have been coincidental. The minimal effect that irrigation type had on soil microbiology and biological suppressiveness suggests that abiotic factors were more likely to be responsible for the nematode suppression that was

observed under the drip irrigation system. Irrigation type significantly affected plant nutrition, but leaf nutrient concentrations were not deficient in either irrigation type. Overall, alteration in soil volumetric water content is the abiotic factor that showed the most potential to be responsible for the differences in nematode population development observed under the different irrigation systems evaluated.

I originally predicted that the drip irrigation system would promote greater plant growth and fruit yield relative to that of the microsprinkler system based upon previous reports of greater plant growth and yield under a drip system, and this was confirmed in the field experiment (Chapter 3). I didn't originally hypothesize that irrigation system would affect *P. penetrans* populations, primarily because no studies to date have evaluated the effects of different irrigation emitter types on populations of *P. penetrans*. Similarly, I didn't hypothesize that irrigation system would have a significant effect on microbial indicators of soil suppressiveness, and for the most part, that was the case; however, consistently greater root colonization by AMF was observed under the drip irrigation system. Although greater soil volumetric water content was observed under the drip irrigation system in this study, as well as other studies, a reverse trend was observed with regard to effects on plant water status, showing lower photosynthetic rate, stomatal conductance, transpiration rate, and stem water potential under the drip irrigation system.

A major strength to the work conducted in this dissertation on low-volume irrigation systems is the concurrent use of five different soil management strategies applied as sub-plots, thereby allowing for evaluation of possible interaction effects. This study is limited to one orchard site with a sandy soil, primarily due to the requirement for field-scale research to effectively evaluate irrigation treatments. Therefore, results from

this study cannot be extrapolated with confidence to other orchard sites with different soil textures and cropping histories.

In this dissertation, the two different irrigation types were only evaluated at a single orchard site; therefore, future research needs to be directed towards evaluating the benefits, particularly with respect to nematodes and AMF, of drip irrigation at additional orchard sites. Factors such as soil texture and management practices, including history of application of organic soil amendments (DeBano, 2000), can have significant influences on soil water dynamics, and it would therefore be expected that irrigation systems might respond differently at other orchard sites. Additionally, understanding the underlying mechanisms behind *P. penetrans* suppression under the drip irrigation system is an area of considerable research interest. The mechanism behind stimulation of *M. xenoplax* populations when the drip irrigation system was used in combination with compost also requires further exploration. Populations of fungal pathogens that may have been affecting roots along with *P. penetrans* were not monitored, and would be an area of useful research focus as well.

5.6 Closing Summary

Chemical fumigants are going to become increasingly less available to tree fruit growers in the future. In many municipalities, a switch to zero green waste is increasing the abundance and local availability of high-quality compost soil amendments. Similarly, increased interest in recycling industrial waste, such as bark chips, is driving interest in utilization of such materials in agricultural productions systems. Water restrictions in many growing regions are influencing a switch to low-volume irrigation systems in tree-fruit production systems. Knowledge of the effects of different irrigation types on tree

establishment under varying soil management practices will help fruit growers utilize the most effective irrigation system given their chosen soil management practices, and vice versa. Overall, my research revealed that utilizing a combination of preplant incorporation of composts and surface application of bark chip mulch, alongside the use of a drip irrigation system provided the best establishment of sweet cherry trees planted into a sandy orchard site in the Okanagan Valley, Canada. This particular strategy may prove to be a useful alternative to soil fumigants in tree-fruit production systems.

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Appendices

Appendix A Microbial Growth Medium Recipes

Phosphate Buffered Saline (PBS)

Eighty grams of NaCl, 2.0 g of KCl, 1.44 g of $Na_2HPO_4*2H_20$, and 0.24 g of KH_2PO_4 were dissolved in 1 L of RO water. The solution was then autoclaved for 45 min at 121°C and 15 psi. After autoclaving, 800 µL of sterile 1N NaOH and 100 mg of streptomycin sulfate were added to the solution through a 0.45 µm pore size syringe filter.

Potato Dextrose Agar (PDA) + Streptomycin

Fifteen grams of potato dextrose powder (Difco, Lawrence, KS) were dissolved in 1 L of RO water, along with 15 g of granulated agar. The solution was brought to boil at 100 °C while stirring then autoclaved for 45 min at 121°C and 15 psi.

Alkaline Water Agar (AWA) + Streptomycin

Eighteen grams of granulated agar were dissolved in 1 L of RO water. The solution was brought to boil at 100 °C while stirring, then autoclaved for 45 min at 121°C and 15 psi. After autoclaving, 800 µL of sterile 1N NaOH and 100 mg of streptomycin sulfate were added to the solution through a 0.45 µm pore size syringe filter.

Tryptic Soy Broth (TSB)

Fifteen grams of tryptic soy powder were dissolved in 1 L of RO water. The solution was then autoclaved for 45 min at 121°C and 15 psi.

Tryptic Soy + Potato Dextrose Agar (TSA+PDA)

Seven and a half grams of potato dextrose powder and tryptic soy powder were dissolved in 1 L of RO water, along with 15 g of granulated agar. The solution was brought to a boil at 100 °C while stirring then autoclaved for 45 min at 121 °C and 15 psi.

Tryptic Soy + Potato Dextrose Broth (TSB+PDB)

Seven and a half grams of potato dextrose powder and tryptic soy powder were dissolved in 1 L of RO water. The solution was brought to a boil at 100 °C while stirring then autoclaved for 45 min at 121 °C and 15 psi.

Tryptic Soy Agar (TSA) + 100 µg mL^{-1} Rifampicin

Fifteen grams of tryptic soy powder were dissolved in 1 L of RO water, along with 15 g of granulated agar. The solution was brought to boil at 100 °C while stirring, then autoclaved for 45 min at 121°C and 15 psi. After autoclaving, 100 mg of rifampicin dissolved in 1 mL of 95% ethanol were added to the solution through a 0.45 µm pore size syringe filter.

Appendix B DNA Sequence Information

***Serratia plymuthica* Isolate 6-5 16S rRNA Sequence Results:**

CGAAAATTAAGGGAATTGCCGGTGCTTCTTCTGCGAGTACGTCAATGCTCAGT
GCTATTAACACTGAACCCTTCCTCCTCGCTGAAAGTGCTTTACAACCCTAAGG
CCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTG
GCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTAC
CCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCGTGAGGCCCGAA
GGTCCCCCACTTTGGTCCGTAGACGTTATGCGGTATTAGCTACCGTTTCCAGT
AGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGC
TCGTCACCCGGAGAGCAAGCTCTCCTGTGCTACCGCTCGACTTGCATGTGTTA
GGCCTGCCGCCAGCGTTCAATCTGAGCCAGGTTCAAACTCAAGCTCTCCTAAT
TTGGTTTAAGCCGGCCCTCCCGGAAGGTTAAGCTACCTACTTCTTTTGCAACC
CACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC
GTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTG
CAGACTCCAATCCGGACTACGACGTACTTTATGAGGTCCGCTTGCTCTCGCGA
GTTCGCTTCTCTTTGTATACGCCATTGTAGCACGTGTGTAGCCCTACTCGTAA
GGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAG
TCTCCTTTGAGTTCCCGACCGAATCGCTGGCAACAAAGGATAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATG
CAGCACCTGTCTCAGAGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCTC
TGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACAT
GCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGG
CCGTACTCCCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGCCTC
AAGGGCACAACCTCCAAATCGACATCGTTTACAGCGTGGACTACCAGGGTAT
CTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAG
GGGGCCGCCTTCGCCACCGGTATTCCTCCCAGATCTCTACGCATTTCACCGCT
ACACCTGGAAATTCTACCCCCCCTCTACAAGACTCTAGCTTGCCAGTTTCAAA
TGCAGTTCCCACGTTAGCGCGGGGAATTTCACATTCTGACTTAAACCAAAACC

CGGCCCCTTG

BLAST Results: >99% identity to *S. plymuthica* strain 265XY5 (GenBank Accession Number KF818650)

***Pratylenchus penetrans* 28S rRNA Sequence Results:**

TGAGGGAAAGTTGCAAAGCACTTTGAAGAGAGAGTTAAAGAGGACGTGAAA
CCGATGAGGTGGAAACGGATAGAGCCAGCGTATCTGGCCTGTATTCAACTGC
GTTGTTGTGAGCAGTTGGGCGCTGCATCTCCAGATTGGGACAGTCCTGGCTTG
CAAGCAACAATGTTGTGCATTTGCAGGTCGAGTGCGCCGAGACTTTCGAGAA
GGCGATATGAGCTCAATTTTGAGGCCAGCTTGCTGGTACCCGGATTGGAGGA
ATGTTGTTCGTTTTGGATGTGAATGGGGGAAAGGTTTTCGGGCTCGTATGGGT
TCGAATTGGTGTGGGGTGGCAGTTGCATGCAACATGTGCCTTCTGCCAATTCG
GTCCTGTGCGAGCTCACAATCCCACTCTCGGCGTAAAAGTTGGTCATCTATCC
GACCCGTCTTGAAACACGGACCAAGGAGTTTATCGTGTGCGCAAGTCATTGG
GTGTTGAAAACTCAAAGGCGCAATGAAAGTAAAGAATCCGCAAGGATACGA
CGTGTGATCTGAGCAATCACGATTGCCTGGAGCAACATGGCCCCATTCTGGTC
GCTTGCGACGGGGTGGAGGAAGAGCTACAACGGCGAGGAGGGAAAGATGGT
GAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGTCCGAA
GCGATTCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGGCGAAAGAC
TAATCGAACCATCTAGTAG

BLAST Results: >99% identity to *P. penetrans* isolate MU2 (GenBank Accession Number KP161612)

***Mesocriconema xenoplax* 28S rRNA Sequence Results:**

CTGCTGCATGCCTGGGCCGAAGCCGCGGCACTCGCATGTCAGCCCGGCACCG
AGCCTTCTCTTTCATTGCGCCTTGGGTTTAACACCCAAAACCGCCTACGCAAA
ACTCCTGGACCCGTCTTGAAACACGGACCAAGGAGTTTAGCGTATGCGCGAG
TCATTGGGTGTTANAACCCACAGGCGCAATGAAAGTGAAGGCTCGGTCGCCG

GGCTGACATGCGAGTGCCGCGGCTTCGGCCCAGGCATGCAGCATGGTCCCAT
TGCAATCGTTTACGATACAGTGGAGACAGAGCGTATGCGCTGAGACCCGAAA
GATGGTGAACTATTCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAA
GTCCGAAGCGATTCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTTCCG

BLAST Results: >99% identity to *M xenoplax* isolate Los Alamos B (GenBank Accession Number FN433871)

***M. hapla* 28S rRNA Sequence Results:**

ACGATCGATTTGCACGTCAGAACCGCTTCGGACTTCCACCAGAGTTTCCTCTG
GCTTCGTCCTGCTCAGGAATAGTTCACCATCTTTCGGGTCTCACCGCGTACGC
TCTTCCGCCACCCTACTGTAAACAGTTAGAATGGGGCCATGATGCACTTTTCT
GAAAAAAGATTGCACATCAGACTCTAAAGAATCATTTACTTTCATTTCGCTTC
TAGGTTTTTAACACCCAAAAACTTGCGCACACGATAAACTCCTTGGTCCGTGT
TTCAAGACGGGTCAA

BLAST Results: >99% identity to *M hapla* isolate ZEW1 (GenBank Accession Number KY587712)

Appendix C Greenhouse Experiment Correlation Matrix

Table C.1 - Correlation between pathogen parameters and plant growth parameters. Values represent: correlation coefficient (*P*-value).

	Plant growth parameter				WinRhizo root analysis		
Pathogen parameter	Shoot length	Shoot weight	Root weight	Biomass	Root length	Root surface area	Root volume
Pratylenchus penetrans 50 mL^{-1}	-0.518 (<0.001)	-0.485 (<0.001)	-0.362 (0.001)	-0.496 (<0.001)	-0.417 (<0.001)	-0.403 (<0.001)	-0.283 (0.014)
P. penetrans g^{-1} root	-0.421 (<0.001)	-0.503 (<0.001)	-0.433 (<0.001)	-0.537 (<0.001)	-0.382 (0.001)	-0.429 (<0.001)	-0.336 (0.003)
P. penetrans pot^{-1}	-0.380 (0.001)	-0.388 (0.001)	-0.343 (0.003)	-0.418 (<0.001)	-0.352 (0.002)	-0.317 (0.006)	-0.276 (0.016)
"*Cylindrocarpon*" sp. C1 (%)	-0.422 (<0.001)	-0.266 (0.021)	-0.145 (0.214)	-0.251 (0.030)	-0.360 (0.001)	-0.239 (0.039)	-0.162 (0.164)
"*Cylindrocarpon*" sp. C2 (%)	-0.135 (0.248)	-0.210 (0.071)	-0.240 (0.038)	-0.247 (0.033)	0.039 (0.738)	-0.225 (0.052)	-0.197 (0.090)
Fusarium sp. F1 (%)	-0.356 (0.002)	-0.183 (0.116)	-0.048 (0.681)	-0.153 (0.191)	-0.368 (0.001)	-0.267 (0.020)	-0.077 (0.514)
Fusarium sp. F2 (%)	-0.145 (0.216)	-0.065 (0.581)	0.009 (0.941)	-0.044 (0.708)	-0.159 (0.174)	-0.038 (0.748)	0.135 (0.250)
Rhizoctonia sp. R1 (%)	-0.153 (0.191)	0.046 (0.698)	-0.007 (0.955)	0.031 (0.793)	0.026 (0.828)	-0.091 (0.440)	0.020 (0.863)

Appendix D Ottawa 3 Rootstock Pathogenicity Bioassay

Table D.1 - Effect of fungal inoculum on shoot extension of Ottawa 3 apple rootstock. Data were analyzed with a one-way ANOVA. Values represent the mean ± standard error. Values sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

	Shoot extension		
Inoculum	28 days *pi*	56 days *pi*	84 days *pi*
Control	0±0	2.1±0.4	4.4±1.2
(N=4)	a	a	a
Fusarium sp. F1-1	0.1±0.1	1.1±0.3	3.5±1.3
(N=4)	a	a	a
Fusarium sp. F1-2	0±0	1.0±0.2	3.3±0.9
(N=4)	a	a	a
Fusarium sp. F2-1	0±0	0.3±0.1	3.0±1.1
(N=4)	a	a	a
Fusarium sp. F2-2	1.6±0.3	4.2±0.7	8.3±1.7
(N=4)	a	a	a
"*Cylindrocarpon*" sp. C1-1	1.6±0.2	3.9±0.2	4.8±0.9
(N=4)	a	a	a
"*Cylindrocarpon*" sp. C1-2	0.6±0.1	2.6±0,3	3.4±1.9
(N=4)	a	a	a
"*Cylindrocarpon*" sp. C2-1	0±0	3.0±0.4	3.9±1.6
(N=4)	a	a	a
"*Cylindrocarpon*" sp. C2-2	0.2±0.1	1.5±0.2	5.9±1.5
(N=4)	a	a	a
Rhizoctonia sp. R1-1	0.4±0.1	1.2±0.3	2.3±1.3
(N=4)	a	a	a
Rhizoctonia sp. R1-2	1.3±0.2	7.5±1.7	10.9±3.9
(N=4)	a	a	a
P-value	0.526	0.339	0.141

Appendix E Supplementary Sampling Depth Experiment

Table E.1 - Effect of sampling depth on root length and *P. penetrans* in the mineral soil layer. Data were analyzed with a one-way ANOVA. Values represent the mean ± standard error. Values sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Mineral soil layer (all soil treatments)		
Factor	Level	Fine root length (cm)	*P. penetrans* 100 mL^{-1} soil	*P. penetrans* g^{-1} root
Depth	Mulch layer	-	-	-
	0 - 7.5 cm (N=60)	90.4±19.1 b	43±9 a	678±254 a
	7.5 - 15 cm (N=60)	142.6±25.2 a	33±7 a	1383±600 a
P-value	SoilTrt	0.357	0.011	0.375
	Irrig	0.071	0.391	0.062
	Depth	0.003	0.200	0.695
	SoilTrt x Irrig	0.936	0.201	0.897
	SoilTrt x Depth	0.936	0.432	0.557
	Irrig x Depth	0.847	0.34	0.981
	SoilTrt x Irrig x Depth	0.432	0.056	0.263

Table E.2 - Effect of sampling depth on root length and *P. penetrans* in the mulch treatments. Data were analyzed with a one-way ANOVA. Values represent the mean ± standard error. Values sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		BCM and BCM+Comp treatments only		
Factor	Level	Fine root length (cm)	*P. penetrans* 100 mL^{-1} soil	*P. penetrans* g^{-1} root
Depth	Mulch layer (N=24)	135.9±23.1 a	17±4 b	1738±543 a
	0 - 7.5 cm (N=24)	90.4±19.8 a	43±6 a	678±234 a
	7.5 - 15 cm (N=24)	142.6±27.4 a	33±4 a	1383±452 a
P-value	SoilTrt	0.001	0.215	0.658
	Irrig	0.796	0.051	0.022
	Depth	0.064	0.013	0.089
	SoilTrt x Irrig	0.001	0.215	0.658
	SoilTrt x Depth	0.796	0.051	0.022
	Irrig x Depth	0.064	0.013	0.089
	SoilTrt x Irrig x Depth	0.871	0.241	0.39

Appendix F Abundance of Other Nematode Populations

Table F.1 - Effect of soil treatment and irrigation type on other plant-parasitic nematode populations. Data were analyzed with a split-plot repeated measures ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	*Paratrichodorus teres* 100 mL^{-1} soil	*Hemicycliophora similis* 100 mL^{-1} soil	*Mesocriconema xenoplax* 100 mL^{-1} soil
Soil treatment	Control (N=72)	3±2 a	3±2 a	1±1 a
	Fum (N=72)	2±1 a	1±1 a	4±2 a
	Comp (N=72)	2±1 a	10±4 a	43±17 a
	BCM (N=72)	2±1 a	1±1 a	6±2 a
	Comp+BCM (N=72)	2±1 a	2±1 a	7±2 a
Irrigation	Drip (N=180)	2±1 a	5±3 a	19±12 a
	MS (N=180)	2±1 a	1±1 a	3±3 a
P-value	SoilTrt	0.656	0.100	0.009
	Irrig	0.880	0.160	0.015
	Date	<0.001	0.319	0.017
	SoilTrt x Irrig	0.190	0.390	0.008
	SoilTrt x Date	0.932	0.090	0.351
	Irrig x Date	0.150	0.065	0.240
	SoilTrt x Irrig x Date	0.844	0.454	0.589

Table F.2 - Effect of soil treatment and irrigation type on populations of *Mononchus* spp. and the total abundance of free-living nematodes. Data were analyzed with a split-plot repeated measures ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

Factor	Level	*Mononchus* spp. 100 mL^{-1} soil	Total nematodes 100 mL^{-1} soil
Soil treatment	Control	1±1	830±35
	(N=72)	a	a
	Fum	4±2	812±29
	(N=72)	a	a
	Comp	1±1	865±34
	(N=72)	a	a
	BCM	7±3	857±38
	(N=72)	a	a
	Comp+BCM	5±3	827±31
	(N=72)	a	a
Irrigation	Drip	3±2	808±28
	(N=180)	a	a
	MS	4±2	868±27
	(N=180)	a	a
P-value	SoilTrt	0.17	0.682
	Irrig	0.218	0.242
	Date	0.279	<0.001
	SoilTrt x Irrig	0.25	0.644
	SoilTrt x Date	0.314	0.208
	Irrig x Date	0.99	0.625
	SoilTrt x Irrig x Date	0.999	0.619

Appendix G Soil Volumetric Water Content

Table G.1 - Effect of soil treatment and irrigation type on soil volumetric water content in 2014. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil volumetric water content (%) 2014					
Factor	Level	May	June	July	August	September	October
Soil treatment	Fum	-	19.4±1.5	20.8±1.6	16.1±2.0	16.7±1.8	17.5±1.7
	(N=6)		a	a	a	b	ab
	Comp	-	20.9±1.4	21.8±2.0	17.2±2.2	18.2±2.0	18.6±1.8
	(N=6)		a	a	a	ab	ab
	BCM	-	19.3±1.4	20.5±1.2	17.5±0.8	17.3±0.7	16.6±0.7
	(N=6)		a	a	a	ab	b
	Comp+BCM	-	22.6±1.4	24.0±1.0	20.8±0.7	20.1±1.0	19.7±1.0
	(N=6)		a	a	a	a	a
Irrigation	Drip	-	20.0±1.1	21.3±1.1	19.1±1.2	19.7±1.1	19.8±1.0
	(N=12)		a	a	a	a	a
	MS	-	21.1±1.0	22.2±1.1	16.7±1.0	16.4±0.7	16.4±0.6
	(N=12)		a	a	a	a	a
P-value	SoilTrt	-	0.066	0.176	0.112	0.028	0.047
	Irrig	-	0.593	0.642	0.330	0.156	0.090
	SoilTrt x Irrig	-	0.052	0.464	0.593	0.260	0.132

Table G.2 - Effect of soil treatment and irrigation type on soil volumetric water content in 2015. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil volumetric water content (%) 2015					
Factor	Level	May	June	July	August	September	October
Soil treatment	Fum	13.4±1.2	15.6±18	15.4±1.1	14.8±1.2	14.8±1.6	16.5±1.0
	(N=6)	a	a	a	a	a	a
	Comp	14.2±1.4	16.2±1.4	15.4±1.4	14.3±1.7	14.2±1.8	15.6±1.5
	(N=6)	a	a	a	a	ab	ab
	BCM	15.4±1.2	16.9±1.8	15.5±1.7	13.7±1.9	13.3±2.0	14.6±1.7
	(N=6)	a	a	a	a	ab	ab
	Comp+BCM	16.6±1.3	17.6±1.4	14.0±1.5	11.4±1.8	11.9±2.4	14.0±2.3
	(N=6)	a	a	a	a	b	b
Irrigation	Drip	15.6±0.9	17.2±1.0	16.0±1.0	15.7±0.9	16.5±1.0	17.4±0.9
	(N=12)	a	a	a	a	a	a
	MS	14.2±0.8	15.9±1.0	14.0±1.0	11.3±1.1	10.6±1.1	13.0±1.1
	(N=12)	a	a	a	a	b	a
P-value	SoilTrt	0.243	0.399	0.351	0.104	0.024	0.020
	Irrig	0.499	0.575	0.370	0.064	0.042	0.070
	SoilTrt x Irrig	0.532	0.233	0.178	0.298	0.023	0.030

Table G.3 - Interaction effect between soil treatment and irrigation type on soil volumetric water content in 2015. Data were analyzed with a one-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		2015 (Interaction effects)	
Irrigation	Soil treatment	September	October
Drip	Fum	17.2±2.0	17.8±2.1
	(N=3)	a	ab
	Comp	16.9±1.9	18.6±2.2
	(N=3)	a	a
	BCM	16.7±2.1	17.7±1.6
	(N=3)	a	ab
	Comp+BCM	15.1±1.9	15.6±1.8
	(N=3)	ab	ab
MS	Fum	11.1±1.7	13.3±2.0
	(N=3)	ab	ab
	Comp	7.0±2.0	9.5±1.5
	(N=3)	b	b
	BCM	12.9±2.2	15.3±1.7
	(N=3)	ab	ab
	Comp+BCM	11.6±2.3	13.7±1.5
	(N=3)	ab	ab
P-value	Trt	0.022	0.044

Table G.4 - Effect of soil treatment and irrigation type on soil volumetric water content in 2016. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil volumetric water content (%) 2016					
Factor	Level	May	June	July	August	September	October
Soil treatment	Fum	15.4±1.2	18.4±1.3	19.9±1.4	21.0±1.6	-	-
	(N=6)	a	a	a	a		
	Comp	16.0±1.5	19.3±1.2	21.4±1.6	24.2±1.6	-	-
	(N=6)	a	a	a	a		
	BCM	15.9±1.4	17.7±1.3	19.5±1.1	19.9±1.3	-	-
	(N=6)	a	a	a	a		
	Comp+BCM	15.3±2.3	17.4±2.8	22.3±2.4	25.1±2.6	-	-
	(N=6)	a	a	a	a		
Irrigation	Drip	17.3±1.0	19.7±1.3	21.1±1.5	21.4±1.7	-	-
	(N=12)	a	a	a	a		
	MS	14.0±0.9	16.7±1.0	20.4±0.7	23.7±0.8	-	-
	(N=12)	a	a	a	a		
P-value	SoilTrt	0.065	0.570	0.467	0.056	-	-
	Irrig	0.189	0.253	0.846	0.367	-	-
	SoilTrt x Irrig	0.100	0.116	0.138	0.203	-	-

Table G.5 - Effect of soil treatment and irrigation type on soil volumetric water content in 2017. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil volumetric water content (%) 2017					
Factor	Level	May	June	July	August	September	October
Soil treatment	Fum (N=6)	-	15.9±1.4 a	16.2±1.5 a	15.7±1.7 a	15.7±1.5 a	14.9±1.2 a
	Comp (N=6)	-	18.5±1.4 a	19.6±1.3 a	20.2±1.9 a	20.2±1.8 a	18.0±1.9 a
	BCM (N=6)	-	16.6±1.2 a	18.1±1.0 a	17.2±1.1 a	16.4±0.8 a	15.0±1.2 a
	Comp+BCM (N=6)	-	17.7±2.5 a	18.5±2.2 a	16.7±1.6 a	15.1±1.6 a	13.9±1.5 a
Irrigation	Drip (N=12)	-	18.9±1.1 a	19.3±1.0 a	18.7±1.0 a	17.0±1.0 a	15.6±0.8 a
	MS (N=12)	-	15.5±1.0 a	17.0±1.2 a	16.2±1.3 a	16.7±1.3 a	15.4±1.3 a
P-value	SoilTrt	-	0.442	0.221	0.783	0.680	0.591
	Irrig	-	0.101	0.330	0.213	0.846	0.851
	SoilTrt x Irrig	-	0.122	0.311	0.612	0.754	0.622

Appendix H Soil Nutrient Analyses

Table H.1 - Effect of soil treatment and irrigation type on soil C, N, and C/N ratios. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Pre-treatment 2014			September 2014			September 2015		
Factor	Level	Soil C content (%)	Soil N content (%)	Soil C/N ratio	Soil C content (%)	Soil N content (%)	Soil C/N ratio	Soil C content (%)	Soil N content (%)	Soil C/N ratio
Soil treatment	Control (N=12)	0.82±0.05 a	0.045±0.005 a	21.4±2.9 a	1.05±0.09 b	0.061±0.008 ab	19.9±2.5 a	1.18±0.09 ab	0.084±0.010 a	15.0±0.9 b
	Fum (N=12)	0.88±0.09 a	0.045±0.006 a	21.5±1.6 a	0.95±0.11 b	0.050±0.007 b	21.2±1.9 a	1.10±0.09 b	0.071±0.007 a	14.7±0.7 b
	Comp (N=12)	0.94±0.07 a	0.051±0.004 a	18.7±0.5 a	1.57±0.08 a	0.097±0.007 a	17.0±0.5 a	1.82±0.17 a	0.124±0.010 a	14.7±0.3 b
	BCM (N=12)	0.91±0.10 a	0.052±0.007 a	19.3±1.5 a	0.96±0.11 b	0.052±0.009 ab	20.9±1.6 a	1.12±0.13 ab	0.068±0.009 a	17.2±0.8 a
	Comp+BCM (N=12	0.86±0.09 a	0.043±0.007 a	25.2±3.6 a	1.45±0.16 ab	0.080±0.010 ab	19.0±1.0 a	1.54±0.10 a	0.093±0.006 a	17.0±1.0 a
Irrigation	Drip (N=30)	0.898±0.06 a	0.047±0.005 a	22.6±1.7 a	1.13±0.07 a	0.063±0.006 b	20.2±1.2 a	1.39±0.11 a	0.090±0.008 a	16.0±0.6 a
	MS (N=30)	0.865±0.04 a	0.047±0.003 a	19.9±1.1 a	1.26±0.10 a	0.073±0.006 a	18.8±1.0 a	1.28±0.07 a	0.086±0.005 a	15.3±0.4 a
P-value	SoilTrt	0.691	0.725	0.332	0.017	0.033	0.185	0.047	0.114	0.045
	Irrig	0.496	0.970	0.163	0.086	0.025	0.196	0.243	0.432	0.379
	SoilTrt x Irrig	0.852	0.870	0.511	0.758	0.775	0.640	0.916	0.710	0.879

Table H.2 - Effect of soil treatment and irrigation type on soil nutrition in September 2015. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, MS refers to microsprinkler, OM refers to organic matter, CEC refers to cation-exchange capacity, and NO_3-N refers to nitrate N. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		Soil parameter (2015)										
Factor	Level	OM (%)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Na (ppm)	pH	CEC (meq $100g^{-1}$)	S (ppm)	Al (ppm)	NO_3-N (ppm)
Soil treatment	Control (N=12)	1.67±0.14 ab	152±12 a	97±5 b	178±7 bc	1413±61 b	26±1 a	7.12±0.07 a	9.6±0.4 b	14±1 ab	423±10 a	21±4 a
	Fum (N=12)	1.42±0.11 b	72±11 b	100±6 b	148±6 cd	1536±136 ab	24±2 ab	7.34±0.07 a	9.6±0.7 b	16±3 a	352±15 b	24±5 a
	Comp (N=12)	2.38±0.18 a	151±27 ab	228±33 a	225±13 a	2053±106 a	27±2 a	7.38±0.06 a	13.0±0.6 a	17±1 a	316±14 b	27±5 a
	BCM (N=12)	1.48±0.18 ab	83±13 b	111±8 b	145±5 d	1473±170 ab	17±1 b	7.39±0.06 a	9.5±0.6 b	9±1 b	360±11 b	6±1 b
	Comp+BCM (N=12)	2.16±0.12 a	104±11 ab	191±22 a	194±11 ab	1796±100 ab	20±1 ab	7.44±0.04 a	11.2±0.6 ab	11±1 b	312±8 b	6±1 b
Irrigation	Drip (N=30)	1.87±0.14 a	120±14 a	138±18 a	170±8 a	1661±83 a	23±1 a	7.31±0.05 a	10.4±0.5 a	14±1 a	351±10 a	17±3 a
	MS (N=30)	1.77±0.09 a	105±9 a	152±11 a	185±7 a	1647±89 a	23±1 a	7.36±0.04 a	10.7±0.4 a	13±1 a	355±11 a	17±3 a
P-value	SoilTrt	0.032	0.012	0.027	0.008	0.007	0.013	0.122	0.03	0.015	<0.001	0.024
	Irrig	0.405	0.381	0.547	0.156	0.843	0.767	0.424	0.622	0.829	0.798	0.827
	SoilTrt x Irrig	0.915	0.308	0.155	0.125	0.827	0.139	0.619	0.793	0.699	0.941	0.299

Appendix I Plant Nutrient Analyses

Table I.1 - Effect of soil treatment and irrigation type on leaf nutrient concentrations in 2014. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		2014									
Factor	Level	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Soil	Control	2.82±0.06	0.36±0.02	1.67±0.10	2.06±0.13	0.41±0.02	64±2	16±1	77±3	69±4	8.5±0.5
	(N=12)	ab	b	b	a	a	a	b	ab	ab	a
treatment	Fum	2.89±0.05	0.31±0.01	2.04±0.10	1.95±0.09	0.32±0.02	62±1	17±1	80±3	87±6	7.4±0.4
	(N=12)	a	b	a	ab	ab	ab	b	a	a	a
	Comp	2.93±0.04	0.42±0.03	2.30±0.09	1.63±0.10	0.28±0.20	62±2	25±1	87±3	54±3	8.5±0.2
	(N=12)	a	b	a	b	b	ab	a	a	b	a
	BCM	2.59±0.09	0.35±0.02	1.56±0.06	2.06±0.10	0.40±0.02	58±1	22±2	58±2	62±5	7.6±0.4
	(N=12)	b	b	b	a	a	b	ab	b	ab	a
	Comp+BCM	2.73±0.09	0.53±0.05	2.12±0.11	1.66±0.08	0.32±0.02	60±1	27±2	67±2	53±4	8.2±0.3
	(N=12)	ab	a	a	ab	ab	ab	a	b	b	a
Irrigation	Drip	2.92±0.03	0.36±0.01	2.08±0.08	1.72±0.07	0.31±0.02	62±1	22±1	70±2	58±4	8.3±0.3
	(N=30)	a	b	a	b	b	a	a	b	b	a
	MS	2.67±0.05	0.43±0.03	1.80±0.07	2.03±0.06	0.38±0.01	60±1	23±1	77±3	73±4	7.8±0.2
	(N=30)	b	a	b	a	a	a	a	a	a	a
P-value	SoilTrt	<0.001	0.002	<0.001	0.036	0.001	0.038	<0.001	<0.001	0.014	0.070
	Irrig	<0.001	0.045	0.020	0.022	0.022	0.258	0.076	0.001	<0.001	0.214
	SoilTrt x Irrig	0.002	0.044	0.363	0.625	0.447	0.080	0.213	0.046	0.470	0.737

Table I.2 - Interaction effect between soil treatment and irrigation type on leaf nutrient concentrations in 2014. Data were analyzed with a one-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		2014 (Interaction effects)		
Irrigation	Soil treatment	N (%)	P (%)	Fe (ppm)
Drip	Control	2.89±0.08	0.359±0.01	71±3
	(N=6)	ab	ab	ab
	Fum	2.94±0.09	0.311±0.02	75±3
	(N=6)	ab	ab	ab
	Comp	2.97±0.05	0.356±0.01	80±2
	(N=6)	ab	ab	ab
	BCM	2.80±0.06	0.351±0.03	56±3
	(N=6)	ab	ab	b
	Comp+BCM	2.98±0.06	0.412±0.01	69±1
	(N=6)	a	ab	ab
MS	Control	2.75±0.10	0.354±0.01	82±3
	(N=6)	ab	ab	ab
	Fum	2.84±0.07	0.306±0.02	84±4
	(N=6)	ab	b	ab
	Comp	2.86±0.09	0.474±0.03	93±3
	(N=6)	ab	ab	a
	BCM	2.37±0.08	0.348±0.02	61±1
	(N=6)	ab	ab	ab
	Comp+BCM	2.49±0.04	0.652±0.04	65±2
	(N=6)	b	a	ab
P-value	Trt	<0.001	<0.001	<0.001

Table I.3 - Effect of soil treatment and irrigation type on leaf nutrient concentrations in 2015. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		2015									
Factor	Level	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Soil	Control	2.41±0.05	0.22±0.01	1.67±0.03	1.25±0.04	0.28±0.01	38±3	16±1	56±3	53±3	6.4±0.2
treatment	(N=12)	b	c	b	a	a	a	b	a	a	b
	Fum	2.49±0.06	0.21±0.01	1.76±0.04	1.18±0.04	0.24±0.01	40±2	15±1	59±4	51±4	6.5±0.3
	(N=12)	ab	c	b	ab	b	a	b	a	a	b
	Comp	2.60±0.06	0.23±0.01	2.28±0.07	1.06±0.05	0.22±0.01	40±1	17±1	62±4	47±3	6.8±0.2
	(N=12)	ab	bc	a	b	b	a	ab	a	a	ab
	BCM	2.58±0.05	0.27±0.02	2.32±0.05	1.08±0.04	0.21±0.01	41±2	22±1	64±4	52±3	7.3±0.3
	(N=12)	ab	ab	a	ab	b	a	a	a	a	ab
	Comp+BCM	2.66±0.05	0.29±0.01	2.63±0.07	1.00±0.04	0.20±0.01	38±1	23±1	74±4	49±2	7.8±0.4
	(N=12)	a	a	a	b	b	a	a	a	a	a
Irrigation	Drip	2.49±0.03	0.24±0.01	2.12±0.07	1.07±0.3	0.22±0.01	41±1	19±1	60±2	49±2	6.8±0.2
	(N=30)	a	a	a	a	a	a	a	a	a	a
	MS	2.61±0.04	0.25±0.01	2.14±0.08	1.16±0.3	0.24±0.01	38±1	19±1	67±3	52±2	7.1±0.2
	(N=30)	a	a	a	a	a	a	a	a	a	a
P-value	SoilTrt	0.032	0.007	<0.001	0.002	<0.001	0.594	0.004	0.224	0.739	0.011
	Irrig	0.079	0.465	0.864	0.094	0.072	0.281	0.778	0.113	0.182	0.403
	SoilTrt x Irrig	0.339	0.521	0.677	0.096	0.223	0.136	0.110	0.343	0.326	0.954

Table I.4 - Effect of soil treatment and irrigation type on leaf nutrient concentrations in 2016. Data were analyzed with a split-plot two-way ANOVA. Fum refers to fumigation, Comp refers to compost, BCM refers to bark chip mulch, Comp+BCM refers to compost and bark chip mulch, and MS refers to microsprinkler. Values represent the mean ± standard error. Factor levels sharing the same letter within a column did not differ significantly (*P*-value > 0.05), according to Bonferroni adjustment.

		2016									
Factor	Level	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Soil	Control	2.24±0.04	0.20±0.01	1.28±0.07	1.61±0.05	0.33±0.01	36±1	11±1	50±2	52±3	4.9±0.2
treatment	(N=12)	b	a	b	a	a	b	a	a	a	a
	Fum	2.33±0.08	0.20±0.01	1.24±0.05	1.68±0.05	0.33±0.01	38±1	8±1	48±2	53±6	4.9±0.2
	(N=12)	ab	a	b	a	a	ab	b	a	a	a
	Comp	2.50±0.04	0.21±0.01	1.81±0.06	1.48±0.07	0.27±0.02	40±1	9±1	52±2	46±2	5.1±0.2
	(N=12)	a	a	a	a	b	a	ab	a	a	a
	BCM	2.17±0.08	0.24±0.01	1.83±0.08	1.42±0.08	0.25±0.02	42±1	11±1	57±3	58±6	4.9±0.2
	(N=12)	b	a	a	a	b	a	a	a	a	a
	Comp+BCM	2.32±0.06	0.23±0.01	2.02±0.06	1.43±0.07	0.25±0.01	41±1	12±1	57±2	55±4	5.0±0.2
	(N=12)	b	a	a	a	b	a	a	a	a	a
Irrigation	Drip	2.38±0.03	0.21±0.01	1.56±0.07	1.55±0.05	0.28±0.01	39±1	10±1	51±1	48±2	4.9±0.1
	(N=30)	a	a	b	a	a	a	a	a	a	a
	MS	2.24±0.05	0.22±0.01	1.72±0.07	1.50±0.04	0.29±0.01	40±1	11±1	55±2	57±3	5.0±0.1
	(N=30)	a	a	a	a	a	a	a	a	a	a
P-value	SoilTrt	0.004	0.107	<0.001	0.129	0.001	0.018	0.01	0.183	0.183	0.853
	Irrig	0.141	0.068	0.017	0.555	0.483	0.438	0.684	0.116	0.078	0.576
	SoilTrt x Irrig	0.899	0.460	0.063	0.872	0.881	0.409	0.490	0.580	0.489	0.070

Appendix J Biocontrol Isolate Library

Table J.1 - Origin of biocontrol isolates.

Origin soil treatment	*Pseudomonas* sp. isolate codes	Number of isolates
Control	P10-61 through P10-80	20
Fum	P10-81 through P10-100	20
Comp	P10-1 through P10-20	20
BCM	P10-41 through P10-60	20
Comp+BCM	P10-21 through P10-40	20

Appendix K Multisoil Compost Experiment ANOVA Table

Table K.1 - ANOVA table for the multisoil compost experiment. Pf/Pi refers to nematode reproductive factor, DAPG+ bacteria refers to 2,4-diacetylphloroglucinol-producing bacteria, and PRN+ bacteria refers to pyrrolnitrin-producing bacteria.

	P-value		
Parameter	Soil treatment	Orchard site	Soil treatment x orchard site
Shoot length	<0.001	<0.001	<0.001
Shoot weight	<0.001	<0.001	<0.001
Root weight	<0.001	<0.001	<0.001
Biomass	<0.001	<0.001	<0.001
Preplant *Pratylenchus penetrans* 100 mL^{-1} soil	<0.001	<0.001	<0.001
Harvest *P. penetrans* 100 mL^{-1} soil	<0.001	<0.001	<0.001
Harvest *P. penetrans* g^{-1} fine root	<0.001	<0.001	<0.001
Harvest *P. penetrans* pot^{-1}	<0.001	<0.001	<0.001
Pf/Pi	0.001	<0.001	<0.001
Soil microbial activity	<0.001	<0.001	0.257
Total bacteria	<0.001	<0.001	0.030
Total fungi	<0.001	<0.001	<0.001
Pseudomonas spp.	<0.001	<0.001	0.172
DAPG+ bacteria	0.131	0.647	0.277
PRN+ bacteria	<0.001	<0.001	0.139

Appendix L Multisoil Biocontrol Experiment ANOVA Table

Table L.1 - ANOVA table for the multisoil biocontrol experiment. Pf/Pi refers to nematode reproductive factor and CFU refers to colony forming units.

	P-value		
Parameter	Soil treatment	Orchard site	Soil treatment x orchard site
Shoot length	<0.001	<0.001	0.001
Shoot weight	<0.001	<0.001	<0.001
Root weight	<0.001	<0.001	<0.001
Biomass	<0.001	<0.001	<0.001
Preplant *Pratylenchus penetrans* 50 mL^{-1} soil	<0.001	<0.001	<0.001
Harvest *P. penetrans* 50 mL^{-1} soil	<0.001	<0.001	<0.001
Harvest *P. penetrans* g^{-1} fine root	<0.001	<0.001	0.086
Harvest *P. penetrans* pot^{-1}	<0.001	<0.001	<0.001
Pf/Pi	<0.001	<0.001	<0.001
log CFU 14 days	<0.001	<0.001	<0.001
log CFU 56 days	<0.001	<0.001	<0.001