12-2016

Forest industry byproducts improve soil quality and increase pepper growth in three soils infested with Phythophthora blight

Xiaojun Zhao
Purdue University

FOREST INDUSTRY BYPRODUCTS IMPROVE SOIL QUALITY AND INCREASE PEPPER GROWTH IN THREE SOILS INFESTED WITH PHYTOPHTHORA BLIGHT

by

Xiaojun Zhao

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Horticulture

West Lafayette, Indiana

December 2016

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor Dr. Lori A. Hoagland for her unwavering understanding, patience and encouragement for helping me refresh my courage and enthusiasm on scientific research, and helping me learn how to manage time and cope with pressure! She helps me go further than I thought I could go and she helps me to be stronger than I thought I could be. To my graduate committee, Dr. Daniel S. Egel, Dr. Timothy T. Filley, Dr. Elizabeth T. Maynard and Dr. Robert J. Joly, without their warm-hearted guidance, I cannot gain so many valuable ideas for our interdisciplinary project. I am also extremely grateful for my teammates Lisseth Zubieta, Sahar Abedelrazek and Judith Martin who are always company with me and always sharing my worries. This work was supported by the Purdue AgSeed Grant Program and Purdue Agriculture Research Programs. We thank Yunfan Li and the Purdue Statistical Consulting Services for their assistance with statistical analyses. We also would like to express our sincere gratitude to Rob Eddy and Michael Russell for their assistance with greenhouse management.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Zhao, Xiaojun. MS
Institution: Purdue University
Degree Received: December 2016
Title: Forest Industry Byproducts Improve Soil Quality and Increase Pepper Growth in Three Soils Infested With Phythophthora Blight
Major Professor: Lori Hoagland.

Phytophthora blight is a serious threat to the Midwest vegetable industry, because the oomycete pathogen responsible for this disease, *Phytophthora capsici*, has a wide host range, can spread quickly in fields, and produces resilient oospores that can survive in soil for years. *Phytophthora capsici* has become resistant to commonly used fungicides and resistant crop varieties are rare. Amending soil with complex organic substrates has potential to improve soil quality and suppress soil-borne pathogens including *P. capsici*. Indiana has a significant forest industry with many residual products that could be used as locally available amendments to meet this goal. However, the mechanisms mediating how amendments induce disease suppressive activity in soil are not well understood, which currently limits their practical application as a disease control strategy. The objective of the experiments described in this thesis were to: (i) determine whether commercially available forest industry byproducts with different compositions and expected rates of decomposition, could suppress Phytophthora blight in pepper, and (ii) determine whether changes in soil physiochemical and biological properties were correlated with the suppressive activity of these amendments. In the first study, four forest industry byproducts were evaluated at a rate of 1% total carbon (w/w) soil. Changes in soil moisture, microbial activity and specific microbial taxa that have previously been associated with disease suppressive activity were monitored during a one month incubation period prior to pepper transplant, and changes in soil chemical properties were quantified at the end of the trial. In the second study, five forest industry byproducts were evaluated, each at one of two rates (1% or 3% total carbon (w/w) soil), and in either a high or low organic matter soil. The soils were amended with *P. capsici*

inoculum to ensure sufficient disease pressure, and a variety of soil physiochemical and biological factors were quantified. The amendments tested in these trials included two biochar products and a locally-available compost derived from woody materials, and kraft pine lignin and sawdust generated as direct byproducts of the forest industry. Both experiments were conducted in the greenhouse using field soil collected from sites with recent outbreaks of Phytophthora blight. Results of these studies indicate that many of the amendments altered soil physiochemical properties including soil moisture, pH, nitrogen, magnesium, potassium, and calcium availability, microbial biomass, and microbial activity, particularly in the low organic matter soil. Several amendments also improved pepper root growth, indicating that they have potential to suppress Phytophthora blight. The suppressive activity of the amendments was not consistently correlated with specific soil physiochemical and biological factors, indicating that different mechanisms may be responsible for the suppressive activity induced by the different types of amendments. Results of these studies indicate that forest industry byproducts have potential to improve soil quality and reduce *Phytophthora* blight, but field trials and cost-benefit analyses will need to be conducted before these products can be recommended to growers. Additional studies that document differences in the biochemical quality of the amendments and quantify changes in microbial community structure using molecular tools are recommended to better understand how these amendments induce disease suppressive activity.

CHAPTER 1. INTRODUCTION: OPPORTUNTIES FOR BIOCONTROL OF PHYTOPHTHORA BLIGHT WITH FOREST INDUSTRY BYPRODUCTS

1.1 Introduction to Soil-Borne Pathogens and *Phytophthora capsici*

Soil-borne pathogens refer to soil organisms that can survive in the soil and infect plants at different stages throughout their life cycles. Soil-borne pathogens mostly tend to affect underground plant tissues, causing root rots, crown rots, seed decay and damping-off of seedlings. However, in some cases, aboveground plant parts can also be affected by soil-borne pathogens resulting in foliar disease symptoms. Most diseases caused by soil-borne pathogens dramatically reduce crop yield, as well as the quality of the produce (Koike et al., 2003). Given the extreme complexity of the soil environment, understanding how to effectively manage soil-borne pathogens is a significant challenge.

One of the most challenging soil-borne pathogens facing Indiana vegetable growers is *P. capsici*, a fungus-like oomycete pathogen which causes Phytophthora blight. In the past, Phythopthora blight was often presumed to be caused by waterlogging of roots because symptoms often occur in low-lying places where surface water cannot be drained away. The first report of *Phytophthora capsici* as the causal agent of Phythophthora blight was presented by L. H. Leonian in Las Cruces, New Mexico in 1922 (Leonian, 1922). In autumn 1918, he noticed a novel species of Phytophthora that was attacking the pods and branches of chili peppers (*Capsicum annuum*). The pathogen was associated with symptoms commonly observed in waterlogged soil. He noted that it was unusual, because the symptoms usually appeared during the early warm, rainy season in June or early summer. He described the distinctive morphological character of this species, which appeared as peculiar tuberous growths on the mycelium resembling sporangia. Since this early discovery, *P. capsici* has become a devastating threat throughout the United States and world (Lamour et al., 2012). This pathogen is particularly difficult to manage, because host plants can be infected by *P. capsici* at any growth stage and infection can result in up to 100% crop loss (Babadoost and Islam,

2003). The pathogen also has a broad host range, which makes crop rotation ineffective, and it produces long-lived spores (oospores) that can survive in soil for years (Babadoost and Pavon, 2013). *Phythophthora capsici* can also spread quickly via long distance movement in water (Ristaino and Johnston, 1999), and it has many isolates that are now resistant to commonly used fungicides (Lamour and Hausbeck, 2001).

1.1.1 *P. capsici* Host Range and Host Susceptibility

Phytophthora capsici has a very wide range of hosts including cultivated crops, ornamentals and even weeds. In 1996, Erwin and Ribeiro reported that 49 species of plants can be infected by this pathogen (Erwin and Ribeiro, 1996), and additional species have since been added to the long list of susceptible hosts. In 2004, Tian and Babadoost first reported that spinach (*Spinacia oleracea*), turnip (*Brassica rapa*), lima bean (*Phaseolus lunatus*) and velvet-leaf (*Abutilon theophrasti*) can be hosts of *P. capsici*. In addition, they reported that most species from the family Chenopodiaceae and almost all species from the Cucurbitaceae and Solanaceae families can be infected by *P. capsici*. As of 2012, 27 families and 71 species have now been confirmed as hosts of *P. capsici* under laboratory and greenhouse conditions (Granke and Ocampo, et al., 2012). This is the widest investigation of host range conducted to date.

Among the wide host range of *P. capsici*, differences in host susceptibility have been observed. For example, Tian and Babadoost (2004) found that more than 50% of seedlings from cucurbits and pepper (*Capsicum annuum*) were infected with disease symptoms by *P. capsici* 12 days after inoculation. In contrast, none of the broccoli (*Brassica oleracea*), cabbage (*Brassica oleracea*), cauliflower (*Brassica oleracea*), kale (*Brassica oleracea*), kohlrabi (*Brassica oleracea*), mustard (*Brassica nigra*), corn (*Zea mays*), wheat (*Triticum aestivum*), basil (*Ocimum basilicum*) and soybean (*Glycine max*) exhibited disease symptoms 12 days after inoculation with *P. capsici* (Tian and Babadoost, 2004). Differences in susceptibility can also be present among different plant parts. For example, compared to the root, the fruit of cucumber are more easily infected by *P. capsici* (Hausbeck and Lamour, 2004).

1.1.2 Disease Symptoms

Phytophthora capsici can strike virtually every part of the host plant causing fruit rot and stunting, leaf defoliation, stem lesions, crown rot and root rot, and it is often observed to result in seedling-blight or damping-off symptoms during emergence (Kingkun et al, 1989). However, while any growth stage of host plants can be infected by this pathogen, studies investigating pepper and tomato (*Solanum lycopersicum*) have revealed that juvenile plants are more easily infected by *P. capsici* than mature plants (Kim, Hwang et al., 1989; Roberts and McGovern, 2000). Typically, the infected stems exhibit soft, water-soaked and brownish lesions close to the soil line (Figure 1.1) and a brownish discoloration in the center of the vascular tissue can be observed (Figure 1.2). Symptoms caused by *P.capsici* are easily confused with those caused by *Verticillium* wilt and white mold (*Sclerotinia*), Tian and Babadoost (2004) suggested that a polymerase chain reaction (PCR) assay should be used to detect *Phytophthora* species in plants rather than rely on visual inspection of symptoms on infected plant tissues.

Figure 1-1 The stem lesion of bell pepper (*Capsicum annuum*) close to the soil line.

Figure 1-2 The brownish discoloration of vascular tissue of bell pepper (*Capsicum annuum*)

1.1.3 Disease Cycle: Dissemination of Zoospores via Water and Long-term Survival of Oospores in Soil

Like other species in the genus Phytophthora, *P. capsici* can be disseminated by both asexual (sporangia and zoospores) and sexual means (oospores), which can result in rapid polycyclic disease development**.** In general, sporangia and zoospores are regarded as ephemeral structures, whereas oospores serve as survival structures.

Swift dissemination of this pathogen generally results from rapid production of sporangia and zoospores under ideal environmental conditions. Temperatures between 25 and 28 C are optimal to produce copious sporangia (Alconero and Santiago, 1972). Lemon-shaped sporangia can release 20 to 40 bi-motile swimming zoospores when immersed in free water (Hausbeck and Lamour, 2004)). Zoospores exhibit negative geotropism and use chemotaxis to locate and contact plant surfaces (Erwin and Ribeiro, 1996). Once they reach the plant, zoospores penetrate the intact cuticle or directly infect the plants through stomata (Katsura and Miyazaki, 1960).

Rainfall and flowing water are often cited as the most critical environmental factor leading to the incidence and progress of Phythopthora blight (Bowers et al., 1990). Disease symptoms are commonly observed to follow water runoff, with symptoms occurring down rows in a field (Café-Filho et al., 1995). In addition, the research conducted by Ristaino and Johnston (1999) served to illustrate that soil moisture,

especially cyclical changes in soil water potential, can also stimulate oospore germination in soil.

Unlike sporangia and zoospores, which generally cannot survive in harsh soil conditions, oospores, produced by sexual recombination, appear to play a critical role in this pathogen's adaptation to the environment. A study conducted by Babadoost and Pavon in 2013 demonstrated that oospores of *P. capsici* can be recovered from Illinois soil samples and remain virulent for more than 36 months. However, after 48 months in a field environment, oospores were no longer viable in this study (Babadoost and Pavon, 2013).

1.1.4 Management Practices

Many fungicides are available for control of Phytophthora blight. However, relying on fungicides alone to control Phytophthora blight is not advised, because *P.capsici* is able to develop fungicide resistance. Methyl bromide was effective against many *P. capsici* isolates; however, this soil fumigant is now banned for use in most vegetable crops (Lamour and Hausbeck, 2001; Parra and Ristaino, 2001). Most growers now rely on the phenylamide class of fungicides including metalaxyl and mefenoxam to combat this pathogen. However, as early as 2004, research conducted by Hausbeck and Lamour, indicated that there was development and increasing incidence of *P. capsici* isolates that were insensitive to mefenoxam in some Michigan fields. They indicated that sexual recombination likely led to the development of mefonoxam insensitive isolates (Hausbeck and Lamour, 2004).

1.2 Soil Health and Disease Suppressive Soil

Soil health, also commonly referred to as soil quality by scientists, is defined as the capacity of a soil to serve as a living ecosystem (Doran and Zeiss, 2000). Only "living" things can be described as healthy or not, so this definition of soil health, stresses the importance of managing our soils correctly, because this ecosystem is dynamic and it is affected by a diversity of living organisms and the environment (Doran & Parkin,

1994). Soil health is very closely related to plant and animal health, environmental quality and biological productivity (Doran and Zeiss, 2000).

1.2.1 Indicators of Soil Health

Soil plays a vital role in sustaining our agricultural production systems by providing nutrients for crop growth, reducing rainwater runoff, detoxifying potential pollutants, and providing a habitat for billions of bacteria, fungi, and other microbes. However, intensive vegetable production can degrade soil health over time. For example, plastic tunnels, which now account for more than 2 million ha of production worldwide, can help farmers improve economic profits (Scarascia- Mugnozza et al., 2012), though long-term continuous cultivation under plastic cover can lead to high electrical conductivity and total soluble salts, and low organic carbon contents (Rudisill et al., 2015). Consequently, management practices that rebuild and maintain soil health are needed to sustain ecosystem services provided by soils.

Because soil is so variable and dynamic, developing an assessment that can be used to quantify soil health is the first step to managing this elegant symbiotic system. Rather than depending on any single parameter, or indicator, a combination of physical, chemical and biological parameters is needed to measure soil health (Doran & Parkin, 1994). For example, Appendix Table 1 shows how soil quality functions can be affected by multiple indicators, as well as how each indicator can be correlated with more than one function.

1.2.2 Relationships between Soil Health and Soil-Borne Pathogens

Diseases caused by soil-borne pathogens are one of the most important limiting factors for plant growth, because they can dramatically reduce crop yield and quality. Outbreak of a disease caused by a soil-borne pathogen requires the joint action of a susceptible plant host, presence of a virulent pathogen, and a favorable environment (Perkins et al., 2011). This relationship is often depicted by the disease triangle (Figure 1.3). While pathologists often think of factors such as adequate temperate and moisture when referring to a favorable environment, soil health can also play a role. In fact, almost all soils have some natural potential to suppress soil-borne pathogens (Mazzola et al., 2001).

However, the degree to which a particular soil can suppress disease is variable and can be affected by management practices (Janvier et al., 2007). Some soils have been found to be highly suppressive to disease and commonly referred to as “disease suppressive soils”. A disease suppressive soil is defined as a soil in which a virulent pathogen fails to persist or cause infection, even with the presence of a susceptible plant host and favorable environment (Darin, 2000; Lazarovits, 2001). Disease suppressive soils are classified based on their potential to contribute to either specific or general disease suppression. Unlike specific suppression, which is attributed to a specific pathogen or parasite and a specific microbial antagonist, and often develops over time in monoculture system, general suppression is attributed to multiple factors and can lead to suppression of multiple pathogens. The level of general disease suppression in soil systems is often highly correlated with biotic factors, especially soil biological activity, but can also be attributed to abiotic factors as well.

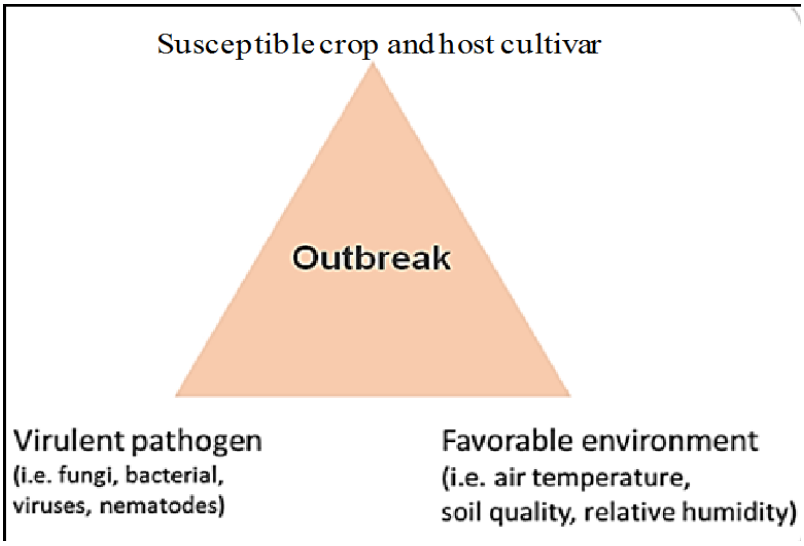


Figure 1-3 Plant disease triangle

1.2.3 Abiotic Factors Associated with Disease Suppressive Soils

Several abiotic factors of soil including pH, available nutrients, organic matter content, clay type, and texture are considered to be able to affect disease incidence and severity. For example, the optimum pH for *Fusarium oxysporum* f. sp. *niveum* activity is between 4.6 and 6.0, and increasing soil pH from 4.0 to 7.5 will result in an obvious

decline of Fusarium wilt incidence (Jones et al., 1975). Mineral nutrition, especially nitrogen, is known to affect the incidence of many plant diseases (Huber and Watson 1974). High applications of N-P-K (10:10:10) fertilizer increased the rate of Fusarium wilt incidence (Wensley and Mckeen, 1965). Heyman and his colleagues found that greater concentration of water-soluble Ca in soil was directly related to lower Aphanomyces root rot disease severity of pea (Heyman et al, 2007). However, Janvier et al., (2007) noted that the correlation between soil physicochemical parameters and disease suppression is not consistent. Similarly, in a comprehensive review by Bonanomi et al. (2010), which summarized over 252 papers investigating the effects of soil amendments, the authors concluded that soil chemical properties were not predictive of pathogen severity or disease suppressive activity. These studies indicate that other factors could play a more significant role in suppressive activity.

1.2.4 Biotic Factors Associated with Disease Suppressive Soils

Studies demonstrating that soil sterilization results in greater disease incidence and severity supports the hypothesis that biological factors play an important role in disease suppression (Borrero et al., 2004). In the comprehensive review by Bonanomi et al., (2010) enzymatic and microbiological parameters such as microbial activity, microbial biomass, and total populations of certain microbial taxa were found to be far more informative than chemical factors in predicting disease suppressive activity (Bonanomi et al., 2010).

Theoretically, increased microbial biomass may lead to greater nutrient competition with pathogens resulting in a direct effect on soil suppressiveness. However, Grünwald et al. (2000) found that the relationship between microbial biomass and disease suppression is not always stable or consistent, indicating that microbial diversity may be a more important factor than biomass. For example, research by Van Elsas et al. in 2002 indicated that severity of the soil-borne potato pathogen *Rhizoctonia solani* AG3, was negatively associated with soil microbial diversity. Other researchers failed to identify correlations between microbial diversity and pathogen suppression, and suggested that instead, microbial community structure could be more effective to predict disease suppressive activity (Jin et al., 2010; Tiquia et al., 2002). Abundance of specific

microbial populations has also been correlated with soil suppressiveness. For example, Bonanomi et al. (2010) pointed out total culturable bacteria, *Fluorescent pseudomonads*, and *Trichoderma* spp. are the most effective features to indicate the extent of soil suppressiveness.

Bonanomi et al. (2010) also pointed out that total microbial activity, as indicated by the fluorescein diacetate (FDA) hydrolysis assay, is often highly correlated with disease suppressive activity. However, this assay has displayed great variability when scientists try to analyze the capacity for a soil to suppress some diseases (Weller et al., 2002). Several microbial activities have been suggested as factors mediating pathogen suppressive activity in soil including competition for nutrients or niches, production of antibiotic compounds, enhancement of plant defenses, production of hydrolytic activities, and predation and parasitism (Weller, 2007). This indicates that other more specific assays of microbial activity could be more predictive for assessing disease suppressive activity. However, different factors may be responsible for suppressive activity when different amendments or pathosystems are under study, and thus researchers should continue to measure a suite of factors in an effort to determine how a particular practice might be inducing disease suppressive activity.

1.3 Potential Effects of Organic Matter Amendments to Increase Disease Suppressive Activity

There are many crop management practices that can improve soil quality, including planting cover crops, practicing crop rotation, reducing tillage, and amending soil with organic inputs (Bailey et al., 2003). Identifying cultural practices that improve soil and plant health, but are of low cost and environment-friendly, are a high priority for contemporary agroecosystems (Martin, 2003). The direct addition of organic matter by organic amendments is experiencing a resurgence in agricultural systems because of its potential to help reduce the need for chemical fertilizers and pesticides (Bailey et al., 2003). External organic matter inputs can improve soil health by improving physicochemical properties such as soil structure, water-holding capacity, and nutrient

availability, as well as biological components - especially the quantity, diversity and functions of soil microbiota (Doran and Zeiss, 2000).

There are many organic waste products that could be used as soil amendments to improve soil health and potentially suppress soil-borne pathogens. In particular, amendments that are harder to decompose, because of a high carbon (C) to nitrogen (N) ratio, are theorized to contribute to greater soil suppressiveness than easily degradable C sources (Senechkin et al., 2014). However, growers will need to be careful when applying soil amendments with high C/N ratios. The C/N ratio of organic matter is a critical parameter influencing mineralization and rate of nutrient release (Parton et al., 2007). In general, when the C/N ratio exceeds the threshold value of 30-35, the amendments can indirectly impair crop growth because the decomposing microbes will integrate N from the surrounding soil into their biomass preventing plants from obtaining adequate N for crop growth (Michelsen et al., 1995).

1.3.1 Compost

Compost can be defined as a mixture of various decaying organic substances such as dead leaves and animal manure, and it is often used for fertilizing soil. Compost has a long history of use as a reliable and effective way to rebuild depleted soil organic carbon (Smith et al., 1997). More importantly, Edwards et al. (2000) reported that amending soil with compost can enhance utilization of complex substrates and lead to greater populations of beneficial soil microbes. Many studies have demonstrated that compost amendments can increase disease suppressive activity in soil, however, results are variable, especially in field trials, given the type of compost applied and the pathosystem under study (Bonanomi et al., 2010). Compost is highly variable given feedstocks and processing conditions, which can dramatically affect its availability for microbial decomposition. In one study, Iovieno et al. (2009) reported that the soil organic carbon recovery by compost was limited, possibly due to a high C/N ratio or presence of other high chemical quality substrates. The presence of certain types of recalcitrant organic materials could favor microbial taxa that are more competitive than soil-borne pathogens, thereby reducing their potential to colonize plant roots (Bonanomi et al., 2013).

1.3.2 Biochar

Biochar is a carbon-rich, recalcitrant and heterogeneous material derived from the pyrolysis process (Bonanomi et al., 2015). Pyrolysis refers to a process of burning organic biomass under low oxygen conditions, with little CO_2 produced (Singh et al., 2010). Biochar has recently received a lot of attention for its potential to improve agricultural productivity and reduce negative environmental impacts such as N leaching (Lehman and Joseph, 2009). The beneficial effects of biochar are thought to result from its high chemical and microbial stability (due to the aromatic structure), which creates a porous structure and large surface area to sorb nutrients (Atkinson et al., 2010). As a soil amendment, biochar is noted for its potential to help sequester atmospheric carbon (Lehmann 2009), provide habitat for beneficial microorganisms (Quilliam et al., 2013), improve soil tilth, enhance nutrient availability (such as N and P), and increase crop growth (Lehmann et al., 2003).

Several recent studies have provided evidence that amending soil with biochar can suppress many soil-borne pathogens, including *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia solani*, as well as foliar pathogens such as *Botrytis cinerea* (gray mold) and *Leveilla taurica* (powdery mildew (Bonanomi et al., 2015). Bonanomi et al. (2015) summarized the potential mechanisms behind the potential disease suppressive effects of biochar: (i) induce systemic resistance of host plant; (ii) aggregate more beneficial microorganisms; (iii) improve soil quality characteristics such as pH and nutrient availability; (iv) secret chemicals that are toxic to fungi; and (v) disrupt chemical signaling between plants and pathogens. Rates used in studies demonstrating disease suppression in response to biochar amendments vary from 1 to 5% (w/w) soil. Achieving such high rates in field trials could be problematic because of the high cost and limited commercial availability of biochar amendments. In addition, because of biochar's physiochemical characteristics, applying this amendment to soil could result in some negative side-effects such as absorbing agrochemicals and reducing the efficacy of herbicides and fungicides.

1.3.3 Lignin

Kraft pine lignin, which is a byproduct of the paper industry, is another organic amendment with potential for improving soil health and suppressing plant disease. In a recent study, Van Beneden et al. (2010), demonstrated that amending soil with kraft pine lignin could suppress *Rhizoctonia solani*. However, the results depended on soil type and appeared to be related to specific changes in resident microbial community structure. Specifically, in the soil that became more suppressive with the amendment, *Trichoderma* spp. and gram negative bacteria were increased.

1.3.4 Sawdust

Sawdust is readily available as a waste product of the forest industry. This material has not been widely tested for its potential to affect soil-borne pathogens, but in one early study, it was found to be a promising amendment for nematode suppression (Muller and Gooch, 1982).

1.4 Summary

Intensive vegetable production can degrade soil quality and potentially cause crop plants to become more susceptible to soil-borne pathogens. Soil organic matter is the most important factor for improving and maintaining soil quality. Amending soil with complex organic substrates has previously been demonstrated to reduce disease severity, but the mechanisms are not well understood and further research is needed before these types of amendments can be used to reliably suppress soil-borne pathogens in vegetable systems. Indiana has a significant forest industry with many residual products that could be used to rebuild soil organic matter and help suppress soil-borne pathogens. Many of these residual products previously ended up as land-fill waste, so using them as soil amendments could have multiple benefits for system-wide sustainability.

CHAPTER 2. IMPACT OF FOREST INDUSTRY RESIDUALS ON SOIL MOISTURE, SOIL MICROBES AND PEPPER GROWTH IN FIELD SOIL INFESTED WITH *PHYTOPHTHORA CAPSICI*

2.1 Abstract

Intensive vegetable production can degrade soil quality and make crops more susceptible to soil-borne pathogens. Indiana has a significant forest industry with residual products that could be used to rebuild soil quality and help suppress soil-borne pathogens. Amending soil with complex organic substrates has previously been demonstrated to reduce disease severity in some trials, though the mechanisms are not well understood. We collected soil from a farm with a recent outbreak of Phytophthora blight and amended it with one of four forest industry residues alongside a control (no amendment) treatment. Results indicate that these amendments differentially impacted soil water holding capacity, enzyme activity, and *Phytophthora*, *Pseudomonas fluorescens*, and *Trichoderma spp*. during a one month incubation period. Soil amended with sawdust had significantly less *P. capsici* root infection and significantly greater root biomass than the control, biochar and compost treatments. Unlike previous studies, biochar amendment did not reduce *P. capsici* infection nor stimulate pepper growth. Future experiments are needed learn more about how these amendments alter physical, chemical and biological properties, which are likely to play a role in pathogen suppression. Results of these studies will have important implications for helping vegetable growers build soil organic matter, and manage soil-borne pathogens.

2.2 Introduction

The soil-borne oomycete pathogen *Phytophthora capsici*, is a major factor limiting crop production during warm and wet seasons. This pathogen is difficult to control with traditional strategies such as crop rotation, resistant cultivars, and chemical fungicides. Crop rotation is ineffective as a control because *P. capsici* has a wide host

range and it produces long-lived spores (oospores) that can survive in soil for years. Using greenhouse studies, Granke and Ocampo (2012) reported that 27 families and 71 species have been confirmed as 'victims' of *P. capsici*. The long list of susceptible host plants includes many economically important crops such as pumpkin, watermelon, zucchini, bell pepper, hot pepper, eggplant, tomato, lima bean and snap beans (Hausbeck and Lamour, 2004). *Phytophthora capsici* is a heterothallic organism which can reproduce via both asexual (sporangia and zoospores) and sexual stages (oospores), and the oospores can survive outside of host tissues for a long time. Babadoost and Pavon (2013) reported that oospores remained virulent in an Illinois field environment for more than three years. Protecting crops from *P. capsici* using resistant cultivars and chemical fungicides is difficult due to the diversity of physiological races of *P. capsici*. The genetic variation in *P. capsici* is rendered by sexual recombination, mutations and outcrossings with other *Phytophthora* species (Babadoost, et al., 2008). Mefenoxam is now the most commonly used fungicide for managing *P. capsici*, however, many isolates have been found to be either insensitive or resistant to this fungicide (Lamour and Hausbeck, 2001; Parra and Ristaino, 2001).

Another obstacle that makes management of *P. capsici* difficult, is the pathogen's polycyclic disease development and rapid dissemination in fields with water. Under optimal environmental conditions of 25 to 28 °C, *P. capsici* can produce large amounts of sporangia (Weber, 1932). Each mature sporangia can release 20 to 40 motile zoospores (Hickman, 1970), which can spread quickly via irrigation water or rain drops that splash water and zoospores onto plants (Schlub, 1983).

An outbreak of plant disease usually can be explained by the "disease triangle", which highlights the interaction of a favorable environment, a virulent pathogen, and a susceptible plant host (Francl, 2001). Since *P. capsici* is difficult to control with traditional disease control approaches and there are few resistant crop varieties available, altering the environment could be an alternative strategy to help manage this pathogen. In this context, organic soil amendments have been proposed as a potentially promising alternative. Adding organic amendments to soil is a comprehensive strategy to rebuild soil quality, provide disease suppression and enhance plant health (Bonilla, et al., 2012). Organic amendments improve soil physical, chemical and biological properties, and in

some cases, induce a more ‘disease suppressive state’. When soil is disease suppressive, a pathogen fails to cause infection despite the presence of a pathogen and a susceptible plant host. Compost is one of the most well studied organic amendments for improving soil quality and suppressing soil-borne pathogens. In a comprehensive review by Bonanomi et al. (2007), the authors noted that many soil-borne plant pathogens including *Fusarium* spp., *Phythophthora* spp., *Sclerotinia* spp., *Verticillium dahlia* and *Rhizoctonia solani* have been suppressed by compost amendments. However, not all composts are disease suppressive and the degree of suppression is often dependent on the composition of the amendment as well as the pathosystem under study (Bonanomi et al., 2010).

Organic amendments derived from woody materials have been suggested as a promising approach to induce disease suppressive soil (Castano et al., 2011; Bonanomi et al., 2013). Indiana has a robust forest industry with many residual products that could be used as amendments to help suppress soil-borne pathogens such as *P. capsici*. Many of these byproducts currently end up as wastes in land-fills, so utilizing these products as soil amendments could increase sustainability in several ways. In addition to compost derived from woody materials, other organic amendments with potential to help suppress soil-borne pathogens include kraft pine lignin, sawdust and biochar. Kraft pine lignin, which is a byproduct of the paper industry, has been reported to induce suppressiveness against *Rhizoctonia solani*, an aggressive soil-borne pathogen with a wide host range that can also survive in the soil for a long time (Van Beneden et al., 2010). In early 1982, sawdust was found to be a promising amendment for nematode suppression (Muller and Gooch, 1982). Many recent studies have reported that biochar can effectively suppress several soil-borne plant pathogens including *Fusarium spp., Rhizoctonia solani, and Phytophthora* spp. (Graber et al., 2015), including *P. capsici* (Shoaf et al., 2016). Biochar is defined as the solid co-product of biomass pyrolysis in the absence of oxygen (Lehmann et al., 2006). As a soil amendment, biochar is often cited for its potential to sequester soil carbon, improve soil tilth, enhance nutrient availability and increase crop productivity (Graber et al., 2010). However, biochar amendment could also result in negative side-effects. For example, biochar amendments could adsorb agrochemicals and reduce their effectiveness (Bonanomi et al., 2015), and introduce environmental contaminants (Montanarella and Lugato, 2013). Biochar amendments are also expensive

and commercial sources are rare (Shoaf et al., 2016). Finally, biochar is difficult to degrade and could persist in the soil indefinitely (Bonanomi et al., 2015), so additional research is needed before this amendment should be tested in field trials.

The objectives of this study were to: 1) test the effectiveness of four forest industry byproducts for their suppressive activity against *P. capsici,* and 2) investigate potential biotic and abiotic factors that might contribute to suppressive activity induced by these amendments.

2.3 Materials and Methods

2.3.1 Soil collection

Soil was collected from a field near Vincennes, IN (lat. 38.47° and long. -87.63°), which contained a poorly drained Ayrshire (fine-loamy, mixed, active, mesic Aeric Endoaqualfs) soil. This field has a history of Phythophthora blight caused by *P. capsici,* and had been planted with watermelon just prior to when the soil was collected. Soil was collected from the top 0 to 20 cm, under diseased plant material. The soil was transported in BPA-free food pails, and stored in the cooler at 4 C in the Purdue University Horticulture Greenhouse, West Lafayette, IN, until the greenhouse experiment was initiated.

2.3.2 Amendments and Treatments

Field moist soil was sieved to 4 mm and allocated into five 40.1*26.1*17.7 cm containers (Rubbermaid Inc., Huntersville, NC), with 5 holes drilled evenly on the bottom of each container to facilitate drainage. Each of the four forest industry byproducts (Appendix Table 2) were ground to a fine powder and the carbon and nitrogen percentage of the byproducts was determined using a FlashEA® 1112 Nitrogen and Carbon Analyzer (CE Elantec, Lakewood, NJ). The soils were amended with the forest industry byproducts and mixed thoroughly within each container to provide the following treatments: 1) unamended control (soil only), 2) biochar, 3) wood fines

compost, 4) kraft pine lignin, and 5) sawdust. All amendments were applied at a rate of 1% total carbon (w/w) soil.

2.3.3 Incubation assay

All treatments were saturated with water and incubated for one month to stimulate decomposition of the forest industry byproducts and alter soil chemical and biological properties in response to the amendments.

Soil volumetric water content (VWC) was monitored regularly using a FieldScout TDR100 soil moisture meter (Spectrum Technologies Inc., Aurora, IL) by taking 10 random readings in each treatment directly from the 40.1*26.1*17.7 cm containers at 0,1,3,7,14,21,28 days after amendment incorporation. One L of water was added immediately after the amendments were incorporated, and 2 L were added after 14 days to maintain soil moisture.

Soil samples were collected from each treatment container at 0,1,3,7,14,21,28 days after amendment incorporation, placed into sterile 50 ml centrifuge tubes, and stored at 4 C until being subject to the following microbial assays. Total populations of *Phytophthora* spp., *Pseudomonas fluorescence*, *Trichoderma* spp., and *Actinomyces* spp. were quantified using serial dilution on selective media. The selective media included PSSM-H (Mazzola et al., 2001) amended with hymexazol for *Phythopthora* spp., modified King's B media (Schaad, 1980) for *P. fluorescence*, Trichoderma semi-selective media (Williams et al., 2003) for *Trichoderma* spp., and starch casein media (Mackay, 1977) for *Actinomycetes* spp. Serial dilutions were conducted as follows. Five grams of moist soil from each treatment was combined with 25 ml of sterile water, with two replicate samples per treatment, and three plates of each media per replicate using the following dilutions. The optimal dilution levels varied among time points, as did the ideal time to quantify colony forming units (CFU) on plates with the different media. PSSM-H media was plated with 10^{-2} dilutions (first reading 5 days after plating), modified King's B media was plated with 10^{-4} to 10^{-6} dilutions (first reading 2 days after plating), Trichoderma semi-selective media was plated with 10^{-2} dilutions (first reading 5 days after plating) and starch casein media was plated with 10^{-2} to 10^{-6} dilutions (first reading

4 days after plating). All plates were incubated at room temperature after plating, with the exception of the starch casein which was incubated at 30 C. Because moist soil was used for the dilutions, the dry weight of soil from each treatment was quantified by putting 25 g soil, with 3 replicates per treatment, into a 70 C oven for 48h. All of the microbial data collected from the plates was adjusted to account for the dry weight of each soil sample. Total microbial activity in each sample was quantified using the fluorescein diacetate hydrolysis (FDA) enzyme assay using methods described in Green et al. (2006).

2.3.4 Greenhouse trials

The susceptible pepper variety ‘Red Knight’ (Johnny’s Selected Seeds, Winslow, Maine) seeds were prepared as follows. Seeds were placed in a beaker for surface sterilization with 8.25% sodium hypochlorite solution containing Tween-20 (two drops per 1 ml), stirred for 20 minutes on a magnetic stir plate, and triple-rinsed in deionized water. Sterilized seeds were placed on 4 layers of autoclaved cheesecloth within a laminar flow hood and allowed to air dry for 30 minutes. Seeds were then stored at 4 C until planting. Surface sterilized seeds were planted into thin plastic trays with 48 cells containing soilless potting mixes (Growing Mix; Fafard, Agawam, MA) which is typically used for germination. This media contains 59-73% Canadian sphagnum peat moss, perlite, vermiculite, dolomite lime, and a wetting agent. After sowing, trays were placed in the mist room at the Purdue University Horticulture Greenhouse, West Lafayette, IN to facilitate germination.

Following the one month incubation, soil from each treatment was distributed into 10.16 cm diameter pots, with 7 replicates per treatment. Twenty-eight-day-old pepper seedlings were transplanted into each pot and pots were arranged in a randomized complete block design on the greenhouse bench. Conditions in the greenhouse were maintained at an average temperature of 20.7 C and relative humidity of 53.09%. All pots received fertilizer water daily to try and prevent potential N immobilization resulting from the high C/N ratio soil amendments. After 37 days, pepper plants were harvested to quantify total biomass and perform root infection assays.

2.3.5 Laboratory Assays

Five grams of roots were randomly collected from each pepper plant along with the adhering rhizosphere soil, and placed in a sterile 50 ml centrifuge tube containing 25 ml sterile DI water. After sonicating the centrifuge tubes for 60 s, roots were removed and washed with DI water. Ten root cuttings (5 mm) were randomly selected and placed on a plate containing PSSM-H (Papavizas et al., 1981) with 3 replicates per plate. Root infection by *P. capsici* was quantified on the plates 3 days later. The 50 ml tubes containing rhizosphere soil were centrifuged at 4300 rpm for 5 min. The water was discarded and the soil was lyophilized, and saved at -20 C for potential future microbial community analysis. Soil was also collected from each pot at harvest and sent to Midwest Laboratories (Omaha, NE) for standard soil chemical analyses. Remaining pepper above and below ground biomass was dried at 50 C for 48 h.

2.3.6 Statistical analysis

Data were checked for model assumptions, and square root or log transformed when normality or equality of variance were not met. Data were back transformed to report means in tables and figures. After validating data standard analysis of variance (ANOVA) was carried out using SAS (SAS VERSION 9.4; SAS Institute Inc., Cary, NC) using PROC GLM, and means separated using Tukey's honestly significant test ($P< 0.05$)

2.4 Results

2.4.1 Soil moisture and microbial dynamics during the incubation

Difference in soil moisture among treatments during the incubation period could not be statistically analyzed because of the experimental design, though the treatments appeared to affect soil moisture (Fig. 2.1). Interestingly, the biochar treatment often had the lowest level of soil moisture relative to the other treatments. In contrast, the compost and sawdust treatments had higher and more stable soil moisture relative to the other treatments.

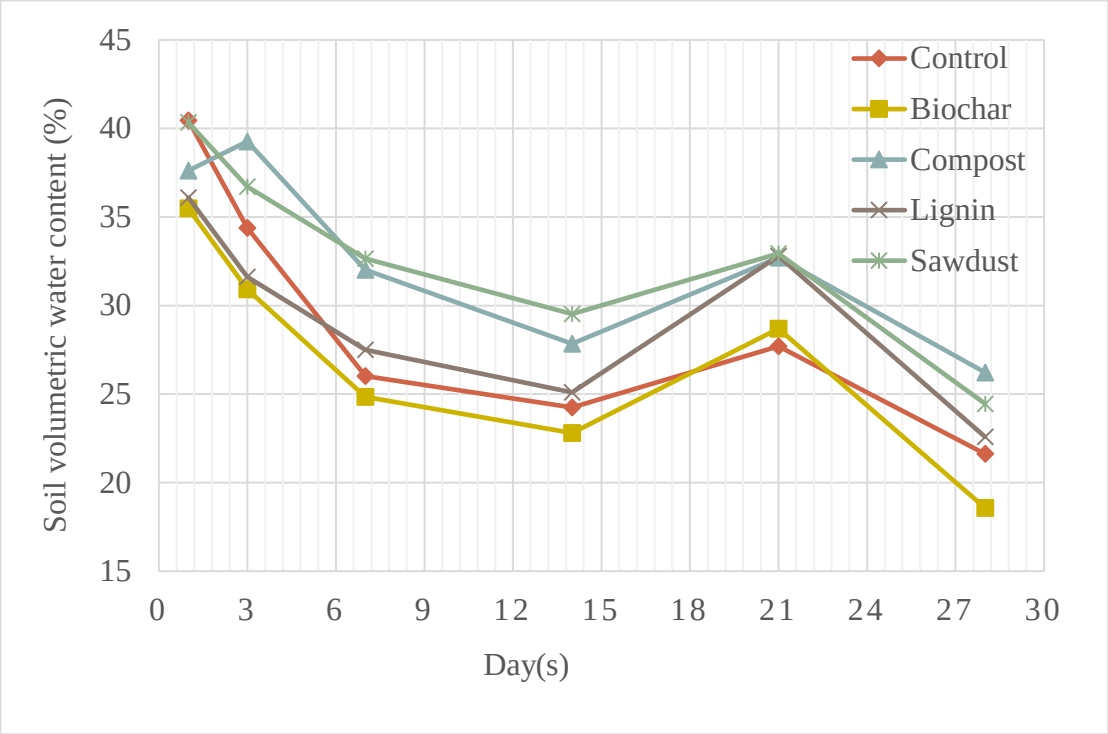


Figure 2-1 Soil moisture (%) dynamics following five forest industry amendment

2.4.2 Soil microbial abundance and microbial activity during the incubation

Microbial abundance and microbial activity also could not be statistically analyzed because of the statistical design, though some trends were evident during the incubation. For example, *P. capsici* abundance spiked in response to the lignin and biochar treatments, while declining steadily over time in the sawdust treatment (Fig. 2.2). *Pseudomonas flourescences* dropped and remained low in all treatments during the incubation period (Fig. 2.3). *Trichoderma* spp. spiked in all treatments during the first few days of the incubation period, with the exception of biochar, which remained low throughout the incubation period (Fig. 2.4). Finally, the *Actinomycetes* spp. spiked in response to soil water in the biochar and control treatments (Fig. 2.5).

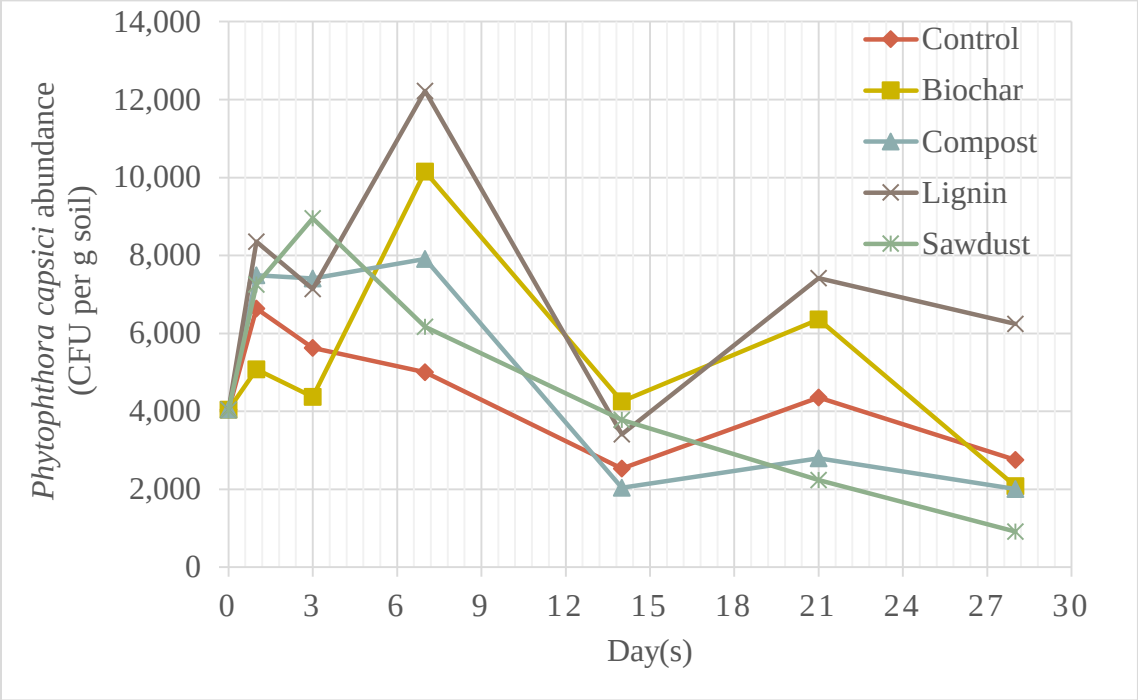


Figure 2-2 Dynamics of *Phytophthora capsici* soil abundance during incubation period following five forest industry amendment.

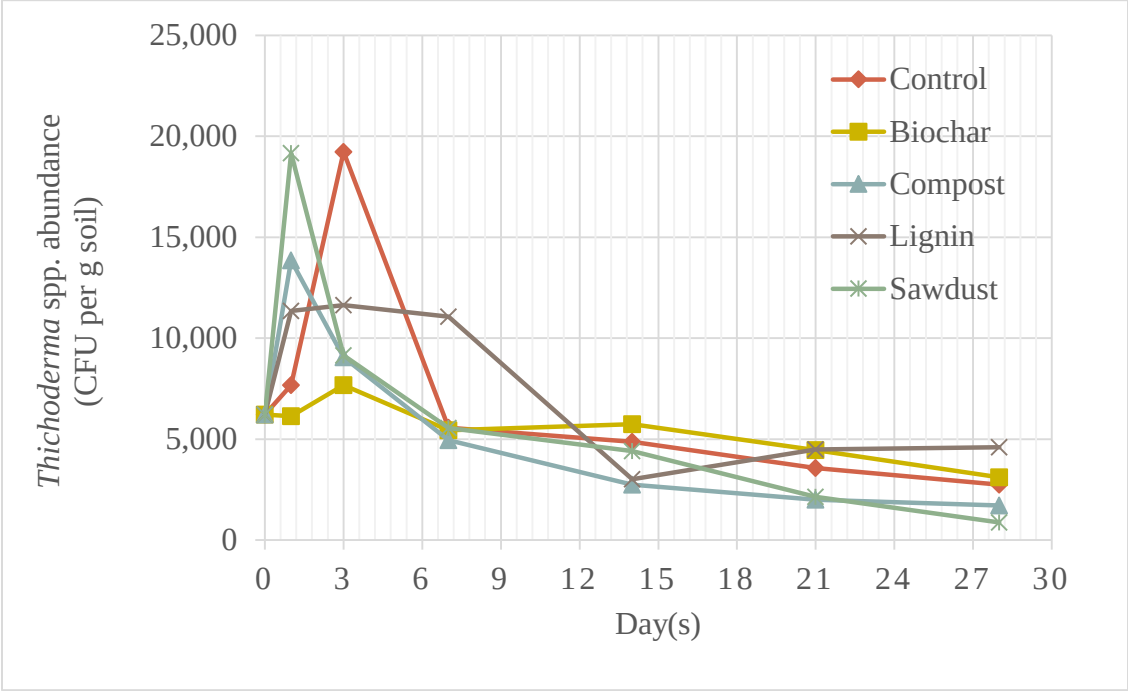


Figure 2-3 Dynamics of Trichoderma spp. soil abundance during incubation period following five forest industry amendment.

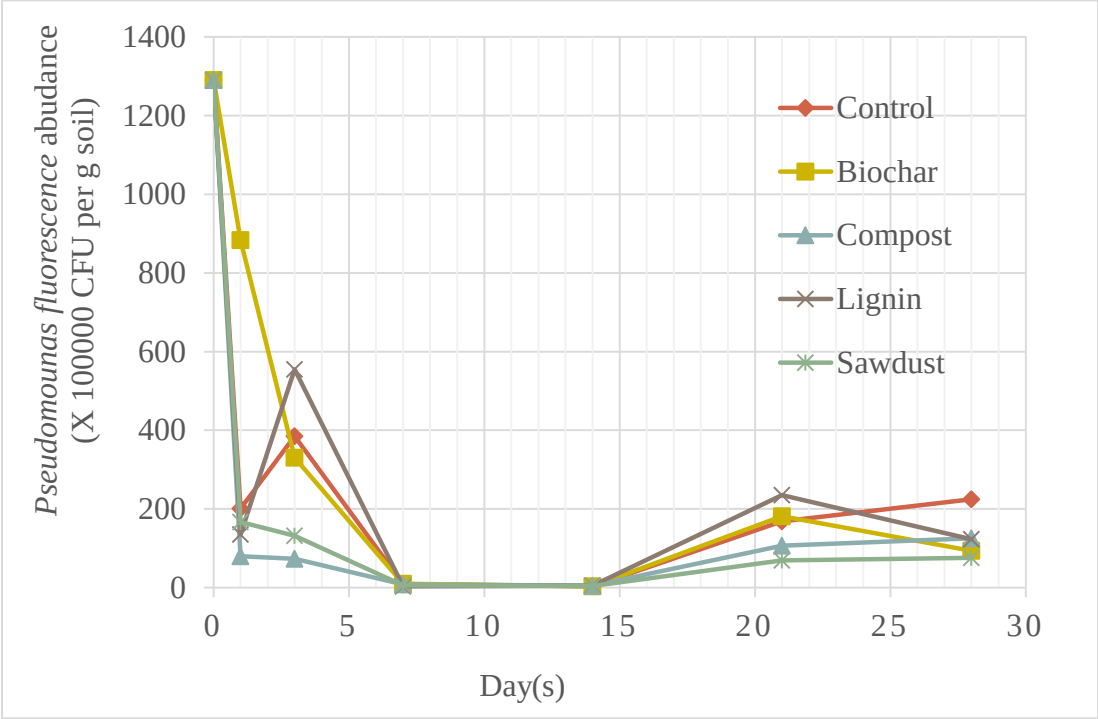


Figure 2-4 Dynamics of *Pseudomounas fluorescence* soil abundance during incubation period following five forest industry amendment

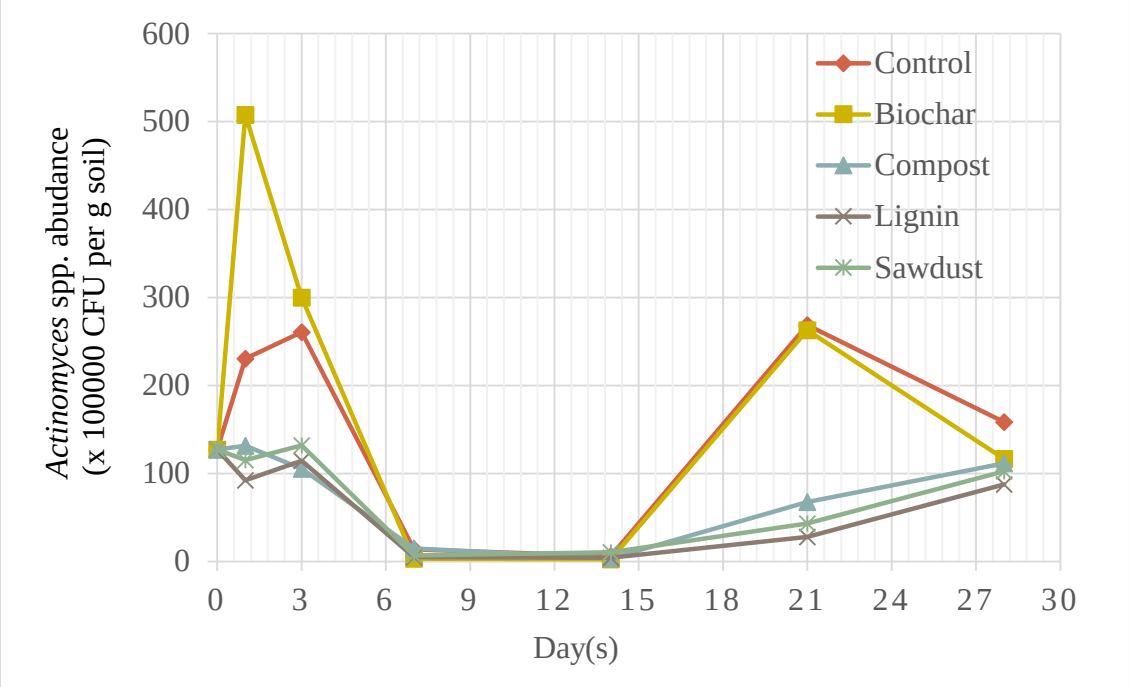


Figure 2-5 Dynamics of *Actinomyces* spp. soil abundance during incubation period following five forest industry amendment.

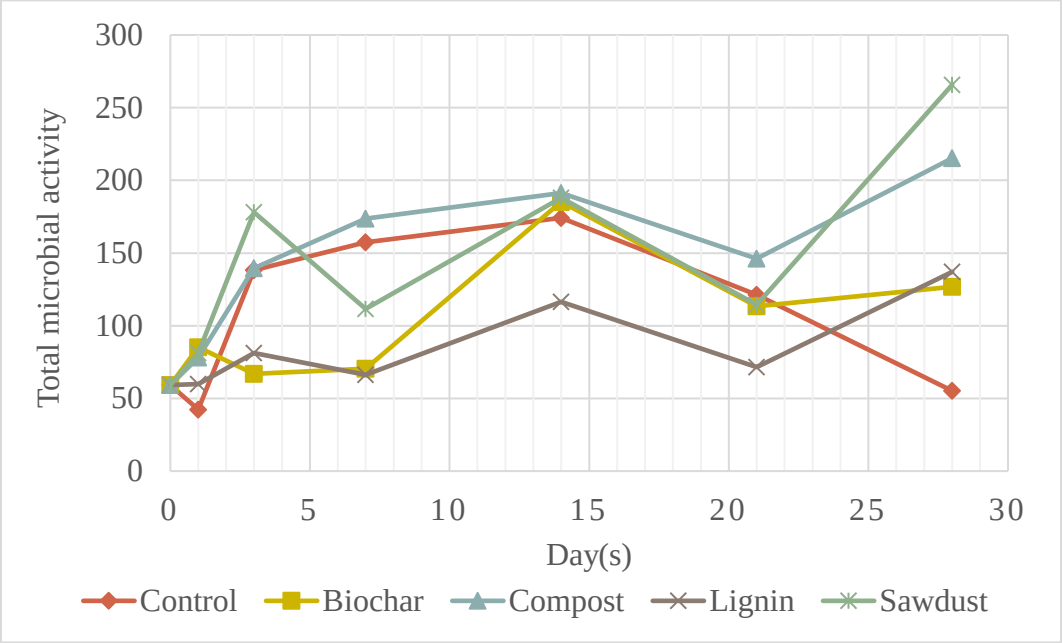


Figure 2-6 Total microbial activity estimated using an FDA enzyme assay

Total microbial activity, estimated using an FDA enzyme assay was greatest in the compost and sawdust treatments, and lowest in the lignin and biochar treatments (Fig.2.6). At the end of the incubation period, microbial activity was lowest in the control treatment.

2.4.3 Percent root infection by *P. capsici*

After growing for one month in treated soils, *P. capsici* root infection was significantly lower in the sawdust treatment relative to the control (Fig. 2.7). However, none of the other treatments differed from the control.

2.4.4 Pepper above and below ground biomass

Aboveground pepper biomass was greatest in the sawdust treatment, but was not significantly different than the control or other treatments (data not shown). Belowground pepper biomass was significantly greater in sawdust treatment relative to the control, biochar and lignin treatments (Fig. 2.8).

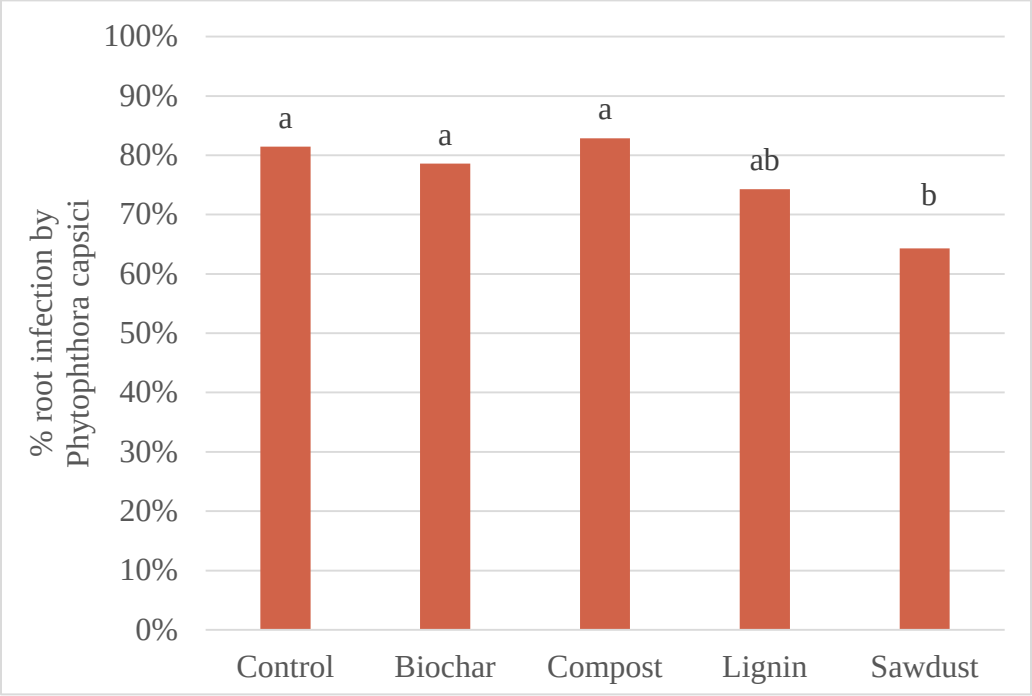


Figure 2-7 Root infection rate of pepper at harvest following five forest industry amendment. Different letters represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

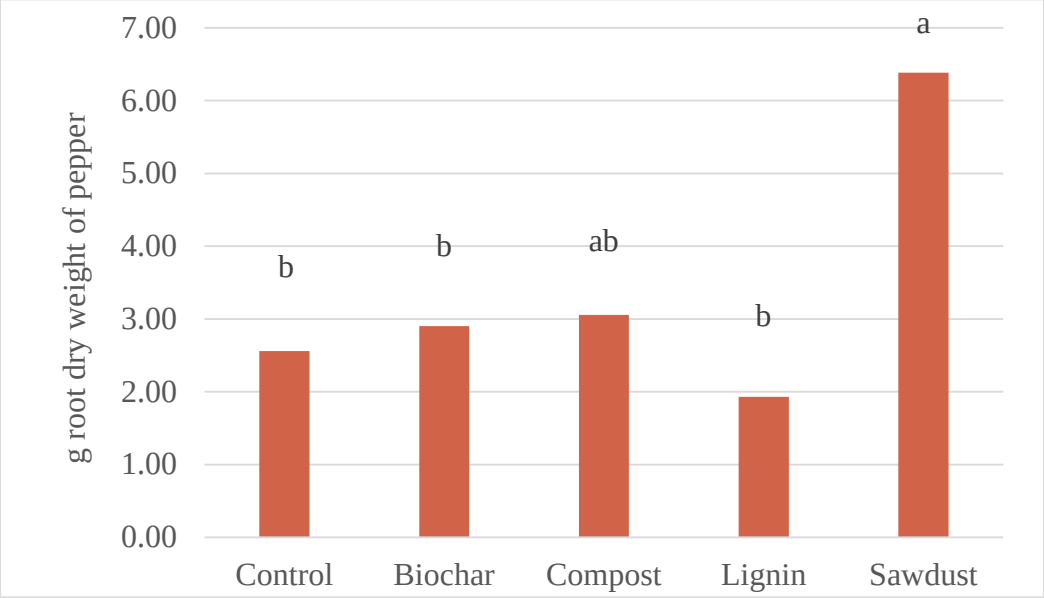


Figure 2-8 Root dry weight of pepper at harvest following five forest industry amendment. Different letters represent significant difference as determined by Tukey's honestly significant difference test ($P< 0.05$).

2.4.5 Soil chemical analyses

Several soil chemical properties were influenced by the amendment treatments (Appendix Table 3). Soil pH was lower in the lignin treatment relative to the control, compost and sawdust treatments. Percent soil organic matter was higher in the lignin relative to the control treatment. Soil Mg was lower in the biochar and sawdust treatments relative to the control and compost treatments, and soil Ca was lower in the biochar, lignin and sawdust treatments.

2.5 Discussion

Results of this study indicate that a locally available sawdust amendment, applied at a rate of 1% total carbon (w/w) soil, can reduce root infection and improve pepper root growth in soils infested with *P. capsici*. This particular amendment is produced as a byproduct of the local forest industry and has often ended up in landfills. Making use of this byproduct as a soil amendment to suppress *P. capsici* disease incidence and severity could provide multiple benefits and increase the sustainability of the Indiana forest and agricultural industry.

Unexpectedly, the biochar amendment tested in this trial did not suppress *P. capsici* root infection, which contradicts results of Shoaf et al. (2016), who observed *P. capsici* suppression with the same biochar amendment in the same soil. Differences in the results between these two trials could be related to the rate at which the biochar was applied. In this trial, the biochar amendment was applied at a rate of 1% total carbon (w/w) soil, whereas in the trial by Shoaf et al. (2016), it was applied at a rate of 3% total biochar (w/w) soil. Disease severity in response to biochar amendments has previously been found to exhibit a U-shaped response curve, with a minimum of disease incidence at some intermediate dose (Graber et al., 2014). Consequently, it is possible that we did not observe *P. capsici* suppression in this study, because the biochar rate was too low. This is unfortunate, because biochar is expensive and it can have negative unintended side effects.

The compost and kraft pine lignin treatments also did not suppress *P. capsici* root infection rate in this trial, which also could be related to the dose. Alternatively, the lack

of response in our study could be due to resident soil microbial community structure. In the study by Van Beneden et al., (2010), the suppressive effect of kraft pine lignin on *R. solani* was dependent on soil type, and was correlated with increased *Trichoderma* and actinomycetes populations in the soil where suppressive activity was observed.

Understanding how soil amendments induce pathogen suppressive activity is important for the development of reliable disease control strategies. Soil moisture is often cited as a factor that promotes survival and infection by oomycete pathogens like *P. capsici*. For example, moisture has been demonstrated to affect almost every stage of the *P. capsici* life cycle, including the development of mycelia, release of zoospores and formation of oospores (Sanogo and Ji, 2013). Consequently, moisture management is expected to be critical for the control of Phytophthora epidemics. Interestingly in our study, soil moisture appeared to be higher with the sawdust treatment, yet *P. capsici* root infection was significantly lower and root growth was greater in this treatment. This indicates that greater soil moisture is not completely consistent with the threat of *P. capsici* abundance and activity in our soil. Other researchers have found that greater soil water content can lead to lower incidence of disease by *P. capsici* (Liu et al., 2008a; Liu et al., 2008b), supporting this hypothesis.

Another interesting finding in this study, was that while biochar amendments are often observed to increase soil water holding capacity (Downie et al., 2009), which could exacerbate oomycete pathogens like *P. capsici*, moisture content was lowest in soil amended with biochar in our trial. One possible reason that soil moisture content was not related to disease incidence in our study, is that it was kept at a relatively high concentration throughout the experiment, and it may not have been at the optimal level to facilitate *P. capsici* disease incidence. In order to better understand how water can affect disease incidence in response to different soil amendments, future researchers might consider using alternative indicators, such as soil matric potential and relative humidity (RH) in the atmosphere, to create optimal conditions of moisture, and help build moisture-based prediction models for disease management. Changes in soil structure, such as the density of pores in soil treated with different amendments, could also be analyzed in future research to more accurately evaluate how organic amendments affect water conditions and disease dynamics.

Changes in soil microbial community structure are often cited as a potential mechanism for induction of disease suppressive activity by soil amendments. In soils with suppressive activity, microbial taxa are suspected to biologically control soil-borne pathogens via competition for space and resources, production of antagonistic compounds, parasitism, and/or induction of induced systemic resistance in plants. In our study, total microbial activity as indicated by FDA, did appear to be higher in sawdust amended soil relative to the control, supporting the hypothesis by Bonanomi et al. (2007), that increased soil microbial activity is related to soil-borne pathogen suppression, and that FDA can be used to predict disease suppressive activity in response to soil amendments. In our trial, abundance of specific microbial taxa that have previously been reported have a role in the suppression of soil-borne pathogens (*Trichoderma* spp., *Pseudomonas fluorescence, and Actinomycetes* spp.*)*, did not appear to be directly related to the suppressive activity observed in response to the sawdust amendment. This could indicate that other microbial taxa that were not measured in this trial could have been responsible for the suppressive activity observed. Higher total microbial activity in the sawdust treatment at the end of the incubation period supports this hypothesis. Alternatively, these microbial taxa could have been active in the rhizosphere of pepper plants grown in sawdust amended soil, which was not measured in this study. Interestingly, *P. capsici* abundance appeared to be greater in the lignin treatment relative to the control throughout the incubation period, but it did not significantly increase root infection or decrease root growth. Consequently, *P. capsici* soil abundance cannot be used alone as a reliable indicator to predict disease incidence.

CHAPTER 3. SUPPRESSIVE EFFECTS OF FIVE FOREST INDUSTRY BYPRODUCTS APPLIED AT TWO RATES ON PHYTOPHTHORA BLIGHT IN A LOW AND HIGH ORGANIC MATTER SOIL

3.1 Abstract

Pythophthora capsici, the pathogen that causes Phytophthora blight, is a serious threat to the Midwest vegetable industry. Phytophthora blight is difficult to control with traditional disease control strategies because of its broad host range, transmission strategies, and fungicide resistance. Amending soil with complex organic substrates has potential to suppress *P. capsici* infection and increase vegetable productivity via biocontrol mechanisms, but the effects could vary given amendment composition and soil type. The goal of this project was to determine how five forest industry byproducts, each with different compositions and expected rates of decomposition, would influence Phytophthora blight in a high and low organic matter soil. Soil was collected from fields with recent outbreaks of *P. capsici*, amended with one of two rates of each amendment, and further inoculated with *P. capsici* zoospores to ensure sufficient disease pressure. Susceptible pepper plants were transplanted into the amended soil and allowed to grow for six weeks, after which root infection, crop biomass and various soil chemical and biological parameters were quantified. Results indicate that several of the amendments improved soil physiochemical and biological properties, and reduced the negative effects of *P. capsici.* The suppressive affects are likely related to changes in these soil properties, but the actual mechanisms could vary given amendment composition. The results of this trial indicate that locally available forest industry byproducts could be used to improve soil quality, reduce Phythopthora blight and increase vegetable crop productivity. However, field trials and cost-benefit analyses will need to be conducted before such practices are recommended to growers.

3.2 Introduction

Organic matter plays a vital role in soil physiochemical quality (i.e. pH, moisture holding capacity, nutrient availability, cation exchange capacity) and biotic factors (i.e. microbial diversity, microbial structure, microbial activity) (Chung et al., 1988). However, intensive agricultural practices such as shortened rotations, reliance on chemical fertilizers and pesticides, and intensive tillage has depleted soil organic matter and potentially made crops more susceptible to soil-borne pathogens (Lazarovits, 2001). Soil-borne pathogens including *Phytophthora capsici*, are one of the main limiting factors affecting vegetable yield and quality. *Phytophthora capsici* is difficult to control with traditional strategies such as planting resistant cultivars, rotating crops and applying fungicides (Shoaf et al., 2016), because it has a wide host range, can survive in soil for years, and has become resistant to common fungicides (Koike et al., 2003).

Adding organic amendments to soil has been reported to control many soil-borne diseases (Bonanomi et al., 2010; Boniulla et al., 2012), and improve crop health and crop yield (Boniulla et al., 2012). Potential mechanisms for the suppressive activity resulting from these amendments include changes in soil physicochemical qualities such as soil moisture, nutrient availability, Ca content and soil pH (Höper and Alabouvette, 1996), as well as changes in soil microbiota such as microbial community structure, microbial diversity and microbial activity (Boniulla et al., 2012). However, the materials' constituents, carbon to nitrogen (C/N) ratio, and degree of decomposition of organic amendments can affect the suppressive activity of these amendments (Papavizas et al., 1968; Janvier et al., 2007; Bonanomi et al., 2010).

Organic soil amendments derived from more complex substrates have been theorized to result in greater disease suppressive activity than those derived from substrates that are more readily available to microbial decomposition (Bonanomi et al., 2013; Senechkin et al., 2014). Organic amendments with a high C/N ratio (>30:1), such as woody or more fibrous amendments, generally provide more stable organic matter to soil and lead to higher cation exchange capacity and greater nutrient-holding capacity (Sarrantonio, 1998). Amendments containing more complex substrates are also expected to result in greater competition among soil microbes and favor taxa with an oligotrophic

growth habit (Bonanomi et al., 2010). The availability of easily degradable carbon has been found to favor pathogen survival in soil, and a high copiotroph to oligotrophic ratio is thought to be associated with poor soil quality and disease conduciveness (Borrero et al., 2004). However, the type of amendment that best suppresses disease activity could depend on the type of pathogen present, as well as the amendment composition and soil type. For example, soil-borne pathogen like *Fusarium*, which are generally more host specific, can be suppressed with labile organic matter substrates, whereas pathogens such as *Rhizoctonia*, *Phytophthora* and *Pythium* are better controlled when complex organic substrates are applied (Bonanomi et al., 2010). Shoaf et al. (2016) observed differences in the potential for several biochar amendments to suppress *P. capsici*, and results also varied given soil type.

Variable application rates is another factor that could affect the disease suppressive activity of organic soil amendments. In a review of several studies investigating the suppressive potential of organic amendments, more than half of studies argued that disease suppression via crop residues, composts and organic wastes would increase with the application rate (Bonanomi et al., 2007). However, the optimal application rate varied with different types of organic amendments. For example, the authors noted that an application rate of less than 5% (v/v) organic wastes were best for disease suppression, while the optimal rate for suppression by crop residues were well under 1.3% (v/v). Further complicating these dynamics is that the optimal rate can depend on the objective. In a recent study, Jaiswal et al. (2014) found that the optimal application rate of biochar for disease suppressiveness is not always consistent with the optimal rate to promote crop growth.

Additional research is needed to better understand the mechanisms mediating the suppressive activity of organic amendments before they can be reliably used by growers to control diseases in the field. The objectives of this study were to: 1) determine how amendment composition and rate would affect *P. capsici* activity in a high and low organic matter soil, and 2) determine whether changes in soil physiochemical and biological properties were correlated with disease suppressive activity of the amendments.

3.3 Materials and Methods

3.3.1 Soil collection

Two soils were collected for use in greenhouse trials from farms with recent outbreaks of Phytophthora blight in pumpkin fields. The farms were located in Knox County, IN. Soil A (with 1.8% organic matter content), collected from a farm at (lat. 38.633196°, long. -87.490869°) containing soil characterized as Aryshire fine sandy loam (deep, poorly-drained soil formed in eolian material). Soil B (with 4% organic matter content), collected from a farm at (lat. 38.602675°, -long. 87.435070°) containing soil characterized as Alford silt loam (deep, well-drained soil formed in loess). Both soil A and soil B were collected from under diseased plant material to a depth of 15 cm, mixed, and stored at 4 C in the cooler at the Purdue University Horticulture Greenhouse, West Lafayette, IN, to limit biological activity until greenhouse experiments were conducted. Both soils were thoroughly mixed and sieved while moist to 4 mm before the trials were initiated. The abundance of *P. capsici* was determined in both soils by plating soil dilutions on PARP-H media.

3.3.2 Amendments and Treatments

Five forest industry byproducts (Appendix Table 4) were obtained for use in greenhouse trials: 1) compost; 2) lignin; 3) sawdust; 4) Biochar A; and 5) Biochar B. All amendments were ground to a fine powder and the C/N ratio of each amendment was determined using a FlashEA® 1112 Nitrogen and Carbon Analyzer (CE Elantec, Lakewood, NJ).

Each soil was amended with one of the forestry industry byproducts at a rate of either 1% or 3% total carbon (w/w) soil and mixed thoroughly. Amended soils from each treatment were distributed into 10.16 cm diameter pots with 6 replicates per treatment, except for biochar B, which only had 4 replicates for each rate because of limited supplies of the amendment.

3.3.3 Plant materials and *Phytophthora capsici* inoculum preparation

Seed from the susceptible pepper variety ‘Red Knight’ (Johnny’s Selected Seeds, Winslow, ME) were prepared as follows. Seeds were placed in a beaker for surface sterilization with a 8.25% sodium hypochlorite solution containing Tween-20 (two drops per 100ml), stirred for 20 minutes on a magnetic stir plate, and then triple-rinsed in deionized water. Surface sterilized seeds were placed on 4 layers of autoclaved cheesecloth within a laminar flow hood and allowed to air dry for 30 minutes. Surface sterilized seeds were planted into 72-cell plastic trays containing soilless potting mix (Growing Mix; Fafard, Agawam, MA) which is commonly used for germination (59-73% Canadian sphagnum peat moss perlite, vermiculite, dolomite lime, wetting agent). The trays were placed in the mist room of Purdue University Horticulture Greenhouse to facilitate germination.

Phytophthora capsici inoculum was prepared using a protocol from Larkin et al. (1995). The pathogen was cultured in petri dishes containing V8 agar media (which contained 200 ml filtered V8 juice, 2g CaCO3, 15g agar and 800 ml distilled water) and allowed to grow at 24C for 7 days. Media in the plates was cut into pieces and flooded with water to injure the mycelia and stimulate zoospore production. The plates were incubated in light at 24C for 72 h, chilled at 5C for 1 hour, and then incubated at 24C for 30 to 60 minutes. Suspensions from the plates were filtered through 8 layers of sterile cheesecloth to remove hyphal and sporangial debris, and zoospore concentration was enumerated using a haemocytometer.

3.3.4 Greenhouse experiment

Pots containing amended soils were distributed on the greenhouse bench in a randomized complete block design. The greenhouse was maintained at an average temperature and relative humidity of 20.7 C and 53.09% for the duration of the experiment. Each pot was watered alternately with exactly 100 ml clean water and fertilizer water every other day to facilitate decomposition and any potential changes in soil properties resulting from the amendments. Moisture content was monitored using a FieldScout TDR100 soil moisture meter (Spectrum Technologies Inc., Aurora, IL) by

taking two random readings in each pot 1, 3, 7, 14, 21, and 28 days after amendment incorporation.

After 28 days, above ground weed biomass was collected from each pot and oven dried at 70 C to obtain the dry weight, and soil in each pot was stirred to simulate tillage and disrupt belowground weed biomass.

One pepper seedling was transplanted into each pot. Each pot was watered with 100 ml of fertilizer every other day to ensure sufficient nutrients and prevent potential effects of immobilization from the amendments. Pots containing soil A were amended with 2450 ppg and soil B 2000 ppg of *P. capsici* zoospores to obtain 3000 ppg *P. capsici* soil abundance in each pot and ensure adequate disease pressure once pepper seedlings were transplanted into each pot. Relative chlorophyll content of pepper leaves was monitored using a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc. 12360 S. Industrial Dr. East Plainfield, IL 60585 USA) by taking three random readings in each pot after 30 days.

Pepper plants were harvested after 37 days. Roots were shaken to remove closely adhering soil and obtain rhizosphere samples. Five grams of roots were collected randomly from each pepper plant and placed in a sterile 50 ml centrifuge tube containing 25 ml sterilized water and stored at 4 C until microbial community analyses described below. The remaining root systems from each plant were washed with water, and root and shoot material was separated and dried at 40 C to obtain above and belowground biomass.

3.3.5 Laboratory analyses

The centrifuge tubes containing plant roots were vortexed for 60s to dislodge rhizosphere soil. Roots were removed and washed thoroughly with DI to remove any remaining soil. Ten root cuttings (5mm) were randomly selected and placed on plates containing PARP-H media (Papavizas et al., 1981) with 3 replicates per plate. Root infection was recorded after 3 days. Rhizosphere soil collected in the 50 ml tubes were centrifuged at 4300 rpm for 5 min. The water was discarded and the soil was lyophilized and stored at -20 C for future potential microbial community analysis.

Soil samples were collected from each pot at harvest, thoroughly mixed and stored at 4 C until being subject to the following analyses. *Phytophthora capsici* abundance was determined by placing 5 grams of moist soil into 25ml sterile water for serial dilutions on selective media (PARP-H amended with hymexazol), with two replicates per pot, and three replicate plates per dilution. Because the dilutions were conducted using moist soil, a 25 g soil sample with 3 replicates per treatment was dried at 50 C for 48 h to determine soil dry weight, and *P. capsici* soil abundance values were adjusted accordingly. Soil microbial biomass was determined by lyophilizing 15 ml of moist soil and sending this soil overnight on ice to WARD Labs (Kearney, NE) for phospholipid fatty acid (PLFA) analyses. WARD labs uses a standard protocol for performing this analyses and references for this procedure can be found at the following website http://www.wardlab.com/WardInfo/ListOfReferences.aspx. Soil microbial activity was estimated using the hydrolysis of fluorescein diacetate [3', 6'-diacetylfluorescein (FDA)] method described in Green et al. (2006). Reacted samples and standards were measured at 490 nm on a BioTek Epoch plate reader (BioTek, Winooski, VT). Soil samples were sent to Midwest Labs (Omaha, NE) for a basic soil test to determine % OM, CEC, pH and availability of nitrate, phosphorous, potassium, magnesium, and calcium.

3.3.6 Statistical analyses

Data were analyzed using standard analyses of variance (ANOVA) procedures in SAS (SAS version 9.2; SAS Institute Inc., Cary, NC) using PROC GLM, and means separated using Tukey's honestly significant difference test ($P < 0.05$). All data were checked for model assumptions. Percent root infection by *P. capsici* was log transformed because the normal distribution was not met, and data was back-transformed to report means in the figure. Data for weed biomass, soil pH and PLFA are reported separately by soil because of significant soil by treatment interactions. All other data was combined across the two soils because the interaction of soil and treatment was not significant.

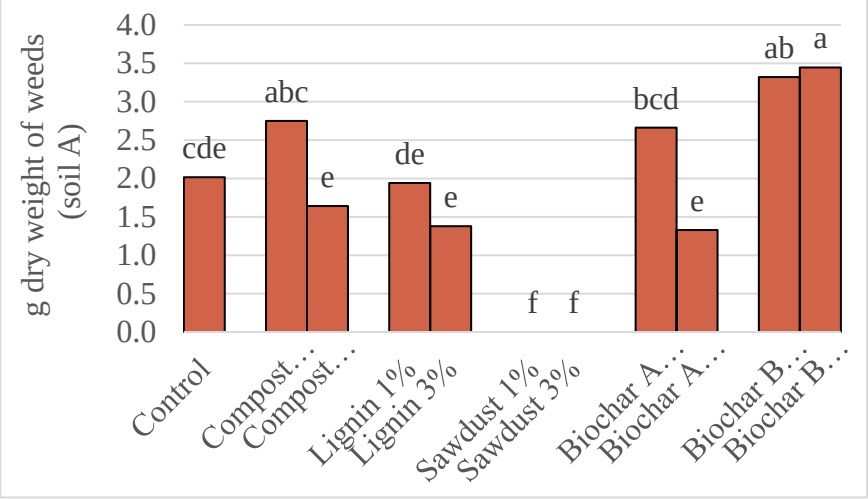
3.4 Results

3.4.1 Soil moisture

Soil moisture was affected by treatment and differences between treatments became more pronounced over the 28 day incubation period (Appendix Table 5). Soils with amendments at 3% were more affected than those receiving the amendments at a rate of 1%. In particularly, compost 3%, lignin 3% and biochar A 3% all increased soil moisture relative to the control. In contrast, the sawdust 3% treatment initially reduced soil moisture, but then resulted in greater soil moisture toward the end of the 28-day period. Biochar B also reduced soil moisture relative to the control early during the 28-day period, but did not result in lower soil moisture later in the experiment. When compared across treatments, soil B had greater soil moisture than soil A (data not shown).

3.4.2 Weed biomass

The soil significantly impacted weed biomass in both soils (Fig. 3.1). The sawdust treatment suppressed weeds relative to the control at both rates in both soils, however, effects of the other amendments depended on the soil. In soil A, biochar B had greater weed biomass relative to the control at both rates, whereas in soil B, weed biomass was greater in the 1% rate. In soil B, both rates of the compost and lignin, and the 1% rate of biochar A had greater weed biomass relative to the control. When compared across treatments, soil B had greater weed biomass than soil A (data not shown).



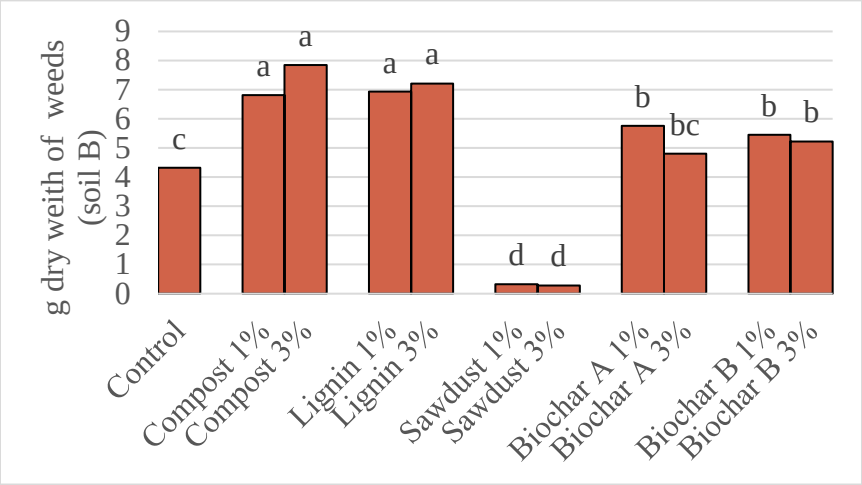


Figure 3-1 Weed biomass one month after soil amendment in two soils infested with *Phytophthora capsici* during incubation period in greenhouse trials. Different letters represent significant difference as determined by Tukey's honestly significant difference test (P< 0.05).

3.4.3 SPAD chlorophyll readings

Relative chlorophyll content of pepper leaves was not significantly affected by the soil amendments with the exception of the sawdust 3% treatment, which had lower relative chlorophyll content that the control (Fig.3.2). When compared across treatments, soil A had greater relative chlorophyll content of pepper leaves than soil B (data not shown).

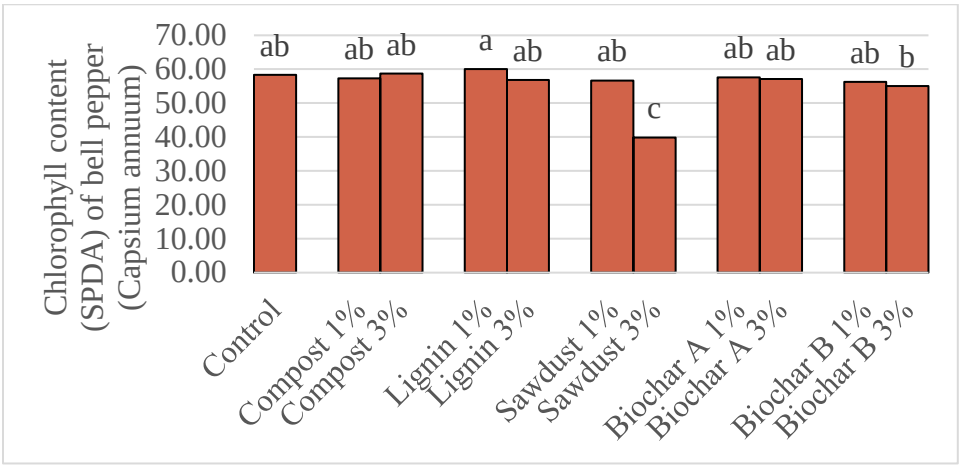


Figure 3-2 Chlorophyll content (SPAD) of bell pepper (*Capsicum annuum*) leaves 30 days after transplanting into soil amendments averaged across two soils. Different letters represent significant difference as determined by Tukey's honestly significant difference test (P< 0.05).

3.4.4 *Phytophthora capsici* pepper root infection and soil abundance

Root infection by *P. capsici* was not significantly affected by any of the amendment treatments (Fig. 3.3). Root infection was greater in soil A than soil B (data not shown). Soil abundance of *P. capsici* (Fig. 3.4) appeared to be reduced in the lignin, sawdust and both biochar treatments at both rates relative to the control, but was not significantly different (Fig. 3.4). There was also no difference in *P. capsici* abundance when compared across the treatments in the two soils (data now shown).

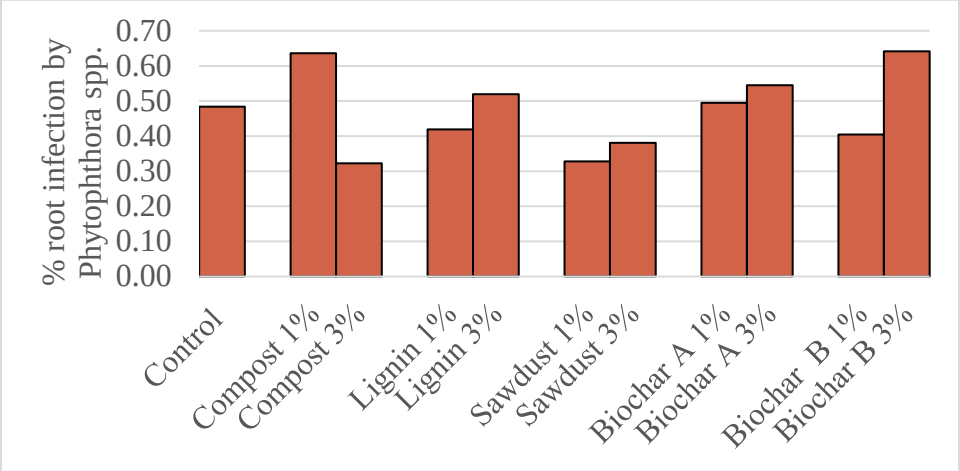


Figure 3-3 Percent root infection of bell pepper (*Capsicum annuum*) with *Phytophthora capsici* six weeks after transplanting into amended soil averaged over two soils. No significant difference was observed across treatments.

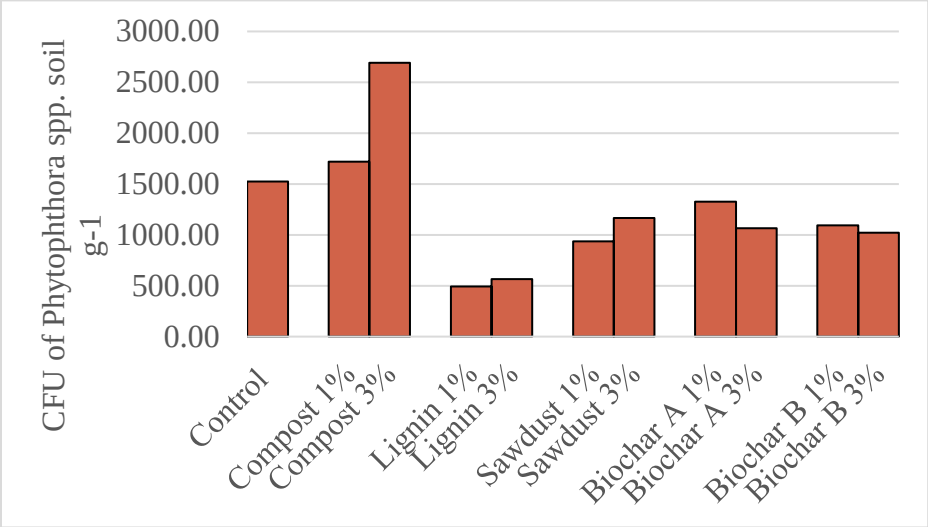


Figure 3-4 Soil abundance of *Phytophthora capsici* at pepper harvest averaged across two soil amended with forest industry byproducts. No significant difference was observed across treatments.

3.4.5 Pepper root and shoot biomass

The lignin, biochar A and biochar B treatments at 3% and the sawdust treatment at 1% all increased root biomass relative to the control treatment (Fig. 3.5). When compared across treatments, there was no difference in root biomass between the two soils (data not shown). In contrast, when compared across treatments, shoot biomass was greater in soil B than soil A (data not shown). None of the treatments improved shoot biomass relative to the control, though the sawdust treatment at 3% reduced biomass relative to the control (Fig. 3.5b).

3.4.6 Soil chemical properties

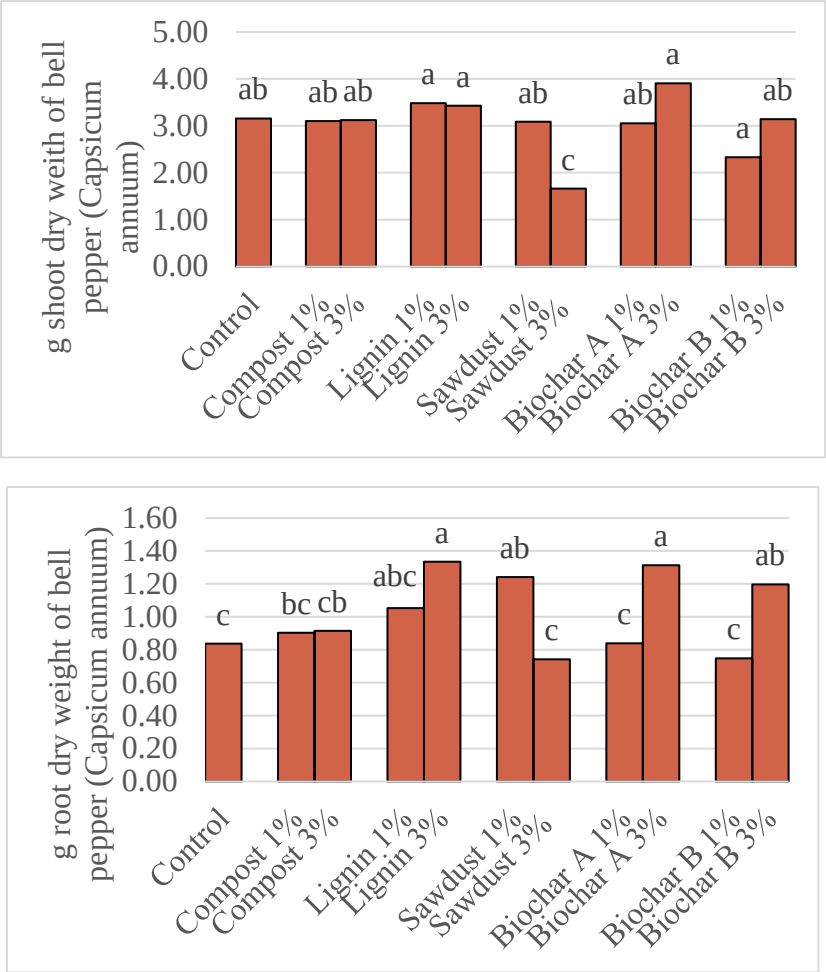


Figure 3-5 Root (a) and shoot (b) biomass of pepper grown in two soils amended with forest industry residuals. Different letters represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

Many of the amendment treatments affected soil pH (Fig. 3.6). In soil A, the compost treatment at 3% and the sawdust and both biochar treatments at both rates had greater soil pH than the control. In soil B, the sawdust and biochar A treatments at both rates, the biochar B at 3%, and the compost at 1% had greater soil pH that the control. The lignin treatment at both rates also had lower soil pH than the control in soil B.

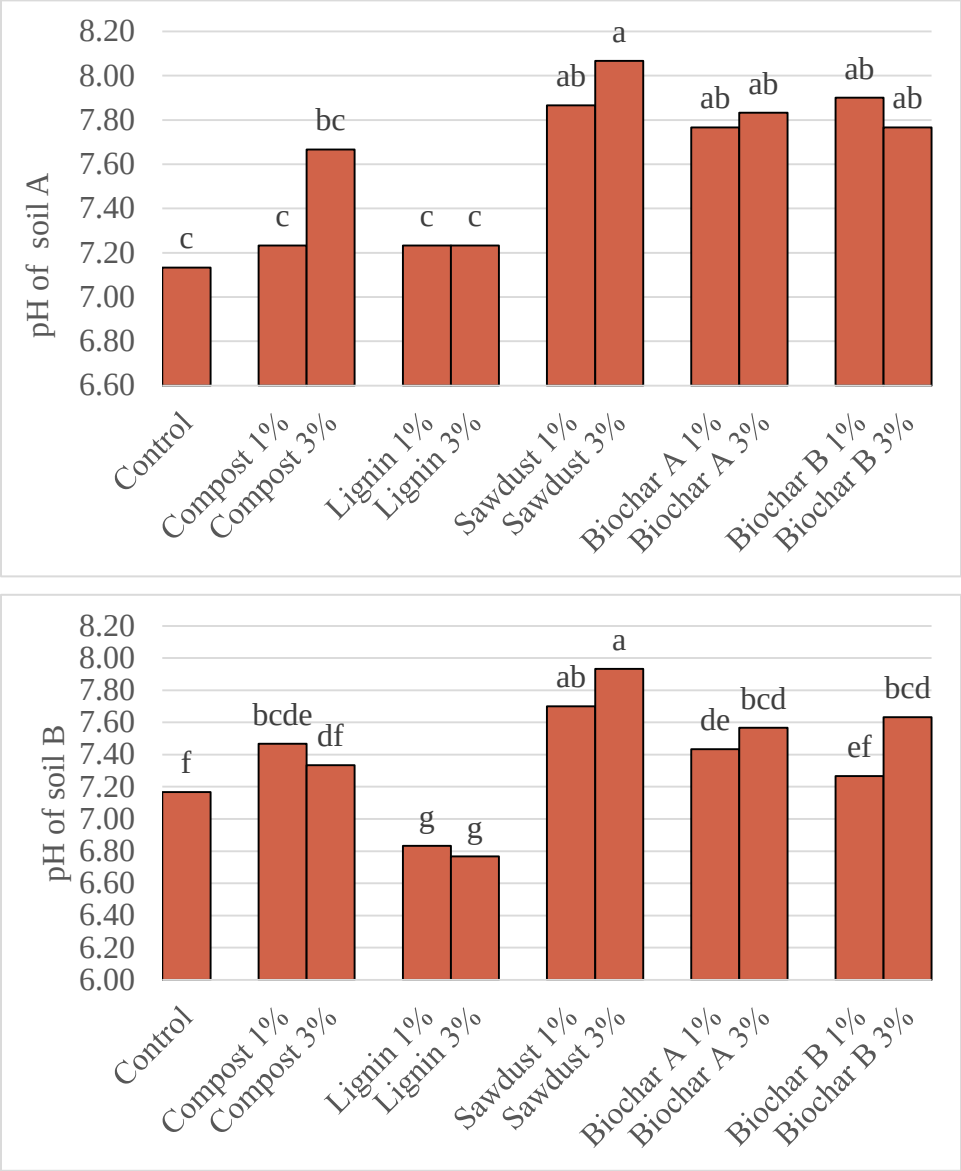


Figure 3-6 Soil pH in two soils amended with forest industry residuals. Different letters represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

The compost treatment at 3% had the greatest positive effect on the other soil chemical properties, with greater % organic matter, cation exchange capacity, and available P, K, Mg, and Ca relative to the control (Appendix Table 6). Several of the other treatments also had greater % organic matter including the lignin and biochar B treatments at both rates and the sawdust treatment at 3% relative to the control. In

contrast only the lignin treatment at both rates had greater cation exchange capacity, and the biochar B treatment at 3% had lower cation exchange capacity than the control. The sawdust treatment at 1% had greater available Mg and the sawdust at 3% had greater available K relative to the control. The lignin and sawdust treatments at both rates had lower available P relative to the control. The sawdust and both biochar treatments at both rates had lower nitrate relative to the control. Biochar B at 3% also had lower available P, K, Mg and Ca relative to the control. When compared across treatments, all soil chemical properties were greater in soil B than A (data not shown).

3.4.7 Soil microbial biomass

In soil A, all amendments dramatically affected soil microbial biomass (Appendix tables 7 and 8). In particular, the compost, lignin and sawdust treatments at both rates had greater diversity index, total biomass, total bacterial biomass, total fungal biomass, undifferentiated biomass, and fungal:bacterial ratio than the control. Most of the amendments also had greater gram +, gram -, actinomyctes, rhizobia, arbuscular mycorrhizal fungi and saprophyte biomass relative to the control. In contrast, both biochar treatments at both rates had greater total fungal biomass, fungi:bacteria ratio, and arbuscular mycorrhizal fungi relative to the control. All amendments had a lower gram + to gram – ratio than the control in soil A. When compared across treatments, microbial biomass was greater in soil B than soil A (data not shown), but there were far fewer effects of the amendments on microbial biomass in soil B (Tables 7 and 8). Interestingly the lignin treatment at rate 3%, and both biochar treatments at 3% had lower biomass of individual microbial groups relative to the control.

3.4.8 Soil microbial activity

Soil microbial activity was greater in the compost treatment at 1%, the sawdust treatment at both rates, and the biochar A treatment at 3% than the control (Fig. 3.7). When compared across treatments, there was no difference between the two soils (data not shown).

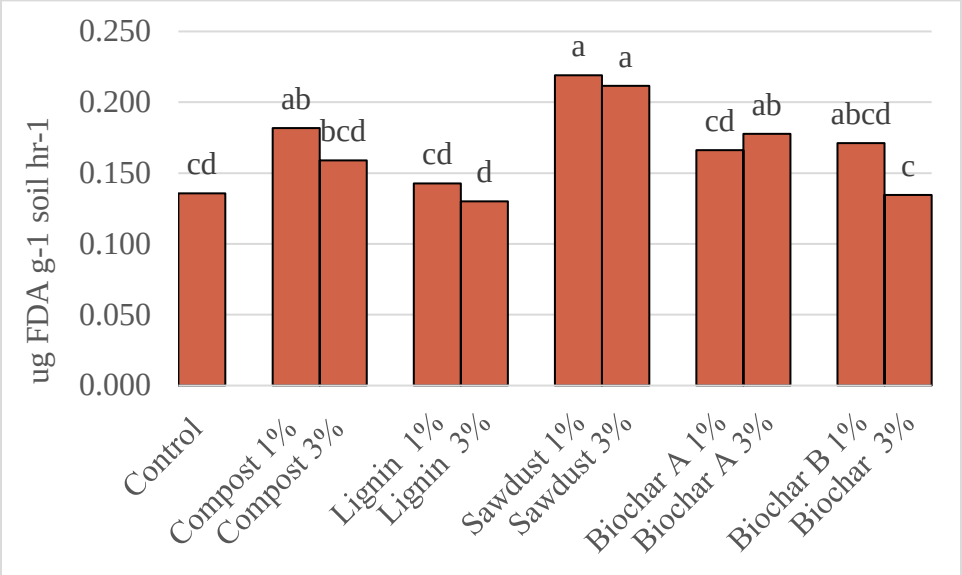


Figure 3-7 Soil microbial activity (FDA) averaged over two soils amended with forest industry residuals. Different letters represent significant difference as determined by Tukey's honestly significant difference test (P< 0.05).

3.5 Discussion

Soil quality is widely regarded to provide the foundation for sustainable crop production and poor soil quality is suspected to contribute disease outbreaks. Amending soil with amendments containing complex carbon-based substrates has been suggested as a viable approach to improve soil quality and reduce negative impacts of soil-borne pathogens such as *Rhizoctonia*, *Pythium* and *Phytophthora* spp. (Bonanomi et al., 2010). Results of this study provide evidence that amending soil with several forest industry byproducts can improve soil quality and reduce *P. capsici* stress in pepper. However, the beneficial effects varied given amendment composition as well as the rate that the amendment was applied, and suppression induced by the different amendments could be the result of alternative mechanisms.

Compost is often used to improve soil quality and in many cases it has also been found to suppress soil-borne pathogens (Bonanomi et al., 2010). In this study, amending soil with a compost derived from woody material at either a 1% or 3% total carbon (w/w) soil, improved several soil quality indicators, particularly in the low organic matter soil.

However, the compost amendment did not appear to reduce *P. capsici* root infection nor improve pepper growth in soils infested with this pathogen. This could be related to the fact that this particular compost amendment had a relatively low C: N ratio for compost, which means it was likely readily available for microbial decomposition. Amendments with labile carbon compounds have generally not been effective in suppressing *Phytophthora* spp. (Bonanomi et al., 2010). The compost amendment did increase plant available nutrients and significantly increased weed biomass in soil B, indicating that this amendment has potential for use as an organic fertility amendment if applied closer to transplanting.

Sawdust amended media has been used in the nursery industry for its potential to control plant parasitic nematodes and Phytophthora and Pythium root rots (Hoitink et al., 2004). In this study, amending soil with a sawdust derived from processing hardwood spp. for furniture at a rate of 1% total carbon (w/w) soil, improved pepper growth in soils infested with *P. capsici*. This amendment also improved several soil quality indicators, including soil pH and microbial biomass in soil A, which could have contributed to the suppressive effects observed. Soil pH is one of the most important factors regulating soil microbial composition and activity in soil (Fierer and Jackson, 2006), and changes in soil pH have previously been found to be highly correlated with disease suppressive activity (Bonanomi et al., 2010). However, previous studies investigating the effects of soil pH on Phythophthora blight have demonstrated that disease severity is greater when soil pH is increased (Muchovej et al., 1980), so changes observed in this study may not be directly related to the suppressive activity observed.

Changes in nitrogen availability is another factor that could have influenced severity of Phytophthora blight in response to the sawdust amendment in this study. High levels of soil nitrates have previously been found to increase host plant susceptibility to pathogens and also could change pathogen virulence (especially in vascular wilt pathogens) (Snyder et al., 1959). Snyder et al. (1959), also found that high C/N ratio organic materials can effectively suppress pathogen infection by stimulating microbial activity and inducing N starvation. However, while reducing N availability could be advantageous in terms of suppressing Phytophthora blight, growers would need to be careful with the amount of sawdust that is applied, because over-application could

prevent plants from obtaining adequate nutrition. For example, nitrogen immobilization may have been responsible for the dramatic reduction in weed biomass in both soils following the sawdust amendments in this trial, as well as the low relative pepper leaf chlorophyll content with the 3% amendment.

Finally, changes in soil microbial biomass could have been related to the suppressive effects observed in response to the sawdust amendment in this trial. However, suppressive effects were greater in soil B, which had a greater microbial biomass to start with and did not exhibit dramatic changes in soil microbial biomass in response to the sawdust amendment. It is not surprising that changes in microbial biomass do not appear to be directly related to disease suppression in this study, given that changes in microbial community structure, rather than changes in total microbial biomass, have previously been reported to be a stronger indicator of disease suppressive potential (Bonanomi et al., 2010). Differences in how the two soils responded to this amendment supports this hypothesis. It has been theorized that greater soil microbial density, and more importantly where a community starts, is likely to have a significant impact on the effectiveness of a particular management practice to induce disease suppressive activity (Kinkel et al., 2011). This is because there is likely to be a greater diversity of genotypes and more potential for mutation and recombination, that could result in more antagonistic phenotypes. This does not mean that a low organic matter soil would not respond at all to an amendment that induces disease suppressive activity in a soil with higher organic matter, but it might take longer to develop suppressive effects.

Amending soil with kraft pine lignin has previously been found to result in a soil dependent reduction of *R. solani* (Van Beneden et al., 2010). In this study, amending soil with kraft pine lignin at 3% total carbon (w/w) soil suppressed the negative effects of *P. capsici* and improved pepper root growth. In Van Beneden et al. (2010) suppressive effects were correlated with greater abundance of actinomycetes, gram – bacteria, and fungi. Lignin degradation by fungi has provided aromatic monomers which can be degraded by bacteria (Vicuna et al., 1993) and many actinomycetes have been found to be able to utilize lignin as a growth substrate (Ball et al., 1989). Actinomycetes are often implicated for their potential to contribute to disease suppressive soil because of their potential to produce compounds that are antagonistic to soil-borne fungi (Janvier et al.,

2007). Many gram – bacteria are oligotrophs, which are also thought to contribute to soil suppressiveness (Bonanomi et al., 2010). In this trial, total fungi, actinomycetes and gram – bacteria were significantly greater in soil amended with lignin relative to the control, however, in soil B, gram – bacteria and actinomycetes were decreased, indicating that changes in individual soil microbial taxa may have been more important that broad groups. Changes in soil pH is another factor that could have contributed to the suppressive effects of lignin observed in this study, since soil pH was reduced in soil B relative to the control, and lower pH has previously been found to reduce the severity of Phyophthora blight (Muchovej et al., 1980). Greater availability of soil K derived from this amendment also could have stimulated weed biomass as well as pepper root growth. Finally, changes in soil moisture availability could have contributed to the suppressive effects of lignin observed in this study. Soil receiving the lignin treatment at 3% had significantly greater soil moisture in the last four of the six moisture measurements quantified in this study. While *P. capsici* activity is generally expected to be favored under greater soil moisture, these conditions also could have been more favorable for root growth.

Many recent studies have provided evidence that biochar amendments can suppress a wide range of soil-borne pathogens (Graber et al., 2015), including *P. capsici* (Shoaf et al., 2016). However, suppressive effects can be dependent on the type of biochar applied (Jaiswal et al., 2014; Shoaf et al., 2016), as well as the rate (Jaiswal et al., 2014; Graber et al., 2015). This is not surprising given that feedstocks and pyrolysis conditions can dramatically alter biochar's physical and chemical properties (Downie et al., 2009). In this study, both biochar amendments applied at the 3% rate, but not the 1% rate reduced the negative effects of *P. capsici* and improved pepper root biomass relative to the control.

Many potential mechanisms for biochar's disease suppressive effects have been proposed including changes in nutrient content, water holding capacity, redox activity, adsorption ability, pH and content of toxic or hormone-like activities, as well changes in the rhizosphere microbiome (Graber et al., 2015). Results of this study indicate that different types of biochars could suppress pathogen activity via alternative mechanisms. For example, both biochar amendments increased soil pH and total fungal biomass in soil

A, but not in soil B, which may or may not have contributed to the suppressive effects observed as discussed above. In contrast biochar A dramatically increased soil moisture relative to the control, while biochar B reduced it. Both changes could have contributed to the plant effects observed, either by promoting root growth in the case of greater soil moisture, or reducing *P. capsici* activity in the case of reduced soil moisture. Greater soil microbial activity, as indicated by FDA hydrolysis in soil amended with biochar A, could have contributed to the suppressive effects observed, as this indicator has previously been found to be highly correlated with disease suppressive effects (Bonanomi et al., 2010). Lower nitrate availability in soil amended with biochar B could have contributed to the suppressive activity with this amendment, as discussed above.

Interestingly, none of the treatments that improved root growth in the presence of *P. capsici* stress appeared to be correlated with lower *P. capsici* soil abundance or root infection. There does appear to be a trend towards lower *P. capsici* abundance in the lignin, sawdust and biochar treatments, and the lack of significant differences could be related to the very high heterogenous nature of soil microbial communities, which could make it difficult to demonstrate differences among treatments. However, it is also not uncommon for soil-borne pathogens such as *Phythophthora* spp. to remain abundant in soils with disease suppressive activity, and abundance has previously been suggested as a poor predictor of disease suppression (Bonanomi et al., 2010). What is more surprising is that there was no difference in root infection despite greater root biomass in the suppressive treatments. Consequently, it is possible that improvements in root growth in response to the suppressive amendments were due to the presence of abiotic factors that stimulated root growth, and/or presence of beneficial plant growth promoting microbes in the rhizosphere.

In conclusion, results of this study provide evidence that forest industry byproducts can help to suppress Phythophthora blight in pepper. However, before recommending this approach to growers, field trials are needed to confirm that suppressive effects will be observed in the field. In addition, cost-benefit analyses should be performed to determine whether these amendments would be cost effective. For example, the common rate of 25 T/ha that has been found to improve crop productivity in field trials with biochar, was estimated to cost over $6000 per ha (Filiberto and Gaunt,

2013). If the benefits of this application are long-term in nature, it is possible that these costs could be recovered, but longer term studies are needed to confirm this assumption. Finally, before these amendments are recommended to growers, studies to investigate potential negative side effects must be conducted. For example, biochar amendments could adsorb agrochemicals, reducing their effectiveness (Bonanomi et al., 2015), or introduce environmental contaminants (Montanarella and Lugato, 2013).

CHAPTER 4. CONCLUSION

4.1 Suppressive Effects of Forest Industry Byproducts on Phytophthora Blight

Results of these studies indicate that commercially available sawdust, lignin and two biochar amendments have the potential to help suppress Phytophthora blight in bell pepper. While field trials are needed before any of these amendments should be suggested as an alternative strategy for managing Phytophthora blight, amending soil with the sawdust at 1% is likely to be the most desirable strategy, because it is readily available, was effective at the low rate in all trials, and it has the least potential for negative long-term side effects.

Many of the amendments tested in this trial including the compost, lignin and sawdust, improved soil quality, particularly in the low organic matter soil, resulting in a greater diversity index, greater total microbial biomass, bacterial and fungal biomass, and a greater fungal to bacterial ratio. These amendments also increased soil pH, soil organic matter, cation exchange capacity and P, K, Mg availability in some cases. The biochar amendments also improved many soil quality parameters, but to a lesser extent than the other amendments. This indicates that these amendments could be helpful in rebuilding soil quality in soils that have been degraded by decades of intensive agricultural practices.

4.2 Correlations between analytical parameters and suppressive capacity of amendments

Understanding how organic soil amendments induce disease suppressive activity is critical to being able to use these amendments to reliably suppress diseases in field trials. In this study, we found that soil moisture holding capacity, soil pH, soil microbial biomass (as indicated by PLFA) and soil microbial activity (as indicated by FDA activity) could be promising parameters. Soil pH has previously been reported to be a strong indicator of disease suppressive activity (Bonanomi et al, 2010). Soil moisture holding capacity has not been widely studied as an indicator of disease suppressive

activity, though it is likely to have a significant impact on root growth and soil microbial communities, and should be further investigated.

FDA activity has been widely investigated for its capacity to predict the suppressive activity of organic amendments (Chen et al., 1988). FDA is a strong indicator of organic matter decomposition, because it quantifies the activity of multiple enzymes (Nannipieri et al., 2003). Organic matter decomposition is thought to be a key factor is soil disease suppressive activity (Bonanomi et al., 2010). In our study, FDA activity did appear to be correlated with the suppressive activity of the sawdust and biochar A amendments.

Total fungal biomass is another promising parameter that appeared to be correlated with some of the suppressive activity observed in our trials. Most agricultural soils have a low fungal to bacterial biomass ratio because they are dominated by bacteria (Postma et al., 2008). Increasing soil fungal biomass could be a significant factor in inducing disease suppressive activity, as Postma et al. (2008) reported a significant correlation between fungal composition and soil suppressiveness against Rhizoctonia.

We did not observe a consistent relationship between *P. capsici* soil abundance, root infection, and plant productivity in this trial. However, this was not surprising given that other studies have observed greater disease suppressive capacity in response to soil amendments, even though *Phytophthora* populations remained high (Szczech and Smolinska, 2001, Widmer et al.,1998).

4.3 Suggestions for future research directions

Results of this study indicate that amending soil with forest industry byproducts could improve soil quality and suppress Phytophthora blight. However, these benefits depended on the type of amendment applied, as well as the initial quality of the soil. In addition, these trials were conducted under controlled conditions in the greenhouse, and results could vary if the amendments were applied in the field given more variable environmental conditions. Consequently, field trials are highly recommended at multiple sites that have variable existing levels of soil quality. These studies should include

evaluation of potential negative side effects, such as N immobilization or adsorption of agrochemicals, as well as cost-benefit analyses.

Greater understanding of the mechanisms mediating how these amendments induce disease suppressive activity is also needed. Results of this and previous studies indicate that changes in soil microbial community structure could be a key factor in disease suppressive activity. Determining whether this is the case will require more advanced microbial community analyses such as next-generation sequencing. Because the composition of the amendments also appears to be a critical factor in the suppressive activity, more advanced assays that better quantify the biochemical quality of the amendments are also needed.

Finally, additional research is needed to develop simple and cost-effective tests and/or ‘kits’ that growers could use to determine whether an amendment has potential to induce suppressive activity, or track whether such amendments are inducing suppressive activity on their farms.

APPENDIX A. TABLES

Table 1 Biological and Chemical Indicators as Related To Soil Quality Functions*.

	Soil Function				
Soil Quality Indicator	Sustain biological diversity activity, and productivity	Regulate and partition water and solute flow	filter, buffer, degrade, detoxify organic and inorganic materials	Store and cycle nutrients and carbon	Physical stability and support for plants and structures associated with human habitation
Earthworms	◯◯◯	–––	◯◯◯	◯◯◯	◯◯◯
Particulate Organic Matter	◯◯◯	◯◯◯	◯◯◯	◯◯◯	◯◯◯
Potentially Mineralizable Nitrogen	◯◯◯	–––	–––	◯◯◯	–––
Phosphorus	◯	◯	–––	–––	–––
Reactive Carbon	◯◯	◯	◯◯◯	◯◯	◯◯
Soil Electrical Conductivity	–––	◯◯◯	–––	–––	–––
Soil Nitrate	◯	◯	–––	–––	–––
Soil pH	◯◯	◯◯◯	◯◯◯	◯◯◯	–––

*Modified by Natural Resources Conservation Service of USDA

Table 2 The Carbon And Nitrogen Percent And Materials Source Of Four Forest Industry Amendments.

Amendment	Carbon (%)	Nitrogen (%)	C/N	Materials source
Compost	35.95	3.2403	11: 1	Wood fines compost, mixed by hardwood spp.; Soilmaker, West Lafayette, IN
Lignin	61.82	0.8449	73: 1	Kraft pine lignin, Indulin AT ®, Westvaco, Co., Charleston, SC
Sawdust	54.24	0.4613	118:1	Wood flour, mixed by oak, maple, & ash; Fiber By-Products Corp., Goshen, IN
Biochar	40.81	0.2980	137:1	Mixed softwood spp, pyrolyzed at 1 hr at temperatures between 450 ° and 550 °C; courtesy North Carolina State University

Table 3 Chemical Properties Across Treatments In Greenhouse Trials.

				ppm			Percent base saturation			Nitrate-N (FIA)
	pH	% Organic matter	Cation exchange capacity	K	Mg	Ca	%K	%Mg	%Ca	ppm
Control	6.9a*	1.375b	8.25	4.93	23.63a	71.45a	4.93	23.63	71.45	45.5
Biochar	6.75ab	1.525b	7.15	6.48	22.08 b	67.75b	6.48	22.08	67.75	59.5
Compost	7.025a	1.525b	8.275	5.08	23.30a	71.63 a	5.08	23.30	71.63	62
Lignin	6.575b	2.4a	7.55	6.20	21.93ab	66.48b	6.20	21.93	66.48	47.75
Sawdust	6.95a	1.475b	6.75	7.18	23.78 b	69.05b	7.18	23.78	69.05	60.5

*Different letters within a column represent significant difference as determined by Tukey's significant difference test ($P < 0.05$).

Table 4 Chemical Properties Across Five Forest Industry Amendments In Greenhouse Trials

Amendment	Carbon (%)	Nitrogen (%)	C/N	Materials source
Compost	35.95	3.2403	11: 1	Wood fines compost, mixed by hardwood spp.; Soilmaker, West Lafayette, IN
Lignin	61.82	0.8449	73: 1	Kraft pine lignin, Indulin AT ®, Westvaco, Co., Charleston, SC
Sawdust	54.24	0.4613	118:1	Wood flour, mixed by oak, maple, & ash; Fiber By-Products Corp., Goshen, IN
Biochar A	40.81	0.2980	137:1	Mixed softwood spp, pyrolyzed at 1 hour at temperatures between 450 and 550 C; courtesy North Carolina State University
Biochar B	31.45	0.1082	291:1	Mixed softwood spp., pyrolyzed for 1 hour at temperatures between 450 and 550 C; Diacarbon, Burnaby, BC).

Table 5 Soil Moisture (%) Following Soil Amendments Over Time Averaged Across Two Soils.

	Soil moisture (%)					
Treatment	Day 0	Day3	Day 7	Day14	Day 21	Day 28
Control	18.55 bcd*	19.27abc	24.33cde	16.05ef	15.81def	16.29d
Compost 1%	19.75 abcd	19.89abc	27.33bc	20.57bcd	19.49cd	19.20dc
Compost 3%	21.92a	22.43a	32.54a	25.44bcd	25.65a	23.10abc
Lignin 1%	13.53e	18.08bcd	26.45bcd	19.49bcde	19.19cd	21.61bc
Lignin 3%	17.38cd	18.99bcd	30.67ab	22.89ab	20.47bc	21.87bc
Sawdust 1%	18.06cd	16.87 cde	22.08ef	17.59def	19.65bcd	24.82ab
Sawdust 3%	13.77e	14.14de	22.63def	22.06abc	23.58ab	27.25a
Biochar A 1%	20.00abc	19.43abc	26.39bcde	18.47cdef	17.19cde	19.57bc
Biochar A 3%	21.11ab	21.15ab	28.64ab	19.86bcd	19.86bc	21.33bc
Biochar B 1%	16.84d	15.98cde	22.35def	15.17f	14.81ef	15.73de
Biochar B 3%	12.30e	13.14e	19.36f	14.99f	12.85f	11.25e

* Different letters within a column represent represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

Table 6 Soil Chemical Properties Averaged Over Two Soils Amended With Forest Industry Residuals.

			ppm						% base saturation		
Treatments	% Organic matter	Cation exchange capacity	Nitrate-N	P1 (weak bray) 1:7	P2 (strong bray)1:7	K	Mg	Ca	%K	%Mg	%Ca
Control	2.90d	11.30de	136.33ab	144.50bc	154.00bc	351.17b	356.50ce	1482.17cd	8.20b	26.57bcd	65.23ab
Compost 1%	4.05bcd	12.93b	118.67abc	167.17ab	169.67ab	389.67bc	401.50b	1700.50b	7.90bc	26.42bcd	65.10ab
Compost 3%	4.98ab	15.05a	139.50a	192.50a	196.00a	450.67bc	466.50a	1913.67a	7.73bc	25.93cd	63.72abc
Lignin 1%	4.28bc	12.60bc	138.67ab	103.83e	108.67e	382.67bc	389.83bc	1541.83bc	7.90bc	26.57bcd	60.17d
Lignin 3%	5.77a	12.98b	101.50ad	115.00de	126.67de	342.67c	385.67bd	1596.33bc	6.75c	25.28d	60.62cd
Sawdust 1%	3.85bcd	12.43bcd	50.33de	111.67de	124.00de	384.83b	429.33ab	1575.50bc	8.15b	29.62a	62.23bcd
Sawdust 3%	6.00a	11.43cde	5.33e	101.00e	130.83cde	440.50a	368.33ce	1447.50cd	9.97a	27.55bc	62.48bcd
Biochar A 1%	3.37cd	10.88e	69.50dc	128.67cde	135.67cd	328.83bc	334.33ef	1454.83cd	7.78bc	26.18bcd	66.03a
Biochar A 3%	3.95bcd	11.42cde	87.50bd	135.00cd	144.33bcd	336.17bc	360.83ce	1510.33c	7.65bc	27.10bcd	65.25ab
Biochar B 1%	4.85ab	10.40ef	109.67dc	125.50cde	139.67cd	339.33b	338.00df	1341.33de	8.42b	28.05bcd	63.53abc
Biochar B 3%	5.58a	9.53f	55.00de	112.50de	127.83cde	260.50bc	293.50f	1280.17e	7.13bc	26.18ab	66.68a

* Different letters within a column represent significant difference as determined by Tukey's honestly significant difference test (P< 0.05).

Table 7 Soil Microbial Biomass (PLFA) Averaged Over Two Soils Amended With Forest Industry Residuals.

Treatments	Soil type	Diversity index	Total Biomass	Protozoa Biomass	Total Bacteria Biomass	Total Fungi Biomass	Fungi: Bacteria	Undifferentiated Biomass
Control	A	1.22e	1950.90 c	3d	936.56 d	78.42 e	0.07 e	932.62 e
Compost 1%	A	1.48abcd	2855.72 b	24d	1341.90 bc	235.01 cd	0.14 cd	1265.98 abc
Compost 3%	A	1.41d	3813.04 b	15d	1856.02 bc	329.69 cd	0.17 d	1612.55 abcd
Lignin 1%	A	1.51abc	3355.57 b	61ab	1614.71 b	309.26 cd	0.17 cd	1407.59 bcd
Lignin 3%	A	1.46ad	5432.66 a	56abc	2758.22 a	566.44 bc	0.20 d	2051.61 a
Sawdust 1%	A	1.54abc	5878.33 a	72a	2823.57 a	1004.17 a	0.35 ab	1979.08 ab
Sawdust 3%	A	1.47bdd	4020.08 b	33bcd	1912.67 bc	720.11 b	0.37 s	1354.65 cde
Biochar A 1%	A	1.55abc	2961.84 bc	28cd	1300.31 cd	391.39 cd	0.29 bc	1241.68 cde
Biochar A 3%	A	1.57a	2884.70 bc	29cd	1340.69 cd	394.59 cd	0.28 bc	1120.46 de
Biochar B 1%	A	1.39d	3030.81 bc	17d	1328.54 cd	290.93 de	0.20 d	1394.81 cde
Biochar B 3%	A	1.57bab	3062.93 bc	30cd	1412.29 bcd	420.26 cd	0.29 bc	1200.57 cde

* Different letters within a column represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

Table 7 Continued

Treatments	Soil type	Diversity index	Total Biomass	Protozoa Biomass	Total Bacteria Biomass	Total Fungi Biomass	Fungi: Bacteria	Undifferentiated Biomass
Control	B	1.59a	7518.60abcde	112a	3539.86abcd	1089.85abcde	0.29bcd	2777.27
Compost 1%	B	1.64a	7714.97abcd	104ab	3731.97abc	1302.08abc	0.34abc	2576.98
Compost 3%	B	1.60a	7783.97abcd	91abc	3550.65abcd	1550.31ab	0.40a	2592.13
Lignin 1%	B	1.56a	7906.99abc	104ab	3727.40abc	1079.59abcde	0.27cde	2995.56
Lignin 3%	B	1.47b	6002.53e	44cd	2891.93cde	642.19e	0.21de	2423.95
Sawdust 1%	B	1.56a	8610.02ab	104ab	4052.03ab	1554.31ab	0.38ab	2899.69
Sawdust 3%	B	1.56a	8960.88a	117a	4267.07a	1716.09a	0.40a	2860.31
Biochar A 1%	B	1.61b	7817.19abc	78abc	3920.06abc	1233.77abcd	0.32abc	2584.92
Biochar A 3%	B	1.42a	5191.81cde	25d	2451.65e	469.86de	0.19e	2244.95
Biochar B 1%	B	1.56a	6552.13bcde	82abc	3171.26bcde	933.80bcde	0.28cde	2365.06
Biochar B 3%	B	1.61a	5445.00de	61bcd	2569.91de	854.47cde	0.32abc	1960.07

* Different letters within a column represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

Table 8 Total Biomass Of Specific Microbial Groups (PLFA) Averaged Over Two Soils Amended With Forest Industry Residuals.

Treatments	Soil type	Gram (+) Biomass	Gram (-) Biomass	Gram(+): Gram(-)	Actinomycetes Biomass	Rhizobia Biomass	Arbuscular Mycorrhizal Biomass	Saprophytes Biomass
Control	A	580.10 f*	356.46 f	1.74 a	161.51 f	0.00 d	11.97 g	66.45 f
Compost 1%	A	752.52 bc	589.38 cde	1.49 bc	209.05 bcde	5.88 cd	63.07 bcd	171.94de
Compost 3%	A	1120.47 ab	735.55 def	1.75 a	302.72 ab	9.25 cd	155.23 ab	174.46 ef
Lignin 1%	A	875.23 bcde	739.48 cd	1.43 cde	249.38 abc	23.67 b	90.42 def	218.84 cd
Lignin 3%	A	921.28 bcd	1836.94 a	0.51 f	276.67 bcd	214.23 a	63.82 efg	502.62 bc
Sawdust 1%	A	1233.10 a	1590.47 ab	0.82 def	363.65 a	86.53 b	201.61 a	802.56 a
Sawdust 3%	A	730.66 def	1182.01 bc	0.64 ef	196.54 def	64.50 bc	97.11 cdef	623.00 ab
Biochar A 1%	A	652.29 ef	648.02 ef	1.05 bcd	180.87 f	32.12 bcd	109.92 bcde	281.46 de
Biochar A 3%	A	700.96 def	639.74 ef	1.19 bc	184.93 ef	48.28 bcd	147.83 abc	246.76 def
Biochar B 1%	A	743.45 cdef	585.09 ef	1.33 b	215.65 cdef	10.07 cd	48.34 fg	242.59 def
Biochar B 3%	A	709.44 def	702.84 def	1.02 cbd	231.20 bcdef	51.19 bcd	90.11 cf	330.15 ce

* Different letters within a column represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

Table 8 Continued

Treatments	Soil type	Gram (+) Biomass	Gram (-) Biomass	Gram(+): Gram(-)	Actinomycetes Biomass	Rhizobia Biomass	Arbuscular Mycorrhizal Biomass	Saprophytes Biomass
Control	B	1821.39abc*	1718.47bcd	1.20abc	585.30abc	115.06	233.95bc	855.90bcde
Compost 1%	B	1967.77ab	1764.20bcd	1.15bcd	647.83a	184.12	279.38ab	1022.70abcd
Compost 3%	B	1824.51abc	1726.14abcd	1.15bcd	571.58abc	169.04	271.67ab	1278.65abc
Lignin 1%	B	1751.19bcd	1976.22abc	0.97cde	576.79abc	198.62	160.20cd	919.39abcde
Lignin 3%	B	1379.95d	1511.99bcde	0.95cde	463.77bcd	106.95	65.04e	577.15de
Sawdust 1%	B	1876.31ab	2175.72ab	0.89de	604.53ab	118.30	256.25ab	1298.06ab
Sawdust 3%	B	1862.74ab	2404.33a	0.79e	574.87abc	178.66	239.44abc	1476.65a
Biochar A 1%	B	2155.63a	1764.42abcd	1.23abc	704.79a	143.08	322.78a	910.99abcde
Biochar A 3%	B	1462.34cd	989.31e	1.49a	413.71d	7.83	132.73de	337.13e
Biochar B 1%	B	1763.14bcd	1408.12cde	1.47a	581.24abc	59.66	221.38bc	712.42bcde
Biochar B 3%	B	1399.47d	1170.43de	1.27ab	461.93cd	93.83	166.82cd	687.66cde

* Different letters within a column represent significant difference as determined by Tukey's honestly significant difference test ($P < 0.05$).

REFERENCES

Adams P B, Lewis J A, Papavizas G C. Survival of Root-Infecting Fungi in Soil. IV. The Nature of Fungistasis in Natural and Cellulose-Amended Soil On Chlamydospores of *Fusarium solani f. sp. Phaseoli* [J]. Phytopathology, 1968, 58: 378-383.

Alconero R, Santiago A. Characteristics of Asexual Sporulation in *Phytophthora palmivora* and *Phytophthora parasitica nicotianae* [J]. Phytopathology, 1972, 62: 993-997.

Babadoost M, Islam S Z. Fungicide Seed Treatment Effects on Seedling Damping-off of Pumpkin Caused by *Phytophthora capsici* [J]. Plant Disease, 2003, 87(1): 63-68.

Babadoost M, Pavon C. Survival of Oospores of *Phytophthora capsici* in Soil [J]. Plant Disease, 2013, 97(11): 1478-1483.

Babadoost M, Tian D, Islam S Z, et al. Challenges and Options in Managing Phytophthora Blight (*Phytophthora capsici*) of Cucurbits [J]. 2008:399-406.

Bailey K L, Lazarovits G. Suppressing Soil-Borne Diseases with Residue Management and Organic Amendments [J]. Soil and Tillage Research, 2003, 72(2): 169-180.

Ball, A.S., Betts, W.B. Mccarthy, A.J., 1989.Degradation of Lignin-Related Compounds by Actinomycetes [J]. Applied and Environmental Microbiology 55:1642-1644.

Bonanomi G, Antignani V, Capodilupo M, et al. Identifying the Characteristics of Organic Soil Amendments that Suppress Soil borne Plant Diseases [J]. Soil Biology and Biochemistry, 2010, 42(2): 136-144.

Bonanomi G, Antignani V, Pane C, et al. Suppression of Soil borne Fungal Diseases with Organic Amendments [J]. Journal of Plant Pathology, 2007: 311-324.

Bonanomi G, Ippolito F, Scala F. A “Black” Future for Plant Pathology? Biochar as a New Soil Amendment for Controlling Plant Diseases [J]. Journal of Plant Pathology, 2015, 97(2): 223-224.

Bonilla N, Gutiérrez-Barranquero J A, Vicente A, et al. Enhancing Soil Quality and Plant Health through Suppressive Organic Amendments [J]. Diversity, 2012, 4(4): 475-491.

Borrero C, Trillas M I, Ordovás J, et al. Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media [J]. Phytopathology, 2004, 94(10): 1094-1101.

Bowers J H, Papavizas G C, Johnston S A. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil [J]. Plant disease, 1990, 74(10): 771-777.

Café Filho A C, Duniway J M. Dispersal of *Phytophthora capsici* And *P. Parasitica* in Furrow-Irrigated Rows of Bell Pepper, Tomato And Squash [J]. Plant Pathology, 1995, 44(6): 1025-1032.

Castaño R, Borrero C, Avilés M. Organic Matter Fractions by SP-MAS 13 C NMR and Microbial Communities Involved in The Suppression of Fusarium Wilt in Organic Growth Media [J]. Biological Control, 2011, 58(3): 286-293.

Chung Y R, Hoitink H A H, Lipps P E. Interactions between Organic-Matter Decomposition Level and Soil borne Disease Severity [J]. Agriculture, Ecosystems & Environment, 1988, 24(1): 183-193.

Doran J W, Parkin T B. Defining And Assessing Soil Quality [J]. Defining Soil Quality for a Sustainable Environment, 1994: 1-21.

Doran J W, Zeiss M R. Soil Health and Sustainability: Managing the Biotic Component of Soil Quality [J]. Applied Soil Ecology, 2000, 15(1): 3-11.

Downie A, Crosky A, Munroe P. Physical Properties of Biochar [J]. Biochar for Environmental Management: Science and Technology, 2009: 13-32.

Edwards L, Burney J R, Richter G, Et Al. Evaluation of Compost And Straw Mulching on Soil-Loss Characteristics In Erosion Plots of Potatoes in Prince Edward Island, Canada [J]. Agriculture, Ecosystems & Environment, 2000, 81(3): 217-222.

Edwards L, Burney J R, Richter G, et al. Evaluation of compost and straw mulching on soil-loss characteristics in erosion plots of potatoes in Prince Edward Island, Canada [J]. Agriculture, ecosystems & environment, 2000, 81(3): 217-222.

Erwin D C, Ribeiro O K. Phytophthora Diseases Worldwide [M]. American Phytopathological Society (APS Press), 1996: 465-562.

Fierer N, Jackson R B. The Diversity and Biogeography of Soil Bacterial Communities [J]. Proceedings of the National Academy of Sciences of the United States of America, 2006, 103(3): 626-631.

Francl L J. The Disease Triangle: A Plant Pathological Paradigm Revisited [J]. Plant Health Instructor DOI, 2001, 10.

Graber E R, Harel Y M, Kolton M, Et Al. Biochar Impact on Development and Productivity of Pepper And Tomato Grown in Fertigated Soilless Media [J]. Plant and Soil, 2010, 337(1-2): 481-496.

Graber E R, Tsechansky L, Lew B, Et Al. Reducing Capacity of Water Extracts Of Biochars and Their Solubilization of Soil Mn And Fe [J]. European Journal of Soil Science, 2014, 65(1): 162-172.

Graber, E.R., O. Frenkel, A.K. Jaiswal, and Y. Elad. How May Biochar Influence Severity of Disease Caused by Soil borne Pathogens? [J] Carbon Management, 2015 (5):169-183.

Granke L L, Quesada-Ocampo L, Lamour K, Et Al. Advances In Research On *Phytophthora capsici* On Vegetable Crops In The United States [J]. Plant Disease, 2012, 96(11): 1588-1600.

Green V S, Stott D E, Diack M. Assay for Fluorescein Diacetate Hydrolytic Activity: Optimization for Soil Samples [J]. Soil Biology and Biochemistry, 2006, 38(4): 693-701.

Grünwald N J, Hu S, Van Bruggen A H C. Short-term cover crop decomposition in organic and conventional soils: characterization of soil C, N, microbial and plant pathogen dynamics [J]. European Journal of Plant Pathology, 2000, 106(1): 37-50.

Hausbeck M K, Lamour K H. *Phytophthora capsici* On Vegetable Crops: Research Progress and Management Challenges [J]. Plant Disease, 2004, 88(12): 1292-1303.

Heyman F, Lindahl B, Persson L, et al. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea [J]. Soil Biology and Biochemistry, 2007, 39(9): 2222-2229.

Hickman C J. Biology of Phytophthora Zoospores [C]. Phytopathology. 1970, 60(7): 1128-1135.

Hointink, HAJ, 2004. Disease Suppression with Compost: History, Principles and Future. International Conference on Soil and Compost Eco-Biology. Http://Www.Soilace.Com/Pdf/Pon2004/15.Hoitink.PDF Accessed, October 28, 2016.

Höper H, Alabouvette C. Importance of Physical and Chemical Soil Properties In The Suppressiveness Of Soils To Plant Diseases [J]. European Journal of Soil Biology, 1996, 32(1): 41-58.

Huber D M, Watson R D. Nitrogen form and plant disease [J]. Annual review of phytopathology, 1974, 12(1): 139-165.

Hwang B K, Kim Y J, Kim C H. Differential Interactions of *Phytophthora capsici* Isolates with Pepper Genotypes at Various Plant Growth Stages [J]. European Journal of Plant Pathology, 1996, 102(4): 311-316.

Jaiswal A K, Elad Y, Graber E R, Et Al. Rhizoctonia Solani Suppression and Plant Growth Promotion in Cucumber As Affected by Biochar Pyrolysis Temperature, Feedstock And Concentration [J]. Soil Biology and Biochemistry, 2014, 69: 110-118.

Janvier C, Villeneuve F, Alabouvette C, Et Al. Soil Health through Soil Disease Suppression: Which Strategy from Descriptors to Indicators? [J]. Soil Biology and Biochemistry, 2007, 39(1): 1-23.

Jones J P, Woltz S S, Everett P H. Effect of liming and nitrogen source on Fusarium wilt of cucumber and watermelon [C]. Proceedings of the Florida State Horticultural Society. 1975, 88: 200-203.

Katsura K, Miyazaki S. Leaf Penetration by *Phytophthora capsici* Leonian [J]. Scientific Reports of the Kyoto Perfectural University, Agriculture, 1960 (12): 65-69.

Kingkun D, Taeyangkun H, Hongsanho J. Expression Of Age-Related Resistance in Pepper Plants Infected with *Phytophthora capsici* [J]. Plant Dis, 1989, 73: 745-747.

Kinkel LL, MG Bakker, DC Schlatter. 2011. A Coevolutionary Framework for Managing Disease Suppressive Soils. Annual Reviews of Phytopathology 49:47-67.

Koike S, Subbarao K, Davis R M, Et Al. Vegetable Diseases Caused by Soil borne Pathogens [M]. UCANR Publications, 2003:11-12.

Lamour K H, Hausbeck M K. The Dynamics of Mefenoxam Insensitivity in a Recombining Population of *Phytophthora capsici* Characterized with Amplified Fragment Length Polymorphism Markers [J]. Phytopathology, 2001, 91(6): 553-557.

Lamour K H, Stam R, Jupe J, Et Al. The Oomycete Broad-Host-Range Pathogen *Phytophthora capsici* [J]. Molecular Plant Pathology, 2012, 13(4): 329-337.

Larkin R P, Ristaino J B, Campbell C L. Detection and Quantification of *Phytophthora capsici* In Soil [J]. Phytopathology, 1995, 85(10): 1057-1063.

Lazarovits G. Management of Soil-Borne Plant Pathogens with Organic Soil Amendments: A Disease Control Strategy Salvaged From the Past [J]. Canadian Journal of Plant Pathology, 2001, 23(1): 1-7.

Lehmann J, Da Silva Jr J P, Steiner C, Et Al. Nutrient Availability and Leaching In an Archaeological Anthrosol and A Ferralsol Of The Central Amazon Basin: Fertilizer, Manure And Charcoal Amendments [J]. Plant and Soil, 2003, 249(2): 343-357.

Lehmann J, Gaunt J, Rondon M. Bio-Char Sequestration in Terrestrial Ecosystems–A Review [J]. Mitigation and Adaptation Strategies for Global Change, 2006, 11(2): 395-419.

Leonian L H. Stem and Fruit Blight of Peppers Caused by *Phytophthora capsici* Sp. Nov [J]. Phytopathology, 1922, 12(9): 401-408.

Liu, B., Gumpertz, M.L., Hu, S.J., Ristaino, J.B. Effect of Prior Tillage and Soil Fertility Amendments on Dispersal of *Phytophthora capsici* And Infection of Pepper. European Journal of Plant Pathology 2008a (120): 273–287.

Liu, X., Zhou, Y., Li, L. Infection of *Phytophthora capsici* On Pepper – Models and Affecting Factors. Frontiers of Agriculture in China, 2008b (2):66–71.

Mackay S J. Improved Enumeration of Streptomyces Spp. On A Starch Casein Salt Medium [J]. Applied and Environmental Microbiology, 1977, 33(2): 227.

Mao W, Lewis J A, Lumsden R D, Et Al. Biocontrol Of Selected Soil borne Diseases Of Tomato And Pepper Plants [J]. Crop Protection, 1998, 17(6): 535-542.

Martin F N. Development of Alternative Strategies for Management of Soilborne Pathogens Currently Controlled with Methyl Bromide [J]. Annual review of phytopathology, 2003, 41(1): 325-350.

Mazzola, M, DM Granatstein, DC Elving, K Mullinix. Suppression of Specific Apple Root Pathogens by Brassica Napus Seed Meal Amendment Regardless Of Glucosinloate Content [J]. Phytopathology 2001(91):673-679.

Montanarella L, Lugato E. The Application of Biochar in the EU: Challenges and Opportunities [J]. Agronomy, 2013, 3(2): 462-473.

Muchovej J J, Maffia L A, Muchovej R M C. Effect Of Exchangeable Soil Aluminum And Alkaline Calcium Salts On The Pathogenicity And Growth Of *Phytophthora capsici* From Green Pepper [J]. Phytopathology, 1980, 70(12): 1212-1214.

Muchovej, J.J., L.A. Maffia, and R. M.C Muchovej. Effect of Exchangeable Soil Aluminum and Alkaline Calcium Salts on the Pathogenicity and Growth of *Phytophthora capsici* From Green Pepper [J]. Phytopathology, 1980, 70:1212-1214.

Papavizas, G. S., Bowers, J. H., and Johnston, S. A. Selective Isolation of *Phytophthora capsici* From Soils [J]. Phytopathology 1981, 71:129-133.

Parra G, Ristaino J B. Resistance to Mefenoxam and Metalaxyl among Field Isolates of *Phytophthora capsici* Causing Phytophthora Blight of Bell Pepper [J]. Plant Disease, 2001, 85(10): 1069-1075.

Perkins L B, Leger E A, Nowak R S. Invasion triangle: an organizational framework for species invasion [J]. Ecology and evolution, 2011, 1(4): 610-625.

Postma J, Schilder M T, Bloem J, et al. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems [J]. Soil Biology and Biochemistry, 2008, 40(9): 2394-2406.

Quilliam R S, Glanville H C, Wade S C, et al. Life in the ‘charosphere’–Does biochar in agricultural soil provide a significant habitat for microorganisms? [J]. Soil Biology and Biochemistry, 2013, 65: 287-293.

Ristaino J B, Johnston S A. Ecologically Based Approaches To Management Of Phytophthora Blight On Bell Pepper [J]. Plant Disease, 1999, 83(12): 1080-1089.

Roberts P D, Urs R R, Mcgovern R J. Age and Varietal Response of Tomato to Infection by *Phytophthora capsici* [J]. Phytopathology, 2000, 90: S65.

Rudisill, M.A., Bordelon, B., Turco, R., and Hoagland, L., 2015. Sustaining Soil Quality in Intensively Managed High Tunnel Vegetable Production Systems; A Role for Green Manures and Chicken Litter [J]. Hortscience 50(3):461-468.

Sanogo S, Ji P. Water Management In Relation To Control of *Phytophthora capsici* In Vegetable Crops [J]. Agricultural Water Management, 2013, 129: 113-119.

Sarrantonio M. Building Soil Fertility and Tilth with Cover Crops [J]. Managing Cover Crops Profitably, 2nd Edn. Sustainable Agriculture Network, Beltsville, Maryland, 1998: 16-24.

Scarascia-Mugnozza G, Sica C, Russo G. Plastic materials in European agriculture: actual use and perspectives [J]. Journal of Agricultural Engineering, 2012, 42(3): 15-28.

Schaad N W, Berthier-Schaad Y, Sechler A, Et Al. Detection Of Clavibacter Michiganensis Subsp. Sepedonicus In Potato Tubers By BIO-PCR And An Automated Real-Time Fluorescence Detection System [J]. Plant Disease, 1999, 83(12): 1095-1100.

Schlub R L. Epidemiology of *Phytophthora capsici* On Bell Pepper [J]. The Journal of Agricultural Science, 1983, 100(01): 7-11.

Semenov AV, E Franz, L Van Overbeek, AJ Termorschizen, AHC Ban Bruggen. 2008. Estimating the Stability of Escherichia Coli O157:H7 Survival In Manure Amended Soils With Different Management Histories [J]. Environmental Microbiology 10:1450-459.

Senechkin, IV, LS Van Overbeek, AHC Van Bruggen. 2014. Greater Fusarium Wilt Suppression after Complex than After Simple Organic Amendments as Affected By Soil pH, Total Carbon And Ammonia-Oxidising Bacteria [J]. Applied Soil Ecology 73:148-155.

Senechkin, IV, LS Van Overbeek, AHC Van Bruggen. 2014. Greater Fusarium Wilt Suppression after Complex than After Simple Organic Amendments As Affected By Soil pH, Total Carbon and Ammonia-Oxidising Bacteria [J]. Applied Soil Ecology 73:148-155.

Shoaf N, Hoagland L, Egel D S. Suppression Of Phytophthora Blight In Sweet Pepper Depends On Biochar Amendment And Soil Type [J]. Hortscience, 2016, 51(5): 518-524.

Snyder W C, Schroth M N, Christou T. Effect Of Plant Residues On Root Rot Of Bean [J]. Phytopathology, 1959, 49(11): 755-756.

Sun R, Jin J, Sun G, et al. Screening and degrading characteristics and community structure of a high molecular weight polycyclic aromatic hydrocarbon-degrading bacterial consortium from contaminated soil [J]. Journal of Environmental Sciences, 2010, 22(10): 1576-1585.

Szczech M, Smolińska U. Comparison of suppressiveness of vermicomposts produced from animal manures and sewage sludge against Phytophthora nicotianae Breda de Haan var. nicotianae[J]. Journal of Phytopathology, 2001, 149(2): 77-82.

Tian D, Babadoost M. Host Range of *Phytophthora capsici* From Pumpkin and Pathogenicity of Isolates [J]. Plant Disease, 2004, 88(5): 485-489.

Tiquia S M, Lloyd J, Herms D A, et al. Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes [J]. Applied soil ecology, 2002, 21(1): 31-48.

Topoliantz S, Ponge J F, Ballof S. Manioc Peel and Charcoal: A Potential Organic Amendment for Sustainable Soil Fertility in the Tropics [J]. Biology and Fertility of Soils, 2005, 41(1): 15-21.

Van Beneden S, Roobroeck D, França S C, Et Al. Microbial Populations Involved In The Suppression Of Rhizoctonia Solani AG1-1B By Lignin Incorporation In Soil [J]. Soil Biology and Biochemistry, 2010, 42(8): 1268-1274.

van Elsas J D, Garbeva P, Salles J. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens [J]. Biodegradation, 2002, 13(1): 29-40.

van Elsas J D, Garbeva P, Salles J. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens [J]. Biodegradation, 2002, 13(1): 29-40.

Vicuna R, Gonzalez B, Seelenfreund D, Et Al. Ability Of Natural Bacterial Isolates To Metabolize High And Low Molecular Weight Lignin-Derived Molecules [J]. Journal of Biotechnology, 1993, 30(1): 9-13.

Weber G F. Blight of Peppers in Florida Caused by *Phytophthora capsici* [J]. Phytopathology, 1932, 22: 775-780.

Weller D M, Raaijmakers J M, Gardener B B M S, et al. Microbial populations responsible for specific soil suppressiveness to plant pathogens [J]. Annual review of phytopathology, 2002, 40(1): 309-348.

Wensley R N, McKeen C D. Some relationship between plant nutrition, fungal populations, and incidence of Fusarium wilt of muskmelon [J]. Canadian journal of microbiology, 1965, 11(3): 581-594.

Williams J, Clarkson J M, Mills P R, Et Al. A Selective Medium for Quantitative Reisolation of Trichoderma Harzianum from Agaricus Bisporus Compost [J]. Applied and Environmental Microbiology, 2003, 69(7): 4190-4191.