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# COVER CROPS, TILLAGE, AND THE SUPPRESSION OF SOIL BORNE DISEASES IN SOYBEAN

BY

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THESIS

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## Abstract

Cover crops have historically been shown to be useful in the management of pathogens of high value crops, such as fruits, vegetables, and nuts. However, the properties of these cover crops and the type of suppressive soil that they induce, can be useful in the production of agronomic crops as well. Disease suppressive soils either do not allow the pathogen to become established, or they reduce the level of disease resulting from the presence of the pathogen, in comparison to the level that would occur in a conducive soil. In this study, five cover crop treatments (cereal rye, vetch, mustard, rye+vetch, and fallow) were evaluated at the University of Illinois South Farms in both 2014 and 2015 for decreasing the incidence and severity of soybean diseases, changing soil microbial community structures, and increasing soybean yield. Two tillage treatments (chisel plow and ridge till) were also evaluated to determine whether these treatments had any effect on the microbial populations. Data of root disease severity and soybean yield were taken over the two seasons to determine the effectiveness of the different treatments. Bulk and rhizospheric soil samples were taken to compare the microbial community structures of the different treatments in relation to disease development. Poor establishment of cover crops in the field plots led to the use of greenhouse bioassays to evaluate the effects of the cover crop treatments.

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# CHAPTER 1

## Introduction and Background

Microbial populations are unique and interesting small ecosystems that have a dramatic impact on the environment in which they live. The role of each microbiome, or microbial population, varies greatly depending on the location of the microbiome as well. The animal microbiome for example, helps with digestion and processing the food we eat. The plant microbiome assists with nutrient uptake and management, as well as many other plant processes (Hill et al., 2000). However, the soil microbiome is even more interesting because it helps to shape the animal and plant microbiomes in many different ways. Plants have direct contact with soil, and many animals feed on other animals or organisms that once lived in the soil. The soil microbiome can be extremely difficult to study because it is so vast and varies widely depending on the location and type of soil environment that is present.

Plant type, soil type, and soil management practices can all influence the microbial populations within any soil. However, when comparing microbial populations from the same soil type, the plant species that are present have a larger influence on the microbial population differences than soil management practices do (Garbeva 2004). The difference in microbial populations also varies depending on where in the soil profile the populations live. The rhizosphere is the layer of soil in direct contact with the growing root system (Smalla 2001). This layer has a large impact on the types of microbes that are allowed to interact with the plant. Depending on the crop species being grown, different populations of bacteria and fungi can be stimulated to grow and flourish. Several groups of rhizospheric organisms have been studied and proven to enhance plant health. Some of those include nitrogen-fixing bacteria, mycorrhizal fungi, and protozoa (Mendes 2013). More research is being done in all areas of the soil

microbiome to find new organisms that are either beneficial to the plant or are able to act antagonistically toward plant pathogens.

Though the amount of space between the rhizospheric and bulk zones of soil is minute, there is a large difference in the types of microbes that live in each environment. In this case, bulk soil can be described as any soil that is not in direct contact with the growing root system. These two soil zones not only vary in their microbial populations, but also in other soil properties. Some soil that was once classified as bulk soil early in the growing season, will become rhizospheric soil later in the season due to the growing root system. The microbial populations will change as well since root exudates stimulate, attract, and deter certain microbes from interacting with the root zone (Smalla 2001). Some microbes that are attracted to, or stimulated by the rhizospheric soil zone, may help to create an antagonistic environment for soilborne pathogens. This, in turn, could result in a disease suppressive soil.

A disease suppressive soil is a soil in which the level of disease that develops is less than it would be in a conducive soil with similar pathogen populations (Baker 1974). However, it is difficult to measure disease suppression because it is not simply present or absent, but rather it falls on a gradient of levels of suppression in different growing environments. A soil cannot be simply be classified as either conducive or suppressive. In many cases suppression can be related to the presence of microorganisms in the soil, and since most soils have some population level of microorganisms, there is also some level of disease suppression present. Two types of disease suppression, general and specific, have been hypothesized to help better understand the theory of disease suppression (Carreea et al., 2007).

General suppression is defined as a reduction in disease levels as a result of a non-specific increase in the level of activity of the microbial population. Essentially, it is the increase

in the activity of the entire microbial community that is important rather than the activity of a few specific organisms. The competition for resources allows the microbial population to thrive, thereby decreasing the pathogens ability to infect. Specific suppression, as the name suggests, results from an increase in certain microbes that act antagonistically toward plant pathogenic organisms (Eastburn 2010). Both methods of suppression can be attained through the addition of organic matter to a cropping system.

The addition of organic matter to soils has received a lot of attention for its potential to increase disease suppression levels. This is also on a gradient scale because not all types of organic matter have been proven to increase disease suppressiveness. The type of organic matter, weather conditions when the organic matter is applied, and the incorporation method can all lead to changes in the levels of disease suppression, but it is not known which method is best.

One method for adding organic matter to soils is the use of cover crops during the off season of a typical field crop such as soybean. However, the use of cover crops as a method of disease control has primarily been used for high-value crops where other disease management strategies cannot be used. There have been a few studies looking at the effect of cover crops as a disease management tool in soybean systems. The addition of cover crop organic matter to the soil would not only help to control serious soilborne plant pathogens, but this addition could also provide relief from foliar disease pathogens. It is believed that microorganisms associated with roots systemically alter the plant's defense systems to help reduce the development of some foliar diseases (Stone et al., 2004).

As was stated previously, the addition of cover crops to a conventional system would not only help with disease suppressiveness, but can also help to increase important soil qualities. The increased soil organic matter levels from cover crops helps to improve compaction, water and

nutrient holding capacity, air and water filtration, erosion, improved soil structure, and improved habitats for beneficial organisms. Cover crops have been used in soybean systems to improve soil biological, chemical and physical properties (Villamil, 2006). Cover crops have also been used as a method of weed control (Moore, 1994; Krishnan, 1998). This was accomplished by reducing either the weed emergence rate, or lowering the overall weed biomass. Additionally, some cover crops have been shown to lower nematode populations. Creech et al (2008), found that annual ryegrass reduced populations of soybean cyst nematode.

There are three main mechanisms involved in soilborne disease suppression through the use of cover crops. The first mechanism involves the use of cover crops as a biofumigant. Cover crops in the Brassica family have been shown to contain high levels of glucosinolates, which can be toxic to soil pathogens (Zukalova & Vasak, 2002). The second mechanism involves the cover crop inducing host resistance to the pathogen. For instance, hairy vetch was proven to induce suppression to Fusarium wilt in watermelon (Zhou & Everts, 2007). Finally, some cover crop species are able to induce a shift in the soil microbial community structure. The addition of a cover crop to a cropping system can induce competition or antagonistic properties toward soilborne pathogens. Clover, ryegrass, and wheat have all been shown to have a significant effect on changing the soil microbial community. In relation to disease suppression, it is important to know more specifically which microbes are present and are having the greatest effect on lowering the overall disease level of the soil. The addition of cover crops to a production system would not only allow new microbes to flourish, but also the overall microbial population would increase. This could allow for more antagonism and parasitism of pathogenic microbes. In agricultural ecosystems, all three mechanisms are likely to be present to some extent, but disease

suppression can have a larger effect on a pathogen population when all mechanisms are in operation concurrently.

The three most common groups of cover crops that are widely used in agricultural systems are legumes, broad leaf species, and small grains or grass species. Depending on the planting date and type of cash crop grown, the choice of cover crop species to plant may change. In the Midwestern United States, the best cover crop would be planted in late fall following the cash crop harvest. Several cover crops fall under these conditions, but in this study cereal rye, hairy vetch, and mustard were evaluated.

Cereal rye (*Secale cereale*), is a rapidly growing grass plant with a dynamic root system and vigorous seedling. This small grain is one of the hardiest grass species and is planted across many acres in the northern United States because of its ability to tolerate harsh winter conditions. Cereal rye is a common winter cover crop species, used to improve soil structure, reduce compaction, and increase soil organic matter (Hancock, 2012). Cereal rye is also used for weed control due to its allelopathic properties (Hoorman, 2009). Additionally, cereal rye has been proven to be effective against several fungal and nematode diseases (Treonis et al., 2010; Zasada et al., 2007).

Hairy vetch (*Vicia villosa*) is a cover crop species that receives much attention due to its ability to fix nitrogen for the following cash crop. This characteristic has allowed for less nitrogen fertilizer to be applied and a small increase in corn yield was also observed (Utomo et al., 1989). This winter annual species is widely adaptable and easy to establish as a cover crop, and species has also been proven to suppress Fusarium wilt in watermelon (Zhou and Everts, 2004).

Mustard, a member of the Brassica family, is commonly used as a cover crop because of its ability to uptake residual nutrients in the soil and produce high levels of glucosinolates (Chew, 1988). The glucosinolates inhibit seed germination and have the potential to suppress soil-borne pests such as weeds, fungal pathogens, and nematodes (Brown and Morra, 1997). Winter survival rates vary greatly depending on the species, but in order for mustard to be a successful cover crop, it needs to be able to tolerate the cold winter temperatures of different growing regions.

Tillage practices have also been shown to alter the microbial populations of soils. The adoption of no-till and conservation tillage practices over the past several years, has had an impact on the severity and incidence levels of soilborne diseases. No-till can be defined as planting crops with no seedbed preparation except for opening the soil to place the seed (Sumner et al., 1981). Conservation tillage practices reduce the loss of soil or water by performing minimal tillage practices. Reduced tillage systems change the availability of certain soil nutrients which can influence pathogen survival as well. Phosphorus, potassium, zinc, manganese, iron, boron, and copper were all found in higher levels in reduced tillage systems compared to conventional tillage systems (Bailey & Lazarovits, 2003). The amount of tillage preformed, changes the structure of the soil and makes it conducive for certain microbes to survive, while others are not able to do so. Soil communities under conventional tillage generally have altered structural, morphological, and functional profiles compared to communities under no-tillage. Tillage practices directly influence soil physical and chemical properties, and these factors, in turn, may influence the viability of plant pathogens to survive and cause disease. Increased functional biodiversity can support provisioning services while simultaneously conserving or

enhancing a range of soil services, including organic matter decomposition and nutrient turnover, soil carbon storage, and pathogen suppression (Williams et al., 2016).

By changing the structure of the soil, tillage practices have been shown to increase certain levels of disease. Lower levels of soil moisture under conventional tillage do reduce fungal:bacterial ratios (Williams et al., 2016). Soil compaction is linked to higher severity of sudden death syndrome (SDS) in soybean (Hartman et al., 1995). The benefits of adding and incorporating organic matter into a soil for its disease suppressive qualities may be minute, but the benefits are cumulative and may last longer than chemical management strategies (Bailey & Lazarovits, 2003).

Evaluation methods of microbial communities have changed significantly in recent years. There are two broad methods that can be used to study microbial diversity in soil: biochemical-based methods and molecular-based methods. Bio-chemical methods include plate counts and fatty acid analysis among others. These methods are fast and relatively inexpensive, but make studying an entire microbial community structure extremely difficult. Molecular-based methods are growing and changing rapidly as the technology that is used is improved (Kirk et al., 2004). One method may not consistently be better than another, but the method used should reflect the type of analysis being performed and the type results one wishes to find. DNA sequencing has become a widely used method as the technology has been improved to enable researchers to look at specific pathogen or microbial groups. DNA sequencing methods have also helped to eliminate the inability to study a group of organisms based on their inability to be cultured. Illumina high-throughput sequencing methods were used for this study with three primer sets; 16s bacterial, fungal ITS 1-4, and archaea. Illumina sequencing offers whole genome sequencing

and is one of the most reliable methods for evaluating microbial community structures (Caporaso et al., 2012).

**Rationale and Significance**

It is well known that soybean diseases cause severe economic losses to producers around the world. Control of these diseases can be difficult because many of them are soilborne, therefore making it difficult to eliminate all of the inoculum that is present throughout different growing seasons. Some of the most important soilborne diseases include, charcoal rot, Phytophthora rot, Rhizoctonia rot, root knot, soybean cyst, and sudden death syndrome (Wrather, 2006). These diseases require different management strategies each year to keep their populations low, and their impact less substantial. However, producers are not using enough variety in their management strategies, and the potential for resistant strains of these pathogens to develop is becoming increasingly large. Crop rotation can be a useful management technique depending on the pathogen present, and if that pathogen is able to survive in the soil from season to season. A common Illinois crop rotation is strictly corn-soybean, and the pathogens are able to overwinter in neighboring fields and continue to infect year after year. Disease resistant varieties are becoming increasingly more widely used, but there are currently only a few that are commercially available for soilborne pathogens. Overuse of this technology will induce changes in pathogen populations that will allow the pathogen populations to eventually become resistant to the disease resistant variety. The same can also be said when it comes to fungicide use, since concern over resistance is already high in this area (Wrather 2006).

Cover crop residues increase the microbial diversity of the soil. This increase in diversity has been proven to affect the level of root and foliar disease pathogens in several cropping systems. By adding a new crop species to the rotation, producers will not only see added benefits

of weed suppression, improved soil properties, and reduced erosion, but disease levels may also decrease (Chellemi, 2002). It has been difficult to characterize microbial populations because they occur on such a minute level. However, if certain microorganisms can be identified and proven to show specific disease suppressive qualities, producers will have a new pest management tool to implement when conditions are favorable. Cover crops may not be for every producer on every field, but if disease levels are high and the management strategies that are currently being implemented are not working, the use of cover crops may be a viable option.

**Overall Project Objective**

The primary objective of this project was to evaluate the combined effect of cover crop treatments and tillage practices on disease development and the microbial community structure of soybean. The final outcome will give a highly informed recommendation on the benefits of cover crops and/or different tillage practices with respect to disease suppression in soybean cropping systems.

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# CHAPTER 2

## Introduction

Control of soybean diseases can be difficult because many are soilborne, therefore making it difficult to eliminate all of the inoculum that is present throughout different growing seasons. Due to the increasing severity of soybean diseases across the United States, new and effective management strategies are needed to help address this problem.

Cover crops have the possibility to help alleviate some of the disease pressure that occurs from increased inoculum levels in the field. Several studies have been published on the disease suppressive qualities of mustard, cereal rye, and hairy vetch cover crops. Mustard and rye were found to decrease fungal pathogens and nematodes, while hairy vetch has been shown to decrease Fusarium wilt in watermelon (Brown and Morra, 1997; Treonis et al., 2010; Zasada et al., 2007; Zhou and Everts, 2004).

Tillage practices can also alter the microbial communities of some soils. Many characteristics within the soil profile are altered between different tillage practices. Some of these changes include temperature, exposure to sunlight, and compaction intensity (Hartman et al., 1995) Because of these cultural changes, phosphorus, potassium, zinc, manganese, iron, boron, and copper were all found in higher levels in reduced tillage systems compared to conventional tillage systems (Bailey & Lazarovits, 2003). Increased functional biodiversity can support downstream processes while also conserving or enhancing a range of soil services, including organic matter decomposition and nutrient turnover, soil carbon storage, and pathogen suppression (Williams et al., 2016).

This study was conducted to determine the effects that cover crops and tillage treatments have on the levels of disease in a soybean production system. This is a broader study compared

to some of the previous work that was cited since both tillage and cover crop effects were evaluated. However, the overall goal of the study was to determine if a specific tillage treatment or cover crop species had the capability of lowering sudden death syndrome (SDS) disease levels in soybean.

## Materials and Methods

**Design of field experiment:** Trials were conducted over two years, starting in 2014, at the Department of Crop Sciences Research and Demonstration Center at the University of Illinois, Urbana-Champaign. There were four replications/blocks of this experiment each year arranged in a randomized complete block design (RCBD). Within each block, two plots measuring 30.5 m by 6.1 m were used for sampling; one with a chisel-plow treatment, and one with a ridge-till treatment. In the ridge till plots, a high-residue cultivator was used to push the soil from the inter-rows against the crop within the crop row at vegetative stage 8 of corn development (8 leaves; approximately 40.6 cm tall). This process buries the base of the crop and any weeds in the crop row, about 5 cm deep. The plots were re-ridged during the soybean phase of the crop rotation as well, around vegetative stage 6; 6 trifoliate leaves (Pedersen 2007).

The plots were then divided into subplots, and each subplot had a different cover crop treatment. The cover crops used in these trials were cereal rye (*Secale cereale* ‘Aroostook’), mustard (*Sinapis alba* ‘Idagold’), and hairy vetch (*Vicia villosa*). There was also a rye-vetch mixture treatment and a non-cover crop control treatment. The rye, vetch, mustard, and rye/vetch subplots all measured 3.0 m by 6.1 m, and the fallow subplots were 18.3 m by 6.1 m. The cover crops were planted in the fall after the previous corn crop was harvested and a fall tillage application. Cover crop seeding rates were as follows: rye at 134.5 kg/ha, mustard at 16.8 kg/ha, and vetch at 16.8 kg/ha.

Cover crops were terminated with glyphosate (Roundup Weathermax) at a rate of 2.3 L/ha in the spring. Soybeans (cultivar P28T33R) were then planted during the third week of May in both 2014 and 2015 at a rate of 395,200 plants per hectare in .76 m row widths. A post-emergence weed control program contained a herbicide mixture of 2.3 L/ha of glyphosate (Roundup Powermax) and 2.3 L/ha of S-metolachor (Dual). Plots were fertilized during the corn phase of the rotation with 200 kg/ha of N supplied as UAN (urea + ammonium nitrate).

**Root disease rating:** Five soybean plants were carefully dug at random from the soil in each experimental subplot at reproductive stage 7 (beginning maturity - one normal pod on the main stem has reached mature pod color). Excess soil was shaken off of the roots, and then the main taproot was split to expose the root xylem tissue. Sudden death syndrome root rot severity was recorded using a rating scale of 1-5 according to the percentage of root discoloration (Figure 2.1). Roots with 0-20% discoloration were assigned a rating of 1; roots with 21-40% discoloration were assigned a rating of 2; roots with 41-60% discoloration were assigned a rating of 3; roots with 61-80% discoloration were assigned a rating of 4; and roots with 81-100% discoloration were assigned a rating of 5 (Farias et al 2008).

**Soil collection:** Soil samples were collected at soybean vegetative stage 3 (three sets of unrolled trifoliate leaves) following the cover crop growing season. Five soil cores were collected randomly from each subplot, about 2.5-5.1 cm away from the soybean stem using a 91.4 cm soil sampler probe. Cores were collected about 15.2 cm deep and placed into 50 mL Eppendorf tubes. The tubes were placed in a cooler and then taken back to the lab and stored at -80°C. Once back in the lab, 2g subsamples from each sample were pooled together to represent each subplot. These samples represented the bulk soil and will be referred to as such from now on.

Five soybean seedlings were selected randomly from each subplot and dug up using a hand trowel at vegetative growth stage 3. The excess soil was shaken off of the roots, and the roots were put into 50 mL Eppendorf tubes. The tubes were then placed in a cooler and brought back to the lab. To extract the rhizospheric soil off of the root systems, all 5 roots from each subplot were combined into 1 Eppendorf tube. Then, 30 mL of a phosphate buffer solution (pH=7) was added to each tube. The tubes were shaken by hand for 1 minute to release the soil from the root systems. The roots were then removed from the tubes and the soil suspension was centrifuged at 8,000 rpm for 10 minutes. The buffer solution was poured off and the soil pellet was collected and used to represent the rhizospheric soil. The washed roots were then placed in new 50 mL Eppendorf tubes. All samples were stored at -80°C until DNA extraction took place.

**Root collection:** After washing the roots in the phosphate buffer solution, the root samples were freeze-dried for 24 hours. Once dried, 0.5 g of root tissue was moved to a 2 mL microcentrifuge tube, and two 0.5 mm diameter balls (Daisy stainless steel bbs) were added to each tube. The tubes were placed in liquid nitrogen for 20 seconds. A BioSpec mini-bead beater homogenizer was then used to pulverize the root tissue for 30 seconds. The pulverized root tissue was stored at -80°C until DNA extraction took place.

**DNA extraction:** Total DNA was extracted from all bulk and rhizospheric soil samples, and from soybean roots taken from the rye and fallow subplots. All DNA was extracted using FastDNA SPIN Kits (MP Biomedicals, Solon, Ohio), following the manufacturers protocol. Upon extraction, all DNA samples were stored at -20˚C until polymerase chain reaction (PCR) and further tests were run. PCR was performed on 10 random samples to check for quality of DNA. A NanoDrop machine was then used to quantify the amount of DNA in each sample. Each

sample was diluted to 30 ng DNA/µl, and a 10 µl aliquot of each sample was arranged on a 96-well PCR plate.

**Sequencing:** The PCR plates were sent to the W. M. Keck Center at the University of Illinois for microbial community analysis using Illumina sequencing based on the 16s RNA sequences. Eight primers were used by the Keck Center in the analysis. The primers are as follows: V3-V5 (ACACTGACGACATGGTTCTACACCTACGGGAGGCAGCAG), Archaea (ACACTGACGACATGGTTCTACAGYGCASCAGKCGMGAAW), fungal ITS 1-4 (ACACTGACGACATGGTTCTACATTCGTAGGTGAACCTGCGG), ammonia monooxygenase bacterial (ACACTGACGACATGGTTCTACAGGGGTTTCTACTGGTGGT), nitrous oxide reductase-typical (ACACTGACGACATGGTTCTACAWCSYTGTTCMTCGACAGCCAG), nitrous oxide reductase-atypical (ACACTGACGACATGGTTCTACASGGCTAYGGCTWYGAYGA), ammonia monooxygenase Archaea (ACACTGACGACATGGTTCTACAATGGTCTGGCTWAGACG), ammonia generating nitrite reductase (ACACTGACGACATGGTTCTACACARTGYCAYGTBGARTA). Each primer was used for evaluating each sample. The primers identify microbes that are present in each sample. Further analysis was then needed to specifically identify and quantify the amount of each microbe in the soil samples. Only the Archaea 16S, Bacterial 16S, and Fungal ITS 1-4 data was analyzed for this project.

## Analysis

**Microbial community analysis:** The workflow from IM-TORNADO (Illinois Mayo Taxon Organization from RNA Dataset Operation) was used to pair the reads produced during the Illumina Sequencing protocol. Once the reads were paired, the QIIME 454 overview

workflow was used for OTU picking and further analysis. OTU graphs were put together based on the QIIME output and the top phyla from each primer were compared between all treatments.

**Statistical analysis**: Analysis of variance (ANOVA) was performed to analyze the effects of cover crops and tillage on SDS disease severity levels and soybean yield using Proc GLM in JMP Version 9.0.2 (SAS Institute Inc., Cary, NC). A model was used to fit the data to the RCBD layout of the experiment and was as follows: rating = tillage|cover crop. The blocking effect was random, while the rest of the effects were fixed. An LS means Tukey adjustment was done to compare treatment effects.

The four replications for each treatment were combined into one DNA extraction sample. This was done to keep the cost for the experiment lower, and to allow for further exploration in the subsequent greenhouse study. For these reasons, statistical analysis of the OTU data is not available.

## Results

**Soybean yield:** Soybean yield was significantly different between tillage treatments within each year, 2014 and 2015 (Table 2.1). In 2014, there was a significant decrease in yield in the ridge tilled plots compared to the chisel plowed plots. This is expected since the ridge tilled plots had more SDS severity (Table 2.2). In 2015, the chisel plowed plots yielded less than the ridge tilled plots (Table 2.1). Sudden death syndrome severity levels were higher in the chisel plowed plots in 2015, which could explain the decrease in yield (Table 2.3).

**Disease rating:** There was significantly more disease in the field in 2014 compared to 2015 (Tables 2.2 and 2.3). Sudden death syndrome disease severity ratings between cover crop treatments were not significantly different in 2014 (Table 2.2). However, there were differences

in 2015. Plants in the rye+vetch plots which had significantly less disease than those in plots with all of the other cover crop treatments (Table 2.3).

**Microbial community results:** Because there were no significant differences between disease severity levels in the cover crop treatments in 2014, analysis of the microbial communities between cover crop treatments was not completed for that year. However, disease severity levels did vary between the cover crop treatments in 2015, so a microbial community analysis was conducted.

Using the Archaea 16S primer, 4 phyla were found in significant levels in the DNA samples. Those 4 phyla are: *Crenarchaeota, Euyarchaeota, Plactomycetes,* and *Verrucomicrobia*. Operational taxonomic unit data shows the percentage of each reported phyla that was found in each sample. In 2014 and 2015, there were no differences between the microbial communities within the respective year between tillage types (Tables 2.4, 2.5, and 2.6). However, when looking within each cover crop treatment between years, several shifts in the microbial community structure occurred within each DNA type (bulk soil, rhizospheric soil, and soybean root). Looking at the bulk soil DNA, almost all of the Archaea reported were in the *Crenarchaeota* phyla in 2014 (Table 2.4). Yet, in 2015, the reported OTUs were split between the *Euyarchaeota* and the *Verrucomicrobia*. The same pattern occurred in the ridge tilled plots, and in the rhizospheric soil and soybean root DNA types (Tables 2.5 and 2.6).

The same 4 Archaeal phyla were detected between the cover crop treatments in 2015. No differences were found within each DNA type between cover crop treatments (Table 2.7). However, there were only slight differences between the microbial communities in the rye and fallow plots in the soybean root DNA. Higher levels of *Verrucomicrobia* were found in the rye plots, and higher levels of *Crenarchaeota* were found in the fallow plots (Table 2.7).

Ten phyla were found to be present in the highest levels in all samples when the 16S Bacterial primer set was used. The 10 phyla are: *Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Proteobacteria, Verrucomicrobia*. In 2014 and 2015, there were no differences between the microbial communities within the respective year between tillage types (Tables 2.8, 2.9, and 2.10). There were also no differences between the microbial communities between years in the bulk soil (Table 2.8), or the rhizospheric soil (Table 2.9). However, a shift in the microbial community occurred in the root DNA samples within tillage treatments, between years. In 2014, the *Proteobacteria* was reported at the highest level, while in 2015, the *Cyanobacteria* were reported at the highest level in both the chisel plowed and ridge tilled plots (Table 2.10). No differences were found between cover crop treatments when the 16S bacterial primer set was used (Table 2.10).

Six phyla were detected when using the ITS 1-4 fungal primer set. Those phyla include: *Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Zygomycota,* and an unclassified group. The unclassified group includes organisms that are being shifted between groups due to the new naming system, or organisms that have been newly discovered but have not yet been placed in a specific phylum. In 2014 and 2015, there were no differences between the microbial communities within the respective year between tillage treatments (Tables 2.12, 2.13, and 2.14). There were also no differences between the fungal microbial communities between years in the bulk soil (Table 2.12). When evaluating the microbial community structure in the rhizospheric soil, there was a difference in the level of *Ascomycota* organisms present between years in both the ridge tilled and chisel plowed plots (Table 2.13). With the soybean root DNA even more differences between the soil communities were detected. In the chisel plowed and ridge tilled

plots, there were higher levels of *Zygomycota* in 2014, and higher levels of *Basidiomycota* in 2015 (Table 2.14).

No differences were found between the cover crop treatments when using the ITS 1-4 Fungal primer set for the bulk and rhizospheric soil (Table 2.15). However, within the soybean root DNA, the mustard plots had higher levels of *Ascomycota* but lower levels of *Zygomycota* compared to the fallow plots.

**Discussion**

The levels of disease and yield varied between tillage treatments in 2014 and 2015. The higher level of disease could explain why the yield was effected so much more in 2015 in the chisel plowed plots. Populations of *Fusarium virguliforme*, the causal agent of SDS, thrived in the cool and wet early part of the growing season, which allowed for high infection rates, and therefore more disease later in the growing season. The ridge tilled plots also had lower soil moisture due to the composition of the soil profile (Williams 2016). This helps to explain why there was less disease in the ridge tilled plots in 2015, even though high rainfall totals accumulated. The microbial community analysis between each primer set can also help to explain these differences.

Due to the large size of each microbial community, the phylum level was chosen to differentiate between samples. The techniques and computer analysis programs allow for differentiation as specific as species, but the differences between treatments become more difficult to understand and interpret. By looking at the phylum level, it was hypothesized that differences between the tillage and cover crop treatments would be evident. By also completing disease severity ratings, I could look for different phyla that are prominent in a higher disease

environment verses a lower disease environment. I could then determine which phyla are responsible for lowering, or increasing, disease severity levels in the field.

When evaluating the microbial communities between years, but within tillage treatments using all three primer sets, there are several differences that can be noted. First, in 2014 there were higher levels of *Crenarchaeota, Proteobacteria,* and *Zygomycota*, as compared to 2015 in all three DNA types. However, in 2015, there were higher levels of *Euyarchaeota, Verrucomicrobia, Cyanobacteria*, and *Basidiomycota* in all three DNA types. Since there was more disease in 2015 in all plots, it is possible that the *Euyarchaeota, Verrucomicrobia, Cyanobacteria, and Basidiomycota* phyla are indicative of a soil that is conducive to SDS in soybean. However, since there were no significant differences between the tillage treatments in any of the DNA types, I cannot hypothesize about which tillage environment is more suppressive toward *Fusarium virguliforme*, the causal agent of sudden death syndrome.

When looking at the microbial populations in the cover crop treatments in 2015, several differences can be found as well. Rye plots had higher levels of *Verrucomicrobia*, mustard plots had higher levels of *Ascomycota*, and fallow plots had higher levels of *Crenarchaeota* and *Zygomycota* all within DNA extracted from the soybean roots. There were no differences in microbial community structure between the cover crop treatments in the bulk or rhizospheric soil, at least at the phylum level. These differences show that there may be disease suppressive mechanisms at work in each tillage and cover crop environment, but the inconsistency of the disease severity results makes those differences difficult to detect. Rye, mustard, and fallow plots all had the same disease severity ratings statistically, so I was not able to determine which cover crop treatment is better at suppressing sudden death syndrome.

Multiple studies have been conducted to evaluate the effect of cover crop treatments on suppressing pathogens, but with very mixed results. Mazzola and Brown (2010) found that mustard and various other brassica species suppressed the development of *R. solani* that causes apple root rot. In a different study in 2009 it was found that only ryegrass had a significant effect on suppressing *S. sclerotiorum*, but none of the other cover crops tested, including mustard, had an effect on suppressing *R. solani* (Martinez, 2013). A study in Washington state found that increased populations of fluorescent *Pseudomonas* spp. bacteria, result in higher levels of take-all decline in wheat (Weller, 2002). All three of these studies merged the academic disciplines of plant pathology with microbiology and molecular techniques. This realization leads to the possibility of dissecting microbial communities and the extremely complex interactions in disease suppressive soils.

Soil suppression to soilborne pathogens was observed in this study, although the effect of the different tillage and cover crop treatments varied, and the result was not consistent over time. The mechanisms of soil suppression induced by cover crops are complex, and there are likely multiple biological factors interacting to produce the suppressive qualities. Additionally, cover crops may not suppress diseases through reducing the total pathogen populations, and general suppression may play a more important role in reducing disease levels. More consistent results may be achieved with greater cover crop biomass and a longer implementation of the cover crop management program.

Furthermore, soil suppressive qualities are not the only benefits that cover crops could bring to a field crop management program. Long term cover cropping will help improve soil structure, increase soil organic matter, and reduce soil erosion among other benefits (Frank and Murphey, 1977; Pedersen and Hughes, 1992).

Further research is needed to better demonstrate the effect of cover crops on suppressing soilborne diseases of soybean. A longer-term cover crop rotation may increase microbial populations for each cover crop species, which could increase differences between the different cover crop treatments. A study that is developed specifically for cover crop aspect rather than the cash crop rotation could also help to ensure timely planting and harvesting of cover crops. This would allow for greater overall biomass which could alter microbial populations as well. As technology also develops and expands, new methods for studying microbial communities may be discovered that will allow for increased credibility and specificity of the results.

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## Tables and Figures

Figure 2.1: sudden death syndrome (SDS) disease rating scale used to rate soybean roots. A 1 to 5 scale was used to rate SDS severity based on root discoloration: 1 = 0-20% discoloration 2 = 21-40% discoloration = 2, 3 = 41-60% discoloration, 4 = 61-80% discoloration5 = 81-100% discoloration.

5 4 3 2 1

Table 2.1. Soybean yield responses to cultivation treatments in 2014 and 2015.

| **Tillage Treatment** | **Soybean Yield Levels* (kg/ha)** | |
|---|---|---|
| | **2014** | **2015** |
| Ridge Till | 275 a | 411 b |
| Chisel Plow | 318 b | 293 a |

* Yield values averaged over cover crop sub-treatments and replications. Values were adjusted to 13% moisture equivalents.

** Values (within years) followed by the same letter are not significantly different based on an LSD test at $\alpha = 0.10$.

Table 2.2. Cultivation cover crop treatment effects on sudden death syndrome severity ratings in 2014.

| **Tillage Treatment** | **Mean Severity Rating*** | |
|---|---|---|
| Ridge Till | 1.45 | a** |
| Chisel Plow | 0.83 | b |
| | | |
| **Cover Crop Treatment** | | |
| Rye | 1.15 | a |
| Vetch | 0.95 | a |
| Mustard | 0.98 | a |
| Rye/Vetch | 1.03 | a |
| Fallow | 1.00 | a |

* A 1 to 5 scale was used to rate SDS severity based on root discoloration: 1 = 0-20% discoloration 2 = 21-40% discoloration = 2, 3 = 41-60% discoloration, 4 = 61-80% discoloration5 = 81-100% discoloration.

** Values followed by the same letter are not significantly different based on an LSD test at $\alpha$=0.10.

Table 2.3: Cultivation and cover crop treatment effects on sudden death syndrome severity ratings in 2015.

| **Tillage Treatment** | **Mean Severity Rating*** |
|---|---|
| Ridge Till | 3.86 a** |
| Chisel Plow | 4.11 b |
| | |
| **Cover Crop Treatment** | |
| Rye | 3.9 ab |
| Vetch | 3.9 ab |
| Mustard | 4.2 a |
| Rye/Vetch | 3.65 bc |
| Fallow | 4.275 a |

*A 1 to 5 scale was used to rate SDS severity based on root discoloration: 1 = 0-20% discoloration 2 = 21-40% discoloration = 2, 3 = 41-60% discoloration, 4 = 61-80% discoloration5 = 81-100% discoloration.
** Values followed by the same letter are not significantly different based on an LSD test at α= 0.10.

Table 2.4: Operational taxonomic unit determinations for the 16s Archaea primer set used on DNA extracted from bulk soil. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Crenarchaeota* | 95.62 | 0.35 |
| | *Euryarchaeota* | 4.03 | 50.31 |
| | *Plactomycetes* | 0 | 0.84 |
| | *Verrucomicrobia* | 0.35 | 48.04 |
| | | | |
| Ridge Till | *Crenarchaeota* | 95.57 | 0.14 |
| | *Euryarchaeota* | 4.07 | 51.29 |
| | *Plactomycetes* | 0 | 0.39 |
| | *Verrucomicrobia* | 0.36 | 47.44 |

Table 2.5: Operational taxonomic unit determinations for the 16s Archaea primer set used on DNA extracted from rhizospheric soil. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Crenarchaeota* | 94.04 | 1.52 |
| | *Euryarchaeota* | 4.63 | 54.81 |
| | *Plactomycetes* | 00.02 | 1.29 |
| | *Verrucomicrobia* | 1.31 | 41.56 |
| | | | |
| Ridge Till | *Crenarchaeota* | 94.08 | 0.94 |
| | *Euryarchaeota* | 4.53 | 54.66 |
| | *Plactomycetes* | 0.02 | 0.93 |
| | *Verrucomicrobia* | 1.37 | 42.98 |

Table 2.6: Operational taxonomic unit determinations for the 16s Archaea primer set used on DNA extracted from soybean root DNA. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Crenarchaeota* | 94.03 | 0 |
| | *Euryarchaeota* | 2.40 | 11.86 |
| | *Plactomycetes* | 0 | 0 |
| | *Verrucomicrobia* | 3.57 | 88.14 |
| | | | |
| Ridge Till | *Crenarchaeota* | 94.05 | 0 |
| | *Euryarchaeota* | 2.37 | 5.94 |
| | *Plactomycetes* | 0 | 2.21 |
| | *Verrucomicrobia* | 0 | 91.16 |

Table 2.7: Operational taxonomic unit determinations for the 16s Archaea primer set. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | | |
|---|---|---|---|---|
| **Cover Crop** | **Phylum** | **Bulk Soil** | **Rhizospheric Soil** | **Soybean Root** |
| Rye | *Euryarchaeota* | 0.04 | 0.49 | 0 |
| | *Crenarchaeota* | 50.05 | 53.89 | 4.43 |
| | *Plactomycetes* | 0.22 | 0.35 | 0.11 |
| | *Verrucomicrobia* | 49.49 | 45.00 | 95.43 |
| | | | | |
| Vetch | *Euryarchaeota* | 0.06 | 1.79 | |
| | *Crenarchaeota* | 50.75 | 54.39 | |
| | *Plactomycetes* | 1.18 | 1.04 | |
| | *Verrucomicrobia* | 46.96 | 42.33 | |
| | | | | |
| Mustard | *Euryarchaeota* | 1.05 | 1.54 | |
| | *Crenarchaeota* | 52.68 | 55.31 | |
| | *Plactomycetes* | 1.07 | 1.27 | |
| | *Verrucomicrobia* | 44.93 | 40.81 | |
| | | | | |
| Rye+Vetch | *Euryarchaeota* | 0.07 | 2.10 | |
| | *Crenarchaeota* | 49.98 | 53.84 | |
| | *Plactomycetes* | 0.36 | 1.69 | |
| | *Verrucomicrobia* | 48.40 | 41.05 | |
| | | | | |
| Fallow | *Euryarchaeota* | 0.01 | 0.23 | 0 |
| | *Crenarchaeota* | 50.53 | 56.24 | 13.36 |
| | *Plactomycetes* | 0.25 | 1.19 | 2.10 |
| | *Verrucomicrobia* | 48.94 | 42.16 | 83.88 |

Table 2.8: Operational taxonomic unit determinations for the 16s Bacteria primer set used on DNA extracted from bulk soil. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Acidobacteria* | 10.71 | 19.08 |
| | *Actinobacteria* | 17.61 | 25.70 |
| | *Bacteriodetes* | 3.15 | 4.63 |
| | *Chloroflexi* | 4.35 | 7.34 |
| | *Cyanobacteria* | 4.47 | 0.67 |
| | *Firmicutes* | 7.32 | 2.27 |
| | *Gemmatimonadetes* | 1.36 | 4.39 |
| | *Nitrospirae* | 0 | 1.51 |
| | *Proteobacteria* | 49.19 | 32.35 |
| | *Verrucomicrobia* | 0 | 0.30 |
| | | | |
| Ridge Till | *Acidobacteria* | 0.39 | 19.91 |
| | *Actinobacteria* | 17.23 | 25.02 |
| | *Bacteriodetes* | 5.61 | 3.59 |
| | *Chloroflexi* | 4.21 | 8.20 |
| | *Cyanobacteria* | 4.42 | 0.67 |
| | *Firmicutes* | 7.14 | 2.10 |
| | *Gemmatimonadetes* | 1.33 | 4.39 |
| | *Nitrospirae* | 0 | 1.62 |
| | *Proteobacteria* | 47.88 | 32.29 |
| | *Verrucomicrobia* | 0 | 0.32 |

Table 2.9: Operational taxonomic unit determinations for the 16s Bacteria primer set used on DNA extracted from rhizospheric soil. Values are percentages of each Phyla in the given sample.

| | | Percentage of Total Population | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Acidobacteria* | 13.42 | 11.63 |
| | *Actinobacteria* | 19.41 | 29.26 |
| | *Bacteriodetes* | 0.38 | 4.61 |
| | *Chloroflexi* | 5.38 | 6.09 |
| | *Cyanobacteria* | 3.71 | 3.83 |
| | *Firmicutes* | 5.28 | 1.65 |
| | *Gemmatimonadetes* | 1.69 | 2.15 |
| | *Nitrospirae* | 0 | 1.16 |
| | *Proteobacteria* | 45.41 | 37.58 |
| | *Verrucomicrobia* | 0 | 0.44 |
| | | | |
| Ridge Till | *Acidobacteria* | 13.35 | 11.83 |
| | *Actinobacteria* | 19.67 | 29.16 |
| | *Bacteriodetes* | 3.77 | 3.10 |
| | *Chloroflexi* | 5.31 | 6.22 |
| | *Cyanobacteria* | 3.73 | 7.85 |
| | *Firmicutes* | 5.26 | 1.57 |
| | *Gemmatimonadetes* | 1.67 | 2.07 |
| | *Nitrospirae* | 0 | 1.11 |
| | *Proteobacteria* | 4.53 | 34.89 |
| | *Verrucomicrobia* | 0 | 0.39 |

Table 2.10: Operational taxonomic unit determinations for the 16s Bacteria primer set used on DNA extracted from soybean root DNA. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Acidobacteria* | 0.61 | 0.32 |
| | *Actinobacteria* | 1.16 | 2.48 |
| | *Bacteriodetes* | 5.26 | 1.17 |
| | *Chloroflexi* | 0.29 | 0.43 |
| | *Cyanobacteria* | 3.65 | 71.95 |
| | *Firmicutes* | 5.03 | 0.07 |
| | *Gemmatimonadetes* | 0.09 | 0.10 |
| | *Nitrospirae* | 0 | 0.01 |
| | *Proteobacteria* | 83.22 | 23.33 |
| | *Verrucomicrobia* | 0 | 0.06 |
| | | | |
| Ridge Till | *Acidobacteria* | 0 | 0.21 |
| | *Actinobacteria* | 0.58 | 2.13 |
| | *Bacteriodetes* | 5.25 | 0.83 |
| | *Chloroflexi* | 0.02 | 0.21 |
| | *Cyanobacteria* | 3.45 | 71.69 |
| | *Firmicutes* | 4.68 | 0.02 |
| | *Gemmatimonadetes* | 0 | 0.05 |
| | *Nitrospirae* | 0 | 0 |
| | *Proteobacteria* | 85.98 | 24.78 |
| | *Verrucomicrobia* | 0 | 0.02 |

Table 2.11: Operational taxonomic unit determinations for the 16s Bacteria primer set. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | | |
|---|---|---|---|---|
| **Cover Crop** | **Phylum** | **Bulk Soil** | **Rhizospheric Soil** | **Soybean Root** |
| Rye | *Acidobacteria* | 20.26 | 11.74 | 0.24 |
| | *Actinobacteria* | 24.00 | 28.10 | 2.07 |
| | *Bacteroidetes* | 4.23 | 3.88 | 0.88 |
| | *Chloroflexi* | 7.77 | 6.00 | 0.30 |
| | *Cyanobacteria* | 0.55 | 8.03 | 73.81 |
| | *Firmicutes* | 2.20 | 1.22 | 0.06 |
| | *Gemmatimonadetes* | 4.32 | 2.05 | 0.07 |
| | *Nitrospirae* | 1.83 | 1.31 | 0.01 |
| | *Proteobacteria* | 32.58 | 35.58 | 22.46 |
| | *Verrucomicrobia* | 0.29 | 0.40 | 0.05 |
| | | | | |
| Vetch | *Acidobacteria* | 19.58 | 11.98 | |
| | *Actinobacteria* | 26.44 | 28.88 | |
| | *Bacteroidetes* | 3.66 | 3.65 | |
| | *Chloroflexi* | 7.54 | 6.57 | |
| | *Cyanobacteria* | 0.61 | 6.59 | |
| | *Firmicutes* | 2.62 | 1.56 | |
| | *Gemmatimonadetes* | 4.37 | 2.28 | |
| | *Nitrospirae* | 1.41 | 1.39 | |
| | *Proteobacteria* | 31.92 | 34.95 | |
| | *Verrucomicrobia* | 0.30 | 0.41 | |
| | | | | |
| Mustard | *Acidobacteria* | 18.49 | 11.71 | |
| | *Actinobacteria* | 26.11 | 29.49 | |
| | *Bacteroidetes* | 4.17 | 3.81 | |
| | *Chloroflexi* | 7.84 | 5.60 | |
| | *Cyanobacteria* | 0.79 | 4.45 | |
| | *Firmicutes* | 2.32 | 1.78 | |
| | *Gemmatimonadetes* | 4.49 | 1.97 | |
| | *Nitrospirae* | 1.57 | 1.00 | |
| | *Proteobacteria* | 32.26 | 37.94 | |
| | *Verrucomicrobia* | 0.25 | 0.58 | |

Table 2.11 (cont.)

| | | **Percentage of Total Population** | | |
|---|---|---|---|---|
| **Cover Crop** | **Phylum** | **Bulk Soil** | **Rhizospheric Soil** | **Soybean Root** |
| Rye + Vetch | *Acidobacteria* | 19.04 | 10.57 | |
| | *Actinobacteria* | 25.43 | 29.35 | |
| | *Bacteroidetes* | 4.82 | 3.51 | |
| | *Chloroflexi* | 7.54 | 5.83 | |
| | *Cyanobacteria* | 0.55 | 8.33 | |
| | *Firmicutes* | 1.93 | 1.60 | |
| | *Gemmatimonadetes* | 4.37 | 2.07 | |
| | *Nitrospirae* | 1.38 | 1.02 | |
| | *Proteobacteria* | 32.70 | 35.88 | |
| | *Verrucomicrobia* | 0.35 | 0.38 | |
| | | | | |
| Fallow | *Acidobacteria* | 20.11 | 12.65 | 0.29 |
| | *Actinobacteria* | 24.82 | 30.22 | 2.55 |
| | *Bacteroidetes* | 3.66 | 4.40 | 1.12 |
| | *Chloroflexi* | 8.13 | 6.78 | 0.35 |
| | *Cyanobacteria* | 0.85 | 1.80 | 69.83 |
| | *Firmicutes* | 1.86 | 1.91 | 0.03 |
| | *Gemmatimonadetes* | 4.40 | 2.20 | 0.08 |
| | *Nitrospirae* | 1.65 | 0.97 | 0 |
| | *Proteobacteria* | 32.14 | 36.82 | 25.66 |
| | *Verrucomicrobia* | 0.34 | 0.32 | 0.04 |

Table 2.12: Operational taxonomic unit determinations for the ITS 1-4 Fungal primer set used on DNA extracted from bulk soil. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Ascomycota* | 64.69 | 58.19 |
| | *Basidiomycota* | 6.99 | 9.62 |
| | *Chytridiomycota* | 0.01 | 0 |
| | *Glomeromycota* | 4.63 | 0.44 |
| | *Zygomycota* | 21.39 | 30.52 |
| | Unclassified | 2.27 | 1.23 |
| | | | |
| Ridge Till | *Ascomycota* | 62.06 | 55.18 |
| | *Basidiomycota* | 6.99 | 0.84 |
| | *Chytridiomycota* | 0.01 | 0 |
| | *Glomeromycota* | 4.23 | 0 |
| | *Zygomycota* | 24.43 | 43.49 |
| | Unclassified | 2.27 | 0.50 |

Table 2.13: Operational taxonomic unit determinations for the ITS 1-4 Fungal primer set used on DNA extracted from rhizospheric soil. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Ascomycota* | 43.63 | 78.79 |
| | *Basidiomycota* | 9.97 | 3.35 |
| | *Chytridiomycota* | 0.89 | 0 |
| | *Glomeromycota* | 6.08 | 0.12 |
| | *Zygomycota* | 37.55 | 17.52 |
| | Unclassified | 1.87 | 0.22 |
| | | | |
| Ridge Till | *Ascomycota* | 43.12 | 76.28 |
| | *Basidiomycota* | 9.97 | 5.50 |
| | *Chytridiomycota* | 0.89 | 0 |
| | *Glomeromycota* | 6.08 | 0 |
| | *Zygomycota* | 38.07 | 17.99 |
| | Unclassified | 1.87 | 0.23 |

Table 2.14: Operational taxonomic unit determinations for the ITS 1-4 Fungal primer set used on DNA extracted from soybean root DNA. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | |
|---|---|---|---|
| **Tillage** | **Phylum** | **2014** | **2015** |
| Chisel Plow | *Ascomycota* | 74.25 | 62.24 |
| | *Basidiomycota* | 0 | 37.76 |
| | *Chytridiomycota* | 0 | 0 |
| | *Glomeromycota* | 0.39 | 0 |
| | *Zygomycota* | 23.93 | 0 |
| | Unclassified | 0 | 0 |
| | | | |
| Ridge Till | *Ascomycota* | 72.11 | 90.91 |
| | *Basidiomycota* | 1.29 | 9.09 |
| | *Chytridiomycota* | 0 | 0 |
| | *Glomeromycota* | 0 | 0 |
| | *Zygomycota* | 22.05 | 0 |
| | Unclassified | 4.55 | 0 |

Table 2.15: Operational taxonomic unit determinations for the ITS 1-4 Fungal primer set. Values are percentages of each Phyla in the given sample.

| | | **Percentage of Total Population** | | |
|---|---|---|---|---|
| **Cover Crop** | **Phylum** | **Bulk Soil** | **Rhizospheric Soil** | **Soybean Root** |
| Rye | *Ascomycota* | 65.23 | 77.64 | 75.31 |
| | *Basidiomycota* | 3.75 | 4.75 | 24.69 |
| | *Glomeromycota* | 0 | 0 | 0 |
| | *Zygomycota* | 31.02 | 17.61 | 0 |
| | Unclassified | 0 | 0 | 0 |
| | | | | |
| Vetch | *Ascomycota* | 50.59 | 71.57 | |
| | *Basidiomycota* | 0 | 5.26 | |
| | *Glomeromycota* | 0.89 | 0 | |
| | *Zygomycota* | 48.04 | 22.59 | |
| | Unclassified | 0.48 | 0.57 | |
| | | | | |
| Mustard | *Ascomycota* | 59.24 | 85.27 | |
| | *Basidiomycota* | 8.62 | 1.71 | |
| | *Glomeromycota* | 0.20 | 0.30 | |
| | *Zygomycota* | 31.94 | 12.72 | |
| | Unclassified | 0 | 0 | |
| | | | | |
| Rye+Vetch | *Ascomycota* | 54.43 | 82.15 | |
| | *Basidiomycota* | 11.19 | 1.84 | |
| | *Glomeromycota* | 0 | 0 | |
| | *Zygomycota* | 30.55 | 16.01 | |
| | Unclassified | 3.83 | 0 | |
| | | | | |
| Fallow | *Ascomycota* | 53.95 | 66.22 | 74.29 |
| | *Basidiomycota* | 2.58 | 9.91 | 25.53 |
| | *Glomeromycota* | 0 | 0 | 0 |
| | *Zygomycota* | 43.47 | 23.85 | 0 |
| | Unclassified | 0 | 0.02 | 0 |

## CHAPTER 3

### Introduction

Previous research has suggested that cover crops may be a viable option for disease control (Brown and Morra, 1997; Treonis et al., 2010; Weller et al, 2002; Zasada et al., 2007; Zhou and Everts, 2004). However, significant cover crop biomass is needed to prove these theories. The more cover crop biomass that is incorporated into the soil, more "food" will become available to the microbial populations. This will allow for antagonistic or synergistic relationships to develop as the microbes compete for the same food source (Eastburn 2010). Due to the poor establishment of the cover crops in the field experiment, a greenhouse assay was developed to further explore the differences in the microbial populations between cover crop treatments. The cover crops in the field experiment were not planted early enough in the fall to allow for adequate growth before the first frost. This late planting did not allow the cover crops to become established well enough to survive the winter months and therefore, the overall cover crop biomass across the plots was quite low. By not having enough biomass, the microbial populations were not significantly different from those detected in the fallow treatment plots. Since many previous studies have proven that cover crops contain disease suppressive qualities, further research was initiated.

The objective of this study was to see if the microbial populations in the soil were altered and better able to control soilborne diseases, by adding more cover crop biomass. By giving the cover crops a full five weeks to grow in optimal growing conditions, an alteration of the microbial community structure would have a better chance of becoming established. This would allow for populations to increase and antagonistic or synergist relationships to develop and thrive, thereby possible producing a soybean disease suppressive environment.

## Materials and Methods

**Field soil collection:** Soil from the Department of Crop Sciences Research and Demonstration Center at the University of Illinois, Urbana-Champaign was collected for use in this assay. Four, 3.8 L samples of soil were collected from each chisel plowed subplot using a hand trowel following the soybean harvest in 2015. Plant debris was removed from the surface and samples were collected randomly from each subplot. Samples were placed into plastic bags, and they were brought back to the lab and stored in a cold room (4-10°C) until used for the greenhouse study.

**Preparation of inoculum**: Clean, red sorghum seed was soaked in water for 24 hours. Floating sorghum debris was removed, and the water was drained. Approximately 2.3 kg of soaked sorghum was moved into a Fisher brand, translucent, 2 milliliter, 61 cm by 91.4 cm bag. Most of the air was removed and the bag was sealed with a 50 mm foam stopper and a 20.3 cm long zip tie about 17.8 cm from the top of the bag. The bag was sealed tightly enough to hold the foam stopper in place but not to impede air exchange. Four bags of sorghum seed were prepared. The bags were autoclaved for 1 hour at 121°C and 18 PSI. The bags were then removed and allowed to cool at room temperature for 24 hours. The autoclave process was then repeated.

An aggressive *Fusarium virguliforme* isolate, Mont 1, was obtained from Dr. Glen Hartman's Lab, University of Illinois Urbana-Champaign (Hartman 1997). The isolate was transferred to potato dextrose agar (PDA) and allowed to grow for two weeks in the dark at 28°C. The colony was sliced into small, 1 $cm^2$ squares under a transfer hood. While under the transfer hood, the tops of the bags, below the foam stopper, were cut off. Pieces of the infested PDA agar were placed into the bags with the sorghum, 1 plate per bag. Bags were then resealed with a new, sterilized foam plug and zip tie. Bags were mixed by hand to distribute the PDA

throughout the bag. Bags were left at room temperature, in normal, lab lighting for 2 weeks, and the contents were mixed every 2 days. Bags were then opened, and the infested sorghum seed was spread out onto a drying tray. Any clumps were broken up, and the trays were placed in a forced-air seed dryer for 3 days. The infested sorghum was ground up using a number 60 power grist mill (C.S. Bell Company Tiffin, OH). The inoculum was then stored in a cold room (4-10°C) until use.

*Rhizoctonia solani* inoculum was collected from Dr. Carl Bradley's lab, University of Illinois Urbana-Champaign, and was used in this assay. Isolate 65L-2 was used for this research (Liu and Sinclair 1991). The preparation of the inoculum was the same as what was used for the *F. virguliforme* inoculum.

**Design of greenhouse assay**: Two replications of this experiment were completed using a randomized complete block design (RCBD). Four bags of each soil treatment were brought into the greenhouse for each trial. Three liters of soil were placed into a 3.8 L pots. The pots were marked with the type of cover crop soil that was used. There were 4 pots of each soil treatment. Cover crops were then planted at the following rates into their respective pots: 3.45g/pot of cereal rye, 0.43g/pot of mustard, and 0.43g/pot of vetch. The remaining soil from each bag, approximately 0.8 L was added to the top of each pot. The cover crops grew for 5 weeks and were watered every 2 days. The greenhouse was set to maintain a 24 hour temperature of 24-26°C and a photoperiod of 14:45 hours. High pressure sodium, 1000 watt bulbs maintained a light threshold of 700 $Wm^2$. After 5 weeks of growth, the cover crops were terminated with glyphosate (Roundup) at a rate of 2.3 L/ha.

One week after the herbicide application, pots were emptied individually onto 61 cm by 25.4 cm flats. The soil was chopped up with a hand trowel and the cover crop biomass was

chopped with shears. This process was done to help incorporate the cover crop biomass into the soil, and to simulate the tillage that occurred in the field. The soil was then watered every 2 days to keep it in good condition before planting soybeans.

Two flats of soil from each cover crop treatment were used for *F. virguliforme* infestation, while the other two flats of soil were used for *R. solani* infestation. Two different infestation rates were used to ensure an acceptable disease incidence level for the assay. Rhizoctonia inoculum was applied at 328 g/flat for the high rate, and 164 g/flat for the low rate. The *F. virguliforme* infestation rate was 14.6 g/flat for the high rate, and 7.3 g/flat for the low rate. Each type of inoculum was mixed into each flat and the soil was then ready for planting of soybeans.

Infested soil (125 mL) was placed into polypropylene Cone-tainers TM (Ray Leach SC-10 Super Cell), measuring 164 mL in volume, 3.8 cm in diameter, and 21 cm in depth. Ten cones were used for each cover crop treatment and each infestation rate, and the cones were place in trays and arranged on a greenhouse bench. Each cone was filled to within 2 cm of the top with the infested soil, and then 2 soybean seeds (Williams 82) were planted. Williams 82 was chosen for use in this study because it is extremely susceptible to both Fusarium and Rhizoctonia. 2.5 cm of infested soil was then placed on top of the soybean seeds, and the cones were watered daily to maintain necessary soil moisture. Upon germination, 1 plant from each cone was pulled so that only 1 plant remained.

**Root collection**: Five plants from each treatment were pulled at 2 weeks and 4 weeks after planting. The last scheduled watering before plant collection was withheld to reduce the soil moisture so that the seedling root systems could be removed from the cones intact. The seedlings were cut off about 10 cm above the soil line, and all 5 root systems from each treatment were

placed into a 50 mL Eppendorf tube. All tubes were kept in a refrigerator at 4°C until the root systems could be washed and the rhizospheric soil could be collected.

**Soil collection**: Root systems were washed with a phosphate buffer solution (pH=7). Thirty milliliters of the buffer solution was added to each Eppendorf tube containing the root systems. The tubes were shaken by hand for 1 minute to release the soil from the roots. The roots were then removed from the tubes and the soil suspension was centrifuged at 8000 rpm for 10 minutes. The buffer solution was poured off and the soil pellet was collected and used to represent the rhizospheric soil. The roots were then gently washed in a tub of water to remove any soil that might still be attached. The washed roots were placed in new 50 mL Eppendorf tubes.

After washing the roots in the phosphate buffer solution, the root samples were freeze-dried for 24 hours. Once dried, 0.5 g of root tissue was moved to a 2 mL microcentrifuge tube, and two 0.5 mm diameter balls (Daisy stainless steel bbs) were added to each tube. The tubes were placed in liquid nitrogen for 20 seconds. A BioSpec mini-bead beater homogenizer was then used to pulverize the root tissue for 30 seconds. The pulverized root tissue and soil samples were stored at -80°C until DNA extraction took place.

**Root disease rating**: After the roots were washed and the rhiozospheric soil was collected, disease severity ratings were taken. Sudden death syndrome (SDS) root rot severity was recorded using a rating scale of 1-5 according to the percentage of root discoloration. Roots with 0-20% discoloration were assigned a rating of 1; roots with 21-40% discoloration were assigned a rating of 2; roots with 41-60% discoloration were assigned a rating of 3; roots with 61-80% discoloration were assigned a rating of 4; and roots with 81-100% discoloration were

assigned a rating of 5 (Farias et al 2008). At the 2 week rating period, no levels of Fusarium were present.

Rhizoctonia root rot severity was recorded using a scale of 0-5 according to the length of lesions on the main taproot. Roots with no lesions were assigned a rating of 0; roots with lesions <2.5 mm were assigned a rating of 1; roots with lesions 2.5-5 mm were assigned a rating of 2; roots with lesions >5 mm were assigned a rating of 3; roots with lesions girdling the plant were assigned a rating of 4; and if the seedling was damping-off or completely dead, a rating of 5 was given (Cardoso and Echanid 1987).

**DNA extraction:** Total DNA was extracted from all rhizospheric soil and soybean root samples. All DNA was extracted using FastDNA SPIN Kits (MP Biomedicals, Solon, Ohio), following the manufacturers protocol. Upon extraction, all DNA samples were stored at -20˚C until polymerase chain reaction (PCR) and further tests were run. PCR was performed on 10 random samples to check for quality of DNA. A NanoDrop spectrophotometer was then used to quantify the amount of DNA in each sample. Each sample was diluted to 30 ng DNA/µl, and a 10 µl aliquot of each sample was arranged on a 96-well PCR plate.

**Sequencing:** The PCR plates were sent to the W. M. Keck Center at the University of Illinois for microbial community analysis using Illumina sequencing based on the 16s RNA sequences. Eight primers were used by the Keck Center in the analysis. The primers are as follows: V3-V5 bacterial (ACACTGACGACATGGTTCTACACCTACGGGAGGCAGCAG), Archaea (ACACTGACGACATGGTTCTACAGYGCASCAGKCGMGAAW), fungal ITS 1-4 (ACACTGACGACATGGTTCTACATTCGTAGGTGAACCTGCGG), ammonia monooxygenase bacterial (ACACTGACGACATGGTTCTACAGGGGTTTCTACTGGTGGT), nitrous oxide reductase-typical

(ACACTGACGACATGGTTCTACAWCSYTGTTCMTCGACAGCCAG), nitrous oxide reductase-atypical (ACACTGACGACATGGTTCTACASGGCTAYGGCTWYGAYGA), ammonia monooxygenase Archaea (ACACTGACGACATGGTTCTACAATGGTCTGGCTWAGACG), Ammonia generating nitrite reductase (ACACTGACGACATGGTTCTACACARTGYCAYGTBGARTA). Each primer was used for evaluating each sample. The primers identify microbes that are present in each sample. Further analysis was then needed to specifically identify and quantify the amount of each microbe in the soil samples. Only V3-V5 bacteria, archaea, and ITS 1-4 fungal primer data will be discussed.

**Analysis**

**Microbial community analysis:** The workflow from IM-TORNADO (Illinois Mayo Taxon Organization from RNA Dataset Operation) was used to pair the reads produced during the Illumina Sequencing protocol. Once the reads were paired, the QIIME 454 overview workflow was used for OTU picking and further analysis. OTU graphs were put together based on the QIIME output and the top ten phyla from each primer were compared between all treatments.

**Statistical analysis**: Analysis of variance (ANOVA) was performed to analyze the effects of cover crops and tillage on SDS disease severity levels and soybean yield using Proc Mixed in JMP Version 9.0.2 (SAS Institute Inc., Cary, NC). A model was used to fit the data to the RCBD layout of the experiment and was as follows: rating = disease|cover crop. The blocking effect was random, while the rest of the effects were fixed. An LS means Tukey adjustment was done to compare treatment effects.

The four replications for each treatment were combined into one DNA extraction sample. This was done to keep the cost for the experiment lower, and to allow for further exploration in this greenhouse study. For these reasons, statistical analysis of the OTU data is not available.

**Results**

**Disease rating**: At the 2-week evaluation period for Rhizoctonia root rot, soybean plants in the vetch treated pots had significantly less disease than the other cover crop treatments at both rates of infestation (Table 3.1). At the 4-week rating, plants in the rye treated and vetch treated pots had significantly less disease than the other cover crop treatments at both rates of infestation (Table 3.1). Overall, Rhizoctonia disease levels were quite low at both the half and full rates of infestation. At the 2-week evaluation period, there were no symptoms of SDS disease present on the soybean plants. However, at the 4-week evaluation period, plants in the vetch, mustard, and rye+vetch treated pots had significantly less disease than those in the rye and fallow treatment pots (Table 3.1).

**Microbial community results**: Using the archaea 16S primer, 4 phyla were found in significant levels in the DNA samples from both the rhizospheric soil and soybean roots. Those 4 phyla were: *Thaumarchaeota, Euyarchaeota, Plactomycetes, and Verrucomicrobia*. Operational taxonomic unit (OTU) data shows the percentage of each reported phyla that was found in each sample. There were no differences in phylum based community structures in the Fusarium treated pots among any of the cover crop treatments (Table 3.2). However, in the Rhizoctonia treated pots, several differences were seen within the rhizospheric soil DNA and soybean root DNA. When evaluating the rhizospheric soil DNA, rhizospheric soil from plants grown in the vetch pots had lower levels of *Thaumarchaeota* than rhizospheric soils from any other cover crop treatment. Rhizospheric soils from plants grown in the fallow pots had the lowest levels of

*Verrucomicrobia* than those of any other cover crop treatment. In the soybean root DNA, several differences were also found. DNA from roots grown in the mustard treatment pots showed higher levels of *Thaumarchaeota*, root DNA from plants in the fallow treatment pots had higher levels of *Planctomycetes*, and root DNA from plants in the rye treatment pots had higher levels of *Verrucomicrobia* compared to the other cover crop treatments (Table 3.2).

When the 16S bacterial primer set was used, 10 phyla were detected in the highest levels in all samples. The 10 phyla were: *Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Proteobacteria, Verrucomicrobia*. No differences in phylum based community structure were seen among any of the cover crop treatments in either disease environment (Table 3.3).

Four phyla were detected when the ITS 1-4 fungal primer set was used. Those phyla included: *Ascomycota, Basidiomycota, Glomeromycota,* and *Zygomycota* (Table 3.4). This data is quite misleading because of the high population of fungal microbes in the soil. Due to the method used of inoculating the soil, the DNA extracts were overpopulated with fungal microorganisms. This saturated the samples quickly in the Illumina sequencing process and did not allow for adequate OTU determination. For this reason, this data will not be discussed.

**Discussion**

This type of research can be difficult to draw conclusions from because there are so many different variables at work. Each one of the phyla that were detected by the different primer sets have the possibility of playing a role in either disease antagonism or suppression. All of the cover crop treatments, rye, vetch, mustard, and rye+vetch produced less disease than the fallow treatment at one or more of the infestation rates. Therefore, the theory of using cover crops as a disease management strategy is a valid one. However, the mechanisms for this suppression and

which cover crop species results in higher levels of suppression, are still unknown. Vetch treatments had less Rhizoctonia root rot consistently across both inoculation rates. When evaluating the microbial communities of the vetch pots, they contained less *Thaumarchaeota* than any of the other cover crop treatments. Therefore, the *Thaumarchaeota* phylum may be associated directly with soils that are more conducive to *Rhizoctonia solani*, the causal agent of Rhizoctonia root rot.

The SDS disease results are more difficult to differentiate since plants receiving the vetch, mustard, and rye+vetch treatments had statistically the same levels of disease. The microbial community results were also not as easily evaluated since there were no clear differences between the treatments. It may be possible that the microbial populations were not as well established in such a short time period, which makes them difficult to distinguish.

Every cover crop species has its own microbial community associated with it. In addition to that, each fungal disease pathogen has a specific set of microbes that are associated with it as well. This means that a cover crop that is suppressive towards Rhizoctonia root rot, does not necessarily have the same suppressive qualities towards sudden death syndrome. As with any disease management program, the strategies used should be specific for the disease or diseases that are present in a production field.

It is difficult to make generalizations or conclusions about the overall effect of cover crops on disease suppression from one study. Much more research is needed in this area to further study how phylum profiles may be indicators of disease suppression. This will allow for greater implementation of this disease management strategy or even new biological agricultural products that could be applied directly to a production field.

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**Tables and Figures**

Figure 3.1: Sudden death syndrome (SDS) disease rating scale used to rate soybean roots. SDS was rated using a 1-5 scale based on the percentage of root discoloration where 1=0-20% discoloration; 2=21-40% discoloration; 3=41-60% discoloration; 4=61-80% discoloration; and 5=81-100% discoloration.

5 4 3 2 1

Table 3.1: Effects of cover crop treatments on root disease severity in the greenhouse assay.

| **Cover crop treatment** | **Rhizoctonia root rot rating at 2-weeks*** | | | |
|---|---|---|---|---|
| | **Half Rate[i]** | | **Full Rate[ii]** | |
| Rye | 1.1 | a | 1.5 | a** |
| Vetch | 0.5 | b | 0.1 | b |
| Mustard | 0.7 | b | 2.3 | a |
| Rye+Vetch | 0.8 | b | 1.1 | a |
| Fallow | 1.5 | a | 1.5 | a |
| | | | | |
| | **Rhizoctonia root rot rating at 4-weeks*** | | | |
| Rye | 1.8 | a | 2.6 | a |
| Vetch | 1.5 | a | 1.7 | b |
| Mustard | 2.7 | b | 2.7 | a |
| Rye+Vetch | 3.1 | c | 2.9 | a |
| Fallow | 2.4 | b | 2.4 | a |
| | | | | |
| | **Sudden death syndrome rating at 4-weeks***** | | | |
| Rye | 2.2 a | a | 2.2 | a |
| Vetch | 1.4 b | b | 1.6 | b |
| Mustard | 0.6 c | c | 1.3 | b |
| Rye+Vetch | 1.2 b | b | 1.5 | b |
| Fallow | 1.8 b | b | 2.5 | a |

*The Rhizoctonia root rot rating scale is as follows: 0= no lesions; 1= <2.5 mm lesions; 2= 2.5-5 mm lesions; 3= >5 mm lesions; 4= lesions girdling the plant; 5= seedling damping-off or completely dead.
**Values followed by the same letter are not significantly different based on an LSD test at $\alpha$= 0.10.
*** Rating scale for sudden death syndrome is as follows: 1= 0-20% discoloration; 2= 21-40% discoloration; 3= 41-60% discoloration; 4= 61-80% discoloration; 5= 81-100% discoloration.
i. Rhizoctonia half rate = 164 g/flat; Fusarium half rate = 7.3 g/flat
ii. Rhizoctonia full rate = 328 g/flat; Fusarium full rate = 14.6 g/flat

Table 3.2: Operational taxonomic unit determinations for the 16s Archaea primer set. Values are percentages of each phylum in the particular DNA sample.

| **Pathogen Treatment** | **DNA Source** | **Phylum** | **Cover Crop Treatment*** | | | | |
|---|---|---|---|---|---|---|---|
| | | | Rye | Vetch | Mustard | Rye+Vetch | Fallow |
| Rhizoctonia | Soil | *Euryarchaeota* | 0 | 1.79 | 0.01 | 0 | 0 |
| | | *Thaumarchaeota* | 62.54 | 58.87 | 71.06 | 73.46 | 75.31 |
| | | *Planctomycetes* | 0.02 | 0.17 | 0.08 | 0 | 0 |
| | | *Verrucomicrobia* | 36.85 | 39.14 | 8.48 | 26.33 | 24.68 |
| | Root | *Euryarchaeota* | 0 | 0 | 0 | 0 | 0 |
| | | *Thaumarchaeota* | 4.38 | 0.56 | 14.38 | 6.90 | 8.56 |
| | | *Planctomycetes* | 0.08 | 0 | 6.33 | 0.38 | 19.11 |
| | | *Verrucomicrobia* | 95.46 | 90.87 | 79.25 | 92.39 | 71.80 |
| Fusarium | Soil | *Euryarchaeota* | 0 | 0 | 0.10 | 0.01 | 0 |
| | | *Thaumarchaeota* | 62.81 | 51.91 | 69.59 | 63.62 | 66.80 |
| | | *Planctomycetes* | 0.01 | 1.15 | 0.84 | 0 | 0 |
| | | *Verrucomicrobia* | 37.13 | 46.73 | 29.41 | 36.34 | 33.20 |
| | Root | *Euryarchaeota* | 0 | 0 | 0 | 0 | 0 |
| | | *Thaumarchaeota* | 0.46 | 6.38 | 0.53 | 0.13 | 2.48 |
| | | *Planctomycetes* | 0.31 | 0.39 | 0.98 | 0 | 0.08 |
| | | *Verrucomicrobia* | 98.88 | 92.75 | 98.37 | 99.74 | 96.78 |

*Cover crops were planted in pots and allowed to grow for 5 weeks before being terminated and incorporated into the soil before soybeans were planted.

Table 3.3: Operational taxonomic unit data for the 16s Bacterial primer set. Values are percentages of each Phylum in the given sample.

| **Pathogen Treatment** | **DNA Source** | **Phylum** | **Cover Crop Treatment** | | | | |
|---|---|---|---|---|---|---|---|
| | | | Rye | Vetch | Mustard | Rye+Vetch | Fallow |
| Rhizoctonia | Soil | *Acidobacteria* | 18.21 | 15.37 | 14.76 | 14.88 | 16.13 |
| | | *Actinobacteria* | 17.58 | 19.72 | 20.06 | 25.37 | 22.39 |
| | | *Bacteroidetes* | 7.17 | 5.98 | 5.66 | 5.26 | 5.64 |
| | | *Chloroflexi* | 4.16 | 4.64 | 4.20 | 4.40 | 5.65 |
| | | *Cyanobacteria* | 1.87 | 1.38 | 1.58 | 0.71 | 1.60 |
| | | *Firmicutes* | 2.25 | 2.44 | 1.99 | 2.67 | 2.05 |
| | | *Gemmatimonadetes* | 2.94 | 3.11 | 3.59 | 4.10 | 2.97 |
| | | *Nitrospirae* | 0.90 | 0.98 | 1.15 | 0.80 | 1.01 |
| | | *Proteobacteria* | 42.36 | 40.35 | 45.34 | 40.50 | 40.61 |
| | | *Verrucomicrobia* | 0.47 | 0.46 | 0.26 | 0.33 | 0.26 |
| | | | | | | | |
| | Root | *Acidobacteria* | 0.23 | 0.18 | 0.15 | 0.20 | 0.15 |
| | | *Actinobacteria* | 5.84 | 6.26 | 5.36 | 6.08 | 5.28 |
| | | *Bacteroidetes* | 17.53 | 16.67 | 17.03 | 16.93 | 17.72 |
| | | *Chloroflexi* | 0.45 | 0.55 | 0.24 | 0.55 | 0.41 |
| | | *Cyanobacteria* | 31.75 | 31.88 | 34.08 | 26.98 | 32.16 |
| | | *Firmicutes* | 0.27 | 0.41 | 0.32 | 0.40 | 0.33 |
| | | *Gemmatimonadetes* | 0.04 | 0 | 0.01 | 0.02 | 0.02 |
| | | *Nitrospirae* | 0 | 0 | 0 | 0 | 0 |
| | | *Proteobacteria* | 43.72 | 43.91 | 42.71 | 48.65 | 43.84 |
| | | *Verrucomicrobia* | 0.07 | 0.05 | 0.05 | 0.06 | 0.02 |

Table 3.3 (cont.)

| **Pathogen Treatment** | **DNA Source** | **Phylum** | **Cover Crop Treatment** | | | | |
|---|---|---|---|---|---|---|---|
| | | | Rye | Vetch | Mustard | Rye+Vetch | Fallow |
| Fusarium | Soil | *Acidobacteria* | 14.54 | 12.90 | 11.32 | 15.67 | 13.71 |
| | | *Actinobacteria* | 18.33 | 19.19 | 22.78 | 15.68 | 19.88 |
| | | *Bacteroidetes* | 9.91 | 9.19 | 9.61 | 5.59 | 5.65 |
| | | *Chloroflexi* | 4.41 | 4.62 | 4.32 | 3.42 | 3.93 |
| | | *Cyanobacteria* | 0.74 | 1.02 | 1.03 | 1.57 | 1.13 |
| | | *Firmicutes* | 1.99 | 2.59 | 1.77 | 0.99 | 1.88 |
| | | *Gemmatimonadetes* | 2.85 | 3.23 | 2.36 | 2.62 | 3.00 |
| | | *Nitrospirae* | 1.53 | 0.94 | 0.92 | 0.96 | 0.77 |
| | | *Proteobacteria* | 43.30 | 44.09 | 44.27 | 51.68 | 48.71 |
| | | *Verrucomicrobia* | 0.49 | 0.47 | 0.46 | 0.54 | 0.22 |
| | | | | | | | |
| | Root | *Acidobacteria* | 0.09 | 0.13 | 0.18 | 0.12 | 0.23 |
| | | *Actinobacteria* | 4.65 | 6.33 | 4.15 | 4.83 | 4.85 |
| | | *Bacteroidetes* | 20.36 | 15.54 | 18.38 | 16.37 | 14.82 |
| | | *Chloroflexi* | 0.20 | 0.56 | 0.30 | 0.17 | 0.35 |
| | | *Cyanobacteria* | 32.71 | 31.19 | 33.37 | 35.76 | 39.08 |
| | | *Firmicutes* | 0.35 | 0.36 | 0.36 | 0.47 | 0.21 |
| | | *Gemmatimonadetes* | 0.01 | 0.06 | 0.02 | 0.01 | 0.04 |
| | | *Nitrospirae* | 0.01 | 0 | 0 | 0 | 0 |
| | | *Proteobacteria* | 41.52 | 45.67 | 43.08 | 42.10 | 40.33 |
| | | *Verrucomicrobia* | 0.06 | 0.07 | 0.06 | 0.13 | 0.07 |

Table 3.4: Operational taxonomic unit data for the ITS 1-4 fungal primer set. Values are percentages of each Phylum in the given sample.

| **Pathogen Treatment** | **DNA Type** | **Phylum** | **Cover Crop Treatment** | | | | |
|---|---|---|---|---|---|---|---|
| | | | Rye | Vetch | Mustard | Rye+Vetch | Fallow |
| Rhizoctonia | Soil | *Ascomycota* | 63.32 | 92.59 | 1 | 39.29 | 0 |
| | | *Basidiomycota* | 20.72 | 0 | 0 | 19.64 | 0 |
| | | *Glomeromycota* | 15.96 | 7.41 | 0 | 41.07 | 0 |
| | | *Zygomycota* | 0 | 0 | 0 | 0 | 1 |
| | | | | | | | |
| | Root | *Ascomycota* | 41.71 | 78.45 | 67.64 | 64.85 | 74.89 |
| | | *Basidiomycota* | 0 | 8.20 | 26.90 | 16.15 | 14.00 |
| | | *Glomeromycota* | 58.29 | 13.35 | 5.46 | 19.00 | 11.11 |
| | | *Zygomycota* | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | |
| Fusarium | Soil | *Ascomycota* | 66.31 | 28.75 | 85.42 | 0 | 0 |
| | | *Basidiomycota* | 17.08 | 3.13 | 6.25 | 0 | 75.00 |
| | | *Glomeromycota* | 14.76 | 68.13 | 0 | 1 | 0 |
| | | *Zygomycota* | 1.85 | 0 | 8.33 | 0 | 25.00 |
| | | | | | | | |
| | Root | *Ascomycota* | 30.94 | 60.13 | 83.08 | 65.55 | 47.25 |
| | | *Basidiomycota* | 11.89 | 33.71 | 7.43 | 24.94 | 46.30 |
| | | *Glomeromycota* | 57.17 | 6.16 | 9.49 | 8.61 | 6.45 |
| | | *Zygomycota* | 0 | 0 | 0 | 0.89 | 0 |