10-28-2022 9:00 AM

Root-Inhabiting and Rhizosphere Mycobiomes and Crop Yield of Corn and Wheat

Marianna E. Wallace, *The University of Western Ontario*

Supervisor: Thorn, R. Greg, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

Abstract

The root mycobiome plays a direct role in plant productivity, and the study of its community composition allows for identification of organisms that influence plant health. To better understand the role of fungal community composition in crop productivity, the root-associated mycobiomes of historically high and low yield sites of corn and wheat planted in rotation were characterized and analyzed along with soil physicochemical variables and crop yield. In each field studied, root and rhizosphere mycobiomes reflected significant differences in fungal species composition. Several soil variables were found to be predictors of differences in composition of sample types including soil texture and pH. The genera *Fusicolla, Epicoccum,* and *Tetracladium* were positively correlated with yield, and *Neonectria, Pythium, Corynespora,* and *Mrakia* were negatively correlated. Identifying differences in the root-associated mycobiome of crops and changes in the soil environment could aid in the development of community management tools that maximize crop productivity.

Keywords

Rhizosphere, root, corn, wheat, microbiome, mycobiome, crop rotation, NGS, metabarcoding, ITS2, LSU

Summary for Lay Audience

Corn and wheat are global staple crops that are used to meet worldwide nutritional and food security needs. To meet the demands of an increasing global population, global agricultural production will need to be increased without exacerbating current environmental problems such as greenhouse gas emissions and loss of biodiversity. Traditional crop and soil management systems may need to be substituted or supplemented with innovative methods of increasing productivity and reducing loss to disease. Compared to intensive farming methods, crop rotation, the sequential planting of crops over time on the same field, is a much more sustainable farming method that increases crop productivity. In Southwestern Ontario, corn, soybean, and wheat are often planted in succession in the same field. The rhizosphere consists of the soil that is in direct contact with the roots of a plant and is rich with microbes, including plant-beneficial organisms and plant pathogens. Increased diversity and composition of bacteria and fungi in the soil have been linked to increased crop productivity and yield. The soil environment, including the organisms present in the soil, can also affect the availability of soil nutrients through various processes. Crop productivity has been linked to the abundance of several nutrients and heavy metals in the soil. Understanding how root-associated fungal communities may be linked to soil management, plant physiological health, and crop productivity is of great interest.

In this project, I sequenced fungal DNA found in the rhizosphere and roots from historically high and low yield sites of corn and wheat planted in rotation. It is common to see sites within the same field that produce high or low crop yields, year after year. I also looked at soil variables in each field to identify which ones may be linked to yield. While I found differences in fungal composition and soil variables of corn and wheat in these sites, the interactions that occur in the samples are complex. Identifying the organisms and soil factors that are driving differences in productivity could help in the development of soil management tools that maximize crop productivity.

Co-Authorship Statement

This work was a part of a larger long-term project of A&L Biologicals Inc. (now a part of Deveron Corp.), directed by Dr. George Lazarovits, Dr. Soledad Saldias, Dr. Saveetha Kandasamy, and Dr. Greg Thorn, with support in the field by various A&L staff and bioinformatic assistance by Nimalka Weerasuriya. In the summer of 2019, I collected and processed wheat samples with the assistance of A&L staff. I conducted all laboratory and data analyses, wrote the thesis with editing by Dr. Greg Thorn, and will be first author on resulting papers, together with the others named above.

Acknowledgments

I express my sincerest gratitude to my supervisor, Dr. Greg Thorn, for giving me the opportunity to earn my Master of Science, and for his support and guidance throughout this journey. I thank A&L Laboratories Inc., especially Dr. George Lazarovits, Dr. Saveetha Kandasamy, Dr. Soledad Saldias, Dr. Shimaila Ali, and the many interns that I encountered during my Master's work. Thank you for lending your facilities, lab space, equipment, materials, and expertise as well as for helping me to collect samples and data. Thank you to Nimalka Weerasuriya for your support and guidance in my bioinformatic analysis. Thank you to my lab peers, and especially to Noor Saeed Cheema for your support, positivity, and encouragement. Also, to my advisory committee, Dr. Kathleen Hill and Dr. Vera Tai, thank you for your participation and insightful advice throughout my research. Thank you Dr. Marc-André Lachance, for your advice and insight in my written work.

Lastly, thank you to all my family and friends that supported me along this journey. Thank you to my parents, Maggie and Steve Wallace, my sisters Victoria and Chiara, and my dog Marty McFly. A special thank you to Chiara for using your graphic design skills to help me with some of my figures. From the bottom of my heart, thank you for being there for me through all the obstacles I have faced over the past few years, for celebrating all the milestones I reached, and for always giving me a reason to smile.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

ALDEx	ANOVA-like differential expression
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
ASV	Amplified sequence variant
BLAST	Basic local alignment search tool
BLASTn	Basic local alignment search tool - nucleotide
CEC	Cation exchange capacity
COX1	Cytochrome *c* oxidase subunit 1
DADA2	Divisive amplicon denoising algorithm 2
DNA	Deoxyribonucleic acid
ENR	Estimated nitrogen release
GPS	Global positioning system
ITS	Internal transcribed spacer
ITS2	Internal transcribed spacer 2
LSU	Large ribosomal subunit
MAFFT	Multiple alignment using Fast Fourier Transform
NDVI	Normalized difference vegetation index
NGS	Next generation sequencing
NJ	Neighbour joining

NMDS	Non-metric multidimensional scaling
OM	Organic matter content
OTU	Operational taxonomic unit
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PGPR	Plant growth-promoting rhizobacteria
RDP	Ribosomal database project
SSU	Small ribosomal subunit
UAV	Unmanned aerial vehicle

Chapter 1

1 Introduction

1.1 Corn and wheat

Corn (*Zea mays*) and wheat (*Triticum aestivum*) are global staple crops. These food staples are used to meet worldwide nutritional and food security needs, and also are of substantial economic value in sectors including livestock feed, biofuel, and industrial use (Robinson *et al.*, 2014). In Canada in 2021, wheat production was estimated at 22.9 million tonnes annually at a yield of 37.2 bushels per acre and corn production was projected to reach 13.7 million tonnes, with yields anticipated at 158.4 bushels per acre (StatsCan, 2021). To meet the demands of an increasing population, global agricultural production may need to be increased by 60%–110% by 2050 (Tilman *et al.*, 2011). A trajectory of global agricultural development that focuses on technological advancements, the preservation of global diversity, the minimization of greenhouse gas effects, and the reduction of land clearing provides a promising path to global sustainability and equitable food supplies (Tilman *et al.*, 2011). It is abundantly clear that traditional crop and soil management practices may need to be substituted or supplemented with innovative methods of increasing crop productivity and reducing loss to disease on land that has already been converted to fields.

1.2 Crop rotation

Current agricultural practices rely heavily on the use of chemical inputs such as fertilizers and pesticides, which can be environmentally damaging as well as threatening to human health. The overuse of such chemicals has resulted in an array of environmental concerns, such as groundwater contamination, increased greenhouse gas emissions and soil acidification (S. Chen *et al.*, 2018; Guo *et al.*, 2010; Liu & Zhang, 2011). In addition to the use of agrochemicals, current agricultural practices are intensive and result in the physicochemical and biological depletion of the soil environment, leading to the loss of nutrients and soil organic matter that are essential to crop productivity (Rashid *et al.*, 2016). Compared to the use of agrochemicals, crop rotation is a much more sustainable

farming method that increases crop productivity. This farming practice involves sequential planting of crops over time on the same field (McDaniel *et al.*, 2014). In Southwestern Ontario, corn, wheat, and soybeans are commonly planted in the same rotation. The benefits of crop rotation include mitigating weed, insect, and pathogen pressure (Smith & Read, 2008). Another advantage of crop rotation is the promotion of agricultural biodiversity, which is lost through monocropping (McDaniel *et al.*, 2014). In prairie grasslands, greater biodiversity has been shown to increase productivity, efficiency of resource use and nutrient availability, and has been linked to greater ecosystem stability (Tilman *et al.*, 2006). There is a well-established correlation between crop rotation and crop productivity (Smith & Read, 2008).

We are only beginning to uncover the complex effects of crop rotation, including how this practice affects the structure and dynamics of the soil along with its associated microbial communities. Crop rotations have been shown to promote agricultural biodiversity, increase soil nutrient concentrations, and increase organic matter content (McDaniel *et al.*, 2014). Crop rotations have also been shown to promote diversity of microorganisms in the rhizosphere, the soil most closely associated with plant roots (Berg *et al.*, 2020). This is important because microbial community diversity has been linked to increased resilience or resistance to disturbance (Griffiths *et al.*, 2000). It has been hypothesized that crop rotations can enhance disease suppressive capacity, either through the influence of plant diversity affecting community composition or through the increased abundance of specific antagonistic microorganisms (McDaniel *et al.*, 2014). Some studies have suggested that crop rotation reduces the abundance of soilborne plant pathogens, and increases the abundance of plant growth-promoting microbes (McDaniel *et al.*, 2014), while another showed that compared to continuous monoculture cropping, crop rotations result in greater average yields for corn and wheat (Lund *et al.*, 1993).

1.3 Root and rhizosphere mycobiomes

The microbiome is a community of microorganisms within a defined habitat with distinct physicochemical properties and interactions with its environment (Berg *et al.*, 2020). The mycobiome refers to the fungal community present in a habitat and its activities (Berg *et*

al., 2020). The root mycobiome plays a direct role in plant productivity, and the study of community composition within the rhizosphere allows for identification of plant-beneficial organisms and plant pathogens that influence plant health (Patel *et al.*, 2015; Rashid *et al.*, 2016). Root-associated mycobiomes are dynamically affected by both the surrounding biotic and abiotic conditions in the soil and the host plant itself (Muneer *et al.*, 2021; Tkacz & Poole, 2015).

The rhizosphere consists of the soil that is in direct contact with the roots of a plant and is a hot spot of microbial diversity and dynamic interactions. Root exudates stimulate the growth of microorganisms in the vicinity of the roots, leading to soil around the roots that is incredibly rich with microbes, including plant-beneficial organisms and plant pathogens (Berg & Smalla, 2009). Through this process of root exudation, the plant feeds the microbial community in the soil, directly influencing its composition and activities. The soil that is not associated with the rhizosphere, bulk soil, is not penetrated by plant roots and typically has less diverse microbial communities and lower organic matter content within it (DeAngelis *et al.*, 2009). The interactions that occur in the rhizosphere can be mutualistic and beneficial to plant growth, with surrounding fungi improving nutrient acquisition and stress tolerance (Philippot *et al.*, 2013). Rhizosphere organisms that have been well studied for their beneficial effects on plant growth and health are nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), biocontrol microorganisms, mycoparasitic fungi, and protozoa. Numerous studies have shown that crops influence the soil microbial communities by shaping their composition and diversity through root exudates and symbiotic association in a species-specific manner (Costa *et al.*, 2006; Garbeva *et al.*, 2008; Staskawicz, 2001). Thus, diversified crop rotations can improve yield through enrichment of microorganisms selectively nurtured by each crop in the rotation that positively affect growth and productivity (Dias *et al.*, 2014). However, in the rhizosphere are also fungal pathogens that elicit harmful effects on plants, which can result in crop losses (Philippot *et al.*, 2013). Rhizosphere organisms that are deleterious to plant growth and health include the pathogenic fungi, oomycetes, bacteria, and nematodes. While fungal pathogens may be present even in high productivity soils, they are less likely to affect plant growth in a highly diverse rhizosphere, as surrounding microbes, fungi, and plant defenses have

developed mechanisms of pathogen resistance (Schnitzer *et al.*, 2011). The need for a clearer picture of the dynamics shaping the community composition of the most common commercial crops has clearly emerged.

Crop productivity has been linked to the abundance of nutrients such as N, P, K, and Fe and heavy metals such as Zn in the soil (Rashid *et al.*, 2016). These nutrients and other physicochemical properties of the soil can affect disease resistance or tolerance (Rashid *et al.*, 2016). While agrochemicals can increase the availability of plant essential nutrients through external input, the soil environment, including the organisms present in the soil, can also affect the availability of soil nutrients through various processes (Rashid *et al.*, 2016). For example, mycorrhizal associations are mutualistic relationships between plant roots and fungi, in which fungi deliver nutrients from the soil in exchange for carbon produced by the plant (M. F. Allen, 2007; M. Brundrett, 2004; Smith & Read, 2008). Mycorrhizae improve plant productivity and keep plant health robust against various abiotic and biotic stresses (Smith & Read, 2008). These interactions are extremely important for development and sustainability of plant communities in ecosystems where nutrient supply is limited (Buscot *et al.*, 2000). Fungal interactions with roots can be classified into various categories, the main categories being arbuscular mycorrhizae and ectomycorrhizae (M. F. Allen, 2007; M. Brundrett, 2004; Smith & Read, 2008). In an arbuscular mycorrhizal association, predominantly formed by members of the phylum Glomeromycota, hyphae penetrate the plant root cells to form arbuscules where nutrient exchange occurs (Smith & Read, 2008). Arbuscular mycorrhizal fungi have been shown to be able to transfer N to plants, and this fungal symbiont-mediated N uptake was stimulated by carbon supplied from the host plant (Fellbaum *et al.*, 2012). Ectomycorrhizal associations arise with many members of the phyla Ascomycota and Basidiomycota and unlike arbuscular mycorrhizae, do not penetrate their host's cell walls and instead the hyphae form a highly branched network with the plant root cells called a Hartig net (M. C. Brundrett, 2009). Both arbuscular mycorrhizal and ectomycorrhizal interactions can involve multiple fungi interacting with the same plant simultaneously (M. F. Allen, 2007). In addition to mycorrhizal fungi, plant roots also interact with endophytic fungi, endophytic bacteria, root pathogens, and bacteria found in the surrounding soil. Endophytes represent a large component of the microbial community of

roots and can interact symbiotically with plants, can become pathogenic, or become mutualistic, helping improve plant growth, health and stress tolerance (Baron & Rigobelo, 2022).

Roots also interact with pathogenic fungi that elicit harmful effects on plants. However, there are relatively fewer parasitic fungi associating with roots than beneficial or neutral fungi as plants have developed diverse mechanisms to protect themselves from most organisms (Staskawicz, 2001).

Plants exhibit a diverse array of interactions with fungal communities present in the soil, which span the full range of ecological possibilities, including competitive, exploitative, neutral, commensal, and mutualistic. Therefore, understanding how the interactions of root-associated fungal communities may be linked to soil management, plant health, and crop yield is of great interest. Many studies have focused on the bacterial composition of root-associated microbiomes, while few have focused on fungi.

1.4 Studying the mycobiome

The advent of high throughput, next-generation sequencing (NGS) technologies has enabled researchers to determine the composition and functions of microbial communities associated with different crops, including corn and wheat. This has allowed the understanding of how different factors affect microbial communities associated with host plants in unprecedented detail (Taylor *et al.*, 2016). The ability of NGS technologies to identify low abundance DNA found in community samples and produce species richness estimates at an affordable cost has led to these methods becoming common practice when studying microbial ecology (Asemaninejad et al., 2016; Tedersoo e*t al.*, 2015). DNA metabarcoding is a valuable tool which couples NGS with taxonomic identification of multiple species extracted from a mixed sample (Caporaso *et al.*, 2012; Taberlet *et al.*, 2012).

Metabarcoding is based upon DNA barcoding, which employs the use of a standardized, short and informative region of DNA (“DNA barcode”) for species identification (Hebert *et al.*, 2003). The development of DNA barcoding has also encouraged extensive

international efforts to build taxonomic reference libraries of the standardized regions. The process of metabarcoding includes collection of an environmental sample, DNA extraction, target gene amplification, high throughput NGS of the amplified community DNA products, sequence processing and statistical analyses (Caporaso *et al.*, 2012; Lynch & Neufeld, 2015; Taberlet *et al.*, 2012).

1.5 Next generation sequencing primers

The sequencing of polymerase chain reaction (PCR) amplicons of specific barcode DNA regions (metabarcoding) is a powerful technique that has led to breakthroughs in our ability to describe, compare and discover new microbial communities across environments (Ursell *et al.*, 2012). Selection of appropriate metabarcoding primers is key to uncovering diversity and community composition in these studies (Taylor *et al.*, 2016). An ideal set of forward and reverse primers must bind to highly conserved sites within one specific group of organisms (Alberdi *et al.*, 2018). To allow for species-specific identification, the primer binding site must border a region with adequate variability (Alberdi *et al.*, 2018). Finally, the amplicon produced must be of an appropriate length for the intended NGS platform (Alberdi *et al.*, 2018).

The region of the mitochondrial cytochrome *c* oxidase subunit 1 (COX1) is commonly used as an animal barcode but has been found to be a poor barcode locus for fungal community studies, because it is difficult to amplify in fungi, often includes large introns, and lacks variability (Dentinger *et al.*, 2011; Schoch *et al.*, 2012). The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA has been proposed as the barcode for molecular identification of fungi (Schoch *et al.*, 2012). The ITS region, which comprises three subregions: ITS1, 5.8S, and ITS2, has many features that make it a strong candidate for a universal fungal DNA barcode region. These include its presence in all fungi, its variability within the ITS1 and ITS2 regions, a high number of copies per cell, and the universality of its flanking regions (Scibetta *et al.*, 2018). Internal transcribed spacer regions have been studied for several decades and are thus well represented in international reference databases (Nilsson *et al.*, 2019; Schoch *et al.*, 2012). The amplification of the entire ITS region has been proposed as a barcode region

for metabarcoding studies, but the length of this region (500 – 1000 bp) makes it unsuitable for many NGS platforms, including Illumina MiSeq (Scibetta *et al.*, 2018). In addition, different fungal species have varying lengths of the ITS region, which causes biased amplification and inaccurate community results (De Filippis et al., 2017; Tedersoo *et al.*, 2014).

New primers targeting the ITS2 region have been shown to result in high-fold taxonomic coverage of fungal communities and are suitable for the Illumina MiSeq platform (Taylor *et al.*, 2016). The ITS2 subregion provides lower length variation and better resolution, resulting in less taxonomic bias than ITS1, and is the recommended target barcode for fungi (Nilsson *et al.*, 2019; Tedersoo *et al.*, 2015). In addition, primers targeting the ITS2 region have been proven to be more selective and thereby advantageous when working with samples that include fungi as a minority, such as with soils and plant tissues (Taylor *et al.*, 2016). However, when using only ITS2 primers, several taxa have been found to be consistently underrepresented (Asemaninejad *et al.*, 2016). Several studies have highlighted the need for new targets and universal primer pairs as secondary DNA barcodes (Schoch *et al.*, 2012). The D1 variable region of the large ribosomal subunit (LSU) (Hassouna *et al.*, 1984) has been proven to be useful in species-level identification and phylogenetic reconstruction of various fungal groups (Kurtzman & Robnett, 1998; Moncalvo *et al.*, 2002). Recent research has shown that the use of both ITS2 and D1 LSU primers recovers a greater diversity of fungi than those only targeting the traditional ITS barcode (Asemaninejad *et al.*, 2016; Poelman *et al.*, 2021). In a study by Asemaninejad *et al.* (2016), D1 LSU primers recovered a greater number and phylogenetic diversity of sequences than ITS2 primers with approximately equivalent read depth. In the same study, operational taxonomic units (OTUs) belonging to 127 genera and 28 species were recovered using D1 LSU primers that were not recovered by ITS2 primers, and OTUs belonging to 10 unique genera and 16 species were recovered by ITS2 primers that were not recovered by LSU primers. Therefore, the combination of LSU and ITS2 primers used for metabarcoding analysis of fungal community data makes for a dataset that more accurately describes the community than either region alone.

1.6 Analysis of next generation sequencing data

Traditionally, the high throughput sequencing of PCR-amplified metabarcoding primers has resulted in the construction of molecular operational taxonomic units (OTUs) (Kopylova *et al.*, 2016; Westcott & Schloss, 2015). OTUs are clusters of reads that differ by less than a fixed dissimilarity threshold selected by the user (Kopylova *et al.*, 2016; Westcott & Schloss, 2015). An OTU table, which features the abundance of each identified OTU in each sample, serves as the basis for all future analyses, with each OTU treated as a species. Recently, new methods have been developed for Illumina amplicon data which eliminate the dissimilarity thresholds needed for constructing OTUs, and instead result in amplicon sequence variants (ASVs) (Callahan *et al.*, 2017). These methods produce a list of ASVs, which are groups of sequences that are unique in length and composition (Callahan *et al.*, 2017). The sensitivity and specificity of ASV methods are as good or better than OTU methods (Callahan *et al.*, 2017). In addition, ASVs are able to capture all biological variation present in the data being analysed, as opposed to OTUs, which only partially capture biological variation (Callahan *et al.*, 2017). Another benefit of working with ASVs is that they are reproducible and can be cross-compared between studies and data sets (Callahan *et al.*, 2017). However, the fine resolution provided by ASV methods comes at a cost, as the resolution may reflect Illumina or PCR artifacts, and there are challenges in merged data sets if the primers used are different from one another (Callahan *et al.*, 2017). Overall, the use of ASVs over OTUs is highly beneficial for generating a more precise, reproducible, and comprehensive analysis of data (Callahan *et al.*, 2017).

1.7 The root mycobiome of corn and wheat

The fungal microbiome of roots and soil from high and low yielding sites has been studied recently. A study by Bandara *et al.* (2021) investigated the microbiome dynamics of bulk soil, rhizosphere soil and root samples of soybeans plants from historically high and low yield sites. They extracted DNA from their samples and performed high throughput sequencing of PCR amplicons from both the fungal ITS1 and SSU (small ribosomal subunit) rRNA gene regions. They constructed ASV tables and performed

network analysis. Their results revealed differences in community composition of soil and roots from the high and low yield sites across eight soybean farms. Networks from high yield sites were found to be more complex compared to those of low yield sites. In their relative abundance analysis, fungal composition was revealed to vary based on site type (high or low) as well as sample type (bulk soil, rhizosphere soil or root soil). A greater relative abundance of ASVs belonging to the genus *Fusarium* and the species *Macrophomina phaseolina* were found in roots of low yield sites compared to high yield sites, and they are both known soybean pathogens. *Corynespora cassiicola*, which has pathogenic properties in some cases and antifungal/antibacterial in others, showed higher relative abundance in high yield sites compared to low yield sites. Additionally, a high abundance of the known beneficial genera *Trichoderma* and *Metarhizium* was seen in the soil of high yield sites. Overall, their findings suggested that spatial variation in yield within a field was associated with community composition (Bandara *et al.*, 2021). Although their study focused on soybean crops and mine focuses on corn and wheat, I may find similarities between their findings and mine, given that they also studied community composition of rhizosphere soil and roots in historically high and low yield sites in the same fields. Additionally, the farms used in my research follow corn-soybean-wheat crop rotations, resulting in possible overlap between the authors' findings and mine. For example, several known soybean pathogens, including species of *Fusarium*, are known pathogens of corn and wheat. In my research, I will be tracking differences in relative abundance of known pathogens of corn and wheat in high and low yield sites.

Wu *et al.* (2022) looked at the impact of long-term fertilization on fungal community composition in the root endosphere, rhizosphere soil, and bulk soil of wheat plants. They found that long-term fertilization significantly influenced fungal community composition, with effects seen strongly in the rhizosphere soil. Results also showed that fungal community composition was significantly correlated with phosphorus and zinc contents. Their analysis showed that although the rhizosphere and root system barriers may buffer stimulation of their fungal communities from long-term fertilization, common agricultural practices such as these can affect not only the rhizosphere soil, but also the roots of the plants. They also found that high levels of phosphorus and zinc were important factors in reducing fungal diversity in all long-term fertilization practices they

studied. This study points to the impact that agricultural practices, including fertilization or crop rotation has on fungal community composition (Wu *et al.*, 2022). It also reinforces the influence of other soil variables on community composition. In my research I will also be assessing the association of soil physicochemical variables, including phosphorus and zinc with fungal community composition.

Recently, Kandasamy *et al.* (2021) looked at the association of the corn root mycobiome with yield and soil physicochemical factors. Using Normalized Difference Vegetation Index (NDVI) maps, the authors identified healthy and stressed sites in 10 corn fields. They sampled rhizosphere soil, bulk soil and corn roots which were then used in DNA extracted and PCR-amplified using ITS2, LSUA, LSUBG, and V4AM primers. Illumina MiSeq data were used to construct OTU tables. They analyzed the soil metadata and determined that the top contributing factors to differences in composition of sites were organic matter %, Al, cation exchange capacity (CEC), pH, soil moisture, and yield. Soil texture (sand and clay) was also important in identifying differences in low yield and high yield sites across all farms and within the same farms. Several ASVs belonging to the genus *Fusarium* were found to be significantly associated with high and low yield sites. In some cases, the *Fusarium* species identified by the authors as being associated with yield may have been pathogenic and in others, they may have acted as saprotrophs. Other genera that were present in high abundance in low and/or high yield sites included *Chalara*, *Penicillium*, and *Trichoderma*. These genera can have varying effects on plant productivity based on species, plant host, and environmental variables. A shortfall of their study was that the fungal LSUBG primers generated a large proportion of *Zea mays* reads from root DNA, which led to poor representation of Basidiomycota in their final results. Overall, their results suggest that the associations between soil physicochemical factors, community composition and productivity are complex and dynamic (Kandasamy *et al.*, 2021). This study, from which I sourced my corn root samples and which used the same farm locations and sites as my research, is an important resource for me and will allow for comparison of results, especially related to soil variables and indicator species. Their study used the same three primer sets I used in my work, along with a fourth one that I did not use, the V4AM primers. This primer set amplifies the 18S region of the ribosomal small subunit and allows for identification of arbuscular mycorrhizal fungi

(Sato *et al.*, 2005). The shortfalls of this study regarding the LSU primers also informed the decision not to perform PCR-amplification of root samples with LSUBG primers, so as not to end up with unusable data.

1.8 Hypothesis and research objectives

I hypothesize that the root and rhizosphere fungal communities will have a strong correlation with crop health and productivity despite varying soil types. I also hypothesize that there will be crop-specific differences in community composition and that there will be indicator taxa present in high and low yield sites across fields. The first objective of my research project is to use Illumina Miseq-compatible fungal barcode primers and apply them to a NGS mycobiome study. This will allow me to identify fungal taxa and profile community compositions of high-yielding and low-yielding samples of corn and wheat roots and rhizosphere soil grown in rotation at the same locations. My second objective is to investigate differences in root mycobiome between samples from high and low yielding sites and identify if shifts in community composition are correlated with differences in crop productivity. My third objective is to investigate differences in corn and wheat root mycobiome and if there are crop-specific differences in community composition. My final objective is to assess potential correlations between fungal community composition and soil physicochemical variables, including yield.

Chapter 2

2 Materials and methods

2.1 Sampling locations

Corn and wheat roots and rhizosphere soil were sampled from three farms in Southwestern Ontario (Table 2.1). All three fields have a history of corn-soybean-wheat rotation, with planting occurring in late spring and harvest occurring in the fall (Figure 2.1). Patchy variation in yield within the fields has been detected, and root- and rhizosphere-associated bacteria (Ali *et al.*, 2020) and root-associated fungi (Kandasamy *et al.*, 2021) of corn in the high- and low-yield patches characterized. A remote sensing unmanned aerial vehicle (UAV) was flown by A&L Biologicals over the fields at the sampling stage in late July to measure healthy and stressed vegetation through Normalized Difference Vegetation Index (NDVI). The cameras on the UAV collected full color imagery as well as infrared imagery, which were used to calculate NDVI. Based on the NDVI maps, high yielding and low yielding patches were identified in each of the fields (Fig. 2.2, Fig. 2.3, Fig. 2.4). These patches, chosen by the team at A&L in 2017 and inherited for my work, were used for sampling corn in 2017 (Ali *et al.*, 2020; Kandasamy *et al.*, 2021) as well as wheat in 2019, and were later harvested by A&L to obtain yield data for each patch sampled.

Table 2.1: Field site locations, planting dates, varieties and sampling information.

Site	Location (Lat/Lon)	Corn (2017)			Wheat (2019)		
		Planting date	Variety planted	Sampling date	Planting date	Variety planted	Sampling date
Field 1	42.836740, −81.101202	May 17	Dekalb 48-56	July 25	October 2018	Soft Red Winter Wheat 25R61	June 24
Field 2	43.139408, −80.837053	May 18	DKC50-78RIB	July 24	October 2018	Pioneer® Soft Red Winter Wheat 25R46	June 27
Field 3	43.289837, −80.545299	May 20	P9526YXR	July 26	October 2018	Pioneer® Soft Red Winter Wheat 25R46	June 27

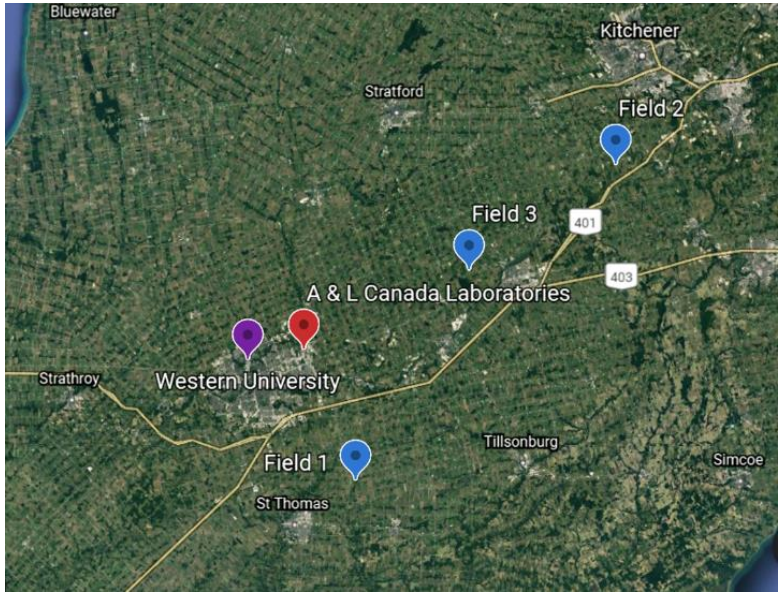


Figure 2.1: Location of farm fields.

Modified Google Earth™ image obtained in June 2020, showing the approximate locations of the three farmer's fields located in Southwestern Ontario, Canada, as well as Western University, and A&L Canada Inc.

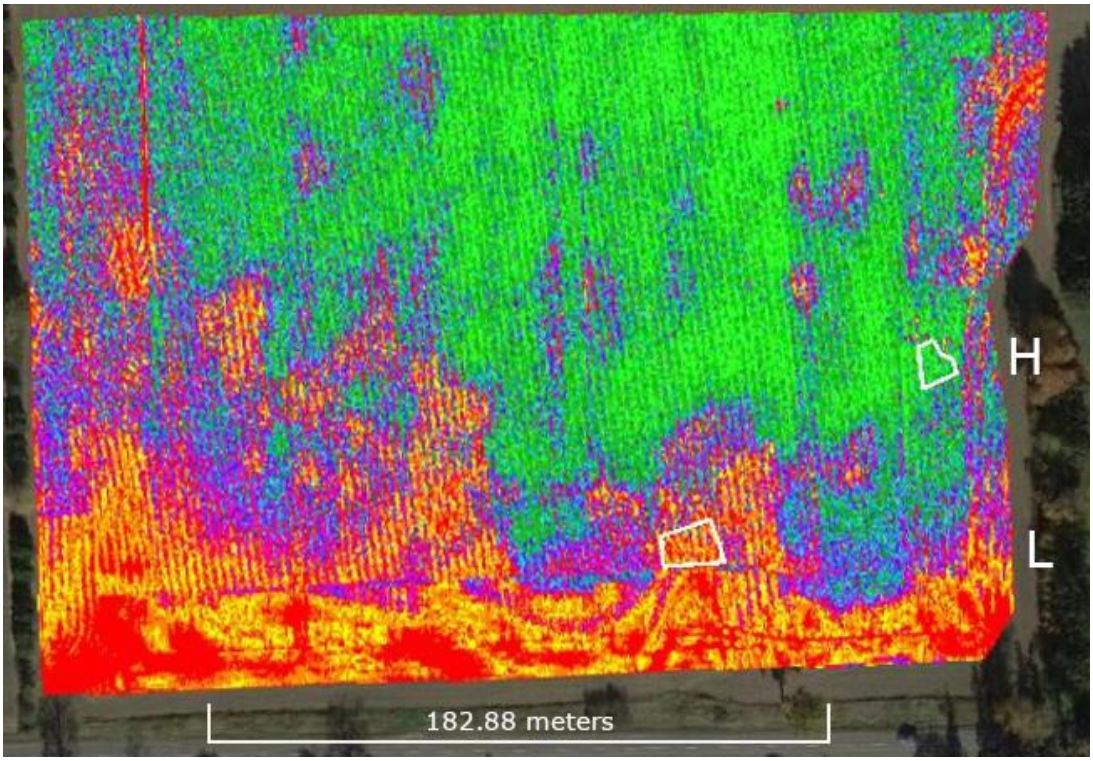


Figure 2.2: Normalized Difference Vegetation Index (NDVI) map of field 1.

A remote sensing unmanned aerial vehicle (UAV) was flown over the farmer's fields, capturing full colour imagery and infrared imagery. NDVI was calculated by A&L Biologicals to create maps to visualize healthy and stressed plants in field 1. As indicated on the map, high yielding (H) and low yielding patches (L), outlined in white, were selected for sampling of corn in 2017 and wheat in 2019.

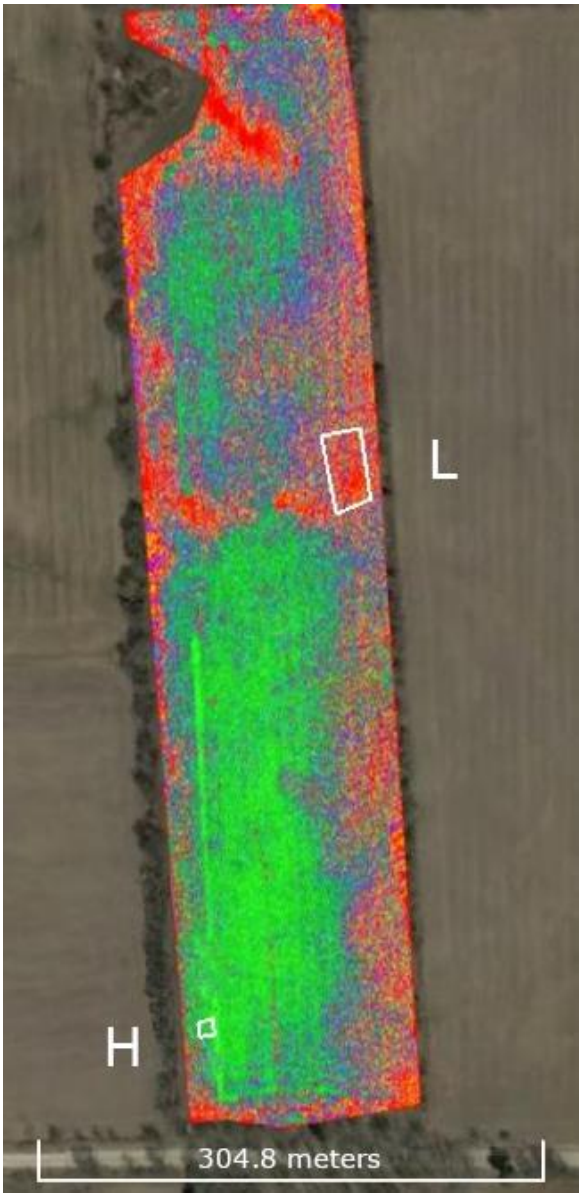


Figure 2.3: Normalized Difference Vegetation Index (NDVI) map of field 2.
A remote sensing unmanned aerial vehicle (UAV) was flown over the farmer's fields, capturing full colour imagery and infrared imagery. NDVI was calculated by A&L Biologicals to create maps to visualize healthy and stressed plants in field 2. As indicated on the map, high yielding (H) and low yielding patches (L), outlined in white, were selected for sampling of corn in 2017 and wheat in 2019.

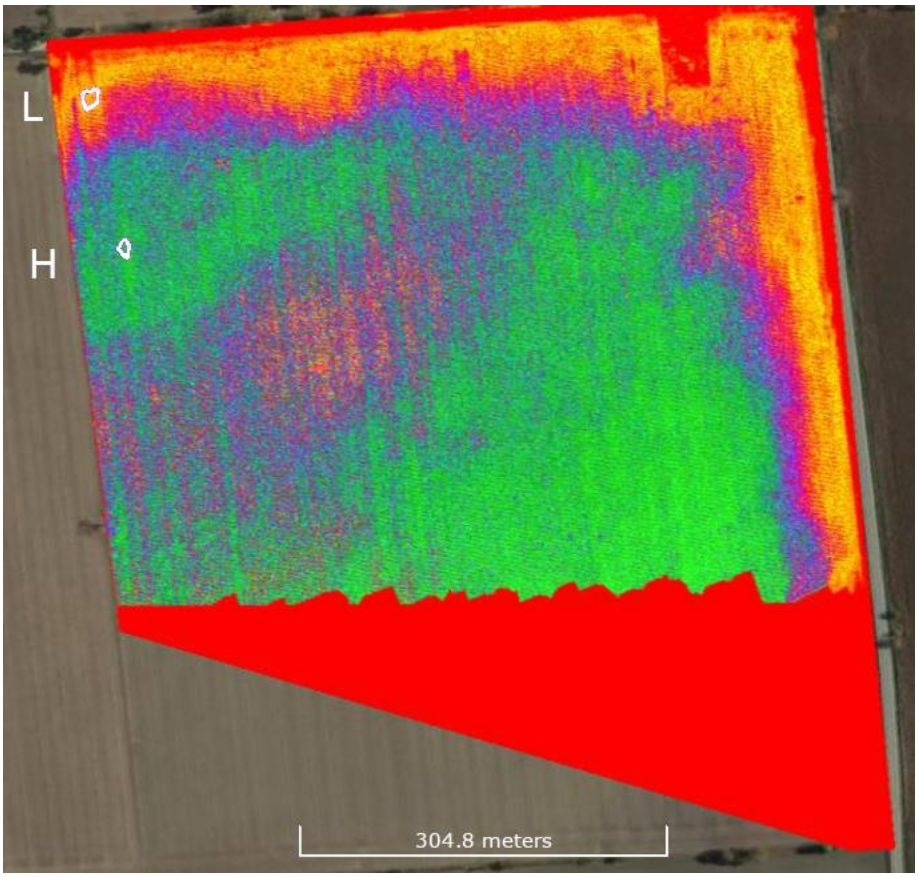


Figure 2.4: Normalized Difference Vegetation Index (NDVI) map of field 3.
A remote sensing unmanned aerial vehicle (UAV) was flown over the farmer's fields, capturing full colour imagery and infrared imagery. NDVI was calculated by A&L Biologicals to create maps to visualize healthy and stressed plants in field 3. As indicated on the map, high yielding (H) and low yielding patches (L), outlined in white, were selected for sampling of corn in 2017 and wheat in 2019.

2.2 Corn sampling and processing

In the summer of 2017, at the V10 stage of the crop cycle, the team at A&L Biologicals collected corn samples at the selected high and low yielding patches on each of the farmer's fields. At each patch, ten corn plants were randomly sampled, each sample site separated by at least 2-3 m. Sampling sites were each about 15 x 15 m. The roots were dug out of the ground carefully, and the plants along with the rhizosphere soil attached to the roots were collected in labelled bags. Root structure and depth was accounted for when sampling corn. Plants were left in a cold room overnight prior to processing.

Rhizosphere soil was removed and shaken off the roots of the plants. Approximately 20 g of soil was frozen and set aside for genomic DNA extraction, and ~ 400 g were sent A&L Canada Inc. for chemical analysis. Roots were then separated from the stalk, washed using tap water, blotted dry, chopped into fine pieces, mixed, and ~ 30 g frozen in bags for root DNA extractions.

2.3 Wheat sampling and processing

Just before full flowering, in late July of 2019, I was part of the team at A&L Biologicals that collected wheat samples from the farmers' fields. At the selected high and low yielding patches, five sample sites were selected from the high-yielding patch and five from the low-yielding patch, 2-3 meters apart. One square foot of wheat was measured at each site, and the roots and rhizosphere soil were dug out of the ground and pooled together. Root structure and depth was accounted for when sampling wheat. Plants were then put into labelled bags and left in a cold room overnight, prior to processing.

Rhizosphere soil was shaken off the roots of the wheat plants. Approximately 20 grams of rhizosphere soil was put into labelled bags and frozen for genomic DNA extraction and ~400 grams of soil were sent to A&L Canada Inc. for soil chemistry analysis. After rhizosphere soil was shaken off the roots, the roots were washed, blotted dry, cut into fine pieces, mixed, and ~ 30 g frozen in bags for root DNA extractions.

2.4 Soil physicochemical analysis

Corn and wheat rhizosphere soil samples were sent to A&L Canada Inc. for physicochemical and particle size analyses, including various metals and metalloids (Jones Jr, 1999). The measures recorded were organic matter content % (OM), Bray-P1, K ppm, Mg ppm, pH (Anderson & Ingram, 1993), CEC meq/100g (cation exchange capacity) (Allen *et al.*, 1974), % K, % Mg, % Ca, % H, % Na (Jones Jr, 1999), S ppm, Zn ppm, Mn ppm, Fe ppm, Cu ppm, B ppm, soluble salts ms/cm, Al ppm, Cl ppm *(*Baird *et al.*, 2017), NO_3-N ppm, ENR (estimated nitrogen release) (Baird *et al.*, 2017), % sand, % silt, % clay, and soil moisture (Appendix A, B). Bray-P1 is a measure of phosphorus (P ppm) and specifically measures phosphorus which is readily available to the plants

(Olsen *et al.*, 1954). % H is a percent base saturation measure of hydrogen ions in the soil. Soluble salts (ms/cm) are a measure of salinity in the soil due to soluble salts that can originate from fertilizer, organic matter or other sources.

2.5 Yield measurements

Corn and wheat sampling sites were revisited by staff of A&L Biologicals, Inc., in mid- to late- October of the sampling year for harvest. Corn and wheat yield was obtained as exact yield (bu/ac) of sampling sites from a yield monitor attached to a combine harvester which tracked the GPS coordinates of the sampling sites (Table 2.2).

Table 2.2: Yield of high and low yielding patches of corn and wheat at three field site locations.

Field site	Patch	Yield (bu/ac)
Field 1	High yielding corn	292
	Low yielding corn	247
	High yielding wheat	99
	Low yielding wheat	67
Field 2	High yielding corn	320
	Low yielding corn	239
	High yielding wheat	24.87
	Low yielding wheat	92.21
Field 3	High yielding corn	263
	Low yielding corn	223
	High yielding wheat	92.64
	Low yielding wheat	85.08

2.6 Molecular protocols

Together, the sites provided 60 corn samples (3 fields x 10 high yielding, 3 fields x 10 low yielding) and 30 wheat samples (3 fields x 5 high yielding, 3 fields x 5 low yielding). To obtain comparable data, five of the ten corn sample sites were randomly chosen to be analyzed in this exploratory analysis.

Soil DNA Isolation Kits (Norgen Biotech Corp.) were used to extract genomic DNA from the frozen samples of rhizosphere soil and root, with minor modifications to the manufacturer's protocols. For extraction, 0.25 grams of rhizosphere soil and 0.17 g of chopped roots were used. Beat-beating was used to facilitate cell lysis for all samples and was carried out in the FastPrep-24™ (MP Biomedicals) instrument at a setting of 6.5 m/s for 60 s. After extraction, the concentration of eluted DNA was quantified using the SpectraMax® QuickDrop™ Micro-Volume Spectrophotometer (Molecular Devices, LLC.). DNA extracts were stored at –20 °C until PCR amplification.

The following three sets of primer combinations were chosen to PCR-amplify the DNA samples to recover a wide range of fungal communities. Each primer set targets different fungal groups, which would be underrepresented if only one primer set was used. The first two primer sets target the D1 variable region of the large ribosomal subunit (LSU) DNA. Basidiomycota and Glomeromycota LSUBG: LSU200-F (5′-AACKGCGAGTGAAGMGGGA−3′) and LSU481-R (5′-TCTTTCCCTCACGGTACTTG−3′) (Asemaninejad *et al.*, 2016); Ascomycota LSUA: LSU200A-F (5′-AACKGCGAGTGAAGCRGYA−3′) and LSU476A-R (5′-CSATCACTSTACTTGTKCGC−3′) (Asemaninejad *et al.*, 2016). The third primer set targets the internal transcribed spacer 2 (ITS2) region of the ribosomal DNA. Fungi ITS2: 5.8S-F (5′-AACTTTYRRCAAYGGATCWCT−3′) and ITS4 -R (5′-CCTCCGCTTATTGATATGCTTAART−3′) (Taylor *et al.*, 2016). The three fungal primers (LSUBG, LSUA, and ITS2) were optimized for the Illumina MiSeq platform. The 5'end of each primer was tagged with Illimuna MiSeq adaptors, a 4 base pair linker (NNNN), and an 8 base pair index sequence that facilitates barcoding (Asemaninejad *et al.*, 2016; Gloor *et al.*, 2010) (Figure 2.5). All three primer sets produce amplicons of approximately 300 bp in length (Asemaninejad *et al.*, 2016; Taylor *et al.*, 2016) (Figure 2.6).

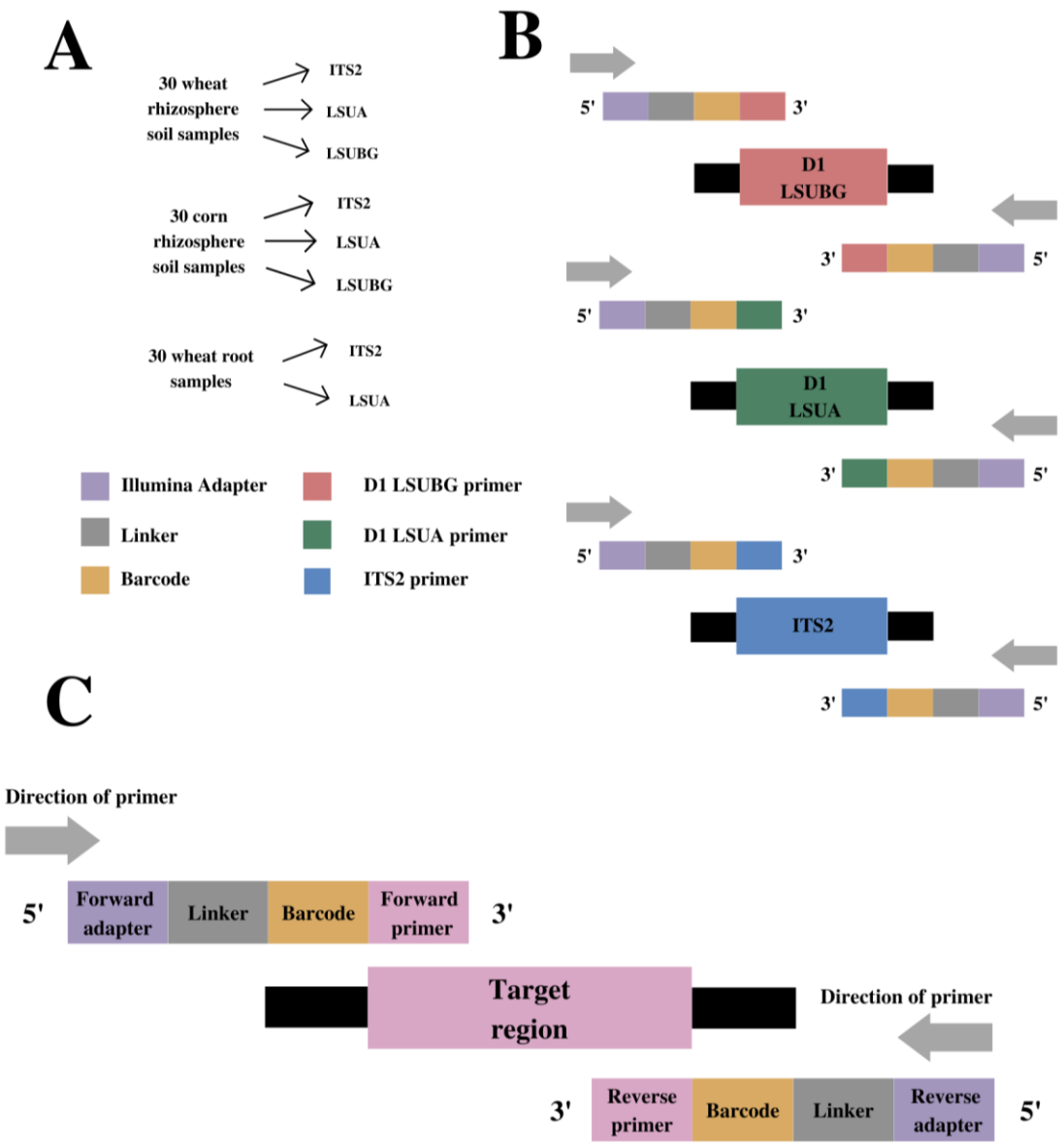


Figure 2.5: Schematic representation of primer set designs for targeting three regions of the ribosomal RNA gene.

A) Primer sets used to amplify DNA from wheat rhizosphere samples, corn rhizosphere samples, and wheat root samples. 30 corn root samples were amplified using LSUA and ITS2 primer sets in 2017 by A&L Biologicals (Kandasamy *et al.*, 2021). B) Design of three primer sets specific for Illumina MiSeq platform. C) Example of primer design. LSU200-F/LSU481-R (LSUBG) primers and LSU200A-F/LSU476A-R (LSUA) primers designed by Asemaninejad *et al.* (2016) and 5.8S-F/ITS4-R (ITS2) primers designed by Taylor *et al.* (2016).

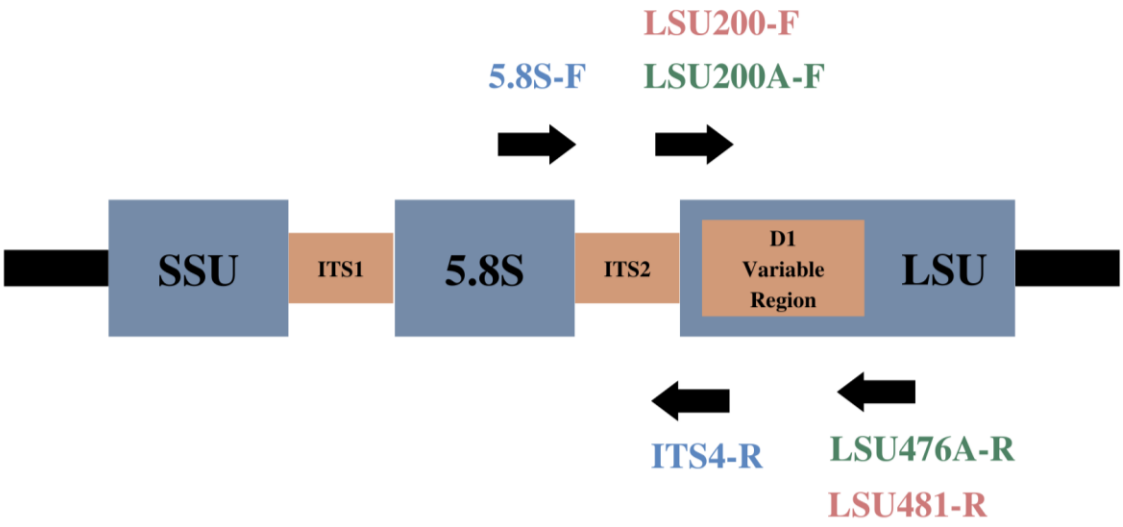


Figure 2.6: Schematic representation of the regions of the ribosomal RNA gene to be amplified.

Approximate location of LSU200-F/LSU481-R (LSUBG, in red), LSU200A-F/LSU476A-R (LSUA, in green) and 5.8S-F/ITS4-R (ITS2, in blue) primers in relation to regions of the ribosomal RNA gene in fungi. LSU200-F/LSU481-R (LSUBG) primers and LSU200A-F/LSU476A-R (LSUA) primers designed by Asemaninejad *et al.* (2016) and 5.8S-F/ITS4-R (ITS2) primers designed by Taylor *et al.* (2016). SSU = small ribosomal subunit, LSU = large ribosomal subunit.

Corn and wheat rhizosphere soil DNA extracts were amplified using all three primer combinations. Wheat root DNA extracts were amplified using only the ITS2 and LSUA primer sets, as amplification of corn root DNA with LSUBG primers was shown to overwhelmingly yield corn root ASVs as opposed to fungal ASVs. In a 2021 study, Kandasamy *et al.* amplified corn root DNA samples with fungal LSUBG primers and found that the majority of reads were 9 OTUs from *Zea mays* (7,220,937 reads) and 5 OTUs from unknown Animalia (949,092 reads). Other non-target OTUs included: Cercozoa, Animalia, Amoebozoa, other Streptophyta, and Ascomycota. Corn root DNA was amplified using the ITS2 and LSUA primers in 2017 and sequence and ASV data for these samples were provided by A&L Biologicals for this project (Kandasamy *et al.*, 2021).

Saccharomyces cerevisiae (Ascomycota) and *Agaricus bisporus* (Basidiomycota) DNA extracts were used as positive controls for PCR amplifications. *Agaricus bisporus* (~10 ng/uL) DNA was used as a positive control for LSUBG primer set reactions, *Saccharomyces cerevisiae* (~50 ng/uL) DNA was used for LSUA primer set reactions, and a combination of DNA of this pair (each at 10 ng/uL) was used for ITS2 primer set reactions.

PCR was carried out using a total volume of 25 µL: 12.5 µL of Toughmix Taq polymerase (Quantabio, Beverly, MA), 1 µL of the forward and reverse primers (10 µM), and 0.5 µL of 50x loading dye. Each sample was amplified twice, using two volumes of DNA (1 µL and 4 µL) to account for the variation in DNA concentrations between samples. Sterile molecular-grade water was used to bring the final volume to 25 µL. PCR amplification was carried out in the T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) using the following parameters: 94 °C for 2 minutes, 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and a hold of 4 °C after cycling. PCR products were confirmed using automated, high-resolution capillary electrophoresis in the QIAxcel Advanced System (Qiagen, Germantown, MD). One high yielding wheat rhizosphere soil sample from Field 1 and one high yielding wheat root sample from Field 1 were excluded due to a lack of PCR product after multiple attempts at DNA extraction and PCR amplification.

PCR products resulting from same template DNA using 1 µL and 4 µL of DNA were pooled and stored at –20 °C until submission to the London Regional Genomics Centre (Robarts Research Institute, London, Ontario, Canada) for paired-end 2 × 300 Illumina Miseq High Throughput Sequencing. A total of 235 samples were pooled into one Illumina run. The presence of barcode sequences in the primers allows for this pooling and resulting reads can be identified by sample based on the corresponding barcode sequence.

2.7 Bioinformatic analysis

Raw FASTQ data were initially processed using a script that allows Illumina MiSeq FASTQ files to be demultiplexed by their primer pairs before sample demultiplexing

(https://github.com/nweerasu/primer_pull). Data were further processed using a MiSeq processing pipeline with minor modifications to account for the correct primer and barcode lengths (https://github.com/ggloor/miseq_bin, https://github.com/nweerasu/primer_pull).

The DADA2 R package was used to carry out the full amplicon workflow: filtering, dereplication, sample inference, chimera identification and removal, and merging of paired-end reads (Callahan *et al.*, 2016). This workflow yields an amplicon sequence variant (ASV) table, an alternative to the more traditionally used operational taxonomic unit (OTU) table. A separate ASV table was made for each primer set. Use of ASVs, compared to OTUs, has been shown to more accurately reconstruct amplicon-sequenced communities, provides a record of the number of times each exact amplicon sequence variant was observed in each sample, and allows direct comparison between different studies using the same gene region (Callahan *et al.*, 2017). Taxonomy was then assigned using DADA2-formatted reference datasets: the UNITE general FASTA release for Fungi for ITS2 data (Abarenkov *et al.*, 2021) and the RDP LSU taxonomic training data formatted for DADA2 (trainingset 11) for LSUA and LSUBG data (Czaplicki, 2017). Taxonomy was then confirmed using RDP Classifier and the Unite Fungal ITS trainset (07-04-2014) and the Fungal LSU training set 11 (Q. Wang *et al.*, 2007) for ITS2 and both LSU datasets, respectively. The RDP Classifier taxonomy was used to identify and remove non-target ASVs. Some unidentified sequences were manually updated after individual BLASTn searches (McGinnis & Madden, 2004). ASVs with total relative abundance less than 0.1% were removed.

2.8 Statistical analysis

Statistical analyses and graphs were done using several packages implemented in R version 4.1.2 (R Core Team, 2021), including phyloseq (McMurdie & Holmes, 2014), vegan (Dixon, 2003), and ggplot2 (Valero-Mora, 2010). Analyses were done separately for each primer set. The VennDiagram package was used to make Venn diagrams of shared ASVs in the three fields for each primer set (H. Chen & Boutros, 2011). An adonis (PERMANOVA) test using Bray-Curtis Dissimilarity indices was performed first

to detect any significant differences between the three fields used in the study (Dixon, 2003). Pairwise adonis (PERMANOVA) analysis was performed using Bray-Curtis Dissimilarity indices and adjustment for multiple comparisons using the Benjamin and Hochberg procedure to identify pairwise differences in fields (Dixon, 2003). Due to significant differences between all fields, analyses were done separately for each field within the data obtained with each primer set. Principal Coordinates Analysis (PCoA) was used to visualize difference in the community structure of the mycobiomes of the three farms (McMurdie & Holmes, 2014).

The alpha diversity of the corn and wheat mycobiome was visualized and calculated using the Shannon diversity index in phyloseq (McMurdie & Holmes, 2014). ASV counts were then transformed into relative abundance counts to visualize the relative abundance of fungal phyla for each sample type: high-yielding corn rhizosphere, low-yielding corn rhizosphere, high-yielding corn root, low-yielding corn root, high-yielding wheat rhizosphere, low-yielding wheat rhizosphere, high-yielding wheat root, and low-yielding wheat root (Valero-Mora, 2010). For LSUA data, relative abundance plots at the level of class were made- since these primers yield amplicons of phylum Ascomycota almost exclusively (Asemaninejad *et al.*, 2016; Valero-Mora, 2010). Non-metric Multi-dimensional Scaling (NMDS) plots using Bray-Curtis dissimilarity were made to visualize differences in community composition of sample types in each farm (McMurdie & Holmes, 2014). ANOSIM tests were performed using the vegan package to identify differences between the fungal communities of the groups of samples (Dixon, 2003). ANOSIM was used as it is a non-parametric test that uses a ranked dissimilarity matrix as opposed to raw data. In ANOSIM tests, there are no assumptions made about the data, making it a good fit for microbial abundance data. Pairwise adonis (PERMANOVA) analysis was then performed using Bray-Curtis Dissimilarity indices and adjustment for multiple comparisons using the Benjamin and Hochberg procedure to identify pairwise differences in sample types (high yield corn rhizosphere, low yield corn rhizosphere, high yield wheat rhizosphere, low yield wheat rhizosphere, high yield corn root, low yield corn root, high yield wheat root and low yield wheat root) (Dixon, 2003).

Heatmaps of the top 10 most abundant ASVs in each field were made for relative abundance of the top 10 ASVs (McMurdie & Holmes, 2014). NMDS of the Bray-Curtis Dissimilarity indices were used to organize rows and columns in the heatmaps. To identify ASVs with significantly different abundance between sample types, an Indicator Species analysis was performed using the indicspecies package (De Cáceres *et al.*, 2010). The data were first split by field to identify ASVs that were indicators for each field. Then, the data were split by crop, rhizosphere/root, and sample type (corn rhizosphere soil, wheat rhizosphere soil, corn roots, wheat roots) groupings within each field to identify ASVs that were indicators for the different sample types. The top ASVs that were found to be significantly different ($p < 0.01$ for ITS2 and LSUA data, $p < 0.05$ for LSUBG data), based on p values adjusted for multiple comparisons using the Benjamin and Hochberg procedure were recorded (De Cáceres *et al.*, 2010).

An ANOSIM test of the ranked dissimilarity matrix (Bray-Curtis Dissimilarity indices) was used to determine whether significant differences existed in the fungal community composition of corn, wheat, rhizosphere and root groupings between high and low yield sites (Dixon, 2003). Based on ANOSIM results, indicspecies analyses were performed to identify ASVs that were significantly associated with high or low yield sites (De Cáceres *et al.*, 2010). The top ASVs that were found to be significantly different ($p < 0.05$), based on p values adjusted for multiple comparisons using the Benjamin and Hochberg procedure were recorded (De Cáceres *et al.*, 2010). These results were visualized using boxplots to show the distributions of ASVs that were statistically differentially abundant between groups (Valero-Mora, 2010).

Mantel tests were performed based on Spearman's rank correlation using the ASV tables for rhizosphere data and soil chemistry and yield data to determine if the differences in community composition between samples are correlated with the differences in soil chemistry or yield (Dixon, 2003). These correlations were visualized using NMDS ordinations overlaid with environmental factors using the envfit function (Dixon, 2003). Generation of NMDS ordinations separately by field greatly impacted the stress values for each plot, decreasing many of the stress values from over 0.2 to below 0.15. Generally, stress values that are greater than 0.2 are considered poor and the plots

generated are potentially uninterpretable (Clarke, 1993). However, reducing ASVs to only the top percentage observed did not decrease stress values and, in some cases, they even increased, which suggests that the rarities were part of the patterns observed for community composition of samples. For the LSUA and LSUBG NMDS ordinations, a few outlier samples that fell outside of the 95 % confidence ellipses were removed to further decrease stress values (Dixon, 2003). Spearman correlation heat plots of soil physicochemical factors were done using the corrplot package in R (Wei & Simko, 2017). Spearman correlation data for the top 25 most abundant taxa identified by each primer set and 20 soil physicochemical factors as well as yield with adjustments for multiple comparisons done with the Benjamin and Hochberg procedure were made using the microbiomeSeq package in R (Ssekagiri, 2022). Correlation heatmaps were made using Complex Heatmap (Gu, 2016).

Chapter 3

3 Results

3.1 Sequencing output

After chimera removal, removal of low relative abundance (<0.1 %) amplified sequence variants (ASVs), removal of non-target ASVs, and combining with 2017 corn root data, a total of 1 471 890 reads of the ITS2 region of the ribosomal DNA were produced from corn and wheat rhizosphere soil and root samples. These sequences comprised 2 188 ASVs, and an average of 11 870 reads per sample was obtained for this primer set. For the D1 LSUA region, the final read count of 5 545 345 with an average of 43 478 reads per sample and a total of 2 330 ASVs was produced from the corn and wheat rhizosphere soil and root samples. The D1 LSUBG region yielded a final read count of 2 902 147 with an average of 25 205 reads per sample and a total of 538 ASVs from the corn and wheat rhizosphere soil samples (Table 3.1). Between 46-305 ASVs were shared among all three fields according to data obtained from each primer set. In addition, fewer than 187 ASVs were shared between any two of the three fields, and between 113-730 ASVs were found in just one field (Fig. 3.1).

Table 3.1: Summary of processing of Illumina Miseq reads through quality control plugin DADA2.

The table includes samples from three fields amplified with ITS2 (5.8S-F/ ITS4 -R), LSUA (LSU200A-F/ LSU476A-R) and LSUBG (LSU200-F/LSU481-R) primers and summarizes retained reads after removing low abundance ASVs, low quality ASVs, and non-fungi ASVs. Corn root data from 2017, marked with an asterisk (*), were provided by A&L Biologicals and combined with the other sample data after chimera removal.

Primer	**5.8S-F/ ITS4 -R**	**LSU200A-F/ LSU476A-R**	**LSU200-F/LSU481-R**
No. samples	30 corn rhizosphere soil, 28 wheat rhizosphere soil, 35 corn root*, 29 wheat root	30 corn rhizosphere soil, 28 wheat rhizosphere soil, 35 corn root*, 29 wheat root	30 corn rhizosphere soil, 28 wheat rhizosphere soil
Demultiplexed reads	2,462,222 + 1,226,639*	9,034,913 + 1,822,568*	5,117,768
Filtered and denoised reads	1,462,907	8,115,582	4,646,972
Non-chimeric reads	1,110,555 + 467,023*	4,995,342 + 925,657*	3,526,450
Final ASVs	2,188	2,443	2,161
Final reads	1,471,890	5,545,345	2,902,147
Target ASVs	2,188	2,330	538
Percent target ASVs	100	91	25
Target reads of % of final reads	100	96	51
Mean target ASVs per sample	90	144	63
Mean target reads per sample	11,870	43,478	25,205

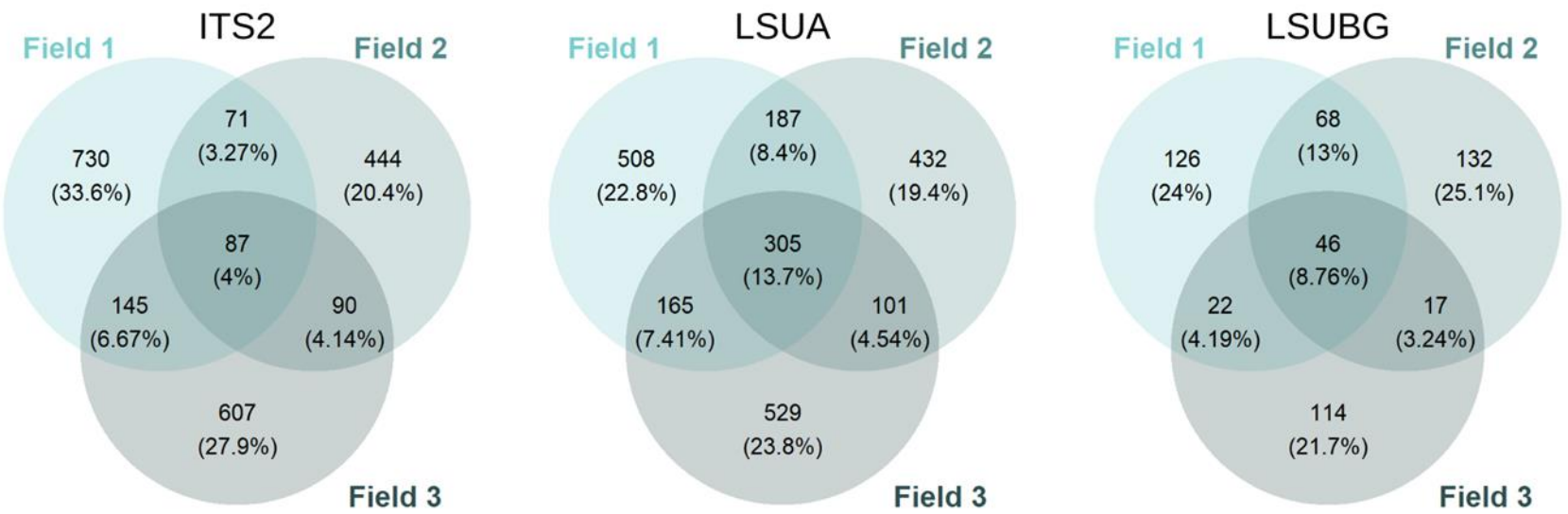


Figure 3.1: Distribution of amplified sequence variants (ASVs) found in each sampled field.

The number and corresponding percentage of all retained ASVs and their distribution across fields were identified within each set of data amplified by the same primer. ASVs were recovered from 122 samples of corn and wheat rhizosphere soil and roots with ITS2 and LSUA primers. LSUBG primers were used to recover ASVs from 58 samples of corn and wheat rhizosphere soil.

Samples were collected from three separate fields with the same crop rotation history. However, it was still important to establish whether the fungal profiles from each site were sufficiently similar to be able to pool the data. Bray-Curtis Dissimilarity indices were calculated from each sample and were pooled by sample type (high and low yield corn rhizosphere, high and low yield corn root, high and low yield wheat rhizosphere, and high and low yield wheat root). The resulting values for each primer set were analyzed using PERMANOVA/ADONIS, revealing significant differences in the fungal composition of the soil mycobiomes between fields (Table 3.2). An additional pairwise PERMANOVA/ADONIS analysis was done using the Bray-Curtis Dissimilarity indices of each sample and pooling by sample type, which revealed significant differences in the fungal composition of soil mycobiomes between all fields using all primers (Table 3.3). PCoA analysis was also used to assess and visualize community similarity among sample types. PCoA plots for all sample types showed similar results, suggesting that there is some similarity between fields, but there is variability in community composition between fields as well (Appendix C). In the PCoA plots for ITS2 and LSUA, which present both rhizosphere and root samples, there is some clustering of fields with overlap on the left side of the plots, and a clear cluster on the right (Appendix C). The cluster on the right was determined to be the high and low yield corn root samples from all three fields. In the PCoA plot for LSUBG, there is clustering by field, with field 3 having the most distinct clustering, as well as some overlap between all three fields (Appendix C). The PCoA analysis overall showed both similarity and variability between community composition of samples from the three fields, and points to clustering based on sample type (Appendix C).

Table 3.2: Differences in mycobiome of corn and wheat rhizosphere soil and roots from three fields.

A PERMANOVA/ADONIS analysis using Bray-Curtis Dissimilarity indices was used to determine whether significant differences existed in the fungal composition of samples from three fields. Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

	Df	Sum Sqs	Mean Sqs	F. Model	R^2	P
5.8S-F/ ITS4 -R						
Field	2	2.8546	1.4273	3.8965	0.06146	0.001***
Residuals	119	43.5899	0.3663		0.93854	
Total	121	46.4446			1.00000	
LSU200A-F/ LSU476A-R						
Field	2	1.6849	0.8424	2.3792	0.03845	0.001***
Residuals	119	42.1379	0.3541		0.96155	
Total	121	43.8228			1.00000	
LSU200-F/LSU481-R						
Field	2	1.5445	0.7723	5.1686	0.15821	0.001***
Residuals	55	8.2178	0.1494		0.84179	
Total	57	9.7624			1.00000	

Table 3.3: *P* values for comparison of corn and wheat rhizosphere soil and root mycobiome from three fields.

A pairwise PERMANOVA/ADONIS analysis using Bray-Curtis Dissimilarity indices was used to determine whether significant differences existed in the fungal composition of samples from three fields.

	Field 1 and Field 2	Field 1 and Field 3	Field 2 and Field 3
5.8S-F/ ITS4 -R	0.0015	0.0020	0.0015
LSU200A-F/ LSU476A-R	0.0255	0.0320	0.0240
LSU200-F/LSU481-R	0.0200	0.0015	0.0015

3.2 The root mycobiome of corn and wheat

Alpha diversity differed among sample types (high and low yield corn rhizosphere, high and low yield wheat rhizosphere, high and low yield wheat rhizosphere, and high and low yield wheat root), with high and low yield corn root having the lowest Shannon diversity index values and greatest variation among samples amplified by ITS2 and LSUA primers (Fig. 3.2). Rhizosphere samples had higher Shannon diversity index values compared to root samples from both crops.

For samples from all fields amplified with ITS2 primers, with the exception of corn root samples from field 2, most of the sequences belonged to the phylum Ascomycota (Fig. 3.3). In field 2 samples from corn roots consisted mostly of sequences belonging to Basidiomycota (Fig. 3.3). Mortierellomycota had the second highest relative abundance in almost all sample types, with Basidiomycota surpassing their abundance in field 1 high-yielding wheat rhizosphere, high-yielding wheat root and low-yielding wheat root, as well as field 2 high yield corn rhizosphere, field 3 corn rhizosphere (high and low yield), field 3 high yield corn root, and field 3 wheat root (high and low yield) (Fig. 3.3). Mucuromycota were most abundant in field 1 and 2 corn root samples, as well as field 1

wheat root samples (Fig. 3.3). In each field, abundance trends were very similar in high and low yield samples from the same crop and type (rhizosphere or root) (Fig. 3.3).

The most abundant class for samples from all fields amplified with LSUA primers was Sordariomycetes for the most part, with Dothideomycetes having the highest relative abundance in field 2 corn root and wheat root samples (Fig. 3.4). Besides those two classes, Lecanoromycetes also had a relatively high abundance of sequences (Fig. 3.4). Eurotiomycetes had higher proportions in corn root samples than in any other samples (Fig. 3.4). As with the results of the analysis with ITS2 primers, similar trends in abundance were observed in high and low yield samples from the same crop and type (rhizosphere or root) (Fig. 3.4).

While a large proportion (a quarter to nearly half) of sequences remain unclassified after amplification with LSUBG primers, the most abundant phyla seen in rhizosphere samples from all three fields were Basidiomycota, Chytridiomycota, and Stramenopiles (Fig. 3.5). Lesser proportions of sequences belonging to Blastocladiomycota and Glomeromycota were also observed in all sample types (Fig. 3.5). A higher relative abundance of Stramenopiles was seen in corn rhizosphere samples in all fields compared to wheat rhizosphere samples (Fig. 3.5). However, overall, similar trends in abundance were seen across all sample types when amplified with the LSUBG primers (Fig. 3.5).

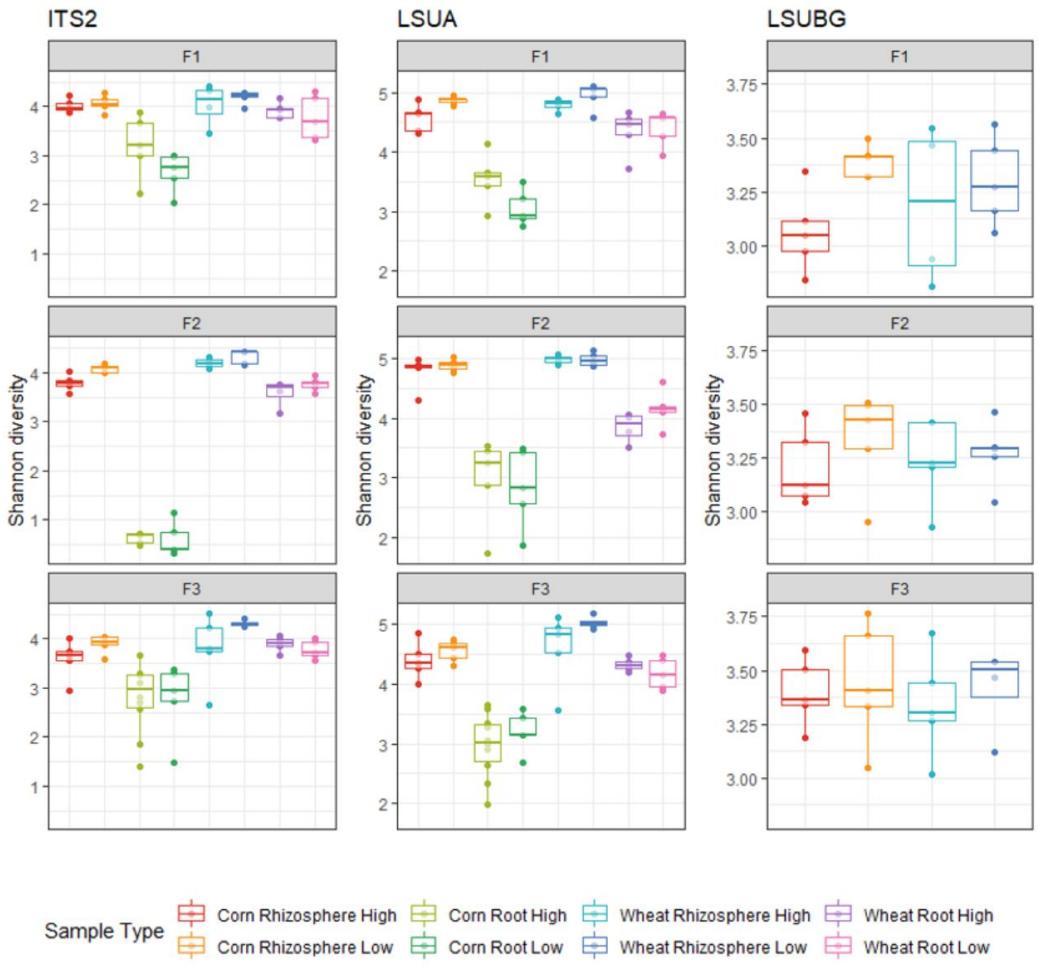


Figure 3.2: Shannon diversity indices of corn and wheat rhizosphere and root fungal microbiomes of three fields.

The coloured dots indicate the calculated Shannon index for each sample. The midline indicates the median and the upper and lower half of the box represents the upper and lower quartile, respectively. Colour indicates sample type.

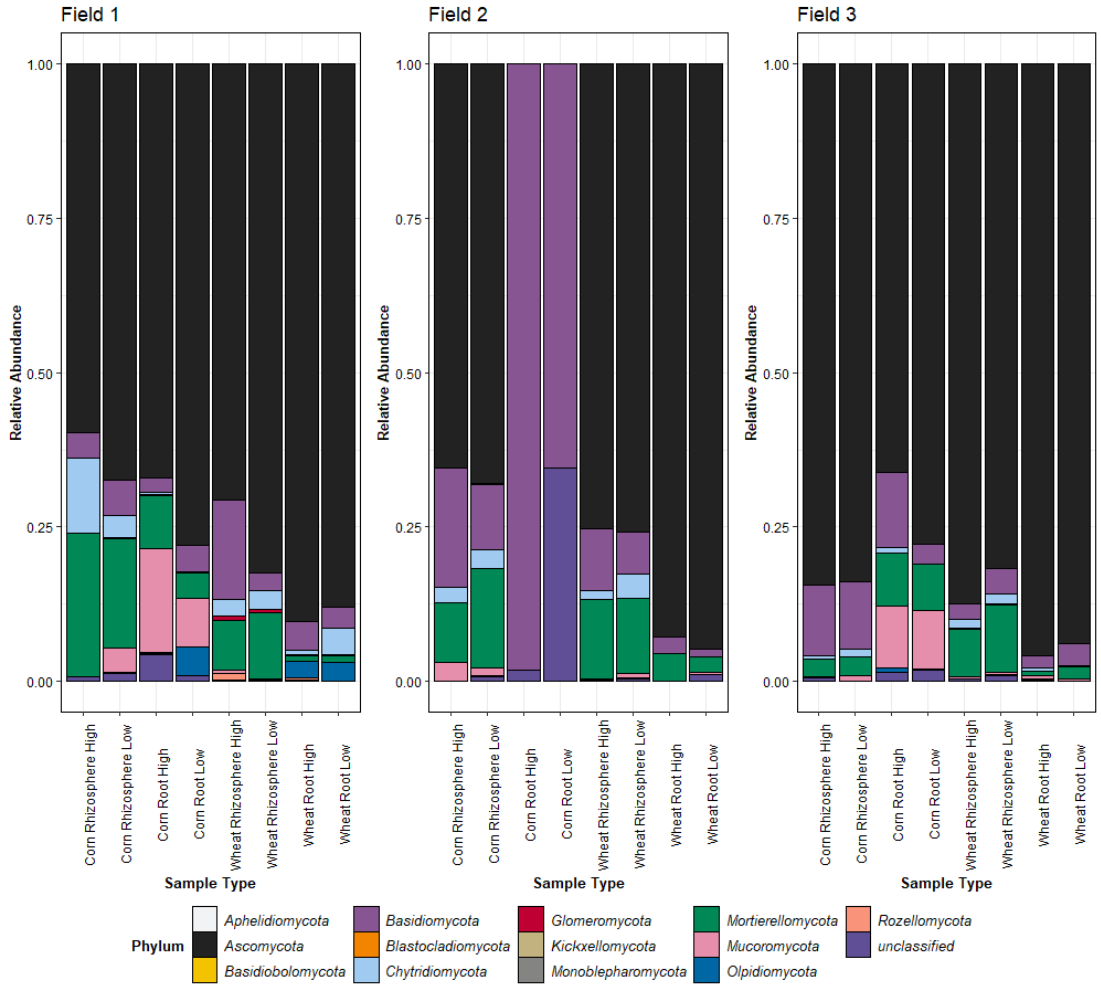


Figure 3.3: Relative abundance of fungal amplified sequence variants (ASVs) obtained using ITS2 primers associated with corn and wheat rhizosphere and root samples.

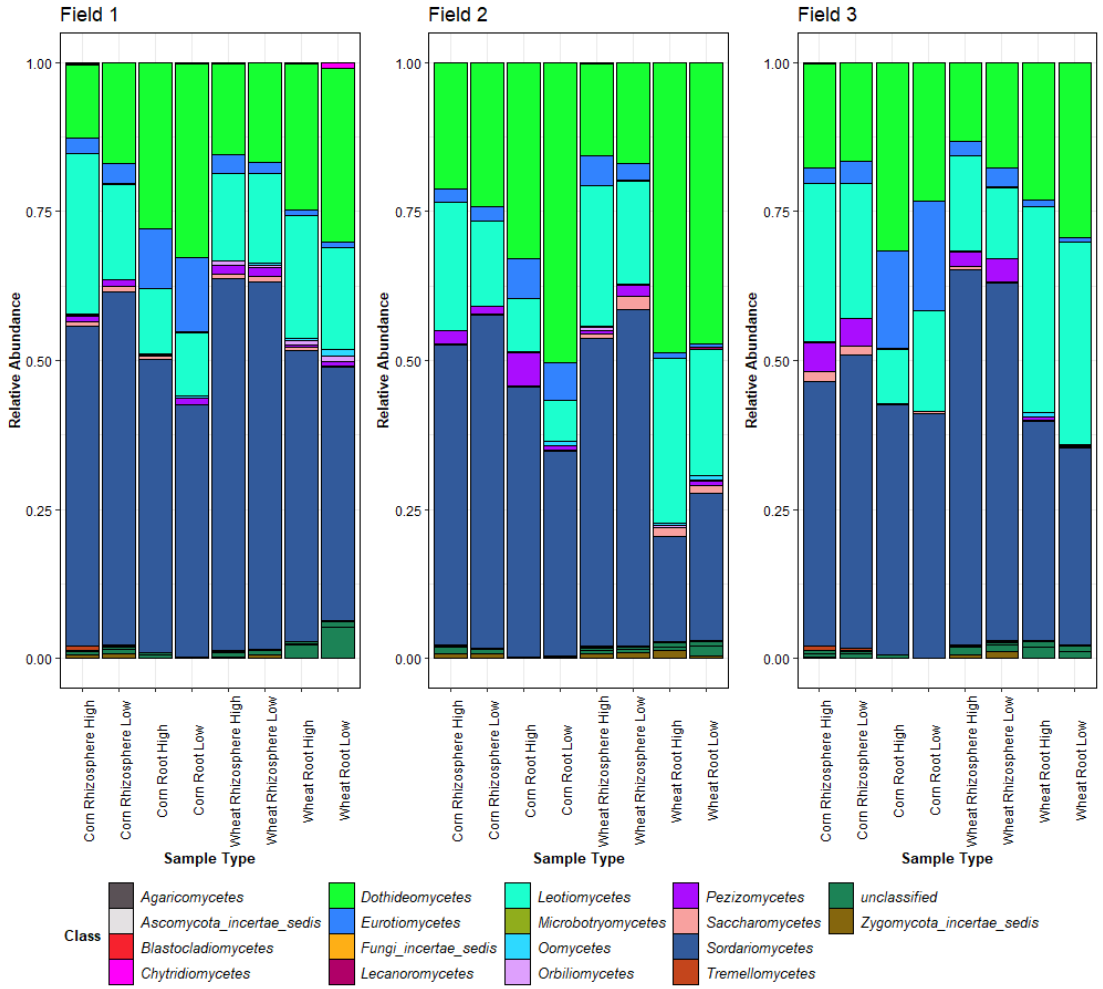


Figure 3.4: Relative abundance of fungal amplified sequence variants (ASVs) obtained using LSUA primers associated with corn and wheat rhizosphere and root samples.

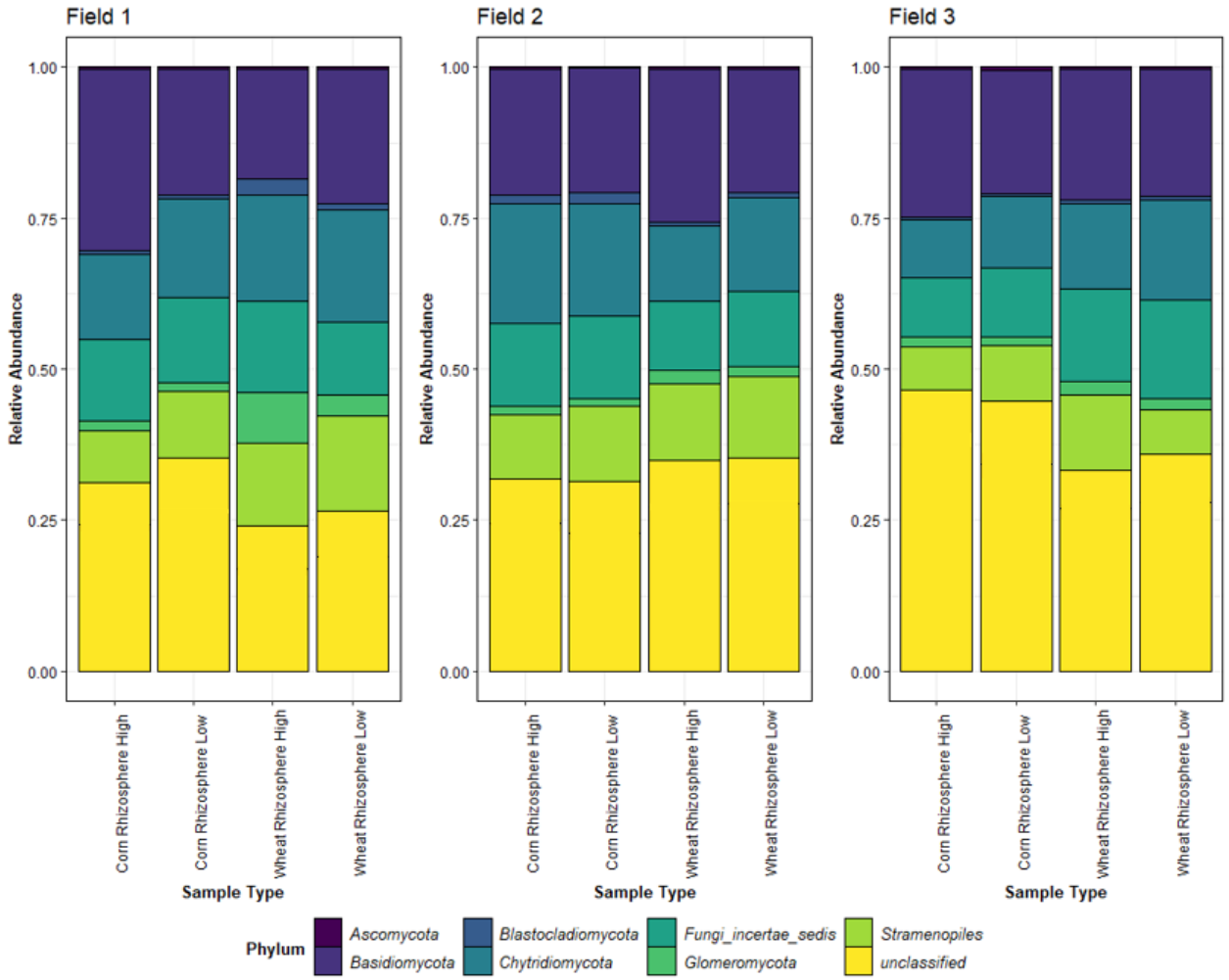


Figure 3.5: Relative abundance of fungal, oomycete and cercozoan amplified sequence variants (ASVs) obtained using LSUBG primers associated with corn and wheat rhizosphere samples.

NMDS analysis was used to assess community similarity among sample types. NMDS plots of sample types from all fields, and with all primers showed similar results, suggesting that community composition differs between sample types and tends to show similarity when the same crop and type (rhizosphere or root) is shared (Fig. 3.6). An ANOSIM test confirmed these results, as sample types were significantly different from one another (Table 3.4). A pairwise PERMANOVA/ADONIS was also performed, which indicated that while most sample types were significantly different in their community composition, some sample types were not significantly different from one another (Table 3.5). For ITS2 and LSUA data, high and low yield corn root samples were not significantly different in fungal community composition in all three fields (Table 3.5). In addition, analysis of samples amplified with LSUA primers also revealed no significant difference in community composition of low yielding corn and wheat rhizosphere soil in field 1, high and low yielding wheat root samples in field 1, high and low yielding corn rhizosphere soil in field 3, and high and low yielding wheat root samples in field 3 (Table 3.5). The analysis performed on LSUBG data revealed no significant difference in the composition of low yield corn and wheat rhizosphere soil in field 3 (Table 3.5). All other pairwise combinations yielded significant differences ($p<0.05$) (Table 3.5).

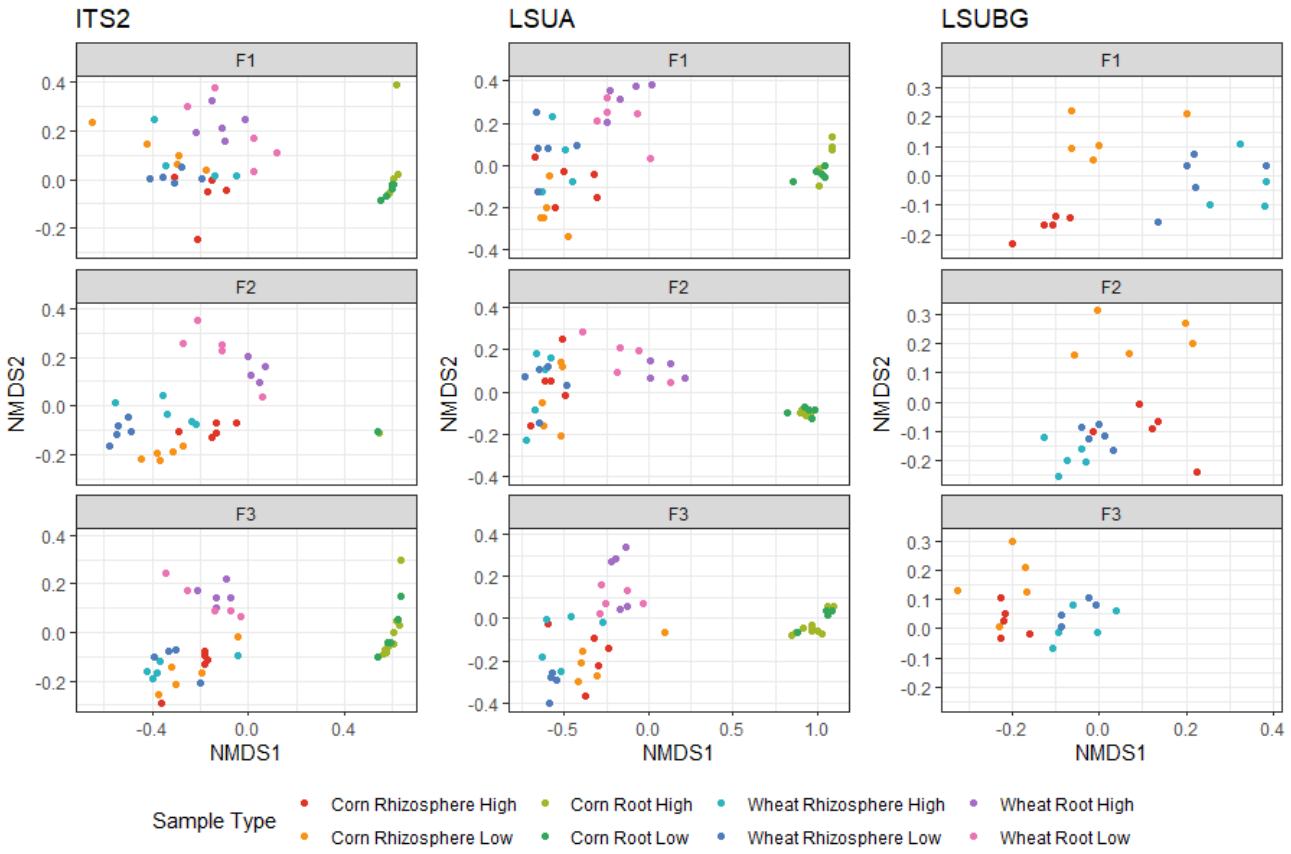


Figure 3.6: NMDS plot of rhizosphere soil and root samples from high and low yield sites from three fields, amplified with ITS2, LSUA and LSUBG primer sets.

Bray-Curtis Dissimilarity indices were plotted in space representing relative relatedness in species composition of the fungal communities of three fields of high and low yielding rhizosphere and root samples from corn and wheat plants collected in summer 2017 and summer 2019, respectively.

Table 3.4: Table 3.4: Differences in fungal community composition of sample types in three fields.

An ANOSIM test of the ranked dissimilarity matrix (Bray-Curtis Dissimilarity indices) was used to determine whether significant differences existed in the fungal community composition of sample types (high yield corn rhizosphere soil, low yield corn rhizosphere soil, high yield wheat rhizosphere soil, low yield wheat rhizosphere soil, high yield corn root, low yield corn root, high yield wheat root, and low yield corn root) from three fields, amplified using three different primer sets. The ANOSIM statistic R compares the mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups. Significant differences in fungal community composition ($p<0.01$) between sample types within the same field are indicated with an asterisk (*).

Primer	Field	**ANOSIM statistic R**	***P* value**
5.8S-F/ ITS4 -R	Field 1	0.64	1.0e-05*
	Field 2	0.57	1.0e-05*
	Field 3	0.55	1.0e-05*
LSU200A-F/ LSU476A-R	Field 1	0.69	1.0e-05*
	Field 2	0.70	1.0e-05*
	Field 3	0.59	1.0e-05*
LSU200-F/LSU481-R	Field 1	0.85	1.0e-05*
	Field 2	0.78	1.0e-05*
	Field 3	0.61	1.0e-05*

Table 3.5: Adjusted P values from a pairwise PERMANOVA/ADONIS analysis of Bray-Curtis Dissimilarity indices comparing fungal community composition of sample types from three fields.

Bray-Curtis Dissimilarity indices were compared from each sample type from each field, separately for sequence data obtained from each primer set. Adjustment for multiple comparisons was done using the Benjamin and Hochberg procedure. Green shading denotes no significant difference between two sample types (p>0.05). (CRSH = high yield corn rhizosphere, CRSL = low yield corn rhizosphere, WRSH = high yield wheat rhizosphere, WRSL = low yield wheat rhizosphere, CRTH = high yield corn root, CRTL = low yield corn root, WRTH = high yield wheat root, WRTL = low yield wheat root)

Sample type 1	Sample type 2	Adjusted *P* value								
		5.8S-F/ ITS4 -R			LSU200A-F/ LSU476A-R			LSU200-F/LSU481-R		
		Field 1	Field 2	Field 3	Field 1	Field 2	Field 3	Field 1	Field 2	Field 3
CRSH	CRSL	0.015	0.014	0.013	0.015	0.039	0.053	0.014	0.012	0.017
	WRSH	0.015	0.014	0.014	0.015	0.016	0.015	0.014	0.012	0.017
	WRSL	0.015	0.014	0.013	0.015	0.016	0.015	0.014	0.012	0.017
	CRTH	0.015	0.014	0.007	0.015	0.016	0.007			
	CRTL	0.015	0.014	0.011	0.015	0.016	0.015			
	WRTH	0.016	0.014	0.013	0.015	0.016	0.015			
	WRTL	0.015	0.014	0.011	0.015	0.016	0.015			
CRSL	WRSH	0.015	0.015	0.013	0.015	0.016	0.027	0.014	0.012	0.017
	WRSL	0.015	0.014	0.013	0.015	0.016	0.016	0.014	0.012	0.022
	CRTH	0.015	0.014	0.007	0.015	0.016	0.009			
	CRTL	0.015	0.014	0.014	0.018	0.016	0.015			
	WRTH	0.015	0.014	0.013	0.015	0.016	0.015			
	WRTL	0.015	0.014	0.013	0.015	0.016	0.015			
WRSH	WRSL	0.015	0.014	0.013	0.089	0.040	0.045	0.018	0.012	0.130
	CRTH	0.015	0.014	0.007	0.015	0.016	0.007			
	CRTL	0.015	0.014	0.013	0.016	0.016	0.015			
	WRTH	0.015	0.014	0.013	0.016	0.016	0.018			
	WRTL	0.015	0.014	0.013	0.015	0.016	0.015			
WRSL	CRTH	0.015	0.014	0.009	0.015	0.016	0.007			
	CRTL	0.015	0.014	0.013	0.015	0.016	0.015			
	WRTH	0.015	0.014	0.013	0.020	0.016	0.015			
	WRTL	0.015	0.014	0.013	0.015	0.016	0.015			
CRTH	CRTL	0.466	0.654	0.800	0.279	0.536	0.187			
	WRTH	0.015	0.014	0.007	0.015	0.016	0.009			
	WRTL	0.015	0.014	0.009	0.016	0.016	0.00700			
CRTL	WRTH	0.015	0.015	0.013	0.015	0.016	0.01467			
	WRTL	0.015	0.014	0.013	0.015	0.016	0.01467			
WRTH	WRTL	0.044	0.027	0.013	0.054	0.029	0.15762			

The top 10 most abundant ASVs in each field, separated by primers used and by field, were identified and used to create abundance heatmaps. The most abundant ASVs were used to build the heatmaps that include all samples, grouped by sample type. NMDS using Bray-Curtis Dissimilarity of sample types was used to organize rows and columns. In the case of ITS2 and LSUA data, which include samples from corn and wheat rhizosphere soil and root samples, the top 10 most abundant ASVs were entirely different in the corn root samples, so corn root heatmaps for each field were done separately from the other sample types.

From the ITS2-amplified samples, ASVs identified as belonging to the genera *Mortierella*, *Trichosporiella*, *Articulospora*, *Tetracladium*, and *Neosetophoma* were abundant in all three fields (Fig. 3.7). *Mortierella* and *Trichosporiella* had higher relative abundance in corn and wheat rhizosphere soil samples than in wheat root samples, while the reverse was true for *Articulospora* and *Neosetophoma* (Fig. 3.7). In many cases for the samples from the ITS2 data, relative abundance of the top ASVs was higher in corn and wheat rhizosphere samples than in wheat root samples. In general, abundance of the top 10 ASVs did not differ much between high and low yield samples from the same crop and type (rhizosphere soil or root). However, *Mortierella* was more abundant in the corn rhizosphere soil from low yield sites in field 2, while *Tetracladium* had higher abundance in high yield corn rhizosphere sites in field 2 (Fig. 3.7). Samples sharing the same crop and type (rhizosphere or root) clustered together, with minimal clustering based on yield site (Fig 3.7). In the corn root samples from all fields, the high and low yield sites did not differ in their abundance of the top 10 ASVs, demonstrated by the lack of clustering (Fig. 3.8). Some ASVs were only present in one or a few samples, such as *Cordana* in field 1, and others showed fairly consistent abundance in all corn root samples from the same field, such as *Setophoma* in field 1 (Fig. 3.8).

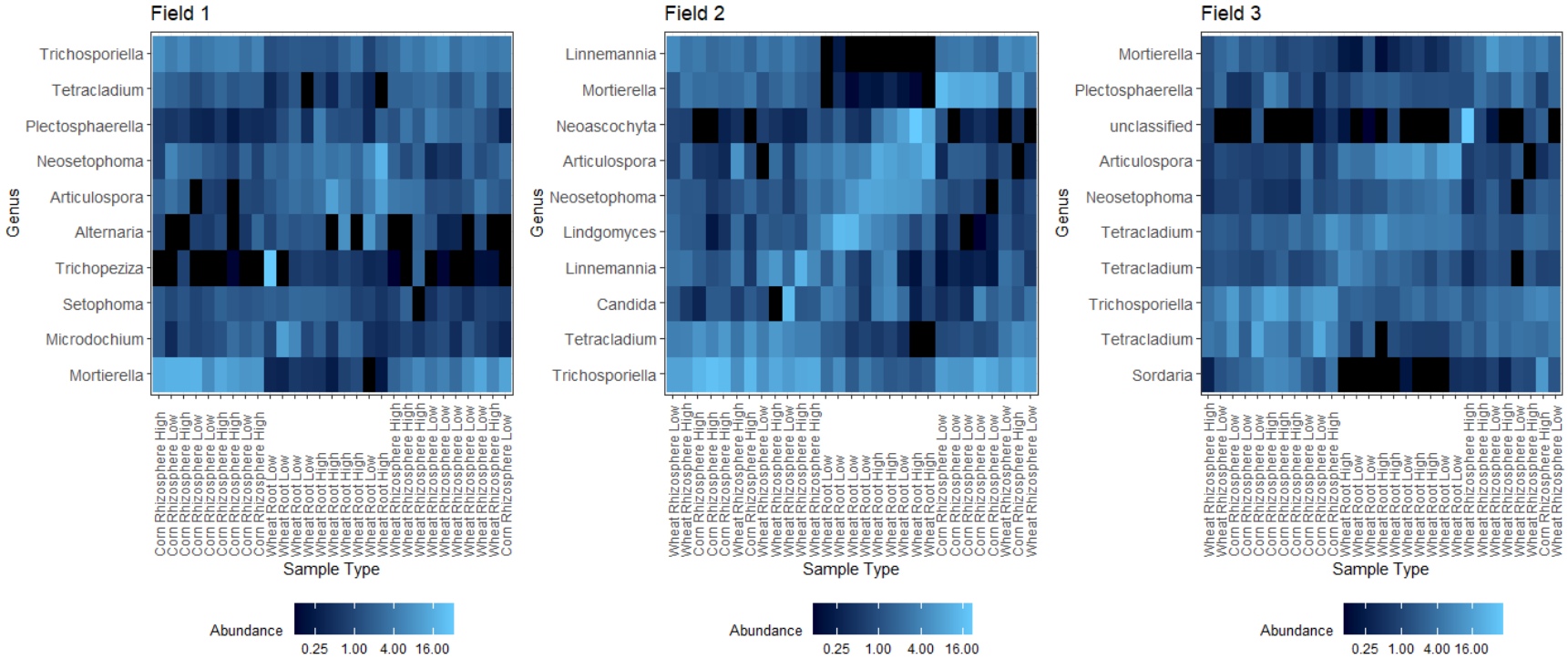


Figure 3.7: Heatmaps of the top 10 ASVs found in corn rhizosphere soil, wheat rhizosphere soil and, wheat root samples amplified with ITS2 primers.

For each field, a heatmap of the top 10 most abundant ASVs was produced, with samples grouped by sample type. NMDS ordination on the Bray-Curtis distances of sample types was used to organize rows and columns. Colour indicates relative abundance.

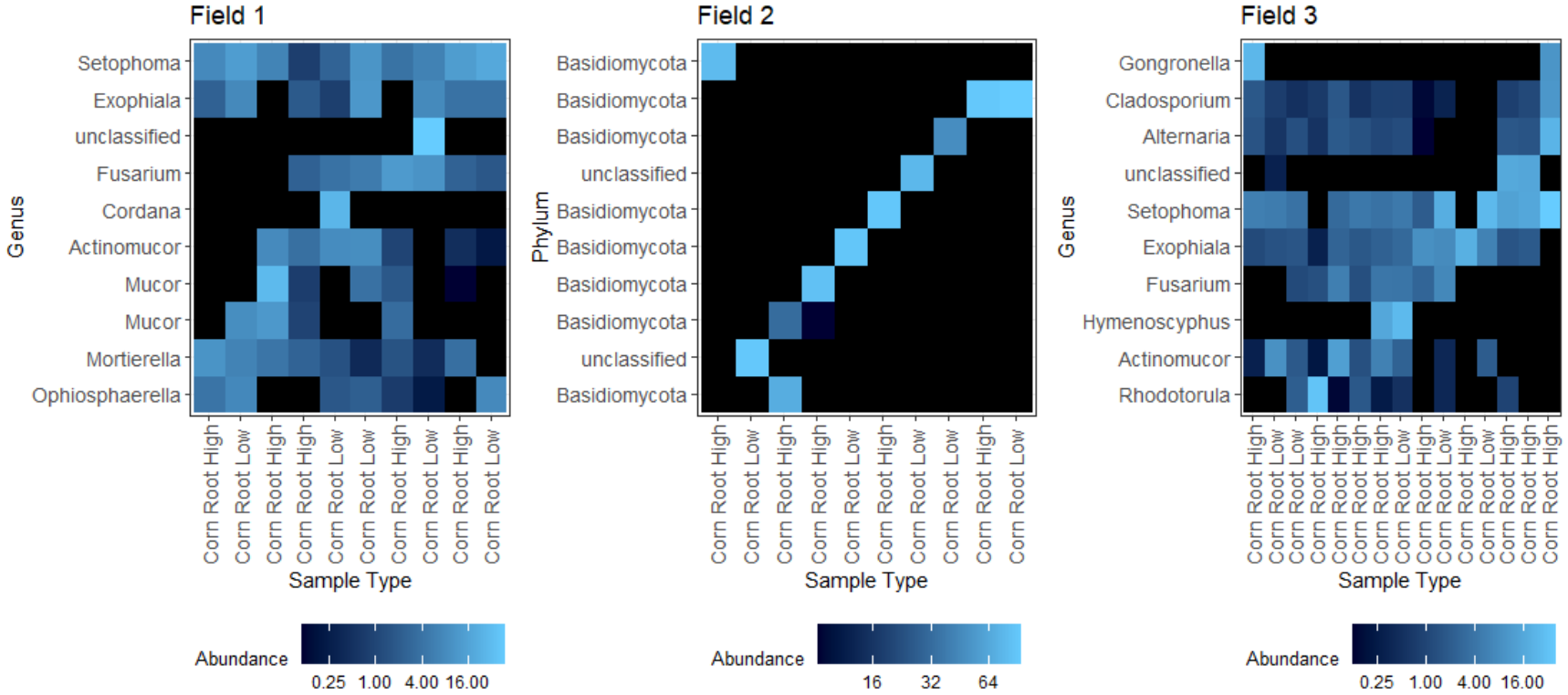


Figure 3.8: Heatmaps of the top 10 ASVs found in corn root samples amplified with ITS2 primers.

For each field, a heatmap of the top 10 most abundant ASVs was produced, with samples grouped by sample type. NMDS ordination on the Bray-Curtis distances of sample types was used to organize rows and columns. Colour indicates relative abundance.

Similar trends to the ITS2 data were observed in data obtained with LSUA primers. In all fields, ASVs belonging to the genus *Tricellula* and several ASVs belonging to the genus *Tetracladium* were shared in corn and wheat rhizosphere soil and wheat root samples among all three fields (Fig. 3.9). *Tricellula* was most abundant in wheat root samples, and some ASVs identified as *Tetracladium* were more abundant in corn and wheat rhizosphere soil samples, while others showed similar abundance in all three sample types (Fig. 3.9). Samples clustering occurred based on crop and type (rhizosphere soil or root) for the most part, with minimal clustering based on yield site (Fig. 3.9) In general, abundance of the top 10 ASVs did not differ much between high and low yield samples from the same crop and type (rhizosphere soil or root) and in most cases, abundance of ASVs was higher in either corn and wheat rhizosphere soil samples or wheat root samples (Fig. 3.9). For the corn root samples, ASVs belonging to the genera Fusarium, *Exophiala* and *Setophoma* were highly abundant in all fields (Fig. 3.10). There was some clustering of corn root samples from the same yield sites in field 3 (Fig. 3.10). However, no clear trends were observed (Fig. 3.10).

In samples amplified with LSUBG primers, ASVs identified as belonging to the genera *Linnemannia*, *Mortierella*, *Tausonia*, and *Mrakia* were highly abundant in all three fields (Fig. 3.11). In contrast to the ITS2 and LSUA heatmaps, most of the top 10 ASVs were either more abundant in corn rhizosphere or in wheat rhizosphere samples, as opposed to consistency in abundance between the two crops. This was clearly demonstrated through the separation of corn and wheat samples in all three fields (Fig. 11). The LSUBG heatmaps demonstrated some clear separation of corn rhizosphere samples from high and low yield sites, differentiated by their abundance of ASVs belonging to genera such as *Tausonia* and *Mortierella* (Fig. 11).

There was some overlap in the top 10 most abundant species identified with each primer set. These include *Tetracladium, Mortierella, Linnemannia, Alternaria* and *Plectosphaerella* in the corn and wheat rhizosphere soil and wheat root samples, as well as *Fusarium, Exophiala* and *Setophoma* in the corn root samples.

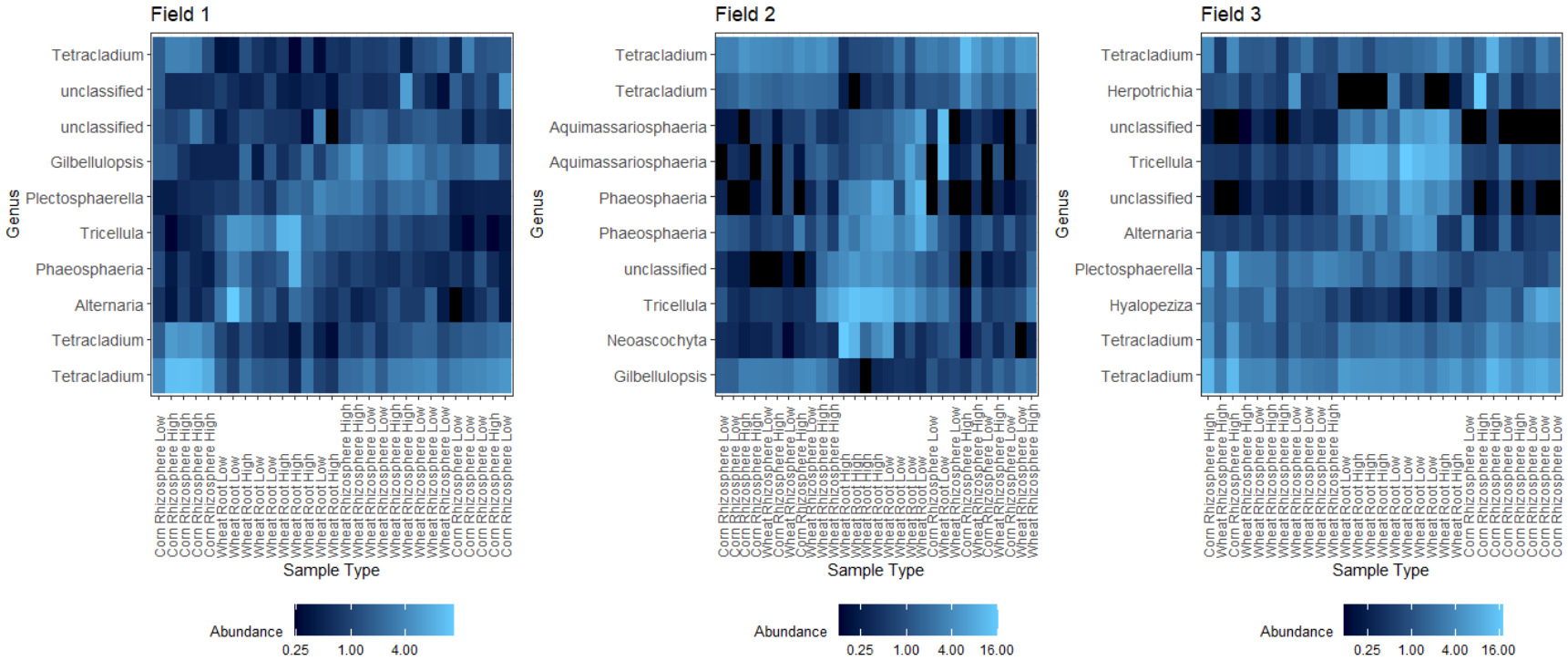


Figure 3.9: Heatmaps of the top 10 ASVs found in corn rhizosphere soil, wheat rhizosphere soil and, wheat root samples amplified with LSUA primers.

For each field, a heatmap of the top 10 most abundant ASVs was produced, with samples grouped by sample type. NMDS ordination on the Bray-Curtis distances of sample types was used to organize rows and columns. Colour indicates relative abundance.

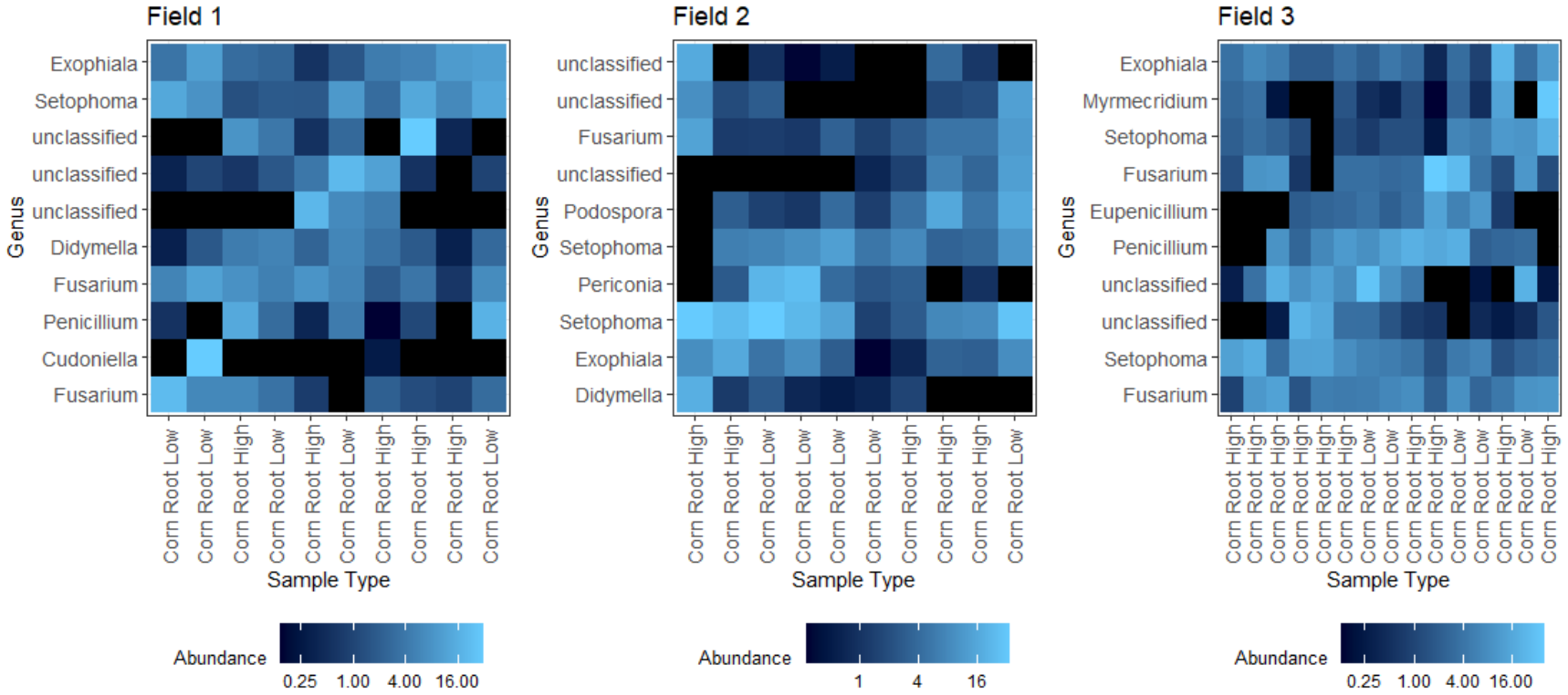


Figure 3.10: Heatmaps of the top 10 ASVs found in corn root samples amplified with LSUA primers.

For each field, a heatmap of the top 10 most abundant ASVs was produced, with samples grouped by sample type. NMDS ordination on the Bray-Curtis distances of sample types was used to organize rows and columns. Colour indicates relative abundance.

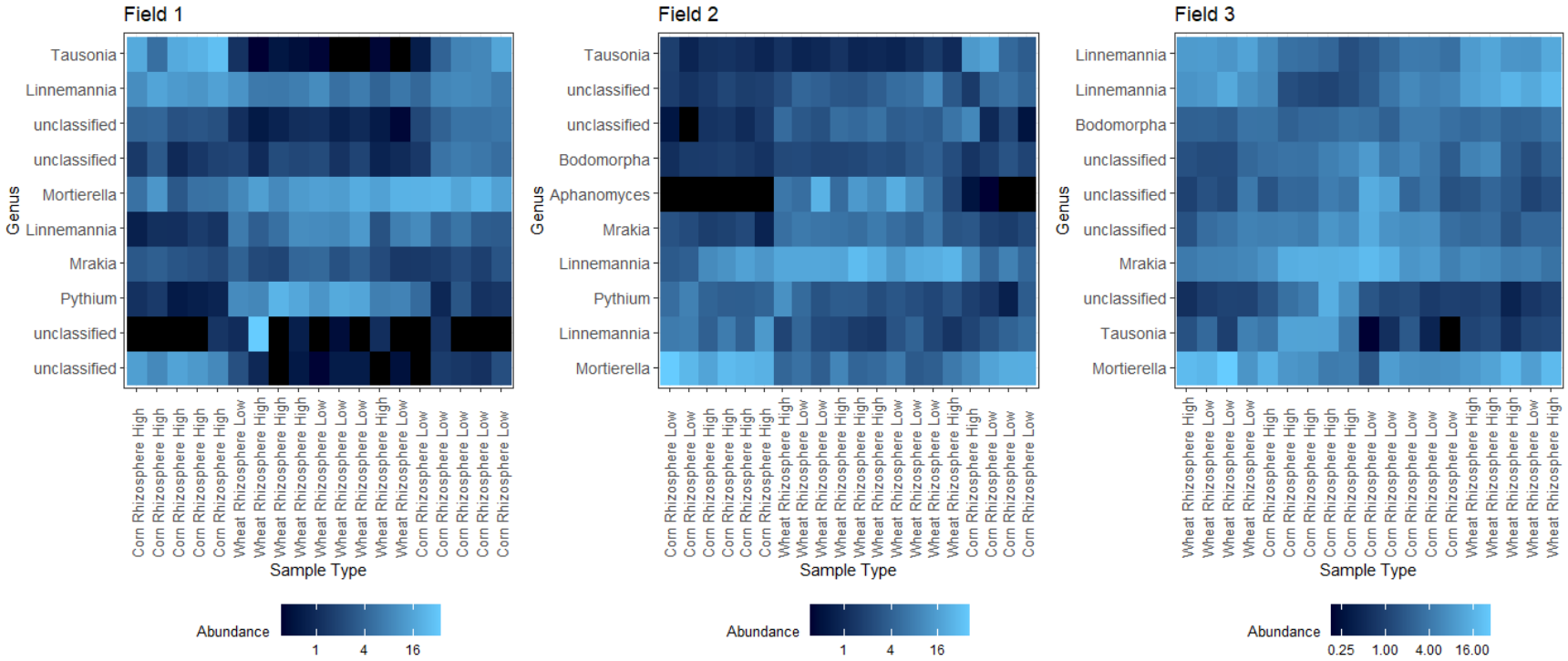


Figure 3.11: Heatmaps of the top 10 ASVs found in corn and wheat rhizosphere soil samples amplified with LSUBG primers. For each field, a heatmap of the top 10 most abundant ASVs was produced, with samples grouped by sample type. NMDS ordination on the Bray-Curtis distances of sample types was used to organize rows and columns. Colour indicates relative abundance.

Several analyses of indicator species were performed in “indicspecies” using various groupings to identify the ASVs that were the most significantly associated with each field as well as with corn, wheat, rhizosphere soil and root samples within each field (Appendix D, E, F, G, H). Analyses were also performed to identify the ASVs that were the most significantly associated with each sample type (corn rhizosphere soil, wheat rhizosphere soil, corn root and wheat root) within each field (Appendix D, E, F, G, H). The *P* value chosen for all analyses of field, crop and sample type for ITS2 and LSUA data was 0.01, as there were a large number of ASVs that were significant at $p<0.05$. With LUSBG data, since there were fewer ASVs to begin with, a *P* value of 0.05 was used as it yielded a reasonable amount of ASVs for each analysis. For the analysis of ITS2 data, 61 ASVs were significantly associated with one or more fields, 63 with corn and/or wheat, 83 with rhizosphere soil and/or root samples, and 285 with one or more sample types (Appendix D, E). For the analysis of LSUA data, 28 ASVs were significantly associated with one or more fields, 90 with corn and/or wheat, 147 with rhizosphere soil and/or root samples, and 315 with one or more sample types (Appendix F, G). And for analysis of LSUBG data, which included only rhizosphere soil samples, 26 ASVs were associated with one or more fields and 39 with corn and/or wheat (Appendix H). In the final ITS2 and LSUA tables, in an effort to condense the lists of ASVs that were significantly associated with one or more sample types, ASVs were grouped together into rows on the tables based on patterns of correlation with sample type and phylogenetic similarity (Appendix D, E, F, G). The similarity was determined using MAFFT sequence alignment and neighbour joining trees made using BioNJ based on Blastn analysis. The ASVs that were significantly associated with one field over the others, and those that were associated with one sample type (corn rhizosphere soil, corn root, wheat rhizosphere soil or wheat root) from a particular field were summarized (Table 3.6).

Table 3.6: Fungal, oomycete and cercozoan ASVs driving community composition differences between three fields.

Indicator species analysis was performed using the indicspecies package in R, and species that were uniquely significantly associated with a field or sample type were recorded. Species identified using ITS2 data = blue, LSUA = green and LSUBG = red. For ITS2 and LSUA data, $p<0.01$ was used, and $p<0.05$ was used for LSUBG data. Species detected in both roots and rhizosphere of one crop within a field or by two primer sets in the same sample type within a field are indicated in bold.

Field 1	**F1 Corn Rhizosphere**	**F1 Corn Root**	**F1 Wheat Rhizosphere**	**F1 Wheat Root**
Podospora	*Peziza*	*Actinomucor elegans*	*Cordana*	*Dioszegia hungarica*
Ramophialophora petraea	*Chysosporium*	*Microdochium*	*Macrophomina phaseolina*	*Periconia macrospinosa*
Rozellomycota	*Pseudomerdarium*	*Rhodotorula graminis*	*Marquandomyces marquandii*	*Setophoma terrestris*
Hymenoscyphus aurantiacus	*Niesslia aurantiaca*	*Acremonium verruculosum*	*Aquanectria submersa*	*Murispora kazachstanica*
	Tausonia pullulans	*Equiseticola fusispora*	*Coprotus ochraeus*	***Pythium biforme***
	Thelebolus	*Fusarium irregulare*	*Globisporangium macrosporum*	*Hyalorbilia spermatophaga*
	Solicoccozyma terrea	*Nectria*	*Neoconiothyrium viticola*	
	Tausonia pullulans	*Staphylotrichum coccosporum*	*Roesleria subterranea*	
			Rhizophlyctis rosea	
			Pythium biforme	
			Staurastromyces oculus	
Field 2	**F2 Corn Rhizosphere**	**F2 Corn Root**	**F2 Wheat Rhizosphere**	**F2 Wheat Root**
Conlarium sacchari	*Berkeleyomyces rouxiae*	*Thelebolus globosus*	*Conichaeta*	*Clohesyomyces aquaticus*
Fusarium verticillioides	*Cyathicula amenti*		*Jennwenomyces navicularis*	*Massariosphaeria*
Cryptococcus watticus	*Paramicrosphaeropsis ellipsoidea*		*Sporidesmiella pini*	*Paraophiobolus plantaginis*
Lachnum	*Pyrenochaetopsis microsporae*		*Aphanomyces cladogamus*	
Linnemannia hyalina	*Pyrenophora sieglingiae*		*Globisporangium viniferum*	
Mortierella fluviae	*Geranomyces tanneri*		*Leucosporidium drummii*	
Solicoccozyma terricola	*Mortierella alpina*			
Fusarium continuum	*Linnemannia exigua*			
Field 3	**F3 Corn Rhizosphere**	**F3 Corn Root**	**F3 Wheat Rhizosphere**	**F3 Wheat Root**
Melanommataceae	*Coniochaeta*	*Cercophora*	*Acremonium rutilum*	*Hymenoscyphus aurantiacus*
Olpidium brassicae	*Coniothyrium*	*Hypocreaceae*	*Ascobolus*	*Sporidesmiella hyalosperma*
Bodomorpha	*Lasiobolidium orbiculoides*	*Penicillium ochrochloron*	*Boubovia luteola*	*Volucrispora aurantiaca*
Psathyrella impexa	*Niesslia exosporioides*	*Sarocladium junci*	*Cadophora gregata*	
	Talaromyces aculeatus	*Trichoderma koningii*	***Linnemannia gamsii***	
	Talaromyces ucrainicus		*Natantispora*	
			Scolecobasidiella	
			Actinomortierella capitata	
			Globisosporangium parvum	
			Linnemannia gamsii	

3.3 Crop yield and community composition

Yield groupings made based on historical data on each field and NDVI maps of the fields were used to identify differences in community composition in high and low yield sites. An ANOSIM analysis was used to evaluate whether significant differences existed in any of the fields between high and low yield sites of different sample groupings (Table 3.7).

Table 3.7: Differences in fungal community composition of high and low yield sites of three fields.

An ANOSIM test of the ranked dissimilarity matrix (Bray-Curtis Dissimilarity indices) was used to determine whether significant differences existed in the fungal community composition of corn, wheat, rhizosphere and root groupings between high and low yield sites. The ANOSIM statistic R compares the mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups. Significant differences in fungal community composition between high and low yield sites within each grouping in the same field (p<0.05) are indicated with an asterisk (*) and green shading.

Primer	Field		Grouping	ANOSIM statistic R	*P* value
ITS2	Field 1	Corn	Rhizosphere and Root	-0.014	0.40
		Wheat	Rhizosphere and Root	0.064	0.15
		Rhizosphere	Corn and Wheat	0.171	0.02*
		Root	Corn and Wheat	-0.040	0.69
	Field 2	Corn	Rhizosphere and Root	0.032	0.20
		Wheat	Rhizosphere and Root	0.119	0.08
		Rhizosphere	Corn and Wheat	0.439	0.0004*
		Root	Corn and Wheat	-0.002	0.44
	Field 3	Corn	Rhizosphere and Root	-0.035	0.72
		Wheat	Rhizosphere and Root	0.057	0.17
		Rhizosphere	Corn and Wheat	0.256	0.006*
		Root	Corn and Wheat	-0.037	0.75
LSUA	Field 1	Corn	Rhizosphere and Root	-0.022	0.52
		Wheat	Rhizosphere and Root	0.077	0.13
		Rhizosphere	Corn and Wheat	0.095	0.13
		Root	Corn and Wheat	-0.040	0.67
	Field 2	Corn	Rhizosphere and Root	-0.035	0.63
		Wheat	Rhizosphere and Root	0.098	0.11
		Rhizosphere	Corn and Wheat	0.102	0.10
		Root	Corn and Wheat	-0.028	0.49
	Field 3	Corn	Rhizosphere and Root	-0.024	0.57
		Wheat	Rhizosphere and Root	0.008	0.34
		Rhizosphere	Corn and Wheat	0.204	0.01*
		Root	Corn and Wheat	-0.032	0.68
LSUBG	Field 1	Corn	Rhizosphere	0.092	0.0076*
		Wheat	Rhizosphere	0.381	0.016*
	Field 2	Corn	Rhizosphere	0.428	0.0078*
		Wheat	Rhizosphere	0.444	0.023*
	Field 3	Corn	Rhizosphere	0.376	0.0077*
		Wheat	Rhizosphere	0.238	0.095

An indicspecies analysis was then performed with various groupings to identify ASVs that were significantly associated ($p<0.05$) with high and/or low yield sites within each grouping. The indicspecies analysis for ITS2 data revealed 13 ASVs that were significantly associated with high or low yield sites from grouping of samples belonging to rhizosphere soil (Fig. 3.12). Of these 13 ASVs, only three were significantly associated with low yield sites: *Leptodontidium camptobactrum*, *Soloacrosporiella* and *Tetracladium* sp. in field 2 rhizosphere soil samples (Fig. 3.12). The rest were all significantly associated with high yield sites in rhizosphere soil samples from their respective field (Fig. 3.12). The indicspecies analysis of various groupings of LSUA data yielded only two ASVs that were significantly associated with yield (Fig. 3.13). An ASV belonging to the family Helotiaceae was significantly associated with low yield sites when all field 2 samples were grouped, and *Penicillium citreonigrum* was significantly associated with high yield sites when field 2 rhizosphere soil samples were grouped (Fig. 3.13). All other field groupings from ITS2 and LSUA as well as all groupings from LSUBG did not yield any ASVs that were significantly associated with high or low yield sites.

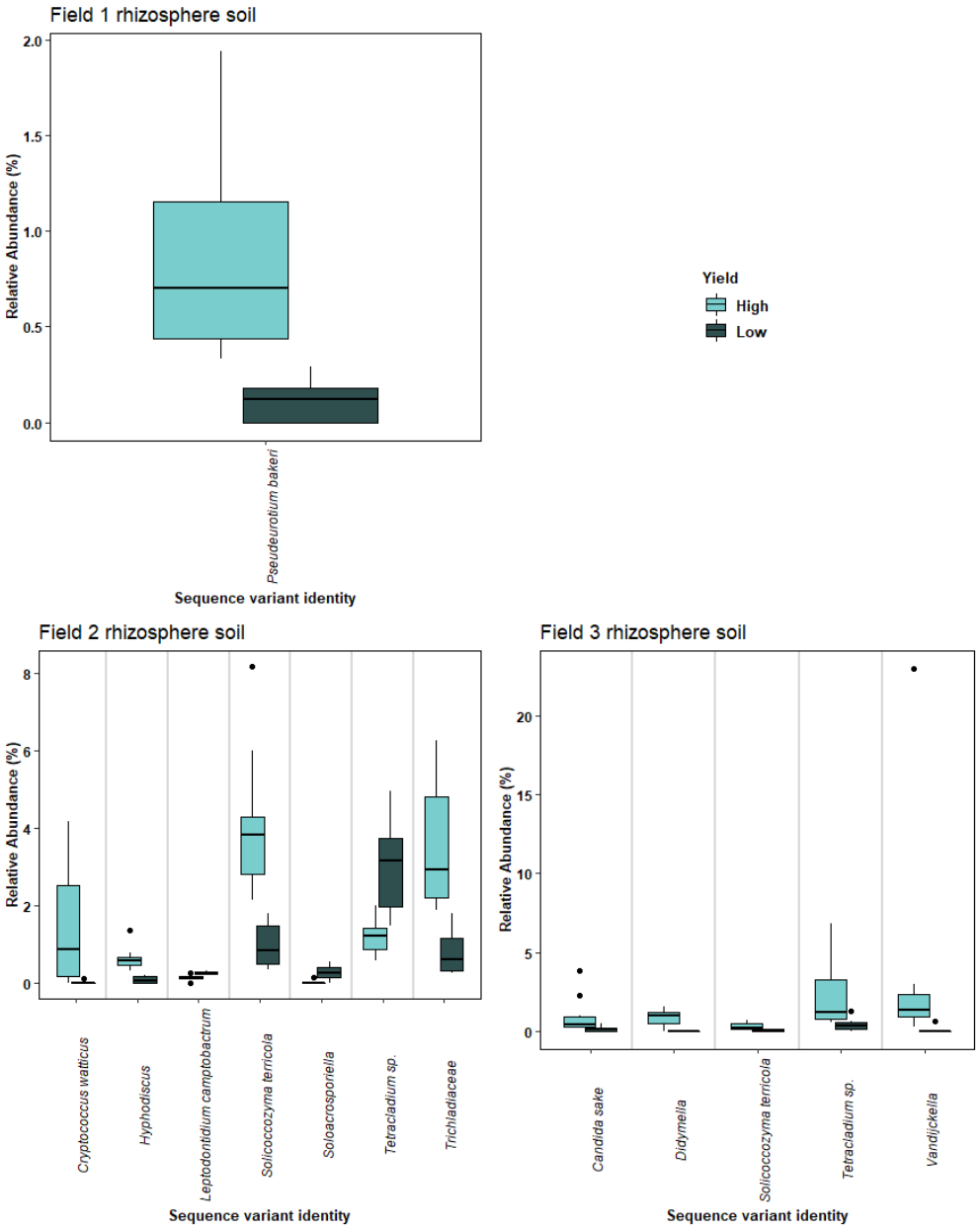


Figure 3.12: Boxplots of relative abundance of ASVs associated with high or low yield sites from rhizosphere soil samples amplified with ITS2 primers.

Relative abundance of ASVs significantly ($p<0.05$) associated with high or low yielding sites based on indicator species analysis using the indicspecies package in R. The midline indicates the median and the upper and lower half of the box represents the upper and lower quartile, respectively. Colours indicate fields. Whisker lines indicate the minimum and maximum values. Black dots indicate outliers. ASVs are arranged alphabetically by genus.

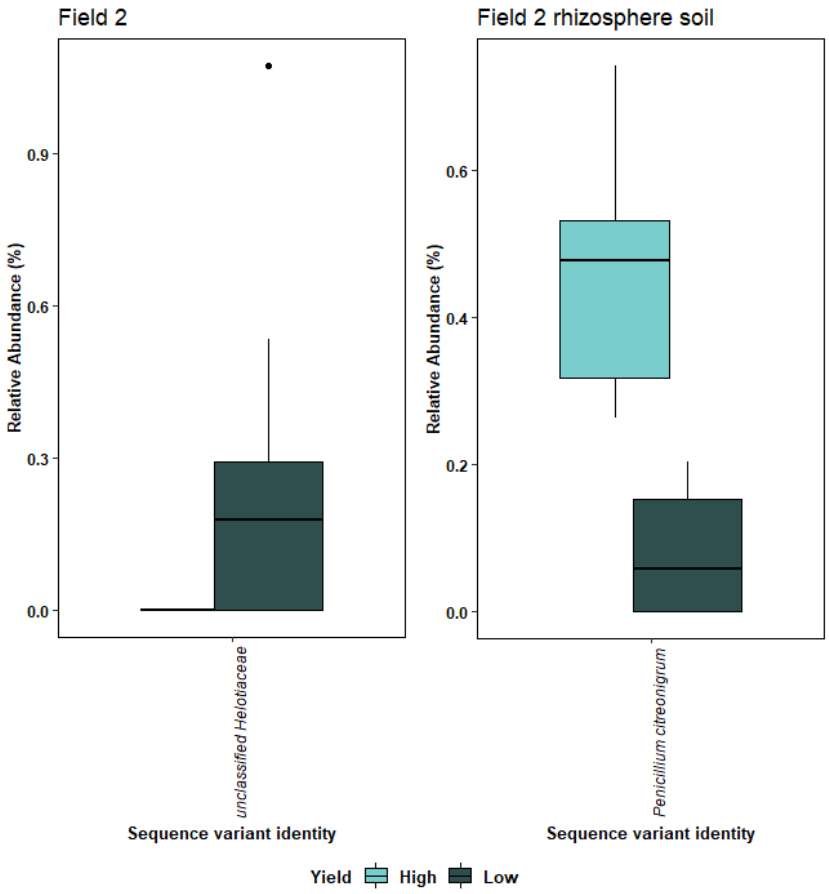


Figure 3.13: Boxplots of relative abundance of ASVs associated with high or low yield sites from field 2 samples amplified with LSUA primers.

Relative abundance of ASVs significantly ($p<0.05$) associated with high or low yielding sites based on indicator species analysis using the indicspecies package in R. The midline indicates the median and the upper and lower half of the box represents the upper and lower quartile, respectively. Colours indicate fields. Whisker lines indicate the minimum and maximum values. Black dots indicate outliers. ASVs are arranged alphabetically by genus.

Actual yield of corn and wheat crops measured with a combine harvester was used in Mantel tests to identify correlation between community composition of samples from each field and yield at harvest (Table 3.8). For ITS2 data, community composition of field 1 corn rhizosphere soil, field 2 wheat rhizosphere soil and field 3 wheat root samples were significantly correlated with yield (Table 3.8). For LSUA data, community composition of field 1 corn rhizosphere soil and field 2 root samples were significantly correlated with yield (Table 3.8). For LSUBG data, community composition of field 1 corn rhizosphere soil and field 3 corn rhizosphere soil samples were significantly correlated with yield (Table 3.8).

Table 3.8: Correlation between yield and fungal community composition of corn and wheat rhizosphere and root samples.

Mantel tests using ranked dissimilarity matrix (Bray-Curtis Dissimilarity indices) for ASV abundance data, Euclidean Distance for combine harvester yield data and Spearman's rank correlation were performed used to determine whether there was significant correlation between community composition of corn, wheat, rhizosphere and root and yield. Significance (p<0.05) is indicated with an asterisk (*) and green shading.

Primer	Field	Sample grouping	**R statistic**	***P* value**
ITS2	Field 1	Corn Rhizosphere	0.55	0.0031*
		Wheat Rhizosphere	0.12	0.22
		Corn Root	-0.04	0.62
		Wheat Root	0.19	0.039
	Field 2	Corn Rhizosphere	-0.04	0.49
		Wheat Rhizosphere	0.33	0.03*
		Corn Root	0.09	0.34
		Wheat Root	0.21	0.096
	Field 3	Corn Rhizosphere	0.26	0.052
		Wheat Rhizosphere	0.19	0.074
		Corn Root	-0.03	0.60
		Wheat Root	0.27	0.041*
LSUA	Field 1	Corn Rhizosphere	0.04	0.016*
		Wheat Rhizosphere	-0.15	0.81
		Corn Root	-0.01	0.52
		Wheat Root	0.20	0.054
	Field 2	Corn Rhizosphere	0.04	0.42
		Wheat Rhizosphere	0.14	0.23
		Corn Root	-0.08	0.84
		Wheat Root	0.39	0.049*
	Field 3	Corn Rhizosphere	0.10	0.23
		Wheat Rhizosphere	0.07	0.28
		Corn Root	0.02	0.34
		Wheat Root	0.18	0.12
LSUBG	Field 1	Corn Rhizosphere	0.32	0.032*
		Wheat Rhizosphere	0.06	0.33
	Field 2	Corn Rhizosphere	-0.19	0.79
		Wheat Rhizosphere	0.24	0.069
	Field 3	Corn Rhizosphere	0.35	0.014*
		Wheat Rhizosphere	0.17	0.15

3.4 Rhizosphere soil physicochemical analysis

Soil metadata, including 28 soil variables from rhizosphere soil (provided by A&L soil testing lab), and yield data (provided by A&L from combine-harvesting) were analyzed using NMDS envfit analysis to assess the significances of correlations between these variables and fungal community composition of rhizosphere soil samples from the sites.

For all three fields, the NMDS analysis for ITS2 data revealed significant correlations of many of the measured variables with community composition when pooling rhizosphere soil samples from high yielding and low yielding sites of both crops (Fig. 3.14, Fig. 3.15, Fig. 3.16). Of the 28 physicochemical variables used in the analyses, 21 were correlated with community composition in field 1, 23 in field 2 and 15 in field 3. Corn yield was significantly correlated (p>0.05) with community composition in all three fields, and its vector line in all three fields showed a strong correlation with corn sample types (Fig. 3.14, Fig. 3.15, Fig. 3.16). Wheat yield was only significantly correlated (p>0.05) with fungal community composition in field 2 and field 3 and did not show a strong correlation with wheat rhizosphere samples (Fig. 3.14, Fig. 3.15, Fig. 3.16). In the NMDS plots for ITS2 datasets for all three fields, corn yield, K ppm, % Mg, % Ca, % Na, Mn ppm, NO_3-N ppm, and clay % were significantly correlated (p>0.05) with community composition of samples, and ENR was not significantly correlated (p<0.05) with community composition (Fig. 3.14, Fig. 3.15, Fig. 3.16). In field 3, there were fewer soil physicochemical variables that were significantly correlated with community composition than in field 1 and 2. Some of the soil variables that showed significant correlations in field 1 and 2 but not in field 3 were P ppm (Bray-P1), Na ppm, pH, Zn ppm, Fe ppm, B ppm, and Cl ppm (Fig. 3.14, Fig. 3.15, Fig. 3.16). In the NMDS plots for ITS2 data, samples clustered by yield sampling site, with most samples contained within the 95% confidence interval ellipses (Fig. 3.14, Fig. 3.15, Fig. 3.16).

The NMDS analysis for LSUA data revealed significant correlations of 19 soil physicochemical variables with community composition in field 1, 20 in field 2 and 21 in field 3 (Fig. 3.17, Fig. 3.18, Fig. 3.19). In all three fields, corn yield and wheat yield were significantly correlated (p>0.05) with fungal community composition, and these vectors showed strong correlations with their respective sample types in the NMDS plots (Fig. 3.17, Fig. 3.18, Fig. 3.19). The community composition of samples in the LSUA dataset from all three fields was significantly correlated (p>0.05) with Mg ppm, Ca ppm, CEC meq/100 g, S ppm, Mn ppm, Fe ppm, and soil moisture (Fig. 3.17, Fig. 3.18, Fig. 3.19). Percent sand composition of rhizosphere soil was not significantly correlated with community composition of samples in any of the fields (p<0.05). Samples from all three fields amplified with LSUA primers clustered by sampling sites, with most samples

contained within the 95% confidence interval ellipses, with some overlap between high and low yielding wheat sites in field 3 (Fig. 3.17, Fig. 3.18, Fig. 3.19).

The analysis of the LSUBG dataset revealed significant correlations of 21 soil physicochemical variables with community composition in field 1, 20 in field 2, and 12 in field 3 (Fig. 3.20, Fig. 3.21, Fig. 3.22). Corn yield was significantly correlated (p>0.05) with community composition in all three fields, and its vector line in all three fields showed a strong correlation with corn sample types (Fig. 3.20, Fig. 3.21, Fig. 3.22). Wheat yield was only significantly correlated (p>0.05) with fungal community composition in field 2 and field 3 and did not show a strong correlation with wheat rhizosphere samples (Fig. 3.20, Fig. 3.21, Fig. 3.22). The soil physiochemical variables that were significantly correlated (p>0.05) with community composition for the LSUBG dataset in all fields were S ppm, Mn ppm, Fe ppm, and soil moisture (Fig. 3.20, Fig. 3.21, Fig. 3.22). Percent sand composition of rhizosphere soil was not significantly correlated with community composition of samples in any of the fields (p<0.05). In field 3, there were fewer soil physicochemical variables that were significantly correlated with community composition than in field 1 and 2. Some of the soil variables that showed significant correlations in field 1 and 2 but not in field 3 were K ppm, Mg ppm, Ca ppm, pH and CEC meq/100 g (Fig. 3.20, Fig. 3.21, Fig. 3.22). Samples from all three fields amplified with LSUBG primers clustered by sampling sites, with most samples contained within the 95% confidence interval ellipses (Fig. 3.20, Fig. 3.21, Fig. 3.22). In field 2 and 3, there was some overlap between high and low yielding sites (Fig. 3.20, Fig. 3.21, Fig. 3.22).

The analysis of data from all three primer sets when looking at each field individually revealed some trends. For field 1, corn yield, Mg ppm, pH, CEC meq/100 g, % K, % Mg, % Na, Mn ppm, Fe ppm, soluble salts ms/cm, Cl ppm, and NO_3-N ppm were significantly correlated with community composition of samples for all primer data (p>0.05) (Fig. 3.14, Fig. 3.17, Fig. 3.20). Some of these soil physicochemical variables were strongly correlated with one sample type in all three datasets, including K ppm and % K which was strongly correlated with wheat samples, Fe ppm which was strongly correlated with low yielding wheat samples, and Cl ppm which was strongly correlated with low yielding

corn samples (Fig. 3.14, Fig. 3.17, Fig. 3.20). In field 1, for several physicochemical variables, the correlation with sample types differed when looking at the ITS2 dataset compared to the LSUA and LSUBG datasets was distinctly different. For example, Mg ppm and % Mg were strongly correlated with low yielding corn samples in the ITS2 dataset and these variables were strongly correlated with low yielding wheat in the LSUA and LSUBG datasets (Fig. 3.14, Fig. 3.17, Fig. 3.20). The reverse was true for soluble salts ms/cm and NO_3-N ppm (Fig. 3.14, Fig. 3.17, Fig. 3.20). While S ppm was only significantly correlated with community composition with LSUA and LSUBG, its vector line was in close proximity to the vector for corn yield, suggesting a potential correlation between these variables (Fig. 3.17, Fig. 3.20).

For field 2, corn yield, wheat yield, P ppm (Bray-P1), K ppm, Mg ppm, Ca ppm, Na ppm, S ppm, Zn ppm, Mn ppm, Fe ppm, Cu ppm, B ppm, % silt, and % clay were significantly correlated with community composition of samples for all primer data (p>0.05) (Fig. 3.15, Fig. 3.18, Fig. 3.21). Of these soil physicochemical variables, K ppm, Mg ppm, Ca ppm, and % clay were strongly correlated with wheat samples in all three primer datasets (Fig. 3.15, Fig. 3.18, Fig. 3.21). Additionally, Zn ppm was strongly correlated with low yielding wheat samples, Fe ppm and Cu ppm were strongly correlated with high yielding corn samples, and B ppm was correlated with high yielding wheat samples (Fig. 3.15, Fig. 3.18, Fig. 3.21). For ITS2, % silt was strongly correlated with wheat samples, however, for LSUA and LSBUG, % silt was strongly correlated with corn samples (Fig. 3.15, Fig. 3.18, Fig. 3.21).

The only soil physiochemical variables that were identified as being significantly correlated with community composition of samples in all primer data for field 3 were corn yield, wheat yield, and Mn ppm (p>0.05) (Fig. 3.16, Fig. 3.19, Fig. 3.22). A strong correlation was observed between Mn ppm and wheat samples in all three primer sets (Fig. 3.16, Fig. 3.19, Fig. 3.22). Overall, corn yield and Mn ppm were the only physicochemical variables that were strongly correlated with community composition of samples from all fields and in all primer data. Field 1 and field 2 also shared K ppm and Mn ppm as being significantly correlated with community composition for all primer datasets, with a strong correlation between K ppm and wheat samples.

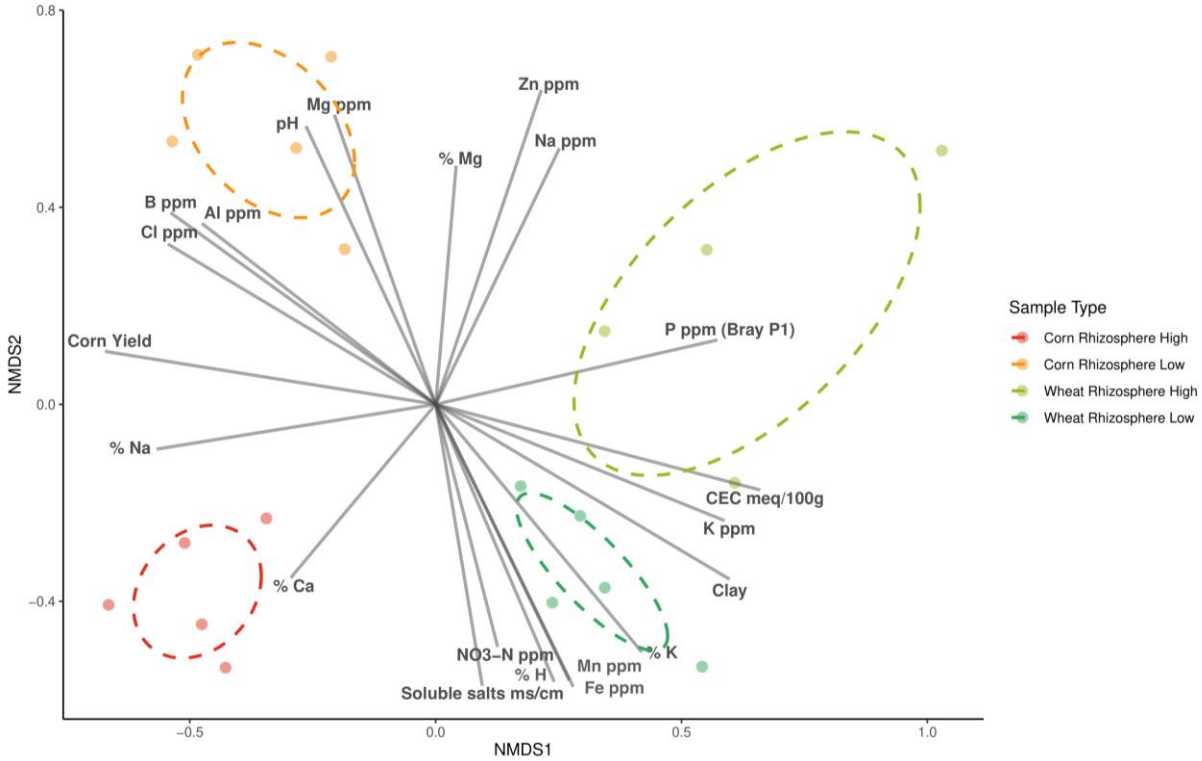


Figure 3.14: NMDS plot of rhizosphere soil samples from high and low yield sites from field 1, amplified with ITS2 primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both Mn ppm and Fe ppm are on the same vector line. Stress: 0.149.

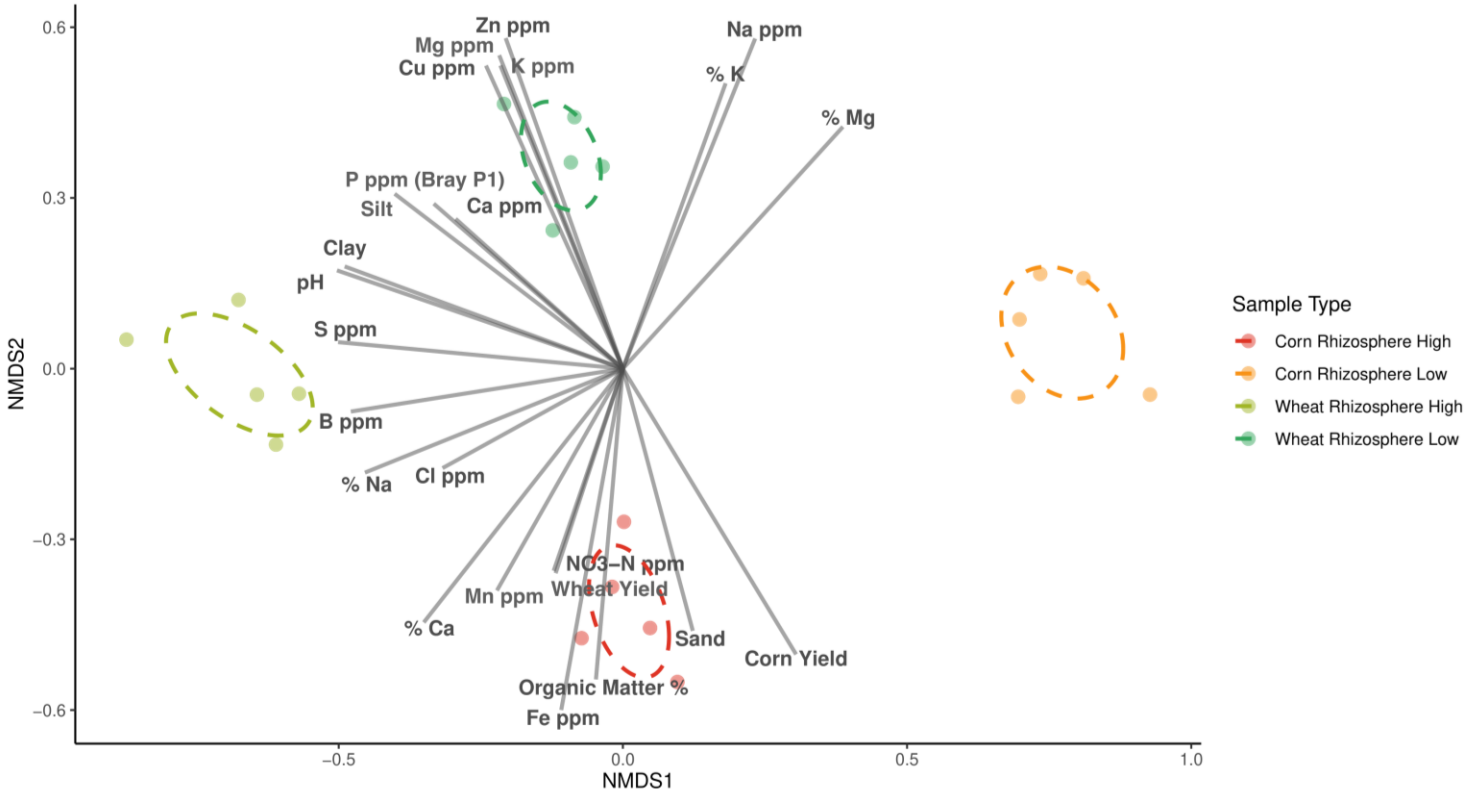


Figure 3.15: NMDS plot of rhizosphere soil samples from high and low yield sites from field 2, amplified with ITS2 primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Stress: 0.105.

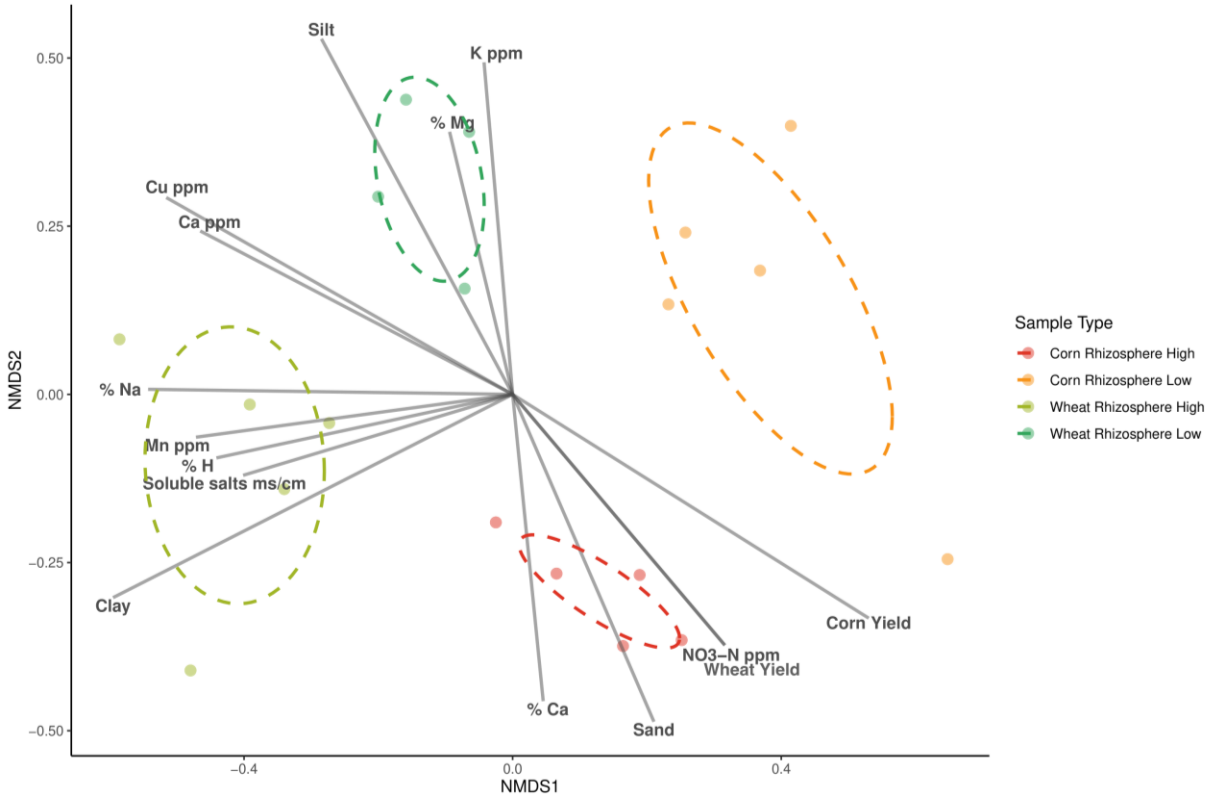


Figure 3.16: NMDS plot of rhizosphere soil samples from high and low yield sites from field 3, amplified with ITS2 primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both NO_3-N ppm and Wheat Yield are on the same vector line. Stress: 0.153.

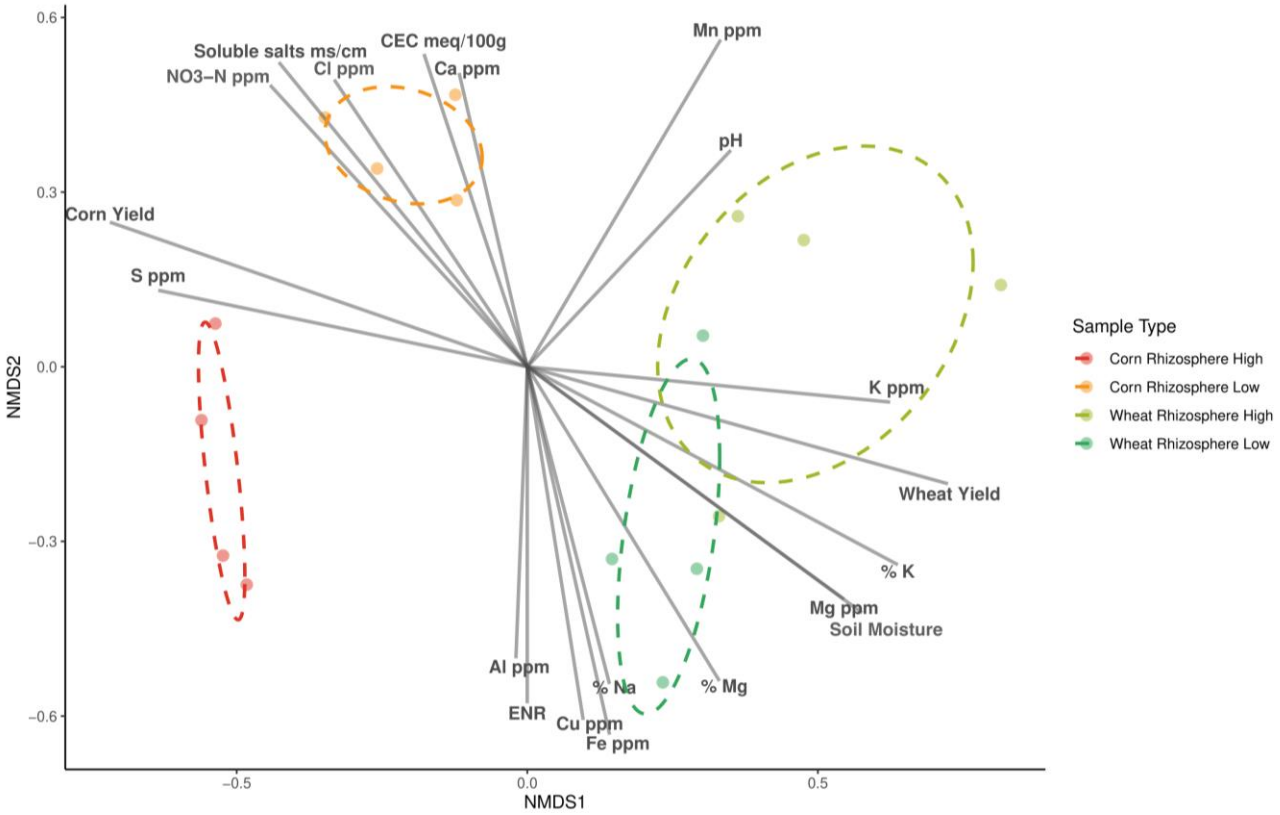


Figure 3.17: NMDS plot of rhizosphere soil samples from high and low yield sites from field 1, amplified with LSUA primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both Mg ppm and Soil Moisture are on the same vector line. Stress: 0.158.

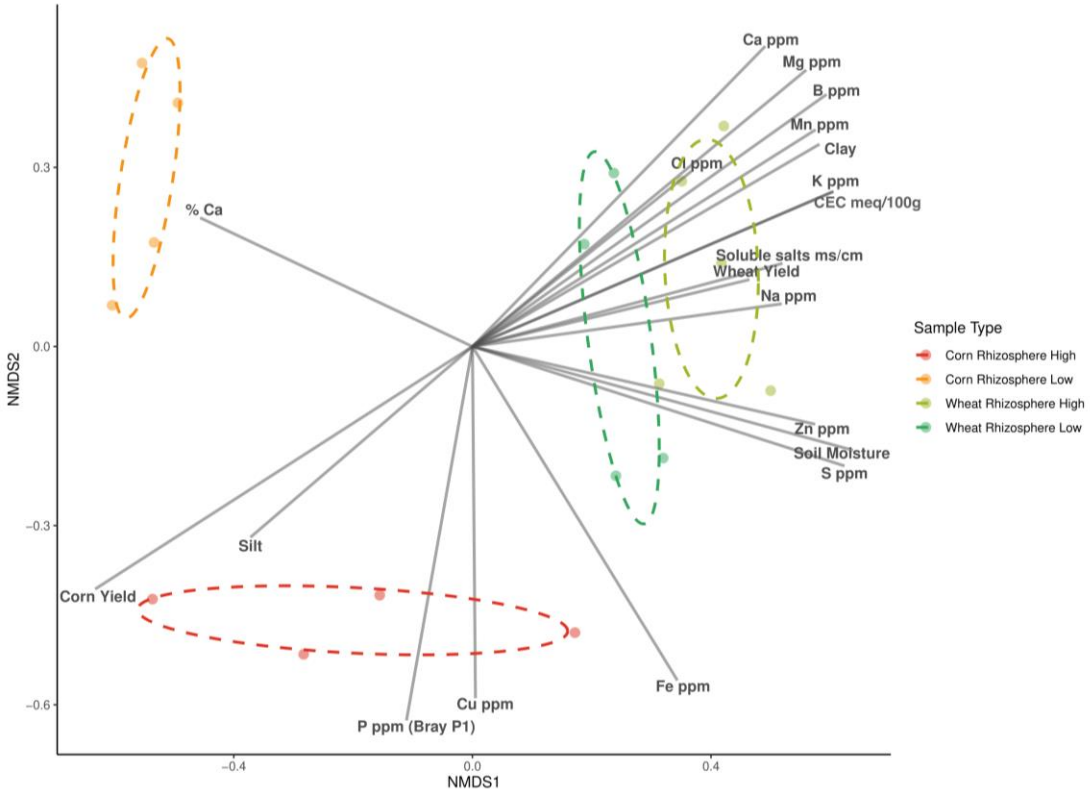


Figure 3.18: NMDS plot of rhizosphere soil samples from high and low yield sites from field 2, amplified with LSUA primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both K ppm and CEC meq/100 g are on the same vector line. Stress: 0.156.

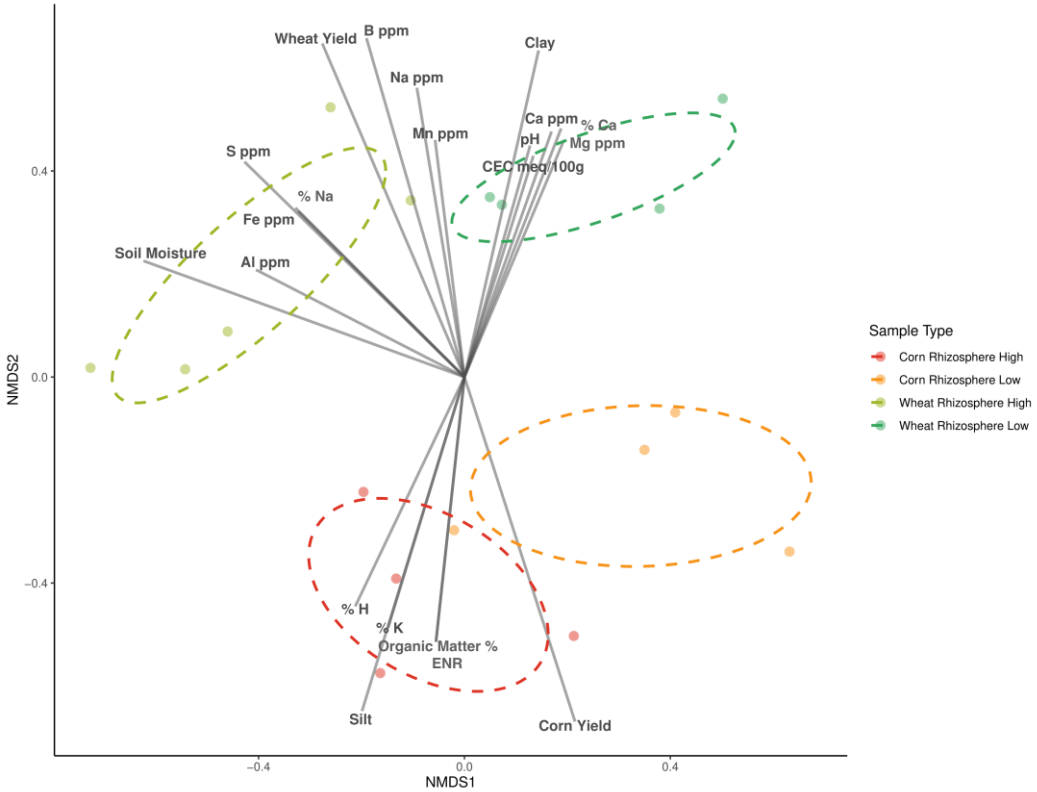


Figure 3.19: NMDS plot of rhizosphere soil samples from high and low yield sites from field 3, amplified with LSUA primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. The pairs of soil variables that are on the same vector line are Organic Matter % and ENR, Fe ppm and % Na, and % K and Silt. Stress: 0.193.

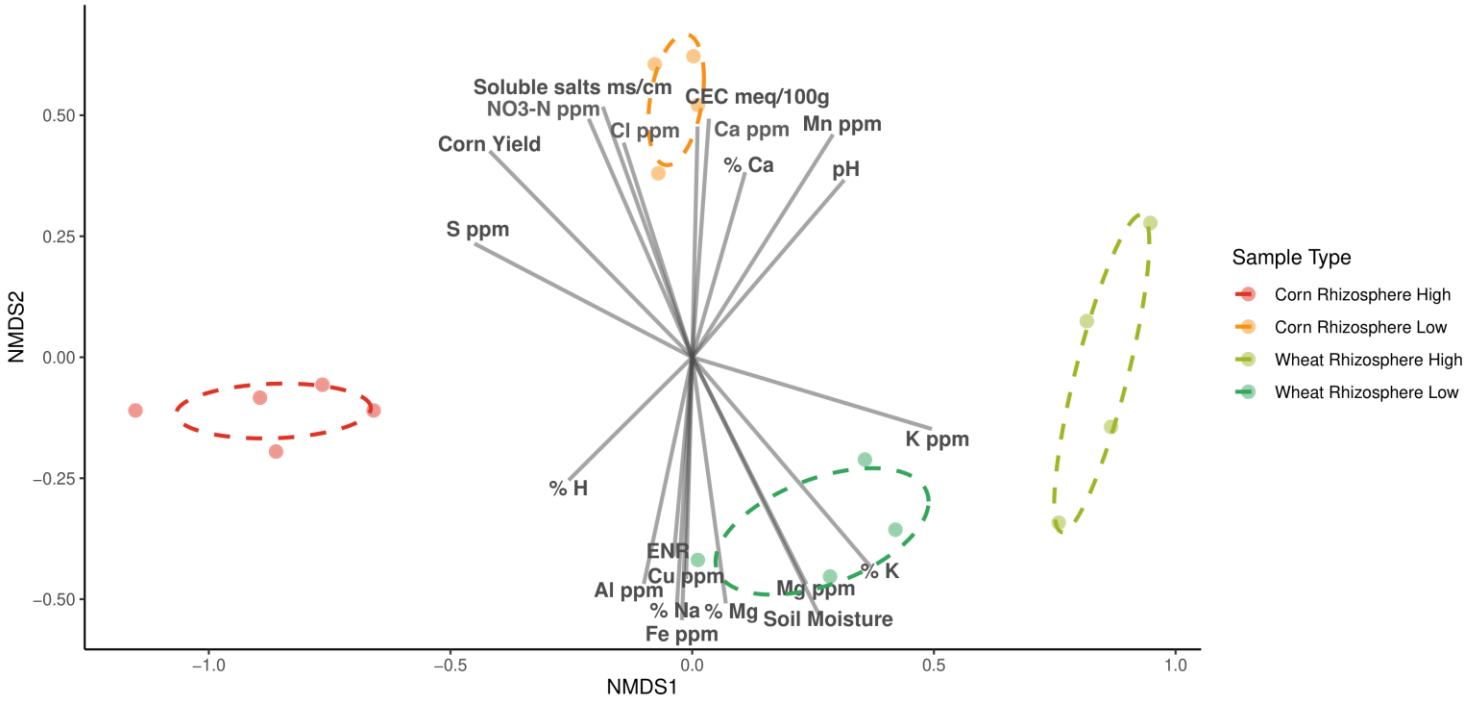


Figure 3.20: NMDS plot of rhizosphere soil samples from high and low yield sites from field 1, amplified with LSUBG primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both Mg ppm and Soil Moisture are on the same vector line. Stress: 0.083.

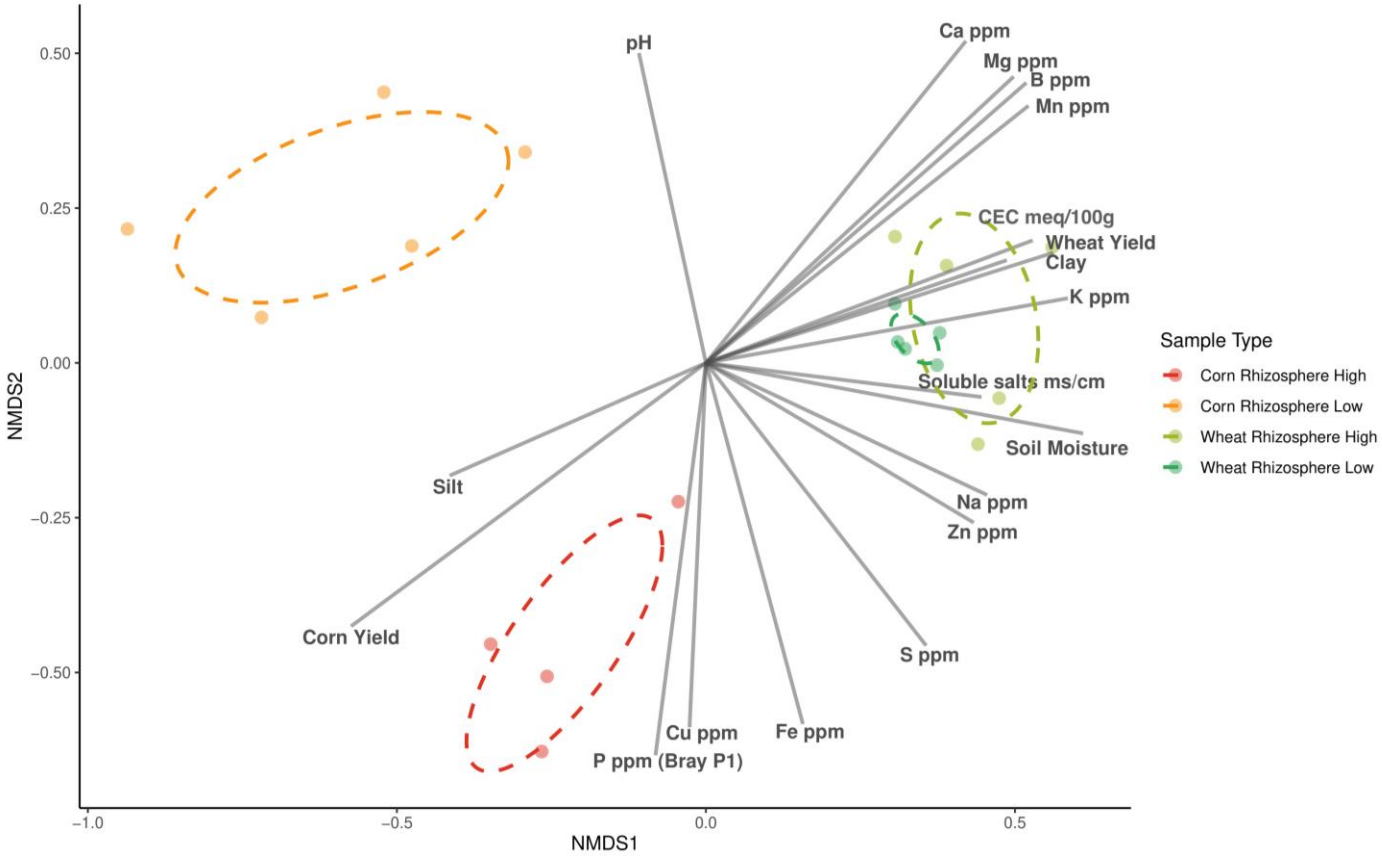


Figure 3.21: NMDS plot of rhizosphere soil samples from high and low yield sites from field 2, amplified with LSUBG primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Stress: 0.112.

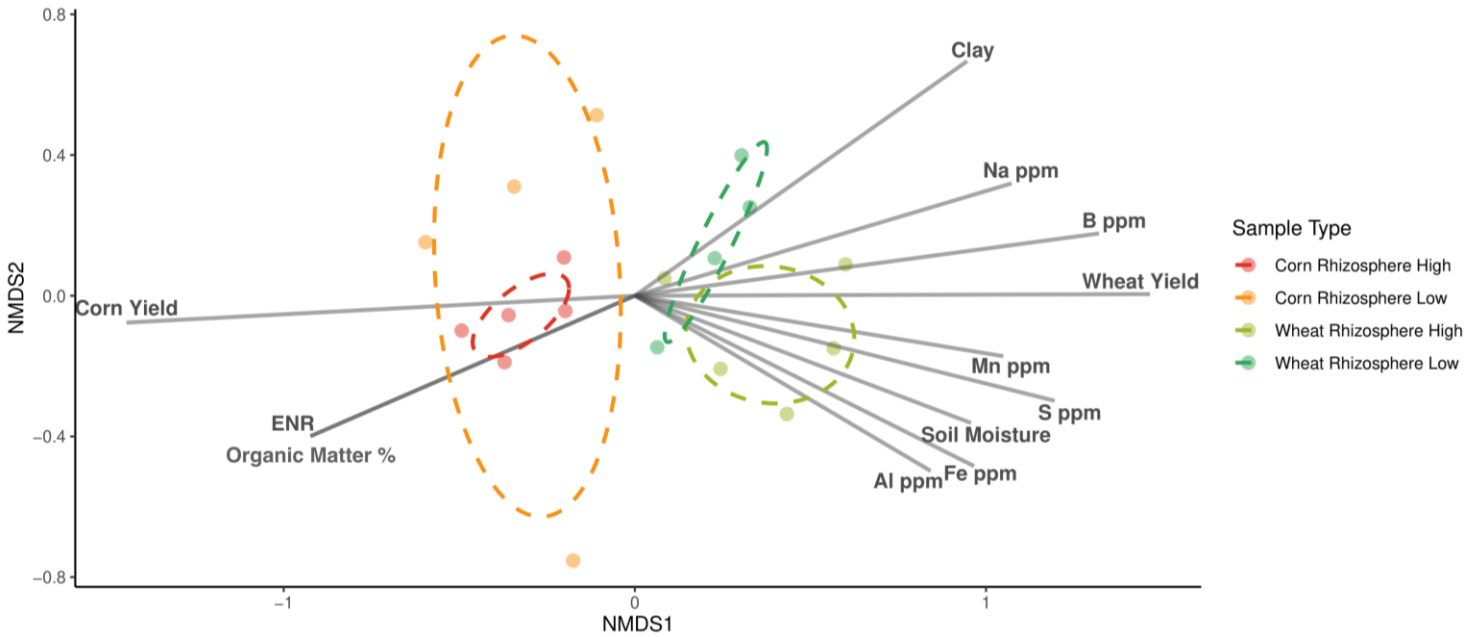


Figure 3.22: NMDS plot of rhizosphere soil samples from high and low yield sites from field 3, amplified with LSUBG primers and overlaid with key rhizosphere physicochemical factors.

Bray-Curtis Dissimilarity indices were plotted in space representing relative similarity in species composition of the fungal communities of corn and wheat rhizosphere soil of three fields. Rhizosphere environmental variables that were significantly associated with community composition of sample types ($p<0.05$) based on envfit analysis were overlaid on the NMDS plot. Colours indicate sample type. Ellipses represent 95% confidence intervals for each sample type. Lines parallel to sample types indicate a strong correlation with that sample type and longer lines indicate a stronger correlation. Both Organic Matter % and ENR are on the same vector line. Stress: 0.157.

Spearman correlation plots combining yield and rhizosphere soil physicochemical factors were used to visualize overall trends in the data (Fig. 3.23). In field 1, K ppm, Ca ppm, pH, CEC meq/100 g (cation exchange capacity), Mn ppm, and clay % were highly negatively correlated (< -0.75) with corn yield (Fig. 3.23). The variables that were highly positively correlated (> 0.75) with corn yield in field 1 were organic matter % (OM), % H, % Na, Fe ppm, Al ppm, ENR (Estimated Nitrogen Release), silt % and soil moisture (Fig. 3.23). In field 2, Mg ppm, Ca ppm, pH, Mn ppm, B ppm, and Al ppm were highly negatively correlated (< -0.75) with corn yield and Bray-P1 (P ppm), % H, Zn ppm, and Fe ppm that were highly positively correlated (> 0.75) with corn yield (Fig. 3.23). In field 3, Mg ppm, pH, and % clay were highly negatively correlated (< - 0.75) with corn yield and K ppm, % H, NO_3-N ppm, % silt and soil moisture were highly positively correlated (> 0.75) with corn yield (Fig. 3.23).

Some differences were observed in the trends seen in the correlation plots with wheat rhizosphere soil physicochemical factors and wheat yield compared to those examining corn rhizosphere soil properties and yield (Fig. 3.23). In field 1, Bray-P1 (P ppm), Na ppm, % Na, Fe ppm, Zn ppm, and clay % were highly negatively correlated (< - 0.75) with wheat yield and Mn ppm and silt % were highly positively correlated (> 0.75) with wheat yield (Fig. 3.23). In field 2, organic matter %, CEC meq/100 g (cation exchange capacity), % H, Al ppm, NO3-N, and ENR (Estimated Nitrogen Release) were highly negatively correlated (< - 0.75) with wheat yield and pH, % Na, and Mn ppm were highly positively correlated (> 0.75) with wheat yield (Fig. 3.23). In field 3, Mg ppm, Ca ppm, NO_3-N, and clay % were highly negatively correlated (< - 0.75) with wheat yield and silt % and soil moisture were highly positively correlated (> 0.75) with wheat yield (Fig. 3.23).

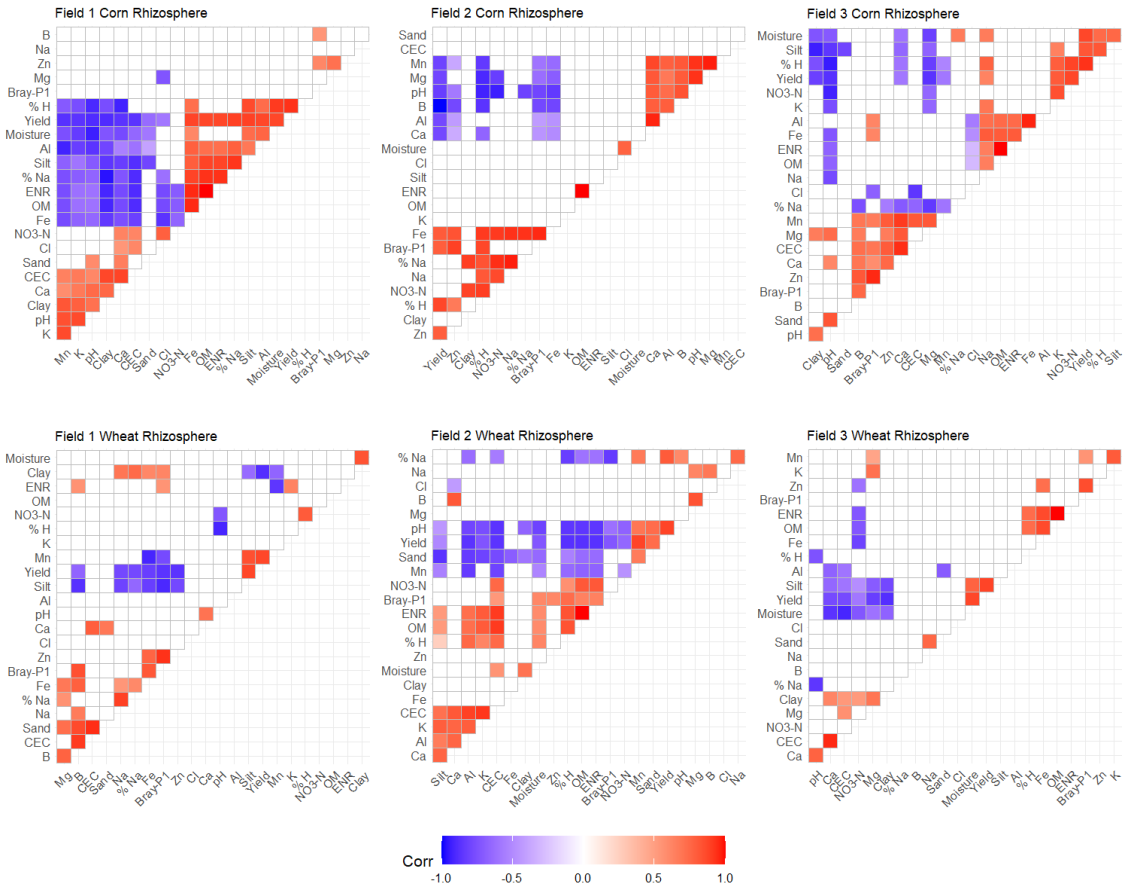


Figure 3.23: Correlation matrix of key rhizosphere soil factors in three fields and yield measured by combine in the field. Correlations were measured using Spearman's correlation coefficient and clustered based on hierarchal clustering. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Correlations that are insignificant ($p>0.05$) are indicated by blank boxes.

The correlation between the top 25 most abundant taxa identified by each primer set and 20 soil physicochemical factors as well as yield was assessed using Spearman's correlation coefficient and adjustments for multiple comparisons were done with the Benjamin and Hochberg procedure. For ITS2 data, *Pleotrichocladium*, *Hyphodiscus*, *Cladosporium*, and *Epicoccum* were significantly positively correlated ($p<0.05$) with yield in field 1, while *Plectosphaerella* and *Neonectria* were significantly negatively correlated ($p<0.01$) with yield (Fig. 3.24). The taxa *Soloacrosporiella*, *Myrmecridium*, and *Sagenomella* were significantly correlated ($p<0.05$) with several soil variables in field 1 (Fig. 3.24). In field 2, none of the top 25 most abundant taxa were significantly positively correlated ($p<0.05$) with yield and *Plectosphaerella* and *Chalara* were significantly negatively correlated ($p<0.05$) with yield (Fig. 3.25). The taxa *Gibellulopsis*, *Sagenomella*, and *Botryotrichum* were significantly correlated ($p<0.05$) with several soil variables in field 2 (Fig. 3.25). In field 3, *Trichosporiella* was significantly positively correlated ($p<0.01$) with yield and *Dactylonectria* and *Linnemannia* were significantly negatively correlated ($p<0.01$) with yield (Fig. 3.26). The only taxon that was significantly correlated ($p<0.05$) to a soil variable in all three fields was *Epicoccum* with soil moisture, which were negatively correlated (Fig. 3.24, Fig. 3.25, Fig. 3.26).

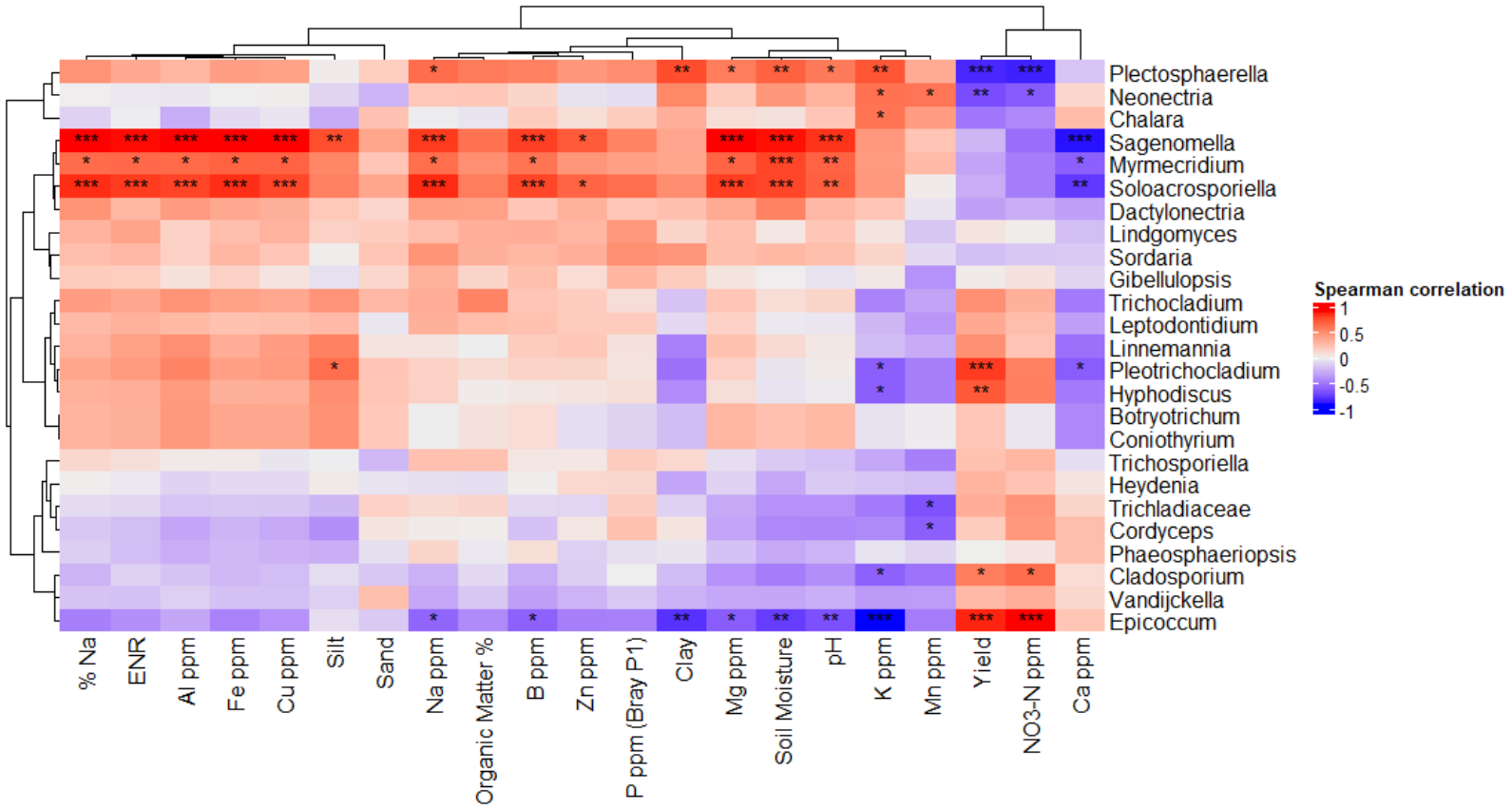


Figure 3.24: Correlation between field 1 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with ITS2 primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

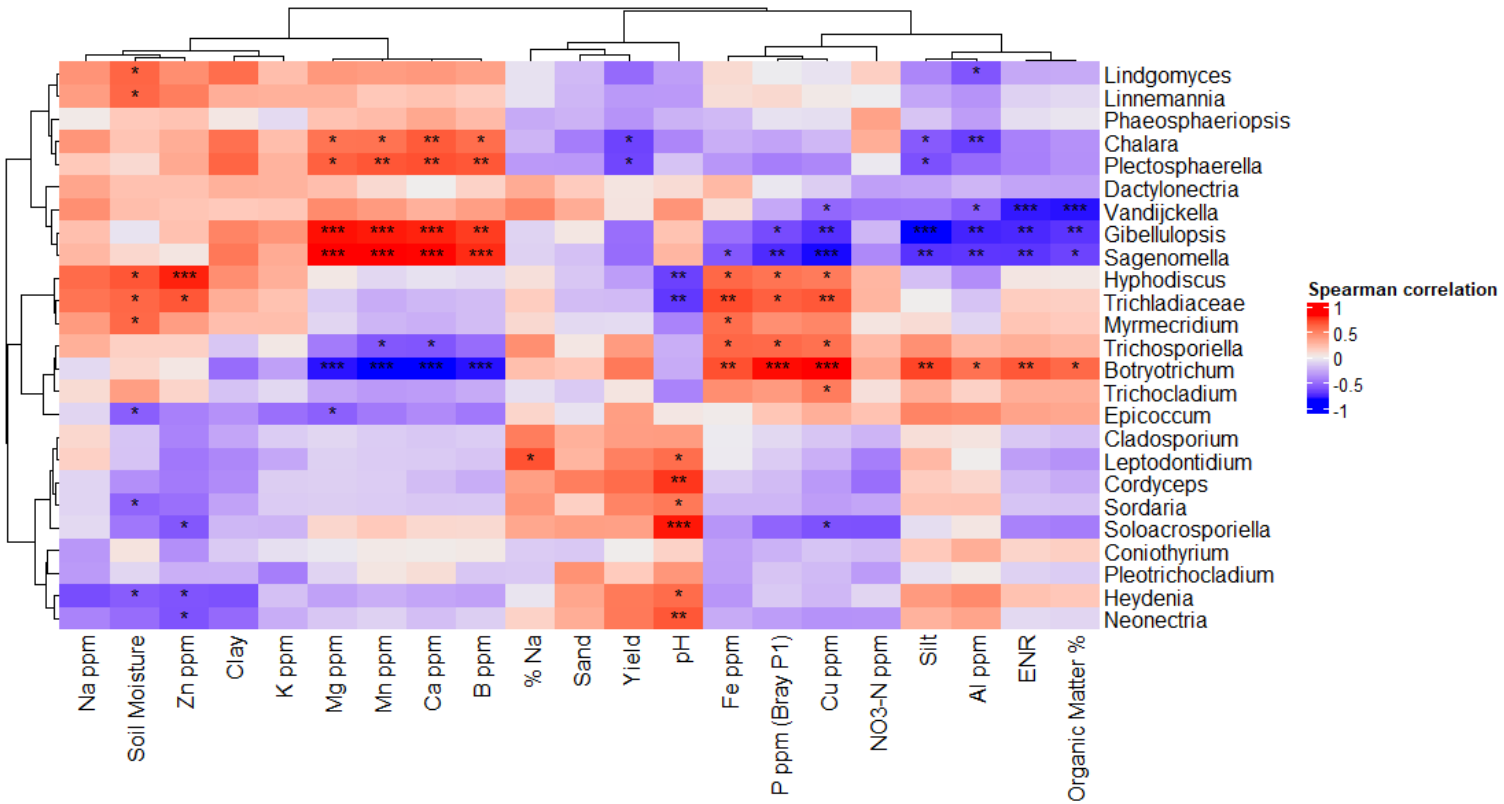


Figure 3.25: Correlation between field 2 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with ITS2 primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

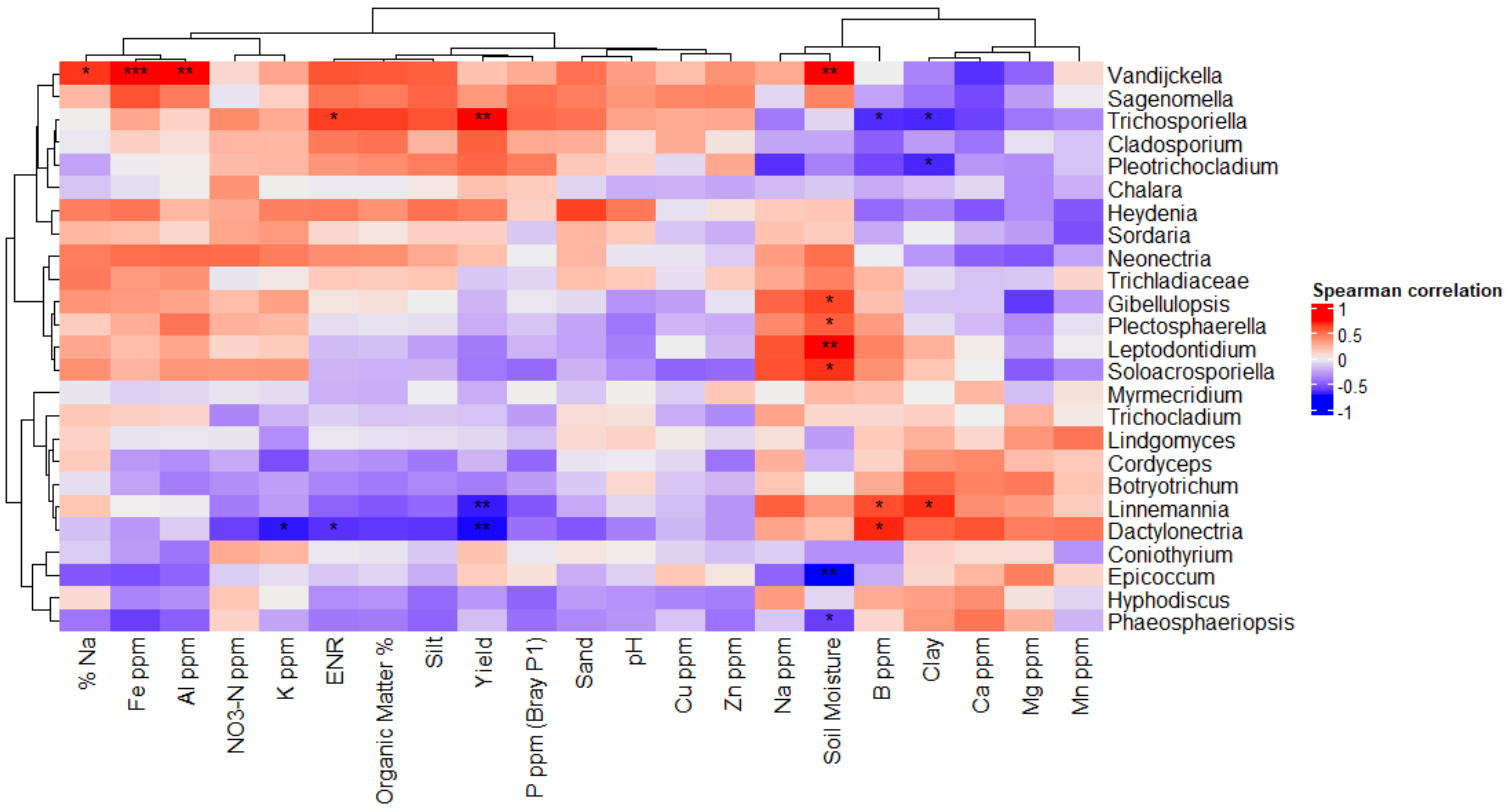


Figure 3.26: Correlation between field 3 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with ITS2 primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

For LSUA data, *Fusicolla* was significantly positively correlated ($p<0.05$) with yield in field 1, while *Plectosphaerella*, *Laetinaevia,* and *Curviclavula* were significantly negatively correlated ($p<0.05$) with yield in field 1 (Fig. 3.27). *Plectosphaerella* and *Fusicolla* were significantly correlated ($p<0.05$) with several soil factors as well as yield, positively for *Plectosphaerella* and negatively for *Fusicolla.* In field 2, *Fusicolla* and *Alternaria* were significantly positively correlated ($p<0.05$) with yield, while *Chloridium*, *Herpotrichia,* and *Aquimassariosphaeria* were significantly negatively correlated ($p<0.05$) with yield (Fig. 3.28). *Aquimassariosphaeria, Herpotrichia, Alternaria,* and *Fusicolla* were also significantly correlated ($p<0.05$) with many soil factors (Fig. 3.28). In field 3, *Tetracladium* was significantly positively correlated ($p<0.01$) with yield and *Corynespora* was significantly negatively correlated ($p<0.01$) with yield (Fig. 3.29). Overall, several taxa were significantly correlated ($p<0.05$) with many of the soil variables, including *Tetracladium, Hyalopezi*a, *Fusicolla*, and *Dactylonectria* (Fig. 3.27, Fig. 3.28, Fig. 3.29).

For LSUBG data, *Guehomyces* was significantly positively correlated ($p<0.001$) with yield in field 1, while *Pythium*, *Funneliformis*, *Tulasnella*, and *Catenomyces* were significantly negatively correlated ($p<0.05$) with yield in field 1 (Fig. 3.30). In field 2, *Mrakia* and *Aphanomyces* were significantly negatively correlated ($p<0.01$) with yield (Fig. 3.31). *Aphanomyces* was also significantly correlated ($p<0.05$) with several other soil factors (Fig. 3.31). In field 3, *Dissophora* and *Rhizophydium* were significantly negatively correlated ($p<0.05$) with yield (Fig. 3.32). In fields 2 and 3, none of the top 25 most abundant taxa were significantly positively correlated ($p<0.05$) with yield (Fig. 3.31, Fig. 3.32). In general, fewer of the top 25 taxa identified by LSUBG primers were significantly correlated with soil variables, positively or negatively, compared to the number of taxa correlated with soil variables from the ITS2 and LSUA datasets.

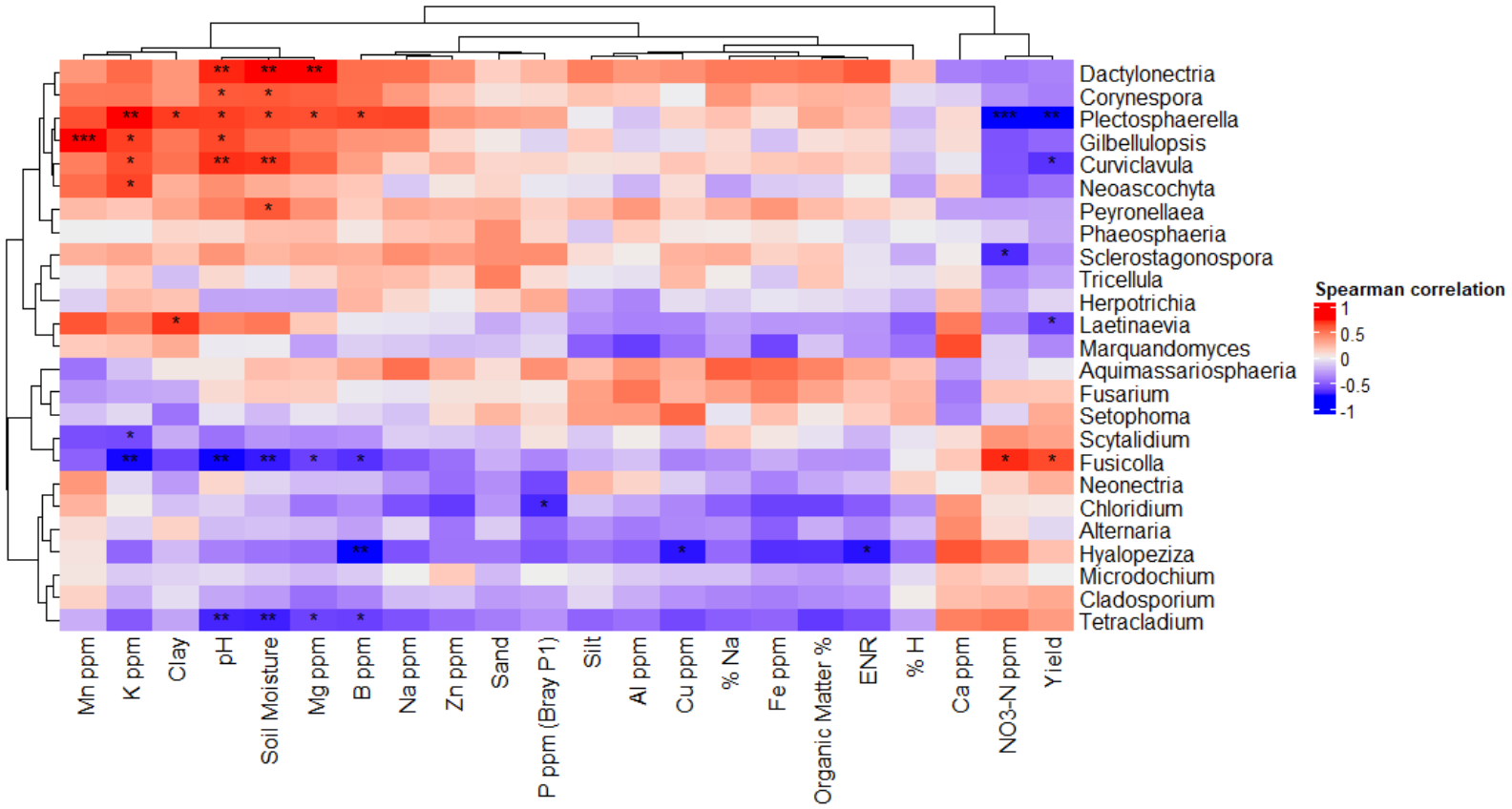


Figure 3.27: Correlation between field 1 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUA primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

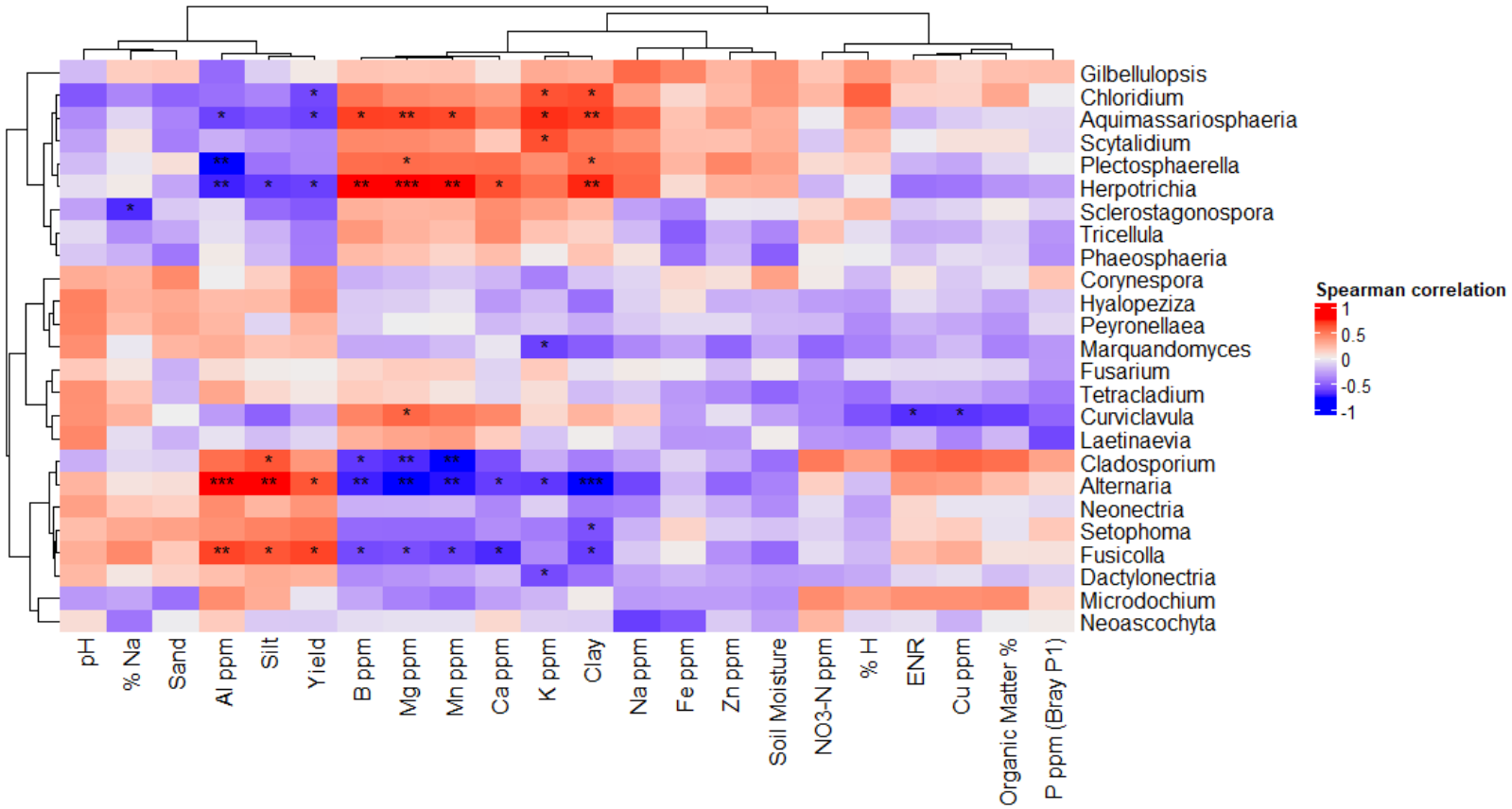


Figure 3.28: Correlation between field 2 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUA primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

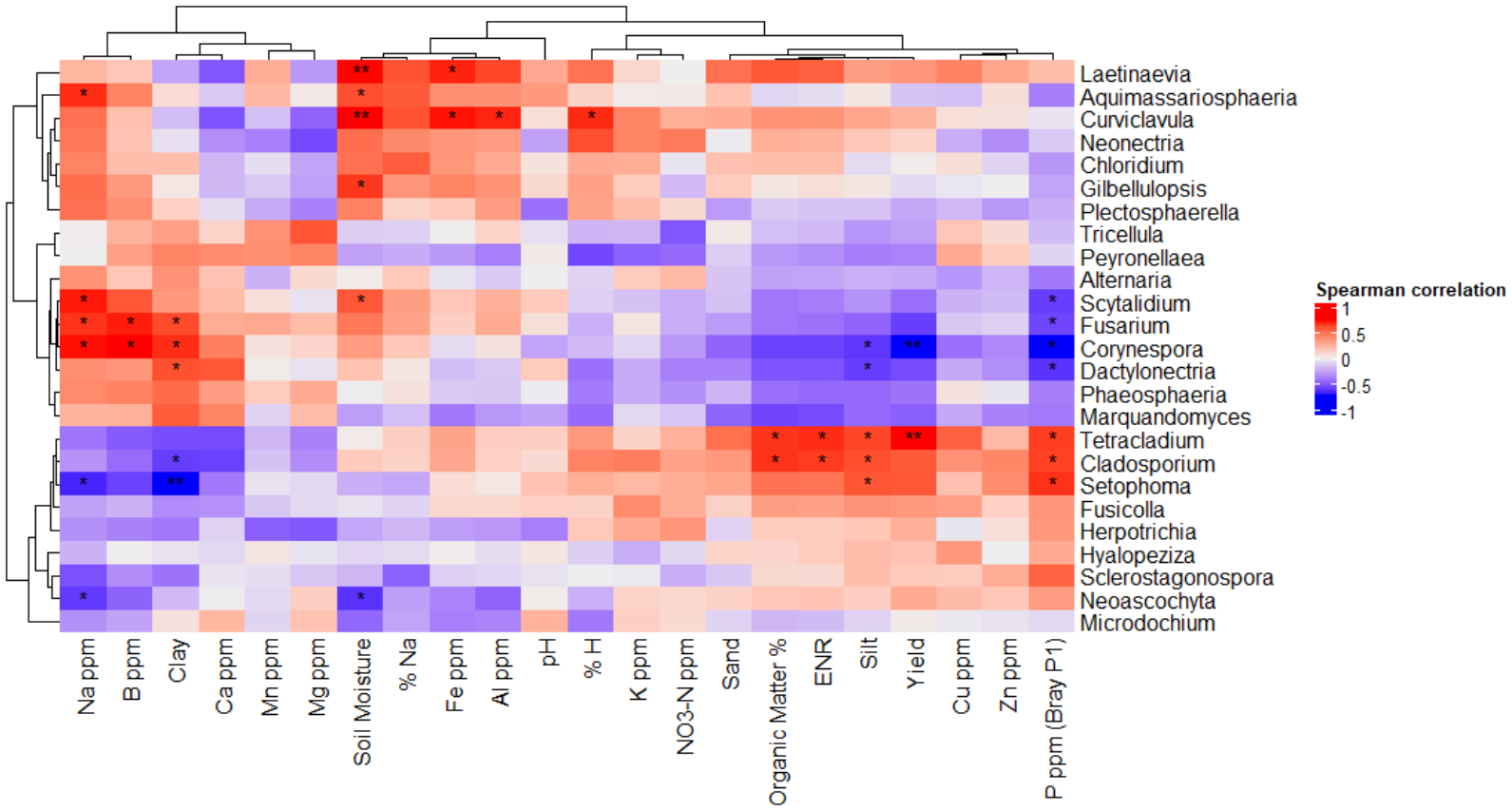


Figure 3.29: Correlation between field 3 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUA primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

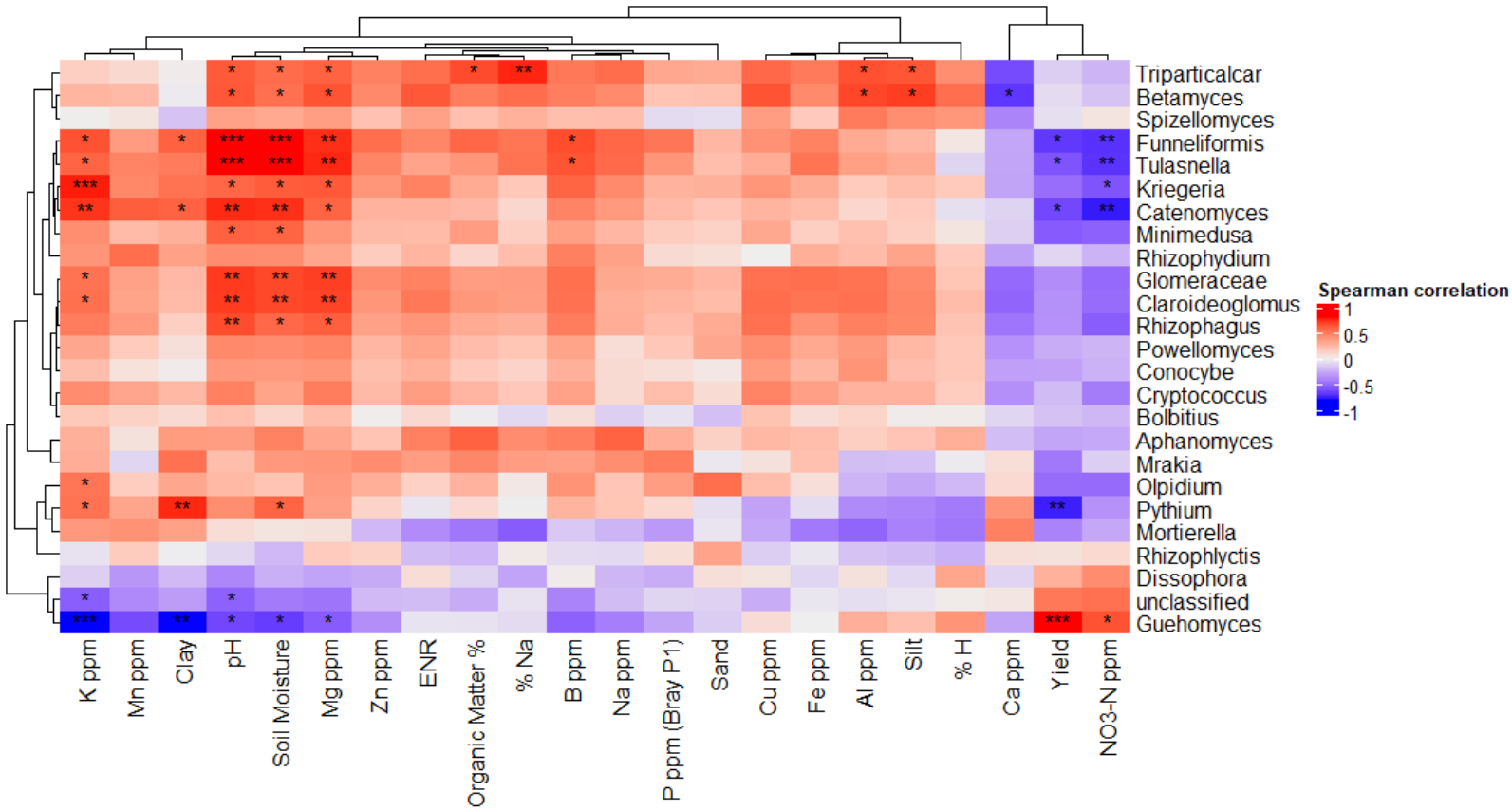


Figure 3.30: Correlation between field 1 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUBG primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

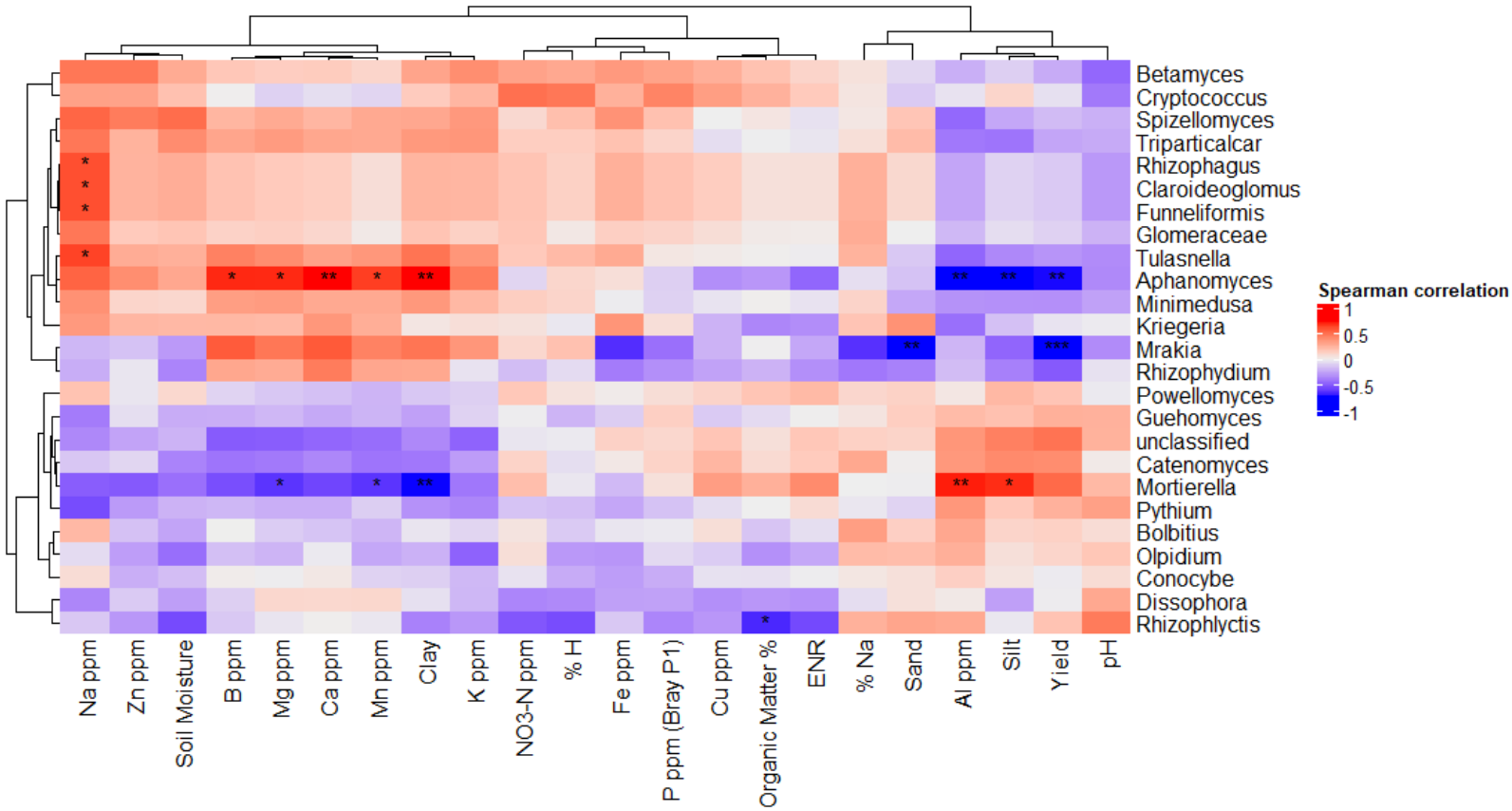


Figure 3.31: Correlation between field 2 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUBG primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

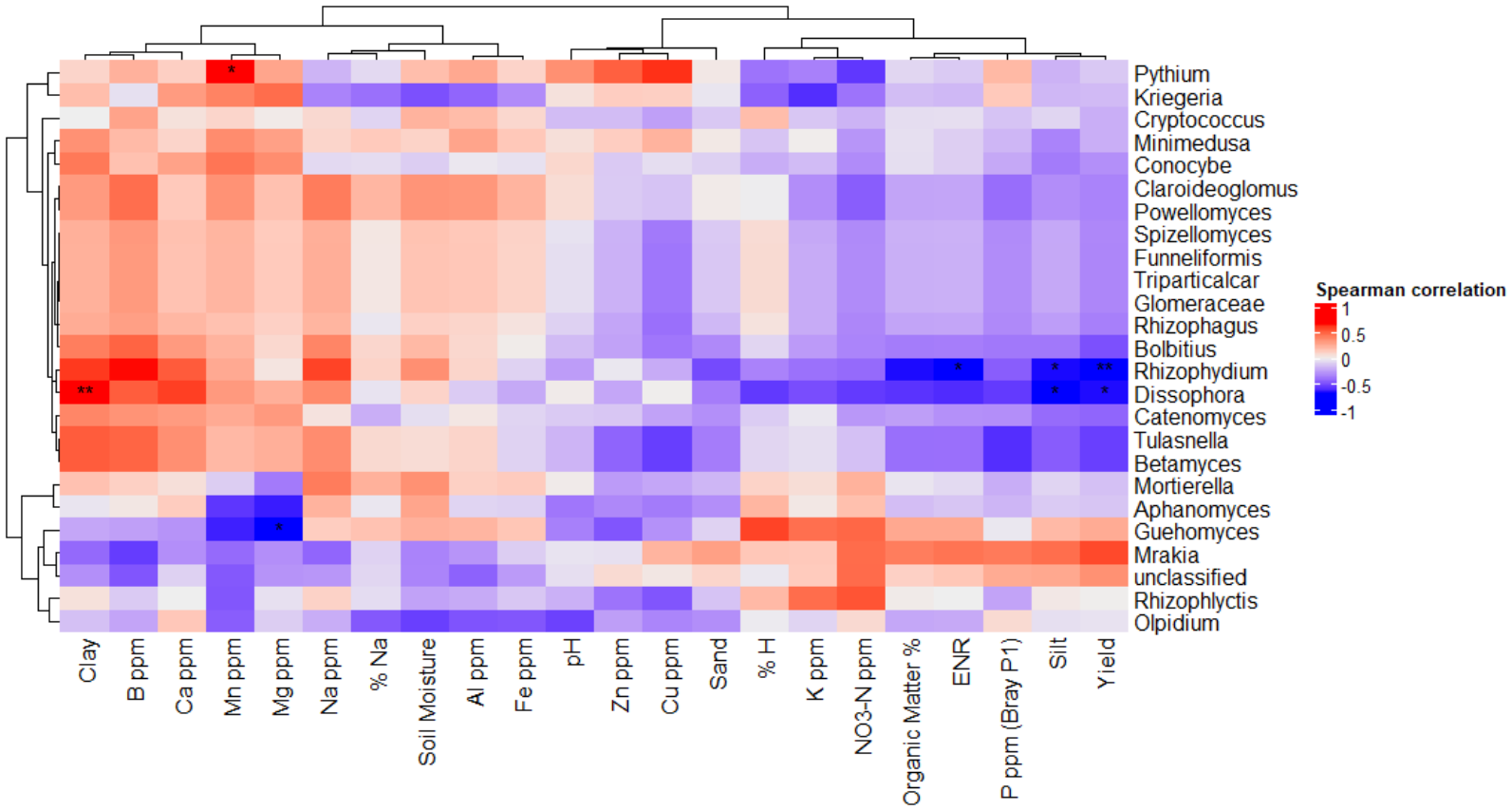


Figure 3.32: Correlation between field 3 key rhizosphere soil factors, including yield at harvest, and the top 25 most abundant taxa from corn and wheat rhizosphere samples from three fields amplified with LSUBG primers.

Correlations were measured using Spearman's correlation coefficient. Positive correlations are displayed in red and negative correlations in blue colour. Colour intensity is proportional to the correlation coefficients. Taxa and soil factors are clustered using the Spearman distance metric. If a p-value was less than 0.05, it was flagged with one star (*). If a p-value was less than 0.01, it was flagged with 2 stars (**). If a p-value was less than 0.001, it was flagged with three stars (***). Adjustment for multiple comparisons was done with the Benjamin and Hochberg procedure.

Chapter 4

4 Discussion

This study focused on characterizing the root mycobiome of corn and wheat planted in rotation using a metabarcoding approach and addresses the association of fungal community composition with soil health variables and yield. The fungal microbiome of rhizosphere soil and root samples from historically high and low yield sites of three fields and soil physicochemical factors within and across the three farms studied were assessed and compared.

4.1 Diversity and composition of the corn and wheat root mycobiome

Despite their geographical proximity and same pattern of crop rotation, the three fields studied were determined to have significantly different fungal community composition from one another, meaning that they must be assessed and compared separately. This points to other soil physical and chemical factors driving the differences between these fields. Many studies claim that plant genotypes are responsible for the composition of root exudates that influence the recruitment of fungi and bacteria in soils and roots (Patel *et al.*, 2015). In this study, three different corn varieties were planted in the three fields, however, fields 2 and 3 were planted with the same variety of winter wheat, which was different from the variety planted in field 1. Some of the variation between fields can therefore be explained by the planting of different genotypic variations of wheat and corn. Crop rotation using genotypically different combinations of corn, soybean, and wheat over many years in the three farms may further explain the variation. Soil characteristics, cropping regimes and soil management practices can have a strong impact on fungal abundance and diversity (Muneer *et al.*, 2021) of the roots and rhizosphere (Tkacz *et al.*, 2015), as well as crop productivity (Rashid *et al.*, 2016). In this study there was no clear or singular soil physicochemical factor or characteristic fungus driving the differences between these fields, meaning that it may be a combination of these factors contributing to differences in community composition of these fields. The study by Kandasamy *et al.* (2021) also concluded that there was no clear, singular mechanism

driving the differences in mycobiome composition in the ten farms they studied. They noted that differences in corn varieties planted in each field may have contributed to differences in community composition of each field (Kandasamy *et al.*, 2021). They also noted that soil physicochemical factors such as pH, soil moisture, soil texture, time of sampling, underlying differences in pathogen pressure depending on farm-specific rotation practices, and differences in community abundance and diversity driven by years of repeated farming practices may be linked to the differences observed in crop productivity across all farms (Kandasamy *et al.*, 2021). In this study, pH, soil moisture, along with other soil variables likely contributed to differences in community composition between fields as well as site-specific differences in crop productivity within the same field.

Greater fungal diversity was seen in corn and wheat rhizosphere soil samples compared to root samples in datasets obtained through PCR-amplification using both ITS2 and LSUA primers. This aligns with claims that while root exudates released by plants recruit fungi to the root mycobiome, most of these root-associated fungi remain in the soil most closely associated with the roots, the rhizosphere, while very few make it into the plant organs (da Silva *et al.*, 2020). The diversity of samples from the crop and type (rhizosphere or root) but from high and low yield sites were very similar to one another, and there were not any stark differences between productivity sites. If anything, across all primers (ITS2, LSUA and LSUBG) and types (rhizosphere or root), median diversity was slightly lower in the high yield sites than in low yield sites. This contradicts previous studies that link diversity of root-associated fungi to higher crop productivity (Kandasamy *et al.*, 2021; Van Der Heijden *et al.*, 1998). Since the differences in the diversity of high and low yield sites are small, the productivity of these sites may be attributed more to composition and abundance rather than diversity. The presence and abundance of beneficial fungi as well as pathogenic fungi, in addition to soil physicochemical factors may be stronger drivers of crop productivity in these farms compared to overall diversity.

PCR-amplification of samples with ITS2 primers resulted in a high relative abundance (> 50%) of ASVs belonging to the phylum Ascomycota, which can be explained by both the

general predominance of this phylum in agricultural soils (Egidi *et al.*, 2019) and because these primers are known to preferentially amplify Ascomycota with shorter ITS2 amplicons over the longer Basidiomycota ones (Bellemain *et al.*, 2010; Taylor *et al.*, 2016). This re-emphasizes the importance of using secondary DNA barcodes in order to better capture community composition in a sample (Schoch *et al.*, 2012). The most abundant class for samples from all fields amplified with LSUA primers was Sordariomycetes for the most part, with Dothideomycetes having the highest relative abundance in field 2 corn root and wheat root samples. Sordariomycetes, which are the second largest class of Ascomycota, has been identified by other microbiome studies as being the most abundant class of fungi in soils (Lauber *et al.*, 2008; Zhang *et al.*, 2018). Dothideomycetes are the largest and most diverse class of fungi, explaining their high relative abundance in all sample types. LSUBG primers developed by Asemaninejad *et al.* (2021), were able to capture a higher proportion of Basidiomycota and Glomeromycota than ITS2 primers, resulting in a more complete composition analysis. A shortfall of the use of LSUBG primers is that reference gene databases are less populated for sequence data obtained using these primers than they are for ITS2 primers. In addition to a greater abundance of non-target ASVs that can be obtained when using LSUBG primers (Kandasamy *et al.*, 2021), which was also seen in this study (49 %), this can result in a less complete picture of composition of Basidiomycota in samples. Such may be the case in my analysis of LSUBG data, which yielded a relative abundance of over 20 % of ASVs that remained unclassified in all samples after taxonomic classification using reference gene databases.

Within each field and using all primers, NMDS analysis, ANOSIM tests and pairwise PERMANOVA/ADONIS analyses revealed significant differences between fungal community composition of crops (wheat or corn, particularly in root samples), types (rhizosphere soil or root) and sample types (high/low yield corn rhizosphere soil, high/low yield corn root, high/low wheat rhizosphere, and high/low wheat root). These analyses supported the hypotheses that there are crop-specific differences in community composition and that root and rhizosphere fungal communities have a strong correlation with crop health and productivity despite varying soil types. There were few exceptions to significant differences between community composition of crops, types, and sample

types. A notable exception was that in all fields, corn root samples from high and low yield sites were not significantly different from one another. All other exceptions were also of samples in high and low yield sites in the same field from the same crop and type (rhizosphere soil and root). It is unsurprising that corn root samples from high and low sites in the same field were not significantly different from one another, as the diversity of these samples was much lower than any other sample type. In addition, many root-associated fungi may not have penetrated the corn roots, leading to differences in rhizosphere soil fungal communities but not in root fungal communities. Given that corn roots are much larger in size compared to wheat roots, this may have affected the quality and quantity of DNA extracted. With proportionally more plant DNA in corn root samples, this could have resulted in less accurate representation of fungal community composition in these samples.

ASVs identified as belonging to the genera *Mortierella*, *Tetracladium*, *Linnemannia*, *Plectosphaerella*, and *Alternaria* were identified as highly abundant in corn rhizosphere soil, wheat rhizosphere soil and wheat root samples when amplified with ITS2, LSUA and LSUBG primers. *Mortierella*, which were found to have high higher relative abundance in corn and wheat rhizosphere soil samples than in wheat root samples, have the potential to act as plant growth-promoting fungi. They have the ability to increase nutrient uptake efficiency in plants, including P and Fe, and can provide positive effects in protecting crops against unfavourable environmental conditions (Ozimek & Hanaka, 2021). For ITS2 data, the abundance of an ASV belonging to this genus was fairly consistent in high and low yield sites in fields 1 and 2. In field 2, *Mortierella* were more abundant in low yield corn rhizosphere soil sites than in high yield sites, pointing to possible unfavourable environmental conditions in low yield sites, in which species belonging to the genus *Mortierella* have the ability to survive. For LSUBG data, *Mortierella* were abundant in all three fields and in high and low yield sites. Their ability to survive in low yield sites that may have unfavourable environmental conditions and the beneficial effects they provide to plants in both high and low yield sites explain their abundance in all sites. Several ASVs belonging to the genus *Tetracladium* were highly abundant in corn rhizosphere, wheat rhizosphere and wheat root samples. *Tetracladium* species, which are root endophytes (Grudzinska-Sterno *et al.*, 2016), have been shown to

have beneficial effects on plant growth (Sati & Pant, 2019), as well as no effect on plant growth (Sati & Arya, 2010). The conflicting evidence regarding this genus suggests that either possibility could exist within the sites observed in this study. *Linnemannia* is a relatively new genus of species formerly belonging to *Mortierella* (Vandepol *et al.*, 2020). The species previously known as *Mortierella elongata* has been shown to have growth promoting effects on plants when associated with plant roots (Vandepol *et al.*, 2022). *Linnemmannia* were present in rhizosphere soil samples amplified with LSUBG with no clear or consistent patterns of abundance in low and high yield sites. There was also no preference between corn and wheat samples, meaning that this genus was abundant and may be providing neutral or growth-promoting effects. The genus *Plectosphaerella* contains many species of plant pathogens (Raimondo & Carlucci, 2018), including *Plectosphaerella cucumerina*, which is a filamentous ascomycete fungus that can survive saprophytically in soil by decomposing plant material (Palm *et al.*, 1995). In ITS2 and LSUA data, there were no clear or consistent patterns of abundance of *Plectosphaerella* in corn and wheat rhizosphere soil and wheat root samples. In high yield sites, the presence or abundance of this genus of mostly pathogenic fungi may be alleviated by other beneficial fungi or beneficial soil characteristics. *Alternaria* were also identified as highly abundant in field 1 and field 3 corn and wheat rhizosphere soil and wheat root with no clear or consistent patterns of abundance in low and high productivity sites. Most *Alternaria* species are common saprophytes while some species are plant pathogens that can cause economically important diseases in a variety of crops (Thomma, 2003). In wheat, some species, including *Alternaria alternata* can cause leaf blight while others have been shown to be non-pathogenic (Mercado Vergnes *et al.*, 2006). Given the lack of patterns in abundance in various sample types, the non-pathogenic species of *Alternaria* have likely been identified in samples used in this study.

Using LSUA primers alone, *Gibellulopsis* were identified as highly abundant in corn and wheat rhizosphere and wheat root samples in more than one field. *Gibellulopsis nigrescens*, formerly known as *Verticillium nigrescens* (Zare *et al.*, 2007), can have pathogenic effects by causing Verticillium wilt (Melouk, 1974). *Gibellulopsis* were more abundant in field 1 corn rhizosphere soil in low yield sites than in high yield sites and were more abundant in field 1 and 2 rhizosphere soil samples than in wheat root samples.

However, in field 2, there was no apparent difference in the abundance of *Gibellulopsis* between low and high productivity sites.

Using LSUBG primers, *Pythium*, *Mrakia*, *Tausonia* and *Bodomorpha* were identified as highly abundant in corn and wheat rhizosphere samples. *Pythium* (Oomycota: Peronosporomycetes), which was highly abundant in field 1 wheat rhizosphere soil samples with no distinction between high and low yield sites, is known to contain many species associated with pathogenic activities, including causing root rot in several crops (Sutton *et al.*, 2006). *Mrakia*, which were most abundant in field 3 high yield corn rhizosphere samples, and *Tausonia*, which were most abundant in field 1 high yield corn rhizosphere samples, are yeasts included in the family Mrakiaceae (Basidiomycota: Tremellomycetes) and are known to be psychrotolerant (Tsuji, 2018). They are able to produce large amounts of enzymes, even at subzero temperatures, meaning they could be important in nutrient cycling in the soil (Tsuji, 2018). Sequences with high identities to *Bodomorpha* (phylum Cercozoa, class Sarcomonadea; Cavalier-Smith and Chao, 2003), showed no clear abundance patterns in fields 2 or 3 corn and wheat rhizosphere soil. These organisms are bacterivorous soil protists and have been shown to perform alongside beneficial bacteria to suppress root rot caused by *Fusarium* (Bahroun *et al.*, 2021).

ASVs belonging to the genus *Setophoma* were identified in ITS2 and LSUA data as being highly abundant in corn root samples in both high and low yield sites. *Setophoma terrestris* is a known fungal pathogen causing red root rot of corn and the genus *Setophoma* belongs to the order Pleosporales, which contains many plant pathogens (Mao *et al.*, 1998). Although this genus is present in both high and low yield sites, the presence of beneficial soil-associated fungi in high yield sites may provide protective benefits against this pathogen. Other genera that were abundant in corn root samples in all fields based on ITS2 and LSUA data include *Fusarium* and *Exophiala*. *Fusarium*, which were most abundant in low yield corn root, is a genus of filamentous fungi that contains many species that are important plant pathogens, including in corn, wheat, and soybeans (Ma *et al.*, 2013). *Exophiala*, which did not show clear patterns of abundance, are cold-adapted plant pathogens that produce cytotoxic metabolites (C. C. Wang *et al.*, 2011). In LSUA

data, the genera *Didymella* and *Penicillium* were identified as highly abundant in corn root samples, which both contain species with a wide range of effects on crop health and productivity. *Penicillium* species are well known for their production of antibiotics, so their activity in corn roots may be beneficial in corn the plants studied.

The relative abundance results of this study showed some similarities to those of Bandara *et al.,* who also saw high relative abundance of *Mortierella* in bulk and rhizosphere soils, particularly in low-yielding sites and high relative abundance of *Exophiala* in high-yielding sites. They also observed high relative abundance of *Fusarium* in low yield sites (Bandara *et al.*, 2021). Conversely, the opposite association was observed in their study compared to this study for the abundance of *Mrakia*, which they identified as being in high abundance in low yield sites (Bandara *et al.*, 2021). The study by Kandasamy *et al.* (2021) also observed significant associations of *Pythium*, *Setophoma, Exophiala*, and *Penicillium* with yield based on ALDEx analysis. However, while *Fusarium* were notably in high abundance in low yield sites in this study, theirs found *Fusarium* to be significantly associated with high yield sites (Kandasamy *et al.*, 2021).

Overall, the ASVs recovered in the three fields of study by the combination of ITS2, LSUA and LSUBG were diverse in their taxonomy and biology. Greater diversity was seen in rhizosphere over root samples. The root mycobiomes in each field, for each crop, and in most cases, for high and low yield sites were different in their community composition.

The indicator species analysis of fields and sample types (corn rhizosphere, corn root, wheat rhizosphere, and wheat root for each field), revealed several species that were uniquely associated with one field, or one sample type within a field. These are likely the drivers of significant differences between fields observed in statistical analyses and NMDS plots. *Tausonia pullulans* was significantly associated with field 1 corn rhizosphere samples and was detected by both ITS2 and LSUBG primers. This species plays an important role in decomposing organic compounds and is frequently found in decaying plant materials (Cadete *et al.*, 2017). *Pythium biforme* was significantly associated with field 1 wheat rhizosphere and wheat root samples. *Pythium biforme*, an

oomycete that was first isolated in freshwater samples in Japan (Uzuhashi *et al.*, 2015), belongs to a genus that includes several species that are known pathogens of various plants (Sutton *et al.*, 2006). *Linnemannia gamsii* was significantly associated with field 3 wheat rhizosphere samples. This species, formerly belonging to the genus *Mortierella*, is commonly found in soils and usually associated with plant rhizospheres or decaying plant matter (Vandepol *et al.*, 2020).

4.2 Corn and wheat mycobiome and yield

An ANOSIM analysis using the groupings of corn rhizosphere and root, wheat rhizosphere and root, corn and wheat rhizosphere, and corn and wheat root for ITS2 and LSUA data revealed that significant differences in fungal community composition between high and low yield only existed when grouping corn and wheat rhizosphere soil samples. This was also true in LSUBG data, where corn and wheat rhizosphere samples were grouped separately. These results suggest that the differences in community composition of high and low yield sites of corn and wheat are driven by rhizosphere soil fungal community composition rather than root fungal community composition. This is unsurprising as greater diversity of rhizosphere soil community composition compared to roots has been seen in this study and in others (Berg & Smalla, 2009; Kandasamy *et al.*, 2021). The release of root exudates from plants and the variety of root associations that can occur in the rhizosphere soil mean that community composition can vary greatly and is overall complex in the rhizosphere, while fungal communities within the roots tend to have lower diversity (da Silva *et al.*, 2020).

Based on indicator species analysis results, *Pseudeurotium bakeri* was associated with high yield sites in field 1 rhizosphere soil. Not much is known in terms of the link between this species and crop productivity, but it is known to tolerate high concentrations of diesel fuel in soils (Ferrari *et al.*, 2011). In field 2 rhizosphere soil, *Cryptococcus watticus, Hyphodiscus,* and *Solicoccozyma terrricola,* were significantly associated with high yield sites. *Cryptococcus watticus* is known to be psychrotolerant and is able to produce large amounts of enzymes, even at sub-zero temperatures, meaning it could be important in nutrient cycling in the soil during late fall, winter, or early spring (Tsuji, 2018). *Hyphodiscus* is a member of the clade Helotiales, which are phylogenetically

closely related to root endophytes and ectomycorrhizal fungi suggesting their strong ecological and evolutionary links (Tedersoo *et al.*, 2009). *Leptodontidium camptobactrum*, *Soloacrosporiella,* and *Tetracladium* sp. were associated with low yield sites in field 2 rhizosphere soil. Not much is known about *Leptodontidium camptobactrum*, other than it is likely a root endophyte. Little is known about *Soloacrosporiella*, other than it has been found on the pods of *Acacia mangium* in Malaysia and it belongs to the class Dothideomycetes, the largest and most diverse class of Ascomycota (Perera *et al.*, 2020). In field 3, *Candida sake, Didymella, Solicoccozyma terrricola, Tetracladium* sp., and *Vandijckella* were significantly associated with high yield sites. *Candida sake,* a soil yeast, has been found to have a positive effect on the growth of corn (Gollner *et al.*, 2006). Soil yeasts, including *Candida sake* can interact with arbuscular mycorrhizal fungi, resulting in increased shoot biomass of corn (Gollner *et al.*, 2006). *Didymella* is a genus that contains several pathogenic species, which can cause disease in a wide variety of crops (Barilli *et al.*, 2016). *Solicoccozyma terrricola* is a soil-borne yeast that is commonly identified in soils worldwide (Mašínová *et al.*, 2017). *Vandijckella* belongs to the class Leotiomycetes, which contains several species that cause plant disease. Others are ectomycorrhizal or neutral/beneficial endophytes. Given the significant association with high yield sites, *Vandijckella* may be acting in a beneficial manner.

Solicoccozyma terrricola, which was identified as being significantly associated with high yield sites in field 2 and 3 rhizosphere soils, is not well-studied in relation to its correlation with crop productivity. However, Bandara *et al.* also observed high abundance of the genus *Solicoccozyma* in the rhizosphere and bulk soil of high yield sites (Bandara *et al.*, 2021). Future studies should attempt to discern the significance of this genus in agriculture.

Overall, the results of the indicator species analysis revealed some key species that may be driving the differences in community composition of low and high yield sites. However, there were some conflicting results based on the biological mechanisms of some of the species identified in high and low productivity sites. Rhizosphere soils of corn and wheat were highly diverse, and in many cases there were no significant

differences in community composition of low and high yield sites in the same field. This suggests that interactions between organisms in the rhizosphere soil, as well as soil physicochemical characteristics are likely driving yield differences in sites over singular species.

Mantel tests were used to identify associations between community composition of sample types (corn rhizosphere soil, wheat rhizosphere soil, corn root, and wheat root) and yield measured in the fields at harvest. The analysis of all primer data revealed several significant associations between rhizosphere communities and crop yield in all fields. Field 3 wheat root communities from ITS2 data and field 2 wheat communities from LSUA data were also significantly associated with yield. This supports the hypothesis that root and rhizosphere fungal communities have a strong correlation with crop health and productivity despite varying soil types. Other factors such as interactions between fungi in the soil and roots, bacterial community composition and their interactions with fungi, nematodes, earthworms, and other abiotic factors not considered in this study could also be contributing to differences in fungal community composition.

4.3 Soil physicochemical variables and community composition

NMDS of ITS2 data revealed a clear separation between rhizosphere sample types (high and low yield corn rhizosphere soil and high and low yield wheat rhizosphere soil). This aligns with other results in this study, including ANOSIM, PERMANOVA/ADONIS, and pairwise PERMANOVA/ADONIS which revealed significant differences not only between fields, but also between sample types (including high/low sample types from the same crops) in the same fields in most cases. Overall, many of the 28 soil physicochemical variables measured were found to be predictors of the separations of sample types based on fungal community composition. This suggests that these biotic and abiotic variables are highly different between sample types in each of the three fields studied and implies that soil physicochemical variables are important predictors of differences between community composition and crop productivity (Chang *et al.*, 2017). Although the three fields studied employ similar cropping regimes and fertilizer, soil characteristics, which can vary in the same field, can greatly influence the microbial

community (Tkacz & Poole, 2015). The variability in soil characteristics identified as significant in the NMDS plots are thereby likely contributing to the differences in community composition that make the sampling sites for high yielding and low yielding corn and high yielding and low yielding wheat distinct from one another. Several other studies have identified environmental factors as being important contributors to fungal diversity (Antoninka *et al.*, 2015; Tedersoo *et al.*, 2014; Tkacz & Poole, 2015; Xu *et al.*, 2009). In one such study by Tedersoo *et al.* (2014), several climactic and edaphic factors were found to be predictors of soil fungi diversity and community composition, including mean annual precipitation, soil calcium concentration, soil pH, soil carbon/nitrogen ratio, and concentration of soil nutrients such as phosphorus. Soil physicochemical variables in fields and in site-specific locations within the fields should continue to be assessed and compared, as their associations with fungal communities may be paramount to understand differences in crop productivity in sites within the same field.

In the NMDS plots for LSUA data, most soil variables were significantly associated with community composition of rhizosphere samples from the three fields. The amount of soil variables that were associated with community composition in field 3 for LSUA data was higher than the amounts for ITS2 and LSUBG data, suggesting that soil variables in this field may have a large influence on composition of the phylum Ascomycota, which is underrepresented by the two other primer sets. While ITS2 primers did yield a high relative abundance of Ascomycota found in sample types originating from all farms, the data from LSUA primers, which selectively amplify sequences belonging to the phylum Ascomycota, suggest that more of these sequences are important when assessing the associations between soil factors and community compositions in the fields studied. These results are consistent with other studies, which have reported a significant correlation of pH and soil elements with particular fungal functional groups (Tedersoo et al., 2012, 2014)

In field 1, wheat yield was not significantly correlated with community composition in the NMDS analysis of ITS2 and LSUBG data but was significantly correlated in the LSUA plot. This suggests that Ascomycota have a larger influence on wheat yield at harvest in this field. Several soil variables were also correlated with community

composition in field 1 and identified as such in all primer data, including K, which was found to be associated with wheat samples, and % K, which was associated with low yielding wheat samples. Potassium is an essential nutrient for plant growth and its involvement in several physiological processes makes it important for crop productivity (Pettigrew, 2008). Availability and uptake of potassium by plants is dependent on several other characteristics, including soil moisture, temperature, and the form of potassium present in the soil, so even though the soil from a low yielding site may contain large amounts of potassium, the plants may not be able to efficiently use it under undesirable conditions (Pettigrew, 2008). The presence of microorganisms, including rhizobacteria in the soil, can also be important in aiding in the uptake of potassium in various forms by crops (Ghadam Khani *et al.*, 2019). Fe ppm, which was correlated with low yielding wheat samples, is essential in crop growth and in the biochemical process of photosynthesis (Rout & Sahoo, 2015). Like with potassium, its uptake by plants is dependent on other conditions, including soil pH, soil texture and temperature (Rout & Sahoo, 2015). Chlorine in the soil was also strongly correlated with low yielding corn in field 1. Chlorine is a plant macronutrient and is important for plant photosynthesis. In the LSUA and LSUBG plots, S was close to the vector line for corn yield, indicating that these vectors are closely related. Sulfur is an important plant nutrient and is needed for higher crop yield and higher crop quality (Aula *et al.*, 2019).

In field 2, several soil physiochemical variables, including corn and wheat yield, were correlated with community composition for all primer data. Potassium was correlated with wheat sample types, along with Ca and % clay. Calcium is an important nutrient, and its availability is tied to pH. Zn was correlated with low yielding wheat samples. Zinc is an essential plant nutrient that is involved in many physiological processes. The uptake and availability of zinc is affected by many soil characteristics, so high amounts of zinc in the soil may not necessarily be taken up by the plant. These characteristics include soil pH, organic matter content, soil texture, and interactions with other soil nutrients *(*Hafeez *et al.*, 2013). Additionally, Wu *et al.* (2022) found that soil with higher zinc content may lead to declines in the fungal diversity in belowground habitats. They found that Zn may limit the reproduction of certain fungi such as Sordariomycetes and Leotiomycetes, as well as suppress the growth of potential phytopathogens. Their results reinforce the

importance of Zn as a key player in recruitment of fungi to the rhizosphere, which results in differences in community composition. In field 2, Fe and Cu were strongly correlated with high yielding corn samples in field 2 and are both associated with improved physiological functions of crops and crop yield (Manthey *et al.,* 1994; Syuhada *et al.,* 2014). Boron in the soil was also strongly correlated with high yielding wheat in field 2. Boron plays a key role in a diverse range of plant functions, including an important role in crop development and growth, resulting in a direct impact on yield (Gupta, 1980).

In field 3, only corn yield, wheat yield, and Mn were identified by NMDS analysis of all primer data as being significantly correlated with community composition of samples. Manganese, which was strongly correlated with wheat sample types, plays a key role in several physiological processes, particularly photosynthesis and is linked to soil texture and pH (Mousavi *et al.,* 2011). Manganese deficiency in soil can result in poor crop yield and quality (Mousavi *et al.,* 2011).

Kandasamy *et al.* (2021) also observed several soil characteristics that contributed to microbiome diversity in their study, including % clay, pH, Fe, K, soil moisture, and Cl. These soil variables were identified as being significantly correlated with differences in community composition of samples in this study. Similarly to the results of the present study, Kandasamy *et al.* (2021) found that Zn, % Na, and pH were contributing to site-specific differences in microbiome diversity within farms.

Several soil physicochemical factors were significantly associated with corn yield in all three fields based on the correlation matrix. Those positively correlated with corn yield in more than one field include % H, Fe, % silt and soil moisture. These are all known components and characteristics of soil that are essential plant health and associated with crop productivity. The soil variables that were significantly negatively correlated with corn yield in more than one field were Ca, pH, Mn, % clay and Mg. Higher levels of these soil variables have been known to be associated with lower yields. Some soil variables that differed in whether or not they were significantly associated with corn yield in each field were % Na, Al, ENR (estimated nitrogen release), organic matter %, Bray-P1, Zn, K, B and CEC (cation exchange capacity). The differences in these variables

between fields could have been contributing to or associated with the differences in community composition between fields. Kandasamy *et al.* (2021) observed pH to be associated with productivity of corn. They similarly found site-specific differences in Mn, Al, CEC, organic matter, B, Zn and soil texture in the same fields. They also observed unique patterns in each of the corn fields they studied, which included the three fields analyzed in this study, along with seven others (Kandasamy *et al.*, 2021). Each farm was unique in the soil chemistry, yield and corn mycobiome trends exhibited (Kandasamy *et al.*, 2021).

Many of the soil physicochemical factors that were significantly associated with wheat yield in the fields were different from those associated with corn yield. This aligns with studies that suggest that crop species and genotype play a large role in recruitment in the microbiome, leading to differences in microbiome composition, which in turn is associated with differences in soil characteristics (Patel *et al.*, 2015; Rashid *et al.*, 2016). The soil characteristics that were positively correlated in with wheat yield in more than one field were Mn and % silt. Manganese is an essential nutrient in the soil, and soil texture, including silt is an important driver of crop productivity. The soil variables that were significantly negatively correlated with wheat yield were Bray-P1, % clay, CEC (cation exchange capacity), and NO_3-N. While higher levels of Bray-P1, % clay and CEC (cation exchange capacity) are beneficial to plant growth, excessive amounts of these soil factors can be detrimental to plants. Some soil variables that differed in whether or not they were significantly associated with wheat yield in each field were pH, %Na, sand, soil moisture, Na, Fe, Zn, organic matter %, K, % H, Al, % silt, Mg, and Ca. The differences in these variables between fields could have been contributing to or associated with the differences in community composition between fields.

Several of the top 25 most abundant taxa identified in each field (corn and wheat rhizosphere soil samples combined) were associated with yield. In field 1, ASVs belonging to the genera *Pleotrichocladium*, *Epicoccum*, and *Fusicolla* were significantly positively correlated with yield. In one study, *Pleotrichocladium* was identified as a keystone taxon for fungal abundance and community structure in forest soils (C. Wang *et al.*, 2022). The positive association of a member of this genus could mean that the

community composition in sites with abundant *Pleotrichocladium* favours high crop productivity. Species of *Epicoccum* have been found to produce antifungal compounds that protect against the soil-borne plant pathogenic oomycetes *Phytophthora* spp. and *Pythium* spp. (Brown *et al.*, 1987). However, some species belonging to this genus can also be pathogenic. The abundance of this taxon and its significant association with yield suggests that it is providing protective and therefore beneficial effects to the crops. Species of *Fusicolla* have been shown to display antifungal activity against *Alternaria alternata* (Li *et al.*, 2021) and plant-growth promoting activity when present in soil microbiomes (Lay *et al.*, 2018). *Plectosphaerella*, *Neonectria*, *Pythium*, and *Funneliformis* were significantly negatively associated with yield in field 1. *Plectosphaerella* and *Pythium* are likely acting as plant pathogens in the rhizosphere soil of low productivity plants in this field (Melouk, 1974; Palm *et al.*, 1995; Seaman *et al.*, 1965; Sutton *et al.*, 2006). Several species of *Neonectria* are also known plant pathogens, causing disease in the roots (Adesemoye *et al.*, 2017; Menkis & Burokiene, 2012). However, despite its correlation with lower yields, the arbuscular mycorrhizal fungus *Funneliformis* may acts as beneficial organisms, providing protection from plant pathogens such as *Fusarium oxysporum* (Qian *et al.*, 2015). The significant correlation of several highly abundant taxa with lower yields in this field may mask the potential protective effects of *Funneliformis.*

In field 2, ASVs belonging to the genera *Fusicolla* and *Alternaria* were significantly positively correlated with yield. *Alternaria* could either be acting neutrally in these soils or as a plant pathogen. However, the even if the species of *Alternaria* is pathogenic, *Fusicolla* has been shown to display antifungal activity against species *Alternaria* (Li *et al.*, 2021) and plant-growth promoting activity when present in soil microbiomes (Lay *et al.*, 2018), thereby potentially masking the negative effects of *Alternaria* in the soil. *Plectosphaerella*, *Chalara*, *Herpotrichia*, *Mrakia*, and *Aphanomyces* were significantly negatively correlated with yield in field 2. *Plectosphaerella* is likely acting as a plant pathogen in low yield crops (Palm *et al.*, 1995; Raimondo & Carlucci, 2018). While the genus *Chalara* contains species that are plant pathogens (Koukol, 2011; Kowalski & Holdenrieder, 2009), it also contains saprobes and beneficial species (Koukol, 2011). Its significant association with lower yields suggests that this may be a pathogenic species.

Some species of *Herpotrichia* are known pathogens of conifers, including *Herpotrichia parasitica* which may be the causal agent of needle browning in silver fir (Kowalski & Andruch, 2012). Its correlation to corn and wheat yield are unclear. *Mrakia* may be important for nutrient cycling in the soil (Tsuji, 2018). *Aphanomyces* is a known plant pathogen and is well studied for causing root rot in pea plants (Pfender, 1983).

In field 3, *Tetracladium* was significantly positively correlated with yield. *Tetracladium* is likely displaying beneficial (Sati & Pant, 2019) rather than neutral effects (Sati & Arya, 2010) *Dactylonectria*, *Linnemannia,* and *Corynespora* were significantly negatively correlated with yield. *Dactylonectria* are potential plant pathogens and part of the family Nectriaceae, a family containing important plant pathogens (Malapi-Wight *et al.*, 2016). Species belonging to the genus *Corynespora* are also well-known plant pathogens (Ma *et al.*, 2013). *Linnemannia,* however, have been shown to promote growth when associated with plant roots (Vandepol *et al.*, 2022).

The taxa that shared the same types of association (positive or negative) with yield in other studies include *Neonectria, Dactylonectria, Pythium* and *Mrakia* (Bandara *et al.*, 2021; Kandasamy *et al.*, 2021). However, the genus *Chalara,* which was significantly negatively associated with yield in this study, was associated with high yield in the study by Kandasamy *et al.* (2021). *Corynespora* were significantly negatively associated with yield in this study. In the study by Bandara *et al.* (2021), *Corynespora cassiicola* was a hub in networks created for both low and high yield soil types with rhizosphere soil and roots. They found *Corynespora* in high abundance in their high yield rhizosphere and bulk soil sites and suggested that the pathogenicity of this genus was decreased in the high yield sites (Bandara *et al.*, 2021).

There were some limitations to the study design of this project. In each farm, a single high and low yielding patch was chosen by the industrial partner with input from the farmers before I started on the project. Ideally, multiple high and low yielding patches sampled in each field would have allowed for a better picture of the dynamics of each field. Sampling several productivity sites would help better identify the key taxa and soil factors driving differences in productivity in the same field and reduce the impact of the

independence of a single high yielding and low yielding patch. Reliance on only two productivity patches from each field may also have accentuated the between-farm differences seen in this study.

The identification of taxa significantly positively associated with yield supports the hypotheses that there are crop-specific differences in community composition and that there are indicator taxa present in high and low yield sites across fields. Overall, the trends in the rhizosphere mycobiome that correlated with actual yield aligned more consistently with knowledge of biological mechanisms of soil fungi when compared to trends seen in high and low yield sites. This emphasizes the importance of measuring fungal community trends against actual yield and other soil physicochemical factors in order to identify species and soil variables that are predictors of crop productivity.

Chapter 5

5 Conclusions and future directions

In this project, I successfully used ITS2, LSUA and LSUBG primers to amplify fungal DNA from corn rhizosphere soil, corn root, wheat rhizosphere soil and wheat root samples from historically and low yield sites of three fields that have a history of corn-soybean-wheat crop rotation. Significant differences in fungal species composition were found between each of the fields, requiring each field to be analyzed separately. Root samples exhibited lower alpha diversity compared to rhizosphere samples but there were no discernible trends between the alpha diversity of corn and wheat rhizosphere samples. The overall fungal community composition of crops (wheat or corn), types (rhizosphere soil or root) and sample types (high/low yield corn rhizosphere soil, high/low yield corn root, high/low wheat rhizosphere and high and low wheat root) were significantly different from one another in the same field. Species that differed in abundance between fields and sample types within each field that may be driving these differences include *Tausonia pullulans, Pythium biforme,* and *Linnemannia gamsii*. Fungal community composition in corn and wheat rhizosphere soils was found to be significantly correlated with yield measured at harvest. The indicator species analysis of rhizosphere soils in each field revealed some key species that may be driving the differences in community composition of high and low yield sites, including *Pseudeurotium bakeri*, *Cryptococcus watticus, Solicoccozyma terrricola,* and *Candida sake,* which were significantly associated with high yield sites, and *Leptodontidium camptobactrum* and *Soloacrosporiella,* which were significantly associated with low yield sites. Differences in fungal community composition of samples was correlated with soil physicochemical variables, including K ppm, Mn ppm, S ppm, Fe ppm, pH, and Mg ppm. Several soil physicochemical variables were significantly associated with yield of wheat and corn, with some soil characteristics differing between the two crops. Soil texture was an important parameter, along with % H, Fe ppm, soil moisture, pH, Ca ppm, CEC (cation exchange capacity), NO_3-N, Mn ppm, Mg ppm, and Al ppm. Genera were identified as being significantly correlated with yield measured at harvest. Notably, *Fusicolla, Epicoccum,* and *Tetracladium* were positively correlated with yield, and

Plectosphaerella, Neonectria, Dactylonectria, Aphanomyces, Pythium, Corynespora, and *Mrakia* were negatively correlated with yield. While there were some limitations to the study design, the results of this study emphasize the importance of comprehensive integrative analysis of soil fungi, soil characteristics, and yield. Future studies should aim to sample multiple high and low yielding locations within a field to achieve a more comprehensive look at community composition and its relation to crop productivity, while reducing the impact of spatial dynamics. Identifying specific compositional shifts and differences in the root-associated mycobiome of crops, as well as the corresponding changes in the abiotic soil environment, could aid in the development of soil inocula or other community management tools that maximize crop productivity. These strategies can be integrated into current soil health management systems to increase agricultural yields and productivity in a low-input and sustainable manner.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U. (2021). *UNITE general FASTA release for Fungi 2* (10.05.2021). UNITE Community. https://doi.org/10.15156/BIO/1280089

Adesemoye, A., Mayorquin, J., Peacock, B., & Moreno, K. (2017). Association of *Neonectria macrodidyma* with dry root rot of citrus in California. *Journal of Plant Pathology & Microbiology*, *08*(01). https://doi.org/10.4172/2157-7471.1000391

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*(1), 134–147. https://doi.org/10.1111/2041-210X.12849

Ali, S., Saldias, S., Weerasuriya, N., Delaney, K., Kandasamy, S., & Lazarovits, G. (2020). Corn microbial diversity and its relationship to yield. *Canadian Journal of Microbiology*, *66*(8), 457–473. https://doi.org/10.1139/cjm-2020-0002

Allen, M. F. (2007). Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal*, *6*(2), 291–297. https://doi.org/10.2136/vzj2006.0068

Allen, S. E., Grimshaw, H. M., Parkinson, J. A., & Quarmby, C. (Eds.). (1974). *Chemical Analysis of Ecological Materials* (p. 252). Blackwell Scientific Publications.

Anderson, J. M., & Ingram, J. S. I. (Eds.). (1993). Chemical analyses. In *Tropical Soil Biology and Fertility: A Handbook of Methods* (2nd ed., pp. 57–93). C.A.B. International.

Antoninka, A. J., Ritchie, M. E., & Johnson, N. C. (2015). The hidden Serengeti—mycorrhizal fungi respond to environmental gradients. *Pedobiologia*, *58*(5–6), 165–176. https://doi.org/10.1016/j.pedobi.2015.08.001

Asemaninejad, A., Weerasuriya, N., Gloor, G. B., Lindo, Z., & Thorn, R. G. (2016). New primers for discovering fungal diversity using nuclear larger ribosomal DNA. *PLOS*

ONE, *11*(7), e0159043. https://doi.org/10.1371/journal.pone.0159043

Aula, L., Dhillon, J. S., Omara, P., Wehmeyer, G. B., Freeman, K. W., & Raun, W. R. (2019). World sulfur use efficiency for cereal crops. *Agronomy Journal*, *111*(5), 2485–2492. https://doi.org/10.2134/agronj2019.02.0095

Bahroun, A., Jousset, A., Mrabet, M., Mhamdi, R., & Mhadhbi, H. (2021). Protists modulate *Fusarium* root rot suppression by beneficial bacteria. *Applied Soil Ecology*, *168*, 104158. https://doi.org/10.1016/j.apsoil.2021.104158

Baird, R., Rice, C., & Eaton, A. (2017). *Standard methods for the examination of water and wastewater* (23rd ed.). American Public Health Association.

Bandara, A. Y., Weerasooriya, D. K., Trexler, R. V., Bell, T. H., & Esker, P. D. (2021). Soybean roots and soil from high- and low-yielding field sites have different microbiome composition. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.675352

Barilli, E., Cobos, M. J., & Rubiales, D. (2016). Clarification on host range of *Didymella pinodes* the causal agent of pea ascochyta blight. *Frontiers in Plant Science*, *7*, 592. https://doi.org/10.3389/fpls.2016.00592

Baron, N. C., & Rigobelo, E. C. (2022). Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology*, *13*(1), 39–55. https://doi.org/10.1080/21501203.2021.1945699

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiology*, *10*(1), 189. https://doi.org/10.1186/1471-2180-10-189

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges.

Microbiome, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Brown, A. E., Finlay, R., & Ward, J. S. (1987). Antifungal compounds produced by *Epicoccum purpurascens* against soil-borne plant pathogenic fungi. *Soil Biology and Biochemistry*, *19*(6), 657–664. https://doi.org/10.1016/0038-0717(87)90044-7

Brundrett, M. (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews of the Cambridge Philosophical Society*, *79*(3), 473–495. https://doi.org/10.1017/S1464793103006316

Brundrett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil*, *320*(1–2), 37–77. https://doi.org/10.1007/s11104-008-9877-9

Buscot, F., Munch, J. C., Charcosset, J. Y., Gardes, M., Nehls, U., & Hampp, R. (2000). Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. *FEMS Microbiology Reviews*, *24*(5), 601–614. https://doi.org/10.1111/j.1574-6976.2000.tb00561.x

Cadete, R. M., Lopes, M. R., & Rosa, C. A. (2017). Yeasts associated with decomposing plant material and rotting wood. In *Yeasts in Natural Ecosystems: Diversity* (pp. 265–292). Springer International Publishing. https://doi.org/10.1007/978-3-319-62683-3_9

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes,

S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, *6*(8), 1621–1624. https://doi.org/10.1038/ismej.2012.8

Cavalier-Smith, T., & Chao, E. E.-Y. (2003). Phylogeny and classification of phylum Cercozoa (Protozoa). *Protist*, *154*(3–4), 341–358. https://doi.org/10.1078/143446103322454112

Chang, H.-X., Haudenshield, J. S., Bowen, C. R., & Hartman, G. L. (2017). Metagenome-wide association study and machine learning prediction of bulk soil microbiome and crop productivity. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00519

Chen, H., & Boutros, P. C. (2011). VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics*, *12*(1), 35. https://doi.org/10.1186/1471-2105-12-35

Chen, S., Wang, F., Zhang, Y., Qin, S., Wei, S., Wang, S., Hu, C., & Liu, B. (2018). Organic carbon availability limiting microbial denitrification in the deep vadose zone. *Environmental Microbiology*, *20*(3), 980–992. https://doi.org/10.1111/1462-2920.14027

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Austral Ecology*, *18*(1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., & Smalla, K. (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiology Ecology*, *56*(2), 236–249.

https://doi.org/10.1111/j.1574-6941.2005.00026.x

Czaplicki, L. (2017). *RDP LSU taxonomic training data formatted for DADA2 (trainingset 11)*. https://doi.org/10.5281/ZENODO.835855

da Silva, L. L., Veloso, T. G. R., Manhães, J. H. C., da Silva, C. C., & de Queiroz, M. V. (2020). The plant organs and rhizosphere determine the common bean mycobiome. *Brazilian Journal of Microbiology*, *51*(2), 765–772. https://doi.org/10.1007/s42770-019-00217-9

De Cáceres, M., Legendre, P., & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos*, *119*(10), 1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

De Filippis, F., Laiola, M., Blaiotta, G., & Ercolini, D. (2017). Different amplicon targets for sequencing-based studies of fungal diversity. *Applied and Environmental Microbiology*, *83*(17), e00905-17. https://doi.org/10.1128/AEM.00905-17

DeAngelis, K. M., Brodie, E. L., DeSantis, T. Z., Andersen, G. L., Lindow, S. E., & Firestone, M. K. (2009). Selective progressive response of soil microbial community to wild oat roots. *The ISME Journal*, *3*(2), 168–178. https://doi.org/10.1038/ismej.2008.103

Dentinger, B. T. M., Didukh, M. Y., & Moncalvo, J.-M. (2011). Comparing COI and ITS as DNA barcode markers for mushrooms and allies (Agaricomycotina). *PLoS ONE*, *6*(9), e25081. https://doi.org/10.1371/journal.pone.0025081

Dias, T., Dukes, A., & Antunes, P. M. (2014). Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. *Journal of the Science of Food and Agriculture*, *95*(3), 447–454. https://doi.org/10.1002/jsfa.6565

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communications*, *10*(1), 2369. https://doi.org/10.1038/s41467-019-10373-z

Fellbaum, C. R., Gachomo, E. W., Beesetty, Y., Choudhari, S., Strahan, G. D., Pfeffer, P. E., Kiers, E. T., & Bücking, H. (2012). Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(7), 2666–2671. https://doi.org/10.1073/pnas.1118650109

Ferrari, B. C., Zhang, C., & van Dorst, J. (2011). Recovering greater fungal diversity from pristine and diesel fuel contaminated sub-antarctic soil through cultivation using both a high and a low nutrient media approach. *Frontiers in Microbiology*, *2*, 217. https://doi.org/10.3389/fmicb.2011.00217

Garbeva, P., van Elsas, J. D., & van Veen, J. A. (2008). Rhizosphere microbial community and its response to plant species and soil history. *Plant and Soil*, *302*(1–2), 19–32. https://doi.org/10.1007/s11104-007-9432-0

Ghadam Khani, A., Enayatizamir, N., & Norouzi Masir, M. (2019). Impact of plant growth promoting rhizobacteria on different forms of soil potassium under wheat cultivation. *Letters in Applied Microbiology*, *68*(6), 514–521. https://doi.org/10.1111/lam.13132

Gloor, G. B., Hummelen, R., Macklaim, J. M., Dickson, R. J., Fernandes, A. D., MacPhee, R., & Reid, G. (2010). Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products. *PLoS ONE*, *5*(10), e15406. https://doi.org/10.1371/journal.pone.0015406

Gollner, M. J., Püschel, D., Rydlová, J., & Vosátka, M. (2006). Effect of inoculation with soil yeasts on mycorrhizal symbiosis of maize. *Pedobiologia*, *50*(4), 341–345. https://doi.org/10.1016/j.pedobi.2006.06.002

Griffiths, B. S., Ritz, K., Bardgett, R. D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S. J., Bååth, E., Bloem, J., De Ruiter, P. C., Dolfing, J., & Nicolardot, B. (2000). Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. *Oikos*, *90*(2), 279–294. https://doi.org/10.1034/j.1600-0706.2000.900208.x

Grudzinska-Sterno, M., Yuen, J., Stenlid, J., & Djurle, A. (2016). Fungal communities in organically grown winter wheat affected by plant organ and development stage. *European Journal of Plant Pathology*, *146*(2), 401–417. https://doi.org/10.1007/s10658-016-0927-5

Gu, Z. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. In *Bioinformatics*. http://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html

Guo, J. H., Liu, X. J., Zhang, Y., Shen, J. L., Han, W. X., Zhang, W. F., Christie, P., Goulding, K. W. T., Vitousek, P. M., & Zhang, F. S. (2010). Significant acidification in major Chinese croplands. *Science*, *327*(5968), 1008–1010. https://doi.org/10.1126/science.1182570

Gupta, U. C. (1980). Boron Nutrition Of Crops. In *Advances in Agronomy* (pp. 273–307). https://doi.org/10.1016/S0065-2113(08)60142-X

Hafeez, B., Khanif, Y. M., & Saleem, M. (2013). Role of zinc in plant nutrition- a review. *American Journal of Experimental Agriculture*, *3*(2), 374–391.

Hassouna, N., Mithot, B., & Bachellerie, J. P. (1984). The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research*, *12*(8), 3563–3583. https://doi.org/10.1093/nar/12.8.3563

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London.*

Series B: Biological Sciences, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Jones Jr, J. B. (Ed.). (1999). *Soil analysis handbook of reference methods* (1st ed.). CRC Press.

Kandasamy, S., Weerasuriya, N., Subramanian, G., Thorn, R. G., Patterson, G., Ali, S., & Lazarovits, G. (2021). Disentangling the association of corn root mycobiome with plant productivity and the importance of soil physicochemical balance in shaping their relationship. *Frontiers in Sustainable Food Systems, 5*, 617332. https://doi.org/10.3389/fsufs.2021.617332

Kopylova, E., Navas-Molina, J. A., Mercier, C., Xu, Z. Z., Mahé, F., He, Y., Zhou, H.-W., Rognes, T., Caporaso, J. G., & Knight, R. (2016). Open-source sequence clustering methods improve the state of the art. *MSystems*, *1*(1), e00003-15. https://doi.org/10.1128/msystems.00003-15

Koukol, O. (2011). New species of *Chalara* occupying coniferous needles. *Fungal Diversity*, *49*(1), 75–91. https://doi.org/10.1007/s13225-011-0092-2

Kowalski, T., & Andruch, K. (2012). Mycobiota in needles of *Abies alba* with and without symptoms of *Herpotrichia* needle browning. *Forest Pathology*, *42*(3), 183–190. https://doi.org/10.1111/j.1439-0329.2011.00738.x

Kowalski, T., & Holdenrieder, O. (2009). Pathogenicity of *Chalara fraxinea*. *Forest Pathology*, *39*(1), 1–7. https://doi.org/10.1111/j.1439-0329.2008.00565.x

Kurtzman, C. P., & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *73*(4), 331–371. https://doi.org/10.1023/A:1001761008817

Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, *40*(9), 2407–2415.

https://doi.org/10.1016/j.soilbio.2008.05.021

Lay, C. Y., Bell, T. H., Hamel, C., Harker, K. N., Mohr, R., Greer, C. W., Yergeau, É., & St-Arnaud, M. (2018). Canola root-associated microbiomes in the Canadian prairies. *Frontiers in Microbiology*, *9*(JUN), 1188. https://doi.org/10.3389/fmicb.2018.01188

Li, W., Long, Y., Mo, F., Shu, R., Yin, X., Wu, X., Zhang, R., Zhang, Z., He, L., Chen, T., & Chen, J. (2021). Antifungal activity and biocontrol mechanism of *Fusicolla violacea* j-1 against soft rot in kiwifruit caused by *Alternaria alternata*. *Journal of Fungi*, *7*(11), 937. https://doi.org/10.3390/jof7110937

Liu, X., & Zhang, F. (2011). Nitrogen fertilizer induced greenhouse gas emissions in China. *Current Opinion in Environmental Sustainability*, *3*(5). https://doi.org/10.1016/j.cosust.2011.08.006

Lund, M. G., Carter, P. R., & Oplinger, E. S. (1993). Tillage and crop rotation affect corn, soybean,and winter wheat yields. *Journal of Production Agriculture*, *6*(2), 207–213. https://doi.org/10.2134/jpa1993.0207

Lynch, M. D. J., & Neufeld, J. D. (2015). Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology*, *13*(4), 217–229. https://doi.org/10.1038/nrmicro3400

Ma, L. J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., Gardiner, D. M., Manners, J. M., & Kazan, K. (2013). *Fusarium* pathogenomics. *Annual Review of Microbiology*, *67*(1), 399–416. https://doi.org/10.1146/annurev-micro-092412-155650

Malapi-Wight, M., Salgado-Salazar, C., Demers, J., Veltri, D., & Crouch, J. A. (2016). Draft genome sequence of *Dactylonectria macrodidyma*, a plant-pathogenic fungus in the Nectriaceae. *Genome Announcements*, *3*(2). https://doi.org/10.1128/genomeA.00278-15

Manthey, J., Crowley, D. E., & Luster, D. G. (1994). *Biochemistry of metal micronutrients in the rhizosphere* (pp. 295–307). CRC Press. https://books.google.ca/books?hl=en&lr=&id=fN5IgfbW2S4C&oi=fnd&pg=PA1&o

ts=cHh_9V2Yo2&sig=tcv55k0Nj_tCdih4SrJiyQoNTnU&redir_esc=y#v=onepage&q&f=false

Mao, W., Carroll, R. B., & Whittington, D. P. (1998). Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. *Plant Disease*, *82*(3), 337–342. https://doi.org/10.1094/PDIS.1998.82.3.337

Mašínová, T., Bahnmann, B. D., Větrovský, T., Tomšovský, M., Merunková, K., & Baldrian, P. (2017). Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiology Ecology*, *93*(2), fiw223. https://doi.org/10.1093/femsec/fiw223

McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecological Applications*, *24*(3), 560–570. https://doi.org/10.1890/13-0616.1

McGinnis, S., & Madden, T. L. (2004). BLAST: At the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Research*, *32*(WEB SERVER ISS.), W20–W25. https://doi.org/10.1093/nar/gkh435

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*(4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Melouk, H. A. (1974). *Verticillium nigrescens* from peppermint. *Phytopathology*, *64*(9), 1267–1268. https://doi.org/10.1094/phyto-64-1267

Menkis, A., & Burokiene, D. (2012). Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery. *Forest Pathology*, *42*(1), 79–83. https://doi.org/10.1111/j.1439-0329.2011.00712.x

Mercado Vergnes, D., Renard, M. E., Duveiller, E., & Maraite, H. (2006). Identification of *Alternaria* spp. on wheat by pathogenicity assays and sequencing. *Plant Pathology*, *55*(4), 485–493. https://doi.org/10.1111/j.1365-3059.2006.01391.x

Moncalvo, J. M., Vilgalys, R., Redhead, S. A., Johnson, J. E., James, T. Y., Catherine Aime, M., Hofstetter, V., Verduin, S. J. W., Larsson, E., Baroni, T. J., Greg Thorn, R., Jacobsson, S., Clémençon, H., & Miller, O. K. (2002). One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution*, *23*(3), 357–400. https://doi.org/10.1016/S1055-7903(02)00027-1

Mousavi, S. R., Shahsavari, M., & Rezaei, M. (2011). A general overview on manganese (Mn) importance for crop production. *Australian Journal of Basic and Applied Sciences*, *5*(9), 1799–1803.

Muneer, M. A., Huang, X., Hou, W., Zhang, Y., Cai, Y., Munir, M. Z., Wu, L., & Zheng, C. (2021). Response of fungal diversity, community composition, and functions to nutrients management in red soil. *Journal of Fungi*, *7*(7), 554. https://doi.org/10.3390/jof7070554

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2019). Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*, *17*(2), 95–109. https://doi.org/10.1038/s41579-018-0116-y

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (939th ed.). US Department of Agriculture.

Ozimek, E., & Hanaka, A. (2021). *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. In *Agriculture (Switzerland)* (Vol. 11, Issue 1, pp. 1–18). https://doi.org/10.3390/agriculture11010007

Palm, M. E., Gams, W., & Nirenberg, H. I. (1995). *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. *Mycologia*, *87*(3), 397–406. https://doi.org/10.1080/00275514.1995.12026545

Patel, J. S., Singh, A., Singh, H. B., & Sarma, B. K. (2015). Plant genotype, microbial recruitment and nutritional security. *Frontiers in Plant Science*, *6*(AUG), 608.

https://doi.org/10.3389/fpls.2015.00608

Perera, R. H., Hyde, K. D., Maharachchikumbura, S. S. N., Jones, E. B. G., McKenzie, E. H. C., Stadler, M., Lee, H. B., Samarakoon, M. C., Ekanayaka, A. H., Camporesi, E., Liu, J. K., & Liu, Z. Y. (2020). Fungi on wild seeds and fruits. *Mycosphere*, *11*(1), 2108–2480. https://doi.org/10.5943/MYCOSPHERE/11/1/14

Pettigrew, W. T. (2008). Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiologia Plantarum*, *133*(4), 670–681. https://doi.org/10.1111/j.1399-3054.2008.01073.x

Pfender, W. F. (1983). Disease progress and yield loss in *Aphanomyces* root rot of peas. *Phytopathology*, *73*(8), 1109. https://doi.org/10.1094/phyto-73-1109

Philippot, L., Raaijmakers, J. M., Lemanceau, P., & Van Der Putten, W. H. (2013). Going back to the roots: The microbial ecology of the rhizosphere. In *Nature Reviews Microbiology* (Vol. 11, Issue 11, pp. 789–799). https://doi.org/10.1038/nrmicro3109

Poelman, A., Weerasuriya, N., Vanderwolf, K. J., Malloch, D., McAlpine, D. F., & Thorn, R. G. (2021). Fungi associated with aeroponic roots in caves and mines of New Brunswick. *Fungal Ecology*, *52*, 101074. https://doi.org/10.1016/j.funeco.2021.101074

Qian, L., Yu, W. J., Cui, J. Q., Jie, W. G., & Cai, B. Y. (2015). *Funneliformis mosseae* affects the root rot pathogen *Fusarium oxysporum* in soybeans. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *65*(4), 321–328. https://doi.org/10.1080/09064710.2015.1009938

Raimondo, M. L., & Carlucci, A. (2018). Characterization and pathogenicity assessment of *Plectosphaerella* species associated with stunting disease on tomato and pepper crops in Italy. *Plant Pathology*, *67*(3), 626–641. https://doi.org/10.1111/ppa.12766

Rashid, M. I., Mujawar, L. H., Shahzad, T., Almeelbi, T., Ismail, I. M. I., & Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate

formation in degraded soils. *Microbiological Research, 183*, 26–41. https://doi.org/10.1016/j.micres.2015.11.007

Robinson, T. P., William Wint, G. R., Conchedda, G., Van Boeckel, T. P., Ercoli, V., Palamara, E., Cinardi, G., D'Aietti, L., Hay, S. I., & Gilbert, M. (2014). Mapping the global distribution of livestock. *PLoS ONE*, *9*(5), e96084. https://doi.org/10.1371/journal.pone.0096084

Rout, G. R., & Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, *3*, 1–24. https://doi.org/10.7831/ras.3.1

Sati, S. C., & Arya, P. (2010). Assessment of root endophytic aquatic hyphomycetous fungi on plant growth. *Symbiosis*, *50*(3), 143–149. https://doi.org/10.1007/s13199-010-0054-6

Sati, S. C., & Pant, P. (2019). Two root endophytic aquatic*Hyphomycetes*, *Campylospora parvula* and *Tetracladium setigerum* as plant growth promoters. *Asian Journal of Agricultural Research, 14*(1), 28–33. https://doi.org/10.3923/ajar.2020.28.33

Sato, K., Suyama, Y., Saito, M., & Sugawara, K. (2005). A new primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. *Grassland Science, 51*(2), 179–181. https://doi.org/10.1111/j.1744-697X.2005.00023.x

Schnitzer, S. A., Klironomos, J. N., HilleRisLambers, J., Kinkel, L. L., Reich, P. B., Xiao, K., Rillig, M. C., Sikes, B. A., Callaway, R. M., Mangan, S. A., van Nes, E. H., & Scheffer, M. (2011). Soil microbes drive the classic plant diversity–productivity pattern. *Ecology*, *92*(2), 296–303. https://doi.org/10.1890/10-0773.1

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K.-D., Bai, F.-Y., Barreto, R. W., Begerow, D., Bergeron, M.-J., Blackwell, M., … Schindel, D. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of*

the National Academy of Sciences, *109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

Scibetta, S., Schena, L., Abdelfattah, A., Pangallo, S., & Cacciola, S. O. (2018). Selection and experimental evaluation of universal primers to study the fungal microbiome of higher plants. *Phytobiomes Journal*, *2*(4), 225–236. https://doi.org/10.1094/PBIOMES-02-18-0009-R

Seaman, W. L., Shoemaker, R. A., & Peterson, E. A. (1965). Pathogenicity of *Corynespora cassiicola* on soybean. *Canadian Journal of Botany*, *43*(11), 1461–1469. https://doi.org/10.1139/b65-154

Smith, S., & Read, D. (2008). Mycorrhizal symbiosis. In *Mycorrhizal Symbiosis*. Elsevier. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

Ssekagiri, A. (2022). *microbiomeSeq: Microbial community analysis in an environmental context.* https://github.com/umerijaz/microbiomeSeq

Staskawicz, B. J. (2001). Genetics of plant-pathogen interactions specifying plant disease resistance. *Plant Physiology*, *125*(1), 73–76. https://doi.org/10.1104/pp.125.1.73

StatsCan. (2021). *Canada: Outlook for principal field crops*. https://www150.statcan.gc.ca/n1/daily-quotidien/210830/dq210830b-eng.htm?indid=812-1&indgeo=0

Sutton, J. C., Sopher, C. R., Owen-Going, T. N., Liu, W., Grodzinski, B., Hall, J. C., & Benchimol, R. L. (2006). Etiology and epidemiology of *Pythium* root rot in hydroponic crops: current knowledge and perspectives. *Summa Phytopathologica*, *32*(4), 307–321. https://doi.org/10.1590/S0100-54052006000400001

Syuhada, N., Sarwar Jahan, M., Moneruzzaman Khandaker, M., Mat, N., Khairi, M., Nozulaidi, M., & Hudzari bin Razali, M. (2014). Application of copper increased corn yield through enhancing physiological functions. *Australian Journal of Basic and Applied Sciences*, *8*(16), 282–286.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Taylor, D. L., Walters, W. A., Lennon, N. J., Bochicchio, J., Krohn, A., Caporaso, J. G., & Pennanen, T. (2016). Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. *Applied and Environmental Microbiology*, *82*(24), 7217–7226. https://doi.org/10.1128/AEM.02576-16

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, H., Hildebrand, F., Bork, P., & Abarenkov, K. (2015). Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys*, *10*, 1–43. https://doi.org/10.3897/mycokeys.10.4852

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios, A. M., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213). https://doi.org/10.1126/science.1256688

Tedersoo, L., Bahram, M., Toots, M., Diedhiu, A. G., Henkel, T. W., Kjoller, R., Morris, M. H., Nara, K., Nouhra, E., Peay, K. G., PÕLME, S., RYBERG, M., SMITH, M. E., & KÕLJALG, U. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, *21*(17), 4160–4170. https://doi.org/10.1111/j.1365-294X.2012.05602.x

Tedersoo, L., Pärtel, K., Jairus, T., Gates, G., Põldmaa, K., & Tamm, H. (2009). Ascomycetes associated with ectomycorrhizas: Molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology*, *11*(12), 3166–3178. https://doi.org/10.1111/j.1462-2920.2009.02020.x

Thomma, B. P. H. J. (2003). Alternaria spp.: From general saprophyte to specific parasite. *Molecular Plant Pathology*, *4*(4), 225–236. https://doi.org/10.1046/j.1364-3703.2003.00173.x

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, *108*(50), 20260–20264. https://doi.org/10.1073/pnas.1116437108

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, *441*(7093), 629–632. https://doi.org/10.1038/nature04742

Tkacz, A., Cheema, J., Chandra, G., Grant, A., & Poole, P. S. (2015). Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. *ISME Journal*, *9*(11), 2349–2359. https://doi.org/10.1038/ismej.2015.41

Tkacz, A., & Poole, P. (2015). Role of root microbiota in plant productivity. *Journal of Experimental Botany*, *66*(8), 2167–2175. https://doi.org/10.1093/jxb/erv157

Tsuji, M. (2018). Genetic diversity of yeasts from East Ongul Island, East Antarctica and their extracellular enzymes secretion. *Polar Biology*, *41*(2), 249–258. https://doi.org/10.1007/s00300-017-2185-1

Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the human microbiome. *Nutrition Reviews*, *70*(SUPPL. 1), S38–S44. https://doi.org/10.1111/j.1753-4887.2012.00493.x

Uzuhashi, S., Okada, G., & Ohkuma, M. (2015). Four new *Pythium* species from aquatic environments in Japan. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *107*(2), 375–391. https://doi.org/10.1007/s10482-014-0336-8

Valero-Mora, P. M. (2010). ggplot2: Elegant Graphics for Data Analysis. *Journal of Statistical Software*, *35*(Book Review 1). https://doi.org/10.18637/jss.v035.b01

Van Der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., & Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature, 396*(6706), 69–72. https://doi.org/10.1038/23932

Vandepol, N., Liber, J., Desirò, A., Na, H., Kennedy, M., Barry, K., Grigoriev, I. V., Miller, A. N., O'Donnell, K., Stajich, J. E., & Bonito, G. (2020). Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. *Fungal Diversity*, *104*(1), 267–289. https://doi.org/10.1007/s13225-020-00455-5

Vandepol, N., Liber, J., Yocca, A., Matlock, J., Edger, P., & Bonito, G. (2022). *Linnemannia elongata* (Mortierellaceae) stimulates *Arabidopsis thaliana* aerial growth and responses to auxin, ethylene, and reactive oxygen species. *PLoS ONE*, *17*(4 April), e0261908. https://doi.org/10.1371/journal.pone.0261908

Wang, C. C., Liu, H. Z., Liu, M., Zhang, Y. Y., Li, T. T., & Lin, X. K. (2011). Cytotoxic metabolites from the soil-derived fungus *Exophiala pisciphila*. *Molecules*, *16*(4), 2796–2801. https://doi.org/10.3390/molecules16042796

Wang, C., Hou, X., Islam, Z. U., Zhang, Z., Zhu, B., & Yang, T. (2022). Driving factors of microbial community abundance and structure in typical forest soils of Sanjiang Plain, Northeast China. *Sustainability*, *14*(13), 8040. https://doi.org/10.3390/su14138040

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wei, T., & Simko, V. (2017). *R package "corrplot": Visualization of a correlation matrix*. https://github.com/taiyun/corrplot

Westcott, S. L., & Schloss, P. D. (2015). De novo clustering methods outperform

reference-based methods for assigning 16S rRNA gene sequences to operational taxonomic units. *PeerJ*, *2015*(12), e1487. https://doi.org/10.7717/peerj.1487

Wu, D., Ma, Y., Yang, T., Gao, G., Wang, D., Guo, X., & Chu, H. (2022). Phosphorus and zinc are strongly associated with belowground fungal communities in wheat field under long-term fertilization. *Microbiology Spectrum*, *10*(2), e0011022. https://doi.org/10.1128/spectrum.00110-22

Xu, Y., Wang, G., Jin, J., Liu, J., Zhang, Q., & Liu, X. (2009). Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biology and Biochemistry*, *41*(5), 919–925. https://doi.org/10.1016/j.soilbio.2008.10.027

Zare, R., Gams, W., Starink-Willemse, M., & Summerbell, R. C. (2007). *Gibellulopsis*, a suitable genus for *Verticillium nigrescens*, and *Musicillium*, a new genus for *V. theobromae*. *Nova Hedwigia*, *85*(3–4), 463–489. https://doi.org/10.1127/0029-5035/2007/0085-0463

Zhang, C., Liu, G., Song, Z., Wang, J., & Guo, L. (2018). Interactions of soil bacteria and fungi with plants during long-term grazing exclusion in semiarid grasslands. *Soil Biology and Biochemistry*, *124*, 47–58. https://doi.org/10.1016/j.soilbio.2018.05.026

Appendices

Appendix A: A&L Canada Laboratories Inc. combine yield and rhizosphere soil test results.

(C = Corn, W = Wheat, F = Field, G = High yield, B = Low yield site, RS = Rhizosphere soil)

Sample ID	Yield (bu/ac)	Organic Matter	Bray-P1	K ppm	Mg ppm	Ca ppm	Na ppm	pH	CEC meq/100g	% K	% Mg	% Ca	% H	% Na
CF1G2RS	292	3.9	30	108	165	1900	10	6.1	12.4	2.2	11.1	76.8	9.5	0.4
CF1G4RS	292	4	25	118	155	1850	10	6.1	12.1	2.5	10.7	76.6	9.8	0.4
CF1G5RS	292	4.1	28	113	165	1880	10	6.1	12.3	2.4	11.2	76.5	9.6	0.4
CF1G7RS	292	3.8	16	109	160	1840	12	6.1	12	2.3	11.1	76.4	9.8	0.4
CF1G9RS	292	3.7	21	112	155	1760	10	5.9	16.4	1.8	7.9	53.6	36.5	0.3
CF1B2RS	247	3.4	25	211	165	3890	11	7.9	21.4	2.5	6.4	90.9	0	0.2
CF1B4RS	247	3.1	24	178	140	4320	10	7.8	23.3	2	5	92.9	0	0.2
CF1B5RS	247	3.5	39	253	165	3960	13	7.9	21.9	3	6.3	90.6	0	0.3
CF1B7RS	247	2.8	15	191	160	5330	12	7.6	28.5	1.7	4.7	93.5	0	0.2
CF1B9RS	247	2.9	17	186	135	4400	10	7.8	23.6	2	4.8	93.1	0	0.2
CF2G2RS	320	3.6	88	36	100	1100	8	5.6	8.8	1	9.4	62.2	27	0.4
CF2G4RS	320	4	109	64	120	1070	8	5.5	10.1	1.6	9.9	52.8	35.4	0.3
CF2G5RS	320	3.7	102	72	130	1050	9	5.4	7.7	2.4	14	67.8	15.3	0.5
CF2G7RS	320	3.6	93	54	115	1120	10	5.6	9.1	1.5	10.5	61.4	26.2	0.5
CF2G9RS	320	4	106	65	125	1100	8	5.6	7.9	2.1	13.1	69.4	15	0.4
CF2B2RS	239	3.6	31	61	145	1370	8	7.1	9.1	1.7	13.3	75.6	8.9	0.4
CF2B4RS	239	3.7	20	52	150	1340	6	7.3	8.1	1.6	15.4	82.8	0	0.3
CF2B5RS	239	3.9	26	62	165	1440	7	7.4	8.7	1.8	15.7	82.3	0	0.3
CF2B7RS	239	3.7	30	61	165	1410	8	7.3	8.6	1.8	16	82	0	0.4
CF2B9RS	239	3.8	25	60	155	1320	6	7.3	8.1	1.9	16	81.9	0	0.3
CF3G2RS	263	2.2	140	112	115	920	7	7	6.7	4.3	14.2	68.2	12.9	0.5
CF3G4RS	263	2.6	209	100	165	1270	7	6.9	9.2	2.8	15	69.1	12.9	0.3
CF3G5RS	263	2.6	189	107	130	1040	8	6.6	7.8	3.5	13.9	66.9	15.2	0.4
CF3G7RS	263	2.6	195	108	135	1210	7	6.8	8.7	3.2	13	69.8	13.7	0.4
CF3G9RS	263	2.5	218	130	145	1160	8	6.7	8.6	3.9	14.1	67.8	13.8	0.4
CF3B2RS	223	1.9	165	69	190	1140	6	7.4	7.5	2.4	21.2	76.4	0	0.3
CF3B4RS	223	2.5	217	105	210	1470	7	7.4	9.4	2.9	18.7	78.4	0	0.3
CF3B6RS	223	2.4	175	69	180	1240	7	7.5	7.9	2.2	19	78.6	0	0.4

Sample ID	Yield (bu/ac)	Organic Matter	Bray-P1	K ppm	Mg ppm	Ca ppm	Na ppm	pH	CEC meq/100g	% K	% Mg	% Ca	% H	% Na
CF3B8RS	223	2.2	194	78	210	1490	6	7.6	9.4	2.1	18.6	79.2	0	0.3
CF3B9RS	223	2.2	248	87	215	1380	7	7.4	8.9	2.5	20.1	77.3	0	0.3
WF1G1RS	99	3.3	20	275	174	2550	12	7.6	14.9	4.7	9.7	85.4	0	0.3
WF1G2RS	99	3.5	15	262	211	2250	12	7.4	13.7	4.9	12.8	82.1	0	0.4
WF1G3RS	99	3.3	18	212	186	2070	9	7.2	13.1	4.2	11.8	79.1	4.6	0.3
WF1G4RS	99	3.9	14	283	171	1860	8	6.7	12.7	5.7	11.2	73.4	9.3	0.3
WF1B1RS	67	3.7	47	270	196	2170	12	7.3	13.2	5.2	12.4	82.2	0	0.4
WF1B2RS	67	37	35	194	195	2310	15	7.4	13.7	3.6	11.8	84.2	0	0.5
WF1B3RS	67	3.7	39	248	205	2230	16	7.3	13.5	4.7	12.6	82.3	0	0.5
WF1B4RS	67	5	61	311	212	2200	13	7.1	15	5.3	11.8	73.5	8.9	0.4
WF1B5RS	67	4.7	66	358	206	2390	14	7.2	15.4	6	11.2	77.8	4.6	0.4
WF2G1RS	24.87	4.6	38	83	225	1760	10	6	16.9	1.3	11.1	52.1	35.3	0.3
WF2G2RS	24.87	4.5	34	85	235	1800	14	5.7	17.2	1.3	11.4	52.3	34.7	0.4
WF2G3RS	24.87	4.5	39	78	210	1660	8	5.8	16.3	1.2	10.8	51	36.8	0.2
WF2G4RS	24.87	4.4	49	81	210	1710	9	5.8	16.5	1.3	10.6	51.7	36.2	0.2
WF2G5RS	24.87	4.4	35	81	205	1740	10	5.9	16.6	1.2	10.3	52.3	35.9	0.3
WF2B1RS	92.21	3	28	80	245	1770	14	7.1	12.2	1.7	16.7	72.3	8.9	0.5
WF2B2RS	92.21	2.7	30	67	210	1500	13	7.3	9.5	1.8	18.5	79.3	0	0.6
WF2B3RS	92.21	2.7	36	64	210	1690	9	7.3	10.4	1.6	16.9	81.4	0	0.4
WF2B4RS	92.21	2.5	16	67	215	1640	10	7.5	10.2	1.7	17.6	80.5	0	0.4
WF2B5RS	92.21	2.8	32	79	220	1600	10	7.2	10.6	1.9	17.4	75.8	4.5	0.4
WF3G1RS	92.64	1.9	154	76	190	1290	12	7.3	8.3	2.4	19.2	78.1	0	0.6
WF3G2RS	92.64	2.3	171	77	195	1190	9	7.2	8.2	2.4	19.9	72.7	4.5	0.5
WF3G3RS	92.64	2.3	181	94	205	1200	11	6.9	9.2	2.6	18.6	65.4	12.8	0.5
WF3G4RS	92.64	1.8	196	93	175	1650	9	7.7	10	2.4	14.6	82.8	0	0.4
WF3G5RS	92.64	1.9	205	80	185	1470	9	7.5	9.1	2.3	16.9	80.6	0	0.4
WF3B1RS	85.08	1.8	158	108	220	1990	13	7.4	12.1	2.3	15.2	82.3	0	0.5
WF3B2RS	85.08	1.4	169	79	205	2070	12	7.5	12.3	1.6	13.9	84.2	0	0.4
WF3B4RS	85.08	2	227	110	255	2250	11	7.7	13.7	2.1	15.5	82.3	0	0.3
WF3B5RS	85.08	1.7	140	106	220	1520	9	7.5	9.7	2.8	18.9	78.2	0	0.4

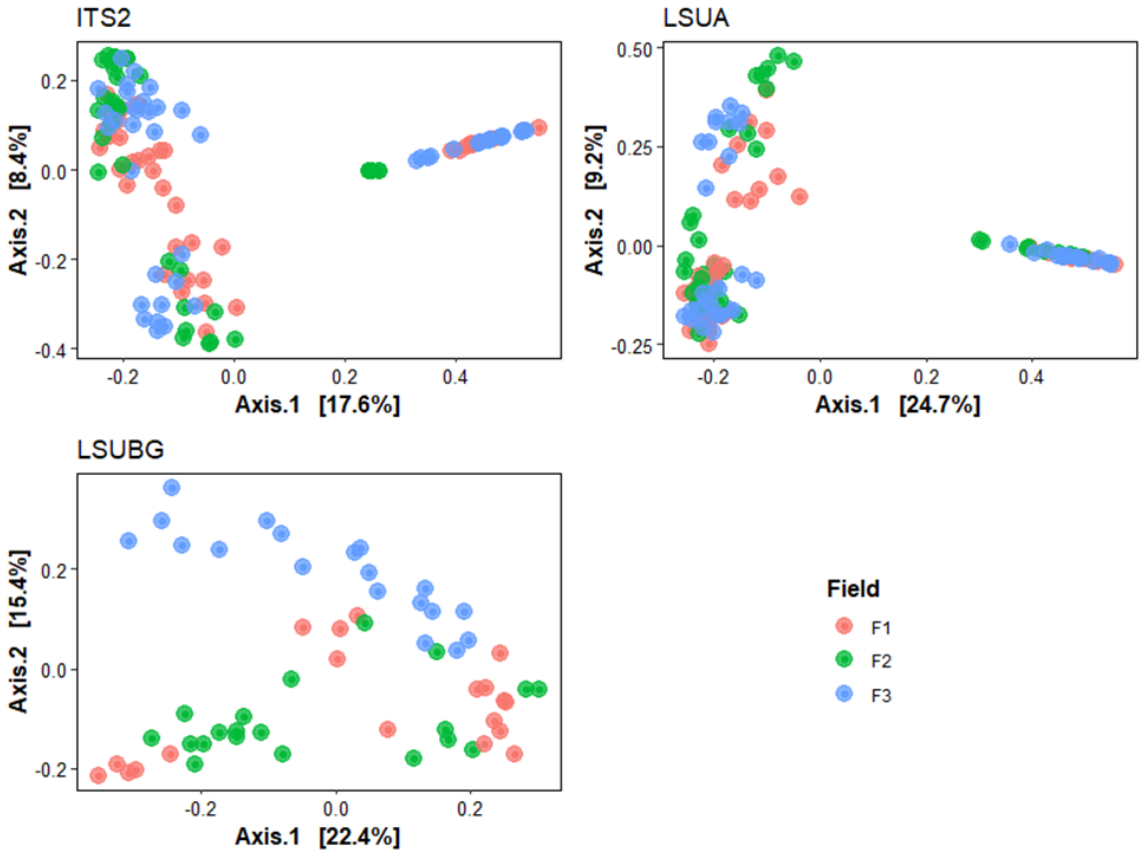
Appendix B: A&L Canada Laboratories Inc. rhizosphere soil test results.

(C = Corn, W = Wheat, F = Field, G = High yield, B = Low yield site, RS = Rhizosphere soil)

SampleID	S ppm	Zn ppm	Mn ppm	Fe ppm	Cu ppm	B ppm	Soluble salts (ms/cm)	Al ppm	Cl ppm	NO_3-N ppm	ENR	Sand %	Silt %	Clay %	Soil Moisture
CF1G2RS	21	4.2	46	64	1.9	0.4	0.35	894	12	16	51	26.9	56	17.1	12.25
CF1G4RS	14	3.1	48	63	1.9	0.5	0.29	881	17	12	52	16.9	66	17.1	12.75
CF1G5RS	15	3.5	46	64	1.8	0.4	0.3	866	8	13	53	16.9	66	17.1	14.55
CF1G7RS	24	3.6	56	59	1.6	0.4	0.41	830	22	23	50	16.9	66	17.1	14.7
CF1G9RS	16	3.1	52	62	1.6	0.4	0.31	856	18	14	49	18.9	62	19.1	16.9
CF1B2RS	11	2.5	88	53	1.2	0.5	0.34	400	16	13	46	22.9	44	33.1	6.5
CF1B4RS	10	2.1	81	51	1.2	0.4	0.52	457	30	36	43	28.9	42	29.1	10.5
CF1B5RS	19	5.8	88	53	1.2	0.6	0.4	431	20	15	47	26.9	46	27.1	6.65
CF1B7RS	12	2.4	87	50	1.1	0.4	0.57	553	24	36	40	22.9	44	33.1	10.2
CF1B9RS	10	1.8	79	49	1	0.4	0.44	454	33	25	41	24.9	44	31.1	8.75
CF2G2RS	11	2.6	7	75	1.9	0.1	0.2	901	10	6	48	36.9	56	7.1	19.25
CF2G4RS	12	4.7	8	73	1.8	0.1	0.22	870	12	8	52	36.9	56	7.1	17.45
CF2G5RS	12	5.2	8	94	2	0.2	0.21	965	13	6	49	36.9	54	9.1	15.1
CF2G7RS	15	3.9	7	78	1.9	0.1	0.24	923	11	8	48	38.9	50	11.1	13.15
CF2G9RS	11	4.9	7	72	1.7	0.1	0.21	891	13	7	52	36.9	52	11.1	15.95
CF2B2RS	8	2.3	30	63	0.9	0.3	0.2	1024	10	7	48	36.9	52	11.1	6.35
CF2B4RS	6	1.7	30	59	0.7	0.3	0.16	986	12	4	49	38.9	52	9.1	10.8
CF2B5RS	8	2.5	33	61	0.8	0.3	0.17	1037	10	3	51	40.9	52	7.1	10.4
CF2B7RS	8	2.8	32	62	0.7	0.3	0.19	1011	13	6	49	36.9	52	11.1	14.95
CF2B9RS	8	1.8	31	61	0.7	0.3	0.17	970	12	4	50	36.9	56	7.1	17.35
CF3G2RS	5	6.5	53	72	1.5	0.1	0.22	576	23	14	34	66.9	29.3	3.8	9.4
CF3G4RS	8	9.1	78	82	2.2	0.3	0.26	758	12	15	38	66.9	29.3	3.8	8.6

SampleID	S ppm	Zn ppm	Mn ppm	Fe ppm	Cu ppm	B ppm	Soluble salts (ms/cm)	Al ppm	Cl ppm	NO_3-N ppm	ENR	Sand %	Silt %	Clay %	Soil Moisture
CF3G5RS	7	7.7	74	85	1.9	0.2	0.25	768	14	16	38	64.9	31.3	3.8	7.7
CF3G7RS	8	8.4	74	81	2	0.2	0.42	712	16	37	38	66.9	27.3	5.8	5.7
CF3G9RS	7	9.4	75	84	2.1	0.2	0.26	771	13	17	37	66.9	29.3	3.8	7.1
CF3B2RS	5	7.7	70	70	1.8	0.2	0.16	551	36	6	31	66.9	27.3	5.8	1.2
CF3B4RS	7	10.5	91	77	2.8	0.3	0.23	660	13	11	37	66.9	25.3	7.8	1.8
CF3B6RS	6	8.1	86	76	2.2	0.2	0.16	621	17	4	36	68.9	23.3	7.8	5.1
CF3B8RS	6	8.6	92	76	2	0.2	0.17	669	10	5	34	68.9	25.3	5.8	0.8
CF3B9RS	7	11.1	95	81	2.7	0.3	0.19	711	13	6	34	66.9	27.3	5.8	4.5
WF1G1RS	6	3.4	90	56	1.6	0.5	0.2	698	11	2	45	20.2	55.2	24.6	26.96
WF1G2RS	7	2.8	82	66	1.7	0.5	0.2	793	8	2	47	22.2	57.2	20.6	26.66
WF1G3RS	6	4	85	56	1.8	0.4	0.2	754	8	2	45	18.2	61.2	20.6	27.6
WF1G4RS	7	2.4	70	58	1.6	0.4	0.2	755	8	3	51	12.3	62.6	25.1	29.98
WF1B1RS	9	7.5	67	69	1.6	0.5	0.2	749	11	2	49	18.2	45.2	36.6	30.92
WF1B2RS	7	4.2	61	67	1.6	0.5	0.2	728	10	2	49	20.2	43.2	36.6	30.08
WF1B3RS	6	4.3	67	66	1.6	0.5	0.2	738	10	2	49	18.2	45.2	36.6	29.7
WF1B4RS	7	7.6	58	69	1.9	0.6	0.2	749	11	3	63	24.2	43.2	32.6	28.9
WF1B5RS	7	5.6	55	70	2.2	0.6	0.2	733	8	2	60	26.2	41.2	32.6	28.14
WF2G1RS	11	5	81	71	1.3	0.7	0.34	984	16	21	59	31.4	52	16.6	19.28
WF2G2RS	14	4.4	80	71	1.1	0.8	0.32	998	15	17	57	18.9	52	29.1	20.22
WF2G3RS	14	5.4	84	70	1.1	0.6	0.29	983	15	13	57	39.4	38	22.6	20.18
WF2G4RS	14	8.5	83	74	1.3	0.6	0.28	994	12	12	56	28.4	50	21.6	19.9
WF2G5RS	13	4.5	76	73	1.4	0.6	0.21	1088	13	3	56	27.4	51	21.6	19.54
WF2B1RS	15	4.7	122	75	0.7	0.8	0.23	884	14	4	42	34.9	43.3	21.8	18.94
WF2B2RS	12	5.5	107	73	0.6	0.6	0.18	819	25	1	39	36.9	41.3	21.8	19.5
WF2B3RS	9	6	107	69	0.6	0.6	0.21	834	12	6	39	46.9	40.3	12.8	19.46
WF2B4RS	9	2.9	105	68	0.6	0.6	0.18	855	15	2	37	46.9	39.3	13.8	18.4
WF2B5RS	13	5	105	68	0.6	0.6	0.22	832	21	5	40	47.9	38.3	13.8	18.64
WF3G1RS	9	8.7	89	87	2.6	0.7	0.18	820	17	4	31	67.9	22.3	9.8	15.

SampleID	S ppm	Zn ppm	Mn ppm	Fe ppm	Cu ppm	B ppm	Soluble salts (ms/cm)	Al ppm	Cl ppm	NO_3-N ppm	ENR	Sand %	Silt %	Clay %	Soil Moisture
															56
WF3G2RS	9	8.7	92	89	2	0.7	0.18	793	12	4	35	67.9	24.3	7.8	16.28
WF3G3RS	11	8.9	94	90	1.9	0.6	0.2	841	9	5	35	66.9	25.3	7.8	14.76
WF3G4RS	9	10.1	92	83	2.1	0.6	0.22	726	20	8	30	66.9	25.3	7.8	14.56
WF3G5RS	9	9.4	94	89	2.4	0.6	0.19	819	14	4	31	64.9	25.3	9.8	15.94
WF3B1RS	9	7.6	95	82	2	0.8	0.3	696	10	16	30	68.9	16.3	14.8	5.24
WF3B2RS	8	6.4	89	82	1.9	0.6	0.27	658	10	13	26	69.9	16.3	13.8	5.2
WF3B4RS	8	10.7	105	91	2.5	0.7	0.21	762	12	4	32	68.9	17.3	13.8	5.67
WF3B5RS	8	6.5	92	83	2	0.6	0.25	827	12	13	29	64.9	19.3	15.8	5.7



Appendix C: PCoA ordination plots of corn and wheat rhizosphere soil and roots from three fields.

Bray-Curtis Dissimilarity indices are plotted for mycobiome data established for all three fields. ITS2 and LSUA primers were used for 122 corn and wheat rhizosphere soil and root samples. LSUBG primers were used to for 58 corn and wheat rhizosphere soil samples.

Appendix D: Indicator species analysis for field, crop and type of ITS2 data.

Indicator species analysis was performed using the indicspecies package in R, and species that were significantly associated (p<0.01) with a field, crop and type are indicated in greeen. F = Field, C = Corn, W = Wheat, RS = Rhizosphere soil, RT = Root. ASVs were combined based on patterns of significance as well as phylogenetic similarity. Taxonomy is arranged alphabetically by genus.

Taxonomy	ASV	F 1	F 2	F 3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Alternaria alternata	75															
Alternaria infectoria	57, 58															
Articulospora proliferata	83															
Articulospora proliferata	13, 126															
Ascobolus sp.	413															
Cadophora	125, 274															
Candida sake	31															
cf. Hyphodiscus	238															
cf. Mycofalcella	66															
cf. Soloacrosporiella	199, 464															
cf. Vandijckella/ Mycoarthris sp.	65, 86, 236															
Chalara sp.	62, 92, 118															
Chloridium	91, 189															
Chytridiomycota	129															
Chytridiomycota	320															
Chytridiomycota	611															
Cladosporium cladosporioides	74, 159															
Cladosporium ramotenellum	93															
Clonostachys rosea	147															
Colletotrichum dematium	98															
Coniochaeta	350															
Coniochaeta canina	485															
Coniothyrium palmicola	393															
Conlarium sacchari	511															
Cordyceps memorabilis	217															
Corynespora cassiicola	105															
Cryptococcus watticus	198															
Dactylonectria torresensis	100															
Dictyosporiaceae	167															
Didymella americana	90, 240															
Dioszegia hungarica	246															

Taxonomy	ASV	F 1	F 2	F 3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Epicoccum nigrum	64, 241			■											■	
Exophiala equina	63, 242						■				■				■	
Exophiala pisciphila	432										■					
Fusarium brachygibbosum	213														■	
Fusarium graminearum	73										■					
Fusarium incarnatum	82						■				■				■	
Fusarium merismoides	89, 261						■				■				■	
Fusarium solani	172										■				■	
Fusarium verticillioides	556		■													
Fusidium	327									■						
Fusidium	333	■				■										
Helotiales	186	■														
Helotiales	244						■								■	
Heydenia alpina/ Lasiobolidium orbiculoides	103, 133			■											■	
Hyaloscyphaceae	685		■													
Hypocreales	257					■										
Hypocreales	403						■									
Lachnum sp.	117		■													
Lasiosphaeris sp.	280														■	
Leptodontidium camptobactrum	191						■				■				■	
Leucosporidium drummii	170										■					
Lindgomyces/ Clohesyomyces	33, 55, 245		■							■				■		
Linnemannia elongata	233						■									
Linnemannia elongata	48, 130	■	■				■			■				■		
Linnemannia exigua	44		■				■				■				■	
Linnemannia hyalina/ Mortierella sarnyensis	94, 184		■													
Macrophomina phaseolina	171	■														
Mariannaea terricola/ punicea	310		■								■					
Melanommataceae	116									■						
Melanommataceae	32										■					
Melanommataceae	324			■												
Metarhizium robertsii	176														■	
Microdochium bolleyi	35													■		
Minimedusa polyspora	148										■				■	
Mortierella alpina	490														■	
Mortierella fluviae	608		■													
Mortierella minutissima	12						■				■				■	
Mrakia cf. frigida/ gellida	72, 144, 278						■			■	■				■	

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Mrakia stelviica	220														■	
Mucor hiemalis	108													■		
Murispora aquatica	561	■					■									
Myrmecridium schulzeri	113					■								■		
Neoascochyta europaea/ graminicola/ desmazieri	22, 79, 208									■						
Neobulgaria sp.	479										■					
Neonectria candida	99						■				■				■	
Neosetophoma samararum	101									■						
Neosetophoma sp./ *samararum cerealis*	36, 17, 160		■							■				■		
Olpidiaceae	153	■														
Operculomyces laminatus	206		■								■					
Ophiosphaerella	97													■		
Penicillium canescens /restrictum	269, 207		■								■				■	
Periconia macrospinosa	193					■										
Phaeosphaeriaceae sp.	88									■						
Plectosphaerella cucumerina/ Gibellulopsis nigrescens	28, 67					■				■	■				■	
Pleosporales	755	■														
Pleotrichocladium opacum	303		■								■					
Podospora	106	■														
Podospora multipilosa	61, 109		■				■				■				■	
Podospora sp.	51									■						
Preussia	330														■	
Pseudeurotium bakeri	271	■					■									
Pyronemataceae	336	■				■										
Ramophialophora petraea	412	■														
Rhizophlyctis rosea	268, 406	■									■					
Rozellomycota	381	■														
Sagenomella oligospora	138						■				■				■	
Setophoma terrestris	162, 398	■														
Setophoma terrestris	19, 71	■														■
Solicoccozyma terrea	127										■				■	
Solicoccozyma terricola	68		■													
Sordaria	328										■				■	
Sordaria fimicola	49			■							■				■	
Sordariales	322	■														
Sordariomycetes	141			■												
Sporormiaceae	302			■											■	
Talaromyces purpureogenus	362										■					
Tausonia pullulans	76										■					

Taxonomy	ASV	F 1	F 2	F 3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Tetracladium	18, 23, 27, 78															
Tetracladium sp.	15, 156															
Tetracladium sp.	53															
Trichocladium griseum/ Botryotrichum spirotrichum/ Chaetomium globosum	85, 132, 335, 411															
Trichosporiella cerebriformis	9															
unclassified Basidiomycota	480, 497															
Vishniacozyma tephrensis/ victoriae	195, 369															
Zymoseptoria tritici	46															

Appendix E: Indicator species analysis of sample types of ITS2 data.

Indicator species analysis was performed using the indicspecies package in R, and species that were significantly associated (p<0.01 with a field, crop and type are indicated in greeen. F = Field, C = Corn, W = Wheat, RS = Rhizosphere soil, RT = Root. ASVs were combined based on patterns of significance as well as phylogenetic similarity. Taxonomy is arranged alphabetically by genus.

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Actinomucor elegans	25												
Alternaria alternata	75												
Alternaria betae-kenyensis	84												
Alternaria infectoria	57, 58												
Articulospora proliferata	83												
Articulospora proliferata	13, 126												
Ascobolus sp.	413												
Cadophora	125, 274												
Candida sake	31												
cf. Hyphodiscus	238												
cf. Soloacrosporiella	199, 464												
cf. Vandijckella/ Mycoarthris sp.	65, 86, 236												
Chalara sp.	62, 92, 118												
Chloridium	91, 189												
Chrysosporium pseudomerdarium	434												
Chytridiomycota	611												
Cladosporium cladosporioides	74, 159												
Cladosporium ramotenellum	93												
Clonostachys rosea	147												
Colletotrichum dematium	98												
Coniochaeta canina	485												
Coniothyrium palmicola	393												
Cordana sp.	323												
Corynespora cassiicola	105												
Cystofilobasidiales carpinicola	230												
Dactylonectria torresensis	100												
Dictyosporiaceae	167												
Didymella americana	90, 240												
Dioszegia hungarica	246												

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Epicoccum nigrum	64, 241	■				■			■	■			■
Exophiala	41		■				■				■		
Exophiala equina	63, 242					■		■	■	■		■	■
Fimicolochytrium alabamae	514							■				■	
Fusarium avenaceum	231								■				■
Fusarium incarnatum	82			■		■		■		■		■	
Fusarium merismoides	89, 261					■				■			
Fusarium oxysporum	52		■				■				■		
Fusarium solani	172					■		■		■		■	
Fusidium	333			■	■								
Helotiales	244	■				■		■		■		■	
Heydenia alpina/ Lasiobolidium orbiculoides	103, 133					■				■			
Hymenoscyphus menthae	205								■				■
Hypocreales	257				■								
Hypocreales	277							■				■	
Hypocreales	385								■				■
Hypocreales	403	■		■									
Lasiosphaeris sp.	280					■		■					
Leptodontidium camptobactrum	191	■						■				■	
Lindgomyces/ Clohesyomyces	33, 55, 245								■				■
Linnemannia elongata	233	■						■				■	
Linnemannia elongata	48, 130	■		■				■	■			■	■
Linnemannia exigua	44	■		■				■				■	
Macrophomina phaseolina	171			■									
Marquandomyces marquandii	714			■									
Melanommataceae	116								■				■
Metarhizium robertsii	176							■				■	
Microdochium	187		■										
Microdochium bolleyi	35								■				■
Minimedusa polyspora	148					■				■			
Mortierella ambigua	643							■				■	
Mortierella exigua	142		■										
Mortierella minutissima	12	■				■		■		■		■	
Mortierella minutissima	70		■				■				■		
Mrakia cf. frigida/ gellida	72, 144, 278	■		■		■				■			
Mrakia stelviica	220			■		■				■			
Mucor hiemalis	108	■											
Myrmecridium schulzeri	113								■				■

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Neoascochyta europaea/ graminicola/ desmazieri	22, 79, 208								■				■
Neonectria candida	99			■		■		■		■		■	
Neosetophoma samararum	101				■				■				■
Neosetophoma sp./ *samararum cerealis*	36, 17, 160				■				■				■
Olpidiaceae	153				■								
Ophiosphaerella	112		■										
Ophiosphaerella sp.	345								■				■
Ophiosphaerella sp.	368				■								
Penicillium canescens/ r estrictum	269, 207					■		■		■		■	
Periconia macrospinosa	193				■								
Peziza sp.	276	■											
Plectosphaerella cucumerina/ Gibellulopsis nigrescens	28, 67	■		■	■	■		■		■		■	
Podospora multipilosa	61, 109	■		■		■		■		■		■	
Preussia	330			■									
Pyronemataceae	336				■								
Rhodotorula graminis	39		■										
Sagenomella oligospora	138			■		■		■		■		■	
Setophoma terrestris	162, 398				■								
Setophoma terrestris	19, 71	■	■		■		■				■		
Sordaria	328							■				■	
Sordaria fimicola	49					■				■			
Sordariomycetes	104							■				■	
Sordariomycetes	501			■									
Sporormiaceae	302					■		■		■		■	
Tausonia pullulans	76					■				■			
Tetracladium	18, 23, 27, 78	■		■	■	■			■	■			■
Tetracladium	146		■				■				■		
Tetracladium sp.	15, 156	■			■	■				■			
Trichocladium griseum/ Botryotrichum spirotrichum/ Chaetomium globosum	85, 132, 335, 411					■		■		■		■	
Trichosporiella cerebriformis	9	■		■		■				■			
unclassified Fungi	691							■				■	
Vishniacozyma tephrensis/ victoriae	195, 369				■				■			■	

Appendix F: Indicator species analysis for field, crop and type of LSUA data. Indicator species analysis was performed using the indicspecies package in R, and species that were significantly associated (p<0.01) with a field, crop and type are indicated in greeen. F = Field, C = Corn, W = Wheat, RS = Rhizosphere soil, RT = Root. ASVs were combined based on patterns of significance as well as phylogenetic similarity. Taxonomy is arranged alphabetically by genus.

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Acremonium persicinum	279, 564															
Alternaria	18, 135, 66															
Alternaria	29															
Aquanectria submersa	444															
Aquimassariosphaeria kunmingensis	35, 217															
Aquimassariosphaeria kunmingensis	39, 196															
Ascobolus	667															
Ascomycota	95, 335, 441															
Cadophora cf. orchidicola	275															
Capnodiales	517															
Cercophora	148, 161															
cf. Aspergillus caninus	569															
cf. Dictyosporella aquatica	81, 124															
cf. Grandibotrys xylophila	329															
cf. Lasiosphaeris hispida	209															
cf. Nimbospora effusa	506															
cf. Otidea	472															
cf. Pleurotheciopsis bramleyi	324, 921															
Chaetomium pilosum	118															
Chloridium aseptatum/ Melanopsammella gonytrichii	43, 133, 304, 152															
Chloridium virescens	210															
Chrysosporium pseudomerdarium	366															
Cladosporium	262, 346															
Cladosporium	98															
Cladosporium crousii	46															

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Clohesyomyces aquaticus	386															
Clonostachys rosea	78															
Colletotrichum	63, 293, 404															
Coniothyrium sp.	316															
Corynespora cf. smithii/ Pleosporales	90, 312, 426															
Cosmospora	126, 443, 536															
Curviclavula/ Cudoniella	26, 137															
Dactylaria fragilis	290															
Dactylaria sparsa	246															
Dactylonectria macrodidyma	100, 264, 499															
Dendryphion nanum	248															
Dothideomycetes	282, 586															
Dothideomycetes	338															
Dothideomycetes	401															
Emericellopsis humicola	295															
Exophiala radicis	7															
Fusarium continuum	357															
Fusarium foetens	6															
Fusarium graminearum/ Gibberella	49, 153															
Fusarium solani/ Nectria	122, 175, 350, 344, 709															
Fusarium tricinctum	40, 128															
Fusicolla merismoides	57, 140															
Fusicolla ossicola	33, 87, 171, 315															
Gilbellulopsis simonii/ Plectosphaerella	14, 42, 58, 71															
Halosphaeriaceae	149															
Halosphaeriaceae cf. Nimbospora effusa	211, 547, 749															
Helotiales	235															

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Herpotrichia	27, 117, 82															
Herpotrichia juniperi	158															
Herpotrichiellaceae	31, 136															
Humicola fuscogrisea	208															
Hyalopeziza raripila/ Tetracladium	24, 105															
Hydropisphaera	155, 394, 466															
Hymenoscyphus aurantiacus	582															
Hypocrea	102															
Hypocreales	64, 198, 383															
Hypocreales	230															
Hypocreales	405															
Hypocreales/ *Neohelicomyces aquaticus*	85, 284, 336															
Jennwenomyces navicularis	296, 840															
Lachnellula arida	195															
Lachnum diminutum	107															
Laetinaevia carneoflavida	91															
Lasiobolidium orbiculoides	241															
Leptodontidium camptobactrum	281															
Linnemannia gamsii	449															
Macrophomina phaesolina	182, 561															
Mariannaea punicea	247, 487															
Marquandomyces marquandii	147															
Massariosphaeria	145															
Melanomma	584															
Melastiza	377															
Metarhizium brunneum	123, 467															
Microdochium bolleyi	32, 75, 183, 276															
Microdochium majus	174, 637															
Mortierella antarctica/ Podila horticola	249, 320															

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Mortierella zonata	214															
Mortierellaceae	225															
Mrakia frigida	370, 654															
Murispora fagicola	116															
Murispora kazachstanica	415															
Nectriaceae	20, 48, 113															
Neoascochyta europaea	22, 73, 93, 237															
Neobulgaria koningiana	256															
Neonectria lugdunensis	47, 132, 291															
Neosetophoma aseptata	391															
Niesslia mucida	203															
Orbicula parietina	168, 365															
Paraphaeosphaeria rosae	261															
Penicillium	226															
Penicillium citreonigrum	236															
Penicillium restrictum	277															
Periconia macrospinosa	34															
Peyronellaea	65, 41, 130, 165															
Peziza sp.	243															
Phaeosphaeria oryzae	38, 13, 53, 69															
Phaeosphaeriaceae	119, 439															
Phialophora expanda	702															
Phomatospora sp.	308															
Plectosphaerella	11, 51, 71, 111															
Pleosporales	61, 309															
Pleosporales	112, 353															
Pleosporales	88															
Pleotrichocladium opacum	286															
Pochonia chlamydosporia	362															

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
var. spinulospora																
Podospora tetraspora	28															
Preussia alloiomera	270															
Preussia grandispora	376															
Pseudeurotium bakeri	141															
Pythium biforme	434															
Remispora quadri-remis	258															
Sclerostagonospora rosae/Phaeosphaeriaceae	99, 327															
Scolecobasidiella	445, 609															
Scytalidium circinatum	382															
Scytalidium multiseptatum	45															
Setophoma terrestris	4, 10															
Setophoma yunnanensis	84, 60															
Sordaria	180, 437, 463															
Sordariales	283															
Sordariomycetes	124															
Sordariomycetes	202, 485, 532															
Sporidesmiella hyalosperma	242															
Sporidesmiella pini	515															
Striaticonidium cinctum	398															
Talaromyces aculeatus	430, 726															
Talaromyces purpureogenus	271															
Talaromyces ucrainicus	513															
Tetracladium globosum	86															
Tetracladium marchalianum/globosum	0, 12, 8															
Thysanorea cantrelliae	188															
Tricellula aquatica	1, 17, 21															
Tricellula aquatica	169															
Tricellula aquatica	170															
Tricellula aurantiaca	190															
Trichoderma afroharziahum	184															
Trichoderma pleuroticola	146															
Trichoderma tomentosum/	79,															

Taxonomy	ASV	F1	F2	F3	Field 1				Field 2				Field 3			
					C	W	RS	RT	C	W	RS	RT	C	W	RS	RT
Hypocreaceae	260, 154															
unclassified Fungi	534															
unclassified Fungi	502															
unclassified Fungi	568, 808															
Valsonectria pulchella	343															
Vermispora spermatophaga	620															
Wongia fusiformis	238, 766															
Zopfiella tabulata	131															
Zymoseptoria brevis	59, 218, 294, 465															

Appendix G: Indicator species analysis for sample types of LSUA data.

Indicator species analysis was performed using the indicspecies package in R, and species that were significantly associated (p<0.01 with a field, crop and type are indicated in greeen. F = Field, C = Corn, W = Wheat, RS = Rhizosphere soil, RT = Root. ASVs were combined based on patterns of significance as well as phylogenetic similarity. Taxonomy is arranged alphabetically by genus.

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Acremonium persicinum	279, 564												
Acremonium rutilum	375												
Acremonium verruculosum	127												
Alternaria	18, 135, 66												
Alternaria	29												
Aquanectria submersa	444												
Aquimassariosphaeria kunmingensis	35, 217												
Aquimassariosphaeria kunmingensis	39, 196												
Ascobolus	667												
Ascomycota	95, 335, 441												
Berkeleyomyces rouxiae	617												
Boubovia luteola	1042												
Cadophora cf. orchidicola	275												
Cadophora gregata	797												
Capnodiales	517												
Cercophora	450												
cf. Aspergillus caninus	569												
cf. Bionectria	94												
cf. Colletotrichum brevisporum	348												
cf. Dictyosporella aquatica	81, 124												
cf. Isthmolongispora ampulliformis	562												
cf. Nimbospora effusa	506												
cf. Otidea	472												
cf. Pleurotheciopsis bramleyi	324, 921												

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Chaetomium pilosum	118		■								■		
Chloridium aseptatum/ Melanopsammella gonytrichii	43, 133, 304, 152	■		■		■		■				■	
Chloridium virescens	210	■		■		■		■		■		■	
Chrysosporium pseudomerdarium	366	■											
Cladosporium	262, 346					■		■		■			
Cladosporium	98	■				■				■			
Cladosporium crousii	46		■								■		
Cladosporium tenellum	120					■				■		■	■
Clohesyomyces aquaticus	386								■				
Colletotrichum	63, 293, 404	■		■				■				■	
Coniochaeta sp.	570							■					
Coniothyrium sp.	316									■			
Coprotus ochraceus	823			■									
Corynespora cf. smithii/ Pleosporales	90, 312, 426	■		■		■		■				■	
Cosmospora	126, 443, 536	■		■		■		■		■		■	
Curviclavula/ Cudoniella	26, 137					■		■					
Cyathicula amenti	160					■							
Dactylaria fragilis	290			■	■								
Dactylaria sparsa	246							■	■				
Dactylonectria macrodidyma	100, 264, 499			■	■	■		■	■			■	■
Dendryphion nanum	248											■	
Didymella	25, 15		■				■				■		
Dothideomycetes	338											■	■
Dothideomycetes	401	■				■		■					
Emericellopsis humicola	295	■								■		■	
Equiseticola fusispora	76		■										

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Exophiala radicis	7		■				■						
Fusarium foetens	6		■				■				■		
Fusarium graminearum/ Gibberella	49, 153	■				■				■		■	■
Fusarium irregulare	3		■										
Fusarium solani/Nectria	122, 175, 350, 344, 709	■		■		■		■		■		■	
Fusarium tricinctum	40, 128	■							■	■		■	■
Fusicolla merismoides	57, 140	■		■						■		■	
Fusicolla ossicola	33, 87, 171, 315	■				■				■		■	
Gaeumannomyces radicicola	108		■				■						
Gibberella	129		■								■		
Gilbellulopsis simonii/ Plectosphaerella	14, 42, 58, 71			■		■		■		■		■	
Globisporangium macrosporum	421			■									
Halosphaeriaceae	149			■									
Halosphaeriaceae cf. Nimbospora effusa	211, 547, 749			■									
Helotiales	5						■						
Helotiales	235	■				■		■					
Helotiales	695								■				
Helotiales	769					■							
Herpotrichia	27, 117, 82	■		■				■					
Herpotrichia juniperi	158												■
Herpotrichiellaceae	31, 136					■		■		■		■	■
Hyalopeziza raripila/ Tetracladium	24, 105	■		■		■		■		■		■	
Hyaloscyphaceae	328			■									
Hyaloscyphaceae	341				■								

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Hydropisphaera	155, 394, 466												
Hymenoscyphus aurantiacus	368												
Hypocrea	102												
Hypocreaceae	297												
Hypocreales	64, 198, 383												
Hypocreales	151												
Hypocreales	230												
Hypocreales	593												
Hypocreales	405												
Hypocreales/*Neohelicomyces aquaticus*	85, 284, 336												
Jennwenomyces navicularis	296, 840												
Laetinaevia carneoflavida	91												
Lasiobolidium orbiculoides	241												
Leptodontidium camptobactrum	281												
Linnemannia gamsii	449												
Macrophomina phaesolina	182, 561												
Mariannaea punicea	247, 487												
Marquandomyces marquandii	147												
Massariosphaeria	145												
Melanomma	584												
Melastiza	377												
Metarhizium brunneum	123, 467												
Microdochium bolleyi	32, 75, 183, 276												
Microdochium majus	174, 637												

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Mortierella antarctica/ Podila horticola	249, 320	■		■		■						■	
Mortierella zonata	214	■						■	■			■	
Mortierellaceae	225							■	■			■	
Mrakia frigida	370, 654							■	■	■			
Murispora kazachstanica	415				■								
Murispora kazachstanica/ Massariosphaeria	682, 920					■							
Myrmecridium schulzeri	23		■				■						
Natantispora sp.	798											■	
Nectria	144		■										
Nectriaceae	20, 48, 113	■		■	■	■		■		■		■	
Nectriaceae	201		■				■				■		
Nectriaceae	121						■						
Neoascochyta desmazieri	240				■								■
Neoascochyta europaea	22, 73, 93, 237	■		■	■				■				■
Neobulgaria koningiana	256	■				■		■					
Neoconiothyrium viticola	253			■									
Neonectria lugdunensis	47, 132, 291	■		■		■		■		■		■	■
Neosetophoma aseptata	391								■				■
Niesslia aurantiaca	407	■											
Niesslia exospoirioides	573									■			
Niesslia mucida	203	■						■		■		■	
Orbicula parietina	168, 365	■											
Paramicrosphaeropsis ellipsoidea	498					■							
Paraophiobolus plantaginis	649								■				
Penicillium	226							■		■		■	
Penicillium citreonigrum	236							■					
Penicillium ochrochloron	2										■		
Penicillium restrictum	277							■		■		■	
Periconia macrospinosa	34		■				■				■		
Peyronellaea	65,	■				■		■		■		■	

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
	41, 130, 165	■				■		■		■		■	
Peziza sp.	243					■							
Peziza subcitrina	224									■			
Phaeosphaeria oryzae	38, 13, 53, 69				■				■				■
Phaeosphaeriaceae	222		■										
Phaeosphaeriaceae	119, 439			■	■							■	■
Phomatospora sp.	308			■		■				■		■	
Phomatospora uniseriata	528				■								
Plectosphaerella	11, 51, 71, 111		■	■	■		■	■		■		■	■
Pleosporaceae	187						■						
Pleosporales	61, 309					■		■	■			■	■
Pleosporales	112, 353	■		■		■		■		■		■	
Pleosporales	492				■				■				
Pleosporales	88	■		■	■	■		■	■			■	■
Pleotrichocladium opacum	286	■				■		■					
Pochonia chlamydosporia var. spinulospora	362					■		■					
Podospora tetraspora	28		■				■						
Preussia alloiomera	270			■									
Preussia grandispora	376									■		■	
Pseudeurotium bakeri	141	■		■		■							
Pyrenochaetopsis microspora	321					■							
Pyrenophora sieglingiae	670					■							
Pythium biforme	434				■								
Remispora quadri-remis	258			■								■	■
Roesleria subterranea	624			■									
Sarocladium junci	70										■		
Sarocladium strictum	44		■								■		
Sclerostagonospora rosae/ Phaeosphaeriaceae	99, 327			■	■				■				■
Scolecobasidiella	445, 609											■	

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Scytalidium circinatum	382												
Scytalidium multiseptatum	45												
Setophoma terrestris	4, 10												
Setophoma yunnanensis	84, 60												
Sodariomycetes cf. Acroceratosphaeria potamia	505, 931												
Sordaria	180, 437, 463												
Sordariomycetes	124												
Sordariomycetes	202, 485, 532												
Sordariomycetes	753												
Sporidesmiella hyalosperma	242												
Sporidesmiella pini	515												
Staphylotrichum coccosporum	134												
Striaticonidium cinctum	398												
Talaromyces aculeatus	430, 726												
Talaromyces purpureogenus	271												
Talaromyces ucrainicus	513												
Tausonia pullulans	446												
Tetracladium globosum	86												
Tetracladium marchalianum/ globosum	0, 12, 8												
Thelebolus	531												
Thelebolus globosus	292												
Thelonectria cidaria	497												
Tricellula aquatica	1, 17, 21												
Tricellula aquatica	169												
Tricellula aquatica	170												
Tricellula aurantiaca	190												
Trichoderma afroharziahum	184												
Trichoderma koningii	372												

Taxonomy	ASV	Field 1				Field 2				Field 3			
		CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT	CRS	CRT	WRS	WRT
Trichoderma pleuroticola	146												
Trichoderma tomentosum/ Hypocreaceae	79, 260, 154												
unclassified	719												
unclassified Fungi	951												
unclassified Fungi	534												
unclassified Fungi	489												
unclassified Fungi	502												
unclassified Fungi	568, 808												
Valsonectria pulchella	343												
Vermispora spermatophaga	620												
Wongia fusiformis	238, 766												
Xylariales	96												
Zymoseptoria brevis	59, 218, 294, 465												

Appendix H: Indicator species analysis for field and sample type of LSUBG data. Indicator species analysis was performed using the indicspecies package in R, and species that were significantly associated (p<0.05) with a field, crop and type are indicated in greeen. F = Field, C = Corn, W = Wheat, RS = Rhizosphere soil. ASVs were combined based on patterns of significance as well as phylogenetic similarity. Taxonomy is arranged alphabetically by genus.

Taxonomy	ASV	F1	F2	F3	Field 1		Field 2		Field 3	
					CRS	WRS	CRS	WRS	CRS	WRS
Actinomortierella capitata	183									
Aphanomyces cladogamus	14									
Bodomorpha	113, 296									
Bodomorpha	196									
Bodomorpha sp.	15, 48, 187, 216									
Bodomorpha sp.	165									
Bodomorpha sp.	85									
Chytridiomycetes	21									
Chytridiomycetes	300									
Curvibasidium pallidicorallinum	180									
Geranomyces tanneri	107									
Globisporangium heterothallicum	45									
Globisporangium macrosporum	9									
Globisporangium parvum	41									
Globisporangium viniferum	142									
Glomeromycota	58									
Hyphochytrium catenoides	51									
Leucosporidium drummii	50									
Linnemannia elongata	2									
Linnemannia exigua	76									
Linnemannia gamsii	7									
Mortierella alpina	54									
Mrakia frigida	6									
Mucor hiemalis	47									
Olpidium	159									
Olpidium brassicae	136									
Operculomyces laminatus	27									

Taxonomy	ASV	F1	F2	F3	Field 1		Field 2		Field 3	
					CRS	WRS	CRS	WRS	CRS	WRS
Psathyrella impexa	502									
Pythium biforme	158									
Pythium graminicola	38									
Pythium monospermum	130									
Rhizophlyctis rosea	128									
Solicoccozyma terrea	30									
Stauratromyces oculus	211									
Tausonia pullulans	5									
unclassified Fungi	278									
unclassified Fungi	328									
unclassified Fungi	34									
unclassified Fungi	22, 59									

Curriculum Vitae

Name:	Marianna Wallace
Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2015-2019 B.Sc.
Honours and Awards:	The Western Scholarship of Excellence 2015
Related Work Experience:	Teaching Assistant The University of Western Ontario 2019-2022
Related Volunteer Experience:	Society of Biology Graduate Students The University of Western Ontario London, Ontario, Canada 2019-2020