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Soil Microbial Community Distributions And Disease Suppressiveness In The Coastal Plain Of Georgia

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SOIL MICROBIAL COMMUNITY DISTRIBUTIONS AND DISEASE SUPPRESSIVENESS IN THE COASTAL PLAIN OF GEORGIA.

by
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(Under the advisement of Tiehang Wu)

ABSTRACT

This study compared the soil microbial communities of three vegetation types in the coastal plain of Georgia: 1. crop land actively in use for agricultural production, 2. transitional grassland in early stages of secondary succession, and 3. pristine unmanaged forest land. Microbial species diversity and quantities of microbial DNA were determined from each of these vegetation types at three separate locations near Statesboro, Georgia. Length heterogeneity PCR(LH-PCR) methods and subsequent analysis of fungal, bacterial, and metazoan communities by analysis of similarity (ANOSIM) revealed high within-group similarity by vegetation type, indicating land management intensity and vegetation cover is a strong determining factor in community similarity. Further analysis of fragments obtained by LH-PCR revealed that fungal and metazoan communities in crop soil included the highest number of common operational taxonomic units (OTUs) represented in all treatments, while forest soils contained the least number of common OTUs of animal and fungi. This trend is not observed in bacterial communities, and may be a function of organism size. Quantitative PCR (qPCR) detection of fungal and bacterial DNA revealed significantly higher concentrations of both fungal and bacterial DNA in forest soils than concentrations in both crop and transitional soils. Despite differences in microbial communities and DNA concentrations, these soils exhibited no significant difference in their suppression of the soil-borne pathogen *Sclerotium rolfsii* in the context of a greenhouse experiment. *S. rolfsii* inoculum was successfully detected through qPCR based methods, however, *S. rolsfii* DNA concentrations lack correlation with Southern Blight disease incidence. In conclusion, vegetation and land management intensity significantly affect soil microbial communities. Forest soils host a fewer number of common animal and fungal OTUs than crop soils. Bacteria had no difference in the occurrence of common OTUs between vegetation types. qPCR methods were successfully employed to detect *S. rolfsii* inoculum. Although *S. rolfsii* DNA concentrations lacked correlation with disease severity, these methods are capable of detecting the potential of soil-borne disease development.

INDEX WORDS: Agriculture, Microbial communities, *Sclerotium rolfsii,* Quantitative PCR, Length heterogeneity PCR

MOLECULAR METHODS FOR THE EXAMINATION OF SOIL MICROBIAL COMMUNITY DISTRIBUTIONS AND DISEASE SUPPRESSIVENESS IN THE COASTAL PLAIN OF GEORGIA.

by

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CHAPTER 1 – REVIEW OF LITERATURE

Ecology is the study of organisms, their relationship with the environment, and their distributions through time and space. Microbial ecology examines these relationships with respect to microorganisms, including bacteria, fungi, algae, and microscopic invertebrate animals. It is important to understand and study these relationships of microbes and their environment because of the ubiquitous nature of microbes and the critical roles they play in biogeochemical processes. Microbes facilitate carbon sequestration and represent the largest known natural carbon reservoir. Microbial communities are also largely responsible for nitrogen fixation and inorganic nutrient cycling (Fenchel, 1998).

One obstacle to studying the microbial world is choosing the appropriate scale at which to examine these communities. Long-standing contention exists as to whether most microbes are evenly and extensively distributed across the globe, or if communities are relatively endemic to specific regions. “Everything is everywhere, but environment selects” (Becking, 1934) is often quoted to serve as a null assumption of microbial distribution. In modern ecology, this idea has found little support and much opposition (Green et al., 2004). It may be the case that some taxa are not bound to a defined biogeography and may indeed be present everywhere the environment allows. If nearly all microbes have a global distribution, choosing a scale for study is less of an issue: regardless of sampling area, diversity should remain constant on average. However, if bacteria are endemic to specific regions, diversity of a sample should increase with sample area size.

Species area relationships (SAR) refer to the increase in number of taxa observed as the sample area increases. This biogeographical concept is often overlooked or ignored in microbial

ecology or microbial community assessment. Only recently has this type of research been in the context of SAR at the level of microbial communities, including algae (Smith et al., 2005), bacteria (Horner-Devine et al., 2004), and fungi (Green et al., 2004). These studies all observed a SAR and provide evidence that microbes have defined biogeography and microbes may be defined as endemic to a certain region. Other meta-analyses have also concluded that differences in microbial distribution can be observed and can be attributed to longstanding geographical isolation and speciation events and not some recent anthropological effect (Martiny et al., 2006). These relatively recent studies are contrary to Becking's tenet of microbial ecology 'everything is everywhere, but, the environment selects'. Applications of this new perspective on microbial distributions have demonstrated that fungi are affected by farming practice and soil alterations on both a local and regional scale (Van der Gast et al., 2011), having major implications for the importance of these studies for agriculture.

Another obstacle to the large-scale study of microorganisms is the methods of detection. Prior to the advent of modern molecular techniques, microbial ecology was reliant on culture-dependent techniques. These techniques lent to a chronic underestimate of microbial biodiversity, as such methods capture less than 10% of microbial diversity calculated by molecular techniques such as cloning and sequencing (Smith et al., 2001). The concept of metagenomics was developed as these modern genetic techniques became available. Metagenomics is defined as studying directly the genomes of all organisms within a community; effectively looking at the genome of an entire environment (Handelsman et al.,1998). The first major revelation of this new field was that, indeed, culture dependent methods had missed the vast majority of biodiversity at the microbial level (Hugenholz et al., 1998). The frontier of

metagenomic research relies on massively parallel pyrosequencing to thoroughly capture all genomes within an environment (Eisen, 2007). While next-generation sequencing does provide high resolution data, many other molecular techniques have been successfully employed to study community-level microbial ecology and infer details of community dynamics. Examples include quantitative PCR (Kim, 2014), Denaturation Gradient Gel Electrophoresis (DGGE) (Senechkin et al., 2014) and Length-heterogeneity PCR (LH-PCR) (Tiirola et al., 2003)

With the recent emergence of high-throughput molecular methods have researchers been able to gain a community-level view of microbial populations. Particularly recent are studies which monitor changes in these communities over time, or monitor changes in microbial communities associated with changes in land usage (Wu et al., 2008). Some basic biogeograpical questions have yet to be addressed regarding the distributions of microbes in different soil environments. Categorizations of environments as either having cosmopolitan or endemic microbial distributions have yet to be firmly established. One aim of this study is to categorize forest soils, agricultural soils, and successional soils with respect to their microbial species diversity and evenness. It is hypothesized that forest soils will host more diverse and more even microbial communities than agricultural soils. This difference could be accounted for by the use of non-local soils used in the course of agricultural practices as well as the inoculation of farmlands with foreign microbes by farm equipment, transplanted crops, and seeds. Conversely, historically forested communities should have a more even distribution as they should be less likely to have been disrupted by foreign microbes. (Litchman, 2010).

An additional aim of this study will be to examine a gradient of environments in transition from farmland back to a forested environment, or conversely a forested environment

cultivated into farmland. This will allow for the examination of whether microbial communities "recover" over time to resemble a pristine forest environment, or if any microbial succession patterns can be identified at all. It has been determined that microbial communities involved in decomposition and nutrient cycling have successional patterns and their community, particularly species richness, changes over time (Jackson, 2003). Similar successional waves of microbes may be present in soils transitioning from farmland back to a forested ecosystem, a process often referred to as afforestation.

The role of some microbes in agriculture is well studied, including bacteria involving organic matter cycling, and nitrogen cycling and fixation (Bloem et al., 1995; Berthrong et al., 2013). By these mechanisms, microbes directly benefit agriculture by increasing the availability of nitrogen-based nutrients. Soil microorganisms, particularly fungi, can more indirectly influence plant health by forming complex biological relationships with crop plants. Mutualistic interactions of arbuscular mycorrizal fungi with crop plants enhances their water retention, nutrient up-take, and possibly disease resistance (Jefferies et al., 2003; Gosling et al., 2006). Soil conditions may vary widely across forested, grassland, and agroecosystems. Environmental factors such as pH, soil mafic quality, and inorganic nutrient availability are significant drivers of variance observed in microbial communities (Esperschutz et al., 2007).

It has been shown that the soil microbial community shifts along with changes in land-usage practices under a wide variety of circumstances including afforestation (Carson, 2010), and intensive agriculture (Jangid, 2008). However it is largely unknown how these shifts in soil microbial community associated with land-management may affect, or correlate with, the susceptibility of that environment to infection by pathogenic microbes. Previous studies have

found that changes in soil type and abiotic chemical differences associated with these various land management practices may affect community structures and also disease incidence (Wakelin, 2007). Many of these pathogenic microbes are host-dependent and suitable hosts may vary in prevalence within these environments. Lastly, 'bio-control' mechanisms may be a significant factor in preventing infection, where specific endemic microbial community members themselves may combat these pathogenic microbes through predation or competition. Bio-control is often praised for its low cost to the user and low impact on the environment (Baker, 1987). it has been shown that a variety of naturally occurring bacteria can be responsible for the control of fungal plant pathogens (Singh et al., 1998), (Duijff et al., 1999). The pathogenic southern blight fungi can be limited by a competitive antagonistic Trichoderma fungus (Mukherjee & Raghu, 1997). With understanding of the population size, richness and community structure of microbes potentially involved in bio-control mechanisms, methods may be developed to help promote community characteristics which inhibit the incidence of these crop pathogens.

In the southeastern United States, a number of microbes negatively impact crop production, including the microbial nematode *Melodogiyne incognita* (southern root-knot nematode)(Sasser, 1977), along with the fungi *Sclerotium rolfsii* (Southern Blight) (Bowen et al., 1992) and *Fusarium oxysporum* (Fusarium Wilt) (Williamson, 2012). Forest communities may also be at risk for invasion by the water mold *Phytopthora ramorum* (sudden oak death) (Oak et al., 2013). Due to their cryptic nature, these harmful microbial species are notoriously difficult to monitor and track compared to macroscopic plant and animal invaders. In recent years, methods involving indirect detection and molecular methods to rapidly assess the extent of

contamination in soils have been utilized. PCR-based detection techniques have successfully been developed for *Sclerotium rolfsii* (Jeeva et al., 2010), *Fusarium* spp. (Horevaj, 2011), *Melodogiyne* spp. (Min et al., 2011) and also *Phytophthora* spp. (Lees et al., 2012). Similar pathogens have been reported in Georgia soils (Gale et al., 2003), (Xu et al., 2006). The development and customization of molecular probes for the detection of these four plant pathogens and their novel application to south eastern Georgia soils would be a useful tool in the diagnosis of crop damage, particularly of tomato, *Solanum lycopersicum* and honeydew melon, *Cucumis melo*. Particularly, the novel development of sound methods which utilize Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR) would allow for rapid detection of these pathogens, as well as collection of data concerning the absolute amount of these pathogens in the soil. Applications of these methods could include diagnosis of disease in an agricultural setting, or the testing of imported foreign soil and substrate for 'hitch-hiking' alien, and potentially invasive, pathogens.

It is hypothesized that bacterial, fungal, and microbial animal communities will exhibit differences in their structure and diversity in conventional farm soils compared to an uncultivated forest environment, and to an ecotome transitional grassland based on intensity of soil disturbance. It is also hypothesized that the differences in their structure and diversity of these communities may impart differing capacities to resist infection by microbial plant pathogens.

CHAPTER 2 – ASSESSMENT OF SOIL MICROBIAL DIVERSITY, MICROBIAL DNA CONCENTRATIONS, AND SOIL CHEMISTRY ACROSS THREE SOIL TYPES

1. INTRODUCTION

Ecological succession can be measured in many ways, including by changes in species diversity, energy flow, and metabolic processes across time (Würtz and Annila, 2010). Regardless of the metrics involved in its measurement, ecological succession can be described as: (1) A directional process that is predictable, (2) community-controlled environmental change, and (3) a process that culminates in an ecosystem with maximum function, diversity, and biomass (Odum, 1969). The process of succession has long been studied in plants and may other macrorganisms (Clements, 1928; DeBruyn et al., 2011). Large-scale investigations of succession and its impacts on microorganisms, or rather the impact of microorganisms on succession, are less common. Microbial communities are essential to soil health in both agricultural and unmanaged ecosystems. Decompositional activity of bacteria is responsible for the formation of humus, which is nutritive to a variety of plants (Lysak, 2014). Phosphorus and nitrogen cycles are primarily driven by microbial action and make nutrients readily available for other organisms (Smith, 2014). Many species of microscopic fungi are capable of forming beneficial symbiotic relationships with plant hosts (Pirinc, 2014). Some studies have also found microbial action directly responsible for the breakdown and removal of environmental toxins from the soil (Garbeva et al. 2004). Ecological disturbance, in the form of land use and management, can have profound effects on the microbial communities of the soil. A wide

variety of agricultural methods including cover cropping, rotation, and fertilizer amendments can significantly alter microbial assemblages (Chellimi, 2012). Not only can these changes be rapid, but they can be long lasting as well. Some studies have found that even centuries later, past land management practices can be a strong predictor of soil bacteria community identity (Gutknecht, 2013). In addition to bacteria, fungi are also known to play an important role in various stages of succession. Studies have found an increase in the biomass of arbuscular mychorrizal fungi in the early and middle stages of succession is important for the establishment of grasslands (Nogueira et al., 2014). Microscopic nematode populations have also been identified to change in response to successional changes and ecological disturbance related to land management (McSorley, 2013). This study attempts to capture in general terms, and at one time point, the interaction of ecological succession and associated soil microbial communities. Three types of vegetation including crops, adjacent transitional grassland and forests were selected to be representative of different levels of ecosystem disturbance and succession. Microbial communities to be investigated include bacteria, fungi, and microscopic metazoa.

2. METHODS

2.1 Sampling design

Three sampling locations were selected in Southeast Georgia. Each of the three sampling locations are comprised of three distinct sampling sites. At each test location, site one (1) is representative of operational, conventional tomato farmland. Site two (2) is a transitional soil, where a secondary successional event is occurring. Site three (3) is representative of a

recently undisturbed forest habitat, containing both deciduous hardwood and coniferous pine forests. At a location, sampling sites were no more than 100 meters apart. Test locations include “Peter’s Farm” (32°34.784, 082°32.313), “Honeydew Farm” (32°32.354, 081°50.053), and “Strickland Farm” (32°19.231, 081°41.554). One approximately 6 x 50 meter plot was randomly selected at each site. A grid with 30 centimeter intervals was established on each plot. 50 (x,y) coordinates were generated, and a stratified random sampling regime was used to collect soil cores. Fifty soil cores (3.4 cm diameter; 10 cm deep) were collected from the rhizosphere of each site with a core sampler and hand mixed in a single sterile plastic bag. 10 soils cores were hand mixed into a single sample, each site yielding 5 samples. With three sites at each of the tree locations, a grand total of 45 samples (representative of 450 soil cores) were collected for this portion of the study. Physical and chemical soil properties were determined including organic carbon content, C:N ratio, P, K, Ca, Mn, Zn, Cu, Fe, S, B, soil pH, and cation-exchange-capacity by Waters Agricultural Laboratories, inc. Camilla, GA.

2.2 DNA extraction

DNA was extracted from each sample using PowerMax® Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) per suggested protocols. This Isolation kit was selected for its ability to purify very low concentrations of microbial DNA. Additionally, this preparation includes reagents which eliminate phenolic soil compounds such as humic acid, which are known to inhibit PCR. DNA was stored at -20°C.

2.3 Assessing microbial community using length heterogeneity polymerase chain reaction (LH-PCR)

Fragments of metazoan 18S rDNA gene were amplified from each sample using metazoan specific primers (Wu et al., 2009 & 2011). The forward primer 18S 11m (5'-GTCAGAGGTTCGAAGRCG-3') labeled with 6FAM corresponds to positions 1037-1054 of the human sequence (NR_003286 in GenBank) and to a region that is relatively constant among metazoans, but has positions that vary considerably in other eukaryotes. The reverse primer 18S0r (5'GGGCATCACAGACCTGTTATTGC-3) corresponds to positions 1480-1502 of the human sequences. The primers amplify approximately a 480 bp segment. Fragments of bacterial 16S rDNA gene were amplified from samples using bacteria-specific primers (forward primer 27F 5'-AGAGTTTGATCMTGGCTCAG-3' labeled with 6FAM and reverse primer 355R 5'-GCTGCCTCCCGTAGGAGT-3') as well as fungal specific primers targeting a section of the ITS region, also labeled with 6FAM (forward primer NSI1: 5'-GATTGAATGGCTTAGTGAGG-3' and reverse primer 58A2R: 5'-CTGCGTTCTTCATCGAT-3'). Length heterogeneity was assessed on an Applied Biosystems 3500 genetic analyzer (Applied Biosystems, Foster City, CA), in the Department of Biology at Georgia Southern University. The size of each fragment was assigned to operational taxonomic units (OTUs) using a ± 0.5 base pair (bp) criterion. Cluster analysis and multidimensional scaling (MDS) ordination was used to compare microbial metazoan, fungal, and bacterial communities in soil samples using PRIMER-E (Plymouth Marine Laboratory, UK) software. Comparisons of community metrics including richness, evenness, and Shannon diversity were completed in JMP statistical software (SAS Institute Inc.).

2.4 Determining total fungal and bacterial DNA amounts using real-time quantitative PCR (qPCR)

qPCR assays to quantify microbial fungal and bacterial DNA in each sample were conducted using the Mastercycler ep realplex real-time PCR system (Eppendorf North America, Inc., Hauppauge, NY) in the Department of Biology at Georgia Southern University. Primer pairs for Bacteria (27F, 355R) and Fungi (NSI1, 58A2R) along with the intercalating dye SYBR green were used to determine the quantity of bacterial and fungal genomes present in each soil sample. Three technical assay replicates were performed for each soil sample. Standard solutions for bacterial and fungal assays were created from DNA extracted from pure cultures of *E. coli* and *S. rolfsii*, respectively. A Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.) was used for initial quantification of these DNA solutions. Serial dilution methods were used to create 6 standard solutions at 10 ng/µl, 1 ng/µl, 0.1 ng/µl, 0.01 ng/µl, 0.001 ng/µl and 0.0001 ng/µl. These standard solutions served as reference solutions for subsequent qPCR assays. Methods used were similar to quantitative PCR protocols outlined in previously published assessments of soil bacterial and fungal communities (Fierer et al., 2005). ANOVA, Kruskal-Wallace and Student's t analyses of these assays were performed in JMP statistical software (SAS Institute Inc.).

2.5 Statistical analysis of microbial biodiversity and community structure

The frequency of each OTU was recorded and organized for diversity and community structure analysis. Diversity indices included richness, evenness, and Shannon diversity using EcoSim (Gotelli and Entsminger, 2008). The microbial metazoan community was analyzed with non-parametric multivariate analysis procedures of multidimentional scaling (MDS), using PRIMER-E statistical software (Primer-E, Plymouth Marine Laboratory, UK). PRIMER-E was used for an analysis of similarity (ANOSIMA) to calculate the significance of differences among

samples from different site and agricultural practices. These methods are described in more detail in Wu et al. 2009. This software was also used to perform a similarity percentage (SIMPER) analysis, used to calculate variation in species abundance. LH-PCR data was used to identify and tally common OTUs, defined as fragments which were common to all treatments in at least one replicate. Contingency analysis was performed based on the frequency of occurrence of common species in each vegetation treatment.

3. RESULTS

3.1 Clustal and ANOSIM analysis of microbial communities

Soil fungal communities showed distinct grouping pattern by vegetation cover (Fig. 2-1). Forest soils showed high separation from crop soils and transition soils R=0.54, P=0.0001. However, crop and transition soils are only weakly separated R=0.175, P=0.0004 (Table 2-1). Fragments obtained with 16S rRNA gene of bacterial primers displayed distinct grouping of forest, transitional, and agricultural soil bacterial communities (Fig. 2-2). Similar to soil fungi, soil bacteria in forest and crop soils were recorded to have the highest separation from each other R=0.73. P=0.0001. In the case of bacterial communities, forest and transition soils shared the most commonality R=0.496, P=0.0001 (Table 2-1). Grouping of soil microbial animals by vegetation type is less distinct than bacteria or fungi (Fig. 2-3). ANOSIM analysis of animal communities reveals a high degree of commonality between transition and forest R=0.231, P=0.0001, transition and crop R=0.222, P=0.0001. In a similar pattern to bacterial and fungal communities, the greatest difference in animal communities is observed between crop and forest treatments R=0.273 P=0.0001.

3.2 Environmental factors affecting microbial communities

Bacterial community patterns were more strongly explained by environmental factors than fungi and animal communities, and had an average environmental factor correlation of 0.545 (Table 2-2). Animal community patterns are explained less by chemical and soil factors than fungal or bacterial community patterns with an average 0.23 percent correlation for animal and 0.46 for fungi (Table 2-3 and 2-4). pH and cation exchange capacity were important influences on fungal, bacterial, and animal communities. NO_3^- was an important factor for Bacteria and animal communities, however was relatively unimportant for fungal community correlations. Factors such as calcium and phosphorus ranked as low correlations for all bacteria, fungi, and animal community correlations (Table 2-2, 2-3, and 2- 4). In addition to being predictors of community similarity, some differences in the environmental nutrient levels between soil treatments were observed. Farm soils were significantly higher in Calcium with 428.2 ppm (P=.0001), Potassium with 152.7ppm (P=.0341), and Phosphorous with 121.5 ppm (P=.0422) than both transition and forest soils (Fig. 2-4). Crop soils had significantly higher average pH 6.41, than forest or transitional soils with 4.94 and 6.12 respectively (P=.0001) (Fig. 2-5).

3.3 Effects of vegetation type on microbial diversity

Metazoan communities exhibited no difference in evenness between vegetation treatments. Crop soils exhibited higher degrees of metazoan community richness with an average index of 64.54 than forest soils with an average index of 49.47 (p=.0195), and also showed no difference in Shannon diversity or evenness (Table 2-5). In regards to bacterial community evenness, crop had an average index of 0.766 and transition displayed an average

index of 0.762, which both differed from forest treatments with an average index of 0.702 (P=.0001, P=.0001 respectively). Conversely, in terms of community richness, forest displayed an average index of 0.978 and transition with an index of 0.928, which both differed from crop treatments with an index of 0.874 (P= .0441, P=.0441). In a similar pattern to evenness, bacterial diversity was similar between transition and crop with indices at 0.050 and 0.021 respectively, which both differed from forest treatments with an index of 0.055 (P=0.0012, P=0.0094) (Table 2-6). No difference in fungal community evenness was observed between vegetation covers. Fungal richness did differ between crop treatments with an index of 84.53 and forest treatments with an index of 68.40 (P=0.0183). No differences in fungal Shannon diversity were observed (Table 2-7).

3.4 qPCR quantification of microbial DNA amounts

Concentrations of forest fungal DNA detected at a mean concentration of 0.0674 ng/µl are significantly higher than both transitional soil concentrations at an average concentration of 0.0183 ng/µl (P=<0.0001) and farm soil concentrations at an average concentration of 0.0274 ng/µl (P=0.0011) (Fig 2-6). Forest bacteria were also detected in highest quantities at 0.881 ng/µl and significantly higher than transitional soil concentrations at 0.507 ng/µl (P=0.0042), but not farm soil concentrations with an average concentration of 0.647 ng/µl (P=0.2652) (Fig 2-7).

3.5 SIMPER analysis of microbial Operational Taxonomic Units

In the top ten contributing taxonomic units for bacterial communities, two common fragments (fragments present occurring at least once in all three soil types), 315 and 317, together make up a high percentage of the total contribution for all treatments: 26.75% in

forest, 22.6% in transitional, and 23.31% in crop soils (Table 2-8). Animal communities display two common taxonomic units in the top ten contributing fragments as well, 381 and 457. These common fragments make up a moderate percentage of the total contribution for all treatments, averaging 6.79% (Table 2-9). For fungal communities, one common taxonomic unit, 341, is represented in the top ten contributing units. Fragment 341 contributes a relatively low percentage, 5.27% on average overall compared to common animal and bacterial taxonomic units (Table 2-10).

3.6 Contingency analysis of common species

A more thorough analysis of all common fragments (fragments present occurring at least once in all three soil types), was performed. Fungal and animal crop soil communities included the highest amount of common OTUs, with 1208 and 835 respectively. Forest soils contained the least number of common fungal and animal OTUs With 979 detected for fungi, and 667 detected for animal communities. While this trend is reversed in bacterial communities with 368 common units detected in crop soils and 391 in forest soils, the differences between treatments were far less (Table 11). Contingency analysis revealed that the number of these common OTUs is dependent on vegetation in the case of fungi ($P=<0.0001$) and animals ($P=<0.0001$) but not bacteria ($P=0.4603$).

DISCUSSION

The dissimilarity between forest fungal communities and those of crop and transitional soils may be due to a higher number of unique species in forest samples, or conversely, a fewer number of common species in forest soils compared to agricultural soils. Indeed, crop soils

contained a higher number of common species in the case of animal and fungal communities while forest soils contained the least number of common species. This idea supports the hypothesis that disturbed agricultural soils are home to more cosmopolitan and widely distributed microbes than forest soils, which may be sanctuaries for rare and less robust microbes (Litchman, 2010). The difference observed in common species between fungi, animals, and bacteria may be a function of organism size and rate of dispersion. Nematodes and fungi are orders of magnitude larger than bacteria, and this may affect how they are transported though the soil by hydrological effect (Finlay, 2002). Bacteria being smaller in size may be more prone to dispersion, and therefore have no difference in the number of common species across relatively local geography.

In regards to ANOSIM metrics, highest within-group similarity is observed in crop-forest comparisons of all three communities. This indicates that crop and forest soils have the most dissimilar communities out of all comparisons made. In the case of fungi, transitional communities bear more resemblance to crop soils. However bacterial communities in transitional soils share more similarity with forest soil communities. This pattern may indicate that while crop and forest soils are distinctly different, bacterial and fungal transitional communities may differ in the rate at which they regain forest community characteristics in the process of succession. Animal communities were relatively similar at all comparisons, which may suggest that these organisms are less impacted by the land management practices. Fungal and bacterial populations were more strongly affected by chemical soil conditions than animal populations, indicating that these populations are heavily driven and shaped mostly by a small number of environmental factors. Indeed, NO_3^- is known to be a strong affecter of soil bacteria

populations (Fenchel, 1998). Fungi are also known to be very sensitive to pH conditions (Esperschutz et al., 2007). pH was found to vary significantly between forest, crop, and transition soils. The combined findings of high pH and high calcium content in agricultural soil is very likely due to the application of agricultural lime (calcium carbonate) as a soil amendment.

Fungal and animal communities both experienced higher species richness under crop vegetation treatment, but no other effects on either evenness or Shannon diversity. This indicates that fungi and animal both have a larger number of different species in crop soils than farm soils, which could be due to introduction of foreign microbes in fertilizers and other soil amendments (Santini et al., 2012).

Regarding bacterial communities, a higher degree of species richness was measured in forest soil than in transitional and crop soils. However, crop and transitional soils displayed higher evenness and ultimately higher Shannon diversity. Higher Shannon diversity in crop soil is counter to the original hypothesis that forest soils would have higher Shannon diversity and evenness due to fewer disturbances (Jangid, 2008).

Higher DNA concentrations of both bacteria and fungi in forested ecosystems compared to transitional and crop soils may be the result of higher rates of carbon-based nutrient input in the form or leaf or plant litter. Low levels of disturbance in forested ecosystems likely create a beneficial environment for fungi, which remain free from hyphae-damaging farm land managements, such as tillage (Alguacil et al., 2008). In an applied sense, agricultural practices could be modified to promote higher levels of microbial biomass as seen in forested environments by using organic plant litter as a soil amendment, thereby mimicking the types of inputs found in forest soils (Hartmann et al., 2014).

4. Conclusion

Length heterogeneity PCR methods and subsequent analysis of fungal, bacterial, and metazoan communities by analysis of similarity revealed high within-group similarity by vegetation type, indicating land management intensity and vegetation cover is a strong determining factor in community similarity. Further analysis of fragments obtained by LH-PCR revealed that fungal and metazoan communities in crop soil included the highest number of common operational taxonomic units (OTUs) represented in all treatments, while forest soils contained the least number of common OTUs of animal and fungi. This trend is not observed in bacterial communities, and may be a function of organism size. Quantitative PCR detection of fungal and bacterial DNA revealed significantly higher concentrations of both fungal and bacterial DNA in forest soils than concentrations in both crop and transitional soils.

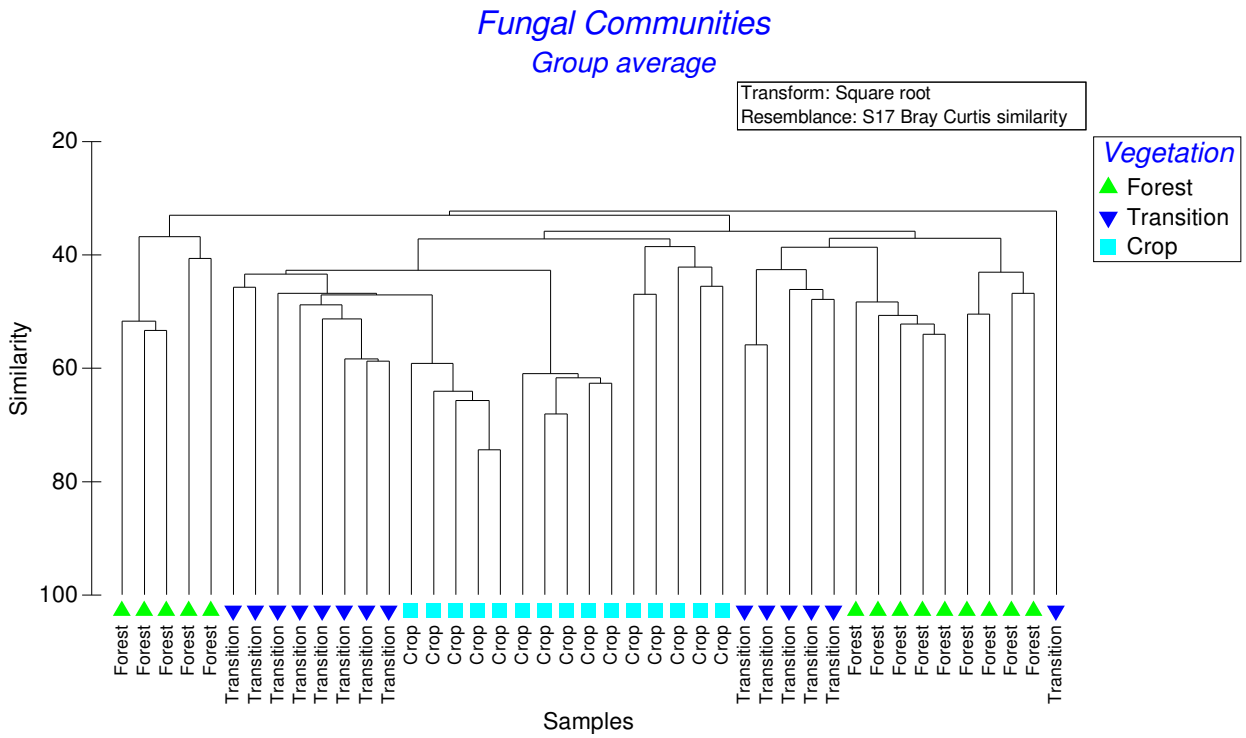

Figure 2-1. Cluster analysis of LH-PCR fragments amplified with fungal 18s ITS targeted primers, arranged by vegetation type. Forest soil communities show the highest degree of dissimilarity from crop and transitional soils, while crop and transitional communities are more closely clustered. Comparisons are based on Bray-Curtis similarity.

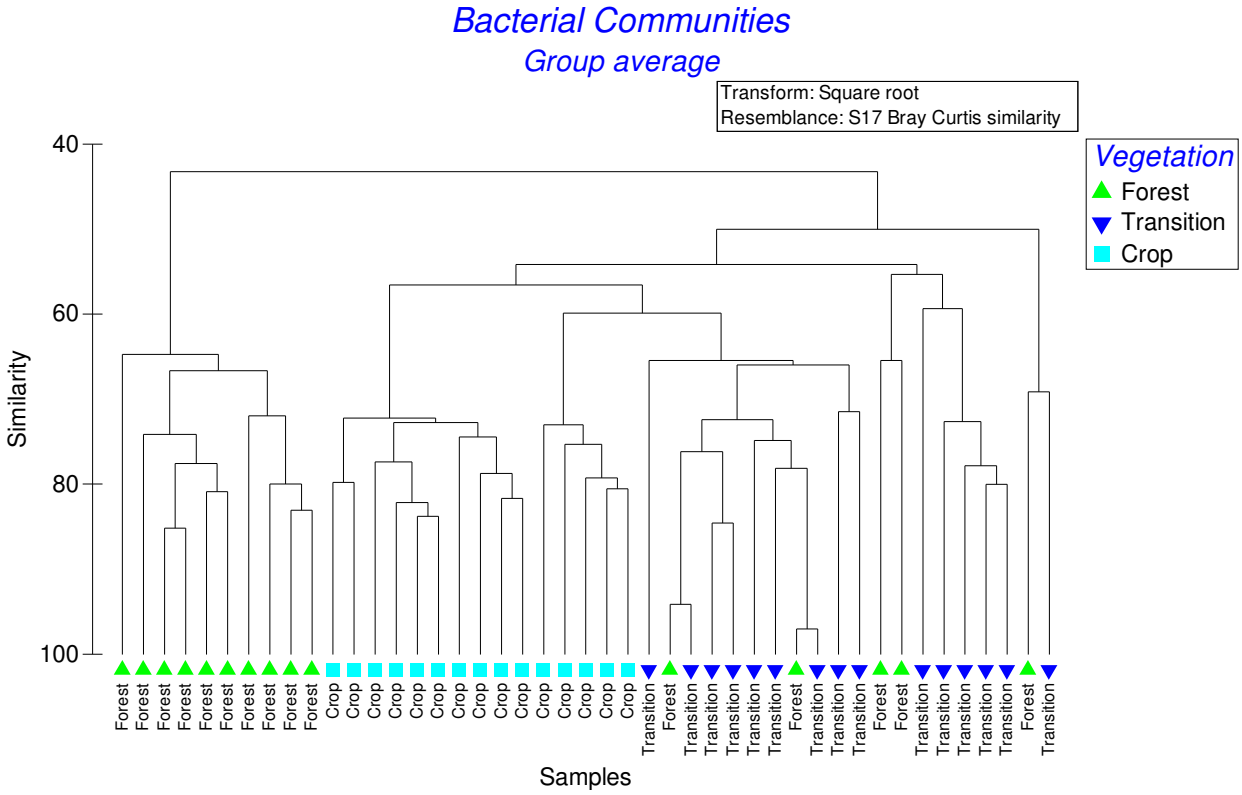

Figure 2-2. Cluster analysis of LH-PCR fragments amplified with bacterial 16s rRNA gene targeted primers, arranged by vegetation type. Transitional and forest soil communities show a higher degree of similarity with each other than to crop soil communities. Comparisons are based on Bray-Curtis similarity.

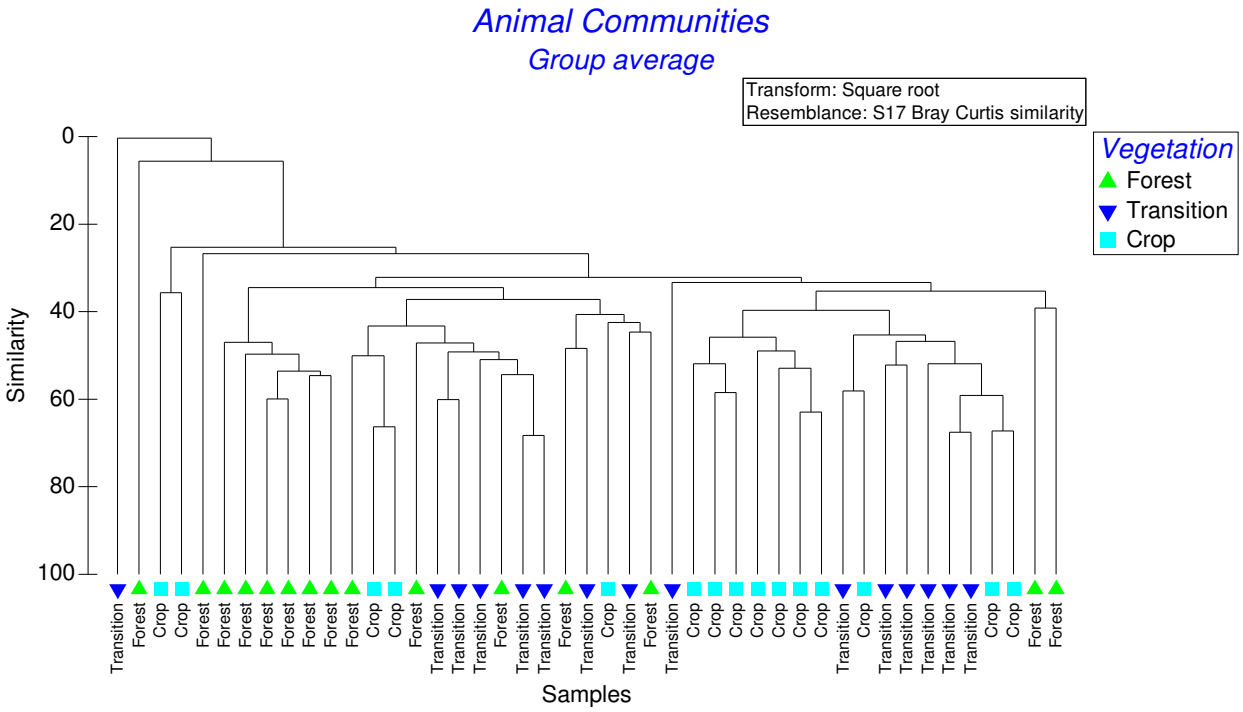

Figure 2-3. Cluster analysis of LH-PCR fragments amplified with animal 18s rRNA gene targeted primers, arranged by vegetation type. Grouping of metazoan communities is much less distinct than fungi or bacteria communities. Comparisons of all three vegetation types bear approximately the same degree of similarity to each other. Comparisons are based on Bray-Curtis similarity.

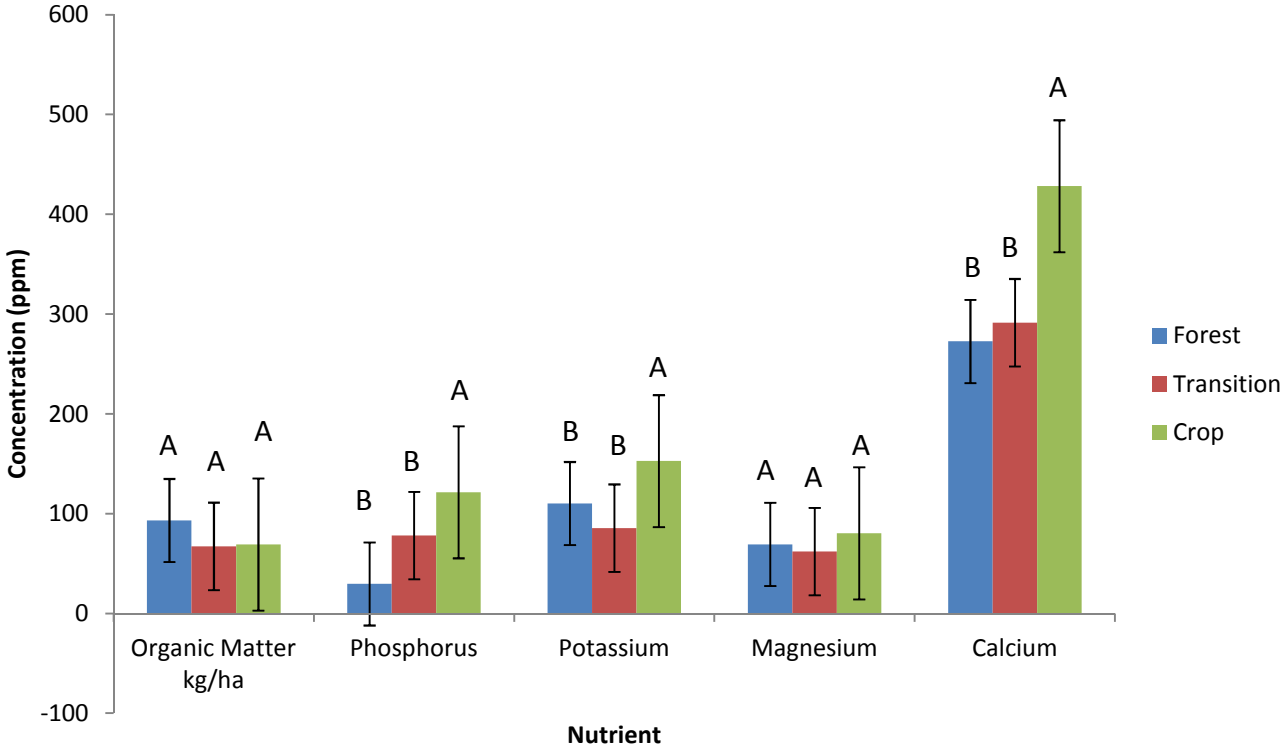

Figure 2-4. Environmental chemical levels recorded in forest, crop, and transition soils. Values recorded in ppm, except for organic matter in $kgha^{-1}$. Bars represent one standard deviation. Differing letters denote significant difference of means (P=<0.05).

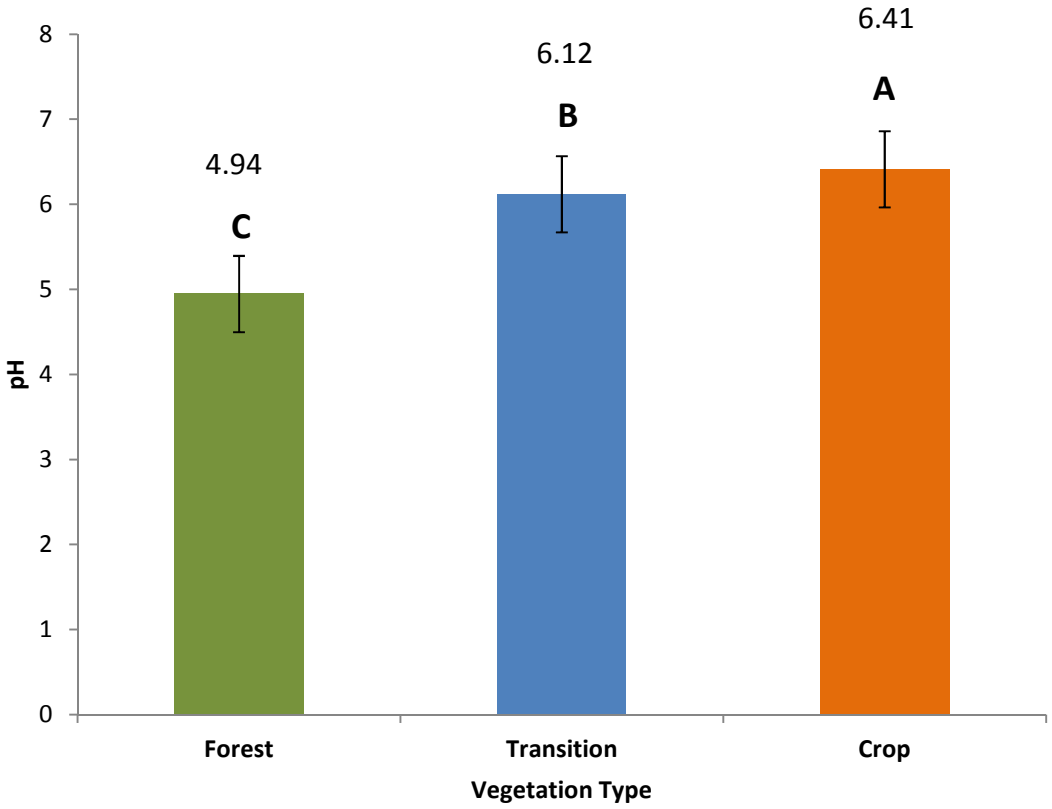

Figure 2-5. Soil pH of forest, transition, and crop vegetation cover. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

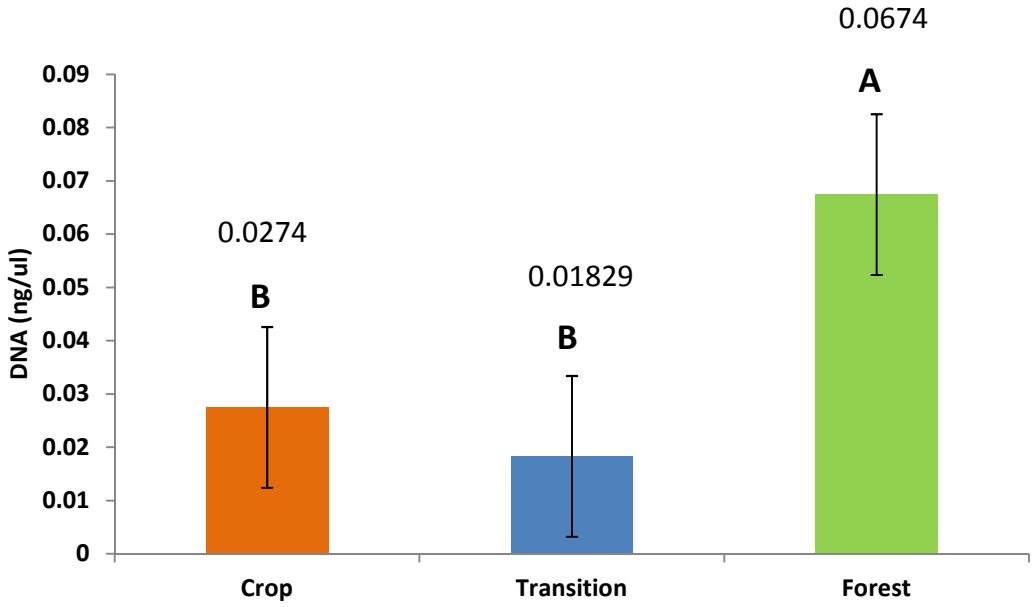

Figure 2-6. Amount of targeted total fungal DNA in ng/µl, detected by qPCR across land management treatments. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

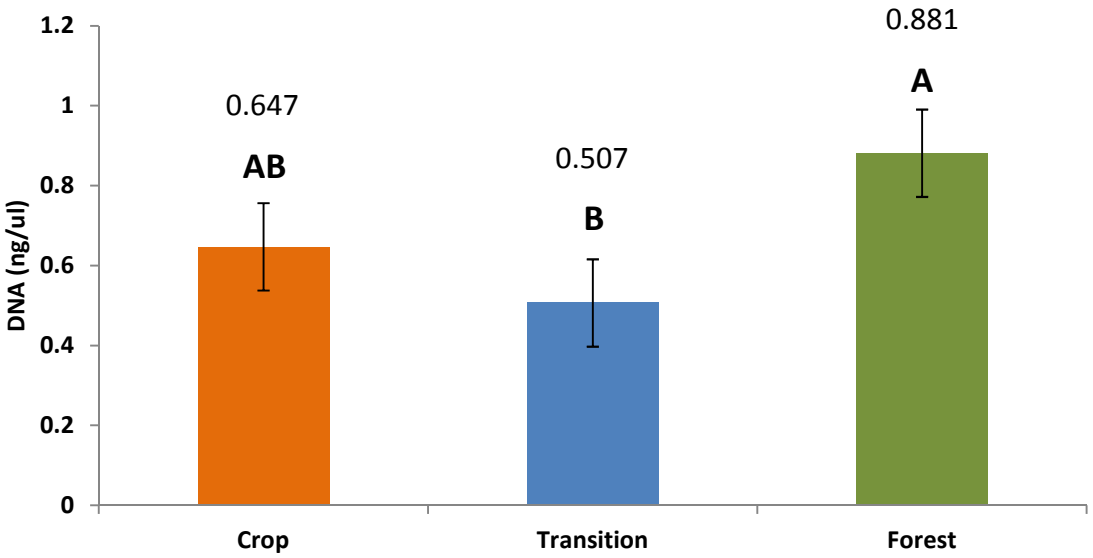

Figure 2-7. Amount of targeted total bacterial DNA in ng/µl, detected by qPCR across land management treatments. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

Table 2-1. ANOSIM results of bacteria , fungi, and animal communities with comparisons between vegetation types. High R values approaching 1 indicate high degree of within group similarity and lower similarity between groups. Conversely, low R values approaching 0 indicate less within group similarity and more similarities between groups compared.

	Comparisons	R Statistic	P Value
Fungi	Forest, Transition	0.459	0.0001
	Forest, Crop	0.54	0.0001
	Transition, Crop	0.175	0.0004
Bacteria	Forest, Transition	0.496	0.0001
	Forest, Crop	0.73	0.0001
	Transition, Crop	0.613	0.0001
Animal	Forest, Transition	0.231	0.0001
	Forest, Crop	0.273	0.0001
	Transition, Crop	0.222	0.0001

Table 2-2. Environmental factors with highest correlations to LH-PCR bacteria community analysis. Bacterial communities most correlate with levels of Mg, H, and NO_3^- combined.

BEST Analysis of Bacteria LH-PCR Fragments

Number of Variables	Percent Correlation	Environmental Factors
1	0.501	pH
2	0.540	Mg, H
3	0.559	Mg, H, NO_3^-
4	0.563	Mg, H, NO_3^-, Cation Exchange
5	0.561	pH, Mg, H, NO_3^-, Cation Exchange
Average	0.545	

Table 2-3. Environmental factors with highest correlations to LH-PCR animal community analysis. Animal communities best correlated with levels of organic matter, pH and NO_3^- combined.

BEST analysis of Animal LH-PCR fragments

Number of Variables	Percent Correlation	Environmental Factors
1	0.214	Organic Matter
2	0.221	Organic Matter, pH
3	0.241	Organic Matter, pH, NO_3^-
4	0.242	Organic Matter, pH, NO_3^-, Mg
5	0.240	Organic Matter, pH, NO_3^-, Mg, Cation Exchange Capacity
Average	0.232	

Table 2-4. Environmental factors with highest correlations to LH-PCR fungi community analysis.

BEST Analysis of Fungi LH-PCR Fragments

Number of Variables	Percent Correlation	Environmental Factors
1	0.45	pH
2	0.49	Organic Matter, pH
3	0.468	Organic Matter, pH, Cation Exchange Capacity
4	0.461	Organic Matter, pH, Cation Exchange Capacity, H
5	0.452	Organic Matter, pH, Cation Exchange Capacity, H, Mg
Average	0.464	

Table 2-5. Mean values of microbial animal community metrics richness, evenness, and Shannon diversity, arranged by vegetation type.

	Vegetation	Mean		Standard Error	Tukey's HSD significance level
Richness (S)	Crop	64.53	±	4.367	A
	Transition	59.00	±	4.587	AB
	Forest	49.47	±	4.196	B
Evenness (J)	Crop	0.44	±	0.023	A
	Transition	0.46	±	0.037	A
	Forest	0.44	±	0.024	A
Shannon Diversity (H)	Crop	1.82	±	0.108	A
	Transition	1.71	±	0.106	A
	Forest	1.73	±	0.099	A

Table 2-6. Mean values of bacterial community metrics richness, evenness, and Shannon diversity, arranged by vegetation type.

	Vegetation	Mean		Standard Error	Tukey's HSD Significance level
Richness (S)	Crop	28.8	±	0.874	B
	Transition	30.9	±	0.928	A
	Forest	30.9	±	0.978	A
Evenness (J)	Crop	0.766	±	0.007	B
	Transition	0.762	±	0.010	B
	Forest	0.703	±	0.012	A
Shannon Diversity (H)	Crop	2.569	±	0.021	B
	Transition	2.613	±	0.050	B
	Forest	2.408	±	0.055	A

Table 2-7. Mean values of fungal community metrics richness, evenness, and Shannon diversity, arranged by vegetation type.

	Vegetation	Mean		Standard Error	Tukey's HSD Significance Level
Richness (S)	Crop	84.533	±	2.765	A
	Transition	74.867	±	5.469	A
	Forest	68.400	±	5.060	A
Evenness (J)	Crop	0.736	±	0.024	A
	Transition	0.789	±	0.025	A
	Forest	0.783	±	0.018	A
Shannon Diversity (H)	Crop	3.252	±	0.089	A
	Transition	3.259	±	0.169	A
	Forest	3.151	±	0.157	A

Table 2-8. Top ten contributing bacterial OTUs per vegetation treatment. OTU number is the base-pair length of that particular LH-PCR amplicon.

Bacterial Species/OTU	Forest Contrib%	Transition Contrib%	Crop Contrib%
311	3.75	-	-
312	-	4.99	7.42
315	10.09	7.19	9.23
317	16.66	15.46	14.08
319	3.1	-	-
328	-	9.28	5.96
329	8.29	-	-
333	–	-	3.79
339	7.75	-	-
340	-	3.59	–
341	7.28	8.3	10.62
343	-	4.15	6.62
344	3.58	-	-
346	5.66	7.77	-
348	–	–	4.58
355	-	5.36	-
356	–	–	4.52
359	4.41	4.82	6.02
Cumulative %	70.6	70.9	72.8

Table 2-9. Top ten contributing animal OTUs per vegetation treatment. OTU number is the base-pair length of that particular LH-PCR amplicon.

Animal Species/OTU	Forest Contrib%	Trans Contrib%	Crop Contrib%
333	2.77	-	-
379	-	-	1.71
374	2.22	-	-
380	-	2.04	1.69
381	3.42	4.01	2.43
389	-	2.07	1.8
408	-	2.19	-
416	-	1.83	-
456	16.56	11.3	-
457	2.6	11.54	16.79
458	2.23	-	7.24
459	2.88	-	4.4
460	3.63	8.38	-
461	15.6	-	4.2
462	-	2.75	2.65
463	-	-	2.66
464	-	2.41	-
466	2.31	-	-
Cumulative %	54.2	48.5	45.6

Table 2-10. Top ten contributing bacterial OTUs per vegetation treatment. OTU number is the base-pair length of that particular LH-PCR amplicon.

Fungi Species/OTU	Forest Contrib%	Transition Contrib%	Crop Contrib%
331	-	2.81	3.17
334	-	-	2.71
335	-	-	2.45
340	-	-	2.73
341	2.68	8.18	4.97
347	2.12	-	2.27
351	-	2.64	-
352	-	2.46	-
353	2.3	-	-
354	2.39	2.18	-
355	-	2.83	2.39
358	2.88	2.63	-
359	-	4.48	-
365	2.73	2.08	-
368	–	–	2.03
374	3.19	-	-
388	–	-	2.09
389	–	2.59	-
391	2.91	–	2.04
394	2.35	–	-
395	2.62	–	–
Cumulative %	26.2	32.9	26.8

Table 2-11. Contingency analysis of common fungal, bacterial, and animal OTU's by vegetation type, weighted by abundance. Criterion for being designated as a common OTU included being present in all treatments in at least one sample replicate.

	Vegetation	Weighted Number of Common Taxonomic Units	Chi Square P Value
Fungi	Crop	1208	<.0001*
	Transition	1047	
	Forest	979	
Bacteria	Crop	368	0.4621
	Transition	397	
	Forest	391	
Animal	Crop	835	<0.0001*
	Transitional	764	
	Forest	667	

CHAPTER 3 – DISEASE INCIDENCE AND DETECTION OF *SCLEROTIUM ROLFSII* BY QUANTITATIVE PCR IN THREE SOIL TYPES

1. INTRODUCTION

Soil-borne phytopthogens are responsible for sizable reductions in crop yield annually (Duveiller et al., 2007; Rovira, 1990). Species belonging to *Rhizoctonia*, *Scelrotium*, and *Fusarium* are notable examples of soil-borne pathogens which affect crop production in the southeastern United States (Franke et al., 1998). These fungal soil-borne pathogens are particularly destructive due in part to their ability to remain in soils even after treatment with pesticides (Neate, 1994). Instances of biologically-based control of these pathogens have been reported (Mazzola, 2004). It has been determined the microbiota of the rhizoshpere surrounding the plant in question may be a factor in the control of soil-borne pathogens, particularly soil-borne fungai (Roget, 1995). Generally, soils with high microbial diversity in their bacterial and fungal communities are better able to repel disease than soils with low microbial diversity (Gupta and Neate, 1999). It is possible that two separate mechanisms may work together to produce resistance to disease. First, pathogenic fungi may compete for resources and space with other fungi and bacteria and be indirectly limited in their growth . Secondly, certain species of the existing microbial community may directly act as antagonists to pathogenic fungi (Ristaino et al., 1991).

Sclerotium rolfsii, also known as southern blight, is a fungal plant pathogen with an extensive range consisting of most warm and subtropical regions of the globe (Harlton et al.,1995) This soil-borne pathogen is capable of infecting a variety of important crop species (Pravi et al., 2014). *S. rolfsii* is particularly hard to be eradicated due to its ability to form

sclerotium which are dense protective structures and can serve as inoculum for further infections. In this manner, *S. rolfsii* populations can persist in the soil in very low concentrations for several seasons. Because of its impact on crop production and its difficulty of detection, sensitive molecular-based assays may be useful in identifying soils which harbor *S. rolfsii* to determine the appropriate management practices.

This pilot study will attempt to answer the following questions: Can *S. rolfsii* innoculum be successfully detected by qPCR methods? What are the background levels of *S. rolfsii* across three different soil environments? Do crop, transitional, and forest soils exhibit differences in their resistance to inoculation? And lastly, is detection of *S. rolfsii* DNA by qPCR methods a good predictor of southern blight symptom severity?

2. METHODS

2.1 Sampling Design and Greenhouse Setup

Soil was collected from three geographic locations: “Peter’s Farm” (32°34.784, 082°32.313), “Honeydew Farm” (32°32.354, 081°50.053), and “Strickland Farm” (32°19.231, 081°41.554). At each of these three sampling locations, about 25kg of soil was collected from three different environments representative of crop land actively in use for agricultural production, transitional grassland in early stages of secondary succession, and pristine unmanaged forest land. The 25kg of soil was divided and placed into 3L potting containers, for a total of 66 pots, 11 pots being assigned to each location and vegetation combination. Pots were arranged in the greenhouse in a completely randomized design. Better Boy F1 tomato seedlings were planted in each 5kg soil sample and placed in a climate controlled greenhouse at Georgia

Southern University under equal watering and light conditions for 6 weeks of maturation. After seedling maturation, 5 of the 11 pots in each location/treatment combination were inoculated with *Sclerotium rolfsii.* The remaining 6 uninoculated pots in each location/treatment combination served as controls. *Sclerotium rolfsii* was isolated from an infected tomato plant found at the Strickland Farm sampling location. *S rolfsii* was grown on approximately 300ml of soaked and autoclaved red wheat seed. *S roflsii* was allowed to develop for 2 weeks prior to inoculation at an incubation temperature of 37°C. Greenhouse soil was inoculated by placing three *Sclerotium*-infected red wheat seeds approximately 3 inches from the base of the tomato plant. Five 3-gram subsamples were taken from each pot at the end of eight weeks for analysis by qPCR. Visual assessment of disease progression was made on weekly intervals, along with tomato plant height, and leaf number. Dry above ground biomass was recorded at the data collection endpoint.

2.2 DNA Extraction

Total DNA was extracted from 0.75 grams of each soil sample using PowerMax® Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). This Isolation kit was selected for its ability to purify very low concentrations of microbial DNA. Additionally, this preparation includes reagents which eliminate phenolic soil compounds such as humic acid, which are known to inhibit PCR. DNA was stored at -20°C.

2.3 Determining total fungal and S. rolfsii DNA amounts using real-time quantitative PCR (qPCR)

qPCR assays to quantify total microbial fungi and *S. rolfsii* DNA in each soil sample were conducted using the Mastercycler ep realplex real-time PCR system (Eppendorf North America, Inc., Hauppauge, NY). Primer pairs for *Sclerotium rolfsii* (SCR-F and SCR-R) and Fungi (NSI1,

58A2R) along with the intercalating dye SYBR green were used to determine the quantity of bacterial and fungal DNA present in each soil sample. Three technical assay replicates were performed for each soil sample. Standard solutions for total fungi and *Sclerotium* detection assays were created from DNA extracted from pure cultures of *S. rolfsii*. A Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.) was used for initial quantification of these standard DNA solutions. Serial dilution methods were used to create 6 standard solutions at 10, 1, 0.1, 0.01, 0.001 and 0.0001 ng μl^{-1}, diluted with ultrapure water. These DNA solutions served as standards for subsequent qPCR assays. Methods used were similar to quantitative PCR protocols outlined in previously published assessments of soil bacterial and fungal communities (Fierer et al. 2005). Statistical analyses including ANOVA, MANOVA, Spearman's correlation, Kruskal-Wallis, and Student's t analyses of these assays were performed in JMP statistical software (SAS Institute Inc.).

2.4 Determining pathogenic fungi DNA amounts using real-time quantitative PCR (qPCR)

The specific primer set of SCR-F(5'-CGTAGGTGAACCTGCGGA-3') and SCR-R (5'-CATACAAGCTAGAATCCC-3') was used to amplify a 540-bp product which contains parts of the ITS1, ITS2 and the entire 5.8S rDNA subunit. This primer pair was designed and tested (Jeeva, 2010) to amplify fragments unique to *Sclerotium rolfsii*. This primer pair was used in conjunction with qPCR methods outlined previously to quantify amounts of *S. rolfsii* DNA in all greenhouse samples. In addition to *S. rolfsii*, total fungi DNA fragments were also quantified using methods described in chapter 1.

2.5. Disease assessment

Disease incidence and severity was determined qualitatively using a scale based on presenting symptoms in the host plant *S. lycopersci*. The scale ranged from 0 to 5, 0 being a plant with no symptoms of basal stem lesions or wilting leaves. Level 1 was classified as a plant with <20% of leaves wilted, and/or <20% of basal stem with lesions. Level 2 included plants with 20-40% of leaves wilted and/or 20-40% of basal stem with lesions. Level 3 plants had 40-60% of leaves wilted and/or 40-60% of basal stem with lesions. Plants determined to be level 4 presented with 60-80% of leaves wilted and/or 60-80% of basal stem with lesions. Lastly, the most severely infected plants were designated as a 5 with >80% of leaves wilted and also included plants determined to be dead from disease.

3. RESULTS

3.1 Total fungal DNA Quantification

Uninoculated forest fungal DNA concentration was significantly higher than all transitional and crop soils, irrespective of inoculation treatment P=0.0076 (Fig. 3-1). Additionally, Inoculation treatments did not differ significantly from their respective controls in any instance P=0.7895.

3.2 Sclerotium DNA Quantification

Significantly higher *Sclerotium* DNA concentrations were detected in the inoculated treatments of both forest and transition soils P=0.0003 (Fig 3-2). Higher concentrations of *Sclerotium* DNA were detected in inoculated crop soils compared to controls, though this difference was not significant. Background levels of *Sclerotium* detected in control plants did not differ significantly.

3.3 Solanum Lycopersicum growth

Solanum lycopersicum growing in both inoculated and uninoculated crop soils had a significantly greater height increase during the study than plants potted in transitional or forest soils, P<0.0001 in all comparisons. Inoculation had no significant effect on plant height (Fig 3-3.) Further analysis by MANOVA revealed trends of significantly higher plant height in crop soils P=<.0001, and no significant effect on change in height from inoculation treatment P=0.1675 and no interaction between vegetation type and inoculation treatment P=0.1623 (Fig. 3-4). Assessment of dry biomass at the conclusion of the study showed significantly higher biomass in both inoculated and uninoculated crop soils compared to all other treatment combinations P=<.0001, and no effect from inoculation treatment P=0.6638 or interaction of inoculation and vegetation type P=0.4897 (Fig. 3-5).

3.4 Disease incidence

Disease incidence of infection by *S. rolfsii* did not vary significantly between soil types P=.7916 (Table 3-1). No damage due to disease was observed in any of the control plants. Disease incidence level also displayed poor correlation with qPCR-detected DNA concentrations of *S. rolfsii,* Spearmans ρ= -0.2064, P=0.207 (Fig. 3-6).

4. Discussion

Uninoculated forest fungi DNA concentration was significantly higher than all fungal DNA concentrations of inoculated transitional and crop soils. This is congruent with qPCR data presented in chapter 2, which also found significantly higher fungal DNA concentrations in forest soils at the beginning of the field study. Inoculation treatment with *S. rolfsii* did not

significantly affect the total amount of fungal DNA detected in the soil. This indicates that the fungal inoculum is present in the soil at relatively minute quantities.

As expected, significantly higher *Sclerotium* DNA concentrations were detected in the inoculated treatments of both forest and transition soils. Inoculated crop soils were found to have a higher mean *Sclerotium* DNA concentration than control crop soils, though this difference was not significant. Background levels of *Sclerotium* between control forest, transition, and crop soils did not differ significantly. These data support the proof of concept and methods by which the pathogen *S. rolfsii* may be detected by qPCR. However, no difference in the ability to suppress *S. rolfsii* was observed in any treatment. Previous studies involving container media soil in a greenhouse setting have successfully identified soils suppressive to *S. rolfsii* and other soil-borne pathogens (Gorodecki and Hadar, 1990), citing antagonistic microbial community members as the likely mechanism responsible for suppression.
Both inoculated and uninoculated plants in crop soils grew significantly more than plants potted in transitional or forest soils. Likely this significantly higher growth in crop soils is due nutrient and fertilizer amendments. Inoculation had no significant effect on plant height. This lack of inoculation effect on overall plant height may be due to the otherwise healthy and unstressed plants used in the study.

Additionally, soil type did not significantly affect the level of southern blight symptom severity. In this case, no soil can be identified as being “suppressive” to *S. rolfsii* growth or southern blight disease progression. Despite differences in diversity and community identified in chapter 2, these soils display no difference in their ability to suppress soil borne pathogens. Other research has indicated that individual genera, not necessarily diversity metrics, may be

responsible for *S. rolfsii* suppression in soils. Microbes documented to be involved in *S. rolfsii* suppression include the bacteria *Pseudomonas fluorescens* (Ganesan and Gnanamanickam, 1987) as well as the fungi *Talaromyces flavus* (Madi et al., 1997) and *Trichoderma konigii* (Tsahouridou and Thanassoulopoulos, 2002). Future study of the suppressive abilities of these soils may involve the simultaneous detection of a select number of these species know for their antagonistic action against *S. rolfsii*.

Southern blight disease symptoms lack correlation with DNA concentrations of the causative pathogen, *Sclerotium rolfsii* in inoculated soils. Plants with low or no symptoms were associated with soils containing a wide range of *S. rolfsii* DNA concentrations. Plants with severe symptoms of southern blight had relatively low amounts of *sclerotium* DNA in their soils. An explanation for this lack of correlation may include patchy distributions of inoculum in the soil, leading to extreme variability in calculated DNA concentrations. Indeed, in agricultural context, distributions of these soil-borne pathogens, including *S. rolfsii*, are highly clustered and not even distributed in the soil (Shew and Campbell, 1984). This spatial arrangement of *S. rolfsii* may be a significant obstacle to accurate detection of this soil-borne pathogen by molecular techniques. Other explanations may include strong, healthy plants, and a weak inoculum produced a situation where *S. lycopercium* had a broad range of responses to soil-borne disease.

5. Conclusion

It was determined that *Sclerotium rolfsii* can be appropriately detected in the soil by qPCR methods. Background levels of *Sclerotium* in control soils are low and do not differ

significantly between vegetation type. Crop, transitional, and forest soils did not vary in their ability to suppress a *S. rolfsii* inoculation. *Sclerotium* DNA concentrations lack correlation with southern blight disease incidence and severity. Although some plants were heavily infected with *S. rolfsii* and developed symptoms, overall inoculation treatment had little impact on plant growth.

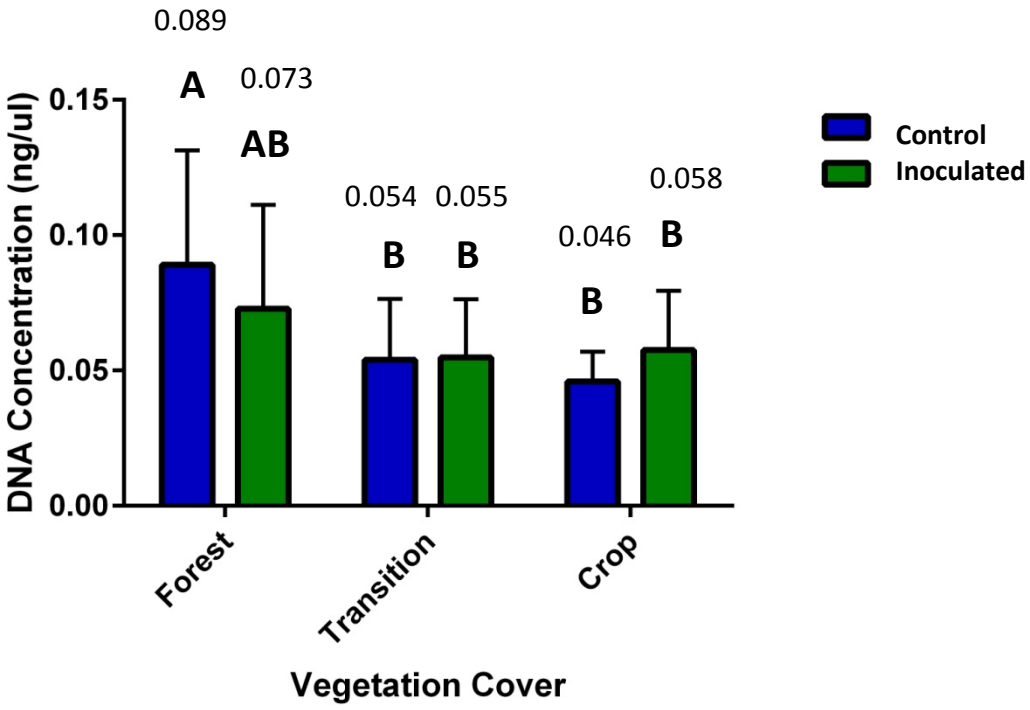

Figure 3-1. Total fungal DNA concentrations between vegetation types and inoculation treatments. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

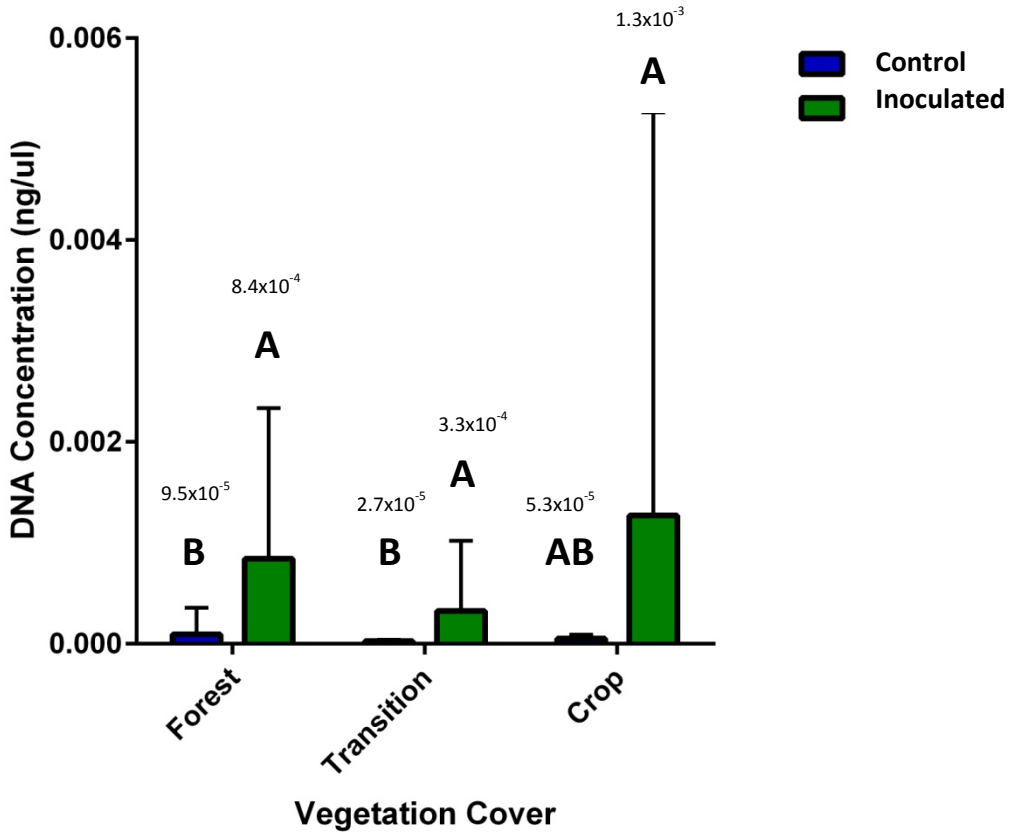

Figure 3-2. *Sclerotium rolfsii* DNA concentrations between vegetation types and inoculation treatments. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

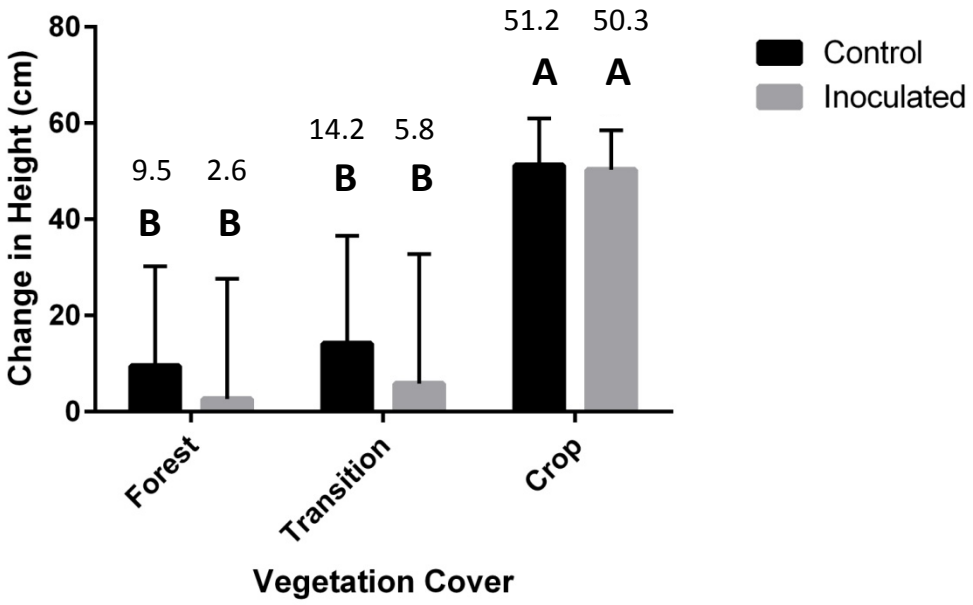

Figure 3-3. Change in height of *S. lycopersicum* under different inoculation treatments and soil types. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

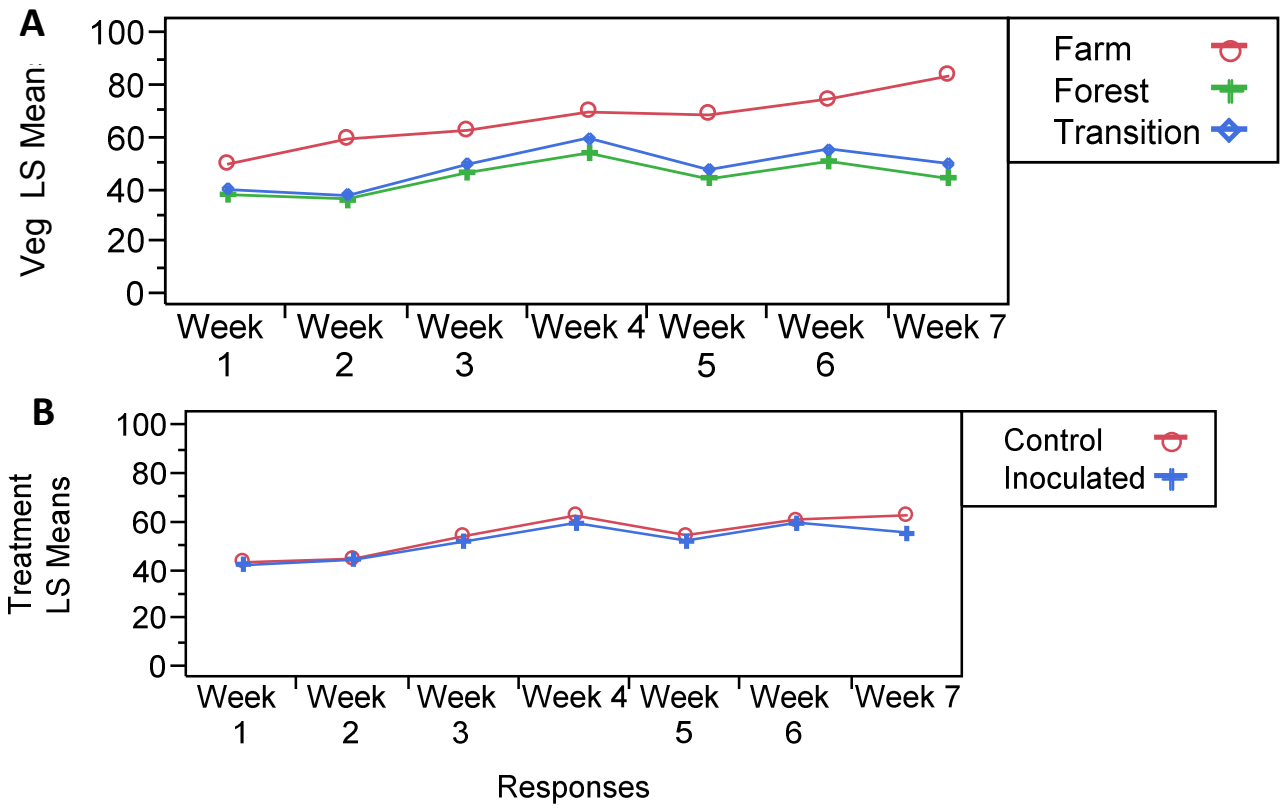

Figure 3-4. *S. lycopersicum* height in centimeters over time. *S. lycopersicum* height over seven weeks by mean crop, transition, and forest soil (A). *S. lycopersicum* height over seven weeks by inoculation treatment (B).

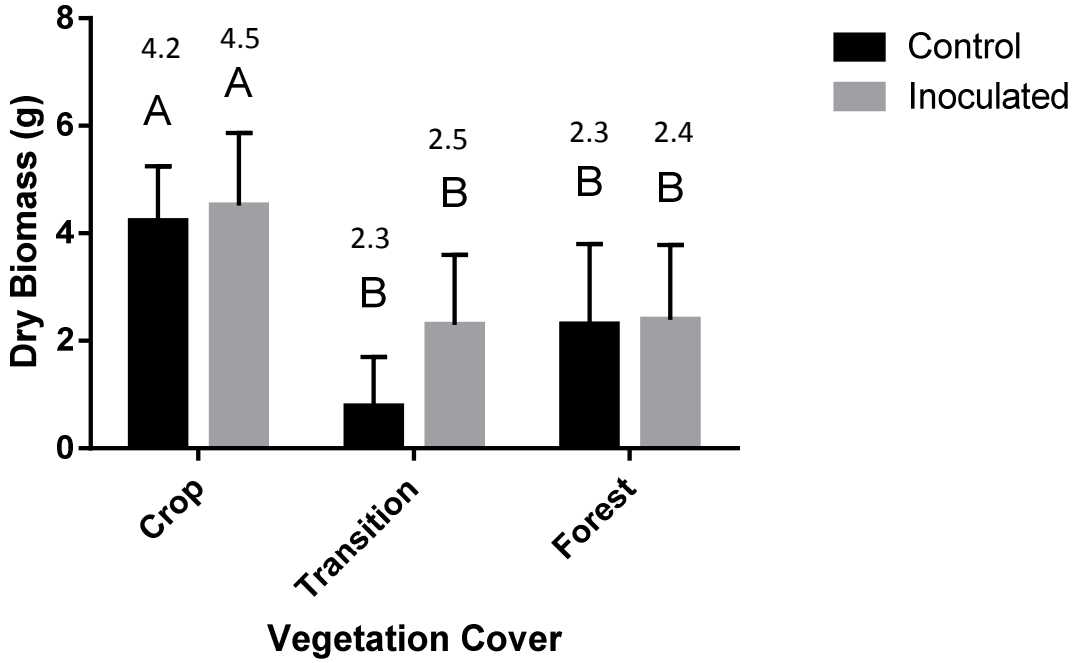

Figure 3-5. Dry-weight biomass measured at end time point. Bars represent standard deviation. Bars represent one standard deviation. Values over bars represent the arithmetic mean. Differing letters denote significant difference of means (P=<0.05).

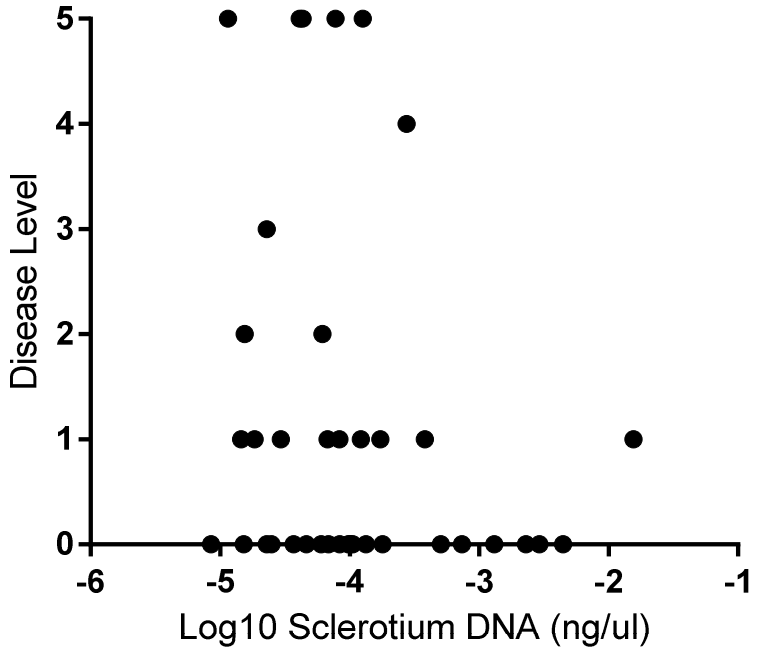

Figure 3-6. Correlation of southern blight disease symptoms with DNA concentrations of the causative pathogen, *Sclerotium rolfsii* in inoculated soils.

Table 3-1. Average southern blight symptom severity of *S. lycopersicum* after inoculation with *S. rolfsii*. Symptom severity was determined qualitatively and recorded on a scale of zero to five.

	Disease incidence of un-inoculated plants	**Disease incidence of inoculated plants**
Forest	0	1.27
Transition	0	1.38
Crop	0.3	0.86

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