IMPACT OF AGRICULTURAL MANAGEMENT AND MICROBIAL INOCULATION ON SOYBEAN (*GLYCINE MAX*) AND ITS ASSOCIATED MICROBIOME

By

Reid Longley

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Microbiology and Molecular Genetics–Doctor of Philosophy

2022

ABSTRACT

IMPACT OF AGRICULTURAL MANAGEMENT AND MICROBIAL INOCULATION ON SOYBEAN (*GLYCINE MAX*) AND ITS ASSOCIATED MICROBIOME

By

Reid Longley

Soybean (*Glycine max*) is a globally important crop with uses as food, cooking oil livestock feed, and biodiesel. Soybean can be considered holobionts because they host diverse microbiomes which extend plant genotypes and phenotypes through various microbial functions such as nitrogen fixation and increased disease resistance. My research focused on assessing the impact of three agricultural management strategies on the soybean holobiont.

Soybean cropping systems can be managed using various strategies, including conventional tillage, no-till, and organic management regimes. These management systems have been shown to impact the microbiomes of soybean-associated soils, however, their impacts on plant-associated microbiomes are still not well understood. In this study, I assessed the impact of conventional, no-till, and organic management treatments on soybean microbiomes at Michigan State's Kellogg Biological Station Long-Term Ecological Research site (KBS LTER). I found that management impacted microbiome composition and diversity in soil, roots, stems, and leaves and that this impact persisted throughout the season. Additionally, when comparing the same soybean genotype grown in conventional and no-till management systems, tillage regime impacted the microbiome throughout the plant and the growing season. This effect impacted microbial taxa which are likely to be plant beneficial, including nitrogen fixing *Bradyrhizobium*.

Another important management tool that is expected to impact plant-associated microbial communities is the application of foliar fungicides. While fungicides are known to protect plants from particular fungal pathogens, non-target impacts of fungicides on crop microbiomes, and the

impact of management on microbiome recovery are not well understood. To address this knowledge gap, I assessed the impact of foliar fungicide application on the maize (Headline® fungicide, 2017) and soybean (Delaro® fungicide, 2018) microbiomes in conventional and no-till plots at the KBS LTER. I found that fungicide applications have a non-target impact on Tremellomycete yeasts in the phyllosphere and this impact was greater in soybean than maize. Co-occurrence network analysis and random forest modelling indicated that changes in fungal communities may lead to indirect impacts on prokaryotic communities in the phyllosphere. Importantly, this work demonstrated that phyllosphere communities of soybeans under no-till management had greater recovery from fungicide disturbance. This novel finding exemplifies how tillage regime can impact phyllosphere microbiomes and their responses to disturbance.

Microbial inoculants in agriculture have long been used for biocontrol of pathogens, but there is also interest in their use to dampen the impacts of abiotic stress including drought. In this study, I tested whether inoculating soybeans with hub taxa identified through network analysis from no-till soybean root microbiome data from the KBS LTER could provide protection against water limitation. Soybean seedlings were enriched in consortia of hub bacteria and fungi and were grown in no-till field soil. Seedlings were then exposed to low-moisture stress, and plant phenotypes, plant gene expression, and amplicon sequencing of microbial DNA and cDNA were assessed throughout the stress period. Inoculation increased plant growth, nodule numbers, and led to increased expression of nodulation-associated genes. 16S sequencing of cDNA revealed higher levels *Bradyrhizobium* in inoculated samples. These results indicate that inoculation with hub microbes can benefit soybean plants, possibly through interaction with other microbes, interaction with the plant, or both. In summary, fungicide, tillage, and inoculation all impact the soybean microbiome, indicating that management choices impact the entire holobiont.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Gregory Bonito for his guidance and consistent support throughout my time at Michigan State. I am particularly thankful that I have had the chance to explore a wide diversity of projects which have helped shape me as a scientist. I would like to thank my committee, Dr. Frances Trail, Dr. Martin Chilvers, Dr. Ashley Shade, and Dr. Sarah Evans for their guidance and helpful advice throughout this process. I would also like to thank my lab mates in the Bonito lab for their unconditional support, advice, friendship, and research help. I am particularly grateful for the mentorship and support of Dr. Nico Benucci, Dr. Zachary Noel, Dr. Marjorie Weber, and Dr. Aaron Robinson. Their advice and mentorship allowed my research to progress at a much faster rate than would otherwise be possible.

I would like to acknowledge the help of the Microbiology and Molecular Genetics department throughout my PhD. Especially, I would like to acknowledge the support and helpful advice of Roseann Bills, Dr. Donna Koslowsky, and Dr. Abramovitch.

I am thankful for the help of Sophia Fitzgerald in sampling and processing of soybean samples used in chapters 2 and 3. I am also grateful for the sampling and sample processing advice of Dr. Kristi Gdanetz MacCready. I am thankful to the KBS agronomy staff for aid and advice in collecting samples which were used in chapters 2,3, and 4. Particularly, I am thankful to Stacey VanderWulp for her willingness to help ensure that my sample collection proceeded smoothly. I am thankful to Julian Liber for his support and bioinformatics advice in processing amplicon sequencing data generated throughout my dissertation work.

I am grateful to the entire Chain lab team at Los Alamos National Laboratory for teaching me bioinformatics techniques and for helpful research and career advice. Particularly, I would like to thank Dr. Patrick Chain, Dr. Aaron Robinson, Dr. Dean Morales, Julia Kelliher,

and Karen Davenport for their advice and support throughout my time in Los Alamos. I would also like to acknowledge Dr. Jessy Labbé for his advice on performing plant inoculations with microbes, and Dr. Robert VanBuren for his advice on performing low-moisture stress experiment in chapter 4. I am thankful for the help of Sophie Gabrysiak in setting up the low-moisture stress greenhouse experiments in chapter 4. I am thankful to the entire greenhouse staff, especially Dr. Chrislyn Patricka, for providing advice for greenhouse experiments. I would like to thank Dr. Tony Schilmiller and Dr. Dan Jones at the MSU Mass Spectrometry and Metabolomics core for their advice on non-target metabolomics. I am thankful to Dr. Kevin Childs, Kevin Carr, and the entire MSU genomics core staff for help and advice regarding sequencing throughout my PhD. I am also thankful to Dr. Aretha Fiebig for her mentorship and advice regarding work with *Caulobacter* for microbial inoculation experiments. I would like to acknowledge the support and mentorship of Dr. Robert Last and Dr. Jyothi Kumar through the Plant Biotechnology for Health and Sustainability (PBHS) program.

Finally, I am grateful to my friends and family for their support throughout my graduate studies. The support of friends and family, both near and far, motivated me to push myself to work hard and to continue to find joy even through stressful times.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

KBS Kellogg Biological Station

LTER Long Term Ecological Research

AMF Arbuscular Mycorrhizal Fungi

IAA Indole-3-Acetic Acid

ITS Internal Transcribed Spacer

OTU Operational Taxonomic Unit

DDT Dichloro-Diphenyl-Trichloroethane

qPCR Quantitative Polymerase Chain Reaction

rDNA Ribsomal DNA

FISH Fluorescence In Situ Hybridization

PCoA Principal Coordinates Analysis

SSU Small Subunit of the ribosome

LSU Large Subunit of the ribosome

BP basepairs

PERMANOVA Permutational Multivariate Analysis of Variance

DPF Days Post Fungicide

QoI Quinone Outside Inhibitor

DMI Demethylation Inhibitor

DI Deionized

fOTU Fungal Operational Taxonomic Unit

pOTU Prokaryotic Operational Taxonomic Unit

CAP constrained analysis of principal coordinates

LB Luria-Bertani (growth medium)

PYE Peptone Yeast Extract (growth medium)

R2A Reasoner's 2A (growth medium)

MEB Malt Extract Broth

CFU Colony Forming Unit

LC -MS Liquid Chromatography – Mass Spectrometry

ZOTUs Zero Radius Operational Taxonomic Units

VST Variance Stabilizing Transformation

GEO Gene Expression Omnibus

GO Gene Ontology

RMD Relative Mass Defect

PCA Principal Components Analysis

ES Extraction Solution

BSA Bovine Serum Albumin

TE Tris EDTA buffer

Chapter 1

Introduction

Problem Statement

As human populations continue to grow globally, it is becoming increasingly clear that agricultural management strategies will need to be adapted to maintain production and minimize environmental impacts of agriculture. In addition to the development of novel crop varieties and management strategies; it is important to assess how current management strategies impact host plants as well as their associated microbiomes. For example, no-till or reduced tillage agriculture have long been used in the United States, and their usage continues to increase [1]. It has been shown that tillage regime impacts the soil microbiome [2, 3] and may have a subtle impact on the plant microbiome [4]. However, there is a knowledge gap on the impact of tillage regime on soybean-associated microbial communities throughout the entire plant growing season. It is particularly important to assess the impact of tillage on soybean-associated microbial communities as soybeans have close relationships with *Bradyrhizobium* for nitrogen fixation, as well as important associations with arbuscular mycorrhizal fungi (AMF).

Foliar fungicides are another common management tool in modern agriculture that are used to control fungal pathogens. In the presence of disease pressure, fungicides are crucial for maintaining crop yield [5]. Yet, fungicide applications are frequently made in the absence of disease pressure to reduce risk of disease development, and in some conditions may provide a potential yield benefit [6]. Applying fungicides when disease pressure is low can account for unnecessary farm expenses, environmental damage, development of fungicide-resistance in the environment, and may also lead to the local extinction of fungal taxa which could benefit plant health and growth or aid in the structuring of the plant microbiome. Although it has been demonstrated that fungicides can harm non-target taxa such as phyllosphere yeasts, further work is needed to determine whether tillage regime alters the impact or recovery of fungal

communities following a fungicide disturbance [7]. Assessing the impact of fungicide applications in multiple crops and tillage regimes will be crucial for understanding how crop, tillage, and management influence community dynamics in the plant microbiome.

In addition to chemical and physical methods of management, the use of microbial inoculants to promote plant growth or protect plants from stress is becoming an increasingly common strategy in agriculture. Initially, biological applications were prepared using single or a few microbial strains that are known to have biocontrol or plant growth promotion effects [8]. However, there is growing interest in the use of microbial consortia that may occupy or function across a larger niche breadth. Yet, strategies for designing or assembling microbial consortia vary between research groups. One strategy for choosing microbes to include within microbial consortia is to focus on microbial taxa which can alter the structure of the microbiome. These microbial taxa, often called "hubs" due to their high levels of connectivity in the microbiome, are hypothesized to be important in structuring microbial communities, but their usefulness as microbial inoculants has not been fully addressed [9, 10]. Researching the impacts on soybeans of inoculation with hub taxa will be crucial for assessing the level to which native microbes can be used to manipulate plant-associated microbiomes.

Background

This chapter serves as a literature review on the impact of various management strategies on microbial (fungal and bacterial) interactions with crops. This chapter is split into four sections that discuss beneficial roles of microbes in plant holobionts and cover the impacts of three different management strategies utilized in modern agriculture: (1) the impact of tillage intensity on field crops and their associated microbiomes; (2) the impact of foliar chemical applications, specifically, fungicides and insecticides, on microbial communities associated with the

phyllospheres of field crops, and; (3) the impact of microbial inoculants in agriculture.

Beneficial Roles of Microbes in Plant Holobionts

With studies on microbiomes becoming increasingly common, it is clear that organisms rarely, if ever, exist in isolation. Instead, macroorganisms typically host a diverse microbiome composed of both prokaryotes and eukaryotes. Plants are no exception to this rule and host microbiomes inside and on the surface of their above and belowground tissues and also have important interactions with soil microbial communities. Knowing this, it is informative to be able to consider plants and their microbiomes as one distinct unit in which microbial genes extend the genetic and functional repertoire of the hosts genome. This unit is referred to as the holobiont, a term that originally referred to a host and a vertically transmitted symbiont, but later was applied more broadly to the host and its closely associated and likely co-evolving microbiome members, whether inherited or not [11, 12]. In this section, a few examples of the many important microbial contributions in the phyllosphere, endosphere, and rhizosphere of plant holobionts will be highlighted.

The plant phyllosphere (aboveground tissue) is colonized by bacteria and fungi on both the surface (epiphytes) and inside plant tissue (endophytes). Phyllosphere microbiota can contribute to functions already present in the plant, for example, bacterial and fungal communities can produce important phytohormones such as indole-3-acetic acid (IAA) [13, 14]. Bacterial strains that have this trait may be enriched by the host plant [13]. In addition to the direct production of phytohormones, microbial communities can also modulate plant functions. One example of this is the priming of the plant immune response by non-pathogens, which can lead to increased pathogen resistance. In *Arabidopsis*, it was demonstrated that bacterial taxa including *Pseudomonas* helped protect hosts from *Botrytis* pathogenesis, and that this function

was not a direct interaction between the microbes in a plate setting but relied on the presence of the plant[15]. In addition to enhancing functions already present in the plant such as hormone production, members of the phyllosphere microbiota can also provide novel functions to the plant. For example, *Epichloe,* a fungal endophyte produces a non-ribosomal peptide that protects ryegrass from insect herbivory [16]. In addition to diverse benefits provided by individual microbial taxa, changes in phyllosphere microbial diversity can impact plant health. For example, it has been demonstrated that increased bacterial diversity on leaves was associated with increased ecosystem productivity in forests [17]. Increased microbial diversity in the phyllosphere may play an important role in increasing functional redundancy (the degree to which a function is performed by multiple taxa); which may in turn provide microbial communities more resilience to disturbance [18].

As in leaves, microbes play crucial roles inside and on the surface of roots by enhancing or modulating plant functions or by adding new functions to the host plant. One clear example of a crucial function provided to the plant that has been known for more than a century is nitrogen fixation by *Bradyrhizobium* in symbioses with soybeans and other plants [19] . Similarly, AMF are a classic example of fungal-root symbiosis in land plants. This group of fungi is known to play important roles in the acquisition of various minerals and nutrients including phosphorous and nitrogen, as well as the acquisition of water for plant hosts [20–22]. Although specific groups of symbiotic organisms have outsized beneficial impacts on their plant hosts, changes in overall microbial community structure, or beta diversity, can also impact host plant health. For example, it appears that watermelon cultivars with lower levels of disease had greater microbial diversity in roots and the rhizosphere. This possibly indicates that greater diversity decreases invasion by *Fusarium oxysporum* [23]. Additionally, in *Arabidopsis* root systems, it was

demonstrated that beyond individual biocontrol bacteria, many bacterial strains appear to have negative correlations with fungi and promote host plant survival in the presence of fungal pathogens [24]. This indicates that there is a level of functional redundancy among biocontrol functions in plant roots.

Although soil microbial communities are not contained within plants, they can still exert a substantial impact on plant health. The importance of the rhizosphere microbiome on plant health is particularly relevant and can be demonstrated by differences between microbial communities and enhanced microbial activity in rhizosphere compared to bulk soils [25]. This indicates that the plants drive microbial activity, and exert selection or enrichment of specific microbial taxa in the rhizosphere, such as through their root exudates [26]. One example is the concept of disease suppressive soils in which crops can be planted into specific soils which are thought to reduce the likelihood of disease development in the plant. In some cases, this may be related to microbial communities as increased microbial diversity can reduce the ability of other microbes to enter the community [27, 28]. High diversity soil bacterial communities were shown to increase the arbuscular mycorrhization of maize roots following inoculation with *Rhizophagus clarus* [29]. These results demonstrate that high diversity microbial communities may prevent the invasion of some but not all taxa into microbial communities. Examples from plant leaves, roots, and associated soils demonstrate that specific beneficial taxa, as well as particular microbial community traits, can have a substantial beneficial impact on the plant holobiont by providing novel functions to the plant or by altering normal plant functioning. Knowing this, it is important to consider how common agricultural management strategies impact plant-associated microbiomes and how these changes affect crop health.

Impact of Tillage Intensity on Crop Plants and Their Associated Microbiomes

Since the Dust Bowl of the 1930s, when massive erosion and loss of topsoil in US occurred as a result of deep tillage farming practices and severe drought, some form of conservation tillage has been practiced in the United States. However, reduced tillage systems began to be increasingly prevalent in the 1960s when herbicides became more common [1, 30]. Reduced tillage systems include reductions in intensity, depth, or area tilled; while no-till systems eliminate tilling completely [31]. Conservation tillage is broadly aimed at reducing soil loss in comparison to traditional tillage methods and is defined as leaving 30% crop residue coverage on the soil surface, although it does not have to include reducing tillage [32]. However, reduced tillage is becoming an increasingly prominent example of conservation tillage. Reduced tillage also provides benefits such as reductions in soil erosion, increase in drought resilience, reductions of greenhouse gas emissions, and fuel cost savings to farmers [33–35]. These benefits may be particularly impactful when used in crops grown on the largest scale such as maize and soybeans which together represent 57% of all crop acreage in the United States [36].

As mentioned, soybean is a major field crop in the United States, and accounts for 90% of oil seed production in the country. The total acreage of soybean grown in the United States continues to increase and was estimated to be 87.6 million acres in 2021[37]. Like other field crops, a substantial portion of soybeans are managed with some form of conservation tillage. In 2012, 70% of US soybeans were managed using conservation tillage; 56% of acreage under conservation tillage was no-till [1]. The proportion of soybean managed under reduced tillage regimes is likely to increase due to negative environmental impacts of tillage cropping practices. Additionally, it has been demonstrated that reductions in tillage intensity can increase yield, but the magnitude of this effect is dependent on soil type and other environmental factors [38]. In

some cases, including at Michigan State University's Kellogg Biological Station Long-Term Ecological Research site (KBS LTER), no-till agriculture has increased crop yield of maize, soybean, and wheat relative to conventional tillage [39]. The long-term yield benefit of tillage reduction is particularly apparent in conventionally tilled fields that have experienced topsoil loss. For example, a study in Indiana demonstrated soybean yield losses of more than 17% in fields that experienced substantial erosion, but there were remaining concerns about no-till systems increasing soil compaction, which could lead to reduced seedling emergence [40].

As reduced or no-till management systems remain common and profitable, it is important to assess their impact on plant physiology and growth as well as the plant-associated microbiome. One important concern regarding the impact of reduced tillage is that without turning over the soil, root growth may be limited. Several studies have demonstrated that soil managed under various forms of reduced tillage can become hardened, which can lead to reduced seedling growth [41, 42]. Tillage regime may also impact root growth. For example, peas grown under conventional tillage had increased root length, but those under no-till management had higher root biomass [43]. In maize, it has been demonstrated that tillage leads to differences in the distribution of root growth with roots in no-till soils growing more horizontally compared to conventionally tilled roots [44] This also appeared to be true in soybean where roots were more abundant in the shallowest soil layers compared to conventional tillage [45]. These studies demonstrate that the impacts of tillage on plant phenotypes appear to be in part mediated through impacts on soil compaction. The extent of this compaction likely relies on the soil texture. The impact of these phenotypic changes on yield likely depends on the crop and on environmental conditions such as precipitation. Assessing microbial communities and plant phenotypes associated with reduced tillage under different environmental conditions

will be important in assessing which areas are well suited to reduced tillage regimes.

Impact of Tillage on Pathogens and Plant Beneficial Microorganisms

Although studies assessing plant-associated microbiomes have become increasingly common, the impact of tillage on microbiomes is not entirely consistent. Several amplicon sequencing studies on wheat, soybean, and maize have demonstrated that tillage regime impacts fungal community composition and beta diversity, particularly in the soil [2, 3, 46–48]. Among fungi, there is concern that tillage may disrupt hyphae leading to the disruption of potentially beneficial activities. It has been demonstrated that there is an increase in hyphal length and fungal biomass in no-till compared to conventionally tilled soils [49, 50]. However, the impact on alpha diversity metrics of fungal communities is not consistent [3, 47, 49, 51]. In addition to comparing overall community metrics, there is a need to compare the impacts of tillage on specific functional groups that may have important interactions with their plant hosts, in particular AMF [52].

Assessing plant pathogens from different tillage regimes is an important aspect in determining which tillage regime to utilize. One of the primary rationales behind more intensive tilling regimes is to reduce pathogen pressure caused by leftover crop residues in fields. No-till fields may also increase pathogen pressure by lowering soil temperature and increasing soil moisture. For example, in studies of soybean sudden death syndrome (SDS) caused by *Fusarium solani*, chisel tillage reduced the foliar disease index by 50% in earlier season sampling and approximately 25% in late season [53]. Similarly in maize, ear rot caused by *Stenocarpella maydis* was reduced by as much as 40% with more intense tilling following a period of reduced tillage compare to other less intense tillage regimes [54]. No-till management may also increase disease severity of oomycete disease caused by *Phytopthora* and *Pythium*, especially in wet

conditions [55, 56]. However, reduced-tillage does not always result in more disease, and in fact there are also examples of pathogens with lower severity or unimpacted severity under reduced tillage practices such as *Fusarium graminearum* in wheat and *Macrophomina phaseolina* in soybean [33, 57]. Reduced tillage may be effective in controlling a small subset of diseases that thrive under drought conditions due to an increase in soil moisture associated with reduced or no-till systems [58]. Together, these studies demonstrated the impact of tillage systems on specific plant pathogens. However, with the development of high-throughput sequencing technologies to sequence the microbiome, the impact of reduced-tillage management on whole communities of microorganisms can now be readily assessed.

Several studies have detected differences by tillage regime in the abundances of specific pathogens [48]. However, trends on the impact of tillage on plant disease were not consistent across all pathogens. This may be partially due to some pathogens not being detected at high enough levels to distinguish between management treatments [2]. Additionally, the detection of tillage-associated differences in pathogen distributions may be hindered by limitations associated with amplicon sequencing. For example, amplicon sequencing can typically only detect taxonomic differences at the genus level or above while detecting pathogens likely requires species or strain-level resolution. Additionally, amplicon sequencing cannot distinguish between DNA from living cells and dead cells, which makes it difficult to assess the activity of pathogens [59]. However, due to the ability of amplicon sequencing to assess the microbiome inclusive of many members and phylogenetically diverse lineages, the distributions of both putative pathogens and other microbiome members can be readily assessed under different tillage regimes.

Crop microbiome studies have increasingly assessed the impact of tillage on groups of

potentially beneficial bacteria and fungi. Since AMF are consistently considered to be beneficial to plants and soils, although their impact is dependent on partner combinations and environmental context [60], the impact of tillage on these fungi will be discussed further. AMF are known to be beneficial to plants in various ways such as through the transport of phosphorous and nitrogen into plant roots and the direct transport of water into plant roots [61]. Due to limitations in culturing AMF given their obligate dependency on plant hosts, various culture-independent methods have been used to study their distributions in soil. For example, a study using spore counting and morphological identification of AMF from soil demonstrated that no-till plots had higher spore densities and species richness compared to those that were conventionally tilled, but this trend was taxa-dependent with some AMF taxa being unimpacted by tillage regime [62]. However, one metanalysis showed that reduced tillage and the use of cover crops increased root colonization of AMF [63].

With advances in high throughput generation sequencing, AMF can be assessed either as part of a survey of the entire fungal community or can be targeted with specific primers which may increase the diversity of recovered taxa in the Glomeromycotina [64]. Although there are limitations to what can be inferred from amplicon sequencing of fungal communities due to skewed abundance estimates from primer biases and variation in internal transcribed spacer (ITS) copy numbers; these studies can still provide valuable insights into shifts in the mycobiome. One amplicon study using AMF specific primers on maize root associated AMF communities demonstrated a significant impact of tillage on AMF communities. Similarly to morphology-based studies, some operational taxonomic units (OTUs) appeared to be associated to tilled plots while others were associated to no-till treatments [65]. Further work is needed to determine the characteristics of AMF taxa that are impacted by tillage compared to those that are

not and their impacts on the host plant. It is also important to note that the impacts of tillage on AMF do not appear to be consistent across all crops. Recent microbiome studies on *Sorghum* demonstrate increases in AMF abundance under conventional tillage, especially when combined with cover cropping [66]. Similar to other fungal communities, advances in sequencing technologies continue to improve the study of AMF. For example, the use of PacBio sequencing technology enables the circular consensus sequencing of longer amplicons, which can improve the detection of strain and species-level differences [67]. These longer amplicons, along with increased study of AMF genomes and transcriptomes, will be useful in revealing specific impacts of tillage on AMF communities.

Similar to plant beneficial fungi, there is interest in determining the impacts of tillage on plant beneficial bacteria. The impact of tillage on overall bacterial alpha diversity appears to be inconsistent and may depend on the crop and environment [47, 68]. The impact of tillage on bacterial communities may be expected to be reduced compared to fungi as bacteria are not characterized by hyphal networks, but this has not always been found to be the case. Previous work has demonstrated that tillage regime impacts bacterial community composition in the soil and roots of soybean, wheat, and maize [3, 47, 69]. As discussed for fungi, it is important to assess the impact of tillage on specific guilds of bacteria that may have important interactions with their host plants. In soybeans, it is especially important to consider the impact of tillage on symbiotic nitrogen fixing bacteria such as *Bradyrhizobium* species, as changing distributions of these taxa could impact yield. Amplicon sequencing work at KBS demonstrated an increase in the relative abundance of *Bradyrhizobium* under no-till management [47]. Other work has demonstrated that conventional tillage reduced *Bradyrhizobium* diversity in soybean fields [70]. This observation correlates with observations of increased soil organic carbon under no-till

management which is thought to increase *Bradyrhizobium* diversity [71, 72]. Further work is needed to ensure that increased *Bradyrhizobium* relative abundance and diversity correlates with increased nitrogen fixation activity in soybean roots and that this increase leads to greater yields.

The impact of tillage on plant-associated microbial communities is expected to be especially pronounced in roots due to the direct contact with soil. However, aboveground plant-associated microbial communities also have important roles in plant health and may be impacted by tillage regime. The impact of tillage regime on phyllosphere microbiomes has been under-assessed compared to belowground compartments. It has been demonstrated that epiphytic and endophytic bacteria and fungi can be transferred from the soil and organic horizon to the phyllosphere, so it can be expected that some of the microbial variation in the soil would be reflected in the phyllosphere [73–75]. However, there is also evidence that aboveground plant tissues select for specific communities [73]. Research on the impact of tillage on phyllosphere microbiomes of soybean has demonstrated that long-term no-till management altered fungal and prokaryotic beta diversity in leaves and stems, but to a lesser extent than soil [47]. However research on wheat did not find the same level of management impact in the phyllosphere, indicating variation by crop and season [4]. Further work is needed to determine if there is a direct impact of no-till management on the crop phyllosphere throughout the growing season or if the phyllosphere microbiome changes because of changes in the soil.

Functional Microbiome Changes Associated with Tillage Regime

As sequencing technologies and methods for assesing microbiomes continue to improve, the functional capacity of microbiomes will become more accessible through shotgun sequencing of the metagenome, metatranscriptomics, proteomics and metabolomics. Differences between tillage managements in microbial functons may be expected to be smaller in comparison to

taxonomic differences due to high levels of functional redundancy, but the extent of functional redundancy may depend on the trait and environment [76, 77]. This was the case in a study on agricultural soils in a rotation that included soybean that demonstrated with shotgun metagenomics that there were differences between tillage managements in carbohydrate metabolism. These could have resulted from differences in organic carbon, but these differences were smaller than differences in bacterial diversity [78]. Similarly, research based on metatranscriptomics of soils demonstrated differences in some areas of microbial metabolism, but again showed some functional redundancy in carbon metabolism [66]. Interestingly, Wipf et al. 2020 demonstrated that the number of AMF transcripts was higher under standard tillage than in a no-till system, an indication of increased AMF activity [66].

Although sequencing techniques such as metatranscriptomics and shotgun metagenomics can reveal the microbiomes functional potential, other techniques such as metaproteomics and metabolomics can be used to provide a snapshot of active microbial functions. However, these methods also have limitations including limited reference databases and difficulties in annotating compounds. Possibly because of these limitations, metabolomics and proteomics studies on the impacts of tillage are rare. Several studies on broad enzymatic activity in maize and vegetable cultivation soils report higher levels of enzymatic activity including protease and urease activity in fields that were not agriculturally managed compared to those that were. Increased enzyme activity was also reported in fields with reduced tillage compared to conventional tillage [79]. Although there are few studies assesing the impact of tillage on plant or soil metabolomes directly, the connection between the soil microbiome and the aboveground metabolome has been assesed, showing that the addition of soil microbes increased production of various compounds such as phenolics in aboveground tissues [80]. In addition to impacts of soil microbiome

changes, it is known that shifts in the root-associated microbiome of *Populus* were associated with changes in at least 10% of detected root secondary metabolites [81]. Since work discussed above demonstrated that tillage impacts the soil and root microbiome, it is possible that these impacts would translate to the plant metabolome. Additional work should address how tillage specifically impacts the metabolome of plant holobionts, and how these changes are associated with changes in yield.

Impact of Tillage on Microbiome Under Different Environmental Conditions

As global climate continues to become increasingly erratic, it is important to analyze the performance of tillage under different environmental conditions. Although there are many environmental stresses relevant to soybeans and other crops which may be altered by tillage, staple crops are particularly sensitive to drought, which is expected to be affected by human-induced climage change and will be discussed further here [82]. Reductions in tillage are thought to be specifically useful under drought conditions as lower tillage increases soil moisture content in various environments including at the KBS LTER [83, 84]. No-till or reduced tillage management systems are thought to be especially beneficial to yield under dry conditions. For example, one study on maize in China demonstrated a nearly 20% increase in yield under no-till conditions during dry years, but a decrease in yield during wet years [85]. Similarly, in the United States, a large scale study on soybean demonstrated that conservation tillage helped prevent yield loss under drought conditions [86]. To further understand differences between tillage systems under drought conditions, it is important to assess impacts on the microbiome under these conditions. Importantly, one metanalysis demonstrated that plant growth promoting bacteria may be more effective under drought conditions [87, 88]. Although it is unclear how tillage impacts diverse classes of plant growth promoting bacteria; this result shows that any

increase in plant growth promoting bacteria from tillage may be even more prominent under drought. As discussed above, reduced tillage frequently appears to increase diversity and colonization of AMF and may be a biological mechanism for drought resilience in reduced tillage systems, as AMF are thought to increase plant growth under drought through the direct transfer of water to plant hosts [89, 90].

Future Directions of Tillage Microbiome Research

Although the impact of tillage on the microbiome has been extensively researched, further work remains to determine the extent of and limits to microbiome impacts on plant hosts. It has been established that tillage regime has an impact on microbial communities associated with agricultural soil and all parts of the plant. However, leveraging microbial genomes and other next generation sequencing techniques will be needed to elucidate the common and unique functions of microbial taxa which benefit from tillage compared to those that do not. Methods for assessing microbial communities associated with different tillage regimes could be one part of the modern toolkit for assessing how tillage regimes impact plant health. Further analyses using field imaging, remote sensing techniques, and spectrosopy-based methods to assess how tillage changes plant health and soil moisture metrics could be paired with microbial community analyses for determining the tillage regime that is best suited for a particular location [91, 92]. Combining these techniques will help guide scaleable methods for maintaining productive and sustainable agriculture.

Impact of Foliar Insecticide and Fungicide Applications on Phyllosphere Microbiomes

Another important agricultural management method critical to modern food and biofuel crops is the use of insecticide and fungicide applications. Such applications can be applied through soil drenching, as seed coatings, or as foliar sprays directly onto aboveground plant

tissue. Foliar sprays are an important method for controlling both insects and fungal pathogens and continue to be widely used in agriculture. However, concerns about undesired environmental consequences including selection for biocide-resistance in microbes, and off-target impacts on the host microbiome and other organisms in ecosystem persist, and warrant further research [93–96]. The leading application type in a survey of 21 crops continues to be herbicides for weed control, but insecticides and fungicides each still accounted for more than 5% by mass of active ingredient applied among the 21 crops in 2008 [97]. The use of insecticides has decreased in cotton and maize due to the introduction of Bt lines which provide reistance to maize borers and other pests through the introduction of resistance from *Bacillus thuringiensis*. However, this is not the case for all crops. For example, fungicides and insecticides were used on nearly 100% of potatoes in 2008 and fungicides were applied to 97% of potatoes in 2016 [97, 98]. Fungicides and insecticides each have various modes of action that should be assesed for off-target microbial impacts and environmental concerns. In addition to assesing off-target impacts of chemical applications on the microbial component of plant holobionts, it is important to assess environmental concerns and resistance development.

Environmental Impacts of Fungicides and Insecticides

It has long been known that drift or runoff of insecticides from agricultural fields can lead to environmental problems. For example, environmental contamination and bioaccumulation of the insecticide dichloro-diphenyl-trichloroethane (DDT) lead to reproductive declines in bald eagles in the United States, which later recovered after the ban of DDT [99]. The environmental impacts of fungicide runoff or environmental persistence and resistence has been less studied. Fungicides have been detected in fresh water ecosystems, but the impact on organisms in these environments has been understudied [100] . However, it is thought that some fungicides may

harm invertebrates, fish, and algae in freshwater [96]. Due to such environmental concerns, there have been approaches for fungicide and insecticide development to mitigate some of these risks. For example, insecticides including pyrethrins are produced by *Tanacetum cinerariifolium* and production can be engineered into other plants [101]. These natural alternatives can be effective, but their environmental impacts need to be assesed frther. Similarly, natural products have been discovered that may have effective uses as fungicides. One such compound, trans-cinnamic acid, isolated from a bacterial symbiont of nematodes (*Photorhabdus luminescens*) was discovered to have antifungal activities against *Fusicladium effusum*, the cauasative agent of pecan scab [102]. These natural alternatives can be effective and may reduce environmental damage, although further work on their environmental persistence and downstream impacts is still needed.

Development of Resistance to Fungicides and Insecticides

Fungicides are generally grouped by their modes of action, and alternating modes of action is thought to help avoid the development of resistance to fungicides. These broad classes include mitosis disrupters, cell mebrane disrupters, respiration inhibitors, oxidative phosphorylation uncouplers, and those that have multiple targets in fungal metabolism [103]. The development of resistance is an important area of concern because it reduces the ability of farmers to control plant pathogens, but it may also introduce fungi which are resistant to antifungal drugs into human populations. For example, there are concerns over the devlopment of triazole resistance in the human pathogen *Aspergillus fumigatus*. One study demonstrated that higher levels of azole fungicides in soils were associated with the increased prevalence of triazole resistant *A. fumigatus*, which can cause clinical infections when resistance develops [93, 94]. Due to similar selective pressures on insects and fungi; insecticides are expected to lead to development of resistances that could impact human health, especially in the case of insect

vectored diseases such as Malaria [104].

As with fungicides, resistance to insecticides can develop and cause concerns about their continued efficacy. The development of resistance makes crops vulnerable to insect pests, but can also jeopardize human health in the case of insect vectors for diseases such as malaria. For example, it has been demonstrated that resitance to DDT insecticides is common among mosquitoes that carry malaria [104]. Insecticide resistance among insect pests of cotton is associated with neonicotonoid seed coatings used on soybean and cotton in the United States. The use of neonicotonoid insecticides on soybean was associated with resistance development in tobacco thrips which are a pest of cotton but not soybean [105]. This highlights an important concern about the development of insecticide resistance in a pest of one crop being associated with insecticide use on another crop that is not impacted by the pest. To avoid the continued development of resistance, it is important to consider chemical applications across crops and growing seasons.

Efficacy and Economic Feasibility of Fungicides and Insecticides

Although concerns such as lasting environmental impacts and development of resistance are imporrtant to consider when using fungicides and pesticides, these products are also effective in reducing populations of their pest targets and maintaining crop yields. Foliar fungicides play an important role in preventing economic loss from reduced crop yields in major crops. For example, soybean diseases were responsible for 95 billion dollars in lost revenue between 1996 and 2016 and more than 12 billion of that loss was caused by foliar pathogens, which are primarily fungal [6, 106]. As expected, foliar fungicide applications are important in preventing yield loss in the presence of foliar disease. For example, one study on winter wheat demonstrated that in a year with increased disease severity, foliar fungicide applications increased monetary

returns by a range of $148 -239/ ha [5]. However, such benefit is dependent on the presence of disease as foliar fungicide applications may not be cost effective in soybean without disease pressure even though there may be a slight yield increase [6]. As with fungicides, yield increases associated with insecticide applications can vary year to year, and is dependent on levels of insect herbivory occuring. In switchgrass, insecticides increased yield in one studied year, but the effect was not consistent between years [107]. However, in wheat grown under high rainfall conditions, insecticides consistently helped to maintain crop yields [108]. These results show that fungicides and insecticides are important tools for maintaining crop yields, but that their impact depends on environmental and biotic factors. The importance of these environmental factors demonstrates the need for tools in agricultural that farmers can use to forecast environmental conditions that may impact pest and disease agents.

Non-Target Impacts of Fungicides and Insecticides

Foliar fungicides are important for controlling foliar pathogens, but the phyllosphere is also enriched in plant growth promoting fungal taxa and fungicides may have off-target impacts on these communities. One non-target fungal group which is frequently found to be impacted by fungicides are basidiomycete yeasts, especially in the Tremellomycetes. *Dioszegia* and *Bullera* may decline after fungicide application [95, 109]. Interestingly, other yeasts, including some *Rhodotorula* and *Sporidiobolus*, have been demonstrated to increase in abundance following fungicide application [7, 95, 110]. This may result from an increase in niche space after the loss of phyllosphere fungal populations, including *Dioszegia* and *Bullera* [95, 109]. These results are particularly important as basidiomycete yeasts appear to be highly abundant in plant phyllospheres and are enriched in plant beneficial functions such as production of phytohormones [111]. It is important to note that concerns regarding off-target impacts of

fungicide applications are primarily when applications occur in the absence of pathogen pressure, as the benefit of reductions in pathogen load would outweigh the benefits of phyllosphere yeasts under high pathogen pressure. Still, off-target impacts on beneficial yeasts should be considered when making management decisions when little or no pathogen pressure is expected.

In addition to concerns regarding off-target impacts of fungicides in the phyllosphere, there are important concerns about fungicides altering microbial communities in environments outside the phyllosphere where fungicides are not being directly applied. The impact of foliar fungicides on other plant organs such as the roots likely depends on the type of fungicide used and its ability to be transported systemically throughout the plant. In some cases, the movement of fungicides through the plant is a desired trait. This is the case for some potato diseases where phosphonic acid sprayed on foliar tissue reduced the infection of tubers by *Phytophthora* [112]. There is particular interest in assesing the impact of foliar fungicides on beneficial root taxa such as arbuscular mycorrhizal fungi. When applied directly to the soil, fungicides were shown to reduce colonization of arbuscular mycorrhizal fungi on daisy plants [113]. Despite this, since many fungicides are applied to leaf tissues and have either no or limited movement through the plant, the impact on AMF taxa in field settings is unlikely [114]. However, fungicide seed treatments may reduce colonization of AMF depending on the fungicide and the identity of the AMF taxa [115]. Foliar fungicides are unlikely to be designed to target soil fungal communities. Despite this, several studies have assesed the impact of foliar fungicide sprays on soil fungal communities. The impact of fungicide application on soil fungal communities is likely to depend on the application rate of the fungicide and the type of crop. Thick foliage, higher planting density, and taller plants are likely to prevent spray from reaching the soil. This has been

demonstrated through varying results among different crops with foliar fungicide applications on cucumber having a substantial impact on soil fungal communities, but applications on soybean having no impact on soil fungal communities [95, 116].

There remain concerns over off-target impacts of insecticides on beneficial insects. Beneficial insects play various important roles in agricultural systems including acting as predators on harmful insects and acting as pollinators. Beneficial insects can be exposed to insecticides through nectar of plants that have been sprayed, through contacts with other plant surfaces, and by the consumption of other insects which have fed on sprayed plants [117]. One study demonstrated that beneficial insects such as parasitic wasps and pollinators, can be killed by consuming secretions from deterimental phloem-feeding insects which fed on citrus trees that had been sprayed with neonicotinoid insecticides [118]. In addition to lethal effects, some insecticide chemistries can have non-lethal impacts which can still harm the reproductive success of important pollinators such as bees [119]. Due to these concerns, novel chemistries and methods are continually being devleoped that can be effective against insect pests while minimizing harm to pollinators or insect predators. These methods can include but are not limited to employing insecticides produced naturally by plants, as discussed above, or utilizing native insect predators to control pest insects [101, 120].

Although insecticides are designed to specifically target insects, it is important to ensure that insecticide application does not impact the microbiome. It has been demonstrated that *Burkholderia* strains found in agricultural soils are capable of degrading fenitrothion insecticides, and that these strains can form symbioses with insect pests leading to increased resistance [121]. Several studies assesing off-target impacts of insecticides on microbial communities have been performed on soil microbiomes. It has been demonstrated that neonicotinoid applications can

alter bacterial community composition, structure and diversity in soils, increasing prevalence of insecticide degrading taxa as well as possible indirect effects [122, 123]. In many cases, it is unclear if impacts on the microbiome are directly a result of the insecticide treatment, or an indirect impact related to changing soil paramaters. Fewer studies have assesed the impact of insecticides on phyllosphere microbial communities, with one study on the cucumber phyllosphere finding an increase in bacterial biomass coupled with a decrease in fungal biomass [124]. Within bacterial communities, it was found that insecticides lead to an increase in gram negative bacteria [125]. Further work is needed to assess the methods by which insecticides may impact plant-associated microbial communities.

Conclusions and Future Directions

Chemical insecticides and fungicides remain important means for managing insect and fungal pests in agricultural systems. However, these applications can have off-target impacts within agricultural ecosystems and beyond targeted areas and organisms. In addition to off-target impacts, these applications can lead to environmental concerns for humans and animals. Due to these concerns, it is important to continue to develop new chemistries that minimize off-target impacts while remaining effective against target pests. Additionally, precision agriculture methods are needed to more reliably sense and forecast pest concerns in fields, and to predict conditions when insecticide and fungicide applications will be cost-effective. For example, remote sensing can be used to detect fungal diseases, which lead to detectable physiological changes in plants [126]. Aerial imaging has been used to detect history of cotton root rot in fields; this information can then be used to create maps for targeted fungicide applications [127]. Using these strategies, as measured by drone, airplane or satellite, will reduce negative impacts associated with chemical applications and will help farmers maintain yields by avoiding

unnessecary chemical applications.

The Use of Microbial Inoculants in Agriculture

In addition to mechanical management using tillage and management using chemical applications, another agricultural management method that is becoming increasingly common is the use of microbial inoculations, known as biologicals. Microbial inoculations can be performed on agricultural soils, seeds, or directly to seedlings and mature plants [8, 128, 129]. In addition to microbial inoculations, potentially plant-beneficial microorganisms can be selected for by engineering plants to have altered exudate profile, which may impact root or rhizosphere microbes [130, 131]. However, there is increasing interest in direct inoculations of microbes to provide microbe-mediated benefit without having to genetically modify the plant. Seed or seedling inoculations are thought to be successful in part due to ecological priority effects, which posits that early arriving species are more likely to occupy a niche and persist due to the lack of competition upon arrival [132]. Priority effects have been shown to have a lasting role in the long-term structuring of microbial communities in diverse environments such as the human gut and agricultural systems [132, 133]. In agricultural systems, small changes such as the arrival order of species can lead to significant differences in community structure, demonstrating that the composition of microbial communities is contingent on many factors [133]. Priority effects have also been demonstrated with the use of a synthetic bacterial community to show that strains which were inoculated early had an effect community structure that persisted, even after the arrival of new taxa [134]. Priority effects have been shown to occur in several different ways, and often result in the competitive exclusion of later arriving species. One mechanism to explain this result is that the inoculant community will be expected to outcompete inhabitants of the natural soil due to their ability to adjust to the niche prior to the arrival of other microbes [135].

Another possibility is that the niche could be modified by the microbial inoculum, making it more difficult for competitors to colonize [136]. Microbial inoculants have been shown to promote plant growth, pathogen resistance, and increase resistance to abiotic stress [8]. Inoculations designed for each of these purposes will be discussed further.

Microbial Inoculant Testing

As described by Kaminsky et al. 2019, designing and assessing the efficacy of microbial inoculants involves analyzing inoculant performance at several stages. First, the microbial taxa with desired traits must be isolated. Next, the taxa must survive storage and formulation. Then the microbes must establish in the soil or plant system and persist until the function can be performed. Finally, the inoculum must avoid unintended ecological consequences associated with persistence passed the desired function [137]. Before inoculations can be applied, microbes with desired traits must be identified and isolated. Many microbial isolation methods select for the fastest growing taxa, but some approaches are able to select for slower growing or unique taxa by lower the nutrient content, extending or modifying growth conditions, baiting media with particular substrates, or by enriching for microbial taxa in their environment [138–140]. These methods allow for the selection of desired microbial functions from a broader range of taxa. Once desired microbes have been isolated, the next step is to ensure that they survive storage to be placed in the soil, on the plant, or coated onto the seed. Various methods can be used to increase the survival of inoculants including encapsulation with a polymer such as alginate, or the selection of strains which are amenable to dry coatings [8, 137, 141]. Following storage, it is important to ensure that the microbes are living and that the desired function is active.

After verifying that inoculant microbes survived storage, the next stage is to ensure that they can establish in the desired environment. As discussed above, it is thought that microbial

inoculants or other early colonizing microbes can establish due to the phenomenon of priority effects, but it is important to assess their establishment. With the number of available fungal and bacterial genomes increasing, strain-specific quantitative polymerase chain reaction (qPCR) markers can be designed from sequenced genomes and these markers can be used to assess the presence of an inoculated strain in plants or associated soil [142]. However, this method could be problematic if closely related strains are present in the same soil. Another method which has long been used to track microbes in the environment is the use of fluorescent reporter strains of bacteria [143]. However, this method relies on the ability to transform the microbe, which is not always possible for bacteria or fungi. Further developments in the tracking of microbial inoculants in the environment will be crucial to the advancement of microbial inoculation methods.

One of the primary concerns in using microbial inoculants in agriculture has to do with the persistence of inoculated taxa. These concerns can be twofold: first, it is important to ensure that the inoculant persists long enough to perform the desired function; second, the persistence of non-native microbial taxa in agricultural ecosystems may be undesirable due to unintended long-term impacts of non-native taxa. Similar methods to those described for assessing establishment can be used for assessing the persistence of microbial inoculants but applied using a time-series sampling approach. One study which used non-native AMF strains that differed in ribosomal DNA (rDNA) sequence from the native taxa demonstrated that AMF inoculants persisted in *Medicago sativa* plants for up to two years [144]. Similarly, rhizobial inoculants were demonstrated to survive in some soybean soils for up to three years following inoculation [145]. Although there are challenges in tracking the establishment and persistence of single isolates, tracking the persistence of large numbers of inoculants can be even more challenging. When

designing and testing synthetic microbial communities in sterile or semi-sterile environments for use as inoculants, their abundance can be tracked through sequencing or culture-based methods, but this is less likely to be viable in complex field environments [146]. Other methods such as fluorescence in situ hybridization (FISH) imaging can be used with probes specific to inoculant members [147]. However, these methods may be most applicable in sterile or controlled conditions due to complications from the presence of closely related strains.

Uses of Microbial Inoculants

It has long been known that bacterial and fungal inocula can be used for general plant growth promotion [148, 149]. However, microbial inocula are also commonly used to combat plant pathogens. These studies have been performed on a wide variety of crops to provide either broad spectrum protection against pathogens or against specific target pathogens [8]. Typically microbes are selected based off of screenings and *in vitro* studies that demonstrate a reduction in pathogen growth in culture [150]. However, with modern sequencing and bioinformatics techniques, taxa can be selected *in silico* based on the potential to produce compounds or activities of interest. For example, one study applied a machine learning framework to detect bacteria with biocontrol activity against pathogens of sorghum and banana, and found that the addition of a machine learning approach improved the number of strains of interest and identified novel strains compared to traditional screening approaches [151]. Once strains with desired biocontrol activities have been identified, studies frequently test a single species of bacteria in the field, greenhouse, or growth chamber against a target oomycete or fungal pathogen [152–154]. However, there is increasing interest in using several strains or a synthetic microbial community to improve plant resilience [155, 156].

Increasingly, concepts used for designing microbial inocula to protect against pathogens

are being applied to protect plants from abiotic stress. As with designing inocula for biotic stress based on noted pathogen inhibition either in plants or in culture, one method for designing microbial inocula for abiotic stress amelioration is to isolate microbes from environments that are extreme for that stress. For example, one method for finding microbes which may protect plants from drought stress is to isolate plant-associated microbes from arid climates, as such microbes are likely to be adapted to drought conditions and may aid in their host plants survival [157]. This strategy has been applied to design synthetic communities that protect plants from drought stress. Previous work demonstrated that inoculating tomato seeds with microbes isolated from a desert cactus increased growth and survival under drought conditions [158]. Some bacterial inoculants have also been found to aid in soybean and tomato responses to flooding through the production or alteration of phytohormones [159, 160]. In addition to drought and flooding stress, fungal and bacterial inoculum have been shown to help protect plants from heavy metal contaminated soil and can reduce the amount of phosphorous fertilization application required [161, 162]. The wide variety of uses for microbial inoculants demonstrates the diversity of plant-beneficial functions that can be harnessed from applications with plant-associated microbes in agriculture.

The impact of inoculants on plant-associated or soil microbial communities are less frequently assessed compared to their impacts on plant performance. However, use of microbial taxa that are thought to be important in structuring the microbiome may be expected to result in changes to the whole microbiome. When microbial community changes have been assessed, the impact of the inoculation appears to depend on the taxa used in the inoculation or the plant host. For example, one study demonstrated a significant impact of *Pseudomonas fluorescens* inoculation on beta diversity in the rhizosphere soil of several plant species including soybean

[163]. However, another study performed on soybean seed inoculated with *Bacillus* did not show a substantial impact of inoculation on the rhizosphere microbiome, although this study did not utilize next generation sequencing so their detection limit was not very sensitive and the inoculation may not have persisted [153]. Further, microbial inocula can also have impacts that appear to be indirect. For example, inoculation with *Metarhizium* increased *Bradyrhizobium* abundance in common bean roots even though inoculation did not increase relative abundance of *Metarhizium* [164].

Conclusions and Future Directions

Microbial inoculants have been demonstrated to be successful in aiding plant responses to various biotic and abiotic stresses, this wide range of use and adaptability makes microbial inoculants a desirable technology for agriculture companies and academic labs alike. For example, in 2015 a group called the BioAg alliance composed of Monsanto and Novozymes tested more than 2000 microbial strains throughout the United States, finding various microbes that increased the yield of maize and soybean [165]. The use of microbial inoculants can also be expected to increase as technologies improve and climate conditions make consistent crop production more difficult. As microbial inoculants become more common, it will be important to assess their in-field performance and impacts on plant health. This can be done using traditional measures such as changes in yield or can be done using remote imaging techniques which can detect changes in plant physiology and performance [126].

In addition to moving microbial inocula from greenhouses or growth chambers to the field, there are other challenges that must be met to continue the widespread use and improvement of microbial inoculants. For example, it is important to ensure that non-native or genetically engineered microbes do not escape beyond research areas. One promising method in

synthetic biology is to engineer microbial inocula with "kill switches" that rely on inputs from the environment to survive and will die when this input is no longer present [166]. However, it will have to be ensured that this can be effective for fungi and for use on a full field scale. Another concern with microbial inoculants is that their broad use may disrupt or replace natural relationships formed between plants and native microbiota over evolutionary time scales [167]. For example, it was demonstrated that invasive plants carried non-native rhizobia that could potentially disrupt symbiotic relationships; this same concern is valid for inoculated microbes [168]. The best way to address these concerns is further testing of microbial inocula, especially in areas where there is low risk of spread to non-target environments. Further testing and development will allow for these concerns to be addressed and will allow microbial inocula to become an important part of creating sustainable and productive agricultural systems.

Research Focus

The three primary goals of my dissertation research were to:

1. *Assess the impact of agricultural management on soybean-associated microbial communities*

Hypotheses: (H1) Crop management will impact bacterial and fungal communities associated with soybean-associated soil, roots, stems, and leaves; with greater impacts in the soil. (H2) Soybean-associated microbial communities will vary throughout the growing season, with greater levels of variation in microbiomes from aboveground plant tissues compared to belowground root and soil communities.

2. *Assess the impact of foliar fungicide applications on microbial communities in the maize and soybean phyllosphere under conventional and no-till management systems.*

Hypotheses: (H3) Fungicide applications will alter microbial diversity and community

structure in soybean and maize leaves, especially in fungal communities. (H4) Fungicides will impact many of the same taxa in each crop, but there will also be crop-specific impacts. (H5) The impact of fungicides and the recovery of microbial communities will differ by crop management system.

3. Assess the impact of inoculation with consortia composed of microbial hub taxa on soybean plant growth during a period of low-moisture stress.

Hypotheses: (H6) Inoculation with hub taxa will alter root-associated microbial communities. (H7) Plants inoculated with microbial hub taxa will have increased growth and aboveground biomass during the low-moisture stress experiment. (H8) Inoculation will lead to increased expression of genes related to plant growth and low-moisture stress survival.

Value of Research

There is increasing interest in assessing the impacts of agricultural management strategies on plant and soil microbiomes. Reduced tillage management is increasingly being utilized for soybeans grown in the United States, has been shown to have benefits including increased soil moisture retention and reduced input costs for farmers. However, since plants are holobionts that host various microbes that provide important functions such as nitrogen fixation, it is important to also assess the impact of tillage on microbial communities. Assessing the impact of tillage on plants without considering the microbiome would provide an incomplete picture of the impact on the holobiont. This research will help to inform how tillage regime impacts microbial communities which along with other data will help to inform how management decisions impact plant holobionts. Similarly, there is interest in assessing the impact of chemical applications such as fungicides on off-target microbial communities to determine potentially detrimental impacts of fungicide applications in the absence of pathogen pressure. Additionally, assessing the

microbial impacts of fungicides under no-till and conventional management systems demonstrates how these management strategies interact to impact the microbiome and microbial recovery from fungicide disturbance. Another agricultural management strategy which is increasingly being utilized is the application of plant-beneficial microbes in agricultural settings to promote plant growth or help plants combat stress. It is well known that applications of plant growth promoting microbes can increase yields and promote crop survival. While many studies have identified hub taxa that are thought to be important to the plant microbiome, few have yet to test whether hub taxa may be important in structuring microbial communities and improving the productivity of the plant holobiont. My dissertation research fills this knowledge gap by demonstrating how hub taxa impact soybean response to low-moisture stress in living soils, provides fundamental knowledge about how hub taxa impact root-associated microbial communities in soybean.

Chapter 2

Crop Management Impacts the Soybean (Glycine max) Microbiome

Source:

This chapter has been published in *Frontiers in Microbiology*: Longley, R., Noel, Z. A., Benucci, G. M. N., Chilvers, M. I., Trail, F., & Bonito, G. (2020). Crop Management Impacts the Soybean (Glycine max) Microbiome. *Frontiers in Microbiology*, *11*, 1116.

Abstract

Soybean (*Glycine max*) is an important leguminous crop that is grown throughout the United States and around the world. In 2016, soybean was valued at $41 billion USD in the United States alone. Increasingly, soybean farmers are adopting alternative management strategies to improve the sustainability and profitability of their crop. Various benefits have been demonstrated for alternative management systems, but their effects on soybean-associated microbial communities are not well-understood. In order to better understand the impact of crop management systems on the soybean-associated microbiome, we employed DNA amplicon sequencing of the ITS region and 16S rRNA genes to analyze fungal and prokaryotic communities associated with soil, roots, stems, and leaves. Soybean plants were sampled from replicated fields under long-term conventional, no-till, and organic management systems at three time points throughout the growing season. Results indicated that sample origin was the main driver of beta diversity in soybean-associated microbial communities, but management regime and plant growth stage were also significant factors. Similarly, differences in alpha diversity are driven by compartment and sample origin. Overall, the organic management system had lower fungal and bacterial Shannon diversity. In prokaryotic communities, aboveground tissues were dominated by *Sphingomonas* and *Methylobacterium* while belowground samples were dominated by *Bradyrhizobium* and *Sphingomonas.* Aboveground fungal communities were dominated by *Davidiella* across all management systems, while belowground samples were dominated by *Fusarium* and *Mortierella.* Specific taxa including potential plant beneficials such as *Mortierella* were indicator species of the conventional and organic management systems. No-till management increased the abundance of groups known to contain plant beneficial organisms such as *Bradyrhizobium* and Glomeromycotina. Network analyses show different highly

connected hub taxa were present in each management system. Overall, this research demonstrates how specific long-term cropping management systems alter microbial communities and how those communities change throughout the growth of soybean.

Introduction

Soybean (*Glycine max L.)* is the third most valuable plant crop worldwide with important uses in feed, as an oilseed crop, and as a nutritional source [169]. Alternative cropping strategies are becoming increasingly common in row crop agriculture in order to manage resource inputs and soil health [1]. For example, the use of no-till and reduced tillage strategies have increased in row crops since the early 2000's in the United States [1]. Reduced tillage strategies are especially prevalent in soybean, representing 70% of planted acreage in 2012 [1]. In addition to time and fuel-cost savings, no-till farming deposits organic carbon closer to the surface of the soil, which acts as an organic mulch and may lead to improved crop growth and health [34, 35]. In wet conditions, however, plant fungal pathogens can sporulate on previous years' vegetation so no-till management regimes may increase disease pressures [2]. In addition to harboring pathogens on plant material, no-till management may allow diseases to persist by increasing soil moisture and slowing soil warming as demonstrated with plant-pathogenic oomycetes, such as *Pythium* and *Phytopthora* [55, 56]. Under drought conditions, no-till maize and soybean crops have shown yield improvements, which has been attributed to increased soil moisture retention [170, 171]. These factors and others may contribute to reports of increased grain yield for no-till managed soybean at several sites, including historically at the KBS LTER site [172].

In addition to reduced-tillage strategies, organic farming is another important alternative management strategy. In 2016, US organic soybeans were valued at more than $78 million US dollars [173]. Acreage of organic field crops has increased since the 1990s, yet the share of total

soybeans considered to be certified organic remained below 1% in 2015 [174]. Although farmers must weigh the considerations mentioned above in determining management strategies, many soybean crops are managed with conventional tillage regimes. Tilling reduces plant material left in fields, which is a source of fungal disease propagules that then can be transferred to live plants; which has been demonstrated with *Rhizoctonia oryzae* [175].

It is also important to consider the effect of management systems on the plant and soil microbiome. Previous studies have investigated the effect of tillage regimes in conventional and organic wheat [3] and maize [176]. These studies found that the management system influenced microbial community composition in roots and soils [3, 177]. In contrast, a whole plant microbiome study on root, stem, and leaf organs of wheat at the KBS-LTER found that the impact of management system was subtle [4]. Studies investigating the impact of management regime on the soybean microbiome have focused on specific bacterial taxa. One such study showed that conventional management reduces the diversity of Rhizobium populations associated with soybean [70], while another study demonstrated that the relative abundance of Acidobacteria was reduced in soybean cultivated soils compared to forest soils [178].

The stage of plant growth at sampling is another important source of microbial community variation that has been observed in agricultural systems including biofuel crops and soybean [179, 180]. For example, it was demonstrated that in the soybean rhizosphere, the relative abundance of *Bacillus*, *Rhizobium*, and *Bradyrhizobium* increased throughout the growing season [177]. In addition to composition shifts, a study on the wheat microbiome found that alpha diversity of prokaryotic communities increased throughout the growing season in both above and belowground plant tissues, but this trend was less clear for fungal communities [4].

Here we characterize the fungal and prokaryotic communities, associated with individual

soybean plants grown as part of a maize-soy-wheat rotation system under conventional, no-till, and organic management systems for nearly 30 years, to determine the impact of cropping management system on the soybean microbiome throughout a growing season. This study is part of a long term field experiment on the effect of agricultural management on plant and soil microbiomes in the maize-soy-wheat rotation at the KBS LTER, and follows previous research on the wheat-associated microbiome [4]. Although the present study is limited by representing a single site and season, results presented here will be available for future longitudinal microbiome studies from the same site under the consistent management provided by the KBS LTER. The organic management plots were planted with a non-genetically modified soybean variety to make it certified organic, while the no-till and conventional management plots were planted with a roundup ready genetically modified variety. Fungal and bacterial communities associated with soil, root, stem, and leaf compartments were characterized at three time points during the 2018 growing season. Management regime and plant developmental stage were hypothesized to impact the structure of the soybean microbiome. More specifically, we expected to see distinct differences between no-till and conventional/organic belowground microbial communities, due to microenvironment changes associated with tilling [181]. In aboveground plant compartments, based on previous work done on wheat at the KBS LTER, we expected that variation in microbial communities would be primarily driven by growth stage [4].To the best of our knowledge, this study represents the first characterization of the effect of agricultural management regime on the soybean microbiome in soil, roots, stems, and leaves across the growing season.

Materials and Methods

Sample Site and Management System

All samples were collected from the Michigan State University (MSU) W.K. KBS LTER crop rotation experiment in Hickory Corners, MI, United States. Soybean seeds were planted into one-hectare plots that have been managed under conventional, no-till, or organic management since 1989 [182]. Six replicate plots of each management system were distributed randomly at the LTER site in order to eliminate bias based on location. The no-till and conventional management plots received fertilizer in the form of potash at a rate of 120 lbs./A (72 lbs./A K2O). In addition to fertilizer, plots within these two management systems received Valor herbicide treatments prior to emergence, at a rate of 3.5 oz/A (Valent Agriculture, United States). Additionally, the two management regimes received mid-season weed control with Roundup Powermax amended with ammonium sulfate at rates of 1 qt/A and 3.4 lbs./A, respectively (Bayer, Germany). Genetically modified soybean and maize have been grown at the LTER site since 2009 and 2011, respectively. The modified varieties provide glyphosate resistance as well as resistance to European maize borer and rootworm in maize [182]. During wheat rotation years, 30 pounds of nitrogen fertilizer/acre is applied to the conventional and no-till management systems in March as well as 43 pounds/A of nitrogen fertilizer and 25 pounds/A of sulfur fertilizer in May. Additionally, during wheat rotation years, conventional and no-till management plots receive herbicide applications in the form of Roundup PowerMax with ammonium sulfate in October and August (1 qt/A,3.4lbs/A) as well as Sharpen (2 oz/A), and maize methylated soybean oil (0.8 qt/A) in August (BASF, Germany; Van Dielst Supply Company, United States). During maize rotation years, nitrogen fertilizer is applied at planting at a rate of 29 lbs./A and in June at a rate of 122 lbs./A, and Lexar EZ herbicide is sprayed at a rate 3.0 qt/A alongside

Roundup Powermax (22 oz/A) in June (Syngenta, United States). The certified organic management system received no chemical inputs or manure but was rotary hoed to control for weeds and has a red clover or annual rye cover crop in the winter season for all crops. The conventional and no-till management systems were planted with Pioneer P22T69R Roundup Ready soybean seed (Pioneer Hi Bred International, United States). The organically managed plots were planted with non-genetically modified Viking O.2188AT12N soybean seed (Albert Lea Seed, United States).

Sampling and DNA Extraction Methods

In 2018, whole soybean plants were sampled at three time points corresponding to the following growth stages: early vegetative (V2 – two sets of unfolded trifoliate leaves), early reproductive (R2 – full flower inflorescence/reproductive stage), and late reproductive (R6 – full pod development) [183]. Within each management system (organic, no-till, conventional), three individual plants in each of four replicate plots were sampled at each of these growth stages (n = 108 plants). Throughout the growing season, samples from the organic management system were delayed 2 weeks due to later planting of the organic system. At each sampling point, independent samples of soil, roots, stems, and leaves were collected. Soil was sampled by removing whole plants from the soil and placing ~2 g of soil from the root zone into a coin envelope which was then dried on silica beads upon return to the lab. Roots were sampled by cutting the entire root system at the soil line and placing the roots into a Whirl-Pak bag (Nasco, United States) containing a 0.1%Tween 20 mixture to remove soil before lyophilizing. The stem section between the first and second true leaves was collected in a 15 ml Falcon tube (Corning, United States) containing 5 mL of CSPL buffer from the Mag-Bind Plant DNA Plus Kit (Omega Biotek, United States). Leaves were sampled by hole punching three 6 mm leaf discs from three leaves

into eppendorf tubes (Eppendorf, Germany) containing 500 µl of CSPL buffer. All samples were placed on ice and transported back to the Michigan State campus for storage at −80◦C. Additionally, plants were sampled for isolations of fungi and bacteria, isolations are described in detail in appendix A.

DNA was extracted from ∼50 mg of soil/sample using the PowerMag Soil DNA Isolation Kit (Qiagen, United States) on the KingFisher Flex system (Thermo Fisher Scientific, United States). DNA was extracted from ∼50 mg of each dried fine roots, stems, and leaves using the Mag-Bind Plant DNA Plus Kit (Omega Biotek, United States) on the KingFisher Flex system (Thermo Fisher Scientific, United States). All extractions included negative controls (extractions containing no sample).

Miseq Library Preparation and Sequencing

Illumina MiSeq amplicon libraries were constructed with the ITS1F – ITS4 primer set to target the ITS region of fungi and the 515F – 806R primer set to target the V4 region of the 16S rDNA of prokaryotes [184–186]. Libraries were prepared following a three step PCR protocol as described previously [187–189]. The PCR cycles used are shown in Table 2.1. Unmodified primer pairs were used in the first step to enrich in target taxa. In the second step, primers incorporating frameshifts into the amplicons were used. In the third step, 10 nucleotide indexing barcodes and Illumina adapters were incorporated following previously used approaches [189, 190]. PNA blocking clamps were incorporated into PCR reactions for steps one and two at a concentration of 0.75 µM to reduce the amplification of chloroplast and mitochondria sequences in plant-associated 16S libraries (PNA Bio Inc., United States). The PCR mixes used are shown in Table 2.2. PCR products were run on an agarose gel to verify amplification. Next, PCR products were normalized to an equal concentration of 1–2 ng/µl using the SequalPrep

Normalization Plate Kit (Thermo Fisher Scientific, United States). Following normalization, eluted samples were combined into one pool and concentrated with Amicon Ultra 0.5 mL 50K filters (EMD Millipore, Germany). Libraries were then cleaned with Agencourt AMPure XP magnetic beads to remove small fragments and primer dimers (Beckman Coulter, United States). Libraries were sequenced at the MSU Genomics Core with the Illumina Miseq V3 600 cycles kit. The produced sequences for the samples analyzed in this study are stored at the NCBI SRA archive under the following accession number: PRJNA603147. Sequences for samples that were not analyzed as part of this study but were sequenced on the same Miseq runs and used for contaminant removal are available under the following accession numbers: PRJNA603199, PRJNA603207.

Table 2.1 - Thermocycling conditions. Used for amplifying fungal (ITS) and bacterial (16S) genes from all sample origins.

Fungi and Soil Prokaryotes Cycling							
Step 1			**Step 2**			**Step 3**	
Time	**Temperature (c)**	**Cycles**	**Time**	**Temperature (c)**	**Cycles**	**Time**	**Temperature (c)**
5:00	95		5:00	95		5:00	95
0:30	95	10X	0:35	95	10X	0:40	95
0:30	50		0:35	50		0:50	63
1:00	72		1:10	72		1:20	72
7:00	72		7:00	72		7:00	72
Infinite	10		Infinite	10		Infinite	10
Plant Tissue Prokaryotes Cycling							
Step 1			**Step 2**			**Step 3**	
Time	**Temperature (c)**	**Cycles**	**Time**	**Temperature (c)**	**Cycles**	**Time**	**Temperature (c)**
5:00	95		5:00	95		5:00	95
0:30	95	10X	0:30	95	10X	0:40	95
0:15	75		0:15	75			
0:30	50		0:35	50		0:50	63
0:45	72		0:50	72		1:20	72
7:00	72		7:00	72		7:00	72
Infinite	10		Infinite	10		Infinite	10

Table 2.2 - PCR Recipes. PCR recipes for amplifying the ITS and 16S markers.

Plant Associated Prokaryotes		**Fungi**
Step 1		**Step 1**
Component	**Volume/Sample (uL)**	**Component**
2X Platinum Green Taq Master Mix (Thermo Fisher, USA)	6.25	2X Dream Taq Green PCR Master Mix (Thermo Fisher)
10 uM 515F Primer (IDT, USA)	0.375	10 uM ITS 1F Primer (IDT, USA)
10 uM 806R Primer (IDT, USA)	0.375	10 uM ITS 4 Primer (IDT, USA)
Bovine Serum Albumin (BSA, 3%)	1	Bovine Serum Albumin (BSA, 3%)
50 uM Mitochondrial PNA clamp (PNA Bio, USA)	0.18	H2O
50 uM Plastid PNA clamp (PNA Bio, USA)	0.18	Extracted DNA
GC Enhancer (Thermo Fisher, USA)	2	**Step 2**
H2O	0.64	**Component**
Extracted DNA	1	2X Dream Taq Green PCR Master Mix (Thermo Fisher)
Step 2		10 uM ITS 1F Primer Frameshift (IDT, USA)
Component	**Volume/Sample (uL)**	10 uM ITS 4 Primer Frameshift (IDT, USA)
2X Platinum Green Taq Master Mix (Thermo Fisher, USA)	6.25	Bovine Serum Albumin (BSA, 3%)
10 uM 515F Primer Frameshift (IDT, USA)	0.375	H2O
10 uM 806R Primer Frameshift (IDT, USA)	0.375	Step 1 Product
Bovine Serum Albumin (BSA, 3%)	0.64	**Step 3**
50 uM Mitochondrial PNA clamp (PNA Bio, USA)	0.18	**Component**
50 uM Plastid PNA clamp (PNA Bio, USA)	0.18	2X Dream Taq Green PCR Master Mix (Thermo Fisher)
GC Enhancer (Thermo Fisher, USA)	2	Barcode Forward Primer
Step 1 Product	2	Bovine Serum Albumin (BSA, 3%)
Step 3		H2O
Component	**Volume/Sample (uL)**	Unique 10 Nucleotide Barcode
2X Platinum Green Taq Master Mix (Thermo Fisher, USA)	8	Step 2 Product
Barcode Forward Primer	0.5	
Bovine Serum Albumin (BSA, 3%)	1	**Soil Prokaryote PCR Mixes are the same as Fungi, but with Platinum Taq**
GC Enhancer (Thermo Fisher, USA)	0.5	
Unique 10 Nucleotide Barcode	1	
Step 2 Product	4	

Bioinformatics Analysis

First, sequences were analyzed for initial quality using FastQC [191]. Following quality analysis, reads were demultiplexed by barcode and assigned to samples using QIIME 1.9.1

[192]. Due to lower quality of the reverse reads, only forward reads were analyzed further. Next, primers, adapters, and the conserved regions including the small subunit of the ribosome, 5.8S ,and large subunit of the ribosome (SSU, 5.8S, LSU) of amplicons were stripped from forward sequences using Cutadapt v2.6 and USEARCH v10 [193–195]. Afterward, library statistics were analyzed using USEARCH for length and quality distributions and reads below 205 basepairs (bp) and above a maximum error of 1% were discarded. Additionally, sequences were de-replicated and singletons were removed prior to clustering OTUs at a 97% threshold using the UPARSE algorithm of USEARCH [194–196]. Following OTU clustering, taxonomy was assigned to fungal OTUs using the UNITE database V10.10.2017 [197] and 16S OTUs using the Silva 16S V123 database [198] with the SINTAX tool [199].

Statistical Analysis

OTU tables, taxonomy tables, mapping files, and OTU sequences were loaded into the R (Version 3.5.2) statistical environment [200] and used to create a phyloseq object for further analysis in the *phyloseq* package [201]. Before analyzing sequence data, OTUs determined to be contaminants in negative controls were removed with the *decontam* package [202]. Samples which produced less than 1000 reads, as well as five soil samples that did not dry properly and were overtaken by mold, were discarded. Alpha diversity (within sample diversity) was estimated for each sample before data was normalized and filtered following recommendations [203]. Alpha diversity was estimated using richness [204] and Shannon diversity [205] within the *BiodiversityR* and *vegan* packages [206, 207]. OTU richness and Shannon diversity were visualized for each plant compartment with boxplots in *ggplot2* [208]. Differences in alpha diversity means due to management system, growth stage, and plant compartment were tested for statistical significance using Kruskal Wallis tests in the *stats* package [200]. In the case of a

significant result ($P < 0.05$), Pairwise Wilcox tests with a false discovery rate (FDR) P-value correction were utilized to determine significance groups by growth stage and management regime [200]. Significance groups for growth stage and management system are denoted on alpha diversity boxplots by letters above boxes where significant differences ($P < 0.05$) were present between means of the same growth stage or the same management system. Following alpha diversity analyses, OTUs with less than five reads in a single sample were placed to zero to account for tag switching and OTUs with less than 10 reads across all samples were removed to account for PCR errors [209, 210]. Rarefaction curves were created to assess the sampling of prokaryotic and fungal communities using the "rarecurve" function in the *vegan* package [207]. Barplots for fungal communities were created in *ggplot2* to show genera having >4% relative abundance [208]; prokaryotic barplots were created to show genera (classes for soil) having >2% relative abundance. Indicator species analysis was performed with the *indicspecies* package to identify taxa which were significantly associated with either one single management system and not the other two or significantly associated with two of three management systems [211]. Following identification of indicator OTUs, p-values were FDR adjusted, and only taxa with adjusted $p < 0.05$ were considered indicators. The top 30 most abundant identified indicator taxa were used to create heatmaps displaying the relative abundance distributions by management regime and growth stage of identified taxa in the *ComplexHeatmap* package in R [212].

Next, data were normalized by cumulative sum scaling in the *metagenomeseq* package [213]. Following normalization, beta diversity was analyzed in the *phyloseq* and *vegan* packages by creating Principal Coordinates Analysis (PCoA) plots with the "ordinate" and "plot_ordination" functions. Community patterns identified in PCoA plots were tested for statistical significance using Permutational Multivariate Analysis of Variance (PERMANOVA)

as implemented by the "adonis" function in *vegan*. Homogeneity of variance between modeled groups was analyzed with the "betadisper" function in *vegan*. To further assess microbial community differences between management systems, random forest models were created to test the accuracy of assigning above and belowground samples to their management system origin using the "randomforest" function in the *randomForest* package in R [214]. Random forest models were optimized by testing different mtry values (number of OTUs randomly sampled from the community to build models). Mtry values of ±10 of the standard value (square root of the number of OTUs in the community) were tested. If the out of bag error did not improve any tested mtry values, the standard value was used. Figures were created from the results of random forest models, displaying the following: the out of bag error plotted against the number of trees, MDS plots created from random forest sample proximities converted to Bray-Curtis distances, and the top 30 OTUs important in assigning samples to their management system. Importance of each individual OTU for distinguishing between management systems was assessed by calculating the mean decrease in model accuracy when that OTU is removed from the community. Significance of random forest models was tested with 999 permutations (random forest models were repeated 999 times) using the "rf.significance" function in the *rfUtilities* package in R [215].

Bipartite co-occurrence networks containing both bacteria and Fungi were created and analyzed using the *SpiecEasi* and *Igraph* packages in R [216, 217]. Networks were constructed with OTUs that were present in 80% of samples or more. Network stability and sparsity were assessed using *SpiecEasi*. Hub taxa were identified as those above the 90th percentile (1.3 standard deviations from the mean) of network OTUs for the measures of degree and betweenness centrality [9]. Additionally, taxa were only considered to be hubs if they were

above the 90th percentile of hub scores (eigenvector centrality) for either fungi or bacteria in that specific network. The betweenness centrality measure was log transformed before determining hubs to account for a non-normal distribution. Following network creation in *Spieceasi* and hub identification, networks were visualized with the attribute circular layout in the Cytoscape program [218]. Random networks with the same number of nodes as experimental networks were generated with the Barbasi-Albert model of the "sample_pa" function in the *igraph* package of R. The degree distributions of 100 random networks were compared to those of experimental networks with a two sample Kolmogorov-Smirnov using the "ks.test" function in the *stats* package of R. All R code and files for producing figures and tables including metadata and OTU tables, as well as example code for building networks and random forest models is available at: https://github.com/longleyr/Management-of-Soybean-Code-and-Files.

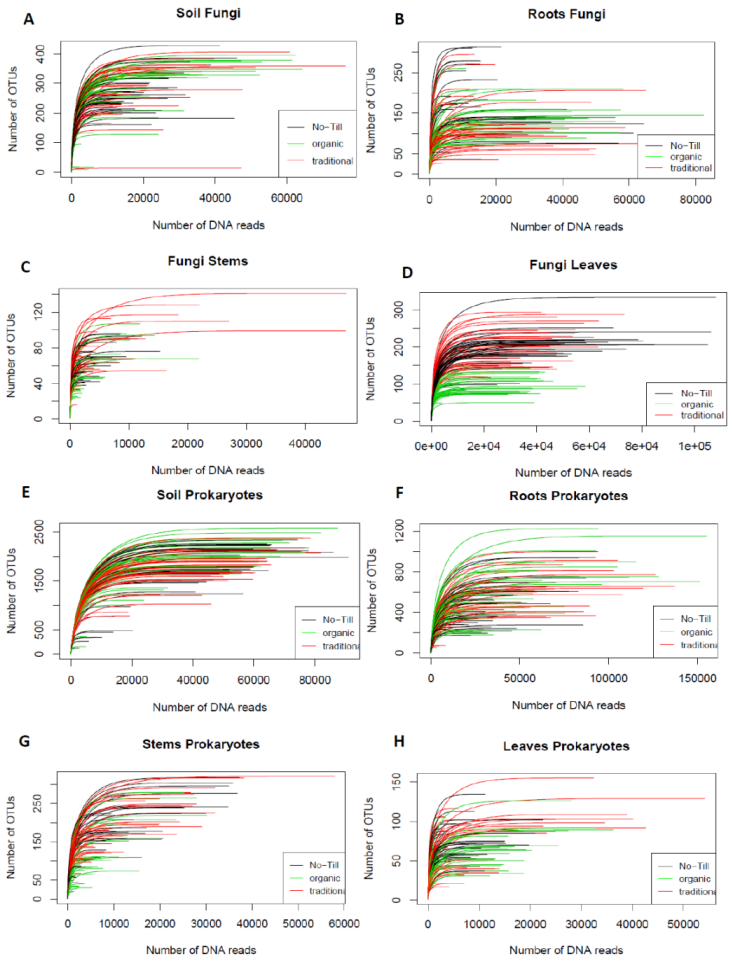
Results

Next Generation Sequencing Results

The final soil fungal library contained 95 samples and 2,562,324 reads for an average depth of 26,972 reads per sample after filtering, removal of contaminants, and removal of samples with less than 1000 reads. Applying the same quality filtering by plant compartment, the root fungal library was composed of 100 samples containing 2,706,574 reads with an average depth of 27,618 reads per sample, the stem fungal library contained 618,697 reads in 93 samples with an average depth of 7,031 reads per sample, and the library for the leaves had 4,572,077 reads in 107 samples for an average read depth of 43,133 reads per sample. Applying these quality filtering criteria to prokaryotic communities the 16S marker produced 6,040,145 reads with an average depth of 59,217 reads in 102 soil samples, 6,378,213 16S reads with an average depth of 60,172 reads from 106 root samples, 1,435,193 reads with an average depth of 14,497

reads per sample from 99 stem samples, and 1,313,368 reads with an average depth 13,402 reads per sample in 99 leaf samples. Rarefaction curves showing the number of OTUs generated against sequencing depth for each sample are shown in Figure 2.1.

Figure 2.1 - Rarefaction curves. Represent the number of OTUs detected per number of reads produced in sequencing of fungal communities (**A**) associated with soil samples, (**B**) associated with soybean root samples, (**C**) associated with soybean stem samples, (**D**) associated with soybean leaf samples and prokaryotic communities (**E**) associated with soil samples, (**F**) associated with soybean root samples, (**G**) associated with soybean stem samples, and (**H**) associated with soybean leaf samples.



Fungal Community Composition

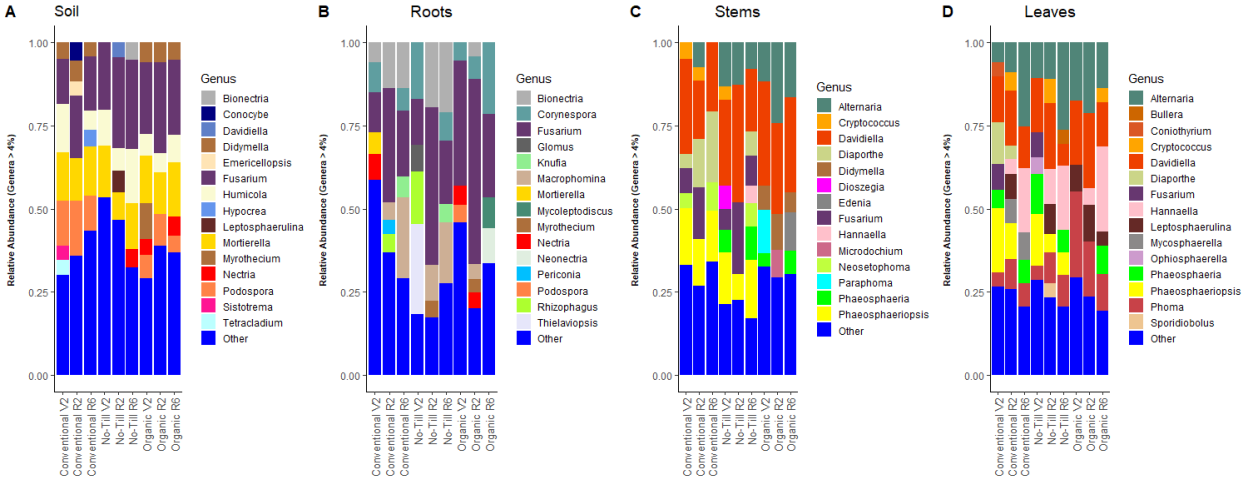
In the soil, Ascomycota were dominant, independent of management system, and accounted for between 75.0 and 81.0% of total reads. In comparison, Mucoromycota and

Basidiomycota abundances ranged between 7.0 and 12.9% in the three management systems (conventional, no-till, and organic). Of note, *Fusarium* was the most abundant fungal genus in the soil across all management systems with a relative abundance range of 15.9–23.7% (Figure 2.2A). All management regimes also contained a high abundance of *Mortierella* in soils with a range of 12.5–14.3%. Ascomycota dominated the fungal community of the roots under all three management systems, accounting for between 82.2 and 85.0% of reads, Glomeromycotina (8.3–13.5%) was the next most abundant lineage. Basidiomycota was present at relative abundances of between 3.7 and 8.1% in the three management systems. As found in the soil, the most abundant genus under all three management systems was *Fusarium*, which represented between 22.1 and 37.7% of all reads (Figure 2.2B). *Fusarium* was followed in relative abundance by *Macrophomina* in the conventional management system (13.2%), *Bionectria* in the no-till management system (13.8%) and *Corynespora* in the organic management system (11.7%).

In stems, Ascomycota and Basidiomycota accounted for nearly 100% of reads in all management regimes with Ascomycota accounting for about 90.0% of the reads. *Davidiella* was the most abundant genus in the stems, with over 20.0% of the reads in all three management systems followed by *Diaporthe* in conventionally managed plots and *Fusarium* and *Alternaria* in no-till and organic management systems (Figure 2.2C). As was found in the stems, Ascomycota and Basidiomycota accounted for nearly 100% of the reads in the leaves of each management system; with ascomycetes accounting for ~75.0% of the reads. *Alternaria* was abundant in aboveground tissues of all management regimes and was the most abundant genus in the conventional and no-till management systems, with relative abundances of 14.9 and 15.5%, respectively. *Davidiella* was omnipresent in aboveground tissues, peaking in relative abundance at 20.0% in the organic management system. This was also true of *Phoma*, which had higher

relative abundance in the organic management regime (Figure 2.2D).

Figure 2.2 - Stacked bar plots showing fungal genera. Genera are shown for each management system at each growth stage (V2 – two sets of unfolded trifoliate leaves, R2 – full flower reproductive stage, and R6 – full pod development) with relative abundance ≥4%, (**A**) present in soil samples throughout the soybean growing season, (**B**) present in soybean root samples, (**C**) present in soybean stem samples, and (**D**) present in soybean leaf samples.



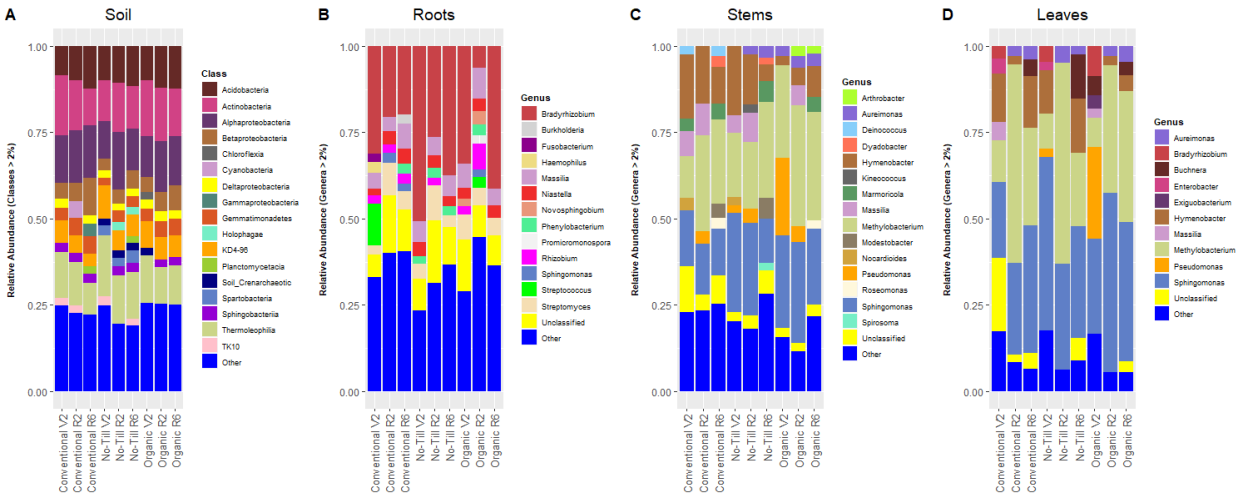
Prokaryotic Community Composition

The prokaryotic community of the soil was relatively consistent across management systems in terms of dominant Phyla. The most abundant phylum in every management system was Actinobacteria, consistently represented by ~30% relative abundance. The next most dominant phylum in each management system was Proteobacteria having relative abundances between 20.0 and 24.0%. In the soil, the most abundant classes were consistent between managements, but differed in their relative abundances (Figure 2.3A). The most abundant genus in every management system was an unclassified member of the Chloroflexi phylum with a range of relative abundances between 5.5 and 7.4%. *Sphingomonas* was the second most abundant genus (4.8%) in conventional managed soils. In contrast, an unclassified Gaiellales genus (6.7%) was the second most abundant in the no-till, while an unidentified genus of Acidobacteria was the second most abundant in the organically managed soils. Soybean roots were dominated by

the same bacteria phyla as the soils, but Proteobacteria were more abundant in roots (57.3–71.7%) compared to 20.0–24.1% in soil. Actinobacteria were the second most abundant bacteria in soybean roots (17.1–21.1%) across management systems. The most abundant genus was *Bradyrhizobium* with relative abundances of 22.9, 40.2, and 33.0% in the conventional, no-till, and organic management regimes, respectively. Following *Bradyrhizobium*, *Streptomyces* was the next highest in relative abundance ranging between 6.4 and 7.1% (Figure 2.3B).

The stem prokaryotic community was also dominated by Proteobacteria, with relative abundances ranging from 60.0 to 77.0%. Actinobacteria were the second most abundant bacteria in no-till (20.8%) and organic (12.7%) management systems. In terms of genera, the stems of soybean in all three management systems were dominated by *Methylobacterium* (24.3–32.0%) and *Sphingomonas* (14.9–25.2%) (Figure 2.3C). The prokaryotic community of soybean leaves was quite like that of the stems. Proteobacteria dominated the community ranging from 78.2%in the conventional management system to 92.6% in the organic management system. The dominant genera in leaves were similar to the stems except that *Sphingomonas* had higher relative abundance in the leaves, ranging from 31.5 to 44.7%. The relative abundance of *Methylobacterium* in the leaves was between 28.1 and 36.1% (Figure 2.3D).

Figure 2.3 - Stacked bar plots showing prokaryotic classes or genera. Taxa are shown for each management system at each growth stage (V2 – two sets of unfolded trifoliate leaves, R2 – full flower reproductive stage, and R6 – full pod development) with relative abundance ≥2%, (**A**) present in soil samples in soybean fields throughout the growing season, (**B**) present in soybean root samples, (**C**) present in soybean stem samples, and (**D**) present in soybean leaf samples.



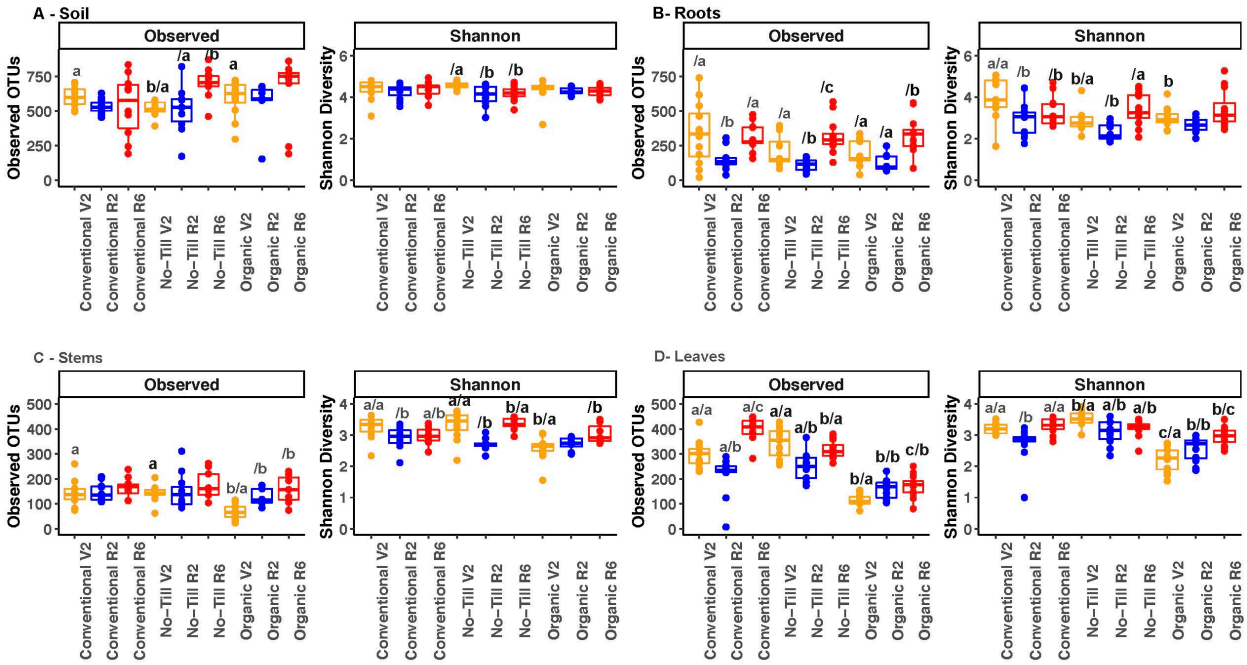
Alpha Diversity of Fungal Communities

Differences in fungal alpha diversity due to management system, plant growth stage, or sample origin were assessed. Fungal alpha diversity was highest in the soil and lowest in the stems with roots and leaves falling between the two (Figure 2.4). Soil had significantly higher species richness compared to roots, leaves, and stems (579 taxa per sample, 237 taxa per sample, 252 taxa per sample, and 140 taxa per sample, respectively). Richness differences between roots and leaves were not significant, but they both had significantly greater richness than stems. The soil also had significantly higher Shannon diversity than roots, stems, and leaves but differences between plant compartments were non-significant. In the soil, the only significant difference in richness between management systems was between the conventional/organic and no-till management systems at the V2 growth stage (Figure 2.4A). Significant differences were detected by growth stage under the no-till management system, with significantly higher fungal richness in the final growth stage but a decrease in Shannon diversity. In the root microbiome, there were

significant differences in Shannon diversity at the early vegetative (V2) growth stage with the organic management regime having significantly lower Shannon diversity mean values (Figure 2.4B). All management systems showed a decrease in fungal richness and Shannon diversity at the early reproductive (R2) stage, which increased again at the late reproductive (R6) stage.

In soybean stems, the conventional and no-till management systems consistently had higher richness than the organic management system, but the difference was only significant at the early vegetative (V2) growth stage (Figure 2.4C). This trend was not consistently reflected in Shannon diversity. All three management systems showed increasing richness throughout the season in the stems, but the trend was only significant for the organic management regime. Alpha diversity trends in the leaves of soybean in each management system were similar to those of their stems, with significantly greater richness in the conventional and no-till management systems throughout the experiment (Figure 2.4D). Fungal richness increased throughout the experiment in organic treatments, but in the other management systems richness and Shannon diversity decreased at the early reproductive (R2) growth stage.

Figure 2.4 - Alpha diversity boxplots showing OTU richness and Shannon diversity metrics for fungal communities. (**A**) present in soil samples, (**B**) present in soybean root samples, (**C**) present in soybean stem samples, and (**D**) present in soybean leaf samples. Colors represent the plant growth stage during sampling (V2 – two sets of unfolded trifoliate leaves, R2 – full flower reproductive stage, and R6 – full pod development). Significance groups are represented by letters above the boxes. The letter before the forward slash (/) represents significance groups within a single growth stage by management system. Letters following the forward slash (/) represent significance groups within a single management system by growth stage. Significance groups were calculated using Kruskal Wallis tests followed by Pairwise Wilcox tests with an FDR P-value correction.



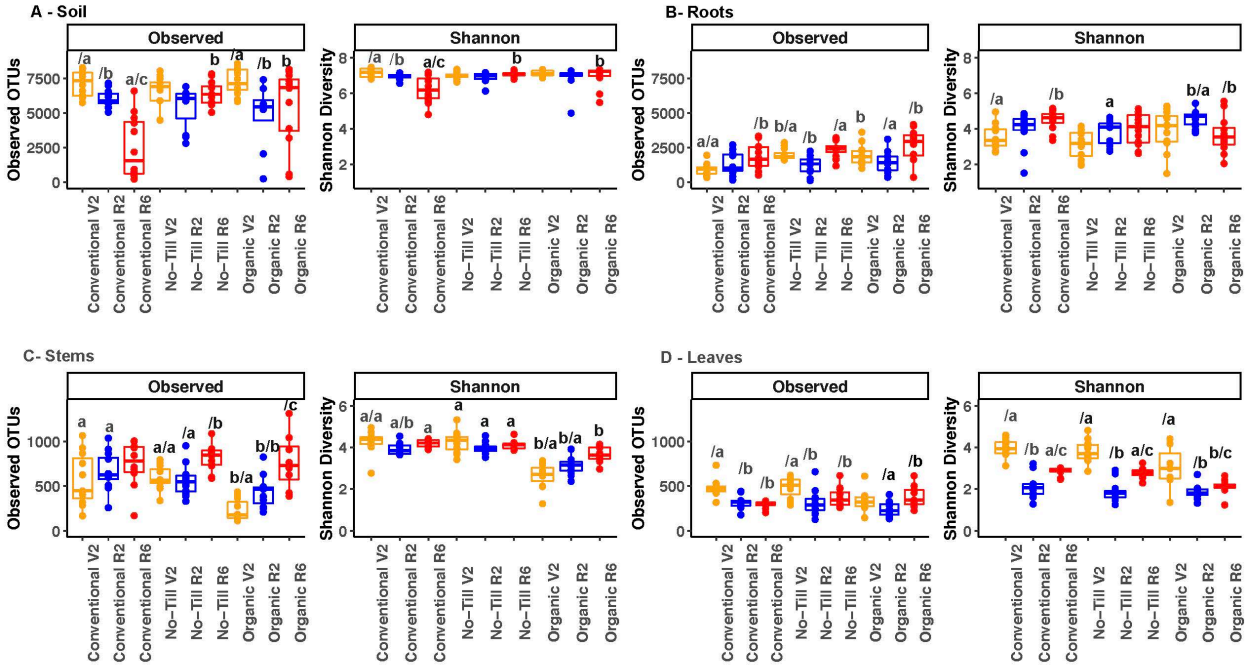
Alpha Diversity of Prokaryotic Communities

In prokaryotic communities, OTU richness was highest in the soil and decreased moving from that of the roots toward distal aerial compartments (Figure 2.5). Soil alpha diversity was significantly greater than the roots, stems, and leaves (5780 OTUs per sample, 1761 OTUs per sample, 597 OTUs per sample, and 358 per sample, respectively). Additionally, the roots had significantly greater alpha diversity compared to stems and leaves, but differences between stems and leaves were not significant. This pattern of statistical significance also held true for Shannon diversity, with a range from 2.7 in the leaves to 6.9 in the soil. In terms of Shannon diversity,

differences between roots and stems were not significant (3.88 and 3.80, respectively). In the soil, at any single growth stage, there were no significant differences between management systems except at the final stage where the conventional management system had significantly lower richness and Shannon diversity compared to the other management systems (Figure 2.5A). Conventional and organic management regimes showed significantly lower richness in the reproductive stages compared to the vegetative (V2) stage. In the roots, the richness was significantly lower in the conventional management system at the first sampling point, but differences were not significant at later stages (Figure 2.5B). The no-till and organic management systems showed significant decreases in richness at the R2 growth stage, but this pattern was not reflected in Shannon diversity.

In the stems, the no-till management system had significantly lower richness in the first growth stage compared to the final stage, and Shannon diversity was significantly lower in the organic management system throughout the season compared to other management systems (Figure 2.5C). Richness increased between the first and last sampling point for all three management systems, but this change was only significant for no-till and organic management regimes. In the leaves, the organic management system had lower richness and Shannon diversity at the early vegetative (V2) growth stage, but this difference was not significant. All three management systems had a significant decrease in richness and Shannon diversity in the leaves at the early reproductive (R2) growth stage (Figure 2.5D).

Figure 2.5 - Alpha diversity boxplots showing OTU richness and Shannon diversity metrics for prokaryotic communities. (**A**) present in soil samples, (**B**) present in soybean root samples, (**C**) present in soybean stem samples, and (**D**) present in soybean leaf samples. Colors represent the plant growth stage during sampling (V2 – two sets of unfolded trifoliate leaves, R2 – full flower reproductive stage, and R6 – full pod development). Significance groups are represented by letters above the boxes. The letter before the forward slash (/) represents significance groups within a single growth stage by management system. Letters following the forward slash (/) represent significance groups within a single management system by growth stage. Significance groups were calculated using Kruskal Wallis tests followed by Pairwise Wilcox tests with an FDR P-value correction.



Beta Diversity of Fungal Communities

When considering all sampling sources together, the soybean-associated fungal communities were most separated by sample source (Figure 2.6A). When considering PCoA ordinations by individual sample origin, distinct clusters by management system are evident in the soil (Figure 2.6B). In the stems and the leaves there is some separation by the management system along both axes, but the management systems are not distinct (Figures 2.6D, E). There is no clear pattern among root samples by PCoA (Figure 2.6C). When samples are colored by growth stage, there are distinct clusters by growth stage along the X axis in the leaves. This axis

accounts for 41% of the variation and primarily separates the V2 growth stage on the left from the R2 and R6 growth stages (Figure 2.7E). There was some clustering by growth stage in the stems, but clusters were not as distinct compared to the leaves (Figure 2.7D). There was no clear pattern of fungal communities by growth stage in the soil or roots (Figure 2.7B, C).

Figure 2.6 - Principal coordinates analysis plots, based on Bray-Curtis dissimilarity, of fungal and prokaryotic communities separated by sample origin and colored by management regime. (**A**) Fungal communities associated with soybean soil, root, stem, and leaf samples, (**B**) associated with soil samples, (**C**) associated with root samples, (**D**) associated with stem samples, (**E**) associated with leaf samples and prokaryotic, (**F**) associated with soil, root, stem, and leaf samples, (**G**) associated with soil samples, (**H**) associated with root samples, (**I**) associated with stem samples, and (**J**) associated with leaf samples. The shape represents the management system, while color represents sample origin in (**A**, **F**). In all others the color represents the management system.

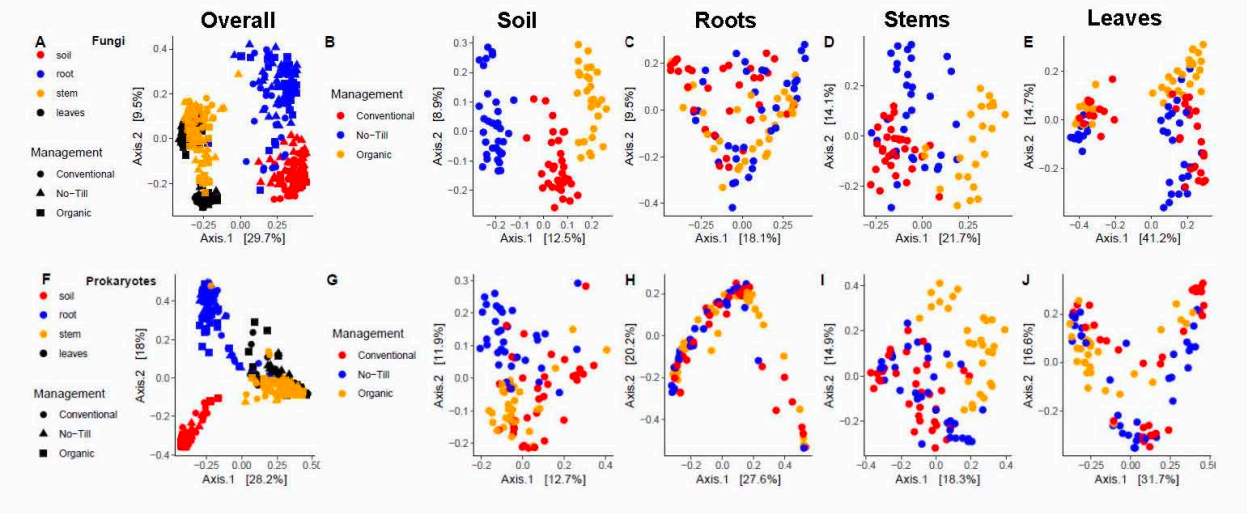
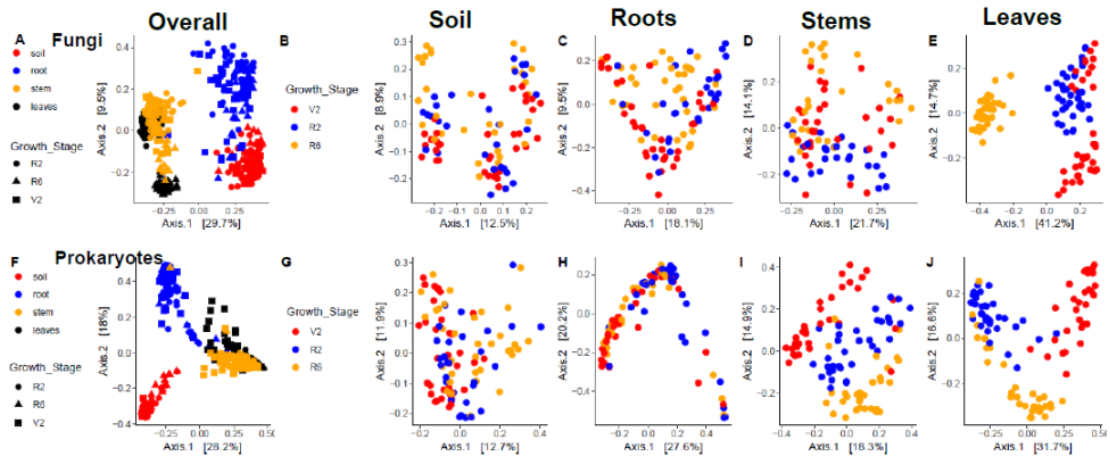


Figure 2.7 - Principal coordinates analysis plots, based on Bray-Curtis dissimilarity, of fungal and prokaryotic communities separated by sample origin and colored by soybean growth stage. (**A**) Fungal communities associated with soybean soil, root, stem, and leaf samples, (**B**) associated with soil samples, (**C**) associated with root samples (**D**) associated with stem samples, (**E**) associated with leaf samples and prokaryotic (**F**) communities associated with soil, root, stem, and leaf samples, (**G**) associated with soil samples, (**H**) associated with root samples, (**I**) associated with stem samples, and (**J**) associated with leaf samples. The shape represents the plant growth stage, while color represents sample origin in (**A**) and (**F**). In all others the color represents the plant growth stage.



The PERMANOVA analysis of fungal communities showed that regardless of sample origin there was a significant ($P < 0.05$) effect of both management system and growth stage (Table 2.3). However, since there was also a significant ($p < 0.05$) effect of the interaction between management regime and growth stage, datasets were split by growth stage and management system to analyze the effects separately (Table 2.3 and Table 2.4). When split by growth stage, the effect of management system was significant across all growth stages and all plant organs (Table 2.4A). This effect accounted for between 13 and 52% of variation. However, at several growth stages in several sample origins, there was a significant effect of dispersion, confounding PERMANOVA results (Soil R2 – P-value: 0.0096, Soil R6 – P-value: 0.023, Roots R2 – P-value: 0.0027, Leaves V2 – P-value: 0.0027). Although there is a significant effect of dispersion for these groups, there is clustering by management system in the PCoA ordination

space for the soil and the leaves, but clustering is less clear for R2 roots (Figure 2.8A, C, G). When split into individual management systems, the effect of growth stage is also significant throughout all management regimes and all plant compartments (Table 2.4B). The no-till roots and the conventional leaves have significant differences in group dispersion (P-values 0.042 and 0.037, respectively), but there do appear to be distinct clusters by growth stage in the ordination space for these groups (Figure 2.8D, H).

Table 2.3 - Permutational multivariate analysis of variance ("adonis") and multivariate homogeneity of groups dispersions analysis ("betadisper") results for fungal and prokaryotic communities split by sample origin. (**A**) fungal communities associated with soybean soil, root, stem, and leaf samples, (**B**) prokaryotic communities associated with soybean soil, root, stem, and leaf samples. Significance values at $p \leq .05$ are indicated in bold.

	Factor	PERMANOVA				DISPERSION	
	A - Fungi	**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
Soil	Growth Stage	2	4.347	0.071	**0.0001**	2.566	0.08580
	Management	2	9.794	0.160	**0.0001**	8.123	**0.00056**
	Growth Stage: Management	4	1.948	0.064	**0.0001**		
	Residuals	86					
	Total	94					
Roots		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	4.310	0.075	**0.0001**	3.525	**0.03337**
	Management	2	4.198	0.073	**0.0001**	0.709	0.49480
	Growth Stage: Management	4	2.286	0.079	**0.0001**		
	Residuals	89					
	Total	97					
Stems		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	8.324	0.138	**0.0001**	0.279	0.7571
	Management	2	8.699	0.144	**0.0001**	4.553	**0.0132**
	Growth Stage: Management	4	1.891	0.063	**0.002**		
	Residuals	79					
	Total	87					
Leaves		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	59.172	0.433	**0.0001**	7.763	**7.24E-04**
	Management	2	15.091	0.110	**0.0001**	0.032	0.9685
	Growth Stage: Management	4	6.998	0.102	**0.0001**		
	Residuals	97					
	Total	105					

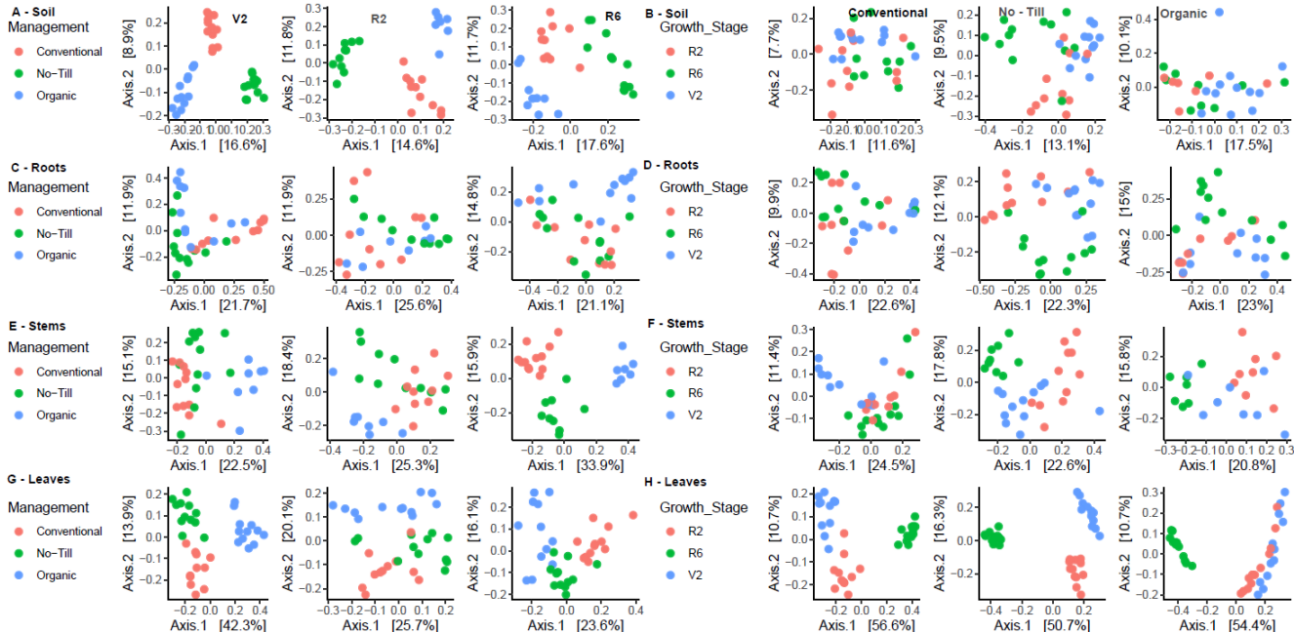
Table 2.3 (cont'd)

	B-Prokaryotes	Df	F-value	R2	P-value	F-value	P-value
Soil	Growth Stage	2	3.829	0.063	**0.0001**	5.086	**0.0079**
	Management	2	7.526	0.119	**0.0001**	8.555	**0.0004**
	Growth Stage: Management	4	1.882	0.060	**0.0002**		
	Residuals	92					
	Total	101					
Roots		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	7.087	0.110	**0.0001**	3.477	**0.0350**
	Management	2	4.405	0.069	**0.0001**	3.055	0.0514
	Growth Stage: Management	4	2.149	0.067	**0.0007**		
	Residuals	97					
	Total	105					
Stems		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	19.511	0.253	**0.0001**	8.146	**8.96E-05**
	Management	2	8.932	0.116	**0.0001**	10.287	**0.0001**
	Growth Stage: Management	4	1.784	0.046	**0.0205**		
	Residuals	90					
	Total	98					
Leaves		**Df**	**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Growth Stage	2	33.654	0.366	**0.0001**	29.286	**1.24E-10**
	Management	2	4.557	0.050	**0.0001**	1.431	0.2442
	Growth Stage: Management	4	4.559	0.099	**0.0001**		
	Residuals	89					
	Total	97					

Table 2.4 - Permutational multivariate analysis of variance ("adonis") and multivariate homogeneity of group dispersion analysis ("betadisper") results for fungal and prokaryotic communities associated with soybean soil, root, stem, and leaf samples split by management and growth stage. (**A**) the effect of agricultural management on individual growth stages (V2 – two sets of unfolded trifoliate leaves, R2 – full flower reproductive stage, and R6 – full pod development), and (**B**) the effect of growth stage on individual agricultural management systems.

A - Growth Stage		**PERMANOVA**				**DISPERSION**	
		F-value	**R2**	**P-value**		**F-value**	**P-value**
Soil	V2	5.23	0.241	**1.00E-04**		3.08	0.052
	R2	4.001	0.242	**1.00E-04**		5.63	**0.0096**
	R6	4.59	0.247	**1.00E-04**		3.63	**0.0396**
Roots	V2	3.6	0.189	**1.00E-04**		0.669	0.519
	R2	2.08	0.138	**5.90E-03**		4.39	**0.023**
	R6	2.85	0.151	**1.00E-04**		0.145	0.866
Stems	V2	4.89	0.259	**1.00E-04**		0.709	0.501
	R2	4.2	0.244	**1.00E-04**		3.15	0.059
	R6	9.98	0.444	**1.00E-04**		1.57	0.227
Leaves	V2	17.6	0.524	**1.00E-04**		7.15	**0.0027**
	R2	5.86	0.268	**1.00E-04**		1.34	0.276
	R6	7.74	0.319	**1.00E-04**		3.27	0.051
B - Management		**PERMANOVA**				**DISPERSION**	
		F-value	**R2**	**P-value**		**F-value**	**P-value**
Soil	Conventional	1.62	0.097	**8.00E-04**		0.728	0.491
	No-Till	2.72	0.149	**1.00E-04**		0.903	0.416
	Organic	1.65	0.116	**0.0065**		0.0401	0.961
Roots	Conventional	2.83	0.163	**1.00E-04**		0.786	0.465
	No-Till	4.18	0.207	**1.00E-04**		3.49	**0.042**
	Organic	2.29	0.14	**1.60E-03**		2.86	0.074
Stems	Conventional	3.67	0.197	**1.00E-04**		1.39	0.264
	No-Till	5.21	0.264	**1.00E-04**		1.19	0.318
	Organic	2.73	0.214	**1.00E-04**		1.77	0.196
Leaves	Conventional	27.6	0.641	**1.00E-04**		3.66	**0.037**
	No-Till	29.1	0.638	**1.00E-04**		1.24	0.302
	Organic	19.9	0.547	**1.00E-04**		3.23	0.052

Figure 2.8 - Principal coordinates analysis plots using Bray-Curtis dissimilarity of fungal communities split by growth stage and management system. (**A**) communities associated with soybean soil split by soybean growth stage, (**B**) communities associated with soybean soil split by agricultural management system, (**C**) communities associated with soybean roots split by soybean growth stage, (**D**) communities associated with soybean roots split by agricultural management system, (**E**) communities associated with soybean stems split by soybean growth stage, (**F**) communities associated with soybean stems split by agricultural management system, (**G**) communities associated with soybean leaves split by soybean growth stage, and (**H**) communities associated with soybean leaves split by agricultural management system.



Beta diversity of no-till and conventional management systems were analyzed together without the organic management regime due to the difference of host genotype. There was a significant effect of management system on beta diversity across all plant compartments and all growth stages with the effect ranging from 9 to 29% (Table 2.5A and Figure 2.9). When split into no-till and conventional management systems, the effect of growth stage was also significant across management systems and sample origins. In the no-till roots, a significant effect of group dispersion (P = 0.016) was found, with separation of growth stages obvious in ordinational space (Figure 2.9D).

Figure 2.9 - Principal coordinates analysis plots using Bray-Curtis dissimilarity, excluding the organic management of fungal communities split by growth stage and management system. (**A**) communities associated with soybean soil split by soybean growth stage, (**B**) communities associated with soybean soil split by agricultural management system, (**C**) communities associated with soybean roots split by soybean growth stage, (**D**) communities associated with soybean roots split by agricultural management system, (**E**) communities associated with soybean stems split by soybean growth stage, (**F**) communities associated with soybean stems split by agricultural management system, (**G**) communities associated with soybean leaves split by soybean growth stage, and (**H**) communities associated with soybean leaves split by agricultural management system.

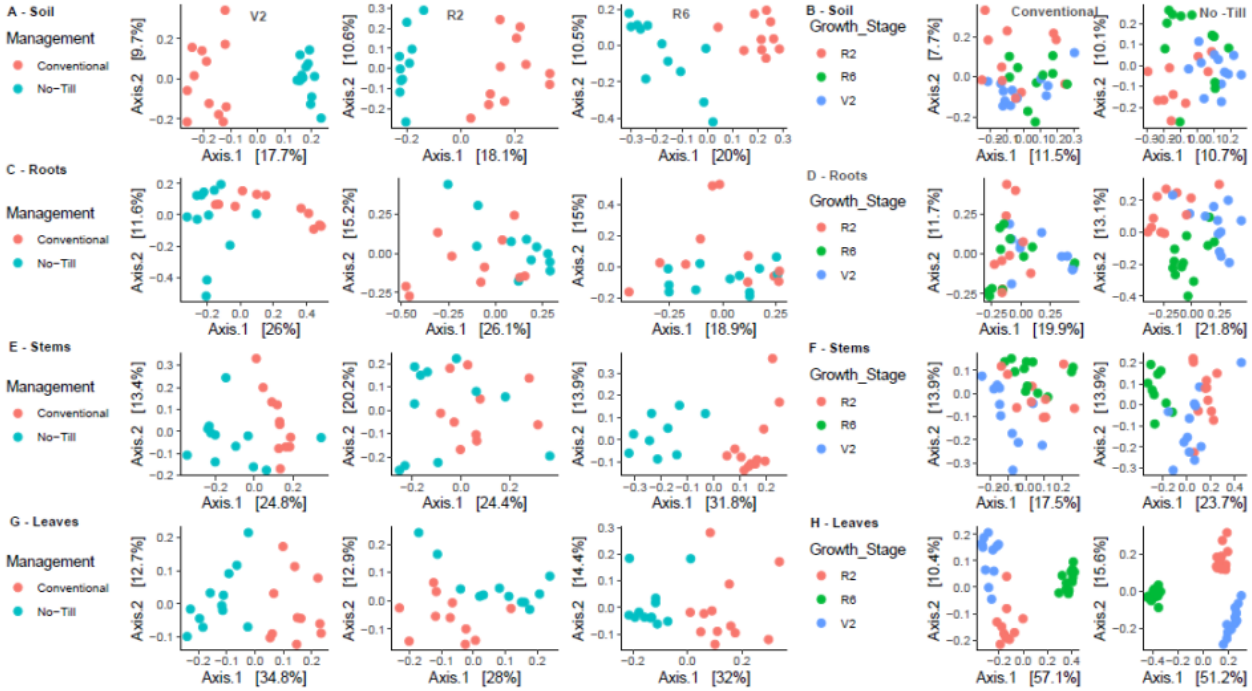


Table 2.5 - Permutational multivariate analysis of variance ("adonis") and multivariate homogeneity of groups dispersions analysis ("betadisper") results for fungal and prokaryotic communities excluding the organic management system and split by growth stage and management. (**A**) fungal communities associated with soybean soil, root, stem, and leaf samples showing the effect of agricultural management on individual growth stages and the effect of growth stage on individual agricultural management systems, and (**B**) prokaryotic communities associated with soybean soil, root, stem, and leaf samples showing the effect of agricultural management on individual growth stages and the effect of growth stage on individual agricultural management systems.

A - Fungi		PERMANOVA			DISPERSION	
Growth Stage		F-value	R2	P-value	F-value	P-value
Soil	V2	4.515	0.17	**1.00E-04**	0.043	0.8377
	R2	3.871	0.17	**1.00E-04**	0.4581	0.507
	R6	4.241	0.17	**1.00E-04**	0.4612	0.505

Table 2.5 (cont'd)

	V2	4.05	0.16	**3.00E-04**	0.831	0.372
	R2	2.16	0.102	**1.60E-02**	4.83	**0.041**
Roots	R6	2.06	0.089	**7.60E-03**	0.778	0.387
	V2	3.96	0.16	**1.00E-04**	0.339	0.567
	R2	2.33	0.11	**9.40E-03**	2.723	0.115
Stems	R6	7.5	0.283	**1.00E-04**	2.394	0.138
	V2	8.62	0.29	**1.00E-04**	1.867	0.186
	R2	3.57	0.15	**8.00E-04**	2.645	0.119
Leaves	R6	7.27	0.25	**1.00E-04**	0.169	0.291
Management		**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Conventional	1.57	0.095	**1.00E-03**	0.453	0.639
Soil	No-Till	2.22	0.125	**1.00E-04**	0.825	0.448
	Conventional	2.79	0.161	**3.00E-04**	0.209	0.812
Roots	No-Till	4.42	0.216	**1.00E-04**	4.712	**0.016**
	Conventional	3.18	0.175	**1.00E-04**	0.997	0.381
Stems	No-Till	4.69	0.244	**1.00E-04**	0.462	0.634
	Conventional	27.35	0.638	**1.00E-04**	2.39	0.108
Leaves	No-Till	29.53	0.642	**1.00E-04**	1.21	0.31
B - Prokaryotes		**PERMANOVA**			**DISPERSION**	
Growth Stage		**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	V2	6.504	0.228	**1.00E-04**	1.656	0.212
	R2	3.124	0.135	**6.00E-04**	0.951	0.341
Soil	R6	3.301	0.13	**1.00E-04**	16.913	**4.58E-04**
	V2	2.731	0.115	**1.69E-02**	2.98	0.099
	R2	0.694	0.032	7.68E-01	0.0309	0.862
Roots	R6	2.213	0.0914	**2.71E-02**	1.523	0.23
	V2	2.864	0.12	**5.30E-03**	1.127	0.3
	R2	3.679	0.143	**6.00E-04**	0.347	0.5621
Stems	R6	3.567	0.151	**1.00E-04**	1.392	0.251
	V2	5.108	0.203	**1.00E-04**	1.709	0.206
	R2	1.418	0.0662	2.15E-01	2.492	0.13
Leaves	R6	2.406	0.0986	**1.21E-02**	0.869	0.361
Management		**F-value**	**R2**	**P-value**	**F-value**	**P-value**
	Conventional	2.972	0.157	**1.00E-04**	29.747	**5.00E-08**
Soil	No-Till	2.259	0.124	**1.30E-03**	1.023	0.371
	Conventional	2.359	0.132	**7.80E-03**	0.165	0.849
Roots	No-Till	3.494	0.175	**6.00E-04**	2.87	0.071
	Conventional	5.33	0.262	**1.00E-04**	1.19	0.319
Stems	No-Till	10.06	0.378	**1.00E-04**	0.627	0.541
	Conventional	15.56	0.509	**1.00E-04**	8.53	**1.20E-03**
Leaves	No-Till	16.79	0.512	**1.00E-04**	5.38	**0.0097**

Beta Diversity of Prokaryotic Communities

When all samples are considered together, prokaryotic communities are clustered by sample origin, although there was not a clear distinction between stems and leaves (Figure 2.6F). When separated by sample origin, there were not clear clusters by management regime in any sample origin, but in the soil the no-till management system did appear slightly separated from the conventional and organic, primarily appearing in the upper left of the ordinational space (Figure 2.6G). In the stems, the organic management system was the most distinct, primarily appearing in the upper right of the PCoA (Figure 2.6I). When samples are colored by growth stage, there are clear clusters for each growth stage in the stem and leaf PCoAs with separation along the X and Y axes (Figure 2.7I, J). Similar to fungal communities, soil and root prokaryotic communities did not show distinct clusters by plant growth stage.

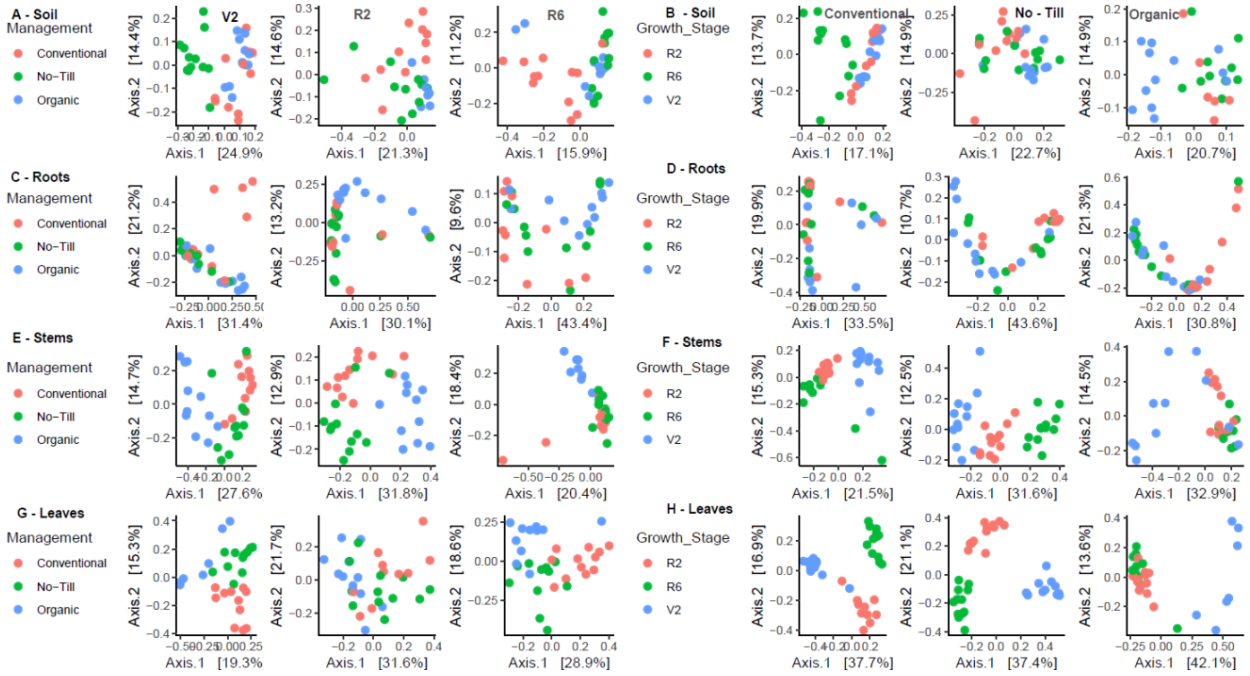
Growth stage and management system had a significant effect ($P < 0.05$) on prokaryotic communities at all sample origins, and the effect of plant growth stage increased moving upwards from the soil to aboveground and distal compartments of the plant (Table 2.3). Since there were significant interactions between growth stage and management system as well as significant differences in group dispersion, datasets were split by management regime and growth stage and analyzed separately (Table 2.6 and Figure 2.10). When split by growth stage, the effect of management system is significant across all sample origins and all growth stages. This effect accounts for between 11.3% (R2 roots) and 30.1% (R2 stems) of the variation. In several groups, there was a significant effect of group dispersion, making PERMANOVA results difficult to interpret. In the soil, at all three growth stages there was a significant ($P = 0.00037$, 0.0417, 0.00271) effect of dispersion, but in the ordinational space, there does seem to be separation by management system (Figure 2.10). In the V2 leaves, where there was also a

significant effect of dispersion (P = 0.045), distinct clusters by management system are visible in the PCoA (Figure 2.10G). When split by management system, there was a significant effect of growth stage in all management systems and all sample origins. This effect accounted for the most variation in the leaves where it accounted for between 42 and 53% of variation (Table 2.6B). However, there was a significant effect of group dispersion (P = 0.0013, 0.0041, 0.00073) in the leaves in all management systems, but samples do cluster by growth stage in the ordinational space (Figure 2.10G). In conventional soil and organic stems, there is also a significant effect of group dispersion (P = 2.5E-8, 7.2 E-5, respectively), but separation by growth stage is less clear in the ordinational space (Figure 2.10B, F).

Table 2.6 - Permutational multivariate analysis of variance ("adonis") and multivariate homogeneity of groups dispersions analysis ("betadisper") results for prokaryotic communities associated with soybean soil, root, stem, and leaf samples split by growth stage and management system. (**A**) the effect of agricultural management on individual growth stages (V2 – two sets of unfolded tri foliate leaves, R2 – full flower reproductive stage, and R6 – full pod development), and (**B**) the effect of growth stage on individual agricultural management systems.

A - Growth Stage		**PERMANOVA**				**DISPERSION**	
		F-value	**R2**	**P-value**		**F-value**	**P-value**
Soil	V2	5.93	0.264	**1.00E-04**		10.1	**3.70E-04**
	R2	3.47	0.211	**1.00E-04**		3.6	**0.0417**
	R6	3.1	0.158	**1.00E-04**		7.11	**2.71E-03**
Roots	V2	3.56	0.182	**2.00E-04**		2.07	0.143
	R2	2.01	0.113	**0.0123**		0.032	0.969
	R6	3.32	0.181	**1.90E-03**		0.739	0.486
Stems	V2	6.17	0.285	**1.00E-04**		3.44	**0.045**
	R2	6.9	0.301	**1.00E-04**		0.247	0.783
	R6	4.49	0.249	**1.00E-04**		1.11	0.344
Leaves	V2	3.94	0.233	**1.00E-04**		3.49	**0.045**
	R2	3.29	0.177	**1.00E-04**		0.119	0.887
	R6	6.25	0.281	**1.00E-04**		0.289	0.751
B - Management		**PERMANOVA**				**DISPERSION**	
		F-value	**R2**	**P-value**		**F-value**	**P-value**
Soil	Conventional	3.1	0.162	**1.00E-04**		31.7	**2.50E-08**
	No-Till	2.21	0.121	**1.10E-03**		1.01	0.376
	Organic	2.55	0.175	**3.00E-04**		0.49	0.619
Roots	Conventional	2.34	0.131	**7.20E-03**		0.175	0.841
	No-Till	4.09	0.209	**1.00E-04**		0.898	0.418
	Organic	3.76	0.186	**1.00E-04**		0.796	0.459
Stems	Conventional	5.2	0.257	**1.00E-04**		1.04	0.367
	No-Till	9.1	0.355	**1.00E-04**		1.07	0.356
	Organic	4.31	0.242	**2.00E-04**		13.9	**7.20E-05**
Leaves	Conventional	13.9	0.481	**1.00E-04**		8.26	**1.30E-03**
	No-Till	18.6	0.537	**1.00E-04**		6.55	**4.12E-03**
	Organic	9.85	0.421	**1.00E-04**		9.56	**7.30E-04**

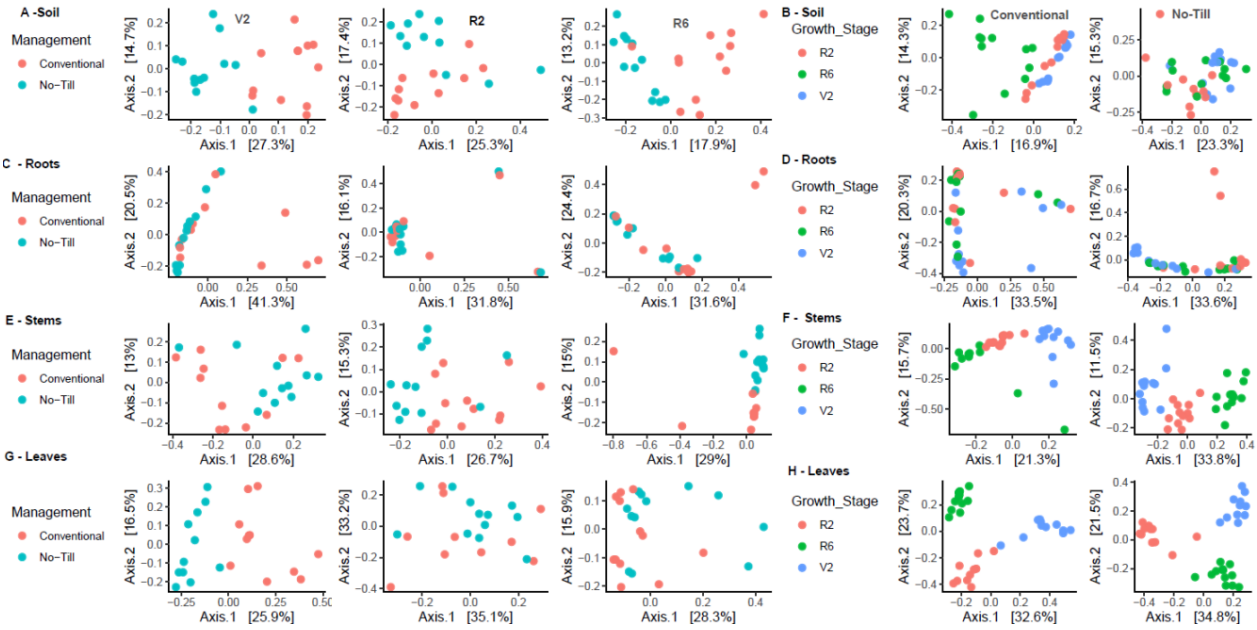
Figure 2.10 - Principal coordinates analysis plots using Bray-Curtis dissimilarity, of prokaryotic communities split by growth stage and management regime. (**A**) associated with soybean soil split by soybean growth stage, (**B**) associated with soybean soil split by agricultural management system, (**C**) associated with soybean roots split by soybean growth stage, (**D**) associated with soybean roots split by agricultural management system, (**E**) associated with soybean stems split by soybean growth stage, (**F**) associated with soybean stems split by agricultural management system, (**G**) associated with soybean leaves split by soybean growth stage, and those (**H**) associated with soybean leaves split by agricultural management system.



As with fungal communities, the no-till and conventional systems were analyzed without the organic system due to the genotypic difference. When split into individual growth stages, the effect of management system is significant ($p < 0.05$) in all growth stages and all sample origins except R2 roots and R2 leaves (Table 2.5B). This effect is the largest in the V2 soil and the V2 leaves (22.8 and 20.3% respectively). In groups where the management system effect is significant, distinct clusters are apparent in the ordinational spaces (Figure 2.11), although clusters are less distinct than those of Fungi. In the R6 soil, there was a significant effect of group dispersion (P = 4.58E-4), but the PCoA reveals separation between no-till and conventional management systems (Table 2.5B). When split into no-till and conventional management

regimes, the effect of growth stage was significant throughout the management systems and sample origins. In the leaves, there were significant differences (P = 0.0012, 0.0097) in group dispersion for both conventional and no-till management systems, but there are distinct clusters by growth stage in both management regimes (Figure 2.11H, Table 2.5B).

Figure 2.11 - Principal coordinates analysis plots using Bray-Curtis dissimilarity, excluding the organic management of prokaryotic communities split by growth stage and management system. (**A**) associated with soybean soil split by soybean growth stage, (**B**) associated with soybean soil split by agricultural management system, (**C**) associated with soybean roots split by soybean growth stage, (**D**) associated with soybean roots split by agricultural management system, (**E**) associated with soybean stems split by soybean growth stage, (**F**) associated with soybean stems split by agricultural management system, (**G**) associated with soybean leaves split by soybean growth stage, and (**H**) associated with soybean leaves split by agricultural management system.



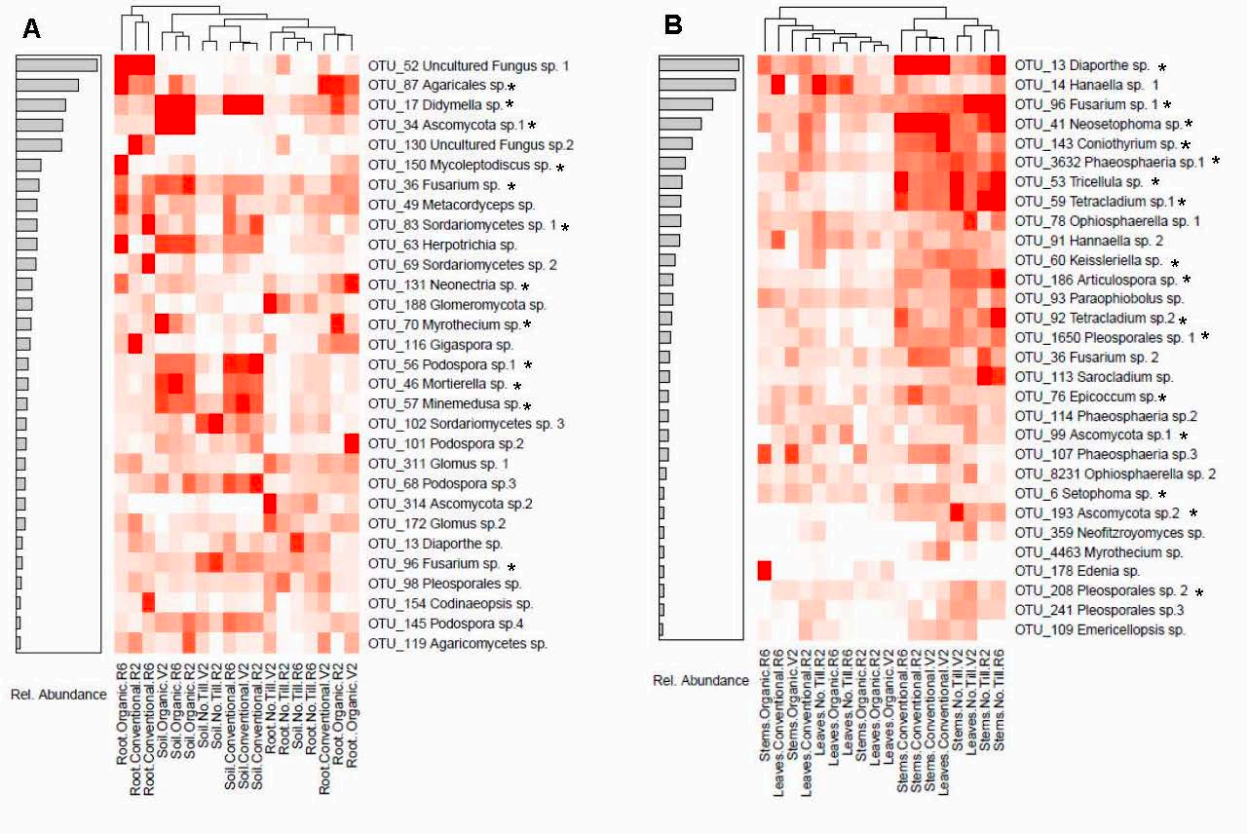
Indicator Species Analysis and Random Forest Modeling of Fungi

Many fungal OTUs were strongly associated with specific management systems. Heatmaps of the top 30 most relatively abundant indicator OTUs in above and belowground samples are shown in Figure 2.12. In belowground fungal communities, many of the indicator

taxa were OTUs which were indicators for conventional and organic soils but were lacking in no-till soils. These indicators were from several genera including *Didymella* OTU 17, *Mortierella* OTU 46, *Podospora* OTU 56, and *Minemedusa* OTU 57 (Figure 2.12A). All these taxa were also identified as being in the top 30 most important taxa for distinguishing between management systems in random forest analysis (Figure 2.13A). Indicators to no-till soils included a Sordariomycetes OTU and *Fusarium* OTU 96 which was also identified by random forest analysis. In the roots, a Glomeromycotina OTU 188 was highly associated to the no-till management system. An unidentified Agaricales OTU 87 was an indicator for the conventional and organic management regimes, and *Mycoleptodiscus* OTU 150 was an indicator for organic root communities (Figure 2.12A). Both taxa were also identified in random forest models as being important in distinguishing between management systems.

In aboveground fungal communities, many of the indicator species for no-till and conventional stems and early vegetative (V2) leaves clustered together. These OTUs included *Diaporthe* OTU 13 which was abundant in conventional stems and leaves, as well as *Fusarium* OTU 96 which was abundant in no-till tissues. Both indicator taxa were also identified as being important in aboveground random forest models. At later growth stages in the leaves, a *Hanaella* sp. was an indicator in the conventional and no-till leaves. The main indicator for the organic management system was an *Edenia* sp. which was most highly abundant in the stems at the late reproductive (R6) growth stage (Figure 2.12B).

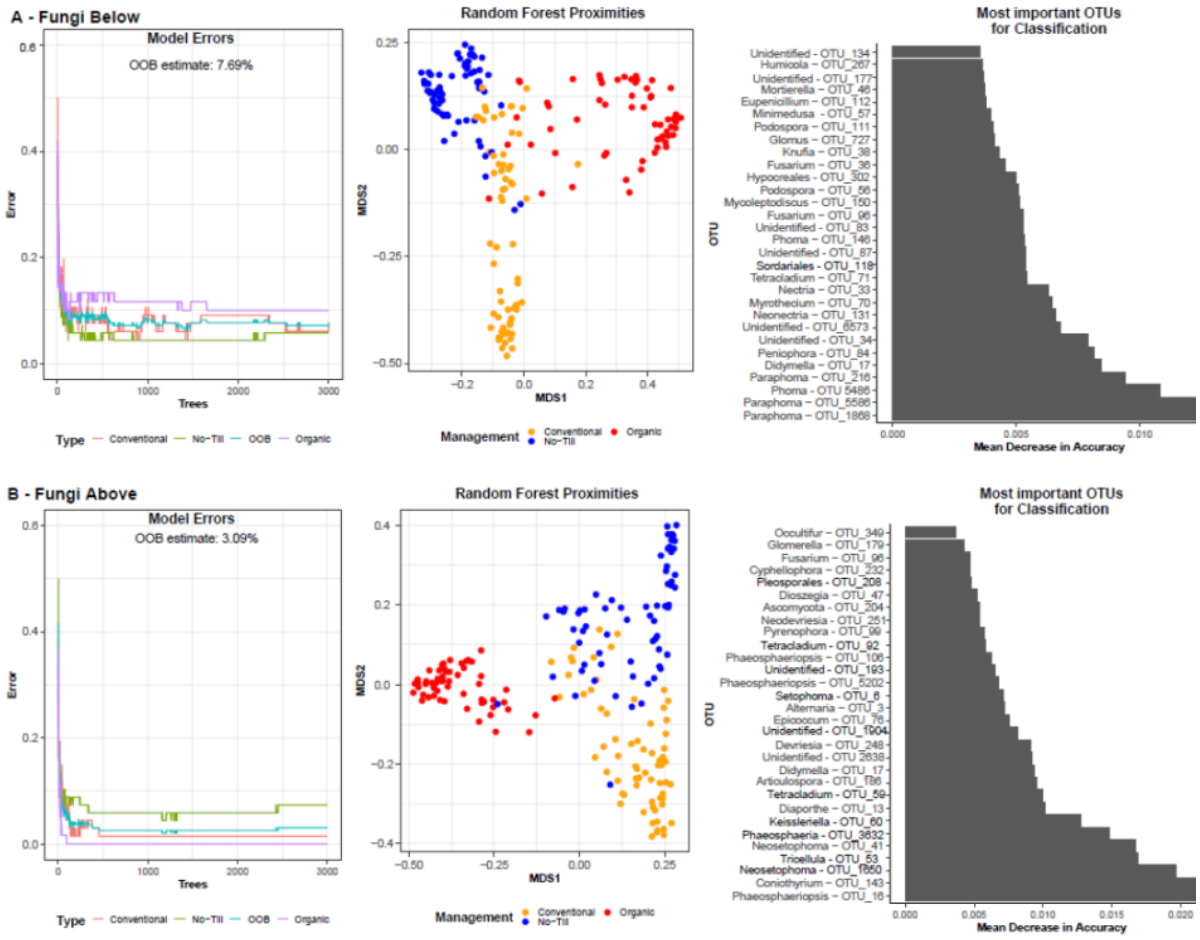
Figure 2.12 - Heatmaps of the relative abundances of the top 30 most abundant indicator taxa of fungi for each above and belowground samples. (**A**) belowground taxa, (**B**) aboveground taxa. Samples are clustered by the displayed dendrogram using Bray-Curtis distances. The associated barplots show the relative abundance among indicator species of the taxa. Taxa that were also among the top 30 most important for distinguishing between managements in Random Forest models of above and belowground samples are indicated with an asterisk (*).



Trends identified through indicator species analysis were further assessed with random forest analysis. Above and belowground fungal communities were assessed, and it was demonstrated that for belowground fungal communities there was an out of bag error for assigning management system to belowground samples of 7.7% (Figure 2.13A). Conventional samples were assigned incorrectly 10.6% of the time, no-till samples were assigned incorrectly 4.3% of the time, and organic samples were assigned incorrectly 10% of the time. For aboveground samples, the out of bag error was 3.1% (Figure 2.13B). The error rate in the organic management system was 0.0%, while the rate for conventional samples was 1.4% and the rate for

no-till samples was 8.8%. Conversion of sample proximities to Bray-Curtis distance allowed for the visualization of clusters of samples by each management regime for above and belowground samples (Figure 2.13A, B). Random forest models identified several *Phoma* and *Paraphoma* taxa which were important in distinguishing management systems but were not identified by indicator species analysis (Figure 2.13A).

Figure 2.13 - Random Forest modelling results of fungal communities. (**A**) associated with belowground samples, (**B**) communities associated with aboveground plant tissues

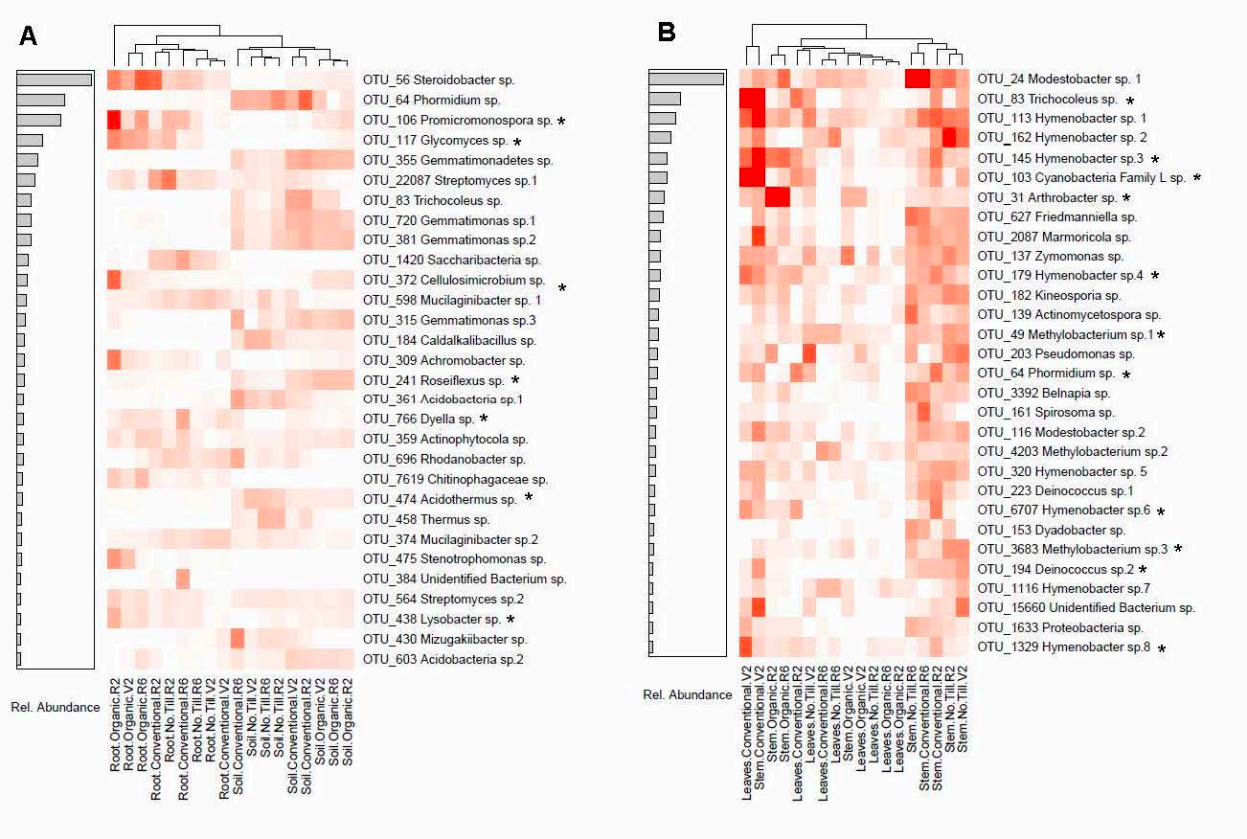


Indicator Species Analysis and Random Forest Modeling of Prokaryotes

Belowground prokaryotic indicator OTUs in root and soil compartments form into groups when clustered by Bray-Curtis distances. In the organic management system, *Steroidobacter* OTU 56 and *Promicromonospora* OTU 106 were indicator bacteria in the soybean root

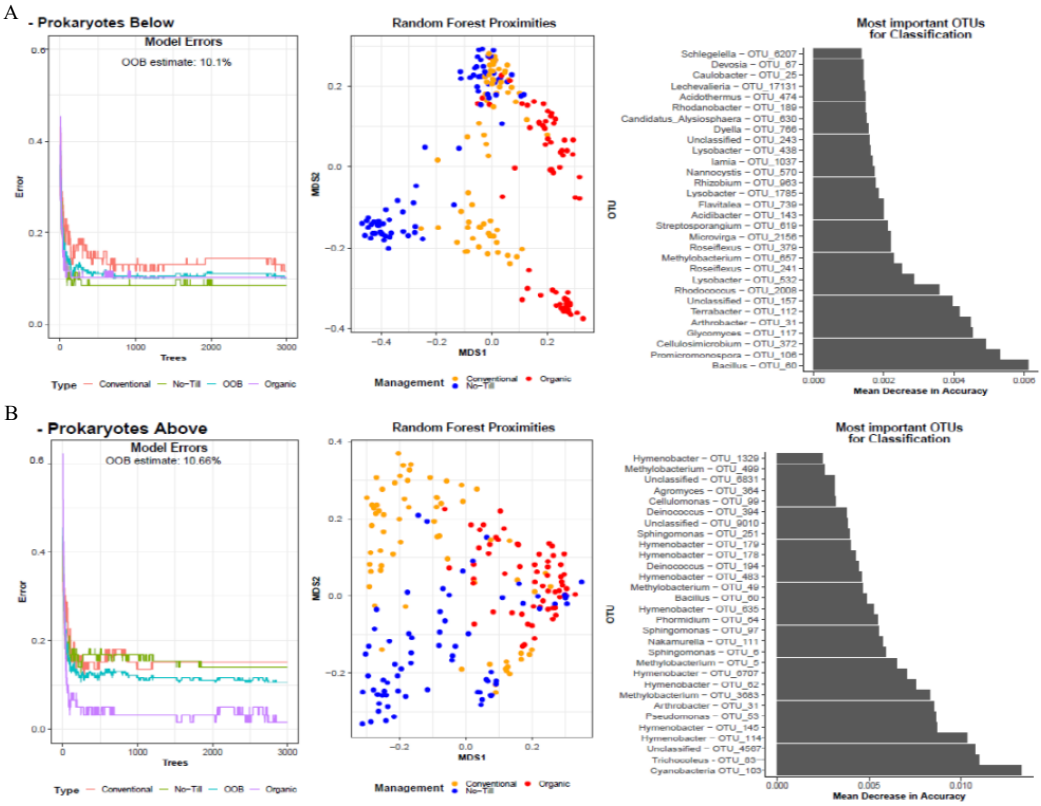
compartment, whereas a Streptomyces OTU was an indicator in roots from conventional and no-till management systems. *Promicromonsopora* OTU 106 was also identified as being among the top 30 most important OTUs in random forest modeling (Figure 2.15A). In the soil, a *Phormidium* OTU was an indicator for conventional and no-till management systems, while *Cellulosmicrobium* OTU 372 was an indicator to the organic soil and roots and was also identified by random forest modeling (Figure 2.14A and Figure 2.15A).

Figure 2.14 - Heatmaps of the relative abundances of the top 30 most abundant indicator taxa of bacteria for each above and belowground samples. (**A**) belowground taxa, (**B**) aboveground taxa. Samples are clustered by the displayed dendrogram using Bray-Curtis distances. The associated barplots show the relative abundance among indicator species of the taxa. Taxa that were also among the top 30 most important for distinguishing between managements in Random Forest models of above and belowground samples are indicated with an asterisk (*).



In aboveground tissues, indicator OTUs clustered based on plant compartments, management regime and growth stage. For example, at the early vegetative (V2) growth stage, stems and leaves from conventional managed soybean shared several indicator OTUs, including Cyanobacteria belonging to *Tricholeus* (OTU 83) and an unidentified Cyanobacteria Family L species (OTU 103). Both taxa were also identified as being important for assigning samples to management systems by random forest analysis. Many of the bacterial indicator taxa were *Hymenobacter* species, the majority of which were associated with no-till and conventional management regimes in both leaves and stems (Figure 2.14B). Many of the *Hymenobacter* taxa were also identified as being important in random forest modeling (Figure 2.15B). A stem associated *Arthrobacter* sp. was an indicator of the organic management system and was identified in random forest modeling. Random forest modeling performed on belowground prokaryotic communities revealed that samples were assigned to the correct management system 89.9% of the time (Figure 2.15A). Samples of the conventional management regime were assigned incorrectly 10.1% of the time, no-till samples 9.9% of the time, and organic samples 10.3% of the time. When proximities between samples were converted to Bray Curtis distance, clustering by management system is visible, but less clear compared to belowground fungal communities. The aboveground prokaryotic random forest model had an out of bag error rate of 10.7% (Figure 2.15B). The conventional management system samples were assigned incorrectly 12.1% of the time, no-till samples were assigned incorrectly 4.2% of the time, while organic samples were assigned incorrectly 0% of the time. In the MDS space, there was separation by management system, but the clusters were less clear than aboveground Fungi (Figure 2.15B).

Figure 2.15 - Random Forest modelling results of bacterial communities. (**A**) associated with belowground samples, (**B**) communities associated with aboveground plant tissues



Core Network Analysis and Hub Species Detection

Microbial networks constructed for above and belowground compartments across each management system differed in their network statistics (Table 2.7). Microbial networks in the no-till management system had the greatest numbers of nodes and edges for both above and belowground networks. Belowground, the network for the organic management system had the next highest number of edges and nodes, but aboveground the organic network was the sparsest in terms of edges and nodes. When compared to 100 random networks, each network except the aboveground organic and belowground conventional networks consistently had a significantly ($p < 0.05$) different degree distribution than 100 random networks (Table 2.7). Since the aboveground organic network and belowground conventional network did not have a

significantly different degree distribution than a random network, they will not be interpreted further. All networks contained a greater number of prokaryotic than fungal nodes and this difference was more pronounced belowground. Overall, networks had a diverse mix of bacterial and fungal phyla but were dominated by Proteobacteria and Actinobacteria with fungal nodes primarily being Ascomycota (Figure 2.16).

Table 2.7 - Summary table of network statistics. Statistics include number of nodes, number of edges, network stability, network sparsity, modularity, number of modules, number of fungal nodes, number of prokaryotic nodes, and number of detected hub species in above and belowground networks of conventional, no-till, and organic management systems.

Network	# Of Nodes	# Of Edges	stability	sparsity	modularity
Belowground Conventional*	139	270	0.047	0.0279	0.44
Aboveground Conventional	96	173	0.0495	0.038	0.61
Belowground No-Till	441	2663	0.0495	0.0274	0.36
Aboveground No-Till	119	270	0.0493	0.0381	0.53
Belowground Organic	424	2232	0.0487	0.0248	0.34
Aboveground Organic*	52	51	0.046	0.0377	0.7
Network	**# Of modules**	**# Fungal Nodes**	**# Prokaryotic Nodes**	**# Of Hubs**	**P Value Range**
Belowground Conventional*	38	26	113	3	0.016 - 0.27
Aboveground Conventional	20	42	54	2	**2.3E-04 - 0.037**
Belowground No-Till	8	36	415	5	**1.3E-03 - 0.027**
Aboveground No-Till	16	46	73	2	**2.2E-05 - 0.031**
Belowground Organic	22	26	398	1	**3.9E-05 - 0.037**
Aboveground Organic*	17	16	36	0	8.5E-03 - 0.57

Figure 2.16 - Bipartite networks constructed for fungal and prokaryotic communities. (**A**) belowground conventional samples, (**B**) aboveground conventional samples, (**C**) belowground no-till samples, (**D**) aboveground no-till samples, (**E**) belowground organic samples, and (**F**) aboveground organic samples.

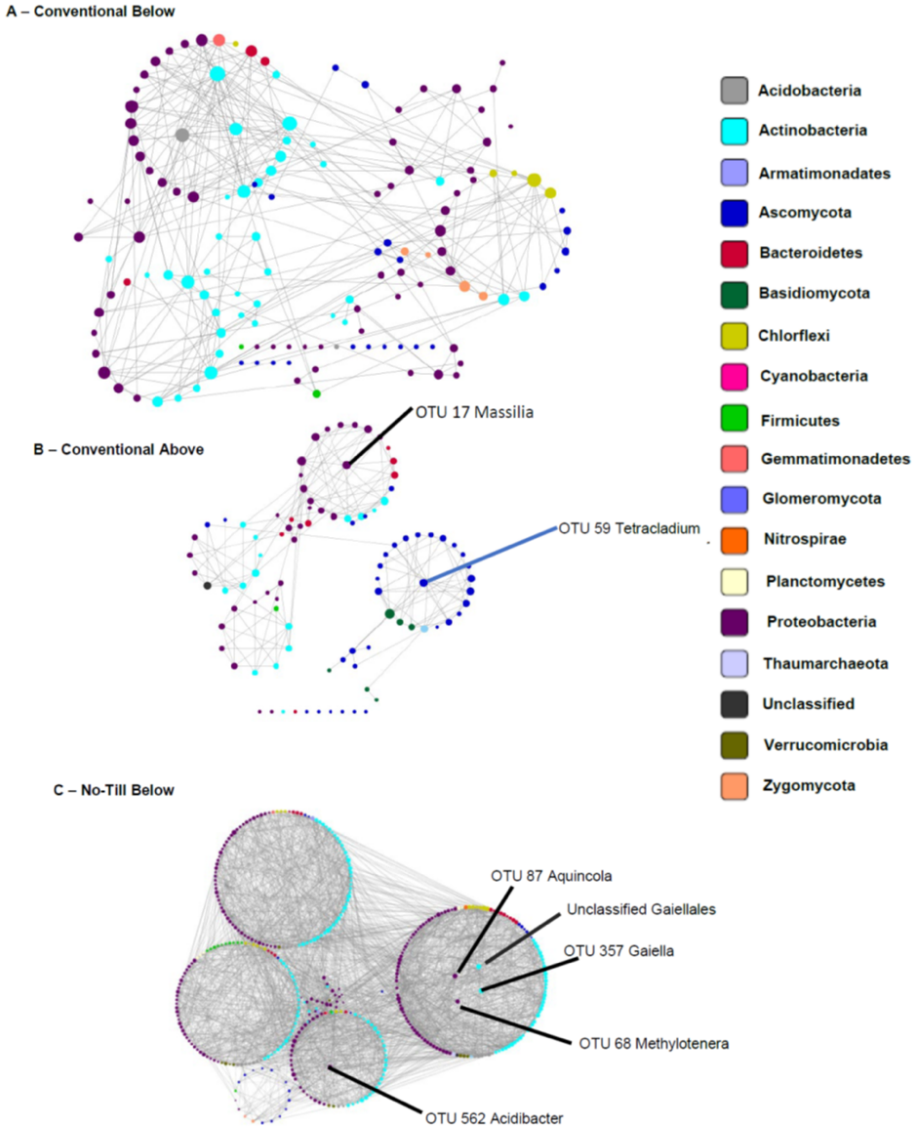
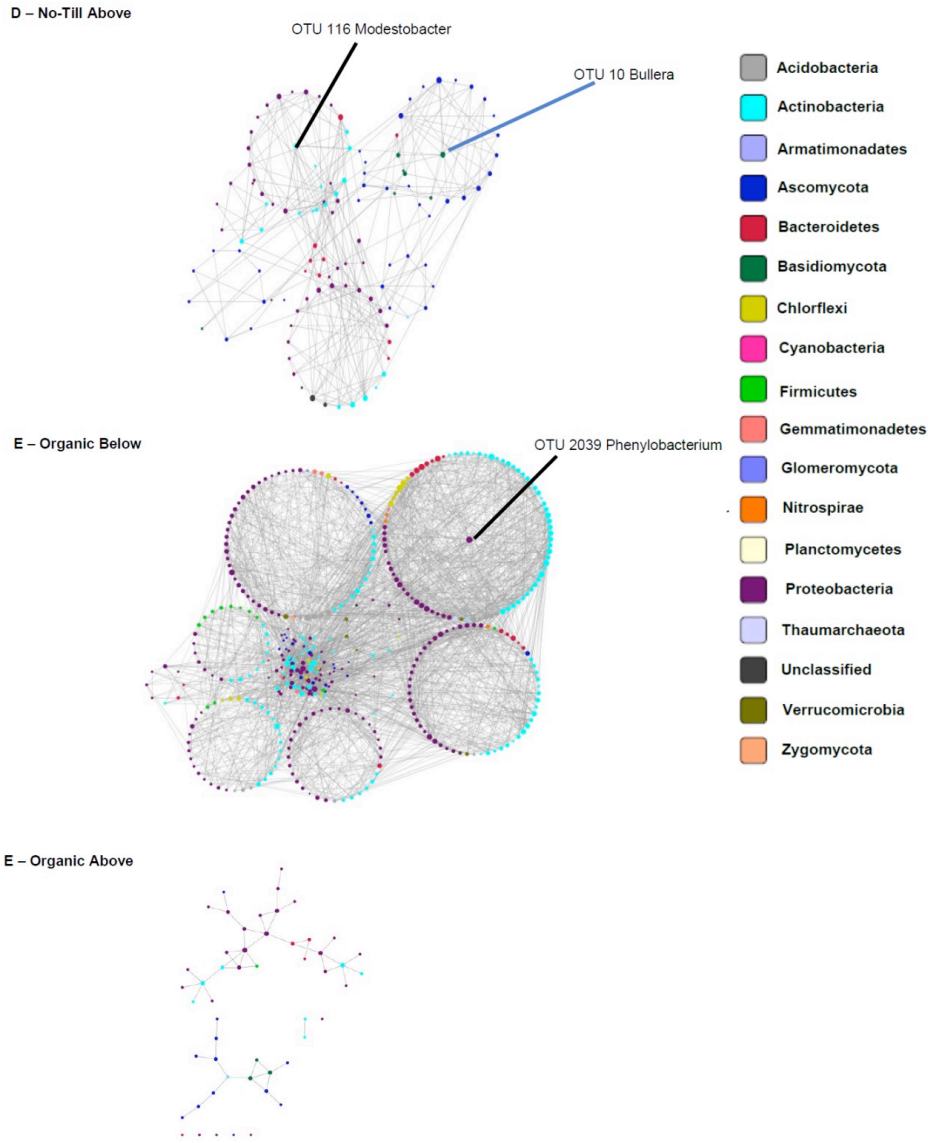


Figure 2.16 (cont'd)



Within above and belowground networks created for each management system, 10 hubs were identified from significant networks to belong to 10 separate fungal and bacterial genera (Figure 2.17A). Most bacterial hubs consisted of Proteobacteria and Actinobacteria while the two fungal hubs were one basidiomycete and one ascomycete. The hub OTUs varied in relative abundance, the *Massilia* OTU 17 and *Bulleria* OTU 10 were dominant among hubs in the roots, stems, and leaves (Figure 2.17B). Some hubs varied in relative abundance by management system, for example, *Tetracladium* OTU 59 was less relatively abundant in organic leaves and

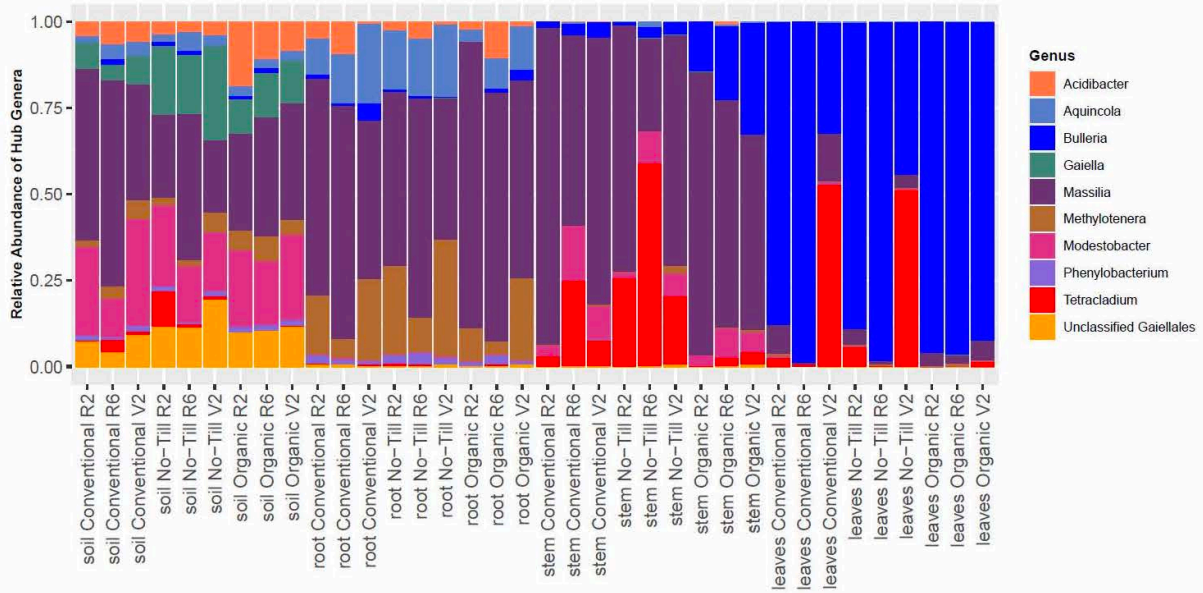
stems compared to no-till and conventional samples. Most hub OTUs were restricted to one compartment or to only above or belowground samples. This was not the case for *Massilia* OTU 17 which was present throughout and *Modestobacter* OTU 116 which was present in the soil and stems (Figure 2.17B).

Figure 2.17 - Summary of hub taxa detected in above and belowground bipartite networks for conventional, no-till, and organic management systems. (**A**) a table of detected hub genera and (**B**) stacked barplot showing the distributions of hub taxa across all managements and sample origins.

A

Hub Genus	Network	Phylum
Acidibacter	Belowground No-Till	Proteobacteria
Aquincola	Belowground No-Till	Proteobacteria
Bullera	Aboveground No-Till	Basidiomycota
Gaiella	Belowground No-Till	Actinobacteria
Massilia	Aboveground Conventional	Proteobacteria
Methylotenera	Belowground No-Till	Proteobacteria
Modestobacter	Aboveground No-Till	Actinobacteria
Phenylobacterium	Aboveground Organic	Proteobacteria
Tetracladium	Aboveground Conventional	Ascomycota
Unclassified Gaiellales	Belowground No-Till	Actinobacteria

B



Discussion

In this study, we assessed impacts of long-term cropping management systems on the soybean microbiome at a unique agricultural LTER site with 30 years of consistent management. We detected differences in the soybean-associated microbiome between management systems and growth stages throughout all sample origins. It is important to note that since plant compartments are not independent of each other; detected differences between managements in non-soil compartments may be largely driven by differences in the soil due to the role of the soil in seeding the microbiome of plant compartments [180]. However, if this is the case, our results demonstrate that differences from the soil persist throughout the plant. Additionally, since the same plots were sampled repeatedly throughout the season, samples from the same plot at different time points are not completely independent. However, when differences by growth stage are the highest (in the leaves and stems) the samples cluster by growth stage even when they are from different management systems; indicating that this is likely a true effect of growth stage, not simply differences between plots that persist due to repeated sampling of that plot (Figure 2.7). Some effect of growth stage may in fact be obscured due to differences between plots that persist because of repeated sampling of the same plots. Future studies performed at multiple sites can identify taxa which are consistently affected by the growth stage of the host plant at multiple sites.

In terms of alpha diversity, there was not a consistent difference between organic and conventional management systems, a pattern that was also observed for maize [219]. Alpha diversity results were consistent with studies that have demonstrated the highest alpha diversity of both Fungi and Prokaryotes in the soil [220, 221]. Additionally, our results were consistent with previous results from the same site which demonstrated that the highest within plant alpha

diversity for prokaryotes could be found in the roots, but for fungi the root alpha diversity was similar to that of the leaves [4]. Interestingly, previous studies have demonstrated higher alpha diversity of fungal communities in *Populus* stems compared to leaves which contradicts our results [222]. This may be due to differences in plants, or the level at which stems were sampled. Within a single compartment, in terms of alpha diversity, the primary pattern in fungal and prokaryotic communities was a decrease in richness in the early reproductive (R2) stage followed by an increase at the late reproductive (R6) growth stage. This differed from a trend of increasing alpha diversity in plant organs throughout the season, as was detected in a previous study on wheat at the KBS LTER [4]. Our results also differed from a previous observation of a reduction in phyllosphere prokaryotic diversity throughout a soybean growing season [223]. Additionally, fungal richness was lower in organic stems and leaves. It is possible that this was due to management but it could also be due to the different plant genotype that was used in the organic system, as has been demonstrated to be an important source of variation in the maize rhizosphere [224]. Taken together, these observations suggest that trends in alpha diversity are not consistent across crops and sites. This may indicate that alpha diversity and other microbial community measures may be altered by unmeasured environmental factors as well as biotic factors such as plant exudates, interspecies competition, and the effects of non-microbial taxa [225].

The structures of the fungal communities were distinct between management regimes in terms of the presence and absence of particular fungal genera. For example, although abundant in other treatments, in no-till soils, *Podospora* and *Didymyella* were below the 4% threshold to be included in bar graphs (Figure 2.2A). *Podospora* has been identified previously as being more abundant in conventionally tilled wheat soils [226]. In the soil, it is postulated that tillage can

alter fungal communities such as AMF by disrupting hyphae [2]. Consistent with this hypothesis, the highest relative abundance of AMF was detected in no-till soils, but mechanistic studies are needed to ensure that this difference is due to tillage at the KBS LTER site.

Indicator species analysis identified taxa such as *Mortierella* and *Minimedusa* that were associated with organic and conventional management systems (Figure 2.12A). These same taxa were identified as being important in assigning samples to management systems in random forest models (Figure 2.13). *Minimedusa polyspora* is of interest because it has been suggested to be plant growth promoting given its ability to solubilize phosphorous [227]. Some *Mortierella* species are also known to solubilize phosphorus [228]. *Mortierella elongata* has been reported to upregulate nutrient uptake and lipid signaling pathways in Populus [229], and are known to break down toxic organic compounds in the soil [230].

Phoma was enriched in aboveground stem and leaf fungal communities in organic managements, while *Fusarium* and *Phaeosphaeriopsis* were conspicuously absent (Figure 2.1C, D). Additionally, various *Phoma* OTUs were identified as being important for separating belowground management systems in random forest models (Figure 2.13A). Interestingly, *Phoma* spp. have been indicated as a possible biocontrol agent for *Fusarium graminearum* in wheat, which may explain the lack of *Fusarium* where *Phoma* was abundant [4]. Indicator species analysis identified Fusarium sp. as statistically associated to aboveground soybean tissues in conventional and no-till managements. This result was interesting because previous work at the same site found Fusarium to be enriched in the phyllosphere of organic wheat [4]. It is also possible that this microbiome difference is due to the difference in soybean cultivar used in the organic system, as host genotype differences have been demonstrated in grape, maize, and poplar phyllospheres [231–233].

In soil prokaryotic communities, *Spartobacteria* were enriched in no-till treatments. *Spartobacteria* has been found to be associated with no -till maize/soybean fields in a previous study, indicating that tillage regime may be specifically disruptive to these bacteria [234]. The no-till prokaryotic community was enriched in *Bradyrhizobium*. Previous studies have found a positive correlation between *Bradyrhizobium* and increased organic carbon caused by not tilling [72]. No-till and organic management regimes have been demonstrated to significantly increase total carbon in surface soils at the KBS LTER, which may explain the enrichment of *Bradyrhizobium* in the no-till management system [71]. However, since soil carbon was not measured as a part of this study, further work is needed to establish this relationship. In aboveground tissues, *Hymenobacter* was enriched in the no-till and conventional management systems. Some *Hymenobacter* species are plant growth promoting bacteria that can increase fatty acid content of plants [235, 236]. Together, these results indicate that management choices may select for beneficial microbes, but strain level identifications of taxa will be needed to assess this hypothesis. The indicator species analysis identified taxa which were tightly associated with roots or soils or tightly associated with specific growth stages (Figure 2.14A). For example, *Aureimonas* appeared only in the early reproductive (R2) and late reproductive (R6) growth stage of the three management systems. This observation is consistent with the idea that plants can recruit diverse microbes throughout their life cycles as they develop and their environment changes [237]. It is important to note that future studies on the effect of management regimes on the soybean microbiome are unlikely to identify the exact same indicator taxa. However, future work and more mechanistic studies may identify classes of microbes likely to be highly impacted by agricultural management. This information could then be used to predict the effect of the microbiome on plant health under alternative agricultural management.

The main explanatory variable of beta diversity in the soybean microbiome appeared to be whether the sample was from above or below ground compartments (Figure 2.6A, F). This result agrees with previous microbiome studies in *Arabidopsis* and wheat which showed different microbial communities are present in above and belowground plant tissues [4, 238]. At a finer resolution, there was separate clustering for leaves and stems and roots and soils, as has been noted in prokaryotic and fungal communities in the *Populus* microbiome [222]. Differences between microbial communities of organic vs. non-organic management systems have been demonstrated in grape and apple [239, 240]. Alternatively, pronounced effects of plant genotype could be driving differences in the phyllosphere fungal community, as has been reported for *Populus* [222, 231]. However, there were also distinct fungal communities between conventional and no-till management systems that persisted throughout the growing season in various plant compartments (Table 2.5 and Figure 2.9). Differences between conventional and no-till management systems were also made clear by the low error rate of random forest analyses in distinguishing agricultural management regimes (Figure 2.13 A, B). Tillage is known to be damaging to fungal mycelial networks in the soil, reducing the ratio of fungal to bacterial cells in soils [241]. Consequently, changes in fungal communities were expected given the substantial differences between tilled and non-tilled soils as has been demonstrated previously [2]. Differences between conventional and no-till management systems were not only in the soil but persisted in the leaves throughout the growing season (Figure 2.9). The effect of no-till vs. conventional agricultural management on the fungal communities of aboveground plant compartments has been understudied but may have an important impact on plant health. Our study found shifts in the phyllosphere community throughout a growing season, and is consistent with previous observations of seasonal phyllosphere shifts in fungal and bacterial communities at

the KBS LTER in wheat, switchgrass and miscanthus [4, 180]. While PERMANOVA results confirm the effect of sampling time-point on aboveground plant microbiome compartments, they also confirm the effects of crop management regime on soil and rhizobiome (Table 2.3, 2.4). Further work is warranted in this area to determine if time- point shifts are driven by deterministic or stochastic effects.

PCoA plots of prokaryotic communities did not show a clear signature of management system on the soybean microbiome (Figures 2.6 G–J), yet a clustering of growth stages is evident in aboveground tissues (Figure 2.7). PERMANOVA results showed that management system played a larger role in the soil and growth stage/sampling point played a larger role in plant tissues, but the effects of both factors were significant in all sample origins (Table 2.3, 2.4). The moderate but significant effect of management regime on soil prokaryotic communities was consistent with results of a previous study that compared organic and conventional management systems [219]. As with fungal communities, changes in aboveground and root prokaryotic communities based on plant growth stage and sampling time-point are consistent with the results of previous studies on maize and rice [242, 243]. Differences in assembly between above and belowground tissues may alter the community's ability to respond to agricultural management and plant growth. Similar to fungi, when the organic management system was not included in analyses, there was still a significant difference between conventional and no-till management systems, although the difference was smaller than in fungal communities (Table 2.5 and Figure 2.11). As with Fungi, this distinction between conventional and no-till agriculture has been demonstrated in soils, but has been understudied within plant compartments [244]. Additionally, in the leaves the effect of management regime was reduced throughout the growing season when analyzing no-till and conventionally managed treatments alone (Figure 2.11G and Table 2.5).

Microbial networks in the long-term no-till management were denser than those of conventional or organic managements. This is undoubtedly related to higher prokaryotic alpha diversity in the no-till management system. We speculate that the increased number of core taxa, and therefore nodes, in the no-till networks may be related to both the lack of disturbance and increased soil carbon quality and quantity associated with no-till [10, 234]. Differences in network density and other network statistics between organic and other management systems may be due to management regime or due to host genotype differences. Further mechanistic studies are needed to assess the effects of more complex networks on host plant health.

Microbial networks detected different hub species in each network. Due to the lack of taxonomic resolution in amplicon sequencing studies, species, and strain level identification of hubs is impossible. However, detection of hub OTUs belonging to particular microbial genera may inform future mechanistic studies. In the no-till belowground network, two detected hubs were from the Gaiellales order, which has been previously shown to be enriched in the roots of rice compared to surrounding soil, their detection as hubs may indicate an important role in structuring the root microbiome [245]. The only hub OTU detected in the belowground organic network was *Phenylobacterium*. This particular OTU seemed to only appear at a low relative abundance among hubs and only appeared in the roots and soil. Species from this genus have been understudied in terms of their effect on plant health, but it's detection as a hub in the roots indicates that it may play a role in structuring the root microbiome.

One hub of the aboveground no-till network was a fungus belonging to the genus, *Bullera*. Similar to many other basidiomycete yeasts, *Bullera* species have been isolated from the phyllosphere of various plants, but their roles in plant health are undetermined [111]. The only other fungal hub detected was a *Tetracladium* OTU which was a hub in the aboveground

conventional network and was previously found to be abundant at the KBS-LTER site [4]. *Massilia* is another aboveground hub taxon of interest. Although studied primarily in the roots, taxa from this genus are potentially beneficial due to their ability to solubilize phosphate [246]. Its presence in aboveground tissues indicates that it may be important in structuring plant microbiomes in both above and belowground phytobiomes. Further research is needed to determine why hub taxa are highly connected to other microbial members and how these connections help assemble soybean-associated microbial communities.

Conclusion

Here we report on the impact of long-term cropping management systems on the soybean microbiome. In doing so, we also addressed whole plant-microbiome changes in above and belowground compartments across the growing season. Our results indicate that the management system and growth stage have significant effects on the soybean microbiome. The effect of management system persisted when comparing conventional and no-till systems, excluding organic samples that were of a different genotype. Our results also indicated that specific indicator taxa varied between management regimes. Some of the indicator taxa such as *Mortierella* and *Hymenobacter* may be beneficial to plants. Additionally, the management system altered the network hub taxa, which may be important in structuring the microbiome. Some hub OTUs, such as *Massilia*, belonged to microbial genera that are known to contain plant beneficial organisms. Taken together, these results indicate that agricultural management practices impact whole-plant microbiomes. How specific management regimes can be employed to select desired microbial traits is still an open question. Further research into taxa identified by indicator species and network analyses may help to elucidate their functional roles to explain why specific taxa may be enriched under different management systems.

Chapter 3

Non-target impacts of fungicide disturbance on phyllosphere yeasts in conventional and no-till management

Source:

This chapter has been published in *ISME Communications*: Longley, R.*, Noel, Z.A.*, Benucci, G.M.N. *et al.* Non-target impacts of fungicide disturbance on phyllosphere yeasts in conventional and no-till management. *ISME COMMUN.* **2,** 19 (2022). https://doi.org/10.1038/s43705-022-00103-w

Abstract

Fungicides reduce fungal pathogen populations and are essential to food security. Understanding the impacts of fungicides on crop microbiomes is vital to minimizing unintended consequences while maintaining their use for plant protection. However, fungicide disturbance of plant microbiomes has received limited attention, and has not been examined in different agricultural management systems. We used amplicon sequencing of fungi and prokaryotes in maize and soybean microbiomes before and after foliar fungicide application in leaves and roots from plots under long-term no-till and conventional tillage management. We examined fungicide disturbance and resilience, which revealed consistent non-target effects and greater resiliency under no-till management. Fungicides lowered pathogen abundance in maize and soybean and decreased the abundance of Tremellomycetes yeasts, especially Bulleribasidiaceae, including core microbiome members. Fungicide application reduced network complexity in the soybean phyllosphere, which revealed altered co-occurrence patterns between yeast species of Bulleribasidiaceae, and *Sphingomonas* and *Hymenobacter* in fungicide treated plots. Results indicate that foliar fungicides lower pathogen and non-target fungal abundance and may impact prokaryotes indirectly. Treatment effects were confined to the phyllosphere and did not impact belowground microbial communities. Overall, these results demonstrate the resilience of no-till management to fungicide disturbance, a potential novel ecosystem service provided by no-till agriculture.

Introduction

Disturbances from chemical applications in agriculture reduce the abundance of pests and pathogens and are common in modern agricultural ecosystems [247–251]. However, applying disturbance concepts to microbial communities can be challenging to assess recovery and analyze the full impacts of crop management. A lack of data on the impacts crop management combined with fungicide disturbances on the plant microbiome hinders developing novel strategies to minimize diversity loss, understand unintended consequences of these applications, and improve crop microbiomes' resilience. Observing fluctuations in taxa abundance and secondary effects mediated through microbial interactions following fungicide application opens the possibility for novel ecologically motivated strategies that promote microbiome stability or resilience following a fungicide application.

Fungicide use has become common in conventional agricultural systems. Yet, concerns remain about direct and indirect effects on non-targeted organisms, consequences (i.e., resistance), and negative impacts on the environment or human health [96, 252, 253]. The rapid evolution of fungicide resistance in plant and human pathogenic fungal populations can cause devastating epidemics in agricultural ecosystems, with spill-over effects to public health [254–257]. For example, there is substantial concern about the overuse of azole fungicides that have been linked to the resistance of Aspergillus fumigatus to antifungals in human infections [256, 257]. Despite concerns, foliar fungicide applications in maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr) are often made without pathogen pressure due to perceived or marketed yield benefits [6, 258] . A meta-analysis of soybeans demonstrated that foliar fungicide application in the absence of disease increased yield by 2.7%, but applications are less profitable without disease pressure [6]. While fungicides are necessary for crop protection, minimizing

non-target effects and unintended consequences is critical in evaluating the sustainability of agricultural production systems.

Studies reporting fungicidal and pesticidal impacts on micro-biomes [259, 260] have focused on soil and aquatic systems [96] rather than effects on foliage microbes. The two most popular fungicide classes used in agricultural field crops are the sterol demethylation inhibitors (DMIs), otherwise known as triazoles, and quinone outside inhibitors (QoI), or strobilurins. Foliar fungicides for maize and soybean are primarily applied as single or premixed QoI and DMI active ingredients [261]. QoI fungicides inhibit fungal respiration by blocking the quinol oxidation site in the cyto-chrome bc1 complex in the electron transport chain. DMI fungicides inhibit CYP51 (encoding 14α-demethylase), an important enzyme in the ergosterol biosynthesis pathway of fungi [18]. Both fungicide classes are highly active against many plant pathogens. From the few studies focused on the plant phyllosphere, a consistent non-target effect is detected against phyllosphere yeasts. One study on grapevine microbiomes reported minimal and transient impacts to the phyllosphere microbiome, including phyllosphere yeast abundance [7]. Similarly, repeated application of broad-spectrum fungicides has been shown through culture-based and culture-independent methods to decrease phyllosphere yeast richness [110, 262–264].

Yeasts that inhabit the phyllosphere are well suited to oligotrophic and dynamic environmental conditions present on leaf surfaces and consequently have been applied for biocontrol of plant pathogens [265]. They are known to produce extracellular polysaccharides and surfactants, which may be necessary for creating or maintaining biofilms [266]. In addition, some phyllosphere yeasts, including species of basidiomycete yeasts in *Cryptococcus* and *Sporidiobolus*, produce carotenoid compounds, which have antioxidant properties and may protect the yeasts and other resident microbes from stress in the phyllosphere [267]. Phyllosphere

yeast communities have also been linked to pollinator insects by altering floral nectary chemistry, and fungicides can modify this relationship [268, 269]. However, few studies have addressed the links between phyllosphere yeasts and other phyllosphere residing microorganisms. One study, which did analyze the links between phyllosphere yeasts and bacteria, found evidence that phyllosphere yeasts have direct interactions with bacterial members of the microbiome [9]. While indirect and collective effects of removing single species or groups of species from ecosystems have been proposed in ecological theory since the 1940s and studied in various macro-organism contexts such as conservation biology, disturbance ecology, and food web ecology, such effects are comparatively understudied in microbiome science [270–272]. In microbiomes, network complexity (i.e., linkage density) has been correlated to ecosystem functioning and stability [273, 274]. Consequently, co-occurrence patterns may reveal indirect effects, which may not be seen using other analyses.

Since the US Dust Bowl of the 1930s, soil conservation efforts have led to the steady adoption of minimum or no-till agriculture management systems [1]. Cropping management systems have been demonstrated to impact phyllosphere microbiomes [4, 47]. Crop management's effect on the resilience of foliar fungal communities following fungicides has not been explored but differing impacts of fungicides in different agricultural managements are probable. In one study performed on soil, agricultural management altered the response of microbial communities to the application of the DMI fungicide tetraconazole [275]. Similarly, a study on wheat demonstrated that crop rotation and wheat variety impacted response to foliar fungicides of various active ingredients, however the crop rotation systems differed between locations, confounding efforts to distinguish fungicide responses in specific rotations from those of location and variety [109]. Long-term experiments circumvent these confounding effects by

applying all treatments at a single location.

Here, we characterize effects of foliar fungicides on the maize and soybean leaf and root microbiomes in no-till and conventional plots of the Long-Term Ecological Research (LTER) Main Cropping Systems Experiment at the Kellogg Biological Station (KBS). Our research objectives were three-fold: (1) to determine whether fungicides alter microbial diversity across plant compartments (e.g., leaves or roots), crop species (e.g., maize or soybean), or tillage management (conventional vs. no-till); (2) to identify non-target and indirect effects of fungicide applications, and (3) determine if crop management alters the resiliency of the microbiome. We hypothesized that fungicides would alter both maize and soybean microbial (fungal and prokaryotic) diversity and network complexity. We predicted that this effect would be most pronounced in the leaves. In addition, given that plant microbiomes have been shown to differ under the two tillage management systems [47], we hypothesize that the response and recovery of plant microbiomes following fungicides would also differ. This LTER site allows for a novel approach by eliminating any differences caused by location bias and assessing the effect of fungicide application under long-term agricultural management. We apply a novel microbiome network analysis approach to determine the impact fungicides have on prokaryote-fungal co-occurrences in the plant microbiome. Finally, we used random forest models to predict prokaryote taxa responsive to altered fungal diversity demonstrating the possible indirect effects of fungicides.

Materials and Methods

Sample Site and Management Systems

Samples were collected from the no-till and conventional tillage management treatments of the main cropping experiment at Michigan State University's KBS LTER site which follows a maize (*Zea mays* L.), soybean (*Glycine max L*. Merr), and winter wheat (*Triticum aestivum* L.) rotation. The site contains six one-hectare replicate plots of no-till or conventional tillage plots consistently managed since 1989 [276] . Fungicide micro-plots (3.05 m wide × 6.10 m long) were established within four replicate plots in no-till and conventional tillage treatments. Control samples were taken from micro-plots of the same size directly next to the fungicide micro-plots. Samples were taken from the middle of plots to minimize the effect of any spray drift. Fungicide applications of Headline® with the quinone outside inhibitor (QoI) active ingredient pyraclostrobin (2017) and Delaro® with the combination of the demethylation inhibitor (DMI) prothioconazole and QoI trifloxystrobin active ingredients (2018) were performed at recommended label rates.

Fungicide Application

In 2017, the fungicide Headline® was applied to maize foliage at a recommended rate of 877 ml ha^{-1} (12 fl oz $acre^{-1}$). Headline® contains the QoI active ingredient pyraclostrobin, which acts as a mitochondrial respiration inhibitor. Pyraclostrobin is a local penetrant fungicide with translaminar movement and is not translocated in the xylem [277]. In 2018, soybean foliage was sprayed with Delaro® fungicide on 3 August 2018 (Bayer, Raleigh, NC, USA) at a recommended rate of 731 ml ha^{-1} (11 fl oz $acre^{-1}$). The active ingredients in Delaro® are a combination of the QoI trifloxystrobin which inhibits mitochondrial respiration, and the DMI prothioconazole, which inhibits ergosterol synthesis. Trifloxystrobin is a local penetrant fungicide with

translaminar movement and is not translocated in the xylem [277]. Prothioconazole has acropetal penetrant activity and has weak basipetal movement [278].

Sample Collection and DNA Extraction

In 2017, maize leaf and root samples were collected at three time points. The first sampling occurred before the fungicide application on 26 June 2017 (V6 growth stage), the second was 9-days post fungicide (dpf) (V8 growth stage), and the final sampling was 35-dpf (V15 growth stage). Leaves and roots from three plants from four replicate control or adjacent fungicide treated plots of each no-till, and conventional management were sampled at each time point. In 2018, soybean leaves were sampled at three time points the first occurred before fungicide spray on 3 August 2018 (R3 growth stage), the second occurred 13-dpf (R4 growth stage), and the final occurred 33-dpf (R6 growth stage) [183].

Sampling and DNA extractions were performed as described previously [4, 47]. Maize leaves were sampled by removing two whole leaves from each plant and placing them into a sterile Whirl Pak (Nasco, Madison, WI, USA) for transport back to the lab where they were stored at -80ºC until they were lyophilized. At the V6 and V8 growth stage, the sixth and seventh leaf was sampled. However, at the V15 growth stage, three leaves above the ear leaf were sampled. Roots were sampled by removing whole plants from the soil and the entire root system to the soil line. Then roots were washed in the field before being transported back to the lab, where roots were washed again with 0.1% tween 20 (ThermoFisher Scientific, USA) and deionized water. Samples were stored at -80ºC before being lyophilized for DNA extraction. Following lyophilization, the fine roots were removed from the root system and used for DNA extraction.

Soybean leaves were sampled with a flamed metal hole punch, washed in 80% ethanol,

and flame sterilized between samples. Three 6-mm leaf discs from three separate leaves were punched directly into an Eppendorf tube (Eppendorf, Germany) containing 500 µl of CSPL buffer (Omega Bio-Tek, Norcross, GA, USA). As with the maize roots, whole soybean plants were removed from soil and soybean roots were removed at the soil line and placed into a new Whirl-Pak (Nasco, Madison, WI, USA) bag containing approximately 50 ml of 0.1% tween 20 to remove the remaining soil. Root samples were transported back to the lab, where roots were washed again with deionized (DI) water, and samples were stored at -80ºC until processing. Maize and soybean leaf and root tissue were pulverized for 2-min at a speed of 30 Hz with two 4-mm stainless balls in a TissueLyser II (Qiagen, Venlo, Netherlands). Total DNA was extracted from plant tissues with the OMEGA Mag-Bind Plant DNA Plus kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions with the aid of a KingFisher Flex™ liquid handling machine (ThermoFisher Scientific, USA). Five or six internal negative extraction controls were included per 96-well plate in each DNA extraction.

Amplicon Library Preparation for ITS and 16S Community Profiling

Amplicon libraries were prepared from a modified three-step PCR protocol as described previously [47]. In brief, fungal libraries were constructed around the ITS and were amplified using the primers ITS1F and ITS4 [185]. Prokaryote libraries targeted the V4 region of 16S rRNA with the primers 515F and 806R [186]. Tables 2.1 and 2.2 describe PCR protocols and cycling conditions in detail. Amplicons were purified with the SequalPrep™ Normalization Plate Kit (ThermoFisher Scientific, USA) and then pooled and concentrated with Amicon® Ultra 0.5 mL filters (EMDmillipore, Germany). Subsequently, the library was purified, and size selected with Agencourt AMPure XP magnetic beads (Beckman Coulter, USA). Amplicon libraries were quantified and checked on the Agilent 4200 TapeStation DNA10000 and Kapa Illumina Library

Quantification qPCR assays. All amplicon libraries were then paired-end sequenced (300 bp reads) on an Illumina MiSeq with a v3 600 cycles kit (Illumina, USA).

Raw sequences for soybean microbial communities used to create figures and tables in this study are available in the NCBI SRA database under the following accession numbers: PRJNA603199 and PRJNA603207. Sequences produced on the same Miseq runs and used to remove contaminants are available in PRJNA603147. Raw sequences for maize microbial communities are available under the following accession numbers: PRJNA739465 and PRJNA739759. Code to generate figures and tables are located on GitHub at https://github.com/noelzach/FungicidePulseDisturbance.

Bioinformatic Sequence Processing

Fungal ITS1 or prokaryotic 16S V4 reads were demultiplexed in QIIME 1.9.1 [192]. Forward and reverse prokaryote reads were merged using QIIME 1.9.1. Only forward fungal ITS1 reads were used since reverse reads did not overlap. After removing primers with Cutadapt 1.8.1 [193], fungal reads were trimmed to remove the conserved SSU and 28S regions. Reads were then quality filtered at an expected error threshold of 0.1 and truncated to equal length (fungi 200 bp; prokaryote 300 bp) in USEARCH 11.0.667 [279]. Singletons and chimeras were removed, and *de novo* OTU clustering was performed at a 97% similarity using the UPARSE algorithm [196]. Using CONSTAX2 [280, 281], the taxonomic classification of fungal and prokaryotic OTU's representative sequences was performed against the UNITE eukaryote database, ver. 8.2 of 04.02.2020 [197] and SILVA, version 138 [198], respectively. To filter out non-target taxa and OTUs unidentified at the Kingdom level, CONSTAX was run twice under different cutoff levels, as previously suggested [282]. Non-target taxa, OTUs not assigned to a Kingdom, and OTUs identified as either chloroplast or mitochondria in either database were

removed from further analysis [283].

Data Import and Preprocessing in R

Data were imported into R 4.0.3 [200] and the R packages *phyloseq* 1.24.2 [201] and *vegan* 2.5.3 [207] were used for most analyses. Samples with low sequencing coverage (less than 1000 reads) were removed from the analysis. Contaminant OTUs (i.e., those prevalent in negative extraction controls) were removed with the R package *decontam* [202]. Before normalization, richness was assessed for Prokaryotes and Fungi in the leaves and roots of each crop using the 'estimate_richness' function in the *phyloseq* package. Results of alpha diversity analyses were plotted using the *ggplot2* package [208]. Then, sample read counts were normalized using the cumulative sum scaling technique within the *metagenomeSeq* R package [213].

Statistical Analysis

Differences in fungal and prokaryotic community composition were tested through PERMANOVA with the 'adonis2' function on Bray-Curtis distances in the R package *vegan* [207]. Variation in multivariate dispersion was tested with the 'betadisper' function in *vegan*. More specific hypotheses were tested based on constrained analysis of principal coordinates (CAP) [284] using the 'capscale' function in *vegan*. Differentially abundant taxa resulting from fungicide application were identified by comparing fungicide treated plots to control plots through an analysis of the composition of microbiomes (ANCOM v 2.1) [285]. For differential abundance analysis, fungal OTUs (fOTU, hereafter) with a mean relative abundance less than 10−5 and fOTUs with zeros present in 95% samples were discarded from the analysis to avoid detecting fOTUs as significantly different based on stochasticity. In addition, fOTUs that were never present in fungicide treated plots were not included. Fungal OTUs were determined to be

significant if the W value was greater than 70% of the taxa tested based on Wilcoxon ranked sum test between additive log-ratio transformed data and a Benjamini-Hochbergj adjusted P value ($\alpha = 0.05$) [285]. Recovered taxa (i.e., transient effects) were defined as fOTUs that were significantly less abundant in the first sampling following fungicide treatment but were not significantly less abundant from non-disturbed plots, after 33- or 34-dpf, for soybean or maize, respectively. Non-recovered taxa were defined as those fOTUs with significantly altered abundance following fungicide application and remained significantly altered for the remainder of the sampling. In addition, a portion of non-recovered taxa was also locally extinct, which were defined as taxa present before fungicide application but having zero relative abundance following fungicide application in fungicide treated plots through the remainder of the sampling while being present in the control plots. Finally, taxa that did not have significantly altered abundance following fungicide application but then had significantly different abundance at a later sampling point (i.e., 33- or 34-dpf) were defined as indirect effects.

The core phyllosphere fungal and prokaryotic taxa from the non-fungicide disturbed no-till or conventional plots were identified based on each abundance and occupancy across space and time. Taxa that contributed to the last 2% increase in Bray-Curtis distances were defined as the core [286].

We built random forest regression models to test the effect of altered prokaryote abundance through fungal diversity by using prokaryote abundances to predict fungal diversity. Random forest models were generated with the 'randomForest' function in the *randomForest* R package [214]. To remove redundant features and avoid overfitting models, we removed redundant OTUs with the 'Boruta' function in the package *Boruta* [287]. The method performs a top-down search for relevant OTUs by comparing the importance of the original OTUs from

those selected at random. Models were tuned to achieve the lowest stable out-of-bag (OOB) error estimate possible, and the best mtry value (number of OTUs sampled at random in the entire pool for each tree at each split) was selected using the 'tuneRF' function in *randomForest* R package.

Network analysis was conducted on soybean and maize leaf samples to estimate co-occurrences among prokaryotic and fungal OTUs in each host and determine whether fungicides altered fungal-prokaryotic co-occurrences and network complexity (i.e., linkage density). For network analyses, soybean and maize fungal and prokaryotic OTU tables were filtered to exclude taxa with mean relative abundance below 10−5. A co-occurrence meta-matrix was estimated using the Meinshausen and Bühlmann algorithm within the *SpiecEasi* R package with the 'nlambda' set to 100 and with 'lambda.min.ratio' set to 10−2 [217]. From this meta-matrix, subnetworks were created from taxa present within each sample. Then, network complexity was calculated for each subnetwork. The contribution of the Bulleribasidiaceae to network complexity was assessed by examining the change in the cumulative edge weights across subnetworks with prokaryotic genera. Bulleribasidiaceae were selected for further analysis because they represent an off-target group of fungi that was substantially impacted by fungicide application.

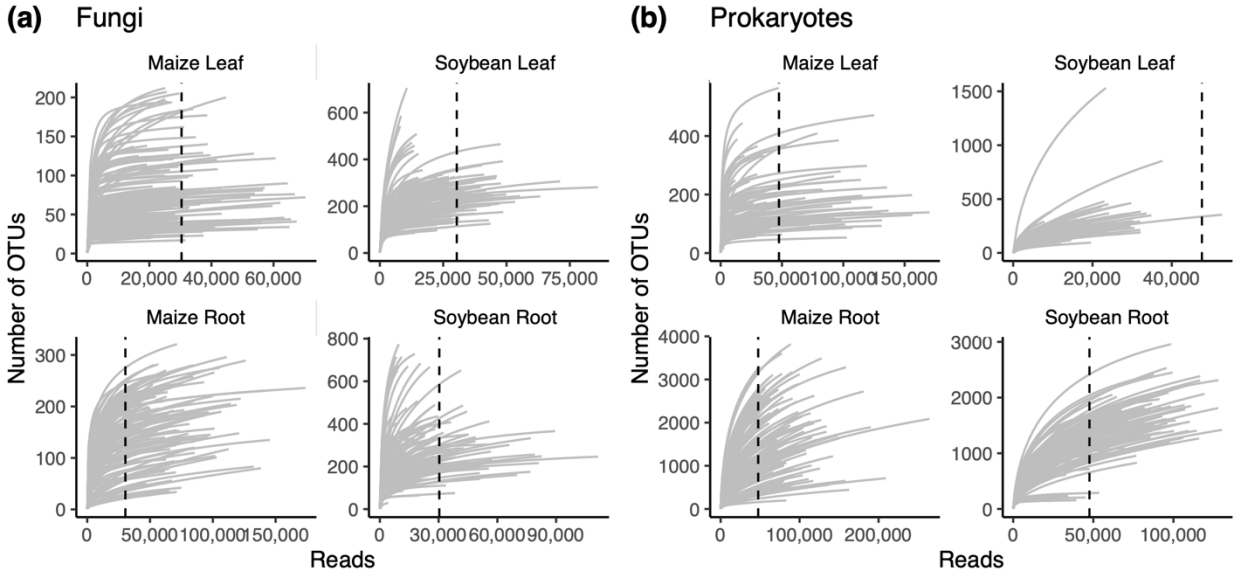
Results

General Sequencing Results

The final fungal OTU table contained 20,844,912 ITS1 reads across 554 samples, including 5,315 fOTUs after filtering 36 contaminant fOTUs detected in negative controls. The median read depth was 30,370 ITS1 reads per sample. Prokaryotes contributed 29,691,681 total reads across 555 samples with a median read depth of 47,590 reads per sample. A total of 14,291

prokaryote OTUs (pOTU, hereafter) were defined after filtering 55 contaminant pOTUs detected in the negative controls. Rarefaction curves verified that the median read depth adequately sampled the diversity present (Figure 3.1).

Figure 3.1 - Rarefaction curves for each sample sequenced in the fungicide study for (**A**) fungi and (**B**) prokaryotes in soybean or maize leaves and roots. The dashed line represents the median sequence depth.



Fungicides alter maize and soybean leaf fungal richness

Following fungicide application, the richness of maize and soybean leaf fungal communities was significantly reduced compared to control plots across managements and crops ($P < 0.05$) except for in no-till maize samples (Figure 3.2). This effect was most pronounced for Dothideomycetes (target) and Tremellomycetes (non-target). However, in other fungal classes such as Sordariomycetes, there was no significant difference in richness between control and fungicide treated samples following fungicide applications. There were no significant differences in fungal richness between fungicide and control plots amongst the assessed fungal classes or in overall richness in the roots of either crop (Figure 3.3). Among prokaryotes, there were no consistent differences between control and fungicide samples in the leaves or roots of either crop

(Figure 3.4).

Figure 3.2 - Fungicidal effects on the richness of different fungal classes. Results shown in (**A)** soybean and (**B)** maize phyllosphere. Black dots are control yellow dots are fungicide samples. Asterisks indicate the level of significance; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

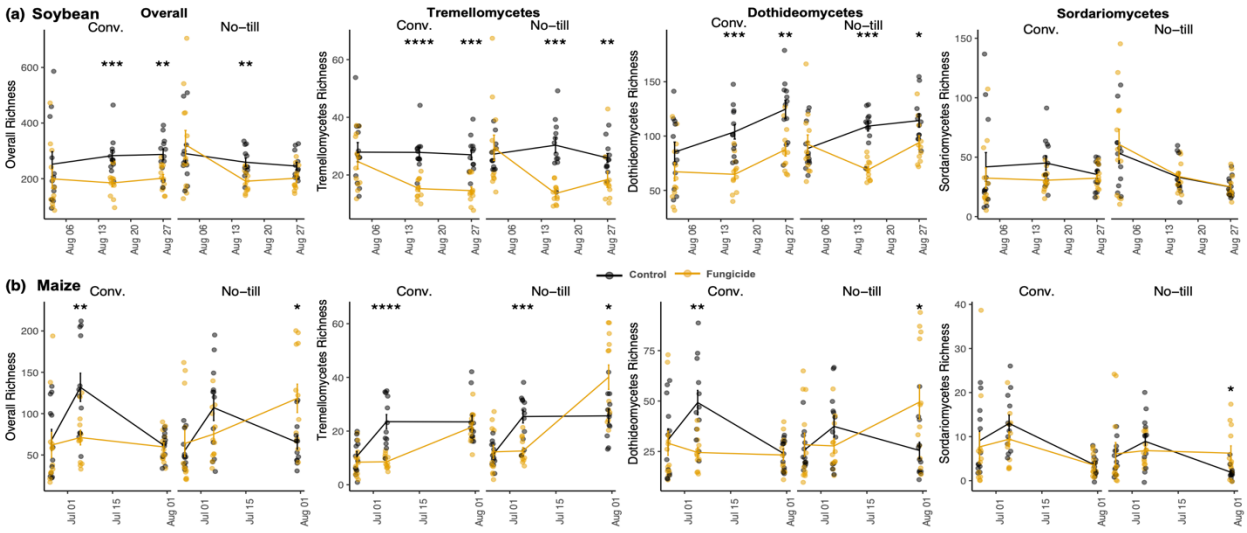


Figure 3.3 - Fungicidal effects on the richness of different fungal classes in the roots. Results shown for (**A**) soybean and (**B**) maize roots. Black dots are control yellow dots are fungicide**.**

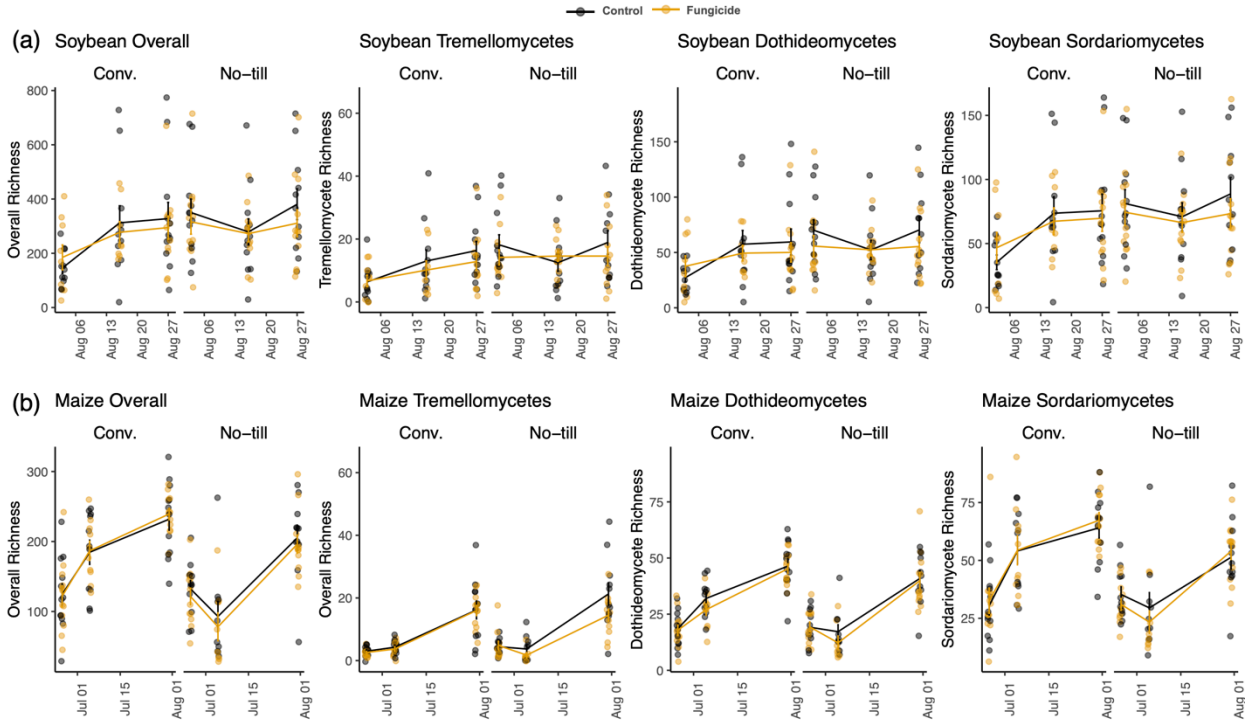
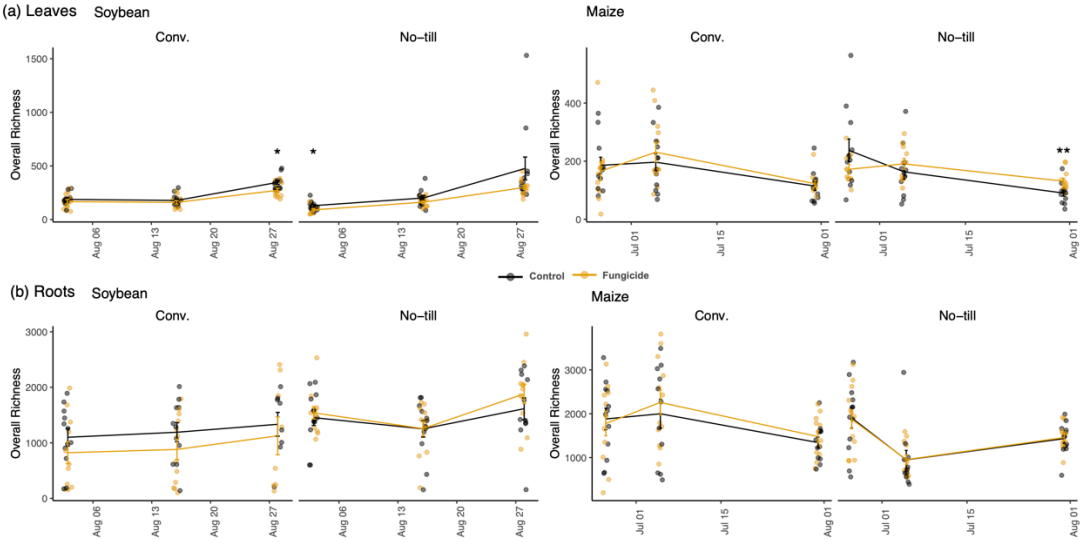


Figure 3.4 - Fungicidal effects on the richness of prokaryotes in soybean and maize. Results shown for (**A**) leaves and (**B**) roots. Black dots are control yellow dots are fungicide. Asterisks indicate the level of significance; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.



Fungicides alter the maize and soybean leaf fungal community composition

Fungal and prokaryote community composition varied significantly due to timepoint (i.e., dpf) and crop management in maize and soybean leaves and roots (Table 3.1; Table 3.2). Notably, before fungicides were sprayed, crop management was shown to have a significant effect on the maize and soybean phyllosphere fungal and prokaryotic communities (maize leaf fungi R2 = 0.050, P = 0.001; maize leaf prokaryotes R2 = 0.038, P = 0.005; soybean leaf fungi R2 = 0.058, P = 0.020; soybean leaf prokaryotes R2 = 0.046, P = 0.049). Furthermore, fungal, and prokaryotic phyllosphere community compositions in control and treatment plots were indistinguishable from each other prior to applying fungicide treatments (maize leaf fungi R2 = 0.032, P = 0.051; maize leaf prokaryotes R2 = 0.023, P = 0.418; soybean leaf fungi R2 = 0.018, P = 0.483; soybean leaf prokaryotes R2 = 0.035, P = 0.128). Despite this, changes to the fungal phyllosphere composition by fungicide treatments differed depending on management (fungicide-management interaction) only in the soybean, but not in the maize leaves (maize leaf fungi 9-dpf R2 = 0.012, P = 0.916; soybean leaf fungi 13-dpf R2 = 0.041, P = 0.017; soybean leaf fungi 33-dpf R2 = 0.039, P = 0.015). There was no substantial evidence that fungicides altered the composition of phyllosphere prokaryote communities, prokaryote root communities, or fungal root communities. Therefore, the variance explained due to the fungicide disturbance was examined for fungal phyllosphere communities before and after fungicide exposure while partitioning out the variation due to crop management by a constrained analysis of principal coordinates (CAP) (Figure 3.5).

Table 3.1 - Permutational multivariate analysis of variance for fungi in maize or soybean in roots or leaves before and after fungicide application. Significant P values (< .05) are shown in bold.

Crop	Tissue	Factor[ab]	Degrees Freedom	Sum of Squares	R^2	Pseudo-F	P
Maize	Leaf	Fungicide	1	0.471	0.013	2.564	**0.005**
		Management	1	0.640	0.018	3.478	**0.002**
		DPF	2	7.628	0.218	20.737	**< 0.001**
		Fungicide x Management	1	0.217	0.006	1.181	0.253
		Fungicide x DPF	2	0.852	0.024	2.317	**0.004**
		Management x DPF	2	0.532	0.015	1.446	0.074
		Fungicide x Management x DPF	2	0.422	0.012	1.148	0.240
		Residual	132	24.276	0.693	-	-
		Total	143	35.038	1.000	-	-
Maize	Root	Fungicide	1	0.345	0.007	1.086	0.295
		Management	1	2.311	0.046	7.283	**< 0.001**
		DPF	2	5.618	0.112	8.852	**< 0.001**
		Fungicide x Management	1	0.381	0.008	1.200	0.235
		Fungicide x DPF	2	0.538	0.011	0.848	0.734
		Management x DPF	2	1.911	0.038	3.010	**< 0.001**
		Fungicide x Management x DPF	2	0.704	0.014	1.109	0.274
		Residual	121	38.401	0.765	-	-
		Total	132	50.208	1.000	-	-
Soybean	Leaf	Fungicide	1	0.816	0.034	6.704	**< 0.001**
		Management	1	0.465	0.020	3.816	**< 0.001**
		DPF	2	4.648	0.196	19.086	**< 0.001**
		Fungicide x Management	1	0.233	0.010	1.910	**0.038**
		Fungicide x DPF	2	0.697	0.029	2.864	**0.002**
		Management x DPF	2	0.470	0.020	1.928	**0.013**
		Fungicide x Management x DPF	2	0.392	0.017	1.608	**0.046**
		Residual	131	15.950	0.674	-	-

Table 3.1 (cont'd)

		Total	142	23.670	1.000	-	-
Soybean	Root	Fungicide	1	0.274	0.007	0.949	0.429
		Management	1	0.890	0.022	3.086	**< 0.001**
		DPF	2	2.330	0.057	4.040	**< 0.001**
		Fungicide x Management	1	0.426	0.010	1.478	0.112
		Fungicide x DPF	2	0.515	0.013	0.893	0.573
		Management x DPF	2	0.563	0.014	0.977	0.439
		Fungicide x Management x DPF	2	0.479	0.012	0.831	0.693
		Residual	122	35.186	0.865	-	-
		Total	133	40.663	1.000	-	-
[a]The management factor refers to no-till or conventional management							
[b]DPF = days post fungicide							

Table 3.2 - Permutational multivariate analysis of variance for prokaryotes in maize or soybean in roots or leaves before and after fungicide application. Significant P values (< .05) are shown in bold.

Crop	Tissue	Factor[ab]	Degrees Freedom	Sum of Squares	R^2	Pseudo-F	P
Maize	Leaf	Fungicide	1	0.278	0.006	0.993	0.384
		Management	1	1.075	0.023	3.834	**0.002**
		DPF	2	7.492	0.160	13.361	**< 0.001**
		Fungicide x Management	1	0.280	0.006	0.997	0.389
		Fungicide x DPF	2	0.523	0.011	0.933	0.511
		Management x DPF	2	0.900	0.019	1.605	**0.043**
		Fungicide x Management x DPF	2	0.639	0.014	1.139	0.240
		Residual	127	35.608	0.761	-	-
		Total	138	46.796	1.000	-	-
Maize	Root	Fungicide	1	0.191	0.005	0.985	0.408
		Management	1	1.568	0.038	8.101	**< 0.001**
		DPF	2	12.291	0.299	31.743	**< 0.001**
		Fungicide x Management	1	0.083	0.002	0.427	0.914
		Fungicide x DPF	2	0.449	0.011	1.161	0.287
		Management x DPF	2	1.225	0.030	3.165	**0.002**
		Fungicide x Management x DPF	2	0.307	0.007	0.793	0.628
		Residual	129	24.976	0.608	-	-
		Total	140	41.091	1.000	-	-
Soybean	Leaf	Fungicide	1	0.138	0.007	1.189	0.281
		Management	1	0.380	0.019	3.276	**0.006**
		DPF	2	3.777	0.185	16.292	**< 0.001**
		Fungicide x Management	1	0.122	0.006	1.048	0.352
		Fungicide x DPF	2	0.234	0.011	1.010	0.446

Table 3.2 (cont'd)

		Management x DPF	2	0.258	0.013	1.112	0.331
		Fungicide x Management x DPF	2	0.298	0.015	1.287	0.181
		Residual	131	15.185	0.745	-	-
		Total	142	20.391	1.000	-	-
Soybean	Root	Fungicide	1	0.197	0.007	0.998	0.370
		Management	1	1.316	0.047	6.672	**< 0.001**
		DPF	2	1.106	0.040	2.805	**0.002**
		Fungicide x Management	1	0.458	0.017	2.321	**0.042**
		Fungicide x DPF	2	0.228	0.008	0.577	0.884
		Management x DPF	2	0.467	0.017	1.185	0.274
		Fungicide x Management x DPF	2	0.298	0.011	0.755	0.689
		Residual	120	23.659	0.853	-	-
		Total	131	27.728	1.000	-	-
[a]The management factor refers to no-till or conventional management							
[b]DPF = days post fungicide							

For soybean leaves, no significant differences were observed prior to fungicide application (P = 0.51), but fungicide treatment had a significant effect on fungal leaf composition after fungicides were applied (13-dpf 12% variation P < 0.001; 33-dpf 11% variation P < 0.001) (Figure 3.5a–c). Similarly, the effect of fungicide disturbance on maize leaf fungal composition was not observed before fungicides were applied (P = 0.075) (Figure 3.5d). However, unlike soybean, there was no evidence the fungicide altered fungal composition longer than nine days (9-dpf 8% variation P < 0.001; 34-dpf 3% variation P = 0.078) (Figure 3.5e, f). The non-significant beta dispersion tests across tillage management at 9-dpf or 34-dpf for maize (9-dpf conventional P = 0.369, no-till P = 0.631; 34-dpf conventional P = 0.364, no-till P = 0.662) and

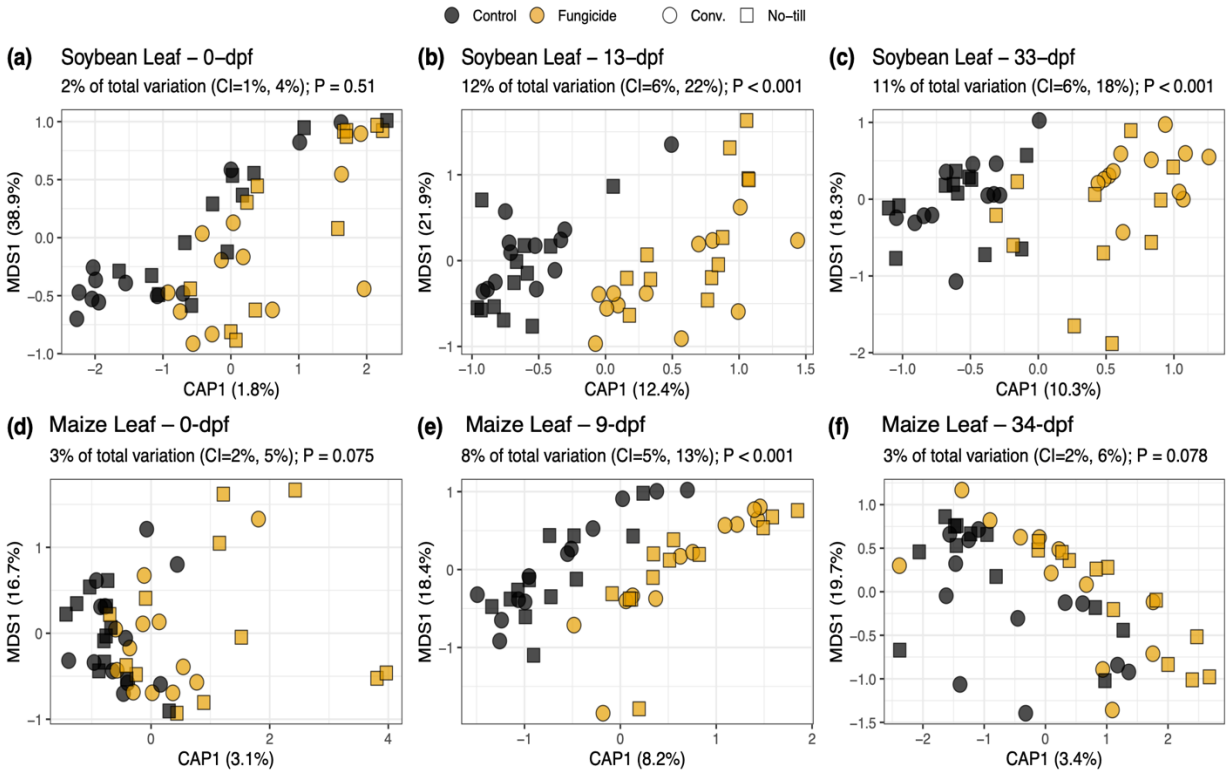
13- and 33-dpf (13-dpf conventional P = 0.742, conventional P = 0.866; 33-dpf conventional P = 0.335, no-till P = 0.123) for soybean, indicate that the effects of fungicide on fungal leaf composition are likely due to true differences in community composition rather than group dispersions (Table 3.3).

Table 3.3 - Effects of fungicide on maize and soybean leaf fungal composition. Significant P values (< .05) are shown in bold

				PERMANOVA			Beta-dispersion	
Crop	Management	Growth Stage	[a]DPF	R^2	Pseudo-F	P-value	Pseudo-F	P-value
Maize	Conventional	V6	0-dpf	0.032	0.724	0.811	0.162	0.679
		V8	9-dpf	0.087	2.108	**0.018**	0.855	0.369
		V15	34-dpf	0.028	0.638	0.821	0.862	0.364
	No-till	V6	0-dpf	0.082	1.959	**0.015**	17.156	**0.002**
		V8	9-dpf	0.104	2.543	**0.003**	0.241	0.631
		V15	34-dpf	0.109	2.697	**0.006**	0.213	0.662
Soybean	Conventional	R3	0-dpf	0.045	1.044	0.357	0.141	0.726
		R4	13-dpf	0.140	3.568	**< 0.001**	0.101	0.742
		R6	33-dpf	0.183	4.912	**< 0.001**	0.979	0.335
	No-till	R3	0-dpf	0.036	0.816	0.494	1.016	0.316
		R4	13-dpf	0.199	5.470	**< 0.001**	0.042	0.866
		R6	33-dpf	0.121	2.900	**0.001**	2.781	0.123

[a]DPF = days post fungicide

Figure 3.5 - Effects of fungicides on fungal leaf composition in maize and soybean. A separate analysis was conducted for soybean (**A**)0- (**B**) 13-or (**C**) 33-days post fungicide (dpf) application or maize (**D**)0-(**E**)9-or(**F**) 34-dpf since there was a significant interaction between dpf and fungicide. Constrained analysis of principal coordinates (CAP) analyses was constrained by the effect of fungicide while partialling out the effect of management. The percentage of total variation due to fungicide is expressed above the plot. The significance was determined based on 1000 permutations.



Fungicidal effects on network properties depend on crop management

In soybean under conventional and no-till management, network complexity was not significantly different before fungicide application (conventional P = 0.13; no-till P = 0.93) but was significantly lower than control plots 13-dpf (conventional P < 0.001; no-till P = 0.01) (Figure 3.6a). However, after one month, the soybean no-till network complexity had recovered (P = 0.12), whereas the conventional treatment was still significantly lower compared to the non-sprayed control plots (P = 0.002) (Figure 3.6a). The loss in network complexity can partially be explained by a reduction in the number of nodes (i.e., OTUs) since the average number of nodes

per network also followed this same trend (Figure 3.6b). Fungicide disturbance was followed by the loss of network complexity mainly through node loss but crops and crop management impacted network properties under fungicide disturbance. These same effects were not observed in maize (Figure 3.7). To investigate these trends more closely, we investigated the specific fungal taxa affected through differential abundance analysis.

Figure 3.6 - Fungicides alter soybean network complexity. A microbial co-occurrence network was constructed using taxa with a mean relative abundance greater than 1−5 and present in greater than 5 % of samples. Subnetworks were generated for each sample based on the OTUs present within those samples, and each point represents a subnetwork. (**A**) Network complexity (i.e., linkage density) and (**B**) number of edges were then calculated for each subnetwork. Comparisons are based on Wilcox ranked sign tests for soybean conventional management and no-till. Asterisks indicate the level of significance; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Comparisons for maize are shown in Figure 3.7

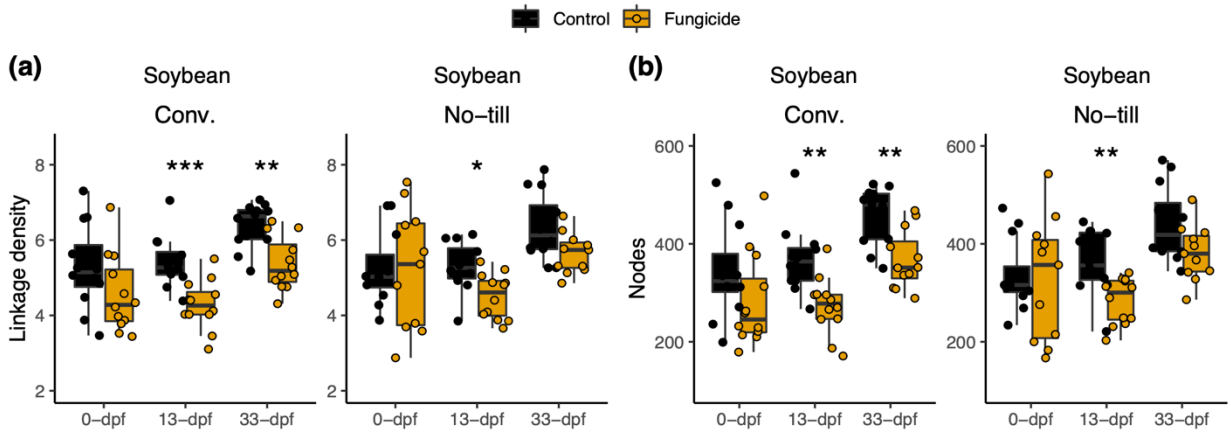
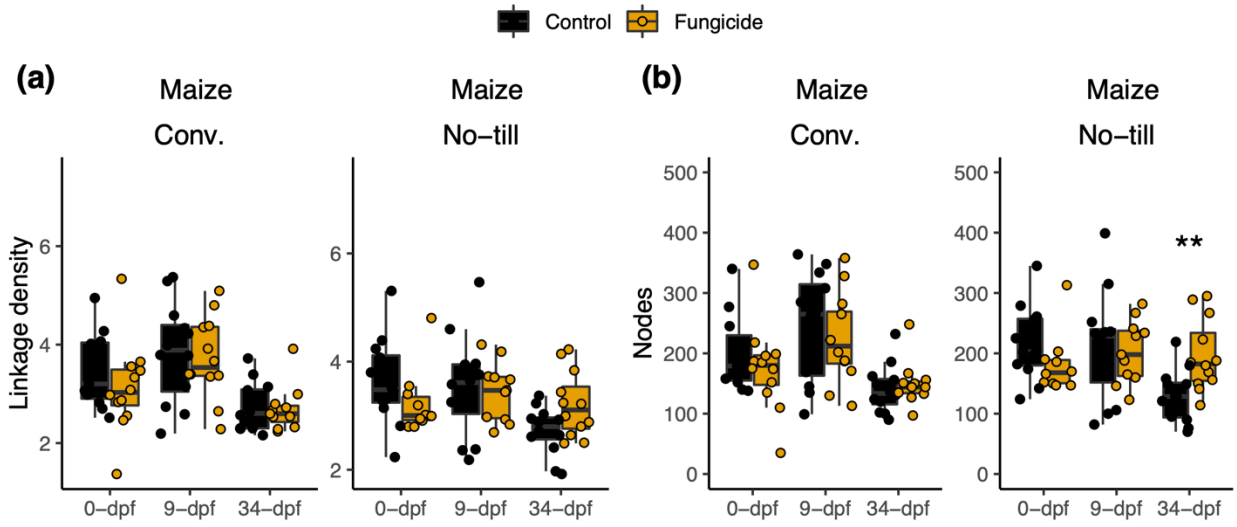


Figure 3.7 - Fungicides do not alter maize network complexity. A microbial co-occurrence network was constructed using taxa with a mean relative abundance greater than 10-5 and present in greater than 5 % of samples. Subnetworks were generated for each sample based on the OTUs present within those samples, and each point represents a subnetwork. (**A**) Network complexity (i.e., linkage density) and (**B**) number of edges were then calculated for each subnetwork. Comparisons are based on Wilcox ranked sign tests for maize conventional management and no-till. Asterisks indicate the level of significance; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

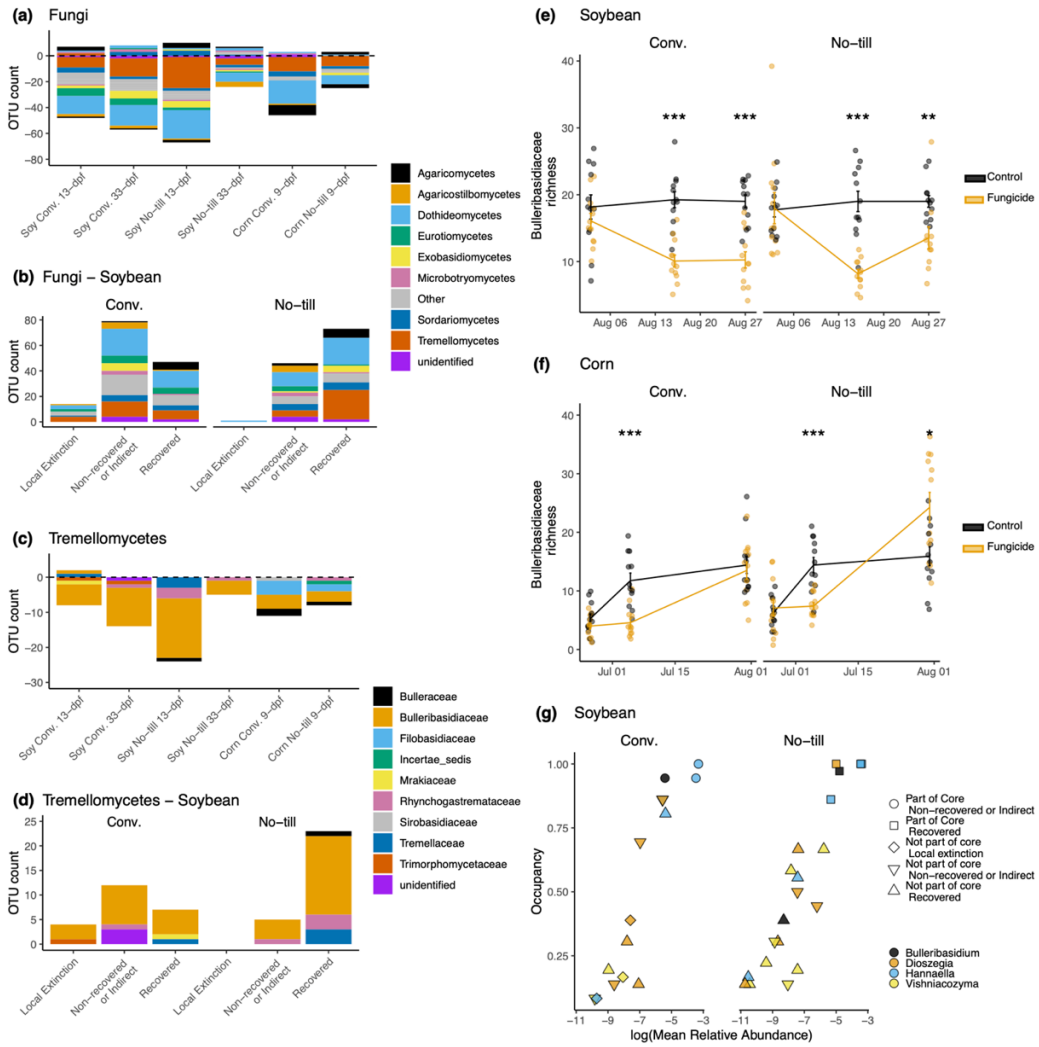


Identification of fungicide-affected fOTUs

To determine which fungal taxa were significantly affected by fungicide application, a differential abundance analysis was conducted with ANCOM (Table B.1). In total, the abundance of 238 unique fOTUs representing 21 fungal classes was altered by fungicide treatments across the two crops. Ascomycota (52.9%) and Basidiomycota (43.3%) fOTUs made up 96.2% of the differentially abundant fOTUs. Within Ascomycota, the Dothideomycetes (28.6%) and Sordariomycetes (9.66%) accounted for the largest percentage of fOTUs that were differentially abundant following fungicide treatment (Figure 3.8a). These fungi may be expected since many foliar plant pathogens fall within these classes of fungi, and fungicides typically target these pathogen groups. Unexpectedly, a large percentage of fOTUs that were differentially abundant included non-target dimorphic clades of fungi that commonly exist as

yeasts such as Agaricostilbomycetes, Cystobasidiomycetes, Exobasidiomycetes, Microbotryomycetes, Spiculogleomycetes, Taphrinomycetes, and Tremellomycetes. A total of 83 fOTUs across these classes were significantly different in abundance following the fungicide application in maize or soybean (Figure 3.8a). Notably, Tremellomycetes made up the second largest class (42 fOTUs, 17.6%) of differentially abundant fungi. Of the Tremellomycetes, 57.1% were concentrated within the Bulleribasidiaceae, accounting for 24 fOTUs that were differentially abundant compared to non-sprayed control. Twenty-three of the Bulleribasidiaceae significantly decreased in abundance. However, not all yeast fOTUs decreased in abundance. For example, *Bulleromyces albus* fOTU10 increased in relative abundance 4.25 times in soybean conventional management 13-dpf but was not significantly different than the control after 33-dpf. In contrast, two *Sporobolomyces* fOTUs (fOTU66 and fOTU94) increased in relative abundance following fungicide application in soybean and remained significantly (7 times) higher in fungicide treated plots than in control plots 33-dpf. *Sporobolomyces patagonicus* fOTU94 was 4.38 times more abundant in the fungicide treated plots than the control 13-dpf in the conventional management and remained significantly higher in fungicide sprayed plots (9.06 times) compared to the control after 33-dpf. *Sporobolomyces roseus* fOTU66 was 15 times more abundant in the conventionally managed fungicide treated plots 33-dpf. This same increase in Sporobolomyces abundance was not observed in maize.

Figure 3.8 - Management scheme alters the recovery dynamics of phyllosphere fungi following fungicide treatment. The composition of fungal operational taxonomic units (OTUs) that were significantly different in abundance, as indicated with analysis of compositions of microbiomes (ANCOM) analysis (n = 12). (**A**) Composition of fOTUs whose abundance was significantly different following a fungicide disturbance. Bars below zero indicate the fOTU decreased in abundance, whereas bars above zero indicate the fOTU increased in abundance. (**B**) Recovery of fungi in soybean leaf samples in conventional (conv.) or no-till management. All fungi recovered in maize leaf samples. (**C**) Composition of fOTUs within the Tremellomycetes whose abundance was significantly altered following a fungicide disturbance. (**D**) Recovery dynamics of Tremellomycetes fOTUs following a fungicide disturbance in conv. or no-till. All Tremellomycete fungi recovered in maize. (**E**) Soybean or (**F**) maize plots subjected to a fungicide treatment compared to non-sprayed control plots. (G) Abundance occupancy relationship with the recovery dynamics of the Bulleribasidiaceae fOTUs significantly affected by the fungicide treatment. All Bulleribasidiaceae fOTUs recovered in maize. A full list of core fungi and prokaryotes for soybean or maize leaves are found in Table B.2. Asterisks indicate the level of significance; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.



Resilience of the Core Mycobiome and Local Extinctions of Accessory Members

Many of the fOTUs affected by a fungicide application were also part of the core phyllosphere taxa (Table B.2). In conventionally managed soybean plots, 22 fOTUs were determined to be core fungal phyllosphere taxa, and the abundances of five of these core members (fOTU 6 *Mycosphaerella* sp., fOTU 10 Tremellales, fOTU 34 *Hannaella* sp., fOTU 13 *Hannaella* sp., and fOTU 643 *Tilletiopsis* sp.) were significantly different following fungicide application. *Hannaella* sp. (fOTU 34), *Hannaella* sp. (fOTU 13), and *Tilletiopsis* sp. (fOTU 643) were also part of the 43 core members of the no-till soybean phyllosphere affected by fungicide application. Of the 40 core members of the conventionally managed maize phyllosphere, the abundance of four Tremellomycetes fOTUs and one unidentified fungal taxon (fOTU 116) were significantly different following fungicide application. These included three yeast taxa that were not members of the soybean core, which included two fOTUs in the genus *Filobasidium* (fOTU 82 *Filobasidium oeirense*, and fOTU 97 *Filobasidium* sp.), one *Bullera crocea* (fOTU 65), and *Vishniacozyma globispora* (fOTU 83). Two of these fOTUs (fOTU 97 *Filobasidium* and fOTU 65 *Bullera crocea*) were also core members of the maize phyllosphere in the no-till management that were significantly altered by the fungicide.

None of the core members of the phyllosphere taxa became locally extinct following fungicide application in the core microbiome of either crop or tillage management. However, among the taxa whose abundance was significantly altered by the fungicide application in soybean, the no-till management had a 61 % recovery compared to the 34 % recovery in the conventional tilled soybean (Figure 3.8b; Table B.3). Fourteen fungal OTUs became locally extinct following fungicide application in soybean with conventional tillage compared to one in the no-till plots (Figure 3.8b). Among the Tremellomycetes fOTUS whose abundances were

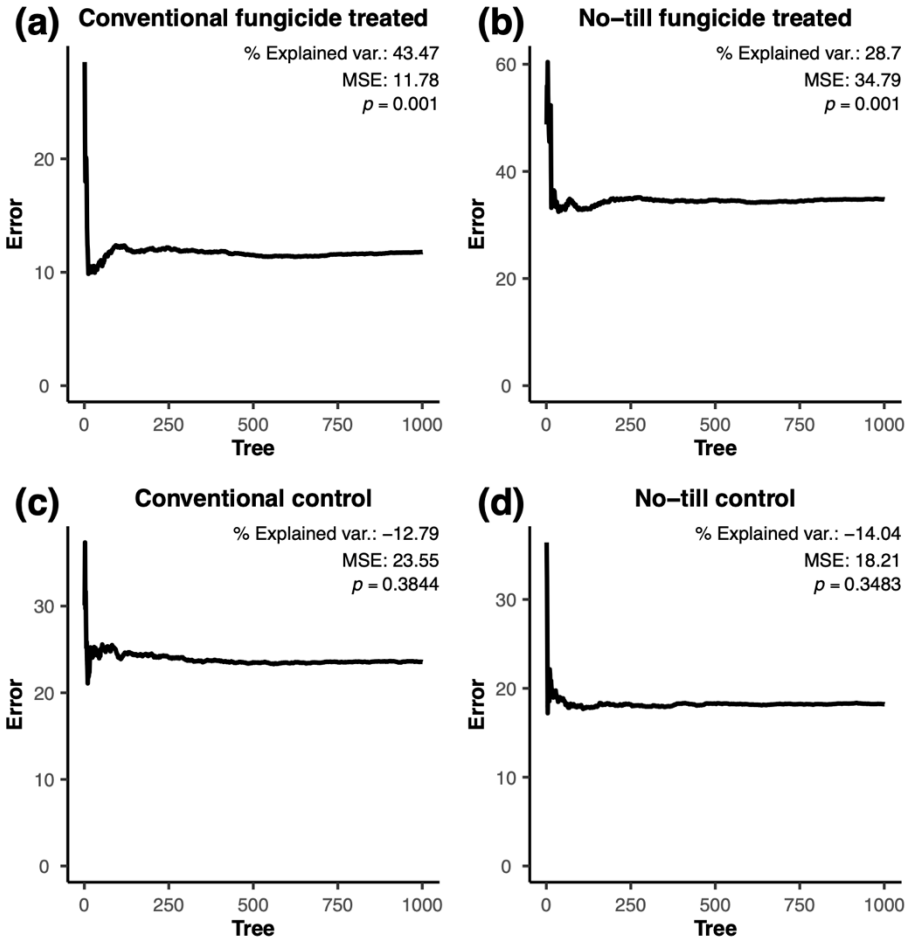
significantly impacted by fungicide applications, the majority were Bulleribasidiaceae (Figure 3.8c). Eighty-two percent of affected Bulleribasidiaceae recovered in no-till managed soybean compared to 30% of the conventionally tilled plots. No Bulleribasidiaceae taxa became locally extinct in the no-till plots; in contrast, three Bulleribasidiaceae fOTUs were never observed again following fungicide application in the conventional tilled management (Figure 3.8d). The trend of increased recovery was also evident in Bulleribasidiaceae richness on the last sampling for maize (33-dpf) and soybean (34-dpf) no-till samples (Figure 3.8e, f). Bulleribasidiaceae in the core of conventional tillage did not fully recover within the study period (Figure 3.8g). In addition, the Bulleribasidiaceae in the conventional tillage management that were locally extinct following fungicide disturbance occupied less than 50% samples in non-sprayed plots indicating that local extinctions caused by fungicides affect the rare, non-core members of the community (Figure 3.8g). No local extinctions among fungal taxa were detected in maize fungicide treated plots; all impacted taxa recovered.

Indirect Effects of Fungicides on Prokaryotes Mediated Through Yeast

Random forest models based on prokaryotic abundance on soybean leaves sprayed with fungicides explained a significant amount of variance ($P < 0.001$) in Bulleribasidiaceae richness in the no-till (28.70%; $R2 = 0.25$) and conventional tillage (43.47%; $R2 = 0.44$) management (Figure 3.10a, c). We then extracted the set of OTUs that were most important for maintaining the model's accuracy in fungicide treated plots. However, there was no evidence ($P \geq 0.05$) those same taxa were able to predict Bulleribasidiaceae richness in control samples indicating the unique effect of the fungicide (Figure 3.9). OTUs classified as *Sphingomonas*, *Methylobacterium*, and *Hymenobacter* were the most important for predicting fungal richness in the no-till management (Figure 3.10c, d). Many taxa from the same genera were important in

predicting Bulleribasidiaceae richness in the conventional tillage management, including the *Sphingomonas* and *Hymenobacter* genera (Figure 3.10a, b). However, other genera were unique by management type, including *Methylobacterium* for the no-till management and *Pseudokineococcus* and *Kineococcus* in the conventional tillage management.

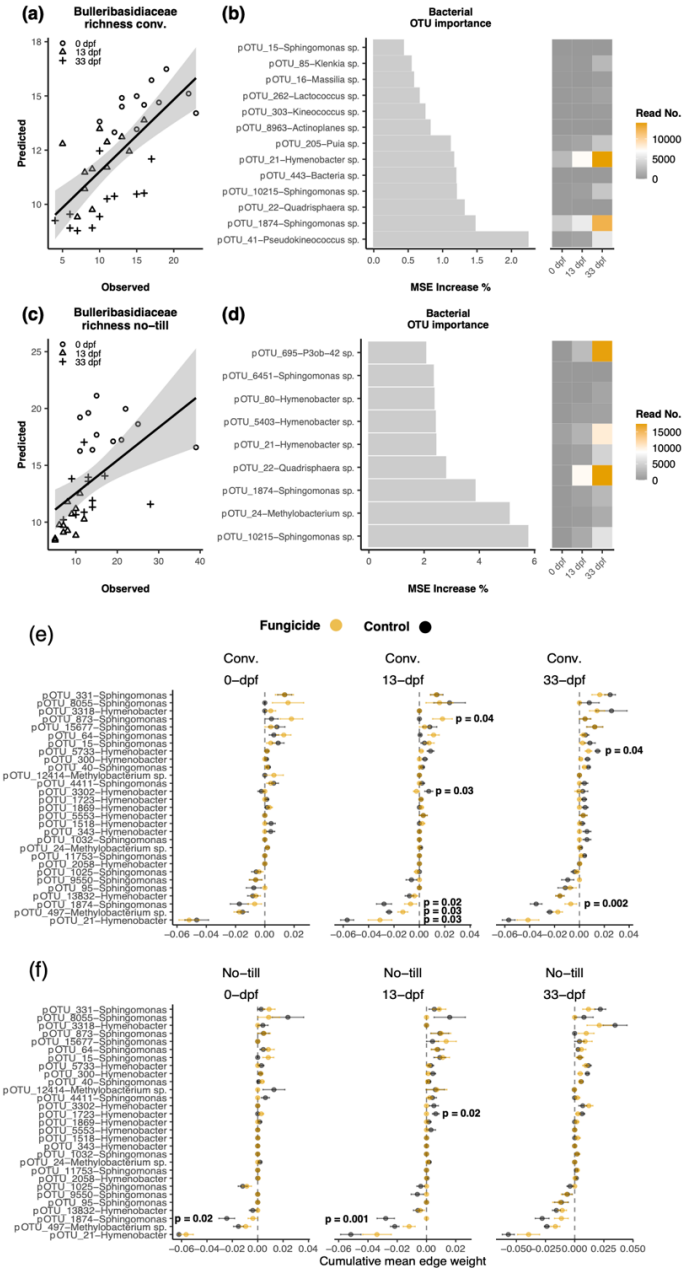
Figure 3.9 - Random Forest models percent explained variance, error, and overall model significance (permutations = 999) for (**A**) conventional management treated with fungicides, (**B**) no-till treated with fungicides, (**C**) conventional management control, and (**D**) no-till control.



Prokaryote OTUs that were important for random forest model accuracy increased in abundance in fungicide treated plots as a response to altering Bulleribasidiaceae diversity and were negatively co-associated with Bulleribasidiaceae (Figure 3.10b, d, e, f). For example, pOTU21 *Hymenobacter* and pOTU1874 *Sphingomonas* abundance increased as

Bulleribasidiaceae richness decreased and was negatively co-associated with the Bulleribasidiaceae (Figure 3.10b, e, f). In addition, the cumulative edge weight between pOTU21 *Hymenobacter*, pOTU1874 *Sphingomonas*, and Bulleribasidiaceae significantly changed when sprayed with fungicides in the conventional tillage management, but not always in the no-till management, indicating that an alteration in Bulleribasidiaceae diversity can indirectly influence the co-occurrence between fungi and bacteria in different crop management schemes. However, not all co-occurrences between the Bulleribasidiaceae and prokaryotes were negative, indicating that positive co-occurrences between prokaryotes and fungi in the phyllosphere may shift as well (Figure 3.10e, f).

Figure 3.10 - Indirect effects of fungicides on prokaryotic communities from altered Bulleribasidiaceae diversity on soybean leaves. Relationship of observed versus predicted Bulleribasidiaceae richness in conventional (**A**) or no-till (**C**) from random forest models using prokaryote OTU abundance in fungicide treated plots. The most important ($P < 0.05$) prokaryote OTUs for random forest model accuracy in fungicide treated conventional (**B**) or no-till (**D**). The cumulative mean edge weight calculated from each sub-network of a meta-network of Bulleribasidiaceae edges between *Sphinogomonas*, *Hymenobacter*, or *Methylobacterium* OTUs and alterations to co-occurrence strength with and without fungicides under (**E**) conventional and (**F**) no-till crop management. Parallel analysis was not conducted with maize due to the lack of evidence to alteration of network structure and complexity (Figure 3.7).



Discussion

To our knowledge, this is the first study to assess the effect of fungicide-imposed disturbance and resiliency under different agricultural management systems. We found that fungicide applications had a substantial effect on target and non-target fungal phyllosphere communities, minor indirect effect on prokaryotic communities in the phyllosphere, and no direct effects on fungal or prokaryotic communities of roots. Soil fungi and prokaryotes were also identified in soybeans, where there was no evidence of fungicidal effects (data not shown). Leveraging the KBS LTER site allowed the direct comparison of long-term crop management impacts to the microbiome without confounding location. Our data demonstrate that the resilience of phyllosphere microbiome depends on the cropping management system, with a greater recovery in the abundance of affected phyllosphere microbiota in long-term no-till compared to annually conventional tilled management. Among the most important results was the commonality in the fungal taxa affected by fungicide treatments. In maize and soybean, fungi in Dothideomycetes (target) and Tremellomycetes (non-target) decreased in abundance following fungicide applications, raising questions on the role of Tremellomycete yeasts; specifically, the Bulleribasidiaceae in phyllosphere microbiomes, and the effects of fungicide use in the absence of disease pressure.

This study observed reductions and local extinctions of yeasts following fungicide application, which may lead to unintended consequences for the host plant. Phyllosphere yeast communities have received less attention in the literature than prokaryote communities [288]. The three Bulleribasidiaceae genera observed in this study were Hannaella, Dioszengia, and Vishniacozyma. Dioszengia, and Hannaella have been demonstrated to produce the plant growth-promoting hormone indole acetic acid (IAA), similar to many plant growth-promoting

phyllosphere prokaryotes [111, 289]. In comparison, Vishniacozyma yeasts have remained understudied but have been isolated from maize kernels [290]. In addition, Dioszegia has been identified as a hub taxon important in maintaining fungal-prokaryote interactions by altering prokaryote diversity in the phyllosphere microbiome of Arabidopsis [9, 111]. As observed in this study, in the absence of disease pressure, fungicide applications may affect populations of beneficial microbes. However, adverse impacts would be expected to be outweighed if the fungicide mitigates the disease, which will be tested in future experiments.

Here, we show for the first time that fungicidal impacts on crop microbiomes are dependent on management, addressing a knowledge gap that previous studies were unable to address specifically [109, 110, 262]. A higher proportion of fOTUs altered by fungicide application in the no-till management system showed improved resilience within the study period, which may be explained by the differences in microbial communities present in the phyllosphere of each management before fungicide applications, as has been demonstrated previously at the KBS LTER site [4, 47]. A previous study from the KBS LTER site demonstrated that aerially dispersed yeasts are enriched in the phyllosphere, but also found in lower abundance in belowground plant organs [291]. Crop residue from previous seasons can harbor fungi that may act as a source to repopulate the phyllosphere following a disturbance like the phenomenon of pathogens transferring from residues [292]. Yeasts that inhabit the phyllosphere are primarily known to disperse through ballistosporic aerial dispersal, and the reassembly of leaves following fungicides may rely heavily on this spore dispersal mechanism. However, not all yeast taxa in the Bulleribasidiaceae have been observed to form ballistocondia in culture [293], leaving arguably less efficient means of dispersal from insects or through wind and rain [294, 295]. Locally extinct taxa were not part of the core microbiome regardless of

tillage management system or spore dispersal mechanism, demonstrating a tight relationship between abundance-occupancy and disturbance. These results indicate that microbiome resilience is improved in no-till crop management, which informs discussion of managing crops for resilience, and demonstrates a potential ecosystem service provided by no-till agriculture in addition to improved nutrient cycling or preservation of habitats for microorganisms and mesofauna [296].

Fungicide applications affected soybean and maize phyllosphere communities differently. These differences may be due to crop, planting year, or fungicide regime. The effect of fungicide was likely reduced in the final sampling of maize due to sampling of new leaves that were not directly sprayed with fungicides, indicating that any effect would have been through systemic activity of the fungicide 34 days after spray. This is unlikely since pyraclostrobin is not easily xylem mobile and mainly works as a translaminar local penetrant [277]. Another critical difference is that the Delaro® fungicide applied to soybeans in 2018 has two modes of action. Application of fungicides having two different modes of action has been shown to have a more significant effect on fungal community composition than a single mode of action in cereal crops [262]. Although the impact of fungicides varied in magnitude between the two crops, the commonality of off-target impacted taxa between crops and fungicides demonstrates that multiple fungicide products on different crops consistently reduce these taxa. This information can be used to inform decisions on the use of fungicides under low pathogen pressure across crops and cropping systems.

Recovery of network complexity is one measure of microbiome resilience. We show that network complexity decreased significantly in the soybean phyllosphere following fungicide treatment. Despite similar affected taxa, the effect of fungicides on maize was moderate

compared to soybean, which saw a reduction in network complexity and local extinctions of some taxa. Therefore, we focused more on fungicidal effects to soybean rather than maize. Other studies have demonstrated that agricultural management alters network complexity. However, the functional consequences of these changes were not directly assessed [10, 297]. In soils, it has been demonstrated that increases in network complexity are positively correlated with various ecosystem functions and increases in the number of unique functions and functional redundancy [274]. The functional consequences of decreases in network complexity remain unexplored in the phyllosphere microbiome. They may provide the rationale for chemical application decisions or novel microbial-based treatments to replace lost taxa.

Notably, fungicide application altered co-occurrences between phyllosphere fungi and prokaryotes, demonstrating the indirect effects of fungicide applications through the loss in the diversity of Bulleribasidiaceae. In support of random forest results, many of the same prokaryotes identified from networks as having changes in cumulative mean edge weight were identified by random forest as predicting Bulleribasidiaceae richness. Disturbance can change cooperation/competition dynamics, and a high level of disturbance can reduce cooperation [298, 299]. In our study, the cumulative mean edge weight between most phyllosphere prokaryotes and Bulleribasidiaceae became more positive, indicating fewer negative associations between particular bacteria and the Bulleribasidiaceae. However, there were exceptions where cumulative edge weights, positive before spray, became neutral following fungicide application likely due to the disappearance of some fungal taxa from samples, and therefore the disappearance of any associations. Loss of negative correlations may also be due to reduced competition between phyllosphere prokaryotes and Bulleribasidiaceae as more niche space is available to phyllosphere prokaryotes following fungicide application.

Shifts in correlations between Bulleribasidiaceae and phyllosphere prokaryotes are of interest due to the unique physiology of many phyllosphere prokaryotes as it relates to plant health. *Methylobacterium* spp. have been demonstrated to be abundant in plants' phyllosphere and have the genes to produce plant growth-promoting auxins and UVA-absorbing compounds [300, 301]. *Hymenobacter* sp., *Methylobacterium* sp., and *Sphingomonas* sp. are core phyllosphere members in switchgrass [180] and are highly abundant in the *Arabidopsis* phyllosphere [302].

A comprehensive view of the phyllosphere organisms is needed to understand microbiome functioning and plant health. This research demonstrates that foliar fungicide treatments alter phyllosphere microbiomes in maize and soybean, and non-target Bulleribasidiaceae yeasts were negatively impacted in both crops. Microbiome complexity was altered partially by decreasing co-occurrence between Bulleribasidiaceae yeasts and dominant phyllosphere prokaryote taxa, demonstrating indirect effects of fungicide applications mediated through the presence of these yeasts. Further, these data support our hypothesis that the recovery of the phyllosphere microbiome differed by tilling management. Together, these results improve our understanding of fungicide impacts on crop microbiomes and their recovery in different managements and inform their rational use to maintain efficacy and intended impacts across different cropping systems.

Chapter 4

Inoculation with Hub Taxa from the Soybean Microbiome Impacts Host Response to Low Moisture Stress

Abstract

Drought is an increasing threat to soybean (*Glycine max*) production worldwide. In addition to methods such as breeding and genetic modification, inoculation of plants or soils with single microbes and microbial consortia are being explored to mitigate the impacts of drought stress on crop plants. One criterion for selecting microbes within a consortium is to select hub taxa, which are those whose abundances appear to be highly correlated with abundances of other taxa in the microbiome. To assess the impact of microbial inoculations with hub taxa on soybean, I identified five hub taxa from soybean roots which were then used to inoculate soybean grown in 75% sand and 25% no-till soil from the KBS LTER. No-till soil was used to match the environment from which hub taxa were identified and isolated. Inoculated and control plants were then exposed to low-moisture stress as seedlings. Selected hub taxa included two fungi (*Humicola* sp., and *Gibellulopsis* sp.) and three bacteria (*Streptomyces* sp., *Massilia* sp., and *Caulobacter segnis*). The impact of inoculation on plant hosts was assessed with phenotype measures (aboveground and belowground) and plant functional measures (transcriptomics and non-target metabolomics of roots). The impact on the microbiome was assessed using amplicon sequencing of DNA as well as cDNA created from RNA extracted from roots. Inoculation had a minor impact on the microbiome that was most apparent when a more active fraction of the microbiome was analyzed using cDNA. cDNA amplicon sequencing demonstrated that *Bradyrhizobium* ZOTUs generally had higher cDNA:DNA ratios in inoculated samples compared to control samples, especially in earlier sampling points. Transcriptomics of soybean roots demonstrated that genes related to nodulation were upregulated in inoculated samples, and non-targeted metabolomics showed differences between the composition of metabolites in inoculated and control plants. Molecular and metabolomics data agreed with phenotypic data

which showed increases in nodulation as well as increased aboveground growth. Experiments using field soils and enriching individual hub taxa revealed that this effect of increased resilience to drought and increased aboveground biomass was reproducible with both *Caulobacter segnis* and *Streptomyces* treatments. Overall, these results support the hypothesis that hub taxa can be used as inocula to improve the performance and resilience of the host plants.

Introduction

Abiotic stresses including flooding, drought, temperature, and nutrition stresses threaten crop production globally. These impacts are likely to become more severe with a changing climate. Drought is among the abiotic stresses that most threatens the yield of crops worldwide, and the frequency of drought is expected to increase [303] . For example, in a global study of crop yields under drought stress, it was determined that drought decreased the yield of maize by 39% and wheat by 21% [304]. Previous work has predicted that soybeans in the United States will be more vulnerable to drought compared to other locations worldwide and that the risk of yield loss due to drought will increase in the future [305]. Severe drought can be particularly damaging to soybean yields due to a reduction in nodule numbers and nitrogen fixation activity [306, 307]. This reduction in nitrogen fixation with severe drought may be caused by a reduced supply of photosynthate from host plants, or possibly due to a loss of oxygen-carrying leghemoglobin [308–310]. In addition to changes in nitrogen fixation activity, there are various phenotypic changes to soybean host plants associated with drought. These changes include increases in the root to shoot ratio due to increased root growth and a reduction in aboveground biomass [308]. Soybean cultivars have been demonstrated to vary in root phenotypic traits including root length and nodulation in response to drought stress. This variation may allow for planting of cultivars with increased drought tolerance when drought conditions are anticipated.

Various approaches can be deployed to enhance the resilience of plants to drought stress. These include molecular breeding, genetic engineering, and the use of remote sensing to monitor and respond to crop stress, such as through irrigation [311–314]. However, each approach comes with its own trade-offs. For example, upregulation of abscisic acid through plant trait manipulation may aid in plant drought response, but could have undesired outcomes in disease resistance [315]. One method to increase crop resilience without altering the crop itself is to enhance natural interactions with classes of beneficial microbes. Many groups of fungi and bacteria can increase plant resilience to drought through diverse mechanisms. Microbial inoculants have been frequently used in prevention of plant disease or to establish *Bradyrhizobium* in soybean fields, but their use is also being explored in the amelioration of the impacts of abiotic stress [157, 316–320]. For example, *Streptomyces chartreusis* has been demonstrated to increase drought tolerance in sugarcane and inoculations with salt-tolerant *Streptomyces* taxa can increase drought tolerance in tomato plants [321, 322]. Other strains have been demonstrated to improve responses to other stresses, such as low nutrient stress and flooding stress [323]. Manipulating these traits through the microbiome adds a method for protecting plants from stress without manipulating the plant.

One challenge in the development of microbial inoculants for plant health and resilience is the selection of microbial taxa to use as inoculants. Frequently, taxa have been selected for their perceived plant growth promoting traits such as plant hormone production or alterations to plant hormones, enhancement of nitrogen or other nutrient acquisition, or increased resistance to pathogens [323–326]. Traditionally, the majority of bioinoculants have been bacterial taxa, but increasingly fungal taxa are being used. These fungal taxa are primarily selected for similar plant growth promoting traits including phytohormone production and nutrient acquisition, as is

provided by AMF [327, 328]. Other fungal taxa such as *Trichoderma harzianum* and *Epichloe* can be utilized in pest control as biopesticides and biofungicides [329, 330] However, applying microbial inoculants based on plant growth promoting traits does not account for how taxa will interact with other members of the microbiome once inoculants are applied.

One method to ensure microbial inoculants will interact favorably with the native microbiome is to select microbes based on interactions with other microbes. Selections of interacting taxa can be done through experimental studies, or they can be predicted bioinformatically. A common bioinformatics approach to predict taxa that may be important in structuring the microbiome is to identify highly connected hub taxa which appear to be important in structuring correlation networks created from amplicon or metagenomic sequence data [9, 10, 331]. Since these hub taxa, identified as operational taxonomic units (OTUs) are only identified through correlation networks, further experimentation is needed to determine their role in the microbiome. Work in the *Arabidopsis* phyllosphere has demonstrated that hub taxa impact the microbiome through microbe-microbe interactions as well as impacts on alpha and beta diversity metrics [9]. Additionally, increased interkingdom microbe-microbe interactions in *Arabidopsis* roots were demonstrated to increase host plant survival in the presence of pathogens [24]. Additional research is needed to determine the impact of hub microbial taxa on plants during periods of abiotic stress.

Here, I assess the impacts of inoculating soybeans with hub microbial taxa identified from the roots of soybean grown under no-till management at the KBS LTER prior to exposing soybean seedlings to a period of low-moisture stress. Five hub taxa were used in the inoculation treatment in this experiment. These included three bacterial taxa (*Streptomyces* sp., *Caulobacter segnis*, *Massilia* sp.) and two fungal taxa (*Humicola* sp., and *Gibellulopsis* sp.). *Streptomyces,*

Caulobacter, and *Massilia* are thought to be plant growth promoting bacteria, but this trait is species and strain-specific [321, 322, 332, 333]. *Humicola* has also been indicated as potentially promoting plant growth fungus, while *Gibellulopis nigrescens* can be a weak phytopathogen of peppermint and other plants [334, 335]. However, plant-impacts of fungi are expected to be species, strain and host-specific as well as being impacted by environmental conditions. Strains used in this study were not assessed for plant growth promotion prior to experiments but were instead selected based off their status as network hubs in soybean roots from no-till management (chapter 1 = Longley et al. 2020).

The objectives of this study were as follows: (1) Determine the impact of microbial hub enrichment to living soils on soybean root and soil associated microbiomes, (2) Assess the impact of microbial consortium inoculation on plant phenotype before, during, and after the low-moisture stress period, (3) Assess the impact of microbial hub enrichment on plant functioning through gene expression and metabolomics analyses. Drought conditions can frequently occur simultaneously with other stressful abiotic conditions such as heat. However, in this study, only low-moisture stress was assessed. I hypothesized that inoculated hub-taxa will persist and impact the plant microbiome in subtle but significant ways. Additionally, it is expected that inoculation will improve plant growth throughout the experiment either through direct benefits from hub microbes or through changes in the microbiome as mediated by hubs. These phenotypic differences will be reflected in differential expression of genes and metabolites related to plant stress response and or growth.

Materials and Methods

Hub Identification

Hub taxa were identified from amplicon sequencing data of the roots of no-till soybeans grown at the KBS LTER in 2018 [47]. Networks were created using OTUs that were detected in more than half of the no-till root samples collected from the three soybean growth stages sampled in 2018 (V2, R3, R6). Methods for hub identification were adapted from Agler et al. 2016 [9]. Hub OTUs were identified by being in the top 10% of the measures of degree and betweenness centrality among all nodes in the co-occurrence network. Sequences of hub taxa were then compared to those resulting from bacterial and fungal culture collections isolated from soybeans in 2018. Isolates were chosen if they had a 100% ITS or 16S rDNA sequence similarity to hub OTUs, and if they had been isolated from no-till soybean roots. Additionally, taxa from genera enriched in pathogens (*Fusarium* etc.) or those whose identities were ambiguous were not included. Using these criteria, the five hub taxa used in this study were selected. A summary of the five taxa identified as hubs and used as inoculum is shown in Table 4.1.

Table 4.1 - Summary of hub taxa used as inocula. Relative abundance values represent relative abundances among taxa included in network analysis. Occupancy represents the number of taxa in which the OTU was detected divided by the total number of taxa included in the construction of the network (n=36).

Isolate Match	Type	Best Taxonomy	Genbank Accession	Relative Abundance (%)	Occupancy (%)
RL269	Fungi	*Gibellulopsis* sp.	MT557270	0.014	61.1
RL661	Fungi	*Humicola sp.*	MT557341	0.035	83.3
RL271	Bacteria	*Caulobacter segnis*	MT653469	0.17	94.4
RL115	Bacteria	*Massilia* sp.	MT653557	0.015	88.9
RL170	Bacteria	*Streptomyces* sp.	MT653560	0.04	86.1

Inoculum Preparation

Microbes used in this study were grown on expanded clay beads (Hydroton, USA) weighing between 0.35g and 0.5g, that were first sterilized with a forced air-dry heat oven (Xingchen Instruments, China) by heating glass jars containing clay beads for 4 hours at 200°C. 75 sterilized clay beads were then placed into 250 mL flasks containing 150 mL of Luria-Bertani (LB) broth for *Streptomyces,* Peptone Yeast Extract (PYE) broth for *Caulobacter*, Reasoner's 2A (R2A) broth for *Massilia*, or Malt Extract Broth (MEB) for fungal isolates. Bacterial strains were grown in flasks with expanded clay beads on a rotating shaker at 100 rpm until they reached a concentration level between $10^7$and 10^9 colony forming units/ml (CFUs). Fungal strains were grown in stationary flasks with expanded clay beads for 10 days. Following the growth of the strains and colonization of expanded clay beads, beads were dipped into a 2% alginate solution, immediately followed by dipping beads in a 2.5% CaCl2 solution to seal the beads. Once the beads were sealed, they were washed in sterile DI H2O to remove any excess CaCl2 and refrigerated until use. Control treatments were made by growing microbes on expanded clay beads and then heating the beads to 200°C for 2 hours in a dry heat oven (Xingchen Instruments, China). This treatment allowed for the addition of nutrients similar to the inoculum but without the addition of live microbial inoculum.

Greenhouse Low-Moisture Experiment Setup

Soil for use in greenhouse experiments was collected from the no-till managed lysimeter fetch field at the Michigan State's Kellogg Biological Station in October 2020. Prior to use in experiments, soil was stored at 4°C. In February of 2021, field soil was mixed with playbox sand (Quikrete, USA) to a proportion of 25% soil: 75% sand to maintain drainage. Prior to adding the soil mixture to 500 mL pots, the bottom of each pot was lined with approximately half an inch of

pea gravel to prevent leaking of the soil mixture out of the pots. Pots were then weighed to ensure that equal amounts of gravel had been added to each pot. Next, the soil/sand mixture was added to each pot to a level of 5.7 cm below the lip of the pot. Inoculated or control expanded clay beads (one bead of each microbe) were then placed on top of this layer of soil mixture and covered with another a 1 cm layer of the soil mixture. Williams 82 soybean seeds were utilized due to the availability of a high-quality transcriptome and genome of the Williams 82 cultivar. Seeds were placed directly on top of the 1 cm soil layer covering the clay beads and covered with the soil mixture to a planting depth of 3.8 cm.

Next, pots were again weighed, and soil mixture was added or removed to ensure that all pots had equal mass. Water was then added to field capacity by watering pots until water began draining from the plot, water was allowed to drain from the pots until dripping stopped. 10 pots were weighed after water had stopped draining. The average mass of these 10 pots was used as the field capacity standard for the remainder of the experiment. Dry pots containing beads, soil, and seeds weighed 600g and the field capacity standard was set to 690g. Pots were randomly placed in flats and each flat contained nine control pots and nine inoculated pots. All pots were then watered to the mass of the field capacity standard. Following planting, pots were watered every other day; water was added until each pot reached the field capacity standard. This method accounted for unequal drying rates in the greenhouse. For example, if a pot had dried relatively more than the others, it would receive more water to reach field capacity. Pots were moved to different positions in each flat at each watering, and the position of flats were changed to ensure randomization in drying rates throughout the experiment. Plants were grown in a greenhouse at Michigan State University, with a 12-hour photoperiod and temperature set to 23.8°C during the day and 20.5°C at night. Daytime temperature is similar to expected temperatures for soybean

planting in Michigan, however nighttime temperatures were likely higher than field conditions. After seedlings reached the VC growth stage (unifoliate leaves fully emerged, 16 days post-planting), the low moisture stress experiment began by reducing watering to 50% of field capacity [183]. Starting at day 16, pots were watered every other day to equal masses that represented 50% of the water added to reach field capacity.

Sample Collection and Phenotyping

Starting two days before the low-moisture stress experiment, plant phenotype collection began. Every other day prior to watering, the height of thirteen control and thirteen inoculated plants were assessed by measuring to the height of the stem node of the most recent emerged leaf, and the same plants were assessed for photosynthesis measurements using the PhotosynQ system [336]. Starting at day 0 of the low-moisture stress period (16 days post planting), five control and five experimental sample root systems were destructively sampled for RNA and metabolites by removing the plant from the soil, briefly washing with sterile DI water, and quickly blot drying with a sterilized paper towel prior to flash freezing the entire root system with liquid nitrogen. Three samples were used in RNA and metabolite extraction, but five samples were collected to have extra tissue for RNA extraction in case of failure to generate useable RNA from a sample. After removal from the soil, samples were processed for a maximum of 15 seconds prior to freezing. Samples were then transported from the greenhouse to the lab where they were stored at -80°C until further processing.

Additionally, eight plants were destructively sampled for root and aboveground phenotyping as well as DNA extraction from roots, leaves, and rhizosphere soil. Rhizosphere soil was sampled by shaking soil from the root system into paper envelopes. Aboveground tissue was sampled by cutting the aboveground plant from the root system and placing it in paper

envelopes. Roots were sampled by placing the entire root system in Whirl pak bags (Whirlpak, United States). Roots were then transported to the lab where they were washed with 0.1% tween 20 and sterile DI water. Roots were carefully washed to avoid the removal of nodules which were then counted by hand for the entire root system. Prior to destructive sampling, plants selected for RNA/metabolites and DNA extraction were analyzed for height and photosynthesis measurements as described above. Non-destructive (height and PhotosynQ) measurements were carried out every two days until the end of the experiment, while destructive samplings for molecular measurements were carried out every six days. The distribution of samples taken and an explanation of the five timepoints is shown in Table 4.2.

Table 4.2 - Summary of collected samples and molecular sampling timepoints.

Sample Type	**Sampling Frequency**	**Number of Samples/treatment/timepoint**	**Total**
Plant Height	Every 2 days	13	365
Photosynthesis Metrics	Every 2 days	13	338
Aboveground Biomass and Area	Every 6 days	13	65
Root Phenotypes	Every 6 days	8	40
Roots for RNASeq, Metabolomics, and cDNA amplicons	Every 6 days	5 (3 analyzed)	30
Roots and soil for amplicon sequencing	Every 6 Days	8	80 roots, 80 soil
Timepoint	**Watering**	**Approximate Growth Stage**	**Days Since Drought**
T1	Field Capacity	VC	0 (pre-drought)
T2	50%	V1	6
T3	50%	V1/V2	12
T4	50%	V2	18
T5	Field Capacity	V2/V3	6 days recovery

Individual Microbe Follow-up Experiments

To assess the impact of individual microbes on soybean seedlings compared to the use of the five inoculants together, experiments were performed with individual microbes. Using the same soil mixes as discussed above, soybeans were inoculated using expanded clay beads as previously. Individual microbe treatments were established by placing five beads of the single microbe below the seed at planting. Controls were established as described above by heat killing individual microbes and placing heat killed clay beads below the seed at planting. To control for the impact of dry heating alginate without the microbes, microbe-free beads coated with alginate were compared to microbe-free, alginate coated expanded clay beads which had been heated to 200°C for 2 hours. This control ensured that phenotypic differences were not due to differences in dry heated alginate vs non-heated alginate. Seven plants of each individual microbe and respective controls were established in addition to the combined inoculum described above. This experiment was performed twice: first, without low-moisture stress by maintaining well-watered conditions throughout, and then with the same low-moisture stress conditions as described above. These experiments were carried out in a Percival GR-36VL growth chamber (Percival Scientific, USA) for a period of 9 or 12 days after plants reached the VC growth stage. Light in the growth chamber ranged from 103 µmol to 118 µmol (measured at 3 points on each of the four chamber shelves). Plants were grown with a 12-hour photoperiod at 23.8°C during the day and 20.5°C at night. Following the 9-day period for well-watered plants and a 12-day period for low-moisture plants, plants were destructively sampled and assessed for dry biomass after drying in a dry oven at 60°C for five days. To ensure complete drying, five samples were weighed after four days and were reassessed after five days, to ensure that masses were stable and not still decreasing. In these low-moisture stress experiments, plants were watered to equal weight every four days instead of every other day due to the greater moisture retention of the growth chamber

environment. Additionally, to reduce the buildup of humidity in the chamber two Petri dishes filled with silica beads were placed on each shelf and were refreshed every other day.

Imaging and Analysis

Following sampling, aboveground plant tissues and washed roots were imaged using an Epson Perfection V700 Photo scanner (Epson, Japan). Root and aboveground tissue scans were pre-processed by removing the background with the canva imaging tool (https://www.canva.com/). The images were then converted to be black on a white background using imagej [337].Aboveground plant tissues (including stems and leaves) were assessed for area using ImageJ with a scale created from a ruler visible in the images. Root images were analyzed with Rhizovision explorer [338] to quantify the number of root tips, total root length, root diameter, and root volume.

Nucleotide Extraction

Following sampling and imaging of the roots, aboveground tissues, and rhizosphere soils were dried with silica beads for two weeks. Five samples were weighed on two consecutive days to ensure that samples were completely dry, and that dry mass was no longer decreasing. Prior to nucleotide extraction, aboveground tissues were weighed, and masses were recorded. DNA was extracted from approximately 50 mg of dried root or leaf tissue using the Mag-Bind Plant DNA Plus Kit (Omega Bio-Tek, United States). DNA was extracted from approximately 50 mg of soil with the PowerMag Soil DNA Isolation Kit (Qiagen, United States) and the Kingfisher Flex System (Thermo Fisher Scientific, United States). RNA was extracted from flash-frozen roots by grinding whole root systems with dry heat sterilized (240c for 4 hours) and RNASE Zap (Sigma-Aldrich, USA) treated mortars and pestles. Liquid nitrogen was added to mortars and pestles before and during grinding to ensure that roots remained frozen. Following grinding,

RNA was extracted from approximately 50 mg of ground root tissue per sample using the RNeasy plant mini kit (Qiagen, United States). RNA was eluted in 50 µl of water. In addition to RNA and DNA, aliquots of cDNA were created from RNA samples using Goscript Reverse Transcriptase with 1 µL of extracted RNA (Promega, United States).

Metabolite Extraction

Prior to extraction, frozen root tissue was ground to a fine powder on liquid nitrogen in heat-sterilized mortar and pestles. Non-target metabolites were extracted with acetonitrile: isopropanol: water in a proportion of 3:3:2. The extraction solvent contained telmisartan at a concentration 0.5 µM as an internal standard. 1.5 mL of extraction solvent was added to a maximum of 0.15g of ground root tissue and samples were left overnight at -20°C. The following day, 200 µL of this solution from each sample was transferred to glass autosampler vials which were stored at -80°C until processing at the MSU metabolomics core.

LC-MS of Non-Target Metabolomics

Samples were analyzed on a Waters G2-XS Q-Tof mass spectrometer interfaced with a Waters Acquity UPLC. Five µl per sample was injected onto a Waters Acquity UPLC HSS-T3 column (2.1x100 mm) at 40°C. Compounds were separated using a binary gradient as follows: initial conditions were 100% mobile phase A (10 mM ammonium formate in water) and 0% mobile phase B (acetonitrile), hold at 100% A for 1 min, linear ramp to 99% B at 16 min, hold at 99% B until 18 min, return to 100% A at 18.01 min and hold until 20 min. The flow rate was 0.3 ml/min. Mass spectra were obtained by electrospray ionization operating in either positive or negative ion mode with a capillary voltage of 3.0 kV (positive) or 2.0 kV (negative), source temperature of 100°C, cone voltage at 35 V, desolvation temperature of 350°C, desolvation gas flow of 600 L/hr and cone gas flow of 50 L/hr. Data were acquired using a data-independent

MS^e method (0.2 second scans with fast switching between no collision energy and using a collision energy ramp of 20-80 V) across an m/z range of 50-1500 while using the dynamic range enhancement setting. Lockmass correction was performed in Masslynx software using leucine enkephalin as the reference compound.

Amplicon Library Preparation

Amplicon libraries were created from extracted DNA and cDNA using a three-step PCR protocol as described previously [47, 339]. Prokaryotes were amplified with the 515F - 806R primer set and fungi were amplified with the ITS1F - ITS4 primer set [185, 186]. The first step in the library construction utilized unmodified primers to enrich in targeted taxa, the second step used primers with frameshifts, and the third step incorporated unique 10 nucleotide barcodes [190]. Details on primers and amplification are available in tables 2.1 and 2.2. Following amplification, samples were run on the Qiaxcel Advanced automated electrophoresis system (Qiagen, United States) to ensure amplification. Next, libraries were normalized to equal concentration with the SequalPrep Plate Normalization Kit (Thermo Fisher Scientific, United States). Following normalization, libraries were combined and concentrated with Amicon Ultra 0.5 mL 50k filters (EMD Millipore, Germany). After libraries were concentrated, they were cleaned with Ampure XP magnetic beads to remove primer dimers (Beckman Coulter, United States). Cleaned libraries were again assessed with Qiaxcel Advanced system to ensure that all small fragments were removed. Amplicon libraries were then submitted to the MSU Genomics Core and sequenced with Illumina MiSeq V3 600 cycles kit (Illumina, United States). Raw amplicon sequencing data is available on NCBI SRA under PRJNA825108.

RNA Quality Analysis and Sequencing

Following RNA extraction, extracts were quantified using the Qubit RNA HS Assay Kit (Thermo Fisher Scientific, United States) and analyzed using the Agilent 2100 Bioanalyzer (Agilent, United States). Samples with concentrations above 50 ng/µl and RIN quality values above 7.5 were submitted for sequencing. Samples that did not reach these thresholds were re-extracted from extra samples taken during sampling. 30 RNA samples were submitted to the MSU genomics core for Illumina Stranded mRNA library prep and sequencing across three HiSeq 4000 lanes using 50 bp single end sequencing. All samples were sequenced across the three lanes to avoid bias based on the HiSeq lane. Raw RNA sequencing data is available on NCBI SRA under the following accession number: PRJNA825532.

Amplicon Data Processing

Raw data was downloaded and assessed for quality with FastQC V 0.11.5 [191]. Due to the lower quality of reverse sequences, only forward reads were analyzed for both loci. Following quality assessment, raw data was demultiplexed by 10 nucleotide barcodes using QIIME V1.9.1 [192]. Next, primers, adapters were removed using Cutadapt V2.6 [193]. Additionally, conserved regions (5.8S, LSU, SSU) of ITS amplicons were removed to improve taxonomic assignments by aligning a subset of 500 sequences with SeaView [340] . Next, length and quality statistics were assessed using USEARCH V10 and reads with an expected error rate above 0.5% were removed [194]. Prokaryotic reads were trimmed to equal length and sequences less than 260 bp in length were discarded. After removing low quality reads, zero radius OTUs (ZOTUs) were created using the UNOISE V3 algorithm of USEARCH [341]. Taxonomy was then assigned to ZOTUs using CONSTAX V2 [280, 281].

Amplicon Sequencing Statistical Analysis and Visualizations

ZOTU tables, sequences, taxonomy tables, and mapping files were transferred to the R statistical environment and combined to create a phyloseq object with the *phyloseq* 1.36.0 package [200, 201]. Prior to further analyses, ZOTU tables were assessed for contamination using the *decontam* 1.12.0 package [202]. Following removal of contaminants, ratios of numbers of cDNA reads to numbers of DNA reads were assessed for each ZOTU. Ratios were created by dividing the number of cDNA reads for a ZOTU by the average number of DNA reads for a ZOTU within a single timepoint and treatment (inoculated and control). ZOTUs with ratios >1 were considered to likely represent taxa which were more active compared to those with ratios of <1. To assess activity of *Bradyrhizobium,* ratios were visualized for the four *Bradyrhizobium* ZOTUs detected by amplicon sequencing.

Prior to beta diversity analyses, OTU tables were normalized by cumulative sum scaling with the *metagenomeseq* 1.34.0 package [213]. Beta diversity was analyzed with the "ordinate" and "plot ordination" functions in the *vegan* 2.5.7 package [207]. Ordinations were visualized using principal coordinate analysis (PCoAs), and visualized patterns were assessed for statistical significance through PERMANOVA with the "adonis" function of *vegan*. Additionally, homogeneity of dispersion between groups was tested with the "betadisper" function in *vegan*. In the case of a significant PERMANOVA for the treatment variable, variation confined only to differences in treatment were visualized using canonical analysis of principal coordinates (CAP) with the 'capscale' function in *vegan* [207, 284].

RNA Data Processing

Initial sequence quality was assessed with FastQC V 0.11.5[191]. Following quality assessment, data from the three separate lanes were then merged for each sample. After merging, reads were trimmed to remove adapter sequences and low quality reads with trimmomatic V 0.39; only reads of 30 bp or longer were retained for further analysis [342]. Next, SortMeRNA V 2.1 was used to remove any residual rRNA reads [343]. Transcripts were then quantified with Salmon and a transcript index was created from the Williams 82 soybean genome published by the Joint Genome Institute (JGI) [344, 345]. Quantified transcripts files were then transferred into the R statistical environment for analysis with *DeSeq2* V 1.32.0 [346]. Multidimensional scaling plots were created to variance stabilizing transformed (VST) data to assess clustering of samples based on plant transcript data. Genes were considered to be differentially expressed if they had an adjusted P value of less than 0.05 and an absolute value of log 2-fold change of greater than 0.585 (a 1.5X change in expression) [347]. Differentially expressed genes for each timepoint (control and inoculated) were annotated based upon the soybase genome annotation tool with the JGI Williams 82 genome [348]. Distributions of differentially expressed genes by timepoint were assessed for control and inoculated samples with *ggVennDiagram* V 1.2.0 [349]. Differentially expressed genes were assessed for their relation to nodulation with the RNAseq atlas tool on SoyBase.org [348, 350, 351]. *DeSeq2* normalized counts for each gene in each sample were deposited to the NCBI Gene Expression Omnibus (GEO) under accession number GSE200609.

To further assess the functions of differentially expressed genes, GO (gene ontology) term functional enrichment analysis was performed with g:profiler [352]. The log10 transformed P values for the top five most significantly enriched GO term in each category at each timepoint were plotted to assess functional categories that were enriched in control and inoculated samples.

Gene model names were converted to match the Severin et al., 2010. RNAseq atlas and nine matched genes which were identified by Severin et al., 2010 as being highly associated with nodulation were assessed further. Genes from the RNAseq atlas were determined to be potentially related to nodulation if a larger number of transcripts mapped to nodules than to any other plant tissue. This gene set was used to determine the proportion of differentially expressed genes (control and inoculated) related to nodulation at each timepoint. Additionally, log10 transformed normalized counts of genes matching the nodulation GO term from gprofiler were used to create a heatmap in *pheatmap* V 1.0.12 that assessed expression patterns of nodulation-related genes [353].

Non-Target Metabolomics Data Processing

Prior to further analysis, metabolites with relative mass defects (RMD) above 1200 were removed as these compounds likely represent non-organic contaminants [354, 355]. Peak areas were normalized to the telmisartan internal standard and sample biomass using the following formula: metabolite peak area/ telmisartan peak area/ sample mass (mg) * 1e8. The metaboanalyst online tool V 5.0 was used to identify metabolites that were significantly differentially abundant between inoculated and control samples at each timepoint [356]. Metabolites were considered to be significantly differentially abundant if there was a T-test P value of less than 0.05 and an absolute value of log 2-fold change of greater than 0.585 (a 1.5X change in expression) to match threshold used in RNASeq results. Principal components analysis (PCA) plots were used to assess clustering of samples by timepoint and inoculation treatment. Distributions of differentially abundant metabolites by timepoint in control and inoculated samples (both positive and negative ion mode) were assessed with Venn diagrams created with *ggVennDiagram* V 1.2.0 [349]. RMD values were used to assess distributions of broad classes of

compounds as previously outlined [354, 355]. The RMD range of 200-350 was assigned as containing phenolics, 350-550 was assigned as containing terpenoid glycosides, and 550 and above was assigned as containing terpenoids and lipids [354, 355]. Histograms were created using *ggplot2* V 3.3.5 to assess the distributions of these groups [208]. Bioinformatics and R code for processing hub inoculation data is available at: https://github.com/longleyr/Hub-Microbe-Inoculation.

Results

Phenotyping Results

Inoculated samples consistently showed increased aboveground biomass and aboveground area compared to plants in control treatments. For aboveground area, this difference was only significant at later timepoints (T4 and T5), but for aboveground biomass the difference was significant throughout the experiment except for the final sampling (Figure 4.1A, B). The same general pattern was true for plant height measurements, but the difference was generally not significant (Figure 4.1C). The Phi2 measurement of photosynthesis, which represents the proportion of incoming light available for photosynthesis, was significantly higher in inoculated plants compared to those in control treatments (Figure 4.1D). The PhiNPQ measurement, which represents the proportion of light, which is non-photochemically quenched, was higher in controls compared to inoculated samples (Figure 4.1E). This pattern was generally consistent throughout the experiment but was not consistently significant (Figure 4.2A,4.2B). Belowground phenotyping did not show a consistent pattern across any of the root phenotypes analyzed except for root nodulation (Figure 4.3). Control and inoculated samples were both nodulated by the second sampling point at vegetative growth stage V1/V2. Inoculated roots consistently had higher nodule numbers than control samples, but this difference was only

significant at the second sampling point (Figure 4.3E). The number of root tips and total root length were higher in inoculated plants at the T4 and T5 sampling points, but these differences were not significant (Figure 4.3A, 4.3B). There was not a consistent pattern for inoculated versus control samples for the measurements of average root diameter or total root volume and there were no significant differences at any time point.

Figure 4.1 - Aboveground phenotyping results of soybean seedlings from image analysis and photosynthesis metrics. Total aboveground area starting at T2 (n = 8/treatment/timepoint) (**A**), aboveground dry biomass (n = 13/treatment/timepoint) (**B**), height measured to the highest emerged leaf node (n = 13/treatment/timepoint) (**C**), and photosynthesis measurements showing the Phi measurement (n =339) (**D**) and PhiNPQ (n =339) (**E**). Comparisons are based on Wilcox ranked sign tests, an asterisk indicates a significant difference; * = $p \leq 0.05$, ** = $p \leq 0.01$.

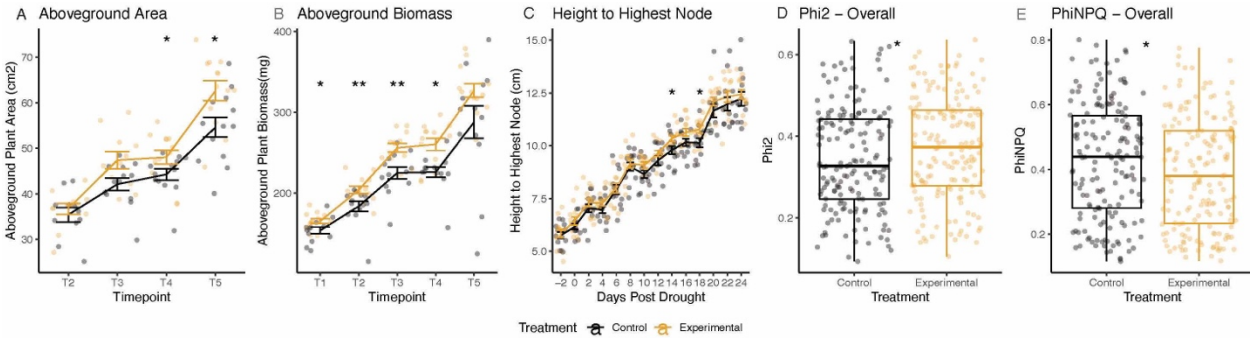


Figure 4.2 - Measurements of photosynthesis metrics at each sampling point. Phi2 (**A**) and PhiNPQ (**B**). Comparisons are based on Wilcox ranked sign tests, an asterisk indicates a significant difference; * = $p \leq 0.05$, ** = $p \leq 0.01$ (n = 13/treatment/timepoint).

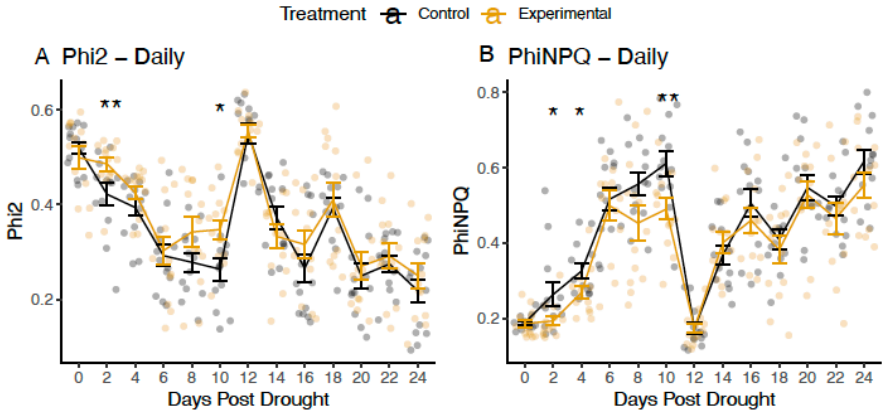
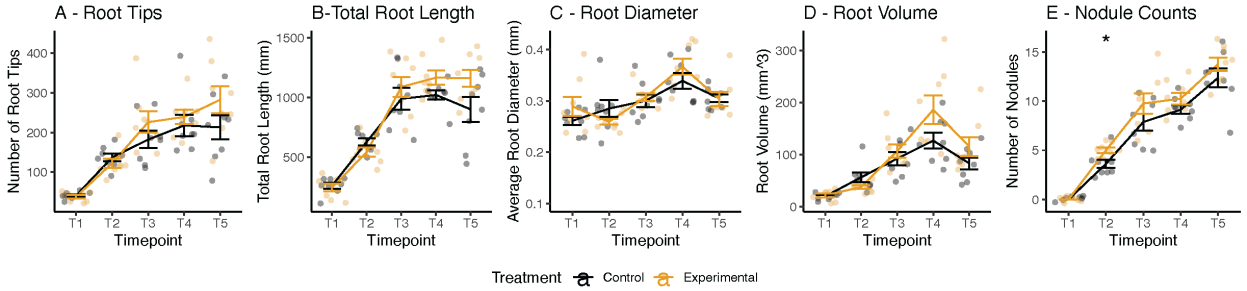


Figure 4.3 - Root phenotyping metrics showing: total number of detected root tips (**A**), total root length across all branches (**B**), average root diameter (**C**), total root volume (**D**), nodule counts (**E**), n = 8/treatment/sampling point.



Amplicon Sequencing Results

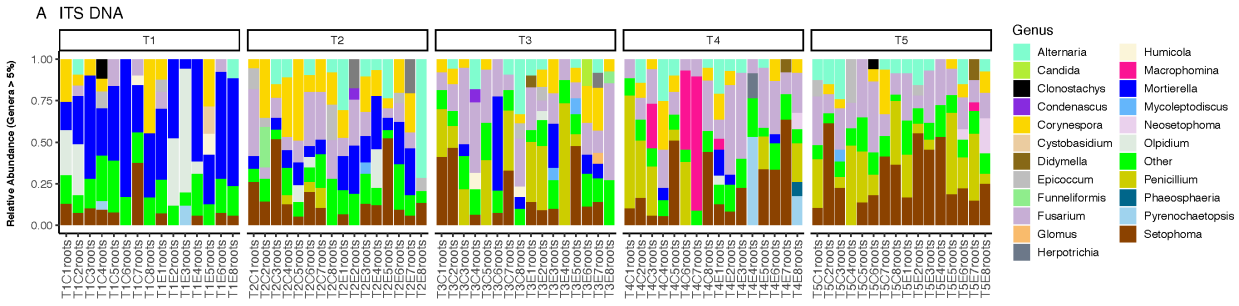
Due to poor amplicon sequencing yields from leaf samples, these samples were used in the removal of contaminants, but were not analyzed further. For 16S rDNA libraries, the soil had an average depth of 45,000 reads per sample across 80 samples and was composed of 13,978 ZOTUs. The root 16S DNA library was composed of 80 samples and had an average depth of 27,000 reads across 9,051 ZOTUs, and the root 16S cDNA library had an average 36,868 reads per sample across 30 samples and 5,974 ZOTUs after the filtering of plastids and non-bacterial sequences. Following filtering and removal of non-fungal sequence, the soil library had an average depth of 11,787 reads per sample across 1528 ZOTUs in 80 samples. Root DNA samples had an average depth of 11,668 reads per sample across 79 samples in 666 ZOTUs. The ITS cDNA was composed of 30 samples and had and average depth of 15,679 reads per sample in 275 ZOTUs.

Fungal Community Composition

DNA amplicons were initially dominated by the *Mortierella* genus at timepoint 1 (T1), accounting for 44% of reads among classified genera. However, beginning at T2 the community was dominated by *Fusarium, Penicillium* and *Setophoma,* which persisted through T5. These taxa accounted for between 12 and 30% of the reads at each of T2 – T5. The relative abundance

of *Mortierella* decreased following the first timepoint and was completely absent from bars by the final sampling point, but fungal communities appeared to be relatively consistent otherwise (Figure 4.4).

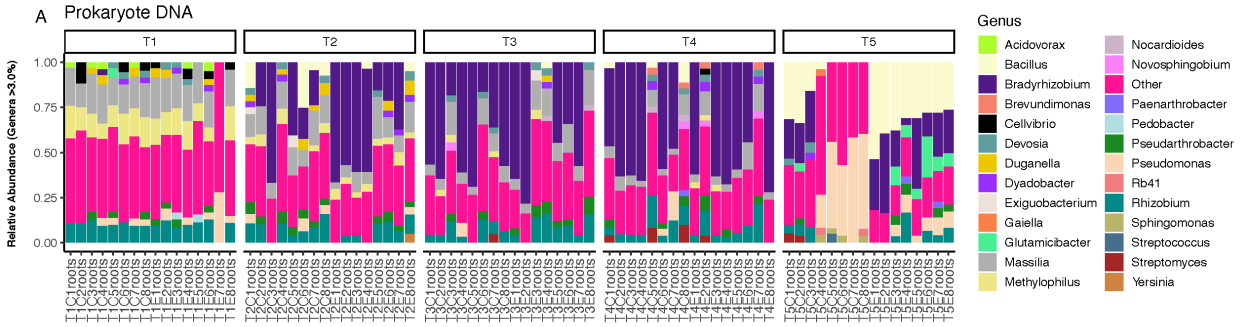
Figure 4.4 - Stacked barplots showing fungal genera in DNA amplicons. Genera > 5% relative abundance present in DNA amplicons.



Bacterial Community Composition

Prior to the low-moisture stress experiment at timepoint 1 (T1), control and inoculated DNA amplicon communities were dominated by *Rhizobium*, *Massilia*, and *Methylophilus*, with *Massilia* having the highest relative abundance (19%). Throughout the low-moisture stress experiments, the majority of both control and inoculated samples were dominated by *Bradyrhizobium,* which accounted for 39% of genus-level classified reads in control samples and 42% in inoculated samples. At the final sampling point, samples continued to be dominated by *Bradyrhizobium*, but several control samples were lacking in *Bradyrhizobium* and were instead dominated by *Pseudomonas* (Figure 4.5).

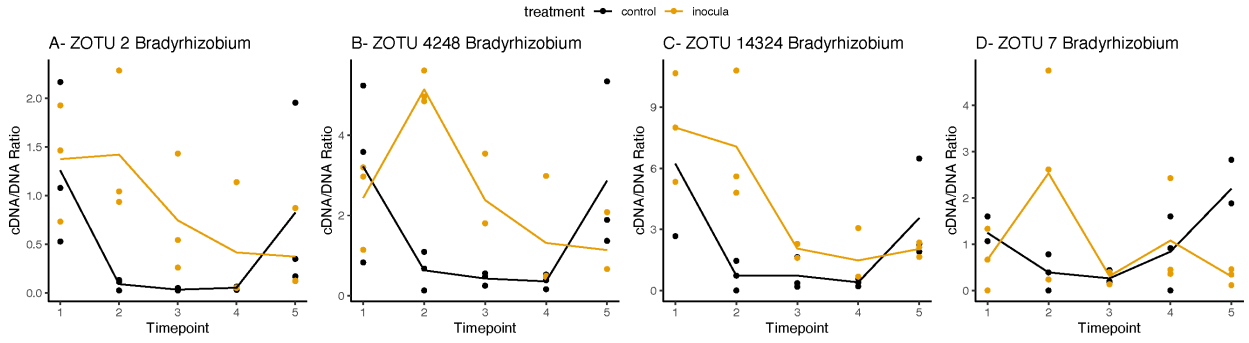
Figure 4.5 - Stacked barplots showing prokaryotic genera in DNA amplicons in roots. Genera > 3% relative abundance present in DNA amplicons.



Activity Assessment of Bradyrhizobium

Four *Bradyrhizobium* ZOTUs were identified from amplicon sequencing. ZOTU 2 was the most abundant *Bradyrhizobium* ZOTU identified and had an average of 7557 reads/sample in the DNA dataset. The other three *Bradyrhizobium* ZOTUs had an average of less than 100 reads/sample. Throughout the low-moisture stress period (T2, T3, and T4), three of the four *Bradyrhizobium* ZOTUs (ZOTU 2, ZOTU 4248, ZOTU 14324) had higher cDNA/DNA ratios in inoculated samples compared to control samples until the recovery period following the low moisture stress period (T5) (Figure 4.6A-C). Differences were especially apparent at T2 where, inoculated samples consistently had cDNA ratios above one, whereas control samples consistently had cDNA:DNA ratios below one (Figure 4.6). Ratios generally decreased in inoculated samples following T2 and in control samples following T1.

Figure 4.6 - cDNA: DNA Ratios for Bradyrhizobium OTUs. Ratios calculated for the four *Bradyrhizobium* ZOTUs detected with amplicon sequencing throughout the experiment.



Beta Diversity Analyses – DNA

PCoA ordinations of soil fungal and prokaryotic communities did not reveal clear clustering by inoculum treatment or timepoint. Samples from all timepoints and both treatments spread throughout ordinational space (Figure 4.7A, C). However, when analyzing root DNA communities, there is clear clustering by timepoint for both fungi and prokaryotes. This variation was primarily spread along the X axis of each ordination, which accounted for 20.9% of variation in fungi and 46.8% of variation in prokaryotes (Figure 4.7B, D). PERMANOVA results confirm patterns present in ordinations as treatment and timepoint were both insignificant for soil communities, but the timepoint factor was significant for root fungi and prokaryotes ($p = 0.0001$). For root fungal communities, there was also a significant difference in group dispersion making PERMANOVA results difficult to interpret, but patterns in the ordinational space appear to confirm PERMANOVA results (Table 4.3).

Figure 4.7 - Principal coordinates analysis of DNA amplicons. Plots based on Bray-Curtis dissimilarity of fungal communities associated with soil (**A**), roots (**B**) and prokaryotic communities associated with soil (**C**) and roots (**D**).

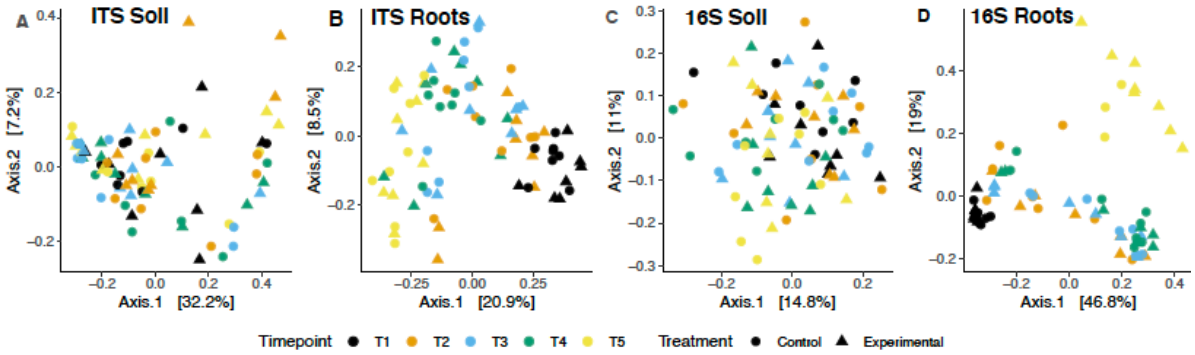


Table 4.3 - Permutational multivariate analysis of variance ("adonis") and multivariate homogeneity of dispersion ("betadisper") analysis results for DNA amplicons of fungal and prokaryotic communities from soil and roots. Significant P values (< 0.05) are shown in bold.

Fungi Soil	**PERMANOVA**				**Dispersion**	
	DF	**F model**	**R2**	**P value**	**F value**	**P value**
Timepoint	4	1.2362	0.06241	0.1571	0.6948	0.5979
Treatment	1	1.3132	0.01657	0.1691	0.0346	0.8528
Timepoint: Treatment	4	0.7434	0.03753	0.9007		
Residuals	70		0.88349			
	Fungi Roots					
Timepoint	4	5.2483	0.21935	**0.0001**	4.4404	**2.85E-03**
Treatment	1	1.1481	0.012	0.2525	0.3195	0.5736
Timepoint: Treatment	4	1.141	0.04769	0.1933		
Residuals	69		0.72096			
	Prokaryote Soil					
Timepoint	4	2.1743	0.10351	**0.0001**	1.4318	0.2318
Treatment	1	1.3042	0.01552	0.1331	0.1799	0.6726
Timepoint: Treatment	4	1.0064	0.04791	0.4376		
Residuals	70		0.83306			
	Prokaryote Roots					
Timepoint	4	14.3406	0.44929	**0.0001**	1.7207	0.1553
Treatment	1	0.7079	0.00554	0.5621	1.194	0.2782
Timepoint: Treatment	4	1.4009	0.04389	0.1425		
Residuals	64		0.50128			

RNA Sequencing Results

Across the three lanes, RNA sequencing produced an average of 30,241,310 reads per sample with a range of 20,962,122 - 41,258,840 reads. Following trimming and discarding of remaining ribosomal RNA reads, the average read depth was 27,212,943 reads per sample with a range of 6,884,446 - 38,903,129 reads. On average, 89.5% of the processed reads mapped to the soybean (*Glycine max*) transcriptome with a range of 72.2% - 92.7%.

Differential Expression Results

An MDS ordination of filtered plant RNA sequencing results showed distinct clustering by both sampling timepoint and inoculation treatment (Figure 4.8A). The two treatments were distinct from each other at each timepoint, but the clusters were most distinct in the T2 and T3 stages (Figure 4.8A). There were 4851 differentially expressed genes upregulated in the experimental treatment and 2008 in the control treatment. The number of differentially expressed genes which were upregulated in the experimental treatment varied by timepoint and ranged from 174 at T4 to 1968 at T2. The number of differentially expressed genes associated to the control treatment varied between 148 at T4 and 785 at T5. There were more shared genes between timepoints among genes that were upregulated in the inoculated samples. For example, between T2 and T3, there were 1093 differentially expressed genes in common (Figure 4.8B). In control samples, the majority of the differentially expressed genes were unique to a single timepoint. The maximum number of genes which were shared between two sampling timepoints was 53 between T1 and T2 (Figure 4.8C). Among inoculated samples, there were two differentially expressed genes identified at all time points. One gene was annotated in the TauE PFAM, which contains sulfite exporters, and the other was identified as being in the AP2 PFAM which contains ethylene responsive transcription factors. Additionally, 17 genes were found to

be differentially expressed in four of the five timepoints. These genes were primarily identified as expansins, nodulation proteins, and copper binding proteins (Table 4.4).

Figure 4.8 - RNA sequencing results of soybean roots. MDS of RNAseq data after variance stabilizing transformation (VST) in DEseq2 (**A**), Venn diagram showing numbers of differentially expressed genes by timepoint in inoculated samples (**B**), and control samples (**C**).

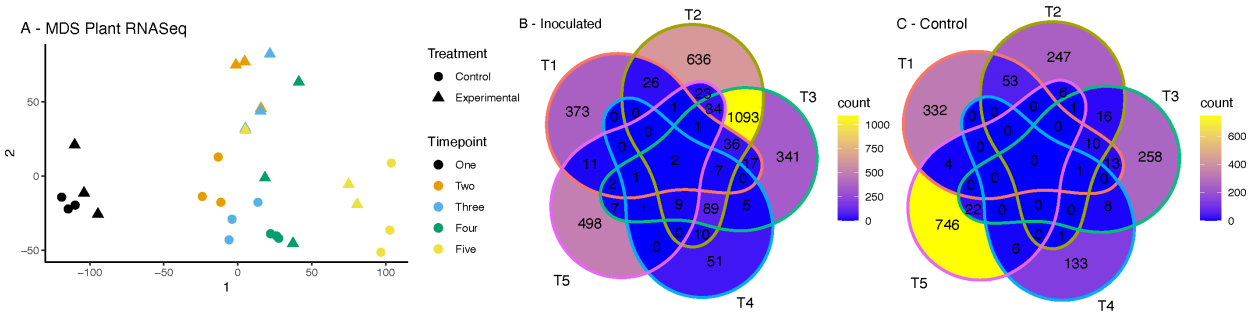


Table 4.4 - Genes that were differentially expressed in inoculated roots at four or more timepoints.

Gene Name	Timepoints	PFAM Name	Uniprot Function
Glyma.01G175200	T1-T5	TauE	Sulfite Exporter
Glyma.07G025800	T1-T5	AP2	Transcription Factor, Ethylene Responsive
Glyma.12G161500	T1-T4	Aminotran_1_2	pyridoxal phosphate binding
Glyma.17G073400	T1-T4	Cu_bind_like	Copper binding
Glyma.08G190700	T1-T4	ABC_membrane; ABC_tran	ABC Transporters
Glyma.02G204500	T1-T4	Cu_bind_like	Copper binding
Glyma.15G260600	T1-T4	TAXi_C	Peptidase
Glyma.06G182700	T1-T4	Pro_CA	Carbonic anhydrase
Glyma.19G251500	T1-T4	Inhibitor_I9; Peptidase_S8	Peptidase
Glyma.15G055900	T1-T4	NA	NA
Glyma.04G222100	T1-T4	DPBB_1; Pollen_allerg_1	Expansin
Glyma.01G050100	T1-T3, T5	DPBB_1; Pollen_allerg_1	Expansin
Glyma.02G132700	T1-T4	VID27	NA
Glyma.13G364400	T1-T4	Nodulin	Nodulation
Glyma.15G045000	T1-T4	Nodulin	Nodulation
Glyma.16G127960	T1-T4	NA	NA
Glyma.13G327500	T1-T4	NA	NA
Glyma.15G048400	T1-T4	zf-XS; XS	Uncharacterized
Glyma.18G153500	T1-T4	DPBB_1; Pollen_allerg_1	Expansin

Functional Enrichment Analysis

Many of the molecular function GO terms enriched in inoculated samples were related to transmembrane transport; with transport GO terms being upregulated in samples at T2, T3, and T5. Additionally, cysteine dioxygenase activity was upregulated at T2 and T3 in inoculated samples, with about 60% of genes in this GO term being presented in differentially expressed genes from T2 and T3 (Figure 4.9A). The most significantly enriched molecular function GO terms in control samples were not shared between timepoints, and many were related to protein folding or associations. Several of the cellular component GO terms enriched in inoculated

samples were shared across multiple timepoints. These terms were shared between T2, T3, and T4 and related to symbiosomes, peribacteroid membranes, and endocytic vesicles. Many of the GO terms enriched in control samples were related to the cell wall or other cellular components such as the cytoplasm (Figure 4.9B). Several biological processes were significantly enriched at multiple timepoints in the inoculated samples. These included transmembrane transport enriched at T2, T3, and T5 and nodulation and anatomical structure formation involved in morphogenesis which were enriched at T2, T3, and T4. The GO term for *de novo* IMP biosynthetic process was enriched at T2 and T3. None of the top enriched GO terms in control sample were shared across multiple timepoints, but many were related to cell wall and cell cycle functions (Figure 4.10A). Several KEGG assignments were shared between the T2 and T3 timepoints in inoculated samples. These assignments were primarily related to secondary metabolism, amino acid biosynthesis, alkaloid biosynthesis, purine metabolism, and nitrogen metabolism. In control samples, pentose and glucuronate interconversions were enriched in T1 and T3. Other enriched functions included various metabolism and biosynthesis functions (Figure 4.10B).

Figure 4.9 - Molecular function and cellular component categories upregulated in control and inoculated plants. Top 5 most highly upregulated functional categories at each timepoint for each control and inoculated plants in the molecular function GO term (**A**), and cellular component GO term (**B**). Point size represents the proportion of the genes in the GO term category found in the differential expression dataset for either control or inoculated samples at that timepoint.

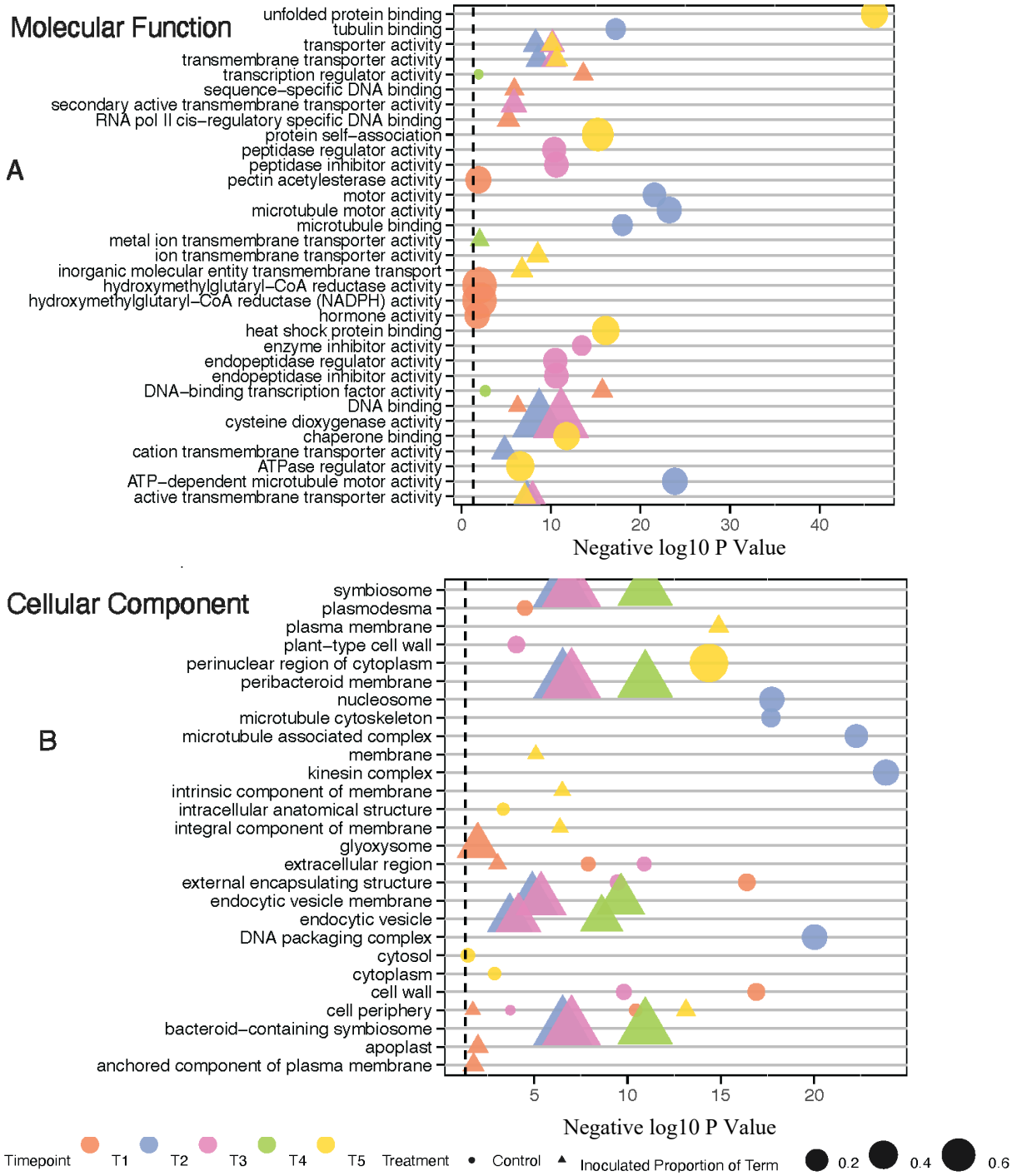
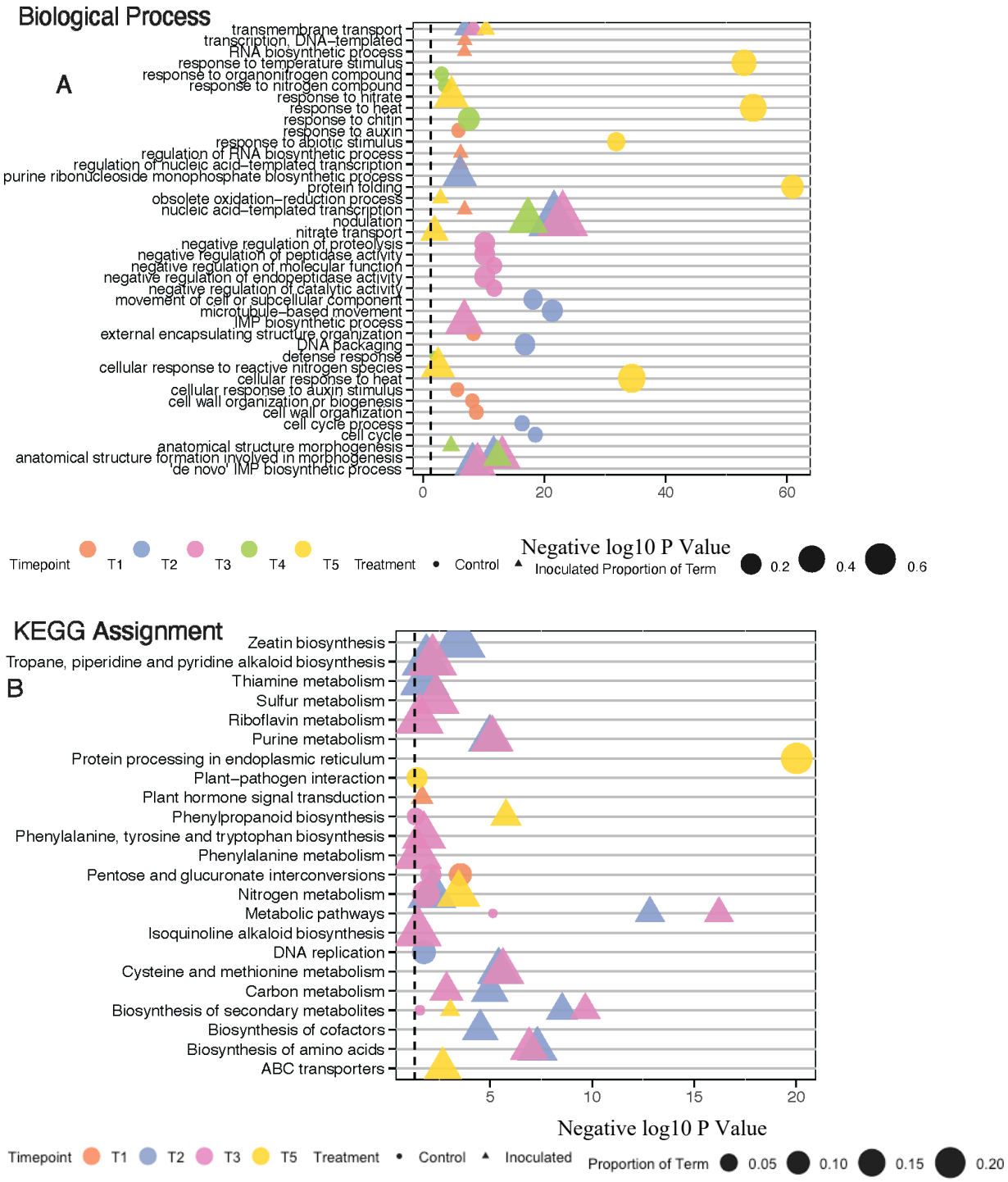


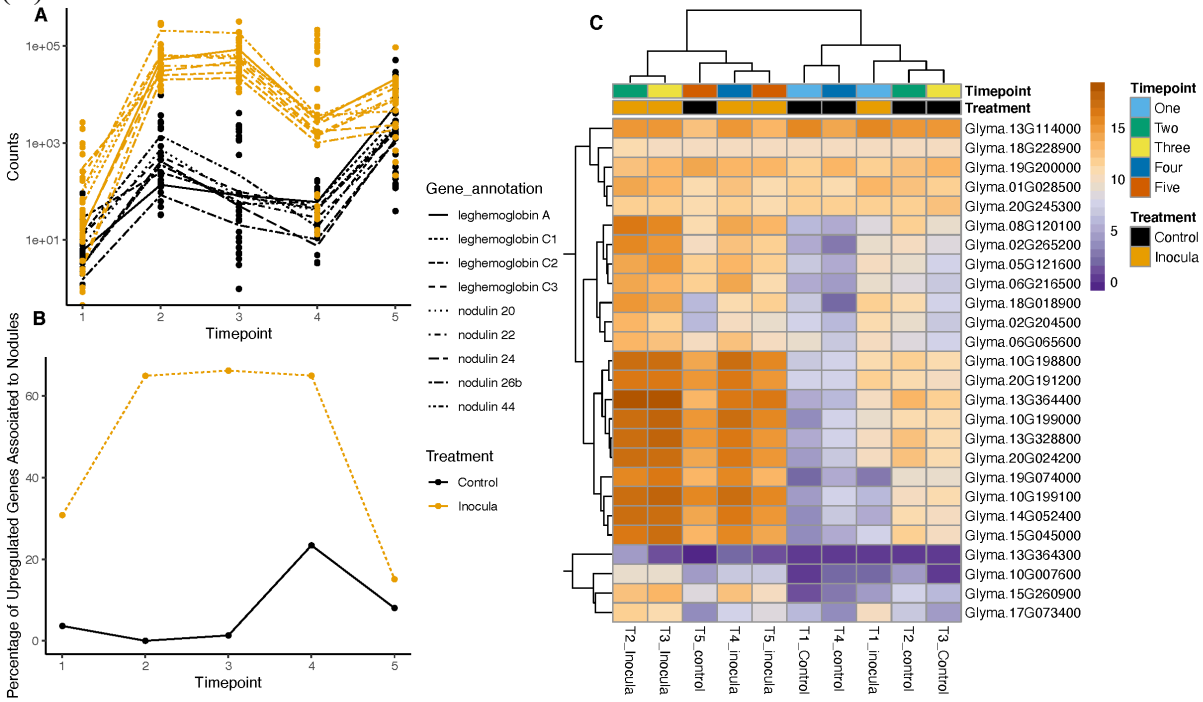
Figure 4.10 - Biological Process and KEGG Assignment categories upregulated in control and inoculated plants. Top 5 most highly upregulated functional categories at each timepoint for each control and inoculated plants in biological processes GO terms (**A**), and KEGG functional categories (**B**). Point size represents the proportion of the genes in the GO term category found in the differential expression dataset for either control or inoculated samples at that timepoint.



Nodulation Specific Results

Nine genes (leghemoglobin and nodulin genes) that had been previously identified as being most highly associated to nodule tissue compared to other tissues in soybean were analyzed for read counts in control vs inoculated samples throughout the experiment [351]. These nine genes had higher counts in inoculated samples compared to controls throughout the experiment, but this difference was larger during the low-moisture stress period (Figure 4.11A). Using the RNASeq atlas, the percentage of differentially expressed genes for each treatment that had the highest counts in the nodules compared to other tissues was assessed. Inoculated samples consistently had a greater percentage of differentially expressed genes, particularly throughout the low moisture stress period (T2, T3, T4). During this period, over 60% of the genes which were upregulated in the inoculated samples were nodule-associated (Figure 4.11B). Genes from the nodulation GO term showed that this group of genes was primarily consistently upregulated in the inoculated samples. This group of nodulation genes were upregulated in inoculated samples beginning at the T1 or T2 sampling point, whereas the expression remained low in the control samples until the T5 sampling point (Figure 4.11C).

Figure 4.11 - Expression of nodulation specific genes. DEseq2 normalized counts for soybean genes identified by Severin et al., 2010 as being most highly associated to nodulation (**A**), percentage of differentially expressed genes for control and inoculated that were identified by Severin et al., 2010 as having higher counts in nodules compared to any other soybean tissue (**B**), heatmap of log10 transformed DEseq2 normalized counts of the genes in the nodulation GO term (**C**).

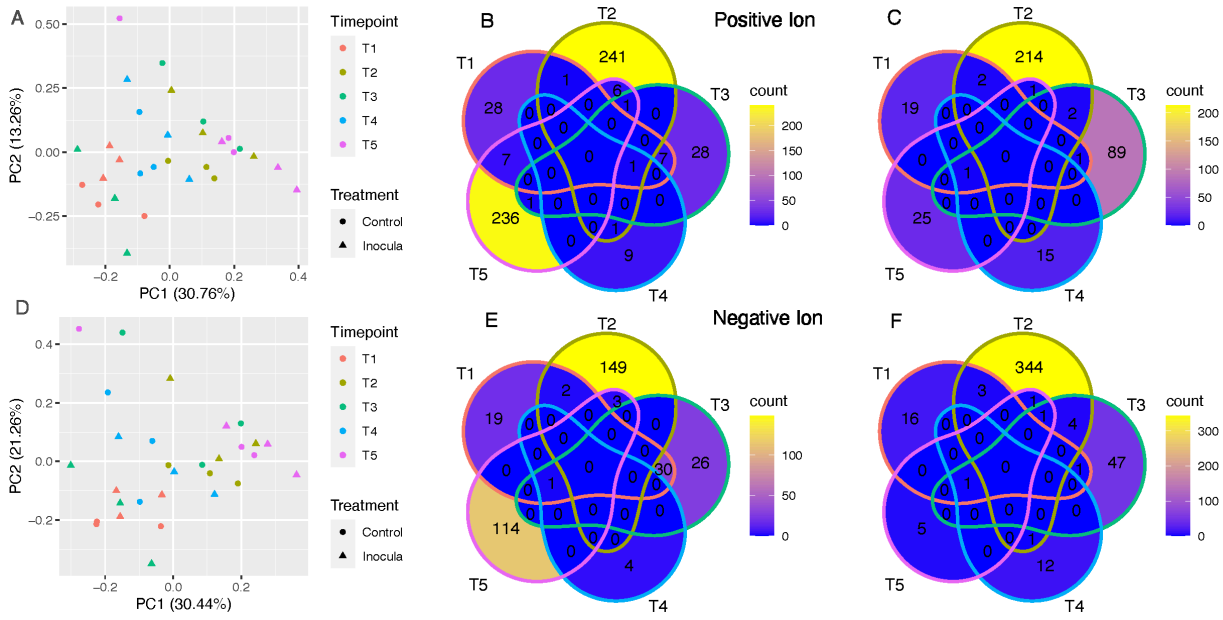


Non-Target Metabolomics

Following filtering of metabolites with RMD values above 1200, negative ion mode results contained 3,512 metabolites while the positive ion mode produced 4,049 metabolites. PCA plots revealed that there was clustering due to both inoculation and timepoint. In positive ion mode, separation of treatments was clearest at T3, but separation was apparent at T1 and T2 as well (Figure 4.12A). In negative ion mode, separation also appeared to be greatest at T3, but there was separation by treatment at T2 as well. At the first sampling point (T1), samples within a treatment appeared to cluster closely together but this dispersion was more substantial at later sampling points (Figure 4.12D). As with results for differentially expressed genes, there were more differentially abundant metabolites at the beginning of low-moisture stress (T2) compared to other timepoints. There was little overlap between differentially abundant metabolites across

timepoints (Figure 4.12B, C, E, F). However, in inoculated samples in negative ion mode, there was substantial overlap between T1 and T3 (Figure 4.12E).

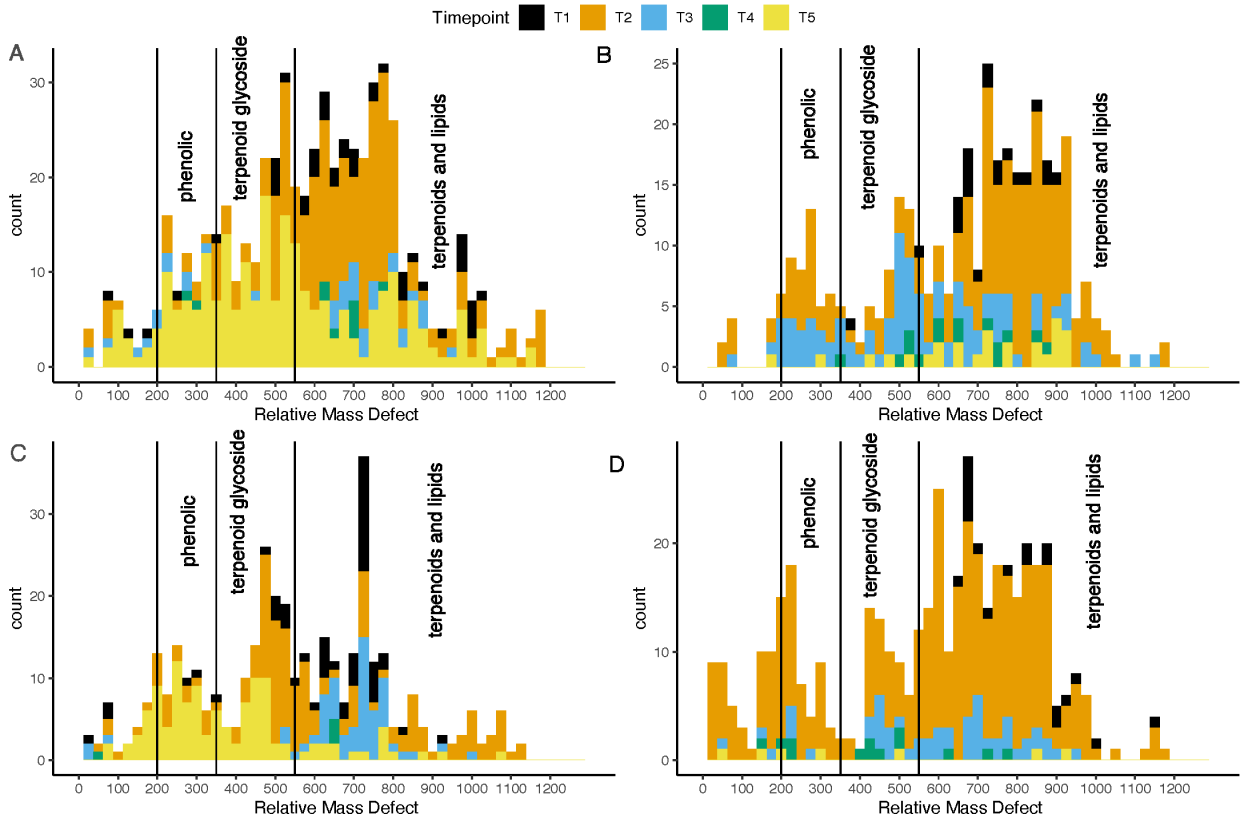
Figure 4.12 - Non-target metabolomics of soybean roots. PCA of metabolomics data after log transformation in positive ion mode (**A**) and negative ion mode (**D**). Venn diagrams showing numbers of differentially abundant positive ion mode metabolites by timepoint in inoculated samples (**B**), and control samples (**C**). Venn diagrams showing numbers of differentially abundant negative ion mode metabolites by timepoint in inoculated samples (**E**), and control samples (**F**).



In positive ion mode, 12% of the metabolites enriched in inoculated samples had RMD values associated with phenolics and 23% were consistent with terpenoid glycosides. For both phenolics and terpenoid glycosides, the majority of these metabolites were significantly enriched at the T5 timepoint (Figure 4.13A). In positive ion, control samples, 14% of metabolites enriched in control samples had RMD values consistent with each phenolics and terpenoid glycosides. The majority of metabolites associated to both groups were differentially abundant at the T2 and T3 timepoints (Figure 4.13B). In negative ion mode, 16% of the metabolites that were significantly enriched in inoculated samples had RMD values consistent with phenolics, while 27% had values consistent with terpenoid glycosides. The metabolites that had RMD values

consistent with phenolics were primarily differentially abundant at T5, while those in the terpenoid glycoside range were from T2 and T5 (Figure 4.13C). In negative ion mode, 11% of metabolites which were significantly enriched in control samples had RMD values consistent with phenolics and 14% had values consistent with terpenoid glycosides. The majority of metabolites consistent with both groups were significantly enriched in control samples at T2 (Figure 4.13D).

Figure 4.13 - Histograms of RMD Distributions. Distributions of RMD values in positive mode for inoculated (**A**) and control (**B**) samples. Distributions of RMD values in negative mode for inoculated (**C**) and control (**D**) samples.

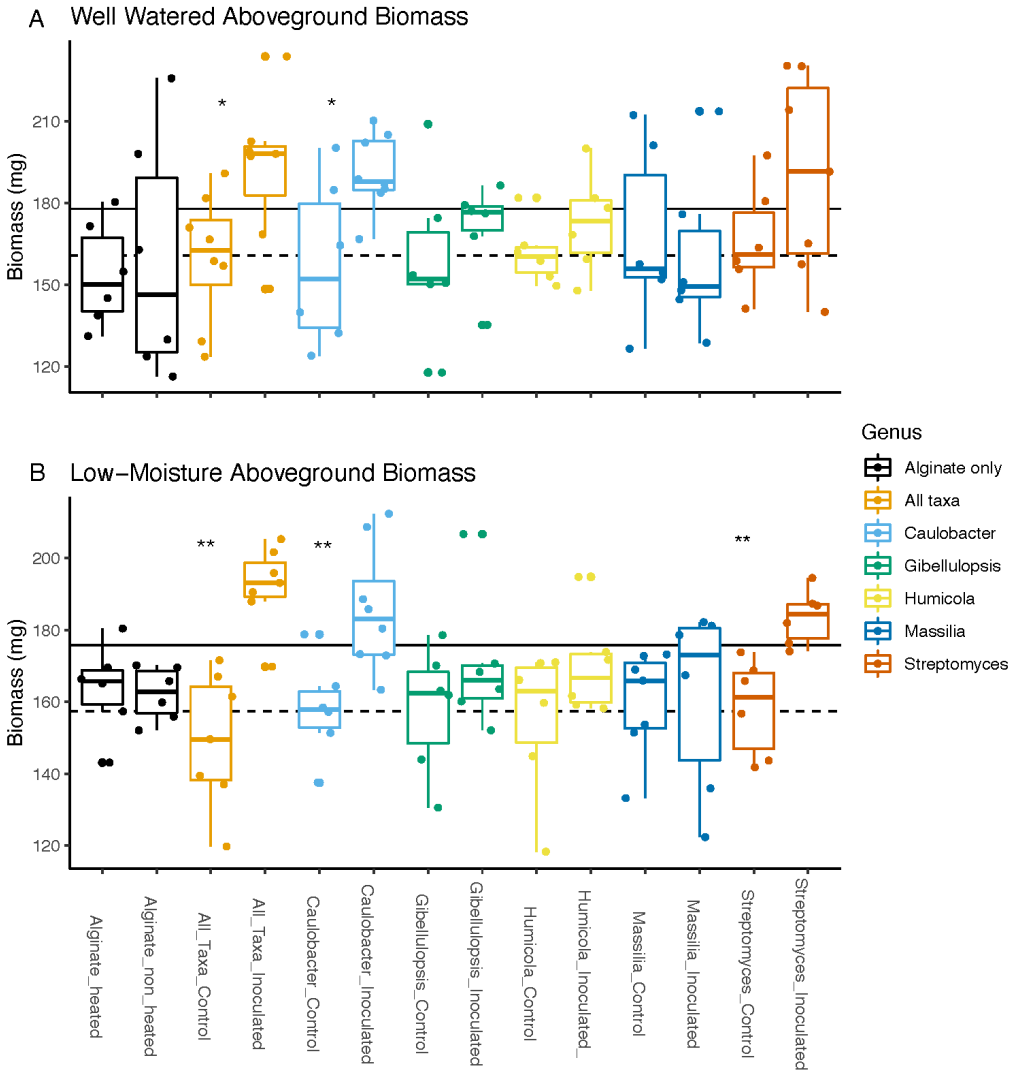


Follow-up Experiments and Individual Microbe Results

In well-watered conditions, each individual inoculant except for *Massilia* appeared to increase aboveground biomass compared to their respective controls. However, this difference was only significant for *Caulobacter* and for the inoculum mix. No significant differences were

found between alginate beads that were heated compared to those that were not (Figure 4.14A). Individual microbe results under low-moisture conditions followed a similar pattern to those under well-watered conditions. Plant biomass in *Streptomyces*, *Caulobacter*, and mixed inoculum treatments were greater in low-moisture conditions compared to respective controls (Figure 4.14B).

Figure 4.14 - Aboveground dry biomass of plants inoculated with individual microbes, combined inoculum, and alginate only controls. Results shown for well-watered (**A**), and low-moisture conditions (**B**). Comparisons are based on Wilcox ranked sign tests, an asterisk indicates a significant difference; * = $p \leq 0.05$, ** = $p \leq 0.01$. Horizontal dashed lines represent the average across all controls, solid line represents average of all inoculated samples



Discussion

Here I tested whether microbial hub taxa could mitigate soybean response to low-moisture stress. My hypotheses that hub-taxa will persist and impact the plant microbiome and phenotype in subtle but significant ways was primarily supported. ZOTUs matching all inoculated taxa (>99% similarity) except for *Humicola* sp. persisted in DNA and cDNA

amplicons at some level, although not in every sample. The *Humicola* inoculant appeared to disappear from most samples at later timepoints. However, assessing the exact persistence of inoculated taxa was difficult as the use of natural soil introduced taxa from the same genera to control roots. I found that the microbial hub taxa had a minor impact on the microbiome, but that a few taxa that were impacted are known to be important to plant health including AMF and *Bradyrhizobium*, which were only apparent through analysis of amplicons made from cDNA. This underlines the importance of assessing microbial activity alongside traditional amplicon sequencing efforts as important community changes may be missed with DNA amplicons. Microbial inoculation was also demonstrated to impact a variety of plant phenotypes including biomass, photosynthesis, and nodule number. RNA sequencing results supported phenotyping measurements and showed increased *Bradyrhizobium* activity through the demonstration of increased expression of nodulation related genes in the soybean host.

Inoculation did not have an obvious impact on microbiomes when assessed with DNA amplicons. This agrees with previous work which showed little substantial change in the rhizosphere microbiome following inoculation [153]. However, other studies have found microbiome changes following inoculation with *Pseudomonas* [163]. Although there was no obvious impact of inoculation on the microbiome, important functional taxa were impacted. Most obviously, three of the four *Bradyrhizobium* ZOTUs had higher cDNA:DNA ratios in inoculated samples compared to control samples, these differences were most obvious in early stages of low-moisture stress (T2). Changes in cDNA reflect microbial RNA differences and therefore are more likely to represent activity changes. This difference was not present in DNA amplicons, indicating the difference was likely related to *Bradyrhizobium* activity within the roots. This agrees with previous work which indicates that soil bacterial changes can alter the

colonization of *Bradyrhizobium* and *Sinorhizobium*. Additionally, this same work demonstrated that some *Bacillus* taxa directly promote *Sinorhizobium* growth [357]. Inoculation with the fungus, *Metarhizium*, was also demonstrated to increase abundance of *Bradyrhizobium* in the common bean rhizosphere [164]. Further work is needed to determine whether the increased activity of *Bradyrhizobium* demonstrated here was a direct effect of *Bradyrhizobium* growth promotion by the microbes or if the effect was mediated through the plant. Importantly, inoculation did not appear to substantially increase the abundance of the inoculated genera. This may be due to a lack of strain-level resolution in amplicons, making it difficult to assess the colonization of inoculated strains. Previous work has shown that inoculation with *Metarhizium* increases the abundance of *Bradyrhizobium* in common bean roots even though *Metarhizium* was not always significantly enriched in inoculated plant roots [164]. Follow-up work is needed in sterile soils or with fluorescently labelled inoculum members to directly assess the colonization of plants by inoculum members. These results underscore the importance of assessing microbial activity in addition to traditional amplicon sequencing in microbiome studies.

Several AMF ZOTUs had higher cDNA:DNA ratios at T1 in control samples compared the ratios of the same ZOTUs in inoculated samples. This is important as it has been demonstrated that earlier AMF colonization can lead to increased plant biomass, but differences in other microbes such as *Bradyrhizobium* may compensate for later AMF colonization [358]. However, ratios at later sampling points appear to demonstrate that levels of AMF activity became more similar between inoculated and control plants.

In addition to increased colonization and activity of *Bradyrhizobium*, it was clear that inoculation increased expression of soybean nodulation genes. This result is line with a metanalysis which demonstrated that co-inoculation of soybeans with *Bradyrhizobium* and other

plant growth promoting bacteria can increase nodule number and other plant phenotypes including root and shoot biomass [359]. Specifically, *Streptomyces* species, including *Streptomyces griseoflavus,* has been demonstrated to increase soybean nodulation and nitrogen fixation activity [360]. However, our result demonstrates a mixed inoculum community can maintain improvements of nodulation under low-moisture stress conditions. This is a crucial finding as low-moisture stress has been demonstrated to reduce nodulation and nitrogen fixation activities in soybeans [306, 307].

Previous work has demonstrated that the presence of Nod factors from rhizobia stimulate AMF colonization [361]. However, it was later demonstrated that this result may depend on specific factors such as simultaneous presence of the Nod factors and AMF colonizers as well as a co-localization of the two. This was demonstrated using a split root design where colonization by AMF on one half of the roots reduced nodule formation in the other half upon later exposure to *Sinorhizobium* [362]. This data appears to be in agreement with trends in cDNA: DNA amplicon ratios which showed early colonization and likely higher activity levels of *Glomus* in control samples, but delayed colonization and reduced nodulation gene expression compared to inoculated plants. Further work is needed to determine whether specific inoculant members used in this study reduce colonization by AMF taxa, and whether the reduction in AMF is related to increased *Bradyrhizobium* colonization.

GO term analysis revealed that many of the most upregulated functions in inoculated plants were related to the observed increase in nodulation related gene expression. In addition, there was upregulation of various transport functions such as transmembrane transport and endocytic vesicle formation. These terms may be related to nodulation such as through the endocytosis of *Bradyrhizobium* [363], but may also be caused by the need to absorb nutrients

from sandy soil as some plants showed foliar signs of low-nutrient stress. Inoculated plants were also enriched in terms related to purine metabolism at T2 and T3, these terms included "*de-novo* IMP biosynthetic process" and "purine metabolism". These terms are crucial as IMP is a precursor to purines and purines are degraded into ureides, which is the form in which fixed nitrogen is transferred to aboveground plant tissue [364, 365]. This enrichment indicates that fixed nitrogen is likely being transferred at an increased level to aboveground tissues of inoculated plants. Additionally, there appeared to be more consistency in upregulated GO terms among inoculated plants with several GO terms shared across multiple timepoints. Additionally, there were several abundant GO terms related to stress in the controls, including responses to heat stress and abiotic stimulus. This indicates that control plants appear to be experiencing greater levels of stress possibly due to a reduction in nitrogen fixation activity resulting in increased nutrient stress.

In addition to impacting plant gene expression, inoculation with hub microbes impacted the root metabolome. This is in line with previous work which has demonstrated that microbial inoculation alters root metabolites and that exposure to different microbiomes alters the composition of root exudates [366, 367]. Additionally, this alternation may depend on stress being experienced by the host plant [366]. Soyasaponins were previously found to be enriched in areas of the root infected by *Bradyrhizobium* [368]. Soyasaponins are triterpenoid glycosides, and RMD values consistent with terpenoid glycosides appeared to be enriched in inoculated samples [369]. Control plant samples were generally enriched in metabolites with larger RMD values that are consistent with lipids [354]. This result appears to be consistent with lower *Bradyrhizobium* colonization as lipids have been previously shown to be more abundant in uninfected areas of roots [368]. More specific annotations will determine which metabolites are

primarily responsible for these differences.

In addition to altering plant gene expression and microbial activity, inoculation also impacted various plant phenotypes, particularly aboveground phenotypes. This impact is likely related to increased nodulation and nitrogen fixation in inoculated plants. These increases in seedling dry biomass and height may lead to increased yield as increases in plant height and seed/seedling vigor measures have been shown to be associated with yield [370, 371]. Inoculated samples also had significantly increased values for the Phi2 measurement of photosynthesis which demonstrates an increased proportion of incoming light going to photosystem II, corresponding to an increased percentage of light which can be converted to sugars [372]. This result, along with increased *Bradyrhizobium* and nodulation activities is in line with previous work demonstrating that rhizobial inoculation enhanced photosynthesis [373]. Interestingly, outside of root nodule numbers, there were no consistent differences in root phenotype measurements assessed with image analysis. This was somewhat unexpected as prior work has demonstrated that plant growth-promoting bacteria including rhizobia can alter root architecture via increases in branching, elongation, and other metrics [374–376]. However, it may be that the root systems were not developed enough in young seedlings to see differences. This underscores the importance of assessing impacts on inoculation throughout the entire plant life cycle in future work.

The only two microbes that significantly increased plant dry biomass when inoculated alone under well-watered or low-moisture conditions were *Streptomyces* sp. and *Caulobacter segnis*. However, under low-moisture stress conditions the impact of the combined inoculum was greater than the impact of individual microbes. The impact of *Streptomyces* inoculation is in line with previous results which have demonstrated that some *Streptomyces* are drought responsive

and can enhance nodulation of soybeans [360, 377]. Plant beneficial impacts of *Caulobacter* strains are understudied, but a *Caulobacter* strain which was closely related to *C. segnis* was shown to promote growth of *Arabidopsis* [332]. More specifically, it has been demonstrated that redox genes were related to the *Arabidopsis* growth promotion in strains of *Caulobacter* including *C. segnis*. When genes related to redox functions were knocked out, plant growth promotion disappeared [378]. Interestingly, genome sequencing revealed that the strain of *C. segnis* used in this study is a riboflavin auxotroph (Appendix C). This may indicate that this strain relies on other microbes or the host plant for riboflavin. Previous work has demonstrated that the production of riboflavin appears to be important in maintaining the relationship between a *Bradyrhizobium* strain and its plant host. Particularly, the production of a riboflavin biosynthesis pathway protein is required for maintaining long-term symbioses, but not important for riboflavin biosynthesis when the bacterium is in a free-living state [379]. The production of riboflavin by *Bradyrhizobium* could form the basis of an interaction between *Bradyrhizobium* and the *Caulobacter segnis* strain utilized in this study, which may be associated with increased *Bradyrhizobium* colonization and activity within nodules. Further studies on the interactions between *Caulobacter, Bradyrhizobium*, soybean and other microbiome taxa will be crucial to determining the basis for the increased root nodulation and aboveground biomass that resulted from *Caulobacter segnis* and hub-consortia inoculations.

Conclusions and Future Directions

In this study, I demonstrated that inoculation with five hub taxa led to increased plant growth under low-moisture and well-watered conditions. Increased colonization by *Bradyrhizobium*, increased expression of nodulation-related genes, and a possible signal of nodulation related shifts in the metabolome seem to indicate that this plant growth promotion

was likely related to increased nitrogen fixation activity. *Caulobacter segnis* and *Streptomyces* appear to be the main driving forces behind this phenotype, as results were replicated through single strain inoculations with these two taxa. In the case of *C. segnis*, a relationship may be formed with *Bradyrhizobium* based on riboflavin autotrophy in *C. segnis* which could be complemented by *Bradyrhizobium*. However, the need of exogenous riboflavin in *C. segnis* could be complimented by other microbes or the host plant. Future studies on interactions between these hub taxa and *Bradyrhizobium* can be used to assess if the impact of hub taxa is directly related to microbial interactions or if the impact is mediated through the plant. Additionally, further work will be needed to determine if impacts of hub taxa inoculation persist throughout the soybean growing season, or if they are confined to seedlings.

Chapter 5

Synthesis

Objectives

This dissertation was composed of three primary objectives: 1) to assess the impact of three agricultural managements on the soybean leaf, stem, root, and soil microbiome (fungi and prokaryotes); 2) to assess the impact of foliar fungicide application on the microbiome of maize and soybean, with particular attention to the microbiome of leaves; and 3) to assess the impact of inoculating soybean plants with hub microbial taxa identified from co-occurrence networks of no-till soybean roots. The detailed results for these three objectives are contained in chapters 2-4 of this dissertation. A brief synthesis of the major findings in each objective as well as future directions for each objective will be provided here.

Impact of Agricultural Management on the Soybean Microbiome

In chapter 2, I assessed how three agricultural managements (conventional, no-till, and organic) impacted the soybean associated microbiome throughout the plant and the growing season. This work revealed that agricultural management impacts fungal and prokaryotic beta diversity of communities associated with all assessed organs of the soybean plant, and this effect persisted throughout the growing season. This finding was novel as previous work demonstrated that agricultural management impacts soil used to grow soybeans but did not assess the microbiome of the plant itself. Additionally, since organic management was planted with a different genotype, no-till and conventional results were analyzed separately. This analysis demonstrated that tillage impacted the soybean microbiome throughout the plant and the growing season.

The results of this study demonstrate that no-till management may cause an increase in the relative abundances of plant beneficial taxa including AMF and *Bradyrhizobium*. These microbial benefits may provide additional motivation for farmers to employ no-till agriculture in

addition to other benefits such as increased soil health, erosion control, and increased drought tolerance. However, since this study employed amplicon sequencing, further work will be needed to ensure that these differences are due to active microbes and not relic or dead DNA. However, my work on the impact of agricultural management on the microbiome is foundational and will provide a basis for studies on the functional consequences of these changes. Additionally, the culture library of soybean-associated microbes will be a resource for studies on soybean-microbe interactions, as was done in chapter four.

This work could be improved by including functional assessments of both the plant host and the associated microbiome. For the plant host, this should include phenotyping of host plants under different management systems and associating this phenotyping data with abundances of microbial taxa using random forest and other modelling methods. Such phenotyping efforts will be particularly valuable if they can be used in a high-throughput manner in field systems [380]. Additionally, transcriptomics and metabolomics could be used to assess the impact of management on plant functioning. From the microbial side, there are many methods that more closely represent microbial function. This could include extracting RNA to assess cDNA amplicons which likely represent a truer reflection of the active community. Additionally, sequencing advances including long-read amplicon sequencing will allow for greater genetic resolution which will provide greater resolution into which species or strains are impacted by management [381]. This will allow the assessment of microbial changes to become part of a broader toolkit used to make agricultural management decisions.

Non-Target Impacts of Foliar Fungicide Applications

In chapter 3, I assessed the impacts of foliar fungicide application on the phyllosphere microbiomes of maize and soybean. Particularly, this study focused on non-target impacts as

there was no obvious pathogen pressure detected in the treated plots. This work revealed that foliar fungicide applications impacted fungal classes such as Dothideomycetes, which contain important soybean pathogens. However, fungicide applications also had a substantial, non-target impact on Tremellomycetes. This class includes many dimorphic species and phyllosphere-residing yeasts are thought to be potentially plant beneficial through the production of carotenoid compounds, which could also protect other phyllosphere microbes or host cells from UV stress [267]. Phyllosphere taxa that were especially impacted by fungicides were concentrated in Bulleribasidiaceae, indicating their sensitivity to fungicide disturbance. This study demonstrated that there were no direct impacts of phyllosphere fungicide applications on soil and communities, nor on phyllosphere bacterial communities. However, in a novel finding, random forest modelling demonstrated that changes in phyllosphere fungal communities may have an indirect impact on phyllosphere prokaryotic communities. These interactions would not be unexpected as the phyllosphere is a dynamic complex of microbial communities that would be expected to respond to changes in niche-space caused by disturbance[288]. Further microbial interaction studies are needed to determine if there are direct interactions between identified bacteria and phyllosphere yeasts.

Additionally, results from this study indicate that the responses to fungicide disturbance differed by crop and agricultural management. Particularly, soybean fungal communities were more highly impacted by fungicide application, but this may have been due to an additional mode of action (DMI) while maize only received a single mode of action (QoI). Effects of fungicides on maize may have been comparatively smaller because the leaves that were directly sprayed had senesced before later sampling, whereas for soybean the sprayed leaves could be sampled repeatedly. Fungal communities in no-till leaves appeared to experience greater

recovery in the study period compared to those in conventionally managed plots. This is a novel finding that indicates that no-till agriculture can increase the recovery of fungal communities following disturbance. This work will lay the foundations for future studies assessing non-target impacts of agricultural chemistries, and the mechanisms and benefits of no-till agriculture to recovery from disturbance.

In addition to assessing the role of no-till management in disturbance recovery, further work should assess the function of impacted basidiomycete yeasts in the plant phyllosphere. Ideally, this work would be done in fields where pathogen pressure is present as the impact of fungicides is likely to outset their costs and off-target impacts when pathogens are highly present [5]. However, in the absence of pathogens it has been shown that fungicide applications are not always economically feasible [6]. This may be particularly true if potentially plant-beneficial yeasts are demonstrated to increase plant biomass or yield. Fungicides will need to continue to be applied to maintain crop yields in the face of pathogen pressure. However, agricultural strategies such as no-till management could be paired with fungicide application to increase recovery of beneficial yeasts. Additionally, other strategies could be paired with fungicide applications to minimize off-target damage. For example, phyllosphere sprays of beneficial microbes such as *Bacillus amyloliquefaciens* have been demonstrated to confer biocontrol activities [382]. This strategy could be used to re-apply phyllosphere yeasts in the weeks following a fungicide application. This combination would allow for an agricultural management system which suppressed the proliferation of phyllosphere pathogens while maintaining the benefits provided by phyllosphere yeasts.

Hub Microbial Inoculation of Glycine max Alters Holobiont Response to Stress

In chapter 4, I assessed the impact of inoculating soybean plants with hub microbes identified from co-occurrence networks of no-till soybean roots assessed in chapter 1. For this assessment, I combined various phenotyping and molecular methods to assess the impact of inoculation on both the plant host and the associated microbiota. Inoculation had an obvious impact on plant growth, and several phenotype measurements including aboveground area, aboveground biomass, nodule numbers, and photosynthesis measurements differed between control and inoculated samples. Additionally, inoculation had a significant effect on gene expression of host plants throughout a low moisture stress period. Primarily, the major category of gene expression that was upregulated in inoculated samples was related to nodulation. The genes most tightly associated to nodulation were consistently upregulated in inoculated samples. This demonstrated that gene expression results mirrored phenotyping results showing higher biomass and nodule numbers in inoculated samples. Subsequent follow-up experiments confirmed the initial results and demonstrated that the *Caulobacter* and *Streptomyces* inoculants were each able to confer an increase in plant biomass. Although the impact on nodule numbers in follow-up experiments was unclear due to fewer replicates and smaller nodules, making them more difficult to count.

Inoculation did not appear to substantially alter the root microbiome, but several important functional taxa were impacted by inoculation. These differences were especially apparent when the root microbiome was assessed using cDNA to reveal a more activity community than traditional DNA amplicon sequencing. Most notably, *Bradyrhizobium* had higher cDNA:DNA ratios earlier in the cDNA from inoculated samples. On the contrary, in fungal communities, AMF taxa belonging to *Glomus* appeared to have higher ratios in control

samples at the first sampling point.

Together these results demonstrate that inoculation with hub microbes can be utilized to ameliorate the impacts of low-moisture stress on soybean seedlings. This work could be complimented by future studies on interactions between hub taxa and other microbes. Especially, to follow-up on results from this study, it will be important to assess how microbes used in inocula interact with both *Bradyrhizobium* and *Glomus*, and soybean. For example, some microbial taxa including *Bacillus* species have been demonstrated to directly enhance the growth of *Bradyrhizobium* [357]. However, another possibility is that this effect is mediated through the plant by making host plants more susceptible to infection by *Bradyrhizobium*. Interaction studies with *Bradyrhizobium* will help begin to disentangle these possibilities. It is also possible that the earlier colonization of control plants by AMF taxa reduced later colonization by *Bradyrhizobium* as has been previously demonstrated [362]. Further microbial interaction studies will be important for assessing if impacts seen here are direct plant growth promoting effects of hub taxa or if they are through microbe-microbe interactions.

Results from inoculation experiments were repeatable in growth-chamber and greenhouse settings. Both settings are useful for studying microbial inoculation. Growth chambers provide an ideal setting due to exact temperature and environmental control. This would allow for the addition of heat to low-moisture stress in future studies of multiple stressors associated with drought. Alternatively, studying microbial inoculation in greenhouses exposes plants to more variation in factors such as light due to changes in cloud cover. This means that greenhouses may provide a more accurate representation of field conditions. Testing inoculum members in multiple settings is one way to assess their potential success before deploying microbial inoculants in the field.

However, further work will be required to assess how these taxa would perform in a field setting. For example, it is clear that inoculants increase seedling aboveground biomass, but it will be important to assess if this increase in seedling biomass correlates with increases in grain yield. For some crops, there does appear to be a relationship between seedling vigor measures such as dry weight and final yield [370]. Plant height measured during soybean reproductive stages was associated with increased yield, therefore if height differences from seedling stages associated with inoculation persist, yield may be expected to increase [371]. Although greenhouse and growth chamber experiments are crucial steps for testing microbial inoculants, it will also be important to test the concept of hub microbes as inoculants in field settings.

Conclusions

Interactions between plants and microbes have long been known to be crucial to the health of the host plant. This is especially true of soybean and other legumes due to the nitrogen fixation benefit provided to the plants by root nodulating Rhizobia. Like all other plants, soybeans interact with a wide diversity of bacteria and fungi throughout the plant, but these interactions are complex and less well characterized compared to relationships with nitrogen fixing bacteria in root nodules. There are various important questions about the functional and taxonomic diversity of these relationships, but one important outstanding goal is to determine the impact of modern agricultural management strategies on soybean-associated microbiomes. Throughout this dissertation I have assessed the impacts of various agricultural management strategies on the soybean microbiome including mechanical tillage, chemical fungicides, and biological consortia.

My dissertation research demonstrated that tillage regime, which is typically selected based off soil moisture, reductions in soil erosion, or reductions in fuel input costs also impacts

the bacterial and fungal microbiome in the soil and throughout the soybean holobiont. My dissertation research provides a foundation for understanding impacts on the microbiome into decisions of which tillage regime to utilize. Incorporating the microbiome into management decisions will also become increasingly streamlined as sequencing methods continue to improve. In addition to tillage regime, foliar fungicide applications are a management technique which can be expected to impact microbial communities by creating a disturbance to fungal communities. Research on soybean and maize microbiomes demonstrated that fungicide applications were particularly detrimental to phyllosphere yeasts. Although, I expect that fungicides will continue to be an important tool for controlling foliar fungal pathogens, the impact on these yeasts should be considered. Studies to assess the plant benefit provided by phyllosphere yeasts and techniques for maintaining these or other microbes in agricultural ecosystems will become more commonplace.

Finally, another common form of agricultural management is to use microbes as inoculants. This has commonly been used as a technique for biocontrol of pathogens but is becoming increasingly common for a variety of abiotic stresses as well. I hypothesized that bioinformatically identified hub taxa would be useful as microbial inoculants as their correlations with other microbes may predict a role in structuring the microbiome. I found that inoculations composed of hub taxa provided a benefit to soybean plants with and without low-moisture stress, and that part of this benefit may have been from an increase in recruitment of beneficial *Bradyrhizobium.* Future analyses will determine if hub taxa create microbial co-occurrence networks which are more stable through time, which may indicate an increase in microbial stability provided by hub taxa inoculation. I expect that microbial inoculations which capitalize on microbe-microbe interactions will become increasingly common as this type of inoculation

may allow for greater flexibility in microbial recruitment compared to inoculation with taxa which are selected for a single plant growth promoting trait. Taken together, these results demonstrate that three diverse agricultural management strategies all impact the soybean associated microbiome. Due to this impact, it is crucial that decisions on agricultural management strategies consider the plant holobiont as a whole, inclusive of the microbial component.

APPENDICES

APPENDIX A: Microbial Isolations

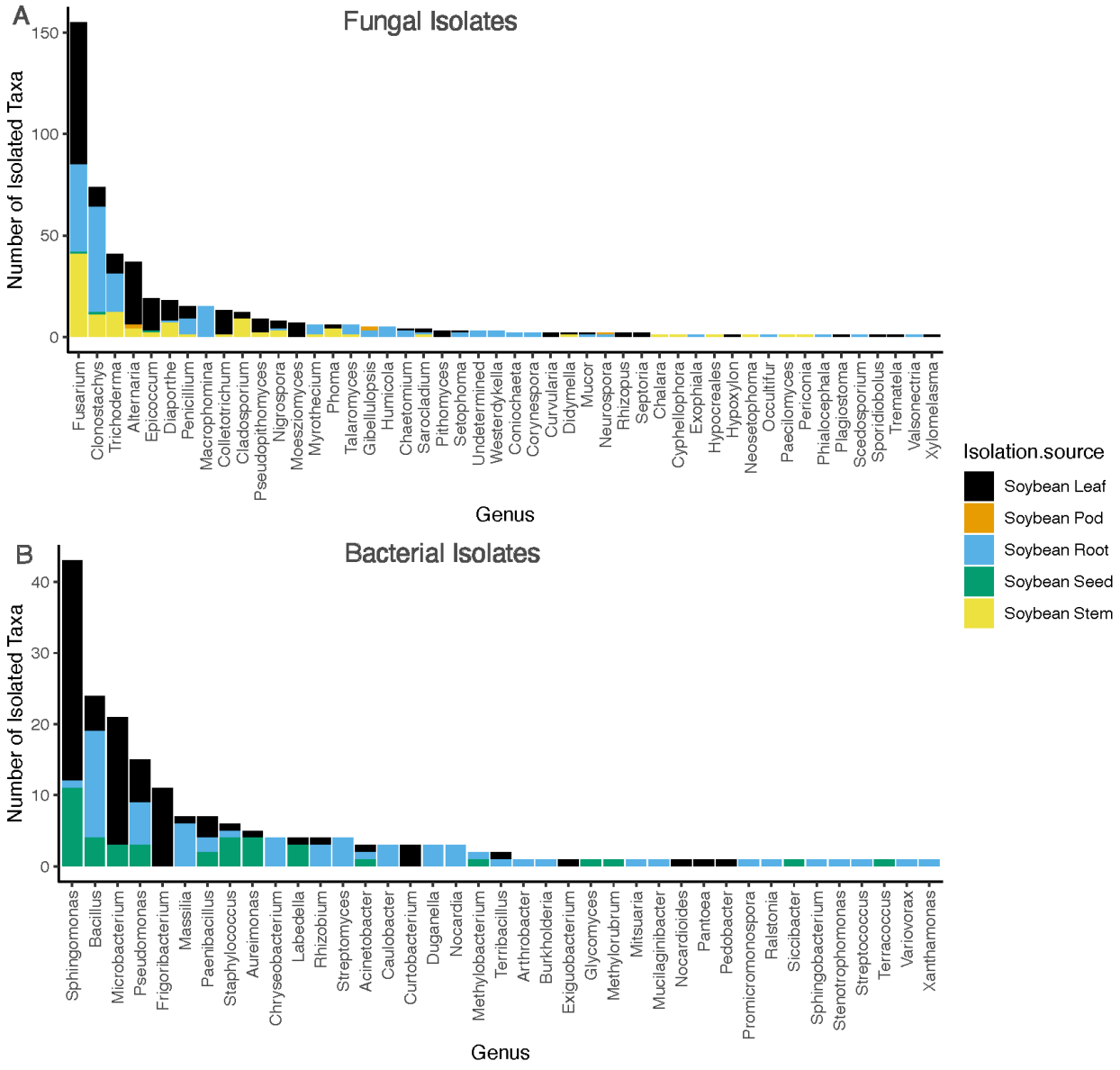
Along with soybean samples taken in chapter two for next-generation sequencing, soybean roots, leaves, stems, and seed pods were collection for isolation and culturing of bacteria and fungi. In addition to plants sampled for next generation sequencing as described in chapter 2, three plants were taken for microbial isolations. Fungal endophytes were isolated from surface sterilized leaves, stems, roots and seed pods as previously described [4]. Briefly, leaves and pods were sampled using a 6 mm hole punch; seven punches were taken from various leaves on each plant and the seven punches from each plant. Roots were processed in the same way, but instead of a hole punch, 1 cm samples of fine roots were taken using flame sterilized scissors. Tissues were then surface sterilized by soaking in a mixture of .5% sodium hypochlorite and .1% tween 20 for 2 minutes, followed by 2 minutes in 70% ethanol, and a rinse with sterile distilled water. Surface sterilized tissues were placed on petri dishes containing 2% MEA amended with rifampicin and chloramphenicol to prevent bacterial growth (two pieces of plant tissue per plate). As fungi emerged from plant tissues, single isolates were transferred to fresh MEA plates. Each isolate was transferred three times to obtain a pure culture.

To isolate endophytic bacteria, plants were sampled, and tissues were surface sterilized as described for fungi. Approximately 100 mg of surface sterilized plant tissue was then ground with a sterile micro pestle in eppendorf tubes containing .9 ml of sterile distilled water. Leaf and pod samples were then serially diluted to a level of 10^{-5} or 10^{-6} and root samples were serially diluted to a level of 10^{-7} or 10^{-8} and 100 ul of each dilution was spread onto petri dishes containing R2A agar or King B agar. After two days of growth at room temperature, single colonies were picked and struck onto fresh plates. Colonies were chosen based off selection of diverse macro morphologies.

DNA was extracted from fungal isolates by removing a small piece of fungal mycelium with flame-sterilized tweezers and placing the tissue into 20 ul of premade extraction solution (ES) in PCR tubes. This solution was then heat lysed by heating to 95c for 10 minutes. Following heat lysis, 60 uL of bovine serum albumin (BSA) was added. Two ul of this solution was then used as template in PCR reactions with the ITS 1F and LR3 primer set [185, 383]. Bacterial 16S sequences from isolate were retrieved by colony PCR with 27F and 1492R primers on single colonies from each isolate [384]. Following PCR, amplification was assessed using gel electrophoresis, and samples which were successfully amplified were sanger sequenced with the forward primer at the MSU genomics core. Following sequencing, isolates were identified using the RDP classifier [385]

Fungal isolations resulted in 820 cultures, from these cultures, 503 ITS sequences were generated using sanger sequencing, representing 47 fungal genera Bacterial isolations resulted in 415 pure cultures. From these cultures, 193 were identified as belonging to 39 genera using 16S sanger sequencing. Fungal sequences are available on genbank under the following accession numbers: MT557064-MT557566. Bacterial sequences are available on genbank under the following accession numbers: MT653370-MT653562.The most prevalent genus among fungal isolates was *Fusarium* (155 isolates) followed by *Clonostachys* and *Trichoderma*. Many of the fungal genera were isolated from multiple parts of the plant, but some genera such as *Macrophomina* were exclusive to the roots (Figure A.1A). The most prevalent bacterial genus among isolates was *Sphingomonas* (43 isolates) followed by *Bacillus* and *Microbacterium*. Similarly, to fungal isolates, many genera were isolated from multiple plant tissues. However, the *Frigoribacterium* genus which was prevalent among isolates was only isolated from soybean leaves.

Figure A.1 - Summary of microbial isolates collected from soybeans. Color represents the isolation source of fungal isolates (**A**) and bacterial isolates (**B**).



APPENDIX B: Fungicide Supplementary Material

Table B.1 - Differentially Abundant Phyllosphere Fungi by Fungicide Treatment

OTU	Growth Stage	Crop	Treatment	Difference in mean % RA	BestMatch	Fungicide abundance response
OTU65	V8	Maize	Conventional	-2.29087%	Bullera crocea	Decrease
OTU65	V8	Maize	No-till	-1.90887%	Bullera crocea	Decrease
OTU83	V8	Maize	No-till	-1.07769%	Vishniacozyma globispora	Decrease
OTU82	V8	Maize	No-till	-0.96538%	Filobasidium oeirense	Decrease
OTU97	V8	Maize	No-till	-0.91537%	Filobasidium sp.	Decrease
OTU97	V8	Maize	Conventional	-0.72116%	Filobasidium sp.	Decrease
OTU34	V8	Maize	Conventional	-0.53438%	Hannaella oryzae	Decrease
OTU1340	V8	Maize	No-till	-0.44156%	Filobasidium wieringae	Decrease
OTU130	V8	Maize	No-till	-0.43680%	Vishniacozyma victoriae	Decrease
OTU2177	V8	Maize	Conventional	-0.33341%	Vishniacozyma sp.	Decrease
OTU155	V8	Maize	Conventional	-0.21524%	Dioszegia sp.	Decrease
OTU118	V8	Maize	Conventional	-0.17354%	Helotiales sp.	Decrease
OTU587	V8	Maize	No-till	-0.16469%	Limonomyces sp.	Decrease
OTU162	V8	Maize	Conventional	-0.16119%	Pleosporales sp.	Decrease
OTU116	V8	Maize	No-till	-0.15336%	Leptospora sp.	Decrease
OTU156	V8	Maize	Conventional	-0.13986%	Filobasidium sp.	Decrease
OTU953	V8	Maize	No-till	-0.12968%	Fibulobasidium inconspicuum	Decrease
OTU421	V8	Maize	Conventional	-0.11637%	Septoriella hirta	Decrease
OTU251	V8	Maize	Conventional	-0.10824%	Papiliotrema frias	Decrease
OTU278	V8	Maize	No-till	-0.10284%	Taphrina sp	Decrease
OTU277	V8	Maize	Conventional	-0.09981%	Coprinellus sp.	Decrease
OTU526	V8	Maize	Conventional	-0.09965%	Holtermanniella festucosa	Decrease
OTU137	V8	Maize	No-till	-0.09663%	Phaeosphaeria sp	Decrease
OTU821	V8	Maize	No-till	-0.09243%	Genolevuria amylolytica	Decrease
OTU134	V8	Maize	No-till	-0.09132%	Bulleribasidium oberjochense	Decrease

Table B.1 (cont'd)

OTU118	V8	Maize	No-till	-0.08603%	Helotiales sp.	Decrease
OTU210	V8	Maize	Conventional	-0.07838%	Alternaria rosae	Decrease
OTU274	V8	Maize	No-till	-0.06528%	Stemphylium sp.	Decrease
OTU254	V8	Maize	Conventional	-0.06453%	Erythrobasidium yunnanense	Decrease
OTU171	V8	Maize	No-till	-0.06026%	Phaeosphaeriaceae sp.	Decrease
OTU487	V8	Maize	No-till	-0.05510%	Phomatospora biseriata	Decrease
OTU368	V8	Maize	No-till	-0.04857%	Xylariales sp.	Decrease
OTU197	V8	Maize	Conventional	-0.04600%	Ophiognomonia sp.	Decrease
OTU664	V8	Maize	No-till	-0.04144%	Agaricomycetes sp.	Decrease
OTU692	V8	Maize	No-till	-0.03397%	Stagonosporopsis sp.	Decrease
OTU678	V8	Maize	Conventional	-0.02891%	Erysiphe sp	Decrease
OTU3375	V8	Maize	No-till	-0.02888%	Dioszegia athyri	Decrease
OTU433	V8	Maize	No-till	-0.02881%	Venturia inaequalis	Decrease
OTU2860	V8	Maize	Conventional	-0.02795%	Cladosporium grevilleae	Decrease
OTU928	V8	Maize	No-till	-0.02602%	Phanerochaete sp.	Decrease
OTU309	V8	Maize	No-till	-0.02492%	Pyrenophora tritici-repentis	Decrease
OTU259	V8	Maize	No-till	-0.02468%	Tubeufiaceae sp.	Decrease
OTU483	V8	Maize	Conventional	-0.02338%	Diaporthe sp.	Decrease
OTU101	V8	Maize	No-till	-0.02266%	Ascomycota sp.	Decrease
OTU2950	V8	Maize	No-till	-0.02258%	Ascochyta sp.	Decrease
OTU1626	V8	Maize	No-till	-0.02218%	Meripilaceae sp.	Decrease
OTU421	V8	Maize	No-till	-0.02193%	Septoriella hirta	Decrease
OTU136	V8	Maize	Conventional	-0.02103%	Aureobasidium sp.	Decrease
OTU836	V8	Maize	No-till	-0.01994%	Trametes trogii	Decrease
OTU309	V8	Maize	Conventional	-0.01870%	Pyrenophora tritici-repentis	Decrease
OTU1324	V8	Maize	No-till	-0.01623%	Physalacriaceae sp.	Decrease
OTU249	V8	Maize	Conventional	-0.01581%	Phaeosphaeria sp.	Decrease
OTU531	V8	Maize	No-till	-0.01503%	Helicoma sp.	Decrease
OTU904	V8	Maize	No-till	-0.01338%	Diaporthe sp.	Decrease
OTU303	V8	Maize	No-till	-0.01319%	Dissoconium eucalypti	Decrease
OTU7134	V8	Maize	No-till	-0.01278%	Filobasidium floriforme	Decrease
OTU643	V8	Maize	Conventional	-0.01227%	Tilletiopsis washingtonensis	Decrease

Table B.1 (cont'd)

OTU366	V8	Maize	No-till	-0.01225%	Leptospora rubella	Decrease
OTU1425	V8	Maize	No-till	-0.01073%	Kondoa yuccicola	Decrease
OTU1242	V8	Maize	No-till	-0.01023%	Agaricomycetes sp.	Decrease
OTU595	V8	Maize	No-till	-0.00875%	Arthrocatena tenebrio	Decrease
OTU483	V8	Maize	No-till	-0.00852%	Diaporthe sp.	Decrease
OTU524	V8	Maize	No-till	-0.00786%	Dissoconium aciculare	Decrease
OTU1504	V8	Maize	Conventional	-0.00774%	Ceratobasidiaceae sp.	Decrease
OTU17	V8	Maize	Conventional	-0.00745%	Tilletiopsis washingtonensis	Decrease
OTU5816	V8	Maize	No-till	-0.00646%	Pleosporales sp.	Decrease
OTU1145	V8	Maize	No-till	-0.00554%	Xylodon erastii	Decrease
OTU5990	V8	Maize	No-till	-0.00461%	Phaeosphaeriaceae sp.	Decrease
OTU3217	V8	Maize	No-till	-0.00426%	Lophiostomataceae sp.	Decrease
OTU5120	V8	Maize	Conventional	-0.00356%	Ceratobasidiaceae sp.	Decrease
OTU245	V8	Maize	No-till	-0.00288%	Occultifur sp	Decrease
OTU2000	V8	Maize	Conventional	0.02734%	Schizoporaceae sp.	Increase
OTU785	V8	Maize	Conventional	0.03981%	Trichaptum biforme	Increase
OTU1318	V8	Maize	No-till	0.05419%	Sporobolomyces sp	Increase
OTU382	V8	Maize	No-till	0.35391%	Chytridiomycota sp.	Increase
OTU776	V8	Maize	No-till	0.47142%	Sporormiella leporina	Increase
OTU489	V8	Maize	Conventional	0.53590%	Preussia tetramera	Increase
OTU13	R4	Soy	Conventional	-4.73367%	Hannaella coprosmae	Decrease
OTU17	R4	Soy	Conventional	-3.15419%	Tilletiopsis washingtonensis	Decrease
OTU34	R4	Soy	Conventional	-2.23774%	Hannaella oryzae	Decrease
OTU643	R6	Soy	No-till	-2.11519%	Tilletiopsis washingtonensis	Decrease
OTU34	R6	Soy	No-till	-2.08712%	Hannaella oryzae	Decrease
OTU13	R6	Soy	No-till	-1.96218%	Hannaella coprosmae	Decrease
OTU643	R6	Soy	Conventional	-1.95997%	Tilletiopsis washingtonensis	Decrease
OTU643	R4	Soy	Conventional	-1.09464%	Tilletiopsis washingtonensis	Decrease

Table B.1 (cont'd)

OTU89	R6	Soy	No-till	-1.04964%	Kondoa sp	Decrease
OTU643	R4	Soy	No-till	-0.92621%	Tilletiopsis washingtonensis	Decrease
OTU27	R4	Soy	Conventional	-0.88111%	Hannaella zeae	Decrease
OTU89	R6	Soy	Conventional	-0.83494%	Kondoa sp	Decrease
OTU120	R4	Soy	Conventional	-0.56930%	Pleosporales sp.	Decrease
OTU134	R6	Soy	No-till	-0.53139%	Bulleribasidium oberjochense	Decrease
OTU52	R4	Soy	Conventional	-0.50497%	Dioszegia sp.	Decrease
OTU154	R6	Soy	No-till	-0.49905%	Leptospora sp	Decrease
OTU130	R4	Soy	Conventional	-0.45301%	Vishniacozyma victoriae	Decrease
OTU502	R6	Soy	Conventional	-0.42456%	Fungi sp.	Decrease
OTU134	R4	Soy	Conventional	-0.41925%	Bulleribasidium oberjochense	Decrease
OTU134	R4	Soy	No-till	-0.39088%	Bulleribasidium oberjochense	Decrease
OTU35	R6	Soy	No-till	-0.35911%	Hannaella luteola	Decrease
OTU6420	R6	Soy	No-till	-0.34788%	Coniothyrium sp	Decrease
OTU155	R6	Soy	Conventional	-0.34465%	Dioszegia sp.	Decrease
OTU95	R4	Soy	No-till	-0.31738%	Neosetophoma rosigena	Decrease
OTU119	R4	Soy	No-till	-0.29512%	Symmetrospora sp.	Decrease
OTU90	R4	Soy	Conventional	-0.24354%	Sporobolomyces phaffii	Decrease
OTU52	R6	Soy	No-till	-0.24351%	Dioszegia sp.	Decrease
OTU27	R4	Soy	No-till	-0.21907%	Hannaella zeae	Decrease
OTU119	R6	Soy	No-till	-0.21377%	Symmetrospora sp.	Decrease
OTU502	R6	Soy	No-till	-0.19236%	Fungi sp.	Decrease
OTU32	R4	Soy	No-till	-0.17986%	Symmetrospora coprosmae	Decrease
OTU120	R6	Soy	Conventional	-0.15907%	Pleosporales sp.	Decrease
OTU119	R4	Soy	Conventional	-0.15562%	Symmetrospora sp.	Decrease
OTU155	R4	Soy	No-till	-0.15023%	Dioszegia sp.	Decrease
OTU89	R4	Soy	No-till	-0.12973%	Kondoa sp	Decrease
OTU278	R6	Soy	No-till	-0.12902%	Taphrina sp	Decrease
OTU6	R6	Soy	No-till	-0.11617%	Mycosphaerella tassiana	Decrease
OTU2746	R6	Soy	Conventional	-0.11591%	Dioszegia sp	Decrease
OTU6350	R4	Soy	Conventional	-0.11100%	Hannaella zeae	Decrease
OTU267	R6	Soy	Conventional	-0.10960%	Cyphellophora sp.	Decrease

Table B.1 (cont'd)

OTU2945	R4	Soy	Conventional	-0.10629%	Vishniacozyma victoriae	Decrease
OTU2746	R4	Soy	No-till	-0.09988%	Dioszegia sp	Decrease
OTU89	R4	Soy	Conventional	-0.09438%	Kondoa sp	Decrease
OTU267	R4	Soy	Conventional	-0.09341%	Cyphellophora sp.	Decrease
OTU258	R4	Soy	Conventional	-0.08320%	Ascomycota sp.	Decrease
OTU3085	R4	Soy	Conventional	-0.07944%	Ophiosphaerella sp	Decrease
OTU124	R4	Soy	Conventional	-0.07813%	Neoascochyta desmazieri	Decrease
OTU222	R6	Soy	No-till	-0.06963%	Dioszegia sp.	Decrease
OTU5225	R6	Soy	No-till	-0.06820%	Coniothyrium sp	Decrease
OTU167	R4	Soy	Conventional	-0.05655%	Parastagonospora sp.	Decrease
OTU195	R4	Soy	No-till	-0.05469%	Dioszegia changbaiensis	Decrease
OTU824	R6	Soy	No-till	-0.05370%	Seimatosporium sp.	Decrease
OTU5171	R4	Soy	Conventional	-0.05314%	Tilletiopsis washingtonensis	Decrease
OTU282	R6	Soy	No-till	-0.04985%	Neodevriesia poagena	Decrease
OTU5171	R4	Soy	No-till	-0.04929%	Tilletiopsis washingtonensis	Decrease
OTU267	R6	Soy	No-till	-0.04884%	Cyphellophora sp.	Decrease
OTU278	R4	Soy	No-till	-0.04864%	Taphrina sp	Decrease
OTU254	R4	Soy	Conventional	-0.04827%	Erythrobasidium yunnanense	Decrease
OTU309	R6	Soy	Conventional	-0.04712%	Pyrenophora tritici-repentis	Decrease
OTU1872	R6	Soy	No-till	-0.04346%	Mycosphaerellaceae sp.	Decrease
OTU186	R6	Soy	No-till	-0.04298%	Zymoseptoria verkleyi	Decrease
OTU195	R4	Soy	Conventional	-0.04070%	Dioszegia changbaiensis	Decrease
OTU325	R4	Soy	Conventional	-0.04012%	Papiliotrema aurea	Decrease
OTU2177	R4	Soy	Conventional	-0.03898%	Vishniacozyma sp.	Decrease
OTU798	R6	Soy	No-till	-0.03873%	Golubevia pallescens	Decrease
OTU354	R4	Soy	Conventional	-0.03844%	Paraphaeosphaeria michotii	Decrease
OTU5171	R6	Soy	No-till	-0.03838%	Tilletiopsis washingtonensis	Decrease
OTU825	R6	Soy	No-till	-0.03736%	Phyllozyma linderae	Decrease
OTU757	R6	Soy	No-till	-0.03735%	Pseudozyma pruni	Decrease

Table B.1 (cont'd)

OTU278	R6	Soy	Conventional	-0.03568%	Taphrina sp	Decrease
OTU4350	R4	Soy	No-till	-0.03247%	Didymella sp.	Decrease
OTU278	R4	Soy	Conventional	-0.03141%	Taphrina sp	Decrease
OTU395	R4	Soy	Conventional	-0.02906%	Vishniacozyma dimennae	Decrease
OTU971	R6	Soy	No-till	-0.02858%	Trichomeriaceae sp.	Decrease
OTU251	R4	Soy	Conventional	-0.02811%	Papiliotrema frias	Decrease
OTU195	R6	Soy	No-till	-0.02801%	Dioszegia changbaiensis	Decrease
OTU254	R4	Soy	No-till	-0.02611%	Erythrobasidium yunnanense	Decrease
OTU825	R4	Soy	No-till	-0.02575%	Phyllozyma linderae	Decrease
OTU65	R4	Soy	Conventional	-0.02544%	Bullera crocea	Decrease
OTU4944	R4	Soy	Conventional	-0.02491%	Cercospora sojina	Decrease
OTU447	R4	Soy	No-till	-0.02336%	Sampaiozyma sp.	Decrease
OTU6184	R4	Soy	No-till	-0.02257%	Diaporthe cotoneastri	Decrease
OTU637	R4	Soy	Conventional	-0.02193%	Pleosporales sp.	Decrease
OTU6163	R4	Soy	Conventional	-0.02148%	Pleosporales sp.	Decrease
OTU222	R4	Soy	No-till	-0.02044%	Dioszegia sp.	Decrease
OTU567	R4	Soy	No-till	-0.02004%	Rachicladosporium cboliae	Decrease
OTU604	R4	Soy	No-till	-0.01986%	Itersonilia perplexans	Decrease
OTU436	R4	Soy	No-till	-0.01935%	Pleosporales sp.	Decrease
OTU436	R4	Soy	Conventional	-0.01907%	Pleosporales sp.	Decrease
OTU361	R4	Soy	Conventional	-0.01783%	Papiliotrema fusca	Decrease
OTU486	R6	Soy	No-till	-0.01590%	Saitozyma paraflava	Decrease
OTU963	R6	Soy	No-till	-0.01588%	Tuber melanosporum	Decrease
OTU222	R4	Soy	Conventional	-0.01521%	Dioszegia sp.	Decrease
OTU2284	R4	Soy	Conventional	-0.01497%	Dissoconium sp.	Decrease
OTU767	R6	Soy	No-till	-0.01455%	Dioszegia sp.	Decrease
OTU642	R4	Soy	Conventional	-0.01405%	Entyloma polysporum	Decrease
OTU1213	R4	Soy	No-till	-0.01386%	Zygophiala inaequalis	Decrease
OTU5990	R4	Soy	Conventional	-0.01288%	Phaeosphaeriaceae sp.	Decrease
OTU293	R6	Soy	No-till	-0.01204%	Papiliotrema sp.	Decrease
OTU28	R4	Soy	Conventional	-0.01147%	Cryptococcus sp.	Decrease

Table B.1 (cont'd)

OTU7117	R4	Soy	Conventional	-0.01141%	Didymella sp.	Decrease
OTU6436	R6	Soy	No-till	-0.01127%	Tilletiopsis sp.	Decrease
OTU944	R4	Soy	Conventional	-0.01114%	Pleosporales sp.	Decrease
OTU741	R4	Soy	No-till	-0.01095%	Strelitziana albiziae	Decrease
OTU869	R4	Soy	Conventional	-0.01081%	Cryptococcus sp	Decrease
OTU536	R4	Soy	No-till	-0.01074%	Knufia sp.	Decrease
OTU740	R4	Soy	Conventional	-0.01041%	Ophiognomonia rosae	Decrease
OTU6108	R4	Soy	No-till	-0.01035%	Uwebraunia communis	Decrease
OTU655	R6	Soy	No-till	-0.00984%	Tremellomycetes sp.	Decrease
OTU83	R6	Soy	Conventional	-0.00981%	Vishniacozyma globispora	Decrease
OTU536	R6	Soy	No-till	-0.00928%	Knufia sp.	Decrease
OTU1488	R6	Soy	No-till	-0.00921%	Ascomycota sp.	Decrease
OTU4350	R4	Soy	Conventional	-0.00904%	Didymella sp.	Decrease
OTU1570	R4	Soy	No-till	-0.00898%	Erythrobasidium sp.	Decrease
OTU4947	R4	Soy	Conventional	-0.00878%	Cryptococcus sp	Decrease
OTU2767	R6	Soy	Conventional	-0.00868%	Basidiomycota sp.	Decrease
OTU518	R4	Soy	No-till	-0.00853%	Tulasnellaceae sp.	Decrease
OTU1747	R6	Soy	No-till	-0.00845%	Crocicreas cyathoideum	Decrease
OTU6748	R6	Soy	No-till	-0.00844%	Tilletiopsis washingtonensis	Decrease
OTU1086	R6	Soy	No-till	-0.00819%	Phaeosphaeria sp	Decrease
OTU1478	R4	Soy	No-till	-0.00804%	Cyphellophora sp.	Decrease
OTU12	R4	Soy	No-till	-0.00780%	Pleosporales sp.	Decrease
OTU513	R4	Soy	Conventional	-0.00750%	Vishniacozyma sp.	Decrease
OTU158	R6	Soy	No-till	-0.00736%	Vishniacozyma dimennae	Decrease
OTU2047	R4	Soy	Conventional	-0.00679%	Rutstroemiaceae sp.	Decrease
OTU158	R4	Soy	Conventional	-0.00677%	Vishniacozyma dimennae	Decrease
OTU358	R4	Soy	No-till	-0.00675%	Neofitzroyomyces nerii	Decrease
OTU2205	R4	Soy	Conventional	-0.00650%	Pleosporales sp.	Decrease
OTU1559	R6	Soy	Conventional	-0.00648%	Kondoa phyllada	Decrease
OTU447	R6	Soy	Conventional	-0.00641%	Sampaiozyma sp.	Decrease
OTU849	R4	Soy	No-till	-0.00628%	Pleosporales sp.	Decrease
OTU381	R4	Soy	Conventional	-0.00596%	Coprinopsis sp.	Decrease
OTU746	R4	Soy	Conventional	-0.00591%	Archaeorhizomyces	Decrease

Table B.1 (cont'd)

OTU224	R4	Soy	No-till	-0.00564%	Exophiala equina	Decrease
OTU1036	R4	Soy	No-till	-0.00564%	Mycosphaerellaceae sp.	Decrease
OTU845	R4	Soy	No-till	-0.00562%	Teratosphaeriaceae sp.	Decrease
OTU279	R4	Soy	No-till	-0.00561%	Hypocreales sp.	Decrease
OTU322	R6	Soy	No-till	-0.00559%	Torula sp.	Decrease
OTU1388	R6	Soy	No-till	-0.00559%	Cyphellophora sp.	Decrease
OTU3417	R6	Soy	No-till	-0.00557%	Helotiales sp.	Decrease
OTU486	R4	Soy	No-till	-0.00525%	Saitozyma paraflava	Decrease
OTU326	R4	Soy	Conventional	-0.00517%	Mycosphaerellaceae sp.	Decrease
OTU3340	R6	Soy	No-till	-0.00498%	Pleosporales sp.	Decrease
OTU347	R6	Soy	Conventional	-0.00495%	Chionosphaeraceae sp.	Decrease
OTU2159	R4	Soy	Conventional	-0.00490%	Xenosonderhenia syzygii	Decrease
OTU3340	R4	Soy	Conventional	-0.00486%	Pleosporales sp.	Decrease
OTU3465	R4	Soy	Conventional	-0.00473%	Phaeosphaeriaceae sp.	Decrease
OTU642	R6	Soy	No-till	-0.00469%	Entyloma polysporum	Decrease
OTU1413	R4	Soy	No-till	-0.00469%	Capnodiales sp.	Decrease
OTU3493	R4	Soy	Conventional	-0.00464%	Bulleribasidium oberjochense	Decrease
OTU246	R4	Soy	No-till	-0.00462%	Sordariales sp.	Decrease
OTU1867	R6	Soy	No-till	-0.00448%	Neosetophoma samararum	Decrease
OTU42	R6	Soy	Conventional	-0.00447%	Pleosporales sp.	Decrease
OTU3340	R6	Soy	Conventional	-0.00446%	Pleosporales sp.	Decrease
OTU522	R6	Soy	No-till	-0.00430%	Microdochium seminicola	Decrease
OTU306	R4	Soy	No-till	-0.00422%	Eucasphaeria sp	Decrease
OTU890	R6	Soy	No-till	-0.00376%	Hannaella sinensis	Decrease
OTU2902	R4	Soy	Conventional	-0.00376%	Spiculogloeomycetes sp.	Decrease
OTU1580	R4	Soy	Conventional	-0.00374%	Chaetothyriales sp.	Decrease
OTU989	R4	Soy	No-till	-0.00373%	Chaetothyriales sp.	Decrease
OTU4459	R4	Soy	Conventional	-0.00364%	Ceriporia humilis	Decrease
OTU1464	R6	Soy	No-till	-0.00364%	Symmetrospora sp.	Decrease
OTU2177	R6	Soy	Conventional	-0.00361%	Vishniacozyma sp.	Decrease

Table B.1 (cont'd)

OTU1358	R4	Soy	No-till	-0.00361%	Kondoaceae sp.	Decrease
OTU1161	R6	Soy	Conventional	-0.00359%	Ramularia sp.	Decrease
OTU4518	R6	Soy	Conventional	-0.00355%	Pleosporales sp.	Decrease
OTU124	R4	Soy	No-till	-0.00355%	Neoascochyta desmazieri	Decrease
OTU4045	R6	Soy	No-till	-0.00332%	Pleosporales sp.	Decrease
OTU233	R6	Soy	No-till	-0.00325%	Penicillium decumbens	Decrease
OTU462	R6	Soy	No-till	-0.00320%	Devriesia pseudoamericana	Decrease
OTU319	R4	Soy	No-till	-0.00319%	Fungi sp.	Decrease
OTU1523	R6	Soy	No-till	-0.00319%	Bannoa sp.	Decrease
OTU661	R4	Soy	Conventional	-0.00314%	Diaporthe sp.	Decrease
OTU4567	R6	Soy	Conventional	-0.00306%	Pleosporales sp.	Decrease
OTU1053	R4	Soy	Conventional	-0.00304%	Thamnolia vermicularis subsp. subuliformis	Decrease
OTU946	R4	Soy	No-till	-0.00297%	Cystobasidiomycetes sp.	Decrease
OTU2945	R6	Soy	No-till	-0.00293%	Vishniacozyma victoriae	Decrease
OTU6163	R6	Soy	No-till	-0.00293%	Pleosporales sp.	Decrease
OTU1614	R6	Soy	Conventional	-0.00271%	Chionosphaeraceae sp.	Decrease
OTU589	R6	Soy	No-till	-0.00264%	Bensingtonia sp	Decrease
OTU362	R6	Soy	No-till	-0.00262%	Ganoderma sp.	Decrease
OTU4802	R4	Soy	Conventional	-0.00260%	Hannaella oryzae	Decrease
OTU1271	R6	Soy	No-till	-0.00256%	Phaeosphaeriaceae sp.	Decrease
OTU1032	R4	Soy	Conventional	-0.00254%	Tilletia sp.	Decrease
OTU2423	R4	Soy	Conventional	-0.00249%	Parastagonospora forlicesenica	Decrease
OTU4045	R4	Soy	No-till	-0.00242%	Pleosporales sp.	Decrease
OTU4518	R6	Soy	No-till	-0.00236%	Pleosporales sp.	Decrease
OTU1563	R4	Soy	No-till	-0.00230%	Rhizophlyctis rosea	Decrease
OTU1584	R6	Soy	Conventional	-0.00226%	Hypoxylon rubiginosum	Decrease
OTU209	R6	Soy	Conventional	-0.00226%	Branch06 sp.	Decrease
OTU5224	R4	Soy	No-till	-0.00224%	Talaromyces sp.	Decrease
OTU4045	R4	Soy	Conventional	-0.00215%	Pleosporales sp.	Decrease
OTU4182	R4	Soy	Conventional	-0.00213%	Dioszegia sp.	Decrease

Table B.1 (cont'd)

OTU141	R6	Soy	Conventional	-0.00213%	Papiliotrema sp.	Decrease
OTU2205	R4	Soy	No-till	-0.00210%	Pleosporales sp.	Decrease
OTU4839	R4	Soy	Conventional	-0.00207%	Pleosporales sp.	Decrease
OTU410	R6	Soy	No-till	0.00225%	Herpotrichiellaceae sp.	Increase
OTU2872	R6	Soy	Conventional	0.00247%	Hypoxylon carneum	Increase
OTU266	R6	Soy	Conventional	0.00313%	Minimedusa polyspora	Increase
OTU744	R6	Soy	No-till	0.00362%	Sordariales sp.	Increase
OTU2984	R4	Soy	No-till	0.00367%	Xylodon flaviporus	Increase
OTU2205	R6	Soy	No-till	0.00380%	Pleosporales sp.	Increase
OTU290	R6	Soy	Conventional	0.00483%	Malassezia sp.	Increase
OTU1561	R4	Soy	No-till	0.00522%	Sistotrema sp	Increase
OTU4924	R4	Soy	Conventional	0.00558%	Hyphoderma sp.	Increase
OTU997	R4	Soy	No-till	0.00641%	Corticiales sp.	Increase
OTU326	R6	Soy	No-till	0.00711%	Mycosphaerellaceae sp.	Increase
OTU409	R6	Soy	No-till	0.00716%	Chaetosphaeriaceae sp.	Increase
OTU731	R4	Soy	Conventional	0.00818%	Bionectriaceae sp.	Increase
OTU1399	R4	Soy	Conventional	0.00861%	Hypocreales sp.	Increase
OTU46	R4	Soy	Conventional	0.00867%	Pleosporales sp.	Increase
OTU531	R4	Soy	No-till	0.00884%	Helicoma sp.	Increase
OTU714	R4	Soy	Conventional	0.01058%	Entylomatales sp.	Increase
OTU1449	R4	Soy	Conventional	0.01220%	Phellinus gilvus	Increase
OTU1487	R4	Soy	Conventional	0.01360%	Coprinellus sp.	Increase
OTU1468	R4	Soy	Conventional	0.01659%	Eutypella sp.	Increase
OTU1895	R6	Soy	Conventional	0.01819%	Paraconiothyrium sp.	Increase
OTU675	R6	Soy	Conventional	0.02323%	Phaeosphaeria typharum	Increase
OTU157	R6	Soy	No-till	0.02560%	Chaetomiaceae sp.	Increase
OTU725	R6	Soy	Conventional	0.03100%	Parmeliaceae sp.	Increase
OTU58	R4	Soy	Conventional	0.06823%	Agaricomycetes sp.	Increase
OTU1509	R4	Soy	No-till	0.10404%	Vishniacozyma victoriae	Increase
OTU94	R6	Soy	Conventional	0.13162%	Sporobolomyces patagonicus	Increase
OTU66	R6	Soy	No-till	0.14794%	Sporobolomyces roseus	Increase
OTU94	R6	Soy	No-till	0.45543%	Sporobolomyces sp.	Increase

Table B.1 (cont'd)

OTU94	R4	Soy	No-till	0.90409%	Sporobolomyces patagonicus	Increase
OTU8	R4	Soy	Conventional	0.96580%	Fusarium sp.	Increase
OTU10	R4	Soy	No-till	12.03078%	Bulleromyces albus	Increase

Table B.2 - Core members of the soybean or maize phyllosphere in no-till and conventional management.

Taxonomy	Treatment	Crop	Kingdom
BOTU_25-Allorhizobium-neorhizobium-pararhizobium-rhizobium sp.	Conventional	Maize	Bacteria
BOTU_19-Aureimonas sp.	Conventional	Maize	Bacteria
BOTU_10796-Burkholderia-caballeronia-paraburkholderia sp.	Conventional	Maize	Bacteria
BOTU_89-Geobacillus sp.	Conventional	Maize	Bacteria
BOTU_21-Hymenobacter sp.	Conventional	Maize	Bacteria
BOTU_130-Leifsonia sp.	Conventional	Maize	Bacteria
BOTU_16-Massilia sp.	Conventional	Maize	Bacteria
BOTU_7-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_17-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_3113-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_497-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_3257-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_24-Methylobacterium-methylorubrum sp.	Conventional	Maize	Bacteria
BOTU_73-Microbacterium sp.	Conventional	Maize	Bacteria
BOTU_296-Noviherbaspirillum sp.	Conventional	Maize	Bacteria
BOTU_481-Pantoea sp.	Conventional	Maize	Bacteria
BOTU_37-Pseudarthrobacter sp.	Conventional	Maize	Bacteria
BOTU_10-Pseudomonas sp.	Conventional	Maize	Bacteria
BOTU_38-Ralstonia sp.	Conventional	Maize	Bacteria
BOTU_5-Sphingomonas sp.	Conventional	Maize	Bacteria
BOTU_1025-Sphingomonas sp.	Conventional	Maize	Bacteria
BOTU_64-Sphingomonas sp.	Conventional	Maize	Bacteria
BOTU_15-Sphingomonas sp.	Conventional	Maize	Bacteria
BOTU_873-Sphingomonas sp.	Conventional	Maize	Bacteria
BOTU_27-Staphylococcus sp.	Conventional	Maize	Bacteria

Table B.2 (cont'd)

BOTU_32-Streptococcus sp.	Conventional	Maize	Bacteria
BOTU_13-Microbacteriaceae sp.	Conventional	Maize	Bacteria
BOTU_11044-Microbacteriaceae sp.	Conventional	Maize	Bacteria
BOTU_211-Sphingomonadaceae sp.	Conventional	Maize	Bacteria
BOTU_14112-Bacteria sp.	Conventional	Maize	Bacteria
BOTU_10618-Bacteria sp.	Conventional	Maize	Bacteria
BOTU_126-Acinetobacter sp.	No-till	Maize	Bacteria
BOTU_25-Allorhizobium-neorhizobium-pararhizobium-rhizobium sp.	No-till	Maize	Bacteria
BOTU_11812-Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium sp.	No-till	Maize	Bacteria
BOTU_91-Allorhizobium-neorhizobium-pararhizobium-rhizobium sp.	No-till	Maize	Bacteria
BOTU_19-Aureimonas sp.	No-till	Maize	Bacteria
BOTU_2-Bacillus sp.	No-till	Maize	Bacteria
BOTU_10796-Burkholderia-caballeronia-paraburkholderia sp.	No-till	Maize	Bacteria
BOTU_94-Corynebacterium sp.	No-till	Maize	Bacteria
BOTU_82-Duganella sp.	No-till	Maize	Bacteria
BOTU_11-Escherichia-shigella sp.	No-till	Maize	Bacteria
BOTU_89-Geobacillus sp.	No-till	Maize	Bacteria
BOTU_21-Hymenobacter sp.	No-till	Maize	Bacteria
BOTU_80-Hymenobacter sp.	No-till	Maize	Bacteria
BOTU_5403-Hymenobacter sp.	No-till	Maize	Bacteria
BOTU_300-Hymenobacter sp.	No-till	Maize	Bacteria
BOTU_314-Hymenobacter sp.	No-till	Maize	Bacteria
BOTU_303-Kineococcus sp.	No-till	Maize	Bacteria
BOTU_85-Klenkia sp.	No-till	Maize	Bacteria
BOTU_130-Leifsonia sp.	No-till	Maize	Bacteria
BOTU_122-Marmoricola sp.	No-till	Maize	Bacteria
BOTU_16-Massilia sp.	No-till	Maize	Bacteria
BOTU_3370-Massilia sp.	No-till	Maize	Bacteria
BOTU_17-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria
BOTU_7-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria
BOTU_3113-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria
BOTU_497-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria
BOTU_3257-Methylobacterium-methylorubrum	No-till	Maize	Bacteria
BOTU_24-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria

Table B.2 (cont'd)

BOTU_53-Methylobacterium-methylorubrum sp.	No-till	Maize	Bacteria
BOTU_73-Microbacterium sp.	No-till	Maize	Bacteria
BOTU_192-Nocardioides sp.	No-till	Maize	Bacteria
BOTU_47-Paenarthrobacter sp.	No-till	Maize	Bacteria
BOTU_5427-Paenibacillus sp.	No-till	Maize	Bacteria
BOTU_34-Paenibacillus sp.	No-till	Maize	Bacteria
BOTU_3180-Paenibacillus sp.	No-till	Maize	Bacteria
BOTU_481-Pantoea sp.	No-till	Maize	Bacteria
BOTU_70-Pedobacter sp.	No-till	Maize	Bacteria
BOTU_347-Polaromonas sp.	No-till	Maize	Bacteria
BOTU_37-Pseudarthrobacter sp.	No-till	Maize	Bacteria
BOTU_10-Pseudomonas sp.	No-till	Maize	Bacteria
BOTU_1039-Pseudomonas sp.	No-till	Maize	Bacteria
BOTU_158-Pseudonocardia sp.	No-till	Maize	Bacteria
BOTU_22-Quadrisphaera sp.	No-till	Maize	Bacteria
BOTU_38-Ralstonia sp.	No-till	Maize	Bacteria
BOTU_5-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_1025-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_64-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_15-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_873-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_10215-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_331-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_167-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_274-Sphingomonas sp.	No-till	Maize	Bacteria
BOTU_115-Spirosoma sp.	No-till	Maize	Bacteria
BOTU_141-Spirosoma sp.	No-till	Maize	Bacteria
BOTU_27-Staphylococcus sp.	No-till	Maize	Bacteria
BOTU_32-Streptococcus sp.	No-till	Maize	Bacteria
BOTU_103-Terrabacter sp.	No-till	Maize	Bacteria
BOTU_78-Thermus sp.	No-till	Maize	Bacteria
BOTU_92-Tumebacillus sp.	No-till	Maize	Bacteria
BOTU_847-uncultured 35 sp.	No-till	Maize	Bacteria
BOTU_5895-Variovorax sp.	No-till	Maize	Bacteria
BOTU_13-Microbacteriaceae sp.	No-till	Maize	Bacteria
BOTU_44-Comamonadaceae sp.	No-till	Maize	Bacteria
BOTU_11044-Microbacteriaceae sp.	No-till	Maize	Bacteria
BOTU_211-Sphingomonadaceae sp.	No-till	Maize	Bacteria

Table B.2 (cont'd)

BOTU_1692-Microbacteriaceae sp.	No-till	Maize	Bacteria
BOTU_10618-Bacteria sp.	No-till	Maize	Bacteria
BOTU_14112-Bacteria sp.	No-till	Maize	Bacteria
BOTU_8-Enterobacteriaceae sp.	No-till	Maize	Bacteria
BOTU_19-Aureimonas sp.	Conventional	Soy	Bacteria
BOTU_3-Bradyrhizobium sp.	Conventional	Soy	Bacteria
BOTU_21-Hymenobacter sp.	Conventional	Soy	Bacteria
BOTU_5403-Hymenobacter sp.	Conventional	Soy	Bacteria
BOTU_80-Hymenobacter sp.	Conventional	Soy	Bacteria
BOTU_2048-Hymenobacter sp.	Conventional	Soy	Bacteria
BOTU_4751-Hymenobacter sp.	Conventional	Soy	Bacteria
BOTU_85-Klenkia sp.	Conventional	Soy	Bacteria
BOTU_7-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_24-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_17-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_3113-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_497-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_3257-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_53-Methylobacterium-methylorubrum sp.	Conventional	Soy	Bacteria
BOTU_41-Pseudokineococcus sp.	Conventional	Soy	Bacteria
BOTU_22-Quadrisphaera sp.	Conventional	Soy	Bacteria
BOTU_109-Roseomonas sp.	Conventional	Soy	Bacteria
BOTU_15-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_5-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_1025-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_64-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_274-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_873-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_10215-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_1874-Sphingomonas sp.	Conventional	Soy	Bacteria
BOTU_5895-Variovorax sp.	Conventional	Soy	Bacteria
BOTU_211-Sphingomonadaceae sp.	Conventional	Soy	Bacteria
BOTU_25-Allorhizobium-neorhizobium-pararhizobium-rhizobium sp.	No-till	Soy	Bacteria
BOTU_19-Aureimonas sp.	No-till	Soy	Bacteria
BOTU_154-Aureimonas sp.	No-till	Soy	Bacteria

Table B.2 (cont'd)

BOTU_14916-Aureimonas sp.	No-till	Soy	Bacteria
BOTU_3-Bradyrhizobium sp.	No-till	Soy	Bacteria
BOTU_26-Buchnera sp.	No-till	Soy	Bacteria
BOTU_108-Corynebacterium sp.	No-till	Soy	Bacteria
BOTU_94-Corynebacterium sp.	No-till	Soy	Bacteria
BOTU_21-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_5403-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_80-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_2048-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_11846-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_4751-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_163-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_322-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_1723-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_300-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_5733-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_8866-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_436-Hymenobacter sp.	No-till	Soy	Bacteria
BOTU_303-Kineococcus sp.	No-till	Soy	Bacteria
BOTU_85-Klenkia sp.	No-till	Soy	Bacteria
BOTU_122-Marmoricola sp.	No-till	Soy	Bacteria
BOTU_16-Massilia sp.	No-till	Soy	Bacteria
BOTU_7-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_24-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_17-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_3113-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_53-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_497-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_7894-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_3257-Methylobacterium-methylorubrum sp.	No-till	Soy	Bacteria
BOTU_73-Microbacterium sp.	No-till	Soy	Bacteria
BOTU_51-Niastella sp.	No-till	Soy	Bacteria
BOTU_33-Novosphingobium sp.	No-till	Soy	Bacteria
BOTU_695-P3ob-42 sp.	No-till	Soy	Bacteria
BOTU_20-Phenylobacterium sp.	No-till	Soy	Bacteria

Table B.2 (cont'd)

BOTU_347-Polaromonas sp.	No-till	Soy	Bacteria
BOTU_41-Pseudokineococcus sp.	No-till	Soy	Bacteria
BOTU_97-Pseudomonas sp.	No-till	Soy	Bacteria
BOTU_10-Pseudomonas sp.	No-till	Soy	Bacteria
BOTU_22-Quadrisphaera sp.	No-till	Soy	Bacteria
BOTU_109-Roseomonas sp.	No-till	Soy	Bacteria
BOTU_15-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_5-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_1025-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_274-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_10215-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_64-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_1874-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_873-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_2407-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_40-Sphingomonas sp.	No-till	Soy	Bacteria
BOTU_99-Spirosoma sp.	No-till	Soy	Bacteria
BOTU_308-Spirosoma sp.	No-till	Soy	Bacteria
BOTU_27-Staphylococcus sp.	No-till	Soy	Bacteria
BOTU_9-Streptomyces sp.	No-till	Soy	Bacteria
BOTU_242-Streptomyces sp.	No-till	Soy	Bacteria
BOTU_10263-Streptomyces sp.	No-till	Soy	Bacteria
BOTU_50-Streptomyces sp.	No-till	Soy	Bacteria
BOTU_847-uncultured 35 sp.	No-till	Soy	Bacteria
BOTU_5895-Variovorax sp.	No-till	Soy	Bacteria
BOTU_110-Variovorax sp.	No-till	Soy	Bacteria
BOTU_211-Sphingomonadaceae sp.	No-till	Soy	Bacteria
BOTU_443-Bacteria sp.	No-till	Soy	Bacteria
BOTU_44-Comamonadaceae sp.	No-till	Soy	Bacteria
BOTU_13-Microbacteriaceae sp.	No-till	Soy	Bacteria
BOTU_18-Oxalobacteraceae sp.	No-till	Soy	Bacteria
BOTU_806-Bacteria sp.	No-till	Soy	Bacteria
BOTU_579-Bacteria sp.	No-till	Soy	Bacteria
FOTU_2-Alternaria sp.	Conventional	Maize	Fungi
FOTU_38-Alternaria sp.	Conventional	Maize	Fungi
FOTU_136-Aureobasidium sp.	Conventional	Maize	Fungi
FOTU_65-Bullera crocea	Conventional	Maize	Fungi
FOTU_10-Bulleromyces albus	Conventional	Maize	Fungi

Table B.2 (cont'd)

FOTU_28-Cryptococcus sp.	Conventional	Maize	Fungi
FOTU_9-Didymella sp.	Conventional	Maize	Fungi
FOTU_21-Dioszegia hungarica	Conventional	Maize	Fungi
FOTU_5526-Dioszegia hungarica	Conventional	Maize	Fungi
FOTU_3-Epicoccum dendrobii	Conventional	Maize	Fungi
FOTU_1725-Epicoccum dendrobii	Conventional	Maize	Fungi
FOTU_1354-Epicoccum dendrobii	Conventional	Maize	Fungi
FOTU_97-Filobasidium sp.	Conventional	Maize	Fungi
FOTU_82-Filobasidium oeirense	Conventional	Maize	Fungi
FOTU_91-Filobasidium floriforme	Conventional	Maize	Fungi
FOTU_13-Hannaella coprosmae	Conventional	Maize	Fungi
FOTU_27-Hannaella zeae	Conventional	Maize	Fungi
FOTU_16-Hannaella oryzae	Conventional	Maize	Fungi
FOTU_154-Leptospora sp	Conventional	Maize	Fungi
FOTU_116-Leptospora sp.	Conventional	Maize	Fungi
FOTU_6-Mycosphaerella tassiana	Conventional	Maize	Fungi
FOTU_96-Neoascochyta sp.	Conventional	Maize	Fungi
FOTU_4-Phoma sp.	Conventional	Maize	Fungi
FOTU_66-Sporobolomyces roseus	Conventional	Maize	Fungi
FOTU_90-Sporobolomyces phaffii	Conventional	Maize	Fungi
FOTU_32-Symmetrospora coprosmae	Conventional	Maize	Fungi
FOTU_119-Symmetrospora sp.	Conventional	Maize	Fungi
FOTU_48-Taphrina caerulescens	Conventional	Maize	Fungi
FOTU_87-Vishniacozyma carnescens	Conventional	Maize	Fungi
FOTU_83-Vishniacozyma globispora	Conventional	Maize	Fungi
FOTU_6430-Dothioraceae sp.	Conventional	Maize	Fungi
FOTU_30-Pleosporales sp.	Conventional	Maize	Fungi
FOTU_14-Pleosporales sp.	Conventional	Maize	Fungi
FOTU_33-Phaeosphaeriaceae sp.	Conventional	Maize	Fungi
FOTU_23-Nectriaceae sp.	Conventional	Maize	Fungi
FOTU_123-Phaeosphaeriaceae sp.	Conventional	Maize	Fungi
FOTU_2-Alternaria sp.	No-till	Maize	Fungi
FOTU_38-Alternaria sp.	No-till	Maize	Fungi
FOTU_65-Bullera crocea	No-till	Maize	Fungi
FOTU_10-Bulleromyces albus	No-till	Maize	Fungi
FOTU_28-Cryptococcus sp.	No-till	Maize	Fungi
FOTU_9-Didymella sp.	No-till	Maize	Fungi
FOTU_21-Dioszegia hungarica	No-till	Maize	Fungi

Table B.2 (cont'd)

FOTU_52-Dioszegia sp.	No-till	Maize	Fungi
FOTU_5526-Dioszegia hungarica	No-till	Maize	Fungi
FOTU_3-Epicoccum dendrobii	No-till	Maize	Fungi
FOTU_1354-Epicoccum dendrobii	No-till	Maize	Fungi
FOTU_1725-Epicoccum dendrobii	No-till	Maize	Fungi
FOTU_82-Filobasidium oeirense	No-till	Maize	Fungi
FOTU_97-Filobasidium sp.	No-till	Maize	Fungi
FOTU_13-Hannaella coprosmae	No-till	Maize	Fungi
FOTU_27-Hannaella zeae	No-till	Maize	Fungi
FOTU_6350-Hannaella zeae	No-till	Maize	Fungi
FOTU_16-Hannaella oryzae	No-till	Maize	Fungi
FOTU_154-Leptospora sp	No-till	Maize	Fungi
FOTU_6-Mycosphaerella tassiana	No-till	Maize	Fungi
FOTU_96-Neoascochyta sp.	No-till	Maize	Fungi
FOTU_4-Phoma sp.	No-till	Maize	Fungi
FOTU_66-Sporobolomyces roseus	No-till	Maize	Fungi
FOTU_90-Sporobolomyces phaffii	No-till	Maize	Fungi
FOTU_32-Symmetrospora coprosmae	No-till	Maize	Fungi
FOTU_83-Vishniacozyma globispora	No-till	Maize	Fungi
FOTU_6430-Dothioraceae sp.	No-till	Maize	Fungi
FOTU_30-Pleosporales sp.	No-till	Maize	Fungi
FOTU_14-Pleosporales sp.	No-till	Maize	Fungi
FOTU_128-Pleosporales sp.	No-till	Maize	Fungi
FOTU_33-Phaeosphaeriaceae sp.	No-till	Maize	Fungi
FOTU_2-Alternaria sp.	Conventional	Soy	Fungi
FOTU_38-Alternaria sp.	Conventional	Soy	Fungi
FOTU_134-Bulleribasidium oberjochense	Conventional	Soy	Fungi
FOTU_10-Bulleromyces albus	Conventional	Soy	Fungi
FOTU_51-Coniothyrium sp	Conventional	Soy	Fungi
FOTU_9-Didymella sp.	Conventional	Soy	Fungi
FOTU_3-Epicoccum dendrobii	Conventional	Soy	Fungi
FOTU_13-Hannaella coprosmae	Conventional	Soy	Fungi
FOTU_16-Hannaella oryzae	Conventional	Soy	Fungi
FOTU_34-Hannaella oryzae	Conventional	Soy	Fungi
FOTU_6-Mycosphaerella tassiana	Conventional	Soy	Fungi
FOTU_4-Phoma sp.	Conventional	Soy	Fungi
FOTU_45-Septoria sp.	Conventional	Soy	Fungi
FOTU_119-Symmetrospora sp.	Conventional	Soy	Fungi

Table B.2 (cont'd)

FOTU_17-Tilletiopsis washingtonensis	Conventional	Soy	Fungi
FOTU_643-Tilletiopsis washingtonensis	Conventional	Soy	Fungi
FOTU_30-Pleosporales sp.	Conventional	Soy	Fungi
FOTU_14-Pleosporales sp.	Conventional	Soy	Fungi
FOTU_6430-Dothioraceae sp.	Conventional	Soy	Fungi
FOTU_33-Phaeosphaeriaceae sp.	Conventional	Soy	Fungi
FOTU_123-Phaeosphaeriaceae sp.	Conventional	Soy	Fungi
FOTU_55-Pleosporales sp.	Conventional	Soy	Fungi
FOTU_2-Alternaria sp.	No-till	Soy	Fungi
FOTU_38-Alternaria sp.	No-till	Soy	Fungi
FOTU_134-Bulleribasidium oberjochense	No-till	Soy	Fungi
FOTU_10-Bulleromyces albus	No-till	Soy	Fungi
FOTU_51-Coniothyrium sp	No-till	Soy	Fungi
FOTU_6420-Coniothyrium sp	No-till	Soy	Fungi
FOTU_5225-Coniothyrium sp	No-till	Soy	Fungi
FOTU_267-Cyphellophora sp.	No-till	Soy	Fungi
FOTU_103-Diaporthe caulivora	No-till	Soy	Fungi
FOTU_9-Didymella sp.	No-till	Soy	Fungi
FOTU_52-Dioszegia sp.	No-till	Soy	Fungi
FOTU_21-Dioszegia hungarica	No-till	Soy	Fungi
FOTU_3-Epicoccum dendrobii	No-till	Soy	Fungi
FOTU_1354-Epicoccum dendrobii	No-till	Soy	Fungi
FOTU_1725-Epicoccum dendrobii	No-till	Soy	Fungi
FOTU_254-Erythrobasidium yunnanense	No-till	Soy	Fungi
FOTU_8-Fusarium sp.	No-till	Soy	Fungi
FOTU_68-Gibberella sp.	No-till	Soy	Fungi
FOTU_13-Hannaella coprosmae	No-till	Soy	Fungi
FOTU_16-Hannaella oryzae	No-till	Soy	Fungi
FOTU_34-Hannaella oryzae	No-till	Soy	Fungi
FOTU_27-Hannaella zeae	No-till	Soy	Fungi
FOTU_35-Hannaella luteola	No-till	Soy	Fungi
FOTU_89-Kondoa sp	No-till	Soy	Fungi
FOTU_6-Mycosphaerella tassiana	No-till	Soy	Fungi
FOTU_106-Ophiosphaerella aquatica	No-till	Soy	Fungi
FOTU_4-Phoma sp.	No-till	Soy	Fungi
FOTU_45-Septoria sp.	No-till	Soy	Fungi
FOTU_90-Sporobolomyces phaffii	No-till	Soy	Fungi
FOTU_94-Sporobolomyces patagonicus	No-till	Soy	Fungi

Table B.2 (cont'd)

FOTU_119-Symmetrospora sp.	No-till	Soy	Fungi
FOTU_32-Symmetrospora coprosmae	No-till	Soy	Fungi
FOTU_17-Tilletiopsis washingtonensis	No-till	Soy	Fungi
FOTU_643-Tilletiopsis washingtonensis	No-till	Soy	Fungi
FOTU_5171-Tilletiopsis washingtonensis	No-till	Soy	Fungi
FOTU_33-Phaeosphaeriaceae sp.	No-till	Soy	Fungi
FOTU_123-Phaeosphaeriaceae sp.	No-till	Soy	Fungi
FOTU_30-Pleosporales sp.	No-till	Soy	Fungi
FOTU_14-Pleosporales sp.	No-till	Soy	Fungi
FOTU_6430-Dothioraceae sp.	No-till	Soy	Fungi
FOTU_55-Pleosporales sp.	No-till	Soy	Fungi
FOTU_120-Pleosporales sp.	No-till	Soy	Fungi
FOTU_5472-Phaeosphaeriaceae sp.	No-till	Soy	Fungi
FOTU_5227-Pleosporales sp.	No-till	Soy	Fungi
FOTU_128-Pleosporales sp.	No-till	Soy	Fungi
FOTU_23-Nectriaceae sp.	No-till	Soy	Fungi

Table B.3 - Recovery status of fungicide-impacted soybean phyllosphere fungal OTUs

OTU	Species	Class	Treatment	Recovery Status
FOTU_27	Hannaella zeae	Tremellomycetes	No-till	Recovered
FOTU_28	Cryptococcus	Tremellomycetes	No-till	Recovered
FOTU_13	Hannaella coprosmae	Tremellomycetes	No-till	Recovered
FOTU_17	Tilletiopsis washingtonensis	Exobasidiomycetes	No-till	Recovered
FOTU_8	Fusarium	Sordariomycetes	No-till	Recovered
FOTU_130	Vishniacozyma victoriae	Tremellomycetes	No-till	Recovered
FOTU_134	Bulleribasidium oberjochense	Tremellomycetes	No-till	Recovered
FOTU_52	Dioszegia	Tremellomycetes	No-till	Recovered
FOTU_34	Hannaella oryzae	Tremellomycetes	No-till	Recovered
FOTU_124	Neoascochyta desmazieri	Dothideomycetes	No-till	Recovered
FOTU_119	Symmetrospora	Other	No-till	Recovered
FOTU_254	Erythrobasidium yunnanense	Other	No-till	Recovered
FOTU_395	Vishniacozyma dimennae	Tremellomycetes	No-till	Recovered

Table B.3 (cont'd)

FOTU_251	Papiliotrema frias	Tremellomycetes	No-till	Recovered
FOTU_258	Ascomycota	unidentified	No-till	Recovered
FOTU_661	Diaporthe	Sordariomycetes	No-till	Recovered
FOTU_167	Parastagonospora	Dothideomycetes	No-till	Recovered
FOTU_325	Papiliotrema aurea	Tremellomycetes	No-till	Recovered
FOTU_3085	Ophiosphaerella sp	Dothideomycetes	No-till	Recovered
FOTU_90	Sporobolomyces phaffii	Microbotryomycetes	No-till	Recovered
FOTU_4944	Cercospora sojina	Dothideomycetes	No-till	Recovered
FOTU_5990	Phaeosphaeriaceae	Dothideomycetes	No-till	Recovered
FOTU_222	Dioszegia	Tremellomycetes	No-till	Recovered
FOTU_65	Bullera crocea	Tremellomycetes	No-till	Recovered
FOTU_195	Dioszegia changbaiensis	Tremellomycetes	No-till	Recovered
FOTU_513	Vishniacozyma	Tremellomycetes	No-till	Recovered
FOTU_869	Cryptococcus sp	Tremellomycetes	No-till	Recovered
FOTU_642	Entyloma polysporum	Exobasidiomycetes	No-till	Recovered
FOTU_714	Exobasidiomycetes	Exobasidiomycetes	No-till	Recovered
FOTU_2945	Vishniacozyma victoriae	Tremellomycetes	No-till	Recovered
FOTU_6163	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_740	Ophiognomonia rosae	Sordariomycetes	No-till	Recovered
FOTU_2284	Dissoconium	Dothideomycetes	No-till	Recovered
FOTU_2902	Spiculogloeomycetes	Other	No-till	Recovered
FOTU_637	Dothideomycetes	Dothideomycetes	No-till	Recovered
FOTU_5171	Tilletiopsis washingtonensis	Exobasidiomycetes	No-till	Recovered
FOTU_361	Papiliotrema fusca	Tremellomycetes	No-till	Recovered
FOTU_2205	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_6350	Hannaella zeae	Tremellomycetes	No-till	Recovered
FOTU_158	Vishniacozyma dimennae	Tremellomycetes	No-till	Recovered
FOTU_2159	Xenosonderhenia syzygii	Dothideomycetes	No-till	Recovered
FOTU_354	Paraphaeosphaeria michotii	Dothideomycetes	No-till	Recovered
FOTU_7117	Didymella	Dothideomycetes	No-till	Recovered
FOTU_326	Mycosphaerellaceae	Dothideomycetes	No-till	Recovered

Table B.3 (cont'd)

FOTU_436	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_4350	Didymella	Dothideomycetes	No-till	Recovered
FOTU_3493	Bulleribasidium oberjochense	Tremellomycetes	No-till	Recovered
FOTU_724	Ascomycota	unidentified	No-till	Recovered
FOTU_2423	Parastagonospora forlicesenica	Dothideomycetes	No-till	Recovered
FOTU_4839	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_4802	Hannaella oryzae	Tremellomycetes	No-till	Recovered
FOTU_4947	Cryptococcus sp	Tremellomycetes	No-till	Recovered
FOTU_2047	Rutstroemiaceae	Other	No-till	Recovered
FOTU_944	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_731	Hypocreales	Sordariomycetes	No-till	Recovered
FOTU_1464	Symmetrospora	Other	No-till	Recovered
FOTU_1468	Eutypella	Sordariomycetes	No-till	Recovered
FOTU_3465	Phaeosphaeriaceae	Dothideomycetes	No-till	Recovered
FOTU_1032	Tilletia	Exobasidiomycetes	No-till	Recovered
FOTU_4182	Dioszegia	Tremellomycetes	No-till	Recovered
FOTU_1449	Phellinus gilvus	Agaricomycetes	No-till	Recovered
FOTU_1399	Hypocreales	Sordariomycetes	No-till	Recovered
FOTU_1487	Coprinellus	Agaricomycetes	No-till	Recovered
FOTU_46	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_1580	Chaetothyriales	Eurotiomycetes	No-till	Recovered
FOTU_381	Coprinopsis	Agaricomycetes	No-till	Recovered
FOTU_1053	Thamnolia vermicularis subsp. subuliformis	Other	No-till	Recovered
FOTU_4045	Pleosporales	Dothideomycetes	No-till	Recovered
FOTU_746	Archaeorhizomyces borealis	Other	No-till	Recovered
FOTU_4924	Hyphoderma	Agaricomycetes	No-till	Recovered
FOTU_58	Agaricomycetes	Agaricomycetes	No-till	Recovered
FOTU_4271	Tapinella atrotomentosa	Agaricomycetes	No-till	Recovered
FOTU_4459	Ceriporia humilis	Agaricomycetes	No-till	Recovered
FOTU_643	Tilletiopsis washingtonensis	Exobasidiomycetes	No-till	Non-recovered or Indirect
FOTU_267	Cyphellophora	Eurotiomycetes	No-till	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_2177	Vishniacozyma	Tremellomycetes	No-till	Non-recovered or Indirect
FOTU_120	Pleosporales	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_278	Taphrina sp	Other	No-till	Non-recovered or Indirect
FOTU_89	Kondoa sp	Agaricostilbomycetes	No-till	Non-recovered or Indirect
FOTU_3340	Pleosporales	Dothideomycetes	No-till	Local Extinction
FOTU_141	Papiliotrema flavescens	Tremellomycetes	No-till	Non-recovered or Indirect
FOTU_94	Sporobolomyces patagonicus	Microbotryomycetes	No-till	Non-recovered or Indirect
FOTU_155	Dioszegia	Tremellomycetes	No-till	Non-recovered or Indirect
FOTU_309	Pyrenophora tritici-repentis	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_266	Minimedusa polyspora	Agaricomycetes	No-till	Non-recovered or Indirect
FOTU_502	unidentified fungus	unidentified	No-till	Non-recovered or Indirect
FOTU_2746	Dioszegia sp	Tremellomycetes	No-till	Non-recovered or Indirect
FOTU_1161	Ramularia	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_2872	Hypoxylon carneum	Sordariomycetes	No-till	Non-recovered or Indirect
FOTU_83	Vishniacozyma globispora	Tremellomycetes	No-till	Non-recovered or Indirect
FOTU_997	Corticiales	Agaricomycetes	No-till	Non-recovered or Indirect
FOTU_675	Phaeosphaeria typharum	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_447	Sampaiozyma	Microbotryomycetes	No-till	Non-recovered or Indirect
FOTU_971	Trichomeriaceae	Eurotiomycetes	No-till	Non-recovered or Indirect
FOTU_1415	Flabellascoma cycadicola	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_725	Parmeliaceae	Other	No-till	Non-recovered or Indirect
FOTU_347	Chionosphaeraceae	Agaricostilbomycetes	No-till	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_290	Malassezia	Other	No-till	Non-recovered or Indirect
FOTU_1337	unidentified fungus	unidentified	No-till	Non-recovered or Indirect
FOTU_1584	Hypoxylon rubiginosum	Sordariomycetes	No-till	Non-recovered or Indirect
FOTU_1559	Kondoa phyllada	Agaricostilbomycetes	No-till	Non-recovered or Indirect
FOTU_1614	Chionosphaeraceae	Agaricostilbomycetes	No-till	Non-recovered or Indirect
FOTU_2435	Chrysozyma griseoflava	Microbotryomycetes	No-till	Non-recovered or Indirect
FOTU_1463	Trichomeriaceae	Eurotiomycetes	No-till	Non-recovered or Indirect
FOTU_1317	Bensingtonia pseudonaganoensis	Agaricostilbomycetes	No-till	Non-recovered or Indirect
FOTU_1695	unidentified fungus	unidentified	No-till	Non-recovered or Indirect
FOTU_1637	Trichomeriaceae	Eurotiomycetes	No-till	Non-recovered or Indirect
FOTU_2767	Basidiomycota	unidentified	No-till	Non-recovered or Indirect
FOTU_1895	Paraconiothyrium	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_1523	Bannoa	Other	No-till	Non-recovered or Indirect
FOTU_42	Pleosporales	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_5204	Pleosporales	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_2060	Microascales	Sordariomycetes	No-till	Non-recovered or Indirect
FOTU_4518	Pleosporales	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_2627	Entomophthorales	Other	No-till	Non-recovered or Indirect
FOTU_4567	Pleosporales	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_1791	Melanommataceae	Dothideomycetes	No-till	Non-recovered or Indirect
FOTU_1083	Protomyces inouyei	Other	No-till	Non-recovered or Indirect
FOTU_209	Sordariomycetes	Sordariomycetes	No-till	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_1261	Hansfordia pulvinata	Sordariomycetes	No-till	Non-recovered or Indirect
FOTU_27	Hannaella zeae	Tremellomycetes	Conv.	Recovered
FOTU_10	Bulleromyces albus	Tremellomycetes	Conv.	Recovered
FOTU_95	Neosetophoma rosigena	Dothideomycetes	Conv.	Recovered
FOTU_124	Neoascochyta desmazieri	Dothideomycetes	Conv.	Recovered
FOTU_32	Symmetrospora coprosmae	Other	Conv.	Recovered
FOTU_531	Helicoma	Dothideomycetes	Conv.	Recovered
FOTU_845	Teratosphaeriaceae	Dothideomycetes	Conv.	Recovered
FOTU_155	Dioszegia	Tremellomycetes	Conv.	Recovered
FOTU_254	Erythrobasidium yunnanense	Other	Conv.	Recovered
FOTU_1358	Kondoaceae	Agaricostilbomycetes	Conv.	Recovered
FOTU_2177	Vishniacozyma	Tremellomycetes	Conv.	Recovered
FOTU_12	Pleosporales	Dothideomycetes	Conv.	Local Extinction
FOTU_358	Neofitzroyomyces nerii	Other	Conv.	Local Extinction
FOTU_604	Itersonilia perplexans	Tremellomycetes	Conv.	Recovered
FOTU_2746	Dioszegia sp	Tremellomycetes	Conv.	Recovered
FOTU_1413	Capnodiales	Dothideomycetes	Conv.	Recovered
FOTU_1509	Vishniacozyma victoriae	Tremellomycetes	Conv.	Recovered
FOTU_997	Corticiales	Agaricomycetes	Conv.	Recovered
FOTU_224	Exophiala equina	Eurotiomycetes	Conv.	Recovered
FOTU_6108	Uwebraunia communis	Dothideomycetes	Conv.	Recovered
FOTU_5224	Talaromyces	Eurotiomycetes	Conv.	Recovered
FOTU_1213	Zygophiala inaequalis	Dothideomycetes	Conv.	Recovered
FOTU_1036	Mycosphaerellaceae	Dothideomycetes	Conv.	Local Extinction
FOTU_518	Tulasnellaceae	Agaricomycetes	Conv.	Recovered
FOTU_781	Plenodomus collinsoniae	Dothideomycetes	Conv.	Recovered
FOTU_989	Chaetothyriales	Eurotiomycetes	Conv.	Recovered
FOTU_447	Sampaiozyma	Microbotryomycetes	Conv.	Recovered
FOTU_567	Rachicladosporium cboliae	Dothideomycetes	Conv.	Recovered

Table B.3 (cont'd)

FOTU_436	Pleosporales	Dothideomycetes	Conv.	Recovered
FOTU_4350	Didymella	Dothideomycetes	Conv.	Recovered
FOTU_849	Pleosporales	Dothideomycetes	Conv.	Recovered
FOTU_6184	Diaporthe cotoneastri	Sordariomycetes	Conv.	Recovered
FOTU_741	Strelitziana albiziae	Eurotiomycetes	Conv.	Recovered
FOTU_319	unidentified fungus	unidentified	Conv.	Recovered
FOTU_1617	Lecanorales	Other	Conv.	Recovered
FOTU_2984	Xylodon flaviporus	Agaricomycetes	Conv.	Recovered
FOTU_1531	Hyphodontia pallidula	Agaricomycetes	Conv.	Recovered
FOTU_946	Cystobasidiomycetes	Other	Conv.	Recovered
FOTU_1478	Cyphellophora	Eurotiomycetes	Conv.	Recovered
FOTU_1570	Erythrobasidium	Other	Conv.	Recovered
FOTU_306	Eucasphaeria sp	Sordariomycetes	Conv.	Recovered
FOTU_1563	Rhizophlyctis rosea	Other	Conv.	Recovered
FOTU_1211	unidentified fungus	unidentified	Conv.	Recovered
FOTU_246	Sordariales	Sordariomycetes	Conv.	Recovered
FOTU_279	Hypocreales	Sordariomycetes	Conv.	Recovered
FOTU_863	Sanchytriaceae	Other	Conv.	Recovered
FOTU_2335	Thecaphora	Other	Conv.	Recovered
FOTU_1561	Sistotrema sp	Agaricomycetes	Conv.	Recovered
FOTU_4259	Pleosporales	Dothideomycetes	Conv.	Recovered
FOTU_4459	Ceriporia humilis	Agaricomycetes	Conv.	Recovered
FOTU_134	Bulleribasidium oberjochense	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_643	Tilletiopsis washingtonensis	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_119	Symmetrospora	Other	Conv.	Non-recovered or Indirect
FOTU_94	Sporobolomyces patagonicus	Microbotryomycetes	Conv.	Non-recovered or Indirect
FOTU_222	unidentified	Tremellomycetes	Conv.	Local Extinction
FOTU_278	Taphrina sp	Other	Conv.	Non-recovered or Indirect
FOTU_195	Dioszegia changbaiensis	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_89	Kondoa sp	Agaricostilbomycetes	Conv.	Non-recovered or Indirect
FOTU_486	Saitozyma paraflava	Tremellomycetes	Conv.	Local Extinction

Table B.3 (cont'd)

FOTU_5171	Tilletiopsis washingtonensis	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_2205	Pleosporales	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_825	Phyllozyma linderae	Other	Conv.	Non-recovered or Indirect
FOTU_536	Knufia	Eurotiomycetes	Conv.	Local Extinction
FOTU_2326	Microbotryomycetes	Microbotryomycetes	Conv.	Non-recovered or Indirect
FOTU_6082	Phyllozyma	Other	Conv.	Non-recovered or Indirect
FOTU_1771	Spiculogloeomycetes	Other	Conv.	Non-recovered or Indirect
FOTU_1053	Thamnolia vermicularis subsp. subuliformis	Other	Conv.	Non-recovered or Indirect
FOTU_1014	Kondoa	Agaricostilbomycetes	Conv.	Non-recovered or Indirect
FOTU_4045	Pleosporales	Dothideomycetes	Conv.	Local Extinction
FOTU_6	Mycosphaerella tassiana	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_13	Hannaella coprosmae	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_35	Hannaella luteola	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_52	Dioszegia	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_34	Hannaella oryzae	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_1225	Tremellales	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_66	Sporobolomyces roseus	Microbotryomycetes	Conv.	Non-recovered or Indirect
FOTU_267	Cyphellophora	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_154	Leptospora sp	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_824	Seimatosporium	Sordariomycetes	Conv.	Local Extinction
FOTU_642	Entyloma polysporum	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_282	Neodevriesia poagena	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_502	unidentified fungus	unidentified	Conv.	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_1271	Phaeosphaeriaceae	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_798	Golubevia pallescens	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_6420	Coniothyrium sp	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_186	Zymoseptoria verkleyi	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_2945	Vishniacozyma victoriae	Tremellomycetes	Conv.	Local Extinction
FOTU_6163	Pleosporales	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_655	Tremellomycetes	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_767	Dioszegia	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_744	Sordariales	Sordariomycetes	Conv.	Non-recovered or Indirect
FOTU_6436	Tilletiopsis	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_293	Papiliotrema	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_963	Tuber melanosporum	Other	Conv.	Local Extinction
FOTU_5225	Coniothyrium sp	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_158	Vishniacozyma dimennae	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_1867	Neosetophoma samararum	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_322	Torula	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_326	Mycosphaerellaceae	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_1220	Taphrina communis	Other	Conv.	Non-recovered or Indirect
FOTU_1388	Cyphellophora	Eurotiomycetes	Conv.	Local Extinction
FOTU_971	Trichomeriaceae	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_362	Ganoderma	Agaricomycetes	Conv.	Non-recovered or Indirect
FOTU_890	Hannaella sinensis	Tremellomycetes	Conv.	Local Extinction
FOTU_1086	Phaeosphaeria sp	Dothideomycetes	Conv.	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_1747	Crocicreas cyathoideum	Other	Conv.	Local Extinction
FOTU_462	Devriesia pseudoamericana	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_1488	Ascomycota	unidentified	Conv.	Non-recovered or Indirect
FOTU_1186	Helotiales	Other	Conv.	Non-recovered or Indirect
FOTU_1165	Curvularia	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_757	Pseudozyma pruni	Other	Conv.	Non-recovered or Indirect
FOTU_1464	Symmetrospora	Other	Conv.	Non-recovered or Indirect
FOTU_3417	Helotiales	Other	Conv.	Non-recovered or Indirect
FOTU_410	Herpotrichiellaceae	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_522	Microdochium seminicola	Sordariomycetes	Conv.	Non-recovered or Indirect
FOTU_6187	Protomyces inouyei	Other	Conv.	Non-recovered or Indirect
FOTU_1463	Trichomeriaceae	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_1317	Bensingtonia pseudonaganoensis	Agaricostilbomycetes	Conv.	Non-recovered or Indirect
FOTU_3340	Pleosporales	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_1637	Trichomeriaceae	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_6748	Tilletiopsis washingtonensis	Exobasidiomycetes	Conv.	Non-recovered or Indirect
FOTU_1284	Tremellomycetes	Tremellomycetes	Conv.	Non-recovered or Indirect
FOTU_1872	Mycosphaerellaceae	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_409	Chaetosphaeriaceae	Sordariomycetes	Conv.	Non-recovered or Indirect
FOTU_1523	Bannoa	Other	Conv.	Non-recovered or Indirect
FOTU_589	Bensingtonia sp	Agaricostilbomycetes	Conv.	Local Extinction
FOTU_1947	Ustilaginaceae	Other	Conv.	Non-recovered or Indirect

Table B.3 (cont'd)

FOTU_2490	Bensingtonia naganoensis	Agaricostilbomycetes	Conv.	Non-recovered or Indirect
FOTU_233	Penicillium decumbens	Eurotiomycetes	Conv.	Non-recovered or Indirect
FOTU_4518	Pleosporales	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_5596	Peroneutypa	Sordariomycetes	Conv.	Non-recovered or Indirect
FOTU_1939	Pleosporales	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_2004	Bensingtonia	Agaricostilbomycetes	Conv.	Non-recovered or Indirect
FOTU_1411	Bambusicola	Dothideomycetes	Conv.	Non-recovered or Indirect
FOTU_1058	Protomyces inouyei	Other	Conv.	Non-recovered or Indirect
FOTU_157	Chaetomiaceae	Sordariomycetes	Conv.	Non-recovered or Indirect
FOTU_3934	Basidiomycota	Other	Conv.	Non-recovered or Indirect
FOTU_699	unidentified fungus	unidentified	Conv.	Non-recovered or Indirect
FOTU_1038	Ascomycota	unidentified	Conv.	Non-recovered or Indirect
FOTU_3552	Pseudocercospora	Dothideomycetes	Conv.	Non-recovered or Indirect

APPENDIX C: Caulobacter Segnis Genome Sequencing

High molecular weight genomic DNA was extracted was using a guanidinium thiocyanate protocol as previously described [386]. Briefly, cells from 1mL of an overnight culture were pelleted and resuspended in .1 ml of Tris EDTA (TE) buffer with added RNAse A. Cells were then lysed with a lysis buffer composed of guanidinium thiocyanate, EDTA, and 0.5% Sarkosyl. Next, samples were incubated at 60c for 15 minutes before adding .25 mL of 7.5M ammonium acetate. Next, samples were placed on ice for 10 minutes prior to adding .5 mL of chloroform. Samples were then centrifuged, and the aqueous phase was then transferred to fresh tubes, mixed with .5 volumes of ice-cold isopropanol, and placed at room temperature for 15 minutes. Following centrifugation, DNA pellets were washed three times with 70% ethanol and resuspended in .1 mL of TE buffer. The *Caulobacter* genome was sequenced at the Microbial Genome Sequencing Center (Pittsburgh, USA) through a combination of long read nanopore (Oxford, UK) sequencing and paired end 150 bp Illumina (San Diego, USA) sequencing for polishing. The genome was then assembled using Pilon and annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (NCBI PGAP) pipeline [387, 388].

The complete, circularized *Caulobacter* genome was 5,687,505 bp long and had 5,287 genes. The isolate used in this study appears to be most closely related to *Caulobacter segnis*. The isolate appears to be a riboflavin auxotroph as it is missing early genes in the riboflavin biosynthesis pathway (*ribD-E-AB-H* locus), although these genes are present in *Caulobacter crescentus* (Figure C.1). Additionally, *C. segnis RL271* had impaired growth in PYE media that had been stored for a month or more where light sensitive riboflavin would be expected to be lacking. The media again supported growth when supplemented with riboflavin. Regardless of

storage age, the media supported growth of *C. crescentus* without riboflavin supplementation (Figure C.2). Additionally, the isolate is lacking genes for the biosynthesis of the polar adhesin holdfast that is characteristic of most *Caulobacter*.

Figure C.1 - Caulobacter segnis RL271 lacks riboflavin biosynthesis genes. **A)** Riboflavin biosynthesis pathway. Riboflavin is synthesized from GTP and ribulose-5-phosphate via the indicated intermediate molecules. The enzymes responsible for each chemical reaction are indicated in color. Riboflavin is further modified to generate FMN and FAD. **B)** Heatmap of the number of enzymes encoded by *Caulobacter crescentus NA1000* (the widely studied model Caulobacter) and *C. segnis RL271*. Full enzyme names with the corresponding enzyme commission numbers are in black. Protein names are in color. Bifunctional enzymes have two activities listed. **C)** Genetic loci encoding riboflavin biosynthesis genes in Caulobacter sps. The locus numbers for each gene are indicated below the operon structures. *C. segnis* lacks the *ribD-E-AB-H* locus. Genes/proteins absent in *C. segnis* are blue; two orthologs of *ribH* (teal) are present in *C. crescentus*, but only one is present in *C. segnis*; the bifunctional *ribFC* (pink) is present in both genomes. Unrelated genes at these loci are grey.

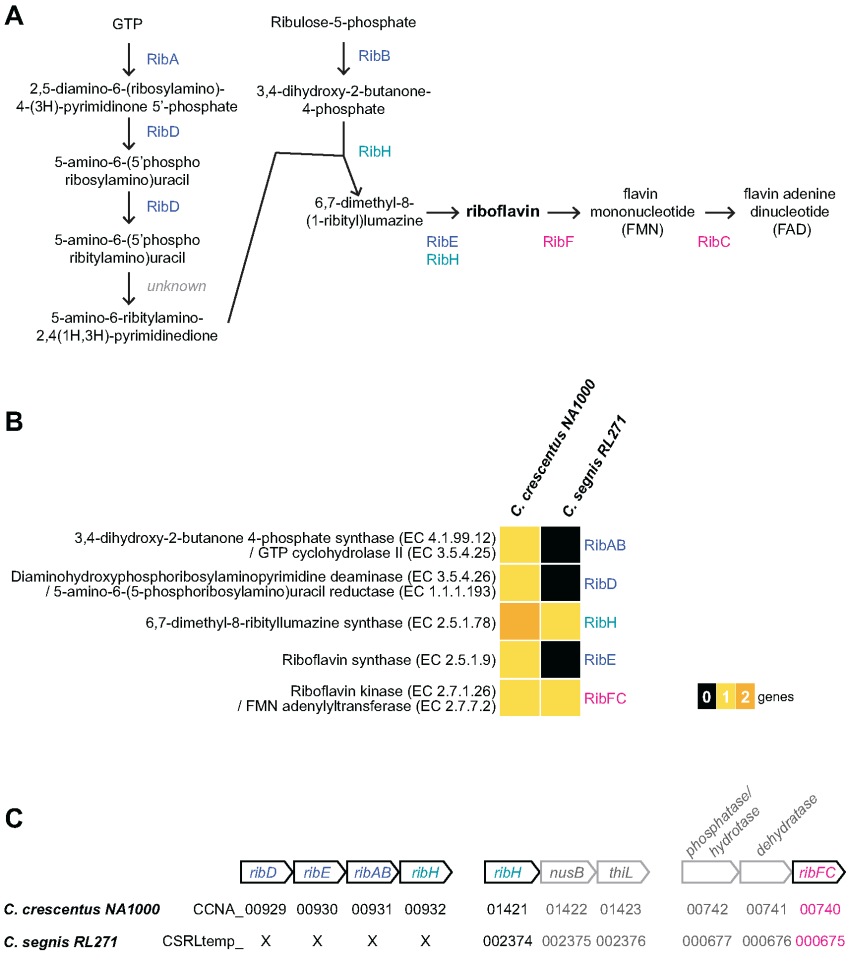
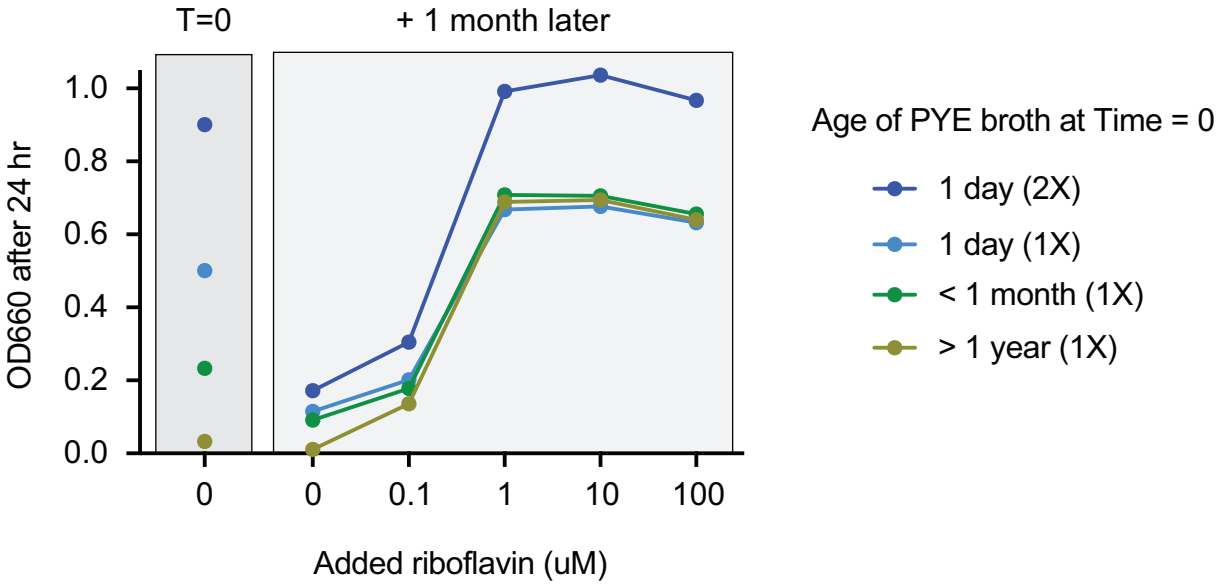


Figure C.2 - Riboflavin supplementation supports growth of Caulobacter segnis RL271. The complex growth medium, peptone-yeast extract (PYE) broth, supports growth of *C. segnis RL271* when freshly prepared (T=0, blue dots). Riboflavin is light sensitive. Over relatively short time scales (~1 month), the same media stored in room light no longer supports robust growth unless supplemented with riboflavin. Double strength (2X) PYE supports more growth than the standard strength recipe. Note that *C. crescentus*, which encodes the genes for riboflavin synthesis, grows robustly in all these batches of PYE medium (not shown).



APPENDIX D: Current Curriculum Vitae

Reid W. Longley

Michigan State University | Department of Microbiology and Molecular Genetics

3285 Molecular Plant Sciences Building | 1066 Bogue St. East Lansing, MI 48824

longleyr@msu.edu

Education:

University of Montana	Missoula	Human Biology	BS,2016
University of Montana	Missoula	Exercise Science	BS,2016

Research Experience**:**

Graduate Researcher/ PhD Candidate: Department of Microbiology and Molecular Genetics, Michigan State University, November 2017- present (research advisor: Dr. Gregory Bonito).

- Working on a project researching crop microbiome effects on crop health and yield. Additionally, studying the importance of the coral microbiome in changing ocean environments. Work includes extraction, next generation sequencing and preparation, PCR, bioinformatics.

PhD Rotation Student: Department of Microbiology and Molecular Genetics, Michigan State University, August 2017 – November 2017, (research advisor: Dr. Matthew Schrenk)

- Performed a 10-week rotation in Matthew Schrenk's laboratory, extracted DNA from deep sea hydrothermal extremophiles. Gained skills in developing extraction techniques.

Undergraduate Research/Laboratory Technician: Department of Biology, University of Montana, January 2015 – August 2017, (research advisor: Dr. Scott Miller).

- Worked on various projects within the field of microbial ecology, Projects included: bacterial genetics, basic microbiology, genome assembly, and bacterial evolution.

Mentorship Experience:

Marcela Tabares Ruiz: PhD rotation student in the Bonito lab in the fall of 2019. Gained skills in bioinformatics analyses and next generation sequencing analyses.

Grace Kuza: Michigan State University undergraduate student Spring/Summer 2019. Assisted in curating culture collection of Fungi and Bacteria isolated from Soybeans. Gained skills in DNA extraction, PCR, and sterile technique.

Sophia Fitzgerald: Denver University undergraduate student Summer 2019. REU student who aided in collection and organization of soybeans in the summer of 2018. Gained skills in DNA extraction, field sampling, and fungal and bacterial isolation.

Sophie Gabrysiak: Michigan State University undergraduate student 2020-2022. Assisted in various tasks including plant care, experimental design, culturing of bacteria and fungi, and DNA extraction.

Publications:

Published (* designates equal contribution)**:**

1. **Longley R***, Noel ZA*, Chilvers MI, Trail F, Bonito G. 2022. Non-target fungicidal disturbance in the soybean and corn phyllosphere lead to loss in network complexity and differential resilience under two management strategies. *ISME Communications* 2:19.
2. Liber J, Minier DH, Stouffer-Hopkins A, Van Wyk J, **Longley R**, Bonito G. 2022. Leaf litter fungal communities reflect pre-senescent leaf communities in a temperate forest ecosystem. *PeerJ* 10:e12701.
3. Chen KH, **Longley R**, Bonito G, Liao HL. 2021. A Two-step PCR Protocol Enabling

Flexible Primer Choice and High Sequencing Yield for Illumina Miseq Meta-Barcoding. *Agronomy* 11:7.

4. **Longley R**, Noel ZA, Benucci GMN, Chilvers M, Trail F, Bonito G. 2020. Crop Management Impacts the Soy (*Glycine max*) Microbiome. *Frontiers in Microbiology* 11:1116.
5. Miller SR, **Longley R**, Hutchins PR, Bauersachs T. 2020. Cellular Innovation of the Cyanobacterial Heterocyst by the Adaptive Loss of Plasticity. *Current Biology* 30:1-7.
6. **Longley R*,** Benucci GMN*, Mills G, Bonito G.2019. Fungal and bacterial community dynamics in substrates during the cultivation of morels (*Morchella rufobrunnea*) indoors. *Fems Microbiology Letters* 366: fnz215.
7. **Longley R***, Benucci GMN*, Zhang P, Zhao Q, Bonito G, Yu F. 2019. Microbial communities associated with the black morel Morchella sextelata cultivated in greenhouses. *PeerJ* 7: e7744.
8. McCoy AG, Roth MG, Shay R, Noel ZA, Jayawardana MA, **Longley RW**, Bonito G, Chilvers MI. 2019. Identification of Fungal Communities Within the Tar Spot Complex of Corn in Michigan via Next-Generation Sequencing. *Phytobiomes J*. 3:3, 235-243.

In Preparation:

1. **Longley R,** Benucci GMN, Pochon X, Bonito V, Bonito G. Post-bleaching microbiome assessment of stony coral survivors and casualties.
2. Zemenick A, **Longley R,** Hughes D, Gordon S, Bonito G, Weber M. Multitrophic community of the phyllosphere influenced by the evolution of a novel leaf trait.
3. Shemanski S, Hatlen RJ, Heger L, Sharma N, Bonito G, **Longley R**, Miles TD.

Blueberry Fruit Microbiome Varies by Tissue Type and Fungicide Treatment.

4. Sultaire SM, Benucci GMN, **Longley R**, Bonito G, Roloff GJ. The influence of retention forestry on richness and composition of ectomycorrhizal fungi in chipmunk diet.
5. **Longley R,** Robinson A, Liber J, Bryson A, LaButti K, Riley R, Barry K, Grigoriev IV, Desiro A, Jones A, Misztal P, Chain P, Bonito G. Comparative genomics of intracellular Mollicutes-related bacterial endosymbionts of Fungi.
6. **Longley R,** Trail F, Chilvers M, Bonito G. Hub Microbe Inoculation Impact on Soybean Response to Low Moisture Stress.

Competitive Honors/Awards

2021: *Michigan State University Dissertation Completion Fellowship*

2021: *Microbiology and Molecular Genetics Ralph Evans Award*

2020: *Department of Energy Office of Science Graduate Student Research Fellowship*

2020: *The James M. Tiedje Graduate Student/Post-doctorate Travel Award in Microbial Ecology*

2020: *Russell B. Duvall Travel Award*

2019: *Dr. C. A. Reddy and Sasikala Reddy Endowed Graduate Award in Microbial Physiology-Ecology*

Fall 2018-2020: *Plant Biotechnology for Health and Sustainability NIH Fellow*

Summer 2018: *Kellogg Biological Station Summer Graduate Fellowship*

2016: *Montana Institute on Ecosystems Summer Fellowship*

Spring 2013-2016: *University of Montana Dean's list*

Fall 2014: *University of Montana Dean's list*

Spring 2014: *Academic All-Big Sky Track and Field*

Fall 2013: *Academic All-Big Sky Cross Country*

Relevant Extracurricular Activities:

Plant Biotechnology for Health and Sustainability Symposium: Serving on organizing committee for 2020 symposium. Helping to plan topics, invite speakers, and organize symposium events.

Smith Foray: Served on the planning committee for the 2019 *Smith Foray* which was a mushroom foray for professional mycologists of the Midwest held at Kellogg Biological Station.

Fascination of Plants Day: Michigan State University community outreach event in May 2019 to teach to community members interested in learning more in plant biology. Volunteered at a booth that displayed the diversity and importance of mushrooms.

STEAM Night: Volunteered alongside other Michigan State University Graduate students in February 2019 at Donley Elementary School to teach elementary schoolers and their parents about plant-associated microbes and their interactions.

Invited Talks/Presentations:

1. *Department of Energy Genomic Science Program 2022*: Poster Presentation – Genomics of Mollicutes Related Endobacteria. **Longley R,** Robinson A, Liber J, Bryson A, LaButti K, Riley R, Barry K, Grigoriev IV, Desiro A, Jones A, Misztal P, Chain P, Bonito G.
2. *MSU Microbiology and Molecular Genetics Work in Progress 2020:* Seminar - The Role of the Microbiome in the Soybean Response to Low-moisture Stress
3. *Mycological Society of America Conference 2021*: Symposium talk- Non-target Fungicidal Effects in the Corn (*Zea mays*) and Soybean (*Glycine max)* Microbiome.

4. *MSU Microbiology and Molecular Genetics Work in Progress 2020*: Seminar- The Microbiome of Bleached and Unbleached Fijian Corals
5. *Mycological Society of America Conference 2019*: Poster Presentation- Soybean Associated Fungal Communities Under Three Different Management Systems. **Reid Longley**, Martin Chilvers, Frances Trail, Gregory Bonito
6. *Predicting and Controlling Microbiomes for Health, Industry, and the Environment 2019*: Poster Presentation- Soybean Associated Root and Soil Fungal Communities Under Three Different Managements. **Reid Longley**, Martin Chilvers, Frances Trail, Gregory Bonito.
7. *International Mycological Congress 2018*: Analysis of coral-associated fungal and microbial communities in Fiji using high throughput amplicon sequencing. **Reid Longley**, Gian Maria Niccolo Benucci, Osama Alian, Xavier Pochon, Victor Bonito, Gregory Bonito
8. *Kellogg Biological Station Undergraduate Symposium 2018*: Poster Presentation- The Culturable Mycobiome of Soybean. Sophia Fitzgerald, **Reid Longley**, Martin Chilvers, Frances Trail.

REFERENCES

REFERENCES

1. Claassen R, Bowman M, Mcfadden J, Smith D, Wallander S. Tillage Intensity and Conservation Cropping in the United States. *USDA Economic Research Service* 2018; **197**: 1–21.

2. Sharma-Poudyal D, Schlatter D, Yin C, Hulbert S, Paulitz T. Long-term no-till: A major driver of fungal communities in dryland wheat cropping systems. *PLoS One* 2017; **12**: e0184611.

3. Hartman K, van der Heijden MGA, Wittwer RA, Banerjee S, Walser J-C, Schlaeppi K. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* 2018; **6**: 14.

4. Gdanetz K, Trail F. The wheat microbiome under four management strategies, and potential for endophytes in disease protection. *Phytobiomes J* 2017; **1**: 158–168.

5. Wegulo S, Stevens J, Zwingman M, Stephen P. Yield response to foliar fungicide application in winter wheat. *Fungicides for Plant and Animal Diseases*. 2012. InTech.

6. Kandel YR, Hunt C, Ames K, Arneson N, Bradley CA, Byamukama E, et al. Meta-analysis of soybean yield response to foliar fungicides evaluated from 2005 to 2018 in the United States and Canada. *Plant Dis* 2021; **105**: 1382–1389.

7. Perazzolli M, Antonielli L, Storari M, Puopolo G, Pancher M, Giovannini O, et al. Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. *Appl Environ Microbiol* 2014; **80**: 3585–3596.

8. O'Callaghan M. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Appl Microbiol Biotechnol* 2016; **100**: 5729–5746.

9. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, et al. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol* 2016; **14**: e1002352.

10. Banerjee S, Walder F, Büchi L, Meyer M, Held AY, Gattinger A, et al. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J* 2019; **13**: 1722–1736.

11. Margulis L, Fester R. Bellagio conference and book. Symbiosis as Source of Evolutionary Innovation: Speciation and Morphogenesis. Conference--June 25-30, 1989, Bellagio Conference Center, Italy. *Symbiosis* 1991; **11**: 93–101.

12. Simon J-C, Marchesi JR, Mougel C, Selosse M-A. Host-microbiota interactions: from holobiont theory to analysis. *Microbiome* 2019; **7**: 5.

13. Brandl MT, Lindow SE. Contribution of indole-3-acetic acid production to the epiphytic fitness of erwinia herbicola. *Appl Environ Microbiol* 1998; **64**: 3256–3263.

14. Limtong S, Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. *World J Microbiol Biotechnol* 2012; **28**: 3323–3335.

15. Ritpitakphong U, Falquet L, Vimoltust A, Berger A, Métraux J-P, L'Haridon F. The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytol* 2016; **210**: 1033–1043.

16. Tanaka A, Tapper BA, Popay A, Parker EJ, Scott B. A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. *Mol Microbiol* 2005; **57**: 1036–1050.

17. Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* 2017; **546**: 145–147.

18. Xiang Q, Chen Q-L, Zhu D, Yang X-R, Qiao M, Hu H-W, et al. Microbial functional traits in phyllosphere are more sensitive to anthropogenic disturbance than in soil. *Environ Pollut* 2020; **265**: 114954.

19. Beijerinck M. Über oligonitrophile Mikroben. *Zbl Backt* 1901; **7**: 561–582.

20. Hodge A, Campbell CD, Fitter AH. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 2001; **413**: 297–299.

21. Raju PS, Clark RB, Ellis JR, Maranville JW. Effects of species of VA-mycorrhizal fungi on growth and mineral uptake of sorghum at different temperatures. *Plant Soil* 1990; **122**: 165–170.

22. Egerton-Warburton LM, Querejeta JI, Allen MF. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *J Exp Bot* 2007; **58**: 1473–1483.

23. Xu L, Nicolaisen M, Larsen J, Zeng R, Gao S, Dai F. Pathogen infection and host-resistance interactively affect root-associated fungal communities in watermelon. *Front Microbiol* 2020; **11**: 605622.

24. Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, et al. Microbial interkingdom interactions in roots promote Arabidopsis survival. *Cell* 2018; **175**: 973-983.e14.

25. Bakker PAHM, Berendsen RL, Doornbos RF, Wintermans PCA, Pieterse CMJ. The rhizosphere revisited: root microbiomics. *Front Plant Sci* 2013; **4**: 165.

26. Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME J* 2014; **8**: 1577–1587.

27. van Elsas JD, Chiurazzi M, Mallon CA, Elhottova D, Kristufek V, Salles JF. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proc Natl Acad Sci U S A* 2012; **109**: 1159–1164.

28. Jousset A, Schulz W, Scheu S, Eisenhauer N. Intraspecific genotypic richness and relatedness predict the invasibility of microbial communities. *ISME J* 2011; **5**: 1108–1114.

29. Ferreira DA, da Silva TF, Pylro VS, Salles JF, Andreote FD, Dini-Andreote F. Soil microbial diversity affects the plant-root colonization by arbuscular mycorrhizal fungi. *Microb Ecol* 2021; **82**: 100–103.

30. Gebhardt MR, Daniel TC, Schweizer EE, Allmaras RR. Conservation tillage. *Science* 1985; **230**: 625–630.

31. Mäder P, Berner A. Development of reduced tillage systems in organic farming in Europe. *Renew Agric Food Syst* 2012; **27**: 7–11.

32. Resource Conservation Glossary. 1976. Soil Conservation Society of America, Ankeny, Iowa.

33. Bockus WW, Shroyer JP. The impact of reduced tillage on soilborne plant pathogens. *Annu Rev Phytopathol* 1998; **36**: 485–500.

34. Pan G, Smith P, Pan W. The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. *Agric Ecosyst Environ* 2009; **129**: 344–348.

35. Powlson DS, Stirling CM, Jat ML, Gerard BG, Palm CA, Sanchez PA, et al. Limited potential of no-till agriculture for climate change mitigation. *Nat Clim Chang* 2014; **4**: 678–683.

36. Spangler K, Burchfield EK, Schumacher B. Past and current dynamics of U.s. agricultural land use and policy. *Front Sustain Food Syst* 2020; **4**.

37. Meyer S, Parsons JL. Acreage. *USDA.gov*. https://www.nass.usda.gov/Publications/Todays_Reports/reports/acrg0621.pdf. .

38. Dick WA, Van Doren DM Jr. Continuous tillage and rotation combinations effects on corn, soybean, and oat yields 1. *Agron J* 1985; **77**: 459–465.

39. Robertson P, Gross KL, Hamilton SK, Landis DA, Schmidt TM, Snapp SS, et al. Farming for ecosystem services: An ecological approach to production agriculture. *Bioscience* 2014; **64**: 404–415.
40. Weesies GA, Livingston SJ, Hosteter WD. Effect of soil erosion on crop yield in Indiana: Results of a 10 year study G. *Schertz Journal of Soil and Water* 1994; **49**: 597–600.

41. Munkholm LJ, Hansen EM, Olesen JE. The effect of tillage intensity on soil structure and winter wheat root/shoot growth. *Soil Use Manag* 2008; **24**: 392–400.

42. Kadžienė G, Munkholm LJ, Mutegi JK. Root growth conditions in the topsoil as affected by tillage intensity. *Geoderma* 2011; **166**: 66–73.

43. Das A, Rangappa K, Basavaraj S, Dey U, Haloi M, Layek J, et al. Conservation tillage and nutrient management practices in summer rice (Oryza sativa L.) favoured root growth and phenotypic plasticity of succeeding winter pea (Pisum sativumL.) under eastern Himalayas, India. *Heliyon* 2021; **7**: e07078.

44. Ball-Coelho B. Tillage alters corn root distribution in coarse-textured soil. *Soil Tillage Res* 1998; **45**: 237–249.

45. Fiorini A, Boselli R, Amaducci S, Tabaglio V. Effects of no-till on root architecture and root-soil interactions in a three-year crop rotation. *Eur J Agron* 2018; **99**: 156–166.

46. Orrù L, Canfora L, Trinchera A, Migliore M, Pennelli B, Marcucci A, et al. How tillage and crop rotation change the distribution pattern of fungi. *Front Microbiol* 2021; **12**: 634325.

47. Longley R, Noel ZA, Benucci GMN, Chilvers MI, Trail F, Bonito G. Crop Management Impacts the Soybean (Glycine max) Microbiome. *Front Microbiol* 2020; **11**: 1116.

48. Wang H, Li X, Li X, Wang J, Li X, Guo Q, et al. Long-term no-tillage and different residue amounts alter soil microbial community composition and increase the risk of maize root rot in northeast China. *Soil Tillage Res* 2020; **196**: 104452.

49. Beare MH, Coleman DC, Pohlad BR, Wright DH. Residue placement and fungicide effects on fungal communities in conventional and no-tillage soils. *Soil Sci Soc Am J* 1993; **57**: 392–399.

50. Frey SD, Elliott ET, Paustian K. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol Biochem* 1999; **31**: 573–585.

51. Wang Z, Li T, Wen X, Liu Y, Han J, Liao Y, et al. Fungal communities in rhizosphere soil under conservation tillage shift in response to plant growth. *Front Microbiol* 2017; **8**: 1301.

52. Lu X, Lu X, Liao Y. Effect of tillage treatment on the diversity of soil arbuscular mycorrhizal fungal and soil aggregate-associated carbon content. *Front Microbiol* 2018; **9**: 2986.

53. Vick CM, Chong SK, Bond JP, Russin JS. Response of soybean sudden death syndrome to subsoil tillage. *Plant Dis* 2003; **87**: 629–632.

54. Flett BC, McLaren NW, Wehner FC. Incidence of ear rot pathogens under alternating corn tillage practices. *Plant Dis* 1998; **82**: 781–784.

55. Broders KD, Lipps PE, Paul PA, Dorrance AE. Characterization of Pythium spp. Associated with corn and soybean seed and seedling disease in Ohio. *Plant Dis* 2007; **91**: 727–735.

56. Licht MA, Al-Kaisi M. Strip-tillage effect on seedbed soil temperature and other soil physical properties. *Soil Tillage Res* 2005; **80**: 233–249.

57. Roth MG, Webster RW, Mueller DS, Chilvers MI, Faske TR, Mathew FM, et al. Integrated management of important soybean pathogens of the United States in changing climate. *J Integr Pest Manag* 2020; **11**.

58. Doupnik B Jr. Ecofallow - A reduced tillage system - and plant diseases. *Plant Dis* 1980; **64**: 31.

59. Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* 2016; **2**: 16242.

60. Klironomos JN. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 2003; **84**: 2292–2301.

61. Berruti A, Lumini E, Balestrini R, Bianciotto V. Arbuscular Mycorrhizal Fungi as natural biofertilizers: Let's benefit from past successes. *Front Microbiol* 2015; **6**: 1559.

62. Säle V, Aguilera P, Laczko E, Mäder P, Berner A, Zihlmann U, et al. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 2015; **84**: 38–52.

63. Bowles TM, Jackson LE, Loeher M, Cavagnaro TR. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *J Appl Ecol* 2017; **54**: 1785–1793.

64. Lekberg Y, Vasar M, Bullington LS, Sepp S-K, Antunes PM, Bunn R, et al. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? *New Phytol* 2018; **220**: 971–976.

65. Higo M, Tatewaki Y, Iida K, Yokota K, Isobe K. Amplicon sequencing analysis of arbuscular mycorrhizal fungal communities colonizing maize roots in different cover cropping and tillage systems. *Sci Rep* 2020; **10**: 6039.

66. Wipf HM-L, Xu L, Gao C, Spinner HB, Taylor J, Lemaux P, et al. Agricultural soil management practices differentially shape the bacterial and fungal microbiome of sorghum bicolor. *Appl Environ Microbiol* 2020; **87**.

67. Kolaříková Z, Slavíková R, Krüger C, Krüger M, Kohout P. PacBio sequencing of Glomeromycota rDNA: a novel amplicon covering all widely used ribosomal barcoding regions and its applicability in taxonomy and ecology of arbuscular mycorrhizal fungi. *New Phytol* 2021; **231**: 490–499.

68. Wang Z, Liu L, Chen Q, Wen X, Liao Y. Conservation tillage increases soil bacterial diversity in the dryland of northern China. *Agron Sustain Dev* 2016; **36**.

69. Srour AY, Ammar HA, Subedi A, Pimentel M, Cook RL, Bond J, et al. Microbial communities associated with long-term tillage and fertility treatments in a corn-soybean cropping system. *Front Microbiol* 2020; **11**: 1363.

70. Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, et al. Nitrogen nutrition of soybean in Brazil: Contributions of biological N2 fixation and N fertilizer to grain yield. *Can J Plant Sci* 2006; **86**: 927–939.

71. Syswerda SP, Corbin AT, Mokma DL, Kravchenko AN, Robertson GP. Agricultural management and soil carbon storage in surface vs. Deep layers. *Soil Sci Soc Am J* 2011; **75**: 92–101.

72. Yan J, Han XZ, Ji ZJ, Li Y, Wang ET, Xie ZH, et al. Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. *Appl Environ Microbiol* 2014; **80**: 5394–5402.

73. Massoni J, Bortfeld-Miller M, Widmer A, Vorholt JA. Capacity of soil bacteria to reach the phyllosphere and convergence of floral communities despite soil microbiota variation. *Proc Natl Acad Sci U S A* 2021; **118**: e2100150118.

74. Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-Marcell J, Lax S, et al. The soil microbiome influences grapevine-associated microbiota. *MBio* 2015; **6**.

75. Jarvis WR. Splash Dispersal of Spores of Botrytis cinerea Pers. *Nature* 1962; **193**: 599–599.

76. Philippot L, Spor A, Hénault C, Bru D, Bizouard F, Jones CM, et al. Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* 2013; **7**: 1609–1619.

77. Tian L, Wang X-W, Wu A-K, Fan Y, Friedman J, Dahlin A, et al. Deciphering functional redundancy in the human microbiome. *Nat Commun* 2020; **11**: 6217.

78. Souza RC, Hungria M, Cantão ME, Vasconcelos ATR, Nogueira MA, Vicente VA. Metagenomic analysis reveals microbial functional redundancies and specificities in a soil under different tillage and crop-management regimes. *Appl Soil Ecol* 2015; **86**: 106–112.
79. Bandick AK, Dick RP. Field management effects on soil enzyme activities. *Soil Biol Biochem* 1999; **31**: 1471–1479.

80. Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM. Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytol* 2013; **198**: 264–273.

81. Mangeot-Peter L, Tschaplinski TJ, Engle NL, Veneault-Fourrey C, Martin F, Deveau A. Impacts of Soil Microbiome Variations on Root Colonization by Fungi and Bacteria and on the Metabolome of Populus tremula × alba. *Phytobiomes J* 2020; **4**: 142–155.

82. Gornall J, Betts R, Burke E, Clark R, Camp J, Willett K, et al. Implications of climate change for agricultural productivity in the early twenty-first century. *Philos Trans R Soc Lond B Biol Sci* 2010; **365**: 2973–2989.

83. Blevins RL, Cook D, Phillips SH, Phillips RE. Influence of no-tillage on soil moisture 1. *Agron J* 1971; **63**: 593–596.

84. Eeswaran R, Nejadhashemi AP, Alves FC, Saravi B. Evaluating the applicability of soil moisture-based metrics for gauging the resiliency of rainfed agricultural systems in the midwestern United States. *Soil Tillage Res* 2021; **205**: 104818.

85. Wang X, Dai K, Zhang D, Zhang X, Wang Y, Zhao Q, et al. Dryland maize yields and water use efficiency in response to tillage/crop stubble and nutrient management practices in China. *Field Crops Res* 2011; **120**: 47–57.

86. Chen B, Gramig BM, Yun SD. Conservation tillage mitigates drought-induced soybean yield losses in the US Corn Belt. *Q Open* 2021; **1**.

87. Rubin RL, van Groenigen KJ, Hungate BA. Plant growth promoting rhizobacteria are more effective under drought: a meta-analysis. *Plant Soil* 2017; **416**: 309–323.

88. French E, Kaplan I, Iyer-Pascuzzi A, Nakatsu CH, Enders L. Emerging strategies for precision microbiome management in diverse agroecosystems. *Nat Plants* 2021; **7**: 256–267.

89. Li J, Meng B, Chai H, Yang X, Song W, Li S, et al. Arbuscular mycorrhizal fungi alleviate drought stress in C3 (Leymus chinensis) and C4 (Hemarthria altissima) grasses via altering antioxidant enzyme activities and photosynthesis. *Front Plant Sci* 2019; **10**: 499.

90. Khalvati MA, Hu Y, Mozafar A, Schmidhalter U. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biol (Stuttg)* 2005; **7**: 706–712.

91. Roper JM, Garcia JF, Tsutsui H. Emerging technologies for monitoring plant health in vivo. *ACS Omega* 2021; **6**: 5101–5107.
92. Babaeian E, Sadeghi M, Jones SB, Montzka C, Vereecken H, Tuller M. Ground, proximal, and satellite remote sensing of soil moisture. *Rev Geophys* 2019; **57**: 530–616.

93. Cao D, Wang F, Yu S, Dong S, Wu R, Cui N, et al. Prevalence of Azole-Resistant Aspergillus fumigatus is Highly Associated with Azole Fungicide Residues in the Fields. *Environ Sci Technol* 2021; **55**: 3041–3049.

94. Snelders E, Huis In 't Veld RAG, Rijs AJMM, Kema GHJ, Melchers WJG, Verweij PE. Possible environmental origin of resistance of Aspergillus fumigatus to medical triazoles. *Appl Environ Microbiol* 2009; **75**: 4053–4057.

95. Noel ZA, Longley R, Benucci GMN, Trail F, Chilvers MI, Bonito G. Non-target impacts of fungicide disturbance on phyllosphere yeasts in conventional and no-till management. *ISME Communications* 2022; **2**: 1–10.

96. Zubrod JP, Bundschuh M, Arts G, Brühl CA, Imfeld G, Knäbel A, et al. Fungicides: An overlooked pesticide class? *Environ Sci Technol* 2019; **53**: 3347–3365.

97. Fernandez-Cornejo J, Nehring RF, Osteen C, Wechsler S, Martin A, Vialou A. Pesticide use in U.s. agriculture: 21 selected crops, 1960-2008. *SSRN Electron J* 2014.

98. 2016 Agricultural Chemical Use Survey. *United States Department of Agriculture National Agricultural Statistics Survey*. www.nass.usda.gov.

99. Grier JW. Ban of DDT and subsequent recovery of Reproduction in bald eagles. *Science* 1982; **218**: 1232–1235.

100. Lundqvist J, von Brömssen C, Rosenmai AK, Ohlsson Å, Le Godec T, Jonsson O, et al. Assessment of pesticides in surface water samples from Swedish agricultural areas by integrated bioanalysis and chemical analysis. *Environ Sci Eur* 2019; **31**.

101. Lybrand DB, Xu H, Last RL, Pichersky E. How plants synthesize pyrethrins: Safe and biodegradable insecticides. *Trends Plant Sci* 2020; **25**: 1240–1251.

102. Bock CH, Shapiro-Ilan DI, Wedge DE, Cantrell CL. Identification of the antifungal compound, trans-cinnamic acid, produced by Photorhabdus luminescens, a potential biopesticide against pecan scab. *J Pest Sci (2004)* 2014; **87**: 155–162.

103. 2020 Fungicide Classification Chart. *Mississippi Soybean Promotion Board*. www.mssoy.org.

104. Antonio-Nkondjio C, Sonhafouo-Chiana N, Ngadjeu CS, Doumbe-Belisse P, Talipouo A, Djamouko-Djonkam L, et al. Review of the evolution of insecticide resistance in main malaria vectors in Cameroon from 1990 to 2017. *Parasit Vectors* 2017; **10**: 472.

105. Huseth AS, Chappell TM, Chitturi A, Jacobson AL, Kennedy GG. Insecticide resistance signals negative consequences of widespread neonicotinoid use on multiple field crops in the U.s. cotton Belt. *Environ Sci Technol* 2018; **52**: 2314–2322.

106. Bandara AY, Weerasooriya DK, Bradley CA, Allen TW, Esker PD. Dissecting the economic impact of soybean diseases in the United States over two decades. *PLoS One* 2020; **15**: e0231141.

107. Gratton C, Casler M, Groves R, Kim TN. Insecticide applications have minor effects on Switchgrass biomass yield. *Agron J* 2015; **107**: 2031–2037.

108. Sassenrath GF, Farney J, Lollato R. Impact of fungicide and insecticide use on wheat production in a high-rainfall environment. *Crop forage turfgrass manag* 2019; **5**: 190008.

109. Karlsson I, Friberg H, Steinberg C, Persson P. Fungicide effects on fungal community composition in the wheat phyllosphere. *PLoS One* 2014; **9**: e111786.

110. Knorr K, Jørgensen LN, Nicolaisen M. Fungicides have complex effects on the wheat phyllosphere mycobiome. *PLoS One* 2019; **14**: e0213176.

111. Wang K, Sipilä TP, Overmyer K. The isolation and characterization of resident yeasts from the phylloplane of Arabidopsis thaliana. *Sci Rep* 2016; **6**.

112. Cooke LR, Little G. The effect of foliar application of phosphonate formulations on the susceptibility of potato tubers to late blight. *Pest Manag Sci* 2002; **58**: 17–25.

113. Gange AC, Brown VK, Aplin DM. Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecol Lett* 2003; **6**: 1051–1055.

114. Hage-Ahmed K, Rosner K, Steinkellner S. Arbuscular mycorrhizal fungi and their response to pesticides. *Pest Manag Sci* 2019; **75**: 583–590.

115. Jin H, Germida JJ, Walley FL. Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. *Appl Soil Ecol* 2013; **72**: 22–30.

116. Santísima-Trinidad ABL, Del Mar Montiel-Rozas M, Diéz-Rojo MÁ, Pascual JA, Ros M. Impact of foliar fungicides on target and non-target soil microbial communities in cucumber crops. *Ecotoxicol Environ Saf* 2018; **166**: 78–85.

117. Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, et al. Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res Int* 2015; **22**: 35–67.

118. Calvo-Agudo M, González-Cabrera J, Picó Y, Calatayud-Vernich P, Urbaneja A, Dicke M, et al. Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. *Proc Natl Acad Sci U S A* 2019; **116**: 16817–16822.

119. Siviter H, Muth F. Do novel insecticides pose a threat to beneficial insects? *Proc Biol Sci* 2020; **287**: 20201265.

120. Losey JE, Vaughan M. The economic value of ecological services provided by insects. *Bioscience* 2006; **56**: 311.

121. Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T. Symbiont-mediated insecticide resistance. *Proc Natl Acad Sci U S A* 2012; **109**: 8618–8622.

122. Zhang H, Zhang Z, Song J, Mei J, Fang H, Gui W. Reduced bacterial network complexity in agricultural soils after application of the neonicotinoid insecticide thiamethoxam. *Environ Pollut* 2021; **274**: 116540.

123. Cycoń M, Markowicz A, Borymski S, Wójcik M, Piotrowska-Seget Z. Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. *J Environ Manage* 2013; **131**: 55–65.

124. Zhang B, Zhang H, Jin B, Tang L, Yang J, Li B, et al. Effect of cypermethrin insecticide on the microbial community in cucumber phyllosphere. *J Environ Sci (China)* 2008; **20**: 1356–1362.

125. Zhang B, Bai Z, Hoefel D, Tang L, Wang X, Li B, et al. The impacts of cypermethrin pesticide application on the non-target microbial community of the pepper plant phyllosphere. *Sci Total Environ* 2009; **407**: 1915–1922.

126. Yang C. Remote sensing and precision agriculture technologies for crop disease detection and management with a practical application example. *Engineering (Beijing)* 2020; **6**: 528–532.

127. Yang C, Odvody GN, Thomasson JA, Isakeit T, Nichols RL. Change detection of cotton root rot infection over 10-year intervals using airborne multispectral imagery. *Comput Electron Agric* 2016; **123**: 154–162.

128. Rogers SL, Burns RG. Changes in aggregate stability, nutrient status, indigenous microbial populations, and seedling emergence, following inoculation of soil withNostoc muscorum. *Biol Fertil Soils* 1994; **18**: 209–215.

129. Preininger C, Sauer U, Bejarano A, Berninger T. Concepts and applications of foliar spray for microbial inoculants. *Appl Microbiol Biotechnol* 2018; **102**: 7265–7282.

130. Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H. Engineering high-level aluminum tolerance in barley with the ALMT1 gene. *Proc Natl Acad Sci U S A* 2004; **101**: 15249–15254.

131. Agoussar A, Yergeau E. Engineering the plant microbiota in the context of the theory of ecological communities. *Curr Opin Biotechnol* 2021; **70**: 220–225.

132. Sprockett D, Fukami T, Relman DA. Role of priority effects in the early-life assembly of the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 197–205.

133. Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, et al. Publisher Correction: Core microbiomes for sustainable agroecosystems. *Nat Plants* 2018; **4**: 733.

134. Carlström CI, Field CM, Bortfeld-Miller M, Müller B, Sunagawa S, Vorholt JA. Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. *Nat Ecol Evol* 2019; **3**: 1445–1454.

135. Weidlich EWA, Nelson CR, Maron JL, Callaway RM, Delory BM, Temperton VM. Priority effects and ecological restoration. *Restor Ecol* 2021; **29**.

136. Fukami T. Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annu Rev Ecol Evol Syst* 2015; **46**: 1–23.

137. Kaminsky LM, Trexler RV, Malik RJ, Hockett KL, Bell TH. The inherent conflicts in developing soil microbial inoculants. *Trends Biotechnol* 2019; **37**: 140–151.

138. Mitchell RT, Deacon JW. Selective accumulation of zoospores of chytridiomycetes and oomycetes on cellulose and chitin. *Trans Br Mycol Soc* 1986; **86**: 219–223.

139. Berdy B, Spoering AL, Ling LL, Epstein SS. In situ cultivation of previously uncultivable microorganisms using the ichip. *Nat Protoc* 2017; **12**: 2232–2242.

140. Stevenson BS, Eichorst SA, Wertz JT, Schmidt TM, Breznak JA. New strategies for cultivation and detection of previously uncultured microbes. *Appl Environ Microbiol* 2004; **70**: 4748–4755.

141. Schoebitz M, López MD, Roldán A. Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. *Agron Sustain Dev* 2013; **33**: 751–765.

142. Hernández I, Sant C, Martínez R, Fernández C. Design of bacterial strain-specific qPCR assays using NGS data and publicly available resources and its application to track biocontrol strains. *Front Microbiol* 2020; **11**: 208.

143. Drahos DJ, Hemming BC, McPherson S. Tracking recombinant organisms in the environment: B–galactosidase as a selectable non–antibiotic marker for fluorescent pseudomonads. *Nat Biotechnol* 1986; **4**: 439–444.
144. Pellegrino E, Turrini A, Gamper HA, Cafà G, Bonari E, Young JPW, et al. Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. *New Phytol* 2012; **194**: 810–822.

145. Albareda M, Rodríguez-Navarro DN, Temprano FJ. Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Res* 2009; **113**: 352–356.

146. Niu B, Paulson JN, Zheng X, Kolter R. Simplified and representative bacterial community of maize roots. *Proc Natl Acad Sci U S A* 2017; **114**: E2450–E2459.

147. Castanheira NL, Dourado AC, Pais I, Semedo J, Scotti-Campos P, Borges N, et al. Colonization and beneficial effects on annual ryegrass by mixed inoculation with plant growth promoting bacteria. *Microbiol Res* 2017; **198**: 47–55.

148. Chiarini L, Bevivino A, Tabacchioni S, Dalmastri C. Inoculation of Burkholderia cepacia, Pseudomonas fluorescens and Enterobacter sp. on Sorghum bicolor: Root colonization and plant growth promotion of dual strain inocula. *Soil Biol Biochem* 1998; **30**: 81–87.

149. Ousley MA, Lynch JM, Whipps JM. Potential of Trichoderma spp. as consistent plant growth stimulators. *Biol Fertil Soils* 1994; **17**: 85–90.

150. Shehata HR, Lyons EM, Jordan KS, Raizada MN. Relevance of in vitro agar based screens to characterize the anti-fungal activities of bacterial endophyte communities. *BMC Microbiol* 2016; **16**: 8.

151. Biggs MB, Craig K, Gachango E, Ingham D, Twizeyimana M. Genomics- and machine learning-accelerated discovery of biocontrol bacteria. *Phytobiomes J* 2021; **5**: 452–463.

152. Ryu C-M, Kim J, Choi O, Kim SH, Park CS. Improvement of biological control capacity of Paenibacillus polymyxa E681 by seed pelleting on sesame. *Biol Control* 2006; **39**: 282–289.

153. Correa OS, Montecchia MS, Berti MF, Fernández Ferrari MC, Pucheu NL, Kerber NL, et al. Bacillus amyloliquefaciens BNM122, a potential microbial biocontrol agent applied on soybean seeds, causes a minor impact on rhizosphere and soil microbial communities. *Appl Soil Ecol* 2009; **41**: 185–194.

154. Fenton AM, Stephens PM, Crowley J, O'Callaghan M, O'Gara F. Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a Pseudomonas strain. *Appl Environ Microbiol* 1992; **58**: 3873–3878.

155. Bennett AJ, Mead A, Whipps JM. Performance of carrot and onion seed primed with beneficial microorganisms in glasshouse and field trials. *Biol Control* 2009; **51**: 417–426.

156. Li Z, Bai X, Jiao S, Li Y, Li P, Yang Y, et al. A simplified synthetic community rescues Astragalus mongholicus from root rot disease by activating plant-induced systemic resistance. *Microbiome* 2021; **9**: 217.

157. Saad MM, Eida AA, Hirt H. Tailoring plant-associated microbial inoculants in agriculture: a roadmap for successful application. *J Exp Bot* 2020; **71**: 3878–3901.

158. Eke P, Kumar A, Sahu KP, Wakam LN, Sheoran N, Ashajyothi M, et al. Endophytic bacteria of desert cactus (Euphorbia trigonas Mill) confer drought tolerance and induce growth promotion in tomato (Solanum lycopersicum L.). *Microbiol Res* 2019; **228**: 126302.

159. Grichko VP, Glick BR. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiol Biochem* 2001; **39**: 11–17.

160. Kim A-Y, Shahzad R, Kang S-M, Seo C-W, Park Y-G, Park H-J, et al. IAA-producing Klebsiella variicola AY13 reprograms soybean growth during flooding stress. *J Crop Sci Biotechnol* 2017; **20**: 235–242.

161. Bisht A, Garg N. AMF species improve yielding potential of Cd stressed pigeonpea plants by modulating sucrose-starch metabolism, nutrients acquisition and soil microbial enzymatic activities. *Plant Growth Regul* 2022.

162. Emami S, Alikhani HA, Pourbabaee AA, Etesami H, Sarmadian F, Motesharezadeh B, et al. Performance evaluation of phosphate-solubilizing fluorescent pseudomonads in minimizing phosphorus fertilizer use and improving wheat productivity: A two-year field study. *J soil sci plant nutr* 2022.

163. Jiménez JA, Novinscak A, Filion M. Inoculation with the plant-growth-promoting Rhizobacterium Pseudomonas fluorescens LBUM677 impacts the rhizosphere microbiome of three Oilseed crops. *Front Microbiol* 2020; **11**: 569366.

164. Barelli L, Waller AS, Behie SW, Bidochka MJ. Plant microbiome analysis after Metarhizium amendment reveals increases in abundance of plant growth-promoting organisms and maintenance of disease-suppressive soil. *PLoS One* 2020; **15**: e0231150.

165. Melchior J, Combest J. The BioAg Alliance targets 250-500 million acres by 2025. *Novozymes.com*. https://www.novozymes.com/en/news/news-archive/2015/11/the-bioag-alliance-targets-250-500-million-acres-by-2025.

166. Chan CTY, Lee JW, Cameron DE, Bashor CJ, Collins JJ. "Deadman" and "Passcode" microbial kill switches for bacterial containment. *Nat Chem Biol* 2016; **12**: 82–86.

167. Jack CN, Petipas RH, Cheeke TE, Rowland JL, Friesen ML. Microbial inoculants: Silver bullet or microbial Jurassic Park? *Trends Microbiol* 2021; **29**: 299–308.

168. Rodríguez-Echeverría S. Rhizobial hitchhikers from Down Under: invasional meltdown in a plant-bacteria mutualism? *J Biogeogr* 2010.

169. Food and Agriculture Organization of the United Nations. FAOSTAT Database. Rome: Food and Agriculture Organization of the United Nations. *FAOSTAT Database*.

170. Verhulst N, Nelissen V, Jespers N, Haven H, Sayre KD, Raes D, et al. Soil water content, maize yield and its stability as affected by tillage and crop residue management in rainfed semi-arid highlands. *Plant Soil* 2011; **344**: 73–85.

171. Daigh ALM, Dick WA, Helmers MJ, Lal R, Lauer JG, Nafziger E, et al. Yields and yield stability of no-till and chisel-plow fields in the Midwestern US Corn Belt. *Field Crops Res* 2018; **218**: 243–253.

172. Pittelkow CM, Linquist BA, Lundy ME, Liang X, van Groenigen KJ, Lee J, et al. When does no-till yield more? A global meta-analysis. *Field Crops Res* 2015; **183**: 156–168.

173. United States Department of Agriculture National Agricultural Statistics Service. Certified Organic Survey 2016 Summary. *usda.gov*.

174. Mcbride W, Greene C. Despite Profit Potential, Organic Field Crop Acreage Remains Low. 2015. United States Department of Agriculture Economic Research Service, Washington, DC.

175. Schroeder KL, Paulitz TC. Root diseases of wheat and barley during the transition from conventional tillage to direct seeding. *Plant Dis* 2006; **90**: 1247–1253.

176. Wattenburger CJ, Halverson LJ, Hofmockel KS. Agricultural management affects root-associated microbiome recruitment over maize development. *Phytobiomes J* 2019; **3**: 260–272.

177. Li R, Khafipour E, Krause DO, Entz MH, de Kievit TR, Fernando WGD. Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. *PLoS One* 2012; **7**: e51897.

178. Navarrete AA, Kuramae EE, de Hollander M, Pijl AS, van Veen JA, Tsai SM. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiol Ecol* 2013; **83**: 607–621.

179. Sugiyama A, Ueda Y, Zushi T, Takase H, Yazaki K. Changes in the bacterial community of soybean rhizospheres during growth in the field. *PLoS One* 2014; **9**: e100709.

180. Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. *Nat Commun* 2019; **10**: 4135.

181. Giller KE, Andersson JA, Corbeels M, Kirkegaard J, Mortensen D, Erenstein O, et al. Beyond conservation agriculture. *Front Plant Sci* 2015; **6**: 870.

182. Robertson GP, Gross KL, Hamilton SK, Landis DA, Schmidt TM, Snapp SS. Farming for Ecosystem Services: An Ecological Approach to Production Agriculture. The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability. 2015. Oxford University Press, New York, NY.

183. Fehr WR, Caviness CE, Burmood DT, Pennington JS. Stage of development descriptions for soybeans, Glycine max (L.) Merrill 1. *Crop Sci* 1971; **11**: 929–931.

184. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols*. 1990. Elsevier, pp 315–322.

185. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. *Mol Ecol* 1993; **2**: 113–118.

186. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* . 2011; **108**: 4516–4522.

187. Benucci GMN, Longley R, Zhang P, Zhao Q, Bonito G, Yu F. Microbial communities associated with the black morel Morchella sextelata cultivated in greenhouses. *PeerJ* 2019; **7**: e7744.

188. Benucci GMN, Bonito V, Bonito G. Fungal, bacterial, and Archaeal diversity in soils beneath native and introduced plants in Fiji, south pacific. *Microb Ecol* 2019; **78**: 136–146.

189. Chen K-H, Liao H-L, Arnold AE, Bonito G, Lutzoni F. RNA-based analyses reveal fungal communities structured by a senescence gradient in the moss Dicranum scoparium and the presence of putative multi-trophic fungi. *New Phytol* 2018; **218**: 1597–1611.

190. Lundberg DS, Yourstone S, Mieczkowski P, Jones CD, Dangl JL. Practical innovations for high-throughput amplicon sequencing. *Nat Methods* 2013; **10**: 999–1002.

191. Babraham bioinformatics - FastQC A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc. Accessed 22 Mar 2022.

192. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; **7**: 335–336.

193. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* . 2011; **17**: 10.

194. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* . 2015. , **31**: 3476–3482

195. Edgar RC. UCHIME2: improved chimera prediction for amplicon sequencing. *bioRxiv* . 2016. bioRxiv.

196. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013; **10**: 996–998.

197. Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, et al. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 2005; **166**: 1063–1068.

198. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013; **41**: D590-6.

199. Edgar RC. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *bioRxiv* 2016.

200. R Core Team. R: A Language and Environment for Statistical Computing.

201. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013; **8**: e61217.

202. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 2018; **6**: 226.

203. McMurdie PJ, Holmes S. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* 2014; **10**: e1003531.

204. Simpson EH. Measurement of diversity. *Nature* 1949; **163**: 688–688.

205. Hill MO. Diversity and evenness: A unifying notation and its consequences. *Ecology* 1973; **54**: 427–432.

206. Kindt R, Coe R. Tree diversity analysis: a manual and software for common statistical methods for ecological and biodiversity studies. 2006.

207. Oksanen, J, Guillaume Blanchet, F, Friendly, M, Kindt, R, Legendre, P, McGlinn, D. Vegan: Community Ecology Package. R Package Version 2.5-4. https://CRAN.R-project.org/package=vegan.

208. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2016. Springer- Verlag, Berlin.

209. Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, et al. Fungal community analysis by high-throughput sequencing of amplified markers--a user's guide. *New Phytol* 2013; **199**: 288–299.

210. Oliver AK, Callaham MA Jr, Jumpponen A. Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. *For Ecol Manage* 2015; **345**: 1–9.

211. De Cáceres M, Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology* 2009; **90**: 3566–3574.

212. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016; **32**: 2847–2849.

213. Paulson JN, Stine OC, Bravo HC, Pop M. Differential abundance analysis for microbial marker-gene surveys. *Nat Methods* 2013; **10**: 1200–1202.

214. Liaw A, Wiener M. Classification and regression by randomforest. *R News* 2002; **2**: 18–22.

215. Murphy MA, Evans JS, Storfer A. Quantifying Bufo boreas connectivity in Yellowstone National Park with landscape genetics. *Ecology* 2010; **91**: 252–261.

216. Csardi G, Nepusz T. The Igraph software package for complex network research. *InterJ, Comp Syst* 2006; **1695**: 1–9.

217. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and compositionally robust inference of microbial ecological networks. *PLoS Comput Biol* 2015; **11**: e1004226.

218. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003; **13**: 2498–2504.

219. Chen H, Xia Q, Yang T, Shi W. Eighteen-year farming management moderately shapes the soil microbial community structure but promotes habitat-specific taxa. *Front Microbiol* 2018; **9**: 1776.

220. Lebreton L, Guillerm-Erckelboudt A-Y, Gazengel K, Linglin J, Ourry M, Glory P, et al. Temporal dynamics of bacterial and fungal communities during the infection of Brassica rapa roots by the protist Plasmodiophora brassicae. *PLoS One* 2019; **14**: e0204195.

221. Suárez-Moo P de J, Vovides AP, Griffith MP, Barona-Gómez F, Cibrián-Jaramillo A. Unlocking a high bacterial diversity in the coralloid root microbiome from the cycad genus Dioon. *PLoS One* 2019; **14**: e0211271.

222. Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, et al. The Populus holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 2018; **6**.

223. Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. Seasonal community succession of the phyllosphere microbiome. *Mol Plant Microbe Interact* 2015; **28**: 274–285.

224. Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, et al. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc Natl Acad Sci U S A* 2013; **110**: 6548–6553.

225. Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D. Plant host-associated mechanisms for microbial selection. *Front Plant Sci* 2019; **10**: 862.

226. Degrune F, Theodorakopoulos N, Colinet G, Hiel M-P, Bodson B, Taminiau B, et al. Temporal dynamics of soil microbial communities below the seedbed under two contrasting tillage regimes. *Front Microbiol* 2017; **8**: 1127.

227. Ceci A, Pinzari F, Russo F, Maggi O, Persiani AM. Saprotrophic soil fungi to improve phosphorus solubilisation and release: In vitro abilities of several species. *Ambio* 2018; **47**: 30–40.

228. Osorio NW, Habte M. Soil phosphate desorption induced by a phosphate-solubilizing fungus. *Commun Soil Sci Plant Anal* 2014; **45**: 451–460.

229. Liao H-L, Bonito G, Rojas JA, Hameed K, Wu S, Schadt CW, et al. Fungal endophytes of Populus trichocarpa alter host phenotype, gene expression, and rhizobiome composition. *Mol Plant Microbe Interact* 2019; **32**: 853–864.

230. Li F, Chen L, Redmile-Gordon M, Zhang J, Zhang C, Ning Q, et al. Mortierella elongata ’s roles in organic agriculture and crop growth promotion in a mineral soil. *Land Degrad Dev* 2018; **29**: 1642–1651.

231. Bálint M, Tiffin P, Hallström B, O'Hara RB, Olson MS, Fankhauser JD, et al. Host genotype shapes the foliar fungal microbiome of balsam poplar (Populus balsamifera). *PLoS One* 2013; **8**: e53987.

232. Mezzasalma V, Sandionigi A, Guzzetti L, Galimberti A, Grando MS, Tardaguila J, et al. Geographical and cultivar features differentiate grape Microbiota in northern Italy and Spain vineyards. *Front Microbiol* 2018; **9**.

233. Chen L, Xin X, Zhang J, Redmile-Gordon M, Nie G, Wang Q. Soil characteristics overwhelm cultivar effects on the structure and assembly of root-associated microbiomes of modern maize. *Pedosphere* 2019; **29**: 360–373.

234. Smith CR, Blair PL, Boyd C, Cody B, Hazel A, Hedrick A, et al. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. *Ecol Evol* 2016; **6**: 8075–8084.

235. Dimitrijević S, Pavlović M, Maksimović S, Ristić M, Filipović V, Antonović D, et al. Plant growth-promoting bacteria elevate the nutritional and functional properties of black cumin and flaxseed fixed oil. *J Sci Food Agric* 2018; **98**: 1584–1590.

236. Aydogan EL, Moser G, Müller C, Kämpfer P, Glaeser SP. Long-term warming shifts the composition of bacterial communities in the phyllosphere of Galium album in a permanent grassland field-experiment. *Front Microbiol* 2018; **9**.

237. Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Gutjahr C, Falter-Braun P. Systems biology of plant-microbiome interactions. *Mol Plant* 2019; **12**: 804–821.

238. Bergelson J, Mittelstrass J, Horton MW. Characterizing both bacteria and fungi improves understanding of the Arabidopsis root microbiome. *Sci Rep* 2019; **9**: 24.

239. Ottesen AR, White JR, Skaltsas DN, Newell MJ, Walsh CS. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *J Food Prot* 2009; **72**: 2321–2325.

240. Schmid F, Moser G, Müller H, Berg G. Functional and structural microbial diversity in organic and conventional viticulture: organic farming benefits natural biocontrol agents. *Appl Environ Microbiol* 2011; **77**: 2188–2191.

241. Beare MH, Hu S, Coleman DC, Hendrix PF. Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. *Appl Soil Ecol* 1997; **5**: 211–219.

242. Manching HC, Carlson K, Kosowsky S, Smitherman CT, Stapleton AE. Maize phyllosphere microbial community niche development across stages of host leaf growth. *F1000Res* 2017; **6**: 1698.

243. Edwards JA, Santos-Medellín CM, Liechty ZS, Nguyen B, Lurie E, Eason S, et al. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. *PLoS Biol* 2018; **16**: e2003862.

244. Piazza G, Ercoli L, Nuti M, Pellegrino E. Interaction between conservation tillage and nitrogen fertilization shapes prokaryotic and fungal diversity at different soil depths: Evidence from a 23-year field experiment in the Mediterranean area. *Front Microbiol* 2019; **10**: 2047.

245. Hernández M, Dumont MG, Yuan Q, Conrad R. Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. *Appl Environ Microbiol* 2015; **81**: 2244–2253.

246. Silva UC, Medeiros JD, Leite LR, Morais DK, Cuadros-Orellana S, Oliveira CA, et al. Long-term rock phosphate fertilization impacts the microbial communities of maize rhizosphere. *Front Microbiol* 2017; **8**: 1266.

247. Rykiel EJ. Towards a definition of ecological disturbance. *Austral Ecol* 1985; **10**: 361–365.

248. Glasby TM, Underwood AJ. Sampling to differentiate between pulse and press perturbations. *Environ Monit Assess* 1996; **42**: 241–252.

249. Sullivan TP, Sullivan DS. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environ Rev* 2003; **11**: 37–59.

250. Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep* 2012; **127**: 4–22.

251. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of microbial community resistance and resilience. *Front Microbiol* 2012; **3**: 417.

252. Hahn M. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. *J Chem Biol* 2014; **7**: 133–141.

253. Schaeffer RN, Vannette RL, Brittain C, Williams NM, Fukami T. Non-target effects of fungicides on nectar-inhabiting fungi of almond flowers. *Environ Microbiol Rep* 2017; **9**: 79–84.

254. Delmas CEL, Dussert Y, Delière L, Couture C, Mazet ID, Richart Cervera S, et al. Soft selective sweeps in fungicide resistance evolution: recurrent mutations without fitness costs in grapevine downy mildew. *Mol Ecol* 2017; **26**: 1936–1951.

255. McDonald MC, Renkin M, Spackman M, Orchard B, Croll D, Solomon PS, et al. Rapid parallel evolution of azole fungicide resistance in Australian populations of the wheat pathogen Zymoseptoria tritici. *Appl Environ Microbiol* 2019; **85**.

256. Riat A, Plojoux J, Gindro K, Schrenzel J, Sanglard D. Azole Resistance of Environmental and Clinical Aspergillus fumigatus Isolates from Switzerland. *Antimicrob Agents Chemother* 2018; **62**.

257. Verweij PE, Snelders E, Kema GHJ, Mellado E, Melchers WJG. Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009; **9**: 789–795.

258. Wise K, Mueller D. Are fungicides no longer just for fungi? An analysis of foliar fungicide use in corn. *APSnet Feature Artic* 2011.

259. Imfeld G, Vuilleumier S. Measuring the effects of pesticides on bacterial communities in soil: A critical review. *Eur J Soil Biol* 2012; **49**: 22–30.

260. Fournier B, Pereira Dos Santos S, Gustavsen JA, Imfeld G, Lamy F, Mitchell EAD, et al. Impact of a synthetic fungicide (fosetyl-Al and propamocarb-hydrochloride) and a biopesticide (Clonostachys rosea) on soil bacterial, fungal, and protist communities. *Sci Total Environ* 2020; **738**: 139635.

261. Morton V, Staub T. A short history of fungicides. *APSnet Feature Artic* 2008.

262. Sapkota R, Knorr K, Jørgensen LN, O'Hanlon KA, Nicolaisen M. Host genotype is an important determinant of the cereal phyllosphere mycobiome. *New Phytol* 2015; **207**: 1134–1144.

263. Southwell RJ, Brown JF, Welsby SM. Microbial interactions on the phylloplane of wheat and barley after applications of mancozeb and triadimefon. *Australas Plant Pathol* 1999; **28**: 139.

264. Dickinson CH, Wallace B. Effects of late applications of foliar fungicides on activity of micro-organisms on winter wheat flag leaves. *Trans Br Mycol Soc* 1976; **67**: 103–112.

265. Freimoser FM, Rueda-Mejia MP, Tilocca B, Migheli Q. Biocontrol yeasts: mechanisms and applications. *World J Microbiol Biotechnol* 2019; **35**: 154.

266. Pter G, Rosa C, Ter GBP (eds). Biodiversity and ecophysiology of yeasts. The yeast handbook. 2006. Springer, New York, NY.

267. Cobban A, Edgcomb VP, Burgaud G, Repeta D, Leadbetter ER. Revisiting the pink-red pigmented basidiomycete mirror yeast of the phyllosphere. *Microbiologyopen* 2016; **5**: 846–855.

268. Cadez N, Zupan J, Raspor P. The effect of fungicides on yeast communities associated with grape berries. *FEMS Yeast Res* 2010; **10**: 619–630.

269. Schaeffer RN, Mei YZ, Andicoechea J, Manson JS, Irwin RE. Consequences of a nectar yeast for pollinator preference and performance. *Funct Ecol* 2017; **31**: 613–621.

270. Tilman D. The ecological consequences of changes in biodiversity: A search for general principles101. *Ecology* 1999; **80**: 1455–1474.

271. Ripple WJ, Beschta RL. Linking wolves and plants: Aldo Leopold on trophic cascades. *Bioscience* 2005; **55**: 613.

272. Sahasrabudhe S, Motter AE. Rescuing ecosystems from extinction cascades through compensatory perturbations. *Nat Commun* 2011; **2**: 170.

273. Zhou J, Deng Y, Luo F, He Z, Tu Q, Zhi X. Functional molecular ecological networks. *MBio* 2010; **1**.

274. Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat Commun* 2019; **10**: 4841.

275. Sułowicz S, Cycoń M, Piotrowska-Seget Z. Non-target impact of fungicide tetraconazole on microbial communities in soils with different agricultural management. *Ecotoxicology* 2016; **25**: 1047–1060.

276. Robertson GP, Hamilton SK. Long-term ecological research at the Kellogg Biolo-gical Station LTER site. The ecology of agricultural landscapes. *Long-term research on the path to sustainability* 2015; 1–32.

277. Latin R. A practical guide to turfgrass fungicides. 2017. The American Phytopathological Society.

278. Augusto J, Brenneman TB. Assessing systemicity of peanut fungicides through bioassay of plant tissues with Sclerotium rolfsii. *Plant Dis* 2012; **96**: 330–337.

279. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; **26**: 2460–2461.

280. Liber JA, Bonito G, Benucci GMN. CONSTAX2: Improved taxonomic classification of environmental DNA markers. *Bioinformatics* 2021; **37**: 3941–3943.

281. Gdanetz K, Benucci GMN, Vande Pol N, Bonito G. CONSTAX: a tool for improved taxonomic resolution of environmental fungal ITS sequences. *BMC Bioinformatics* 2017; **18**: 538.

282. Bowsher AW, Benucci GMN, Bonito G, Shade A. Seasonal dynamics of core fungi in the Switchgrass phyllosphere, and co-occurrence with leaf bacteria. *Phytobiomes J* 2021; **5**: 60–68.

283. Zhang J, Liu Y-X, Zhang N, Hu B, Jin T, Xu H, et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat Biotechnol* 2019; **37**: 676–684.

284. Anderson MJ, Willis TJ. Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology* 2003; **84**: 511–525.

285. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis* 2015; **26**: 27663.

286. Shade A, Stopnisek N. Abundance-occupancy distributions to prioritize plant core microbiome membership. *Curr Opin Microbiol* 2019; **49**: 50–58.

287. Kursa MB, Rudnicki WR. Feature selection with the Boruta package. 2010. Journal of Statistical Software.

288. Lindow SE, Brandl MT. Microbiology of the phyllosphere. *Appl Environ Microbiol* 2003; **69**: 1875–1883.

289. Sun P-F, Fang W-T, Shin L-Y, Wei J-Y, Fu S-F, Chou J-Y. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant Drosera indica L. *PLoS One* 2014; **9**: e114196.

290. Yurkov AM, Kurtzman CP. Three new species of Tremellomycetes isolated from maize and northern wild rice. *FEMS Yeast Res* 2019; **19**.

291. Gdanetz K, Noel Z, Trail F. Influence of plant host and organ, management strategy, and spore traits on microbiome composition. *Phytobiomes J* 2021; **5**: 202–219.

292. Sommermann L, Geistlinger J, Wibberg D, Deubel A, Zwanzig J, Babin D, et al. Fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions analyzed by high-throughput ITS-amplicon sequencing. *PLoS One* 2018; **13**: e0195345.

293. Li A-H, Yuan F-X, Groenewald M, Bensch K, Yurkov AM, Li K, et al. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: Proposal of two new orders, three new families, eight new genera and one hundred and seven new species. *Stud Mycol* 2020; **96**: 17–140.

294. Gilbert DG. Dispersal of yeasts and bacteria by Drosophila in a temperate forest. *Oecologia* 1980; **46**: 135–137.

295. Starmer WT, Peris F, Fontdevila A. The transmission of yeasts by Drosophila buzzatii during courtship and mating. *Anim Behav* 1988; **36**: 1691–1695.

296. Murrell EG. Can agricultural practices that mitigate or improve crop resilience to climate change also manage crop pests? *Curr Opin Insect Sci* 2017; **23**: 81–88.

297. Schmidt JE, Kent AD, Brisson VL, Gaudin ACM. Agricultural management and plant selection interactively affect rhizosphere microbial community structure and nitrogen cycling. *Microbiome* 2019; **7**: 146.

298. Brockhurst MA, Buckling A, Gardner A. Cooperation peaks at intermediate disturbance. *Curr Biol* 2007; **17**: 761–765.

299. Brockhurst MA, Habets MGJL, Libberton B, Buckling A, Gardner A. Ecological drivers of the evolution of public-goods cooperation in bacteria. *Ecology* 2010; **91**: 334–340.

300. Kwak M-J, Jeong H, Madhaiyan M, Lee Y, Sa T-M, Oh TK, et al. Genome information of Methylobacterium oryzae, a plant-probiotic methylotroph in the phyllosphere. *PLoS One* 2014; **9**: e106704.

301. Yoshida S, Hiradate S, Koitabashi M, Kamo T, Tsushima S. Phyllosphere Methylobacterium bacteria contain UVA-absorbing compounds. *J Photochem Photobiol B* 2017; **167**: 168–175.

302. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, et al. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proc Natl Acad Sci U S A* 2009; **106**: 16428–16433.

303. Masson-Delmotte V, Zhai P, Pörtner HO, Roberts D, Skea J, Shukla PR, et al. Intergovernmental Panel on Climate Change (IPCC). Global Warming of 1.5◦ C: An IPCC Special Report on the Impacts of Global Warming of 1.5◦ C Above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change. *Sustainable Development, and Efforts to Eradicate Poverty Available online: https://www ipcc ch/sr15/(accessed on 18 October 2019)* 2018.

304. Daryanto S, Wang L, Jacinthe P-A. Global Synthesis of Drought Effects on Maize and Wheat Production. *PLoS One* 2016; **11**: e0156362.

305. Leng G, Hall J. Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future. *Sci Total Environ* 2019; **654**: 811–821.

306. Larrainzar E, Molenaar JA, Wienkoop S, Gil-Quintana E, Alibert B, Limami AM, et al. Drought stress provokes the down-regulation of methionine and ethylene biosynthesis

pathways in Medicago truncatula roots and nodules. *Plant Cell Environ* 2014; **37**: 2051–2063.

307. Marquez-Garcia B, Shaw D, Cooper JW, Karpinska B, Quain MD, Makgopa EM, et al. Redox markers for drought-induced nodule senescence, a process occurring after drought-induced senescence of the lowest leaves in soybean (Glycine max). *Ann Bot* 2015; **116**: 497–510.

308. Kunert KJ, Vorster BJ, Fenta BA, Kibido T, Dionisio G, Foyer CH. Drought stress responses in soybean roots and nodules. *Front Plant Sci* 2016; **7**: 1015.

309. Arrese-Igor C, González EM, Marino D, Ladrera R, Larrainzar E. Physiological response of legumes nodules to drought. *Plant Stress* 2011; **5**: 24–31.

310. King CA, Purcell LC. Genotypic variation for shoot N concentration and response to water deficits in soybean. *Crop Sci* 2006; **46**: 2396–2402.

311. Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor Appl Genet* 2012; **125**: 625–645.

312. Zafar SA, Zaidi SS-E-A, Gaba Y, Singla-Pareek SL, Dhankher OP, Li X, et al. Engineering abiotic stress tolerance via CRISPR/ Cas-mediated genome editing. *J Exp Bot* 2020; **71**: 470–479.

313. Martignago D, Rico-Medina A, Blasco-Escámez D, Fontanet-Manzaneque JB, Caño-Delgado AI. Drought Resistance by Engineering Plant Tissue-Specific Responses. *Front Plant Sci* 2019; **10**: 1676.

314. Khanal S, Fulton J, Shearer S. An overview of current and potential applications of thermal remote sensing in precision agriculture. *Computers and Electronics in Agriculture* . 2017. , **139**: 22–32

315. de Vries FT, Griffiths RI, Knight CG, Nicolitch O, Williams A. Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science* 2020; **368**: 270–274.

316. Köhl J, Kolnaar R, Ravensberg WJ. Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. *Front Plant Sci* 2019; **10**: 845.

317. de Almeida Lopes KB, Carpentieri-Pipolo V, Fira D, Balatti PA, López SMY, Oro TH, et al. Screening of bacterial endophytes as potential biocontrol agents against soybean diseases. *J Appl Microbiol* 2018; **125**: 1466–1481.

318. Gdanetz K, Trail F. The wheat microbiome under four management strategies, and potential for endophytes in disease protection. Phytobiomes 1: 158--168. 2017.

319. Singh DP, Singh V, Gupta VK, Shukla R, Prabha R, Sarma BK, et al. Microbial inoculation in rice regulates antioxidative reactions and defense related genes to mitigate drought stress. *Sci Rep* 2020; **10**: 4818.

320. Ulzen J, Abaidoo RC, Mensah NE, Masso C, AbdelGadir AH. Bradyrhizobium inoculants enhance grain yields of soybean and Cowpea in Northern Ghana. *Front Plant Sci* 2016; **7**: 1770.

321. Wang Z, Solanki MK, Yu Z-X, Yang L-T, An Q-L, Dong D-F, et al. Draft genome analysis offers insights into the mechanism by which Streptomyces chartreusis WZS021 increases drought tolerance in sugarcane. *Front Microbiol* 2018; **9**: 3262.

322. Abbasi S, Sadeghi A, Safaie N. Streptomyces alleviate drought stress in tomato plants and modulate the expression of transcription factors ERF1 and WRKY70 genes. *Sci Hortic (Amsterdam)* 2020; **265**: 109206.

323. Lopes MJ dos S, dos Santos Lopes MJ, Dias-Filho MB, Gurgel ESC. Successful Plant Growth-Promoting Microbes: Inoculation Methods and Abiotic Factors. *Frontiers in Sustainable Food Systems* . 2021; **5**

324. Shi T-Q, Peng H, Zeng S-Y, Ji R-Y, Shi K, Huang H, et al. Microbial production of plant hormones: Opportunities and challenges. *Bioengineered* 2017; **8**: 124–128.

325. Dellagi A, Quillere I, Hirel B. Beneficial soil-borne bacteria and fungi: a promising way to improve plant nitrogen acquisition. *J Exp Bot* 2020; **71**: 4469–4479.

326. Enebe MC, Babalola OO. The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach. *Appl Microbiol Biotechnol* 2019; **103**: 9–25.

327. Imran M, Abulreesh HH, Monjed MK, Elbanna K, Samreen, Ahmad I. Multifarious functional traits of free-living rhizospheric fungi, with special reference to Aspergillus spp. isolated from North Indian soil, and their inoculation effect on plant growth. *Ann Microbiol* 2021; **71**.

328. Zai X-M, Fan J-J, Hao Z-P, Liu X-M, Zhang W-X. Effect of co-inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing fungi on nutrient uptake and photosynthesis of beach palm under salt stress environment. *Sci Rep* 2021; **11**: 5761.

329. Tronsmo A. Biological and integrated controls of Botrytis cinerea on apple with Trichoderma harzianum. *Biol Control* 1991; **1**: 59–62.

330. Kauppinen M, Saikkonen K, Helander M, Pirttilä AM, Wäli PR. Epichloë grass endophytes in sustainable agriculture. *Nat Plants* 2016; **2**: 15224.

331. Poudel R, Jumpponen A, Schlatter DC, Paulitz TC, McSpadden Gardener BB, Kinkel LL, et al. Microbiome Networks: A Systems Framework for Identifying Candidate Microbial Assemblages for Disease Management. *Phytopathology* . 2016; **106**: 1083–1096.

332. Luo D, Langendries S, Mendez SG, De Ryck J, Liu D, Beirinckx S, et al. Plant growth promotion driven by a novel Caulobacter strain. *Mol Plant Microbe Interact* 2019; **32**: 1162–1174.

333. Zheng B-X, Bi Q-F, Hao X-L, Zhou G-W, Yang X-R. Massilia phosphatilytica sp. nov., a phosphate solubilizing bacteria isolated from a long-term fertilized soil. *Int J Syst Evol Microbiol* 2017; **67**: 2514–2519.

334. Alisaac E, Götz M. First report of Gibellulopsis nigrescens on peppermint in Germany. *J Plant Dis Prot (2006)* 2021.

335. Radhakrishnan R, Khan AL, Kang SM, Lee I-J. A comparative study of phosphate solubilization and the host plant growth promotion ability of Fusarium verticillioides RK01 and Humicola sp. KNU01 under salt stress. *Ann Microbiol* 2015; **65**: 585–593.

336. Kuhlgert S, Austic G, Zegarac R, Osei-Bonsu I, Hoh D, Chilvers MI, et al. MultispeQ Beta: a tool for large-scale plant phenotyping connected to the open PhotosynQ network. *Royal Society Open Science* . 2016. , **3**: 160592.

337. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012; **9**: 671–675.

338. Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM. RhizoVision Explorer: open-source software for root image analysis and measurement standardization. *AoB Plants* 2021; **13**: lab056.

339. Benucci GMN, Longley R, Zhang P, Zhao Q, Bonito G, Yu F. Microbial communities associated with the black morel cultivated in greenhouses. *PeerJ* 2019; **7**: e7744.

340. Galtier N, Gouy M, Gautier C. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* 1996; **12**: 543–548.

341. Edgar RC. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* . 2016. bioRxiv.

342. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014; **30**: 2114–2120.

343. Kopylova E, Noé L, Touzet H. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* 2012; **28**: 3211–3217.

344. Valliyodan B, Cannon SB, Bayer PE, Shu S, Brown AV, Ren L, et al. Construction and comparison of three reference-quality genome assemblies for soybean. *Plant J* 2019; **100**: 1066–1082.

345. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 2017; **14**: 417–419.

346. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**: 550.

347. Vandepol N, Liber J, Yocca A, Matlock J, Edger P, Bonito G. Linnemannia elongata (Mortierellaceae) stimulates Arabidopsis thaliana aerial growth and responses to auxin, ethylene, and reactive oxygen species. *PLoS One* 2022; **17**: e0261908.

348. Grant D, Nelson RT, Cannon SB, Shoemaker RC. SoyBase, the USDA-ARS soybean genetics and genomics database. *Nucleic Acids Res* 2010; **38**: D843-6.

349. Gao C-H, Yu G, Cai P. GgVennDiagram: An intuitive, easy-to-use, and highly customizable R package to generate Venn diagram. *Front Genet* 2021; **12**: 706907.

350. Brown AV, Conners SI, Huang W, Wilkey AP, Grant D, Weeks NT, et al. A new decade and new data at SoyBase, the USDA-ARS soybean genetics and genomics database. *Nucleic Acids Res* 2021; **49**: D1496–D1501.

351. Severin AJ, Woody JL, Bolon Y-T, Joseph B, Diers BW, Farmer AD, et al. RNA-Seq Atlas of Glycine max: a guide to the soybean transcriptome. *BMC Plant Biol* 2010; **10**: 160.

352. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Research* . 2019. , **47**: W191–W198.

353. Kolde R. Pheatmap: pretty heatmaps. *https://cran.r-project.org/web/packages/pheatmap/index.html* 2012; **1**: 747.

354. Ekanayaka EAP, Celiz MD, Jones AD. Relative mass defect filtering of mass spectra: a path to discovery of plant specialized metabolites. *Plant Physiol* 2015; **167**: 1221–1232.

355. Li X, Sarma SJ, Sumner LW, Jones AD, Last RL. Switchgrass metabolomics reveals striking genotypic and developmental differences in specialized metabolic phenotypes. *bioRxiv* . 2020. bioRxiv.

356. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* 2018; **46**: W486–W494.

357. Han Q, Ma Q, Chen Y, Tian B, Xu L, Bai Y, et al. Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. *ISME J* 2020; **14**: 1915–1928.

358. Sohn BK, Kim KY, Chung SJ, Kim WS, Park SM, Kang JG, et al. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. *Sci Hortic (Amsterdam)* 2003; **98**: 173–183.

359. Zeffa DM, Fantin LH, Koltun A, de Oliveira ALM, Nunes MPBA, Canteri MG, et al. Effects of plant growth-promoting rhizobacteria on co-inoculation with Bradyrhizobium in soybean crop: a meta-analysis of studies from 1987 to 2018. *PeerJ* 2020; **8**: e7905.

360. Htwe AZ, Moh SM, Moe K, Yamakawa T. Effects of co-inoculation of Bradyrhizobium japonicum SAY3-7 and Streptomyces griseoflavus P4 on plant growth, nodulation, nitrogen fixation, nutrient uptake, and yield of soybean in a field condition. *Soil Sci Plant Nutr* 2018; **64**: 222–229.

361. Xie ZP, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ, et al. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. *Plant Physiol* 1995; **108**: 1519–1525.

362. Catford J-G, Staehelin C, Lerat S, Piché Y, Vierheilig H. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. *J Exp Bot* 2003; **54**: 1481–1487.

363. Loh J, Stacey G. Nodulation gene regulation in Bradyrhizobium japonicum: a unique integration of global regulatory circuits. *Appl Environ Microbiol* 2003; **69**: 10–17.

364. Kohl DH, Schubert KR, Carter MB, Hagedorn CH, Shearer G. Proline metabolism in N2-fixing root nodules: energy transfer and regulation of purine synthesis. *Proc Natl Acad Sci U S A* 1988; **85**: 2036–2040.

365. Atkins CA, Ritchie A, Rowe PB, McCairns E, Sauer D. De Novo Purine Synthesis in Nitrogen-Fixing Nodules of Cowpea (Vigna unguiculata [L.] Walp.) and Soybean (Glycine max [L.] Merr.). *Plant Physiol* 1982; **70**: 55–60.

366. Schillaci M, Kehelpannala C, Martinez-Seidel F, Smith PMC, Arsova B, Watt M, et al. The metabolic response of Brachypodium roots to the interaction with beneficial bacteria is affected by the plant nutritional status. *Metabolites* 2021; **11**: 358.

367. Korenblum E, Dong Y, Szymanski J, Panda S, Jozwiak A, Massalha H, et al. Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proc Natl Acad Sci U S A* 2020; **117**: 3874–3883.

368. Agtuca BJ, Stopka SA, Evans S, Samarah L, Liu Y, Xu D, et al. Metabolomic profiling of wild-type and mutant soybean root nodules using laser-ablation electrospray ionization mass spectrometry reveals altered metabolism. *Plant J* 2020; **103**: 1937–1958.

369. Zhang W, Popovich DG. Chemical and biological characterization of oleanane triterpenoids from soy. *Molecules* 2009; **14**: 2959–2975.

370. TeKrony DM, Egli DB. Relationship of seed vigor to crop yield: A review. *Crop Sci* 1991; **31**: 816–822.

371. Li M, Liu Y, Wang C, Yang X, Li D, Zhang X, et al. Identification of traits contributing to high and stable yields in different soybean varieties across three Chinese latitudes. *Front Plant Sci* 2019; **10**: 1642.

372. Kramer DM, Johnson G, Kiirats O, Edwards GE. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth Res* 2004; **79**: 209.

373. Peng S, Biswas JC, Ladha JK, Gyaneshwar P, Chen Y. Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Agron J* 2002; **94**: 925–929.

374. Mantelin S, Desbrosses G, Larcher M, Tranbarger TJ, Cleyet-Marel J-C, Touraine B. Nitrate-dependent control of root architecture and N nutrition are altered by a plant growth-promoting Phyllobacterium sp. *Planta* 2006; **223**: 591–603.

375. Grover M, Bodhankar S, Sharma A, Sharma P, Singh J, Nain L. PGPR mediated alterations in root traits: Way toward sustainable crop production. *Front Sustain Food Syst* 2021; **4**.

376. Concha C, Doerner P. The impact of the rhizobia-legume symbiosis on host root system architecture. *J Exp Bot* 2020; **71**: 3902–3921.

377. Xie J, Dawwam GE, Sehim AE, Li X, Wu J, Chen S, et al. Drought stress triggers shifts in the root microbial community and alters functional categories in the microbial gene pool. *Front Microbiol* 2021; **12**: 744897.

378. Berrios L, Ely B. Genes related to redox and cell curvature facilitate interactions between Caulobacter strains and Arabidopsis. *PLoS One* 2021; **16**: e0249227.

379. Nouwen N, Arrighi J-F, Gully D, Giraud E. RibBX of Bradyrhizobium ORS285 plays an important role in intracellular persistence in various Aeschynomene host plants. *Mol Plant Microbe Interact* 2021; **34**: 88–99.

380. Fahlgren N, Gehan MA, Baxter I. Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Curr Opin Plant Biol* 2015; **24**: 93–99.

381. Karst SM, Ziels RM, Kirkegaard RH, Sørensen EA, McDonald D, Zhu Q, et al. High-accuracy long-read amplicon sequences using unique molecular identifiers with Nanopore or PacBio sequencing. *Nat Methods* 2021; **18**: 165–169.

382. Lee GH, Ryu C-M. Spraying of Leaf-Colonizing Bacillus amyloliquefaciens Protects Pepper from Cucumber mosaic virus. *Plant Dis* 2016; **100**: 2099–2105.

383. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS One* 2012; **7**: e40863.

384. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 2008; **74**: 2461–2470.

385. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007; **73**: 5261–5267.

386. Varesio LM, Willett JW, Fiebig A, Crosson S. A carbonic anhydrase pseudogene sensitizes select Brucella lineages to low CO2 tension. *J Bacteriol* 2019; **201**.

387. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 2014; **9**: e112963.

388. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016; **44**: 6614–6624.