THESIS

A COMPREHENSIVE MICROBIOME ANALYSIS OF WHEAT AND ITS WILD RELATIVES

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ABSTRACT

A COMPREHENSIVE MICROBIOME ANALYSIS OF WHEAT AND ITS WILD RELATIVES

Microbiomes are diverse assemblages of endophytic and free-living microorganisms that can confer competitive advantages to their plant hosts such as water acquisition, nutrient mobilization, drought tolerance, salt tolerance, and disease resistance (Chaparro et al., 2012; Sherameti et al., 2008; Zolla et al., 2013). Plant domestication and selective breeding have altered the composition of these plant-microbe interactions in several crops. It is thought that the progenitors of the A, B, and D genomes in modern hexaploid wheat (*Triticum aestivum*) manage environmental stress in their native environment by establishing symbioses with a consortium of beneficial microbes (Iannucci et al., 2017). However, these microbial communities are not well understood.

The goal of this study is to better understand the core community of microbes in wild wheat relatives and how they differ from the microbiome of cultivated wheat. This study compares the bacterial and fungal taxa found in and on the leaves, roots, and rhizosphere of three accessions of hard winter wheat and 14 accessions of eight wild relative species grown in a common soil. These plants and the agricultural soil they inhabit were sampled from a randomized complete block design with two replications, grown in well-watered and water-limited treatments in Fort Collins, Colorado. DNA was extracted and barcoded amplicon sequencing of the 16S-V4 (bacteria/archaea) and ITS2 (fungi) small subunit ribosomal RNA (rRNA) genes was used to describe the diversity of the microbial community associated with the root, rhizosphere and leaves of each accession.

The results indicate that while there were limited differences in microbial communities among plant species, plant tissue type appears to be a strong predictor of microbial community structure. Across all plant genotypes, the rhizosphere consistently contained the most diverse and abundant microbiomes, followed by roots, and lastly leaves, which were the least diverse tissue type. When these three tissue types were analyzed independently (PERMANOVA), there was a significant difference in rhizosphere communities between the wet and dry treatments. Wet treatments contained a greater number of facultative anaerobes and bacteria common to cold, saturated soils. The wet treatment received an additional 13 mm of water, applied five days prior to collection. Overall, while plant host genotypes did not differ significantly in their microbiomes, some unique symbioses among different plant accessions indicate evolutionary adaptation. An initial look at the core microbiome shared among representatives of the five plant genomes in this study showed few shared sequence variants (<2% of total microbial SV's). However, this was largely explained by the use of high-resolution SV's that do not necessarily equate to different taxonomic assignment, suggesting an inflated number of actual microbial taxa. Coarser taxonomic overviews depicted a more realistic, and narrow, number of participating taxonomic groups in the phytobiome. Plant tissue type remained a chief driver of microbiome composition. Soil moisture and fertility may have also played a role in determining microbial community structure, but since they were not measured in this study, claims cannot as yet be made. The close genetic relationships among plant species in this study may have reduced the observable differences in microbial community structure. Additionally, common garden experiments limit the pool of potential plant-microbe interactions. Despite the advancement and evolution of modern wheat, the microbiome remains essentially the same as the microbiomes of wild relatives, when grown in the same soil. This indicates that modern winter wheat retains the

same ability to recruit and sustain its microbiome as its wild relatives. In the future, microbiome consensus studies in these hosts' centers of origin could broaden our understanding of long-evolved microbial symbioses.

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Literature Review

The Plant Microbiome: Who lives there and where do they come from?

Over the course of three billion years, microorganisms have colonized every observable ecosystem on our planet. The earliest known plant-microbe interactions formed 400 million years ago in the early Devonian period, between endophytic fungi and simple bryophytic land plants (Brundrett, 2002). Today, higher plants associate with bacteria, fungi, archaea, and protists across a gradient of symbioses. Pathogens, commensalists, and mutualists interact with plants above and below ground, within and around plant tissues, influencing the fitness of their plant hosts (Andreote et al., 2014; Lebeis, 2014). Plants provide habitats for these consortia of microbes in three broadly classified ecosystems: the rhizosphere (the soil immediately surrounding roots), endosphere (interior root, stem, and leaf tissue), and phyllosphere (the plant surface) (Berg et al., 2016).

Plant-associated microbes can be recruited through horizontal transmission (from the environment) or vertical transmission (from parent to offspring via seed) (Frank et al., 2017). Most vertically-transmitted endophytes are obligate mutualists, which ensures perpetuation of the symbiotic relationship. Pollen is a common vector of seed-born endophytes, bearing bacteria both on the inside and outside of pollen grains (Frank et al., 2017). Some examples of this action in nature include microbes that help plants germinate and grow in hostile environments by fixing atmospheric nitrogen, or breaking seed dormancy through the production of cytokines, as is seen in high plains ryegrass (*Lolium* spp.)(Frank et al., 2017). However, most endophytes are acquired horizontally from the environment.

The most abundant and diverse ecosystem in the plant microbiome exists in the roots and rhizosphere, where root exudates chemically signal the recruitment of specific microbial taxa from soil (Broeckling et al., 2008). This relationship can benefit plants in myriad ways, such as the conveyance of nutrients and stress tolerance from endophytic microbes, while microbes receive a steady supply of exuded carbon. Plant root exudates constitute 2-10% of the total carbon fixed by plants, constituting an abundance of food for soil microbiota, and are large dictators of the microbiome community structure (Broeckling et al., 2008). However, the rhizosphere is a highly competitive environment, implying resource limitation due to high microbial cell counts and activity. The components of root exudates differ among plant species, and are therefore capable of recruiting (through selective pressure or chemotaxis) distinct subsets of microbes from the bulk soil flora population tailored to the needs of each plant (Broeckling et al., 2008; Hartmann et al., 2008).

Functions of Microbiomes in Plants

The significant role of rhizosphere ecology to plant health was discovered in the early 20th century by the German botanist Lorenz Hiltner (Hartmann et al., 2008). Hiltner recognized that not only were distinct root exudates responsible for the development of distinct microbial communities, but that the roots of healthy plants were being influenced by the presence of endophytic bacteria (Hartmann et al., 2008). Hiltner's research focused mainly on leguminous crops, but since then the study of plant-microbe interactions has exploded with interest, leading to the discoveries of beneficial microbial associations among many plant clades (Andreote et al., 2014). In order to harness the plant microbiome for crop improvement, enhanced food security,

and increased farmer profitability, the ecological role of these plant growth-promoting microbes and their function in plant development must be explored.

Stress Tolerance

Abiotic stress is responsible for devastating crop losses worldwide, with drought stress being among the most destructive forces. With the climate growing less predictable and increasingly hostile toward healthy plant growth, the plant microbiome presents a promising frontier for combating abiotic stresses such as drought, cold, and salinity (Andreote et al., 2014; Gaiero et al., 2013; Mendes et al., 2013).

Many taxonomically diverse bacteria are capable of interrupting production of the stress hormone ethylene by digesting its pathway precursor, 1-aminocyclo-propane-1-carboxylic acid (ACC), as a source of nitrogen (Mendes et al., 2013). Under drought conditions, plants release the volatile hormone ethylene to initiate senescence and abscission (Jha et al., 2012). When this pathway is interrupted by ACC-deaminase activity, ethylene production is reduced and plants may continue to grow roots deeper into the soil moisture profile where they may escape drought and remain productive (Jha et al., 2012). Many bacteria possessing ACC-deaminase activity also produce the plant growth auxin indole acetic acid (IAA), including the taxa "*Brachybacterium saurashtrense* sp. nov., *Zhihengliuella* sp., *Brevibacterium casei*, *Haererehalobacter* sp., *Halomonas* sp., *Vibrio* sp., *Cronobacter sakazakii*, *Pseudomonas* spp., *Rhizobium radiobacter*, and *Mesorhizobium* sp.," (Mendes et al. 2013) isolated from the roots of the halophytic oil seed crop *Salicornia brachiate* (Jha et al., 2012; Mendes et al., 2013). When ACC-deaminase activity and IAA production occur in concert, a remarkable stress tolerant plant profile may be achieved (Jha et al., 2012).

Certain fungal endophytes are also capable of conferring drought tolerance to their plant hosts. The basidiomycete *Piriformospora india*, originally isolated from plant roots in the Indian Thar desert, can endow the model species *Arabidopsis thaliana* (among many other less documented species) with drought tolerance by upregulating a diverse set of dehydration-response genes in the leaf tissue (Sherameti et al., 2008). While the communication pathway between these root endophytes and their host plants' shoots is not well understood, experimental evidence demonstrates a significant correlation between the *P.indica*-inoculated roots of *Arabidopsis*, *Zea mays* (maize), *Brassica oleracea* var. *capitate* (cabbage), and *Nicotiana tabacum* (tobacco) and a rapid sensitivity to drought response (Sherameti et al., 2008). These inoculated plants respond much faster to drought conditions than untreated plants, with increased production of myo-inositol (an auxin precursor that enhances stress tolerance and improves plant growth) and antioxidant enzymes, among other stress-mediating biosynthesis pathways (Kumar et al., 2009; Sherameti et al., 2008; Zhai et al., 2016). Since this primed stress response courtesy of *P. indica* has been observed in *Arabidopsis*, maize, cabbage, tobacco, and myriad wild desert species, it is likely that this action relies on general (as opposed to plant-specific) mechanisms, and may be amenable to association with other crop species (Kumar et al., 2009; Sherameti et al., 2008).

Another mechanism of drought tolerance conferred to plants by symbiotic microbes is the accumulation of compatible solutes in plant tissues that regulate osmotic pressure. Instead of experiencing destructive plummets in leaf water potential, plant tissues containing compatible osmolytes like glycine betaine and choline maintain turgor pressure and physiological function when confronted with drought, cold, and salinity (Hussain Wani et al., 2013). The Gram-positive soil bacterium *Bacillus subtilis* GBO3 is one such microbe whose volatile organic compounds

have been shown to upregulate plant genes responsible for the production of such osmolytes (Zolla et al., 2013).

Abiotic stress tolerance in plants is a complex quantitative trait and difficult to select for through conventional breeding methods. By continuing to annotate the microbiomes of successful plants in challenging climates, we may discover plant-microbe interactions with the potential to translate into low input, sustainable solutions for crop improvement.

Disease Resistance

Plant pathogens present another global threat to food security, often overwhelming years of breeding efforts by evolving weapons not recognized by host resistance genes. Beneficial microbes can help defend plants against pathogenic invaders by occupying niches to prevent invader establishment, producing antimicrobial compounds, and inducing systemic immune responses in plants for faster reactions to infections (Mendes et al., 2013; Ramamoorthy, 2001). Understanding and harnessing the plant microbiome (see section below) may help ward off future disease epidemics, reduce the reliance on synthetic pesticides, and prevent crop losses.

Many bacterial phyla contain taxa classified as plant growth-promoting rhizobacteria (PGPR) that serve a variety of functions benefiting plant growth; these phyla include Actinobacteria, Bacterioidetes, Firmicutes, and Proteobacteria (Chaparro et al., 2012). Phylum Proteobacteria has garnered the most interest for its role in a healthy plant microbiome, particularly the class Gamma-Proteobacteria which contains the notable PGPR genus *Pseudomonas* (Ramamoorthy, 2001). Certain *Psueodomonas* pathovars can produce anitifungal chlorinated lipopeptide molecules in the rhizosphere of *Beta vulgaris, Solanum tuberosum,* and *Oryza sativa* plants confronted with the economically devastating root rot pathogen *Rhizoctonia*

solani (Mendes et al., 2012). Another species in this exceptional genus, *Pseudomonas fluorescens* str. CHA0, has demonstrated abilities to produce cyanide and the antibiotic 2,4-diacetylphloroglucinol in the rhizosphere of tobacco that protects against black root rot (*Thielaviopsis basicola*) (Keel et al., 1990).

Diverse assemblages of PGPR can fortify disease resistance by inducing systemic immune responses in their plant hosts. Seed treatments containing various *Pseudomonas* spp. have shown a remarkable ability to prime plants for faster immune responses when confronted with myriad diseases, insects, and nematodes (Ramamoorthy, 2001). Early microbe-associated molecular patterns and elicitors perceived by the plant from their *Psuedomonas* symbionts induce systemic resistance mechanisms such as hypersensitive response (necrosis), cell wall fortification, and salicylic acid-/jasmonic acid-dependent immune pathways that combat foreign invaders (Ramamoorthy, 2001).

However, many disease resistance mechanisms bestowed by the microbiome cannot be demonstrated by isolated microbial endophytes; plant host fitness is usually improved through the cooperation of a consortia of microbes (Hu et al., 2016). Genera in the Proteobacteria, including *Burkholderia, Rhizobium,* and *Methylophilus*, have been significantly correlated with the phyllosphere (outer surface) of *Arabidopsis* mutants that exhibit strong resistance to the fungal pathogen *Botrytis cinerea* (Ritpitakphong et al., 2016). In the rhizosphere of tomato, greater diversity of *Pseudomonas* sp. is associated with increased resistance to the bacterial plant pathogen *Ralstonia solanacearum* (Hu et al., 2016). Synergistic effects among antifungal-producing *Streptomyces* and *Bacillus* sp. and antibiotic-producing *Burkholderia* and *Pseudomonas* sp. can create enhanced suppression of rice sheath blight (*Rhizoctonia solani*) when found together in the plant rhizosphere (Ramamoorthy, 2001). The concept of a diverse

microbiome enhancing disease suppression is important to maintain when considering the manufacture of synthetic microbiomes for improved crop production.

Water and Nutrient Uptake

One of the best documented examples of beneficial plant-microbe interactions is fungal mycorrhizae mantling or colonizing plant roots for increased moisture and nutrient absorption (Augé, 2004; Brundrett, 2002; Safir, et al., 1972). Most notably, members of the phylum Glomeromycota contain non-pathogenic arbuscular mycorrhizae that penetrate root cells, increasing plant root surface area and facilitating a more effective transfer of soil moisture and carbon-rich exudates (Safir et al., 1972). Soil mycorrhizae can improve stomatal conductance, allowing plants to transpire and fix carbon when soil water potential is low, even if the plants themselves are not mycorrhizal (Augé, 2004). A greenhouse study in tomato inoculated the roots of one treatment with PGPR and mycorrhizal fungi, applied 75% less fertilizer than the uninoculated treatment, and achieved identical yields to the uninoculated, full-rate fertilizer treatment (Chaparro et al., 2012).

Increasing the bioavailability of soil nutrients is a vastly important role many PGPR play in the plant rhizosphere. Myriad taxa in the phyla Actinobacteria, Proteobacteria, and Firmicutes fix atmospheric nitrogen, but the most ubiquitous nitrogen-fixing genera across different soil types are *Arthrobacter*, *Rhizobium*, and *Bacillus* (Patel & Archana, 2017). The Gram-negative genera *Mesorhizobium* and *Rhizobium* (C. Alphaproteobacteria) form tightly symbiotic nodules on the roots of leguminous plants in the Fabaceae family (Elkoca et al., 2008). Soil nitrate residues from the roots of leguminous crops like clover and alfalfa are often in such surplus that they reduce required fertilization regimens for the following crop (Lenssen et al., 2007). Even

certain non-nodulating diazotrophs can penetrate the root cortex of economically important cereal crops like wheat, sorghum, and maize to enrich their tissues with bioavailable forms of nitrogen (Elkoca et al., 2008). In addition to endophytic diazotrophs, there are numerous examples of free-living nitrogen fixers across the bacterial kingdom, including the noteworthy genera *Azospirillum, Azotobacter, Bacillus, Enterobacter, Pseudomonas, Serratia*, and *Streptomyces* (Elkoca et al., 2008). Some of these bacteria are capable of performing several plant growth-promoting activities simultaneously, like producing plant growth factors, suppressing disease resistance, and improving nutrient availability. *Bacillus* sp. can solubize organic phosphorus in the soil from plant residues and animal waste into the plant bioavailable phosphate form (Elkoca et al., 2008). As prices for synthetic fertilizers continue to rise and their negative environmental impacts become more apparent, biofertilizers containing PGPR and mycorrhizal isolates may become more attractive as a sustainable alternative to conventional fertilizers.

Breeding for Microbiomes

In recognizing the pivotal role microbiomes play in the expression of plant traits such as abiotic/biotic stress resistance and nutrient use efficiency, the next generation of plant breeding could adopt an approach that targets the recruitment of beneficial microorganisms. Some genes that drive the establishment of microbial symbionts have already been characterized, including those that facilitate arbuscular mycorrhizae and leguminous root nodule development (Elkoca et al., 2008; Hohmann & Messmer, 2017). However, many of the genetic mechanisms underlying rhizodeposition characteristics remain elusive. This study, along with greater bodies of microbial census data from different plant genotypes, lays the groundwork for further genome-wide

association studies that correlate microbiome community structure with their associated recruitment genes. In the meantime, endophyte seed treatments and soil transplant experiments performed in the private and public sector have provided proof of concept for the new wave of resilient, productive crops that rely less on synthetic inputs (Gopal & Gupta, 2016).

Phytobiome research consistently suggests that a small number of microbial phyla dominate the plant rhizosphere and are largely conserved among plant species (Bulgarelli et al., 2015; Fierer, 2017). However, crop wild relatives have long been viewed as reservoirs for genetic diversity among plant breeders, and host genotype does appear to drive the differentiation of a small subset of plant microbiota (Bulgarelli et al., 2015; Hale et al., 2014). However, the case for strong genotype-dependent microbial diversity remains hotly debated. A 2015 study of the root microbiota in three subspecies of barley (*Hordeum vulgare* ssp.) noted that any significant variation in root community structure was purely quantitative and could be explained by differences in abundance, as opposed to the presence of unique microbial taxa (Bulgarelli et al., 2015). Additionally, many studies that compare the microbiomes of different plant species do so across a biogeographical range, with varying biotic and abiotic conditions (Coleman-Derr et al., 2015). Regardless, plant domestication remains a factor of interest in microbiome dissimilarity for its potential impact on plant breeding. An examination of bacterial communities within wild and domesticated strains of sunflower (*Helianthus annuus*) determined that differences in microbiomes were relatively minor across a plant domestication gradient, but fungal rhizosphere communities demonstrated greater differences (Leff et al., 2016). Where these fungal differences indicate mycorrhizal symbiosis or other physiologically-beneficial mechanisms, gene introgression opportunities for crop improvement may exist (Hohmann & Messmer, 2017; Mendes et al., 2013).

Wheat was chosen as a model species for this project due to its traceable hybridization history, living wild relatives, and importance as a human food source. Modern wheat cultivars are polyploids that acquired their genomes over thousands of years in the Middle East and Western Asia. *Triticum aestivum* (bread wheat) is a hexaploid species that contains an A, B, and D genomes (2n=6x=42 chromosomes), and *Triticum durum* (pasta wheat) is a tetraploid species that contains the A and B genomes (2n=4x=28 chromosomes). The A-genome progenitors are well-documented as *Triticum monococcum* and *Triticum urartu* (Gustafson et al., 2009). The B-genome's origin remains shrouded in controversy, but many accept the claim of *Aegilops speltoides* as the purported progenitor (Gustafson et al., 2009). These two species hybridized to form the AB-tetraploids *T. durum* and *T. dicoccoides* (Gustafson et al., 2009). Later, the D-genome progenitor *Aegilops tauschii* wildly introgressed to form the first generation of hexaploid wheat (Gustafson et al., 2009). Following these spontaneous speciation events, selective breeding and domestication by humans generated even greater genetic distance between these related species (Gustafson et al., 2009).

The narrowing genetic background of *T. aestivum* has led to interest in wild species introgression in recent years (Warburton et al., 2006). It is speculated that wild wheat relatives mitigate biotic and abiotic stresses in their native environments by recruiting microorganisms that enhance plant defense systems (Lebeis, 2014). One study found marked differences in the amount of microbial biomass carbon and nitrogen in the rhizosphere soils of 24 different wheat genotypes (Corneo et al., 2016). Another experiment found strong distinctions in bacterial richness in diversity across different maize fields, and a small but highly heritable variation in bacterial diversity among 27 inbred maize lines (Peiffer et al., 2013). These findings suggest strong differences in microbial communities among different soil types, and a small but

potentially meaningful opportunity for plant breeding (Peiffer et al., 2013). Plants possessing rhizodeposition genes that foster microbial communities capable of fixing atmospheric nitrogen have a competitive advantage in nutritionally depleted soils. Wild wheat relatives continue to grow in their center of origin, and their persistence in the gene pool creates unique opportunities for plant breeding experiments (Warburton et al., 2006). This foot print of living evolution provides a way to observe the effects of domestication on the microbiome of a vital global food crop.

Goal and Objectives

This basic research project is a critical early step toward breeding for microbiomes in the next generation of commercial wheat. The overall goal of this study is to better understand the core community of microbes in wild wheat relatives and how they differ from the microbiome of cultivated wheat. Specific objectives are as follows:

1. Identify and compare the bacterial and fungal taxa in the leaves, roots, and rhizospheres of three accessions of wheat and 14 accessions of its CWRs (crop wild relatives).
2. Determine the greatest driver(s) of microbiome variation from among the four variables in this study (i.e. host genotype, plant tissue, planting season, moisture treatment).
3. Define the core microbiome of wheat and its wild relatives by ploidy and tissue type.

Chapter 2

A Comprehensive Comparison of the Microbiomes in Wheat and Its Wild Relatives

Summary

The importance of microbiomes to plant growth and health has motivated research on the efficient characterization of these communities in numerous crops. Some studies indicate genotype-dependent microbial diversity across different wheat (*Triticum aestivum*) accessions, and suggest wild relative species might mitigate stress in their native environments through microbial interactions (Corneo et al., 2016; Iannucci et al., 2017). The narrowing genetic base of many modern crops has brought interest in wild relative introgression to plant breeding so such competitive advantages may be incorporated into modern germplasm (Gopal & Gupta, 2016). The goal of this study was to broaden the current knowledge of plant-microbe interactions and predictors of microbial community structure in wheat and wild wheat relatives (*Aegilops* and *Triticum* spp.) by quantifying the significance of several comprehensive variables including planting season, water treatment, plant tissue type, and plant genotype.

The host genotypes in this study were planted as a common garden experiment containing two treatments, wet and dry, in a randomized complete-block design in Fort Collins, Colorado. Leaves, roots, and rhizosphere soil were sampled from each plant at the pre-flowering stage following winter vernalization. DNA was extracted and sequenced on a two-lane Illumina MiSeq platform with primers designed to amplify the DNA of prokaryotes (bacteria and archaea) and fungi (Appendix Table 4.2). Raw sequence reads were processed through the bioinformatics pipelines DADA2 (for quality trimming and filtering) and Phyloseq (for community analyses) using R software. The results indicated minimal differences in microbial communities between

plant genotypes (PERMANOVA $P > 0.05$), and significant differences in plant tissue, especially in the rhizosphere (PERMANOVA $P > 0.05$). One notable, though not statistically significant, difference was the increased abundance of arbuscular mycorrhizae in the roots of the Colorado-adapted wheat cultivars and the D-genome progenitor *Aegilops tauschii*. Consistent with existing literature, soil moisture and soil fertility appear to be stronger predictors of rhizosphere ecology than plant genotype. The increased moisture of the wet treatment, as well as nutrient-enriched soils as suggested by cropping history records, may have led to greater diversity and abundance of aquatic and facultative-anaerobic bacterial species in rhizosphere samples (PERMANOVA $P < 0.05$). One of the discriminative fungal taxa (Tremellales) found in the dry treatment is a known decomposer of cellulose and raw plant matter, the presence of which was consistent with a recent cropping history of canola (*Brassica napus* L.) and plant residue.

While not all of the functional roles of soil and plant-associated microbes are well understood, evidence suggesting the most important influencers of microbial community structure will help drive more efficient research goals in the future. Experiments containing more distantly-related host genotypes may provide an opportunity to see significant differences among plant microbiomes, and demonstrate opportunities to breed for microbial-recruitment genes. The results from this research appear to indicate, however, that manipulating soil characteristics may be the most effective way to exploit soil microbes to enhance crop performance.

Introduction

The first person to recognize the pivotal role of rhizosphere ecology to plant health and development was an early 20th century German botanist named Lorenz Hiltner (Hartmann et al., 2008). Hiltner discovered the presence of endophytic bacteria in the root nodules of his most

vigorous leguminous plants, identifying a beneficial nitrogen-fixing symbiosis that is now well documented across the plant family Fabaceae (Hartmann et al., 2008). Microbiomes, defined as consortia of bacteria, fungi, and other microorganisms within a larger organism, influence their plant hosts' physiology by enhancing water uptake, nutrient availability, stress tolerance, and disease resistance (Lebeis, 2014; Mendes et al., 2013). The most diverse microbial ecosystem in the phytobiome is the rhizosphere, or thin interface of soil and carbon-rich root exudates that chemically signal the recruitment of specific microbial taxa suited to meet plant demands (Broeckling et al., 2008). Since Hilter's discoveries, the study of plant-microbe interactions has expanded, largely in the agricultural realm where the promise of microbial-mediated crop improvement has become a tantalizing frontier.

Ideas for strategies to integrate this new deluge of microbial metagenomic data into practical agricultural applications have included plant breeding for rhizosphere recruitment genes, inoculation-based seed treatments, and soil quality manipulation experiments (Berg et al., 2016; Gopal & Gupta, 2016). There is evidence to suggest that plant species plays a role in the structure of phytobiome communities (Coleman-Derr et al., 2015). In barley (*Hordeum vulgare*), an economically important row crop and close cousin to wheat, host genotype has demonstrated a small but highly significant effect on microbiome composition, and appears to shift as domestication steers away from these closely-evolved biogeographical symbioses (Bulgarelli et al., 2015). Since the metabolic composition of root exudates is known to be highly heritable and strongly differentiated among wheat relatives of differing ploidy, it prompts the question of whether crop wild relatives hold the key to manipulating the plant microbiome (Iannucci et al., 2017).

Crop wild relatives have long been known to plant breeders as reservoirs of genetic diversity. If beneficial microbial-recruitment genes exist in crop wild relatives in their centers of origin, where they have co-evolved over countless generations with microbes to mitigate myriad environmental stresses, they might be the easiest genes to integrate into the genome of an adapted crop cultivar due to their close genetic relationship (Gopal & Gupta, 2016; Hale et al., 2014). Common garden experiments, where wild plants are grown ex-situ (in agricultural soils, for example), provide a preliminary glimpse into the potential symbioses that can be formed from an ambient soil microbiome by these crop wild relatives using their intrinsic genetic diversity (Hale et al., 2014).

The diversity of wheat and wild relative germplasm analyzed in a common soil makes this study unique. Three modern wheat cultivars and 14 accessions of landraces and wild wheat relatives of varying ploidy were chosen for planting in a common garden experiment with a randomized complete block design, containing both a wet and dry treatment. The multivariate approach to this experiment provided a look into a general census of wheat microbiome data, as well as important predictors of microbial community structure. The overall goal of this study is to better understand the core community of microbes in wild wheat relatives and how they compare to the microbiome of cultivated wheat. The specific objectives of this study were to:

1. Identify and compare the bacterial and fungal taxa in the leaves, roots, and rhizospheres of three accessions of wheat and 14 accessions of wheat CWRs (crop wild relatives).
2. Determine the factors driving microbiome variation from among the four variables in this study (i.e. host genotype, plant tissue, planting season, moisture treatment).

Materials and Methods

Germplasm

Three wheat cultivars, Byrd, RonL, and Kharkov, represent plants with "domesticated" microbiomes in this study. Byrd is a hard red winter wheat released by the Colorado Agricultural Experiment Station in 2010, purported to have excellent yield performance in both dryland and irrigated environments, tolerance to stripe and stem rust (*Puccinia striiformis* Westend and *Puccinia graminis* Pers.:Pers. F. sp. *tritici* Eriks. E. Henn, respectively), and favorable bread making qualities (Haley et al., 2012). RonL is a hard white winter wheat released by the Kansas Agricultural Experiment Station in 2006, adapted for dryland production in Kansas, and with excellent resistance to wheat streak mosaic virus (WSMV) (Martin et al., 2007). Kharkov is a much older hard winter wheat variety with origins in western Russia, but was grown widely across the American Great Plains in the early twentieth century (Charest & Phan, 1990). The seed of 14 landraces and wild relatives of wheat (*Triticum aestivum*) was sourced from the Wheat Genetics Resource Center at Kansas State University (https://www.k-state.edu/wgrc/), and the source of these accessions is shown in Figure 2.1. This latter group of plants includes diploid, tetraploid, and hexaploid genotypes (Table 2.1).

Field Trial

This experiment was planted at the Agricultural Research, Development and Education Center (ARDEC) near Fort Collins, Colorado (DMS Lat: 40° 35' 6.9288" N; DMS Long: 105° 5' 3.9084" W; Elevation (m): 1,526). This arid plains region has an average temperature of 10.1°C, average annual rainfall of 40.8 cm, and alkaline sandy clay loam soil (pH 7.9) (Appendix Table 1). Seeds were sown on October 15, 2015. Two seeds of each accession were planted 12

cm apart in a randomized complete block design with two replications, each in well-watered and water-limited treatments separated by about 5 m. The water-limited treatment was planted where canola (*Brassica napus* L.) was harvested about 30 days previously, whereas the well-watered treatment area was fallow the previous year. The plants grew to the 2-3 leaf stage, went dormant by early winter, and resumed growth in early March. Because the overwintering ability of these wild accessions was unknown, a second planting was done on February 13, 2016, which is traditionally early enough in Colorado to allow a minimum of 6 weeks of cool weather for plants to vernalize. Eight seeds of each accession were planted, in the same rows as the fall-planted materials in wet and dry treatments. Five of the 17 accessions were planted in October only, seven of the 17 were planted in February only, and five of the 17 were planted in both October and February. The region was not under drought stress at any point in the growing season, per the Palmer Drought Severity Index provided by NOAA (https://www.ncdc.noaa.gov/temp-and-precip/drought/historical-palmers/). Due to abundant spring rainfall, the "wet" plot was irrigated just once (13 mm) in June, five days prior to the sampling of October-seeded plants, and 16 days prior to the sampling of February-seeded plants. Soil moisture content analysis was not performed. The October-seeded plants were sampled on June 8 and February-seeded plants were harvested on June 19. This corresponded to the pre-heading to heading stage. Plants were not allowed to ripen to full maturity because several of the goatgrass species are considered potentially invasive in Colorado. A basic soil analysis was performed on a sample collected between the two fields at the time of harvest.

One healthy plant per plot (44 plants total) was dug up (15 cm deep) and the root ball shaken to release loose soil. Any soil that remained adhered to the root ball after vigorous shaking constituted the "rhizosphere", which was collected and stored at -80°C until DNA

extraction. Two bulk soil samples from the wet and dry fields were also collected for DNA sequencing. Three undamaged, mature leaf blades (non-flag leaf) were collected from each plant, along with roots from a diverse range of size classes. The roots were surface sterilized in a 0.05 M NaClO solution. Leaves were not sterilized in order to include the "phyllosphere" microbes that colonize the leaf surface. Leaves and roots were lyophilized, sealed in centrifuge tubes, and stored at room temperature until DNA extraction.

DNA Extraction

Leaf DNA was extracted with a ThermoScientific Plant DNA Kit (Thermo Fisher Scientific, Waltham, MA, USA). Root and rhizosphere DNA were extracted with a MoBio PowerMag Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). Both kits were optimized for the KingFisher™ Flex Purification System. Once extracted, the DNA concentration was calibrated using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The three 96-well plates (leaf, root, and rhizosphere) containing the extracted DNA were stored at -20°C until they were shipped on dry ice to the University of Minnesota Genomics Center for PCR-amplification, library preparation, and two-lane Illumina MiSeq sequencing.

Next-Generation Amplicon Sequencing

The ITS2 and 16S-V4 small subunit ribosomal RNA (rRNA) operons were sequenced to identify the fungi and bacteria/archaea in each sample, respectively. Primers were barcoded to identify which sequences correspond to which samples. These primers amplify DNA fragments 260-290 bp in length and are ideal candidates for amplification because they are highly

conserved, but possess sufficient variability to distinguish closely related taxa (Op De Beeck et al., 2014; Walters et al., 2015; Yao et al., 2010)(Appendix Table 4.2).. Additionally, the 16S-V4 primer refines the detection of *Thaumarchaeota*, a prokaryotic phylum known to play an important role in terrestrial nitrogen and carbon cycling (Walters et al., 2015). The ITS2 operon possesses superior detection of several ectomycorrhizal fungal species, including *Sistotrema sp., Rhizopogon luteolus*, and *Wilcoxina mikolae,* as well as arbuscular mycorrhizae in the Glomeromycota (Op De Beeck et al., 2014). Since Illumina MiSeq sequencers read 300 bp in each direction, these shorter fragments allow for almost complete double-stranded overlap, increasing base call accuracy (Goodrich et al., 2014). A downside to using primers for shorter genetic elements is that the sequences generated are less informative than reads with longer single-stranded tails (Kozich et al., 2013). Longer fragment sequences can generate higher resolution OTU's, but are prone to higher error rates (Quail et al., 2012). The resultant sequences were delivered digitally as ".fastq" files for taxonomic identification and statistical analysis.

Data Analysis

The open-source software package DADA2 was used to model and correct Illumina amplicon sequencing errors for all raw .fastq reads in this study (Long et al., 2016). This high-throughput bioinformatics pipeline, built for use in R, improves the structure and quality of amplicon sequences so they can be passed downstream for taxonomic assignment and hypothesis-based analyses. The sequence cleaning pipeline for this project required five steps: filtering, dereplicating, denoising, chimera-removing, and merging. First, forward (5'→3') and reverse (3'→5') reads are trimmed where Phred quality scores fall below 20; a score of 20 or higher ensures base call accuracy of at least 99% (Bokulich et al., 2013). The tails of reads and

reverse reads in general have lower quality scores (Long et al., 2016). Next, a dereplication function inputs the filtered reads and outputs a list of unique sequences and their abundances. After dereplication, the unmerged sequences are passed to a denoising algorithm that identifies total number of true sequence variants through sample inference. More abundant sequences are also checked for chimeras by identifying sequence mismatches and indels. The chimeric model flags these offending sequences, and chimeras in this project were removed to improve accuracy. Finally, the filtered forward and reverse reads are merged to create paired-end sequences poised for clustering and taxonomic assignment (Long et al., 2016).

Another common filtering tool employed in metagenomics projects is rarefaction (McMurdie & Holmes, 2014). Rarefaction creates consistent sequencing "depth" by reducing the number of sequences in every sample to the number of sequences present in the sample with the fewest unique sequences. However, a downside to this method is that it does not account for differentially abundant taxa and wastes information in smaller studies (McMurdie & Holmes, 2014). Since this experiment contained only 268 samples (few compared to animal microbiome studies), rarefaction was not used.

After performing quality control in DADA2, the cleaned sequences were classified as true sequence variants, or SV's (based on 100% sequence similarity, as opposed to OTU's that are typically clustered by 97% similarity), and assigned taxonomy using the RDP database for bacteria and the UNITE database for fungi (Koljalg et al., 2014; Wang et al., 2007). This taxonomic information was passed to the open-source R package Phyloseq for pre-processing and all further analyses (McMurdie & Holmes, 2013).

Pre-processing began by looking at the total number and distribution of reads across SV's and samples (Appendix Fig. 4.1). These graphs depicted similar distributions between bacterial

and fungal reads; however, there are approximately twice as many bacterial taxa as fungal taxa. Next, the sequencing depth (or evenness) was summarized in general and category-specific histograms to evaluate the balance in this experiment (Appendix Fig. 4.2; Appendix Fig. 4.3). These graphs depicted normally distributed sequencing depth for both bacterial and fungal reads. Imbalance exists in the tissue type category because only two bulk soil samples were taken; bulk soil was only collected as a check, and the main tissues of interest (leaf, root, and rhizosphere) were sampled evenly. Imbalance also exists in the plant species category, since some species (i.e. *Aegilops tauschii* and *Triticum aestivum*) contained more accessions than other species. This imbalance was remedied by including an independent variable in the metadata spreadsheet titled "Genotype" that allowed analyses to be performed on the more evenly sampled plant accessions. Next, histograms of taxa prevalence were observed to better understand the depth of the data, specifically, how many reads of each taxon were found in each sample (Appendix Fig. 4.4). These graphs indicated that most taxa were not highly prevalent across all samples. To reduce the length of this skewed tail and to minimize noise from taxa with extremely low prevalence, the taxa table was filtered to exclude phyla that were observed less than 10 times across the entire dataset, as well as any kingdoms besides Fungi, Bacteria, and Archaea (the last of which is low abundance, but included with bacteria for the purposes of this project). Understanding the depth and distribution of sequencing data is vital when drawing conclusions from downstream analyses.

Analyses of microbial ecosystems typically look at two types of diversity: alpha (within-sample) diversity and beta (among-sample) diversity. Alpha diversity was evaluated using the Chao1 and Shannon indices, due to their comparability and reproducibility among microbiome studies. Beta diversity was interpreted by examining a combination of stacked bar charts, faceted

violin plots, and distance ordinations. Stacked bar charts provide ideal visualizations for coarsely differing abundances of microbial taxa, while violin plots depict varying abundances of a specific taxon across multiple plant categories (i.e. genotype and tissue). The ordinations included principle coordinate analyses and non-metric multidimensional scaling analyses, which work well with datasets containing null values. Additionally, both ordination methods work well with the Bray-Curtis index, which allows ordination models to calculate compositional dissimilarity between samples without the use of phylogenetic trees, which are currently unavailable for fungal taxa (unlike the UNIFRAC index, commonly seen in strictly bacterial studies). Data was transformed for principle coordinate analysis by using weighted Bray-Curtis distances. Phylogenetic-based metrics (such as cladograms) cannot yet be made with fungal sequence data because the ITS operon is not amenable to alignments across all fungal communities (Goodrich et al., 2014; Op De Beeck et al., 2014)**.** Finally, plant tissue types were analyzed individually between planting season and treatment type using PERMANOVAs, or ANOVAs using permutations of distance metrics, to generate *P*-values and identify potential drivers of community diversity among samples. This technique was performed using the 'adonis' tool in the Vegan package for R and is recommended for "explaining communities with environmental variables" (Oksanen et al., 2018; R Core Team, 2017). All graphics were generated using the Phyloseq and Ggplot2 packages in R (McMurdie & Holmes, 2013; Wickham, 2009; R Core Team, 2017).

Results and Discussion

Microbiome Diversity Associated with Wheat and Wild Wheat Relatives

The bacterial and fungal communities associated with three tissue types taken from 17 accessions of wheat and wild wheat progenitors were analyzed through Illumina sequencing on a MiSeq platform. After performing quality control and filtering out phyla with a prevalence of <10 across the entire dataset, there remained 2,662 prokaryotic (bacterial and archaeal) taxa and 1,323 fungal taxa. As evidenced by alpha diversity boxplots and violin plots of the most highly represented microbial phyla, faceted by each independent variable (plant species, plant genotype, season, treatment, and tissue type), within-sample microbial diversity appears to be strongly driven by plant tissue type (Figs. 2.2-2.5, Appendix Figs. 4.4-4.5). For this reason, the focus of this study's results and discussion will be on community structure differentiation based on tissue type. Large sample size (n = 268) provides the freedom to interpret results through alpha/beta diversity graphs and distance ordinations.

Alpha diversity was measured using the Chao1 and Shannon diversity indices and is summarized in boxplots (Fig. 2.2-2.5). While both measurements describe within-sample diversity, Chao1 reports richness (number of SV's per sample) and Shannon reports diversity (number of SV's and their evenness in a sample). In general, the median alpha diversity scores for bacteria and fungi between treatments (wet and dry) did not indicate any remarkable ecological distinction (Fig. 2.2). When planting seasons were compared, within-sample diversity was marginally higher in the spring than in the fall for both bacteria and fungi (Fig. 2.3). No significant trends were detected in the alpha diversity medians among plant species (Fig. 2.4). While Chao1 scores were similar for bacteria and fungi among all plant species, the highest prokaryotic Shannon score was in the diploid species *Triticum monococcum monococcum* (A-

genome relative). In contrast, fungal alpha diversity was highest in the diploid species *Aegilops speltoides ligustica* (closely related to the B-genome progenitor), but only slightly higher than the adapted modern cultivars of hexaploid *Triticum aestivum* (ABD-genome). Alpha diversity among plant tissue types was highly stratified (Fig. 2.5). The rhizosphere samples contained the highest alpha diversity scores, slightly higher than bulk soil samples, which confirms previous findings suggesting that plant-root exudates or other plant-related factors may lend greater microbial richness to root-zone soil (Coleman-Derr et al., 2015). Most often, however, plants select a subset of the total microbial community, so rhizospheres can have lower diversity and richness compared to bulk soil (Peiffer et al., 2013; Santoyo et al., 2017). Root tissue, rhizosphere soil, and bulk soil (below-ground samples) all contained higher levels of bacterial than fungal richness and diversity, as is also demonstrated by abundance bar plots (Figs. 2.6-2.7). Proteobacteria was the most abundant bacterial phylum (23% of total sequences), and the plant growth promoting rhizobacteria (PGPR) genus *Pseudomonas* was well-represented in the rhizosphere. Other phyla found among plant tissues containing PGPR taxa include Planctomycetes (18% of sequences), Bacteroidetes (11% of sequences), Actinobacteria (9% of sequences), Acidobacteria (6% of sequences), Firmicutes (4% of sequences), and Verrucomicroba (4% of sequences). As discussed in Chapter 1, many of the beneficial properties of these microbial symbionts are still putative and environment-specific, but their documented abundance in this experiment further reinforces their purported roles in plant health and development.

Leaf tissue, in contrast to below-ground samples, contained higher fungal richness and diversity, with a higher proportion of the phylum Basidiomycota than below-ground samples. This is in large part due to the high prevalence of the basidiomycete genus *Puccinia* found on

stripe rust-infected leaf tissue (Fig. 2.8); pesticides were not used in this project. The larger vase-shaped polygons encompassing the leaf samples in Figure 2.8 demonstrate the greater abundance of *Puccinia* in above-ground versus below-ground tissues; presence of *Puccinia* in below-ground tissue indicates contamination. Overall, the largest represented fungal phylum was the phylum Ascomycota (61% of sequences), whose class Dothidiomycetes contained the largest number of taxa across all tissue types (Fig. 2.9). Dothidiomycetes is the largest and most diverse ecological class of Ascomycota, containing numerous plant pathogens and decomposers important to soil carbon cycling (Mendes et al., 2013). A small quantity of arbuscular mycorrhizae from the phylum Glomeromycota (1% of total fungal reads) was found in root tissue (Fig. 2.6b). At least one root sample in every wild and domesticated wheat species contained Glomeromycota, but the hexaploid *Triticum aestivum* cultivars contained the largest amount of mycorrhizal root associations, followed by *Aegilops tauschii* (D-genome relative)(Fig. 2.10). With very few exceptions, the dominant Glomeromycete taxon found in roots was *Funneliformis mosseae*. This arbuscular mycorrhizal taxon is associated with enhanced water and nutrient uptake in diverse crop species, and may contribute to the robust growth of the three Colorado-adapted hexaploid cultivars in this study (Bitterlich et al., 2018; Chen et al., 2017). It also suggests that the D-genome may be important for recruiting mycorrhizae and possessing root architecture conducive to such associations. *Aegilops tauschii*, the diploid D-genome progenitor, can grow roots deep into the soil moisture profile and be colonized by mycorrhizae, both traits of which contribute to the species' drought tolerance (Bektas et al., 2017). While all wild and domesticated wheat species possessed at least one root sample containing a member of order Glomeroales, this limited representation of Glomeromycete diversity is indicative of tilled agricultural soils where fungal hyphal networks are routinely disturbed (Schalamuk & Cabello, 2010). Another

remarkable group of fungi found in large abundance within the root tissue is phylum Chytridiomycota (Fig. 2.6b). Within this phylum, the overwhelming number of root samples contained fungi from class Chytridiomycetes. This class contains many infamous parasites; however, non-hyphal Chytrids in plant roots are purported to be capable of enhancing plant nutrient uptake in some arid rangeland species (Barrow et al., 1997).

Factors Driving Microbial Community Diversity

Beta diversity metrics were analyzed to elucidate major drivers of microbial community composition. Principle coordinate analyses (PCoA) using the Bray-Curtis dissimilarity index consistently indicated plant tissue type as the vector with the largest eigenvalue (responsible for the greatest amount of variability). In the bacterial PCoA, tissue type accounted for 8.6% of the among-sample variability, while tissue type accounted for 12.2% in the fungal PCoA (Fig. 2.10). Planting season was secondarily evaluated in the same graphs to determine whether fall- and spring-planted samples should be analyzed separately; the resulting lack of significant clustering between fall and spring suggested that separate analyses were not needed. This is not surprising, since both fall- and spring-planted plants underwent vernalization, flowered with a short delay for spring-planted materials, and were sampled within 11 days of one another. Non-metric multidimensional scaling (NMDS) ordinations also demonstrated pronounced clustering of tissue types and their respective microbial phyla (Fig. 2.11).

To further investigate any underlying factors influencing the significant differences among tissue types, tissues were analyzed individually by planting season, treatment, plant species, plant genotype, and plant ploidy through permutational analyses of variance (PERMANOVA)(Table2.2). When plant tissue types were analyzed individually, nearly half of

the total samples were found to differ significantly in microbial community structure for treatment (wet/dry), particularly in the rhizosphere (*P*-values: 0.046 [Fall, 16S], 0.002 [Spring, 16S], 0.002 [Fall, ITS2]). Due to an exceptionally rainy spring, the wet treatment only received one additional irrigation (13 mm) five days prior to tissue collection. Therefore, these significant PERMANOVA values suggested an additional, unaccounted variable, such as soil fertility, texture, or pH (Fierer, 2017). The cropping history of the field site indicated that the "wet" site was fallow the year before this study, while the "dry" site was planted immediately following a canola (*Brassica napus* L.) harvest. A fallow period allows moisture to accumulate in the soil profile, and the decomposing canola residue would return nutrients to soil (Kabir et al., 1999; Lenssen et al., 2007). Recently harvested sites are more likely to be depleted of key nutrients, such as nitrogen and carbon and to have a reduced soil moisture level (Kabir et al., 1999; Lenssen et al., 2007). The only soil test performed in this project was on a sample of bulk soil taken directly between the wet and dry sites (Appendix Table 4.1). Soil moisture status was not measured, so we cannot say whether it was a primary driver in microbiome composition. However, other studies have found that soil moisture and fertility, particularly the resultant effect on soil pH, play an enormous role on soil microbial biomass and diversity (Fierer, 2017; Leff et al., 2015).

A random forest regression was used to classify rhizosphere-sampled SV's in their order of importance for discriminating between wet and dry treatments (Figure 2.12). The most significant bacterial sequence variant found (SV21) was a member of the phylum Chloroflexi, in the proposed class Ellin 6529 (Figure 2.13). This taxon was found in higher abundance among wet samples across all wild and domesticated species in this study (Figure 2.14). Other members of the phylum Chloroflexi have been found in similarly cold, water-saturated soils in meadows

throughout the nearby Colorado Rocky Mountains (Costello et al., 2006). The second most significantly differentiating bacterial sequence variant (SV30) was *Flavobacterium succinicans*, a Gram-negative facultative anaerobe in the phylum Bacteroidetes whose presence has also been documented amongst frozen soils in northern latitudes (Zhang et al., 2006). *F. succinicans* was also found in higher abundance among wet rhizosphere samples, particularly in the plant species *Triticum aestivum*, *Triticum dicoccoides*, and *Triticum urartu* (Figure 2.14). The most significant fungal taxon (SV47) for distinguishing between wet and dry rhizosphere samples was *Trichocladium asperum* (Figure 2.13). This ascomycete is a slow-growing microfungi (loosely classified as "black yeast") that is resilient to environmental stresses and commonly found in soils and aquatic environments (Christian, 2014). *T. asperum* was most abundant in wet samples, predominately within the rhizosphere of the wild wheat relative *Aegilops tauschii*. The second most important fungal taxon (SV32) was another ascomycete in the family Tremellales (Figure 2.13). Members of the Tremellales are notable for their ability to decompose cellulose, and their populations tend to proliferate in soils with high organic litter content (Kuramae et al., 2013). Unlike the other top three significantly discriminative microbial taxa in this study, Tremellales was most represented among dry rhizosphere samples. This is consistent with the cropping history known of the dry site, which included a recent canola harvest and subsequent plant litter residue. The presence of distinct microbial taxa in wet and dry rhizosphere samples suggests that moisture, and likely soil conditions (e.g. pH, texture, organic matter content), play an important role in microbial community structure.

Conclusions

The initial goal of this study was to compare the microbiomes of wheat and its wild relatives through a multivariate approach, including an analysis of different plant tissue types. Ultimately, no significant differences were found among the microbiomes of three hexaploid wheat cultivars and 14 landrace and wild relative accessions of different ploidy levels. This is consistent with results in contemporary literature that indicate differences in the plant microbiome are relatively minor across a plant domestication gradient (Leff et al., 2016). Broader literature reviews agree that plant species identity is not a strong predictor of soil microbial communities (Gous et al., 2015). Alpha and beta diversity metrics suggest that plant tissue type, however, was the greatest driver of within-sample diversity. Overall, rhizosphere samples hosted the most complex microbial communities, with greater abundance and diversity than other tissue types. Upon deeper examination of among-sample diversity, treatment (wet/dry) influenced some underlying differences among tissue types. The different moisture regimes between sites, as well as an imbalanced cropping history that may have caused variable soil fertility, resulted in the notable presence of certain discriminative microbes.

This study confirms results from the current literature's understanding of distinct microbial communities among plant tissue types, as well as the critical role of soil conditions. The close phylogenetic relationships among the host germplasm in this study may have limited the ability to observe genotype-dependent microbial diversity. Biogeography is a known driver of microbial diversity, and that effect is diminished in common garden experiments such as this one. Future innovations in phytobiome technology can benefit from this study by the corroborative evidence demonstrating similar microbiome functionality among closely related

plant species, and the distinct microbial communities that occupy leaves, roots, and rhizosphere soil.

Tables

Table 2.1 Plant genotypes included in this study, by planting date.

	Planted 15 October 2015			
Entry	**Taxa**	**Acc. No.[1]**	**Ploidy[2]**	**Genome**
1	*Aegilops sharonensis*	TA 1998	2x	B
2	*Aegilops speltoides ligustica*	TA 1772	2x	B
3	*Triticum monococcum aegilopoides*	TA 177	2x	A
4	*Triticum monococcum monococcum*	TA 142	2x	A
5	*Triticum turgidum dicoccoides*	TA 61	4x	AB
6	*Triticum turgidum durum*	TA 10451	4x	AB
7	*Triticum urartu*	TA 739	2x	A
8	*Aegilops tauschii*	TA 2374	2x	D
9	*Aegilops tauschii*	TA 2536	2x	D
10	*Aegilops tauschii*	TA 10106	2x	D
	Planted 13 February 2016			
Entry	**Taxa**	**Acc. No.**	**Ploidy**	**Genome**
1	*Aegilops tauschii*	TA 1707	2x	D
2	*Aegilops tauschii*	TA 2458	2x	D
3	*Aegilops tauschii*	TA 2536	2x	D
4	*Aegilops tauschii*	TA 10144	2x	D
5	*Aegilops tauschii*	TA 10330	2x	D
6	*Triticum aestivum*	Kharkov	6x	ABD
7	*Triticum aestivum*	Byrd	6x	ABD
8	*Triticum aestivum*	RonL	6x	ABD
9	*Triticum urartu*	TA 739	2x	A
10	*Triticum monococcum monococcum*	TA 142	2x	A
11	*Aegilops speltoides ligustica*	TA 1772	2x	B
12	*Triticum turgidum dicoccoides*	TA 61	4x	AB

[1] Accession number of Wheat Genetics Resource Center, Manhattan, KS (http://www.k-state.com/wgrc/).
[2] "x" refers to the number of chromosomes in a basic set, which is 7 for wheat and its wild relatives.

Table 2.2 Tissue type PERMANOVA *P*-values generated by 'adonis' tool in Vegan package for R (R Core Team, 2018).

Primer	Season	Tissue	Variable	*P*-value
16S	Fall	Leaf	Genome	0.092
16S	Fall	Leaf	Genotype	0.501
16S	Fall	Leaf	Plant Species	0.360
16S	Fall	Leaf	Ploidy	0.160
16S	Fall	Leaf	Treatment	0.065
16S	Fall	Root	Genome	0.472
16S	Fall	Root	Genotype	0.557
16S	Fall	Root	Plant Species	0.223
16S	Fall	Root	Ploidy	0.116
16S	Fall	Root	Treatment	0.613
16S	Fall	Rhizosphere	Genome	0.389
16S	Fall	Rhizosphere	Genotype	0.313
16S	Fall	Rhizosphere	Plant Species	0.606
16S	Fall	Rhizosphere	Ploidy	0.278
16S	Fall	Rhizosphere	Treatment	**0.046***
16S	Spring	Leaf	Genome	0.425
16S	Spring	Leaf	Genotype	0.359
16S	Spring	Leaf	Plant Species	0.340
16S	Spring	Leaf	Ploidy	0.651
16S	Spring	Leaf	Treatment	0.856
16S	Spring	Root	Genome	0.293
16S	Spring	Root	Genotype	0.784
16S	Spring	Root	Plant Species	0.468
16S	Spring	Root	Ploidy	0.256
16S	Spring	Root	Treatment	0.139
16S	Spring	Rhizosphere	Genome	0.627
16S	Spring	Rhizosphere	Genotype	0.538
16S	Spring	Rhizosphere	Plant Species	0.693
16S	Spring	Rhizosphere	Ploidy	0.497
16S	Spring	Rhizosphere	Treatment	**0.002****
ITS2	Fall	Leaf	Genome	0.223
ITS2	Fall	Leaf	Genotype	0.137
ITS2	Fall	Leaf	Plant Species	0.147
ITS2	Fall	Leaf	Ploidy	0.223
ITS2	Fall	Leaf	Treatment	**0.003****
ITS2	Fall	Root	Genome	0.179
ITS2	Fall	Root	Genotype	0.099
ITS2	Fall	Root	Plant Species	0.102
ITS2	Fall	Root	Ploidy	0.164
ITS2	Fall	Root	Treatment	**0.041***
ITS2	Fall	Rhizosphere	Genome	0.132

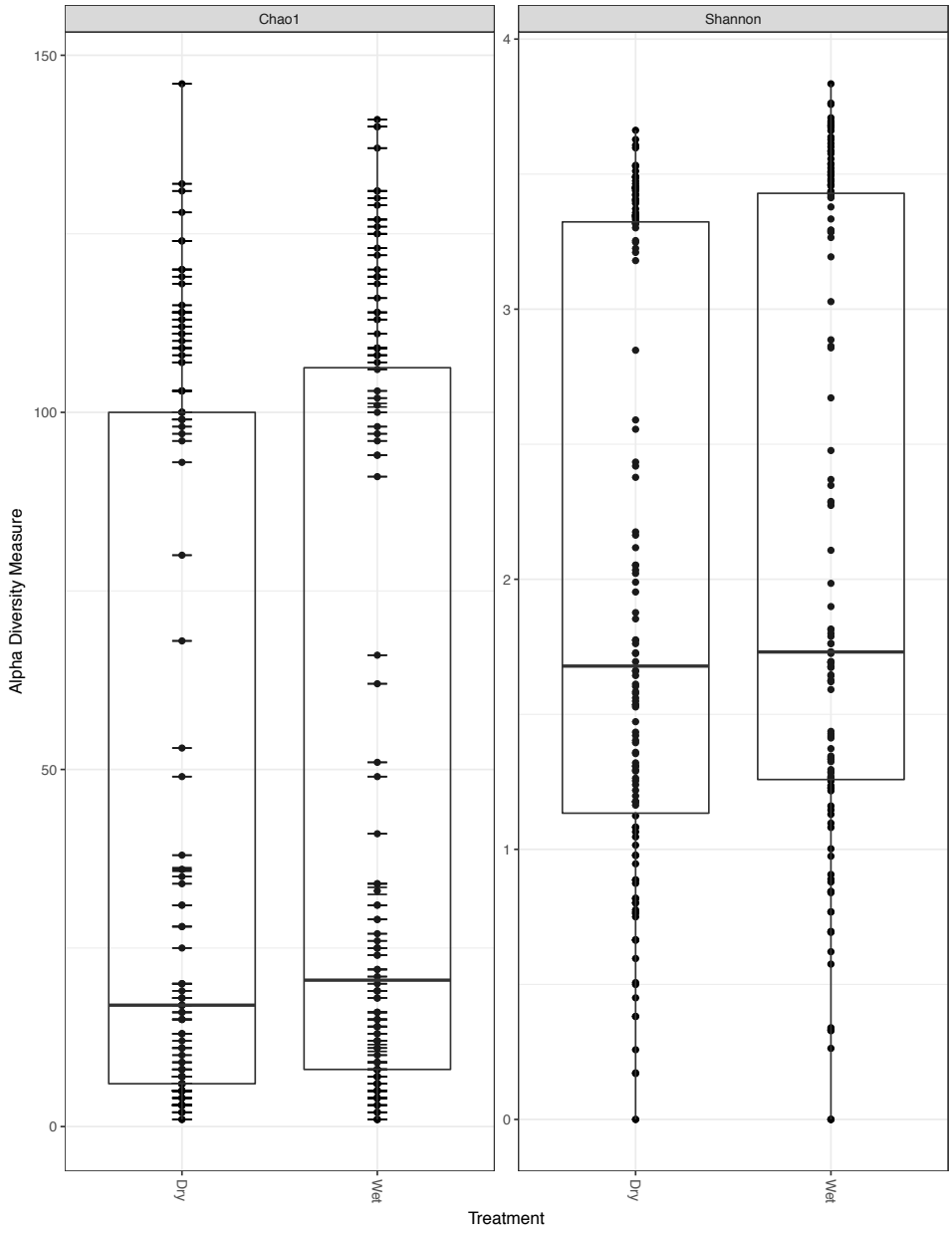
ITS2	Fall	Rhizosphere	Genotype	0.594
ITS2	Fall	Rhizosphere	Plant Species	0.112
ITS2	Fall	Rhizosphere	Ploidy	0.149
ITS2	Fall	Rhizosphere	Treatment	**0.002****
ITS2	Spring	Leaf	Genome	0.936
ITS2	Spring	Leaf	Genotype	0.907
ITS2	Spring	Leaf	Plant Species	0.100
ITS2	Spring	Leaf	Ploidy	0.215
ITS2	Spring	Leaf	Treatment	0.225
ITS2	Spring	Root	Genome	0.693
ITS2	Spring	Root	Genotype	0.094
ITS2	Spring	Root	Plant Species	0.379
ITS2	Spring	Root	Ploidy	0.407
ITS2	Spring	Root	Treatment	0.910
ITS2	Spring	Rhizosphere	Genome	0.239
ITS2	Spring	Rhizosphere	Genotype	0.353
ITS2	Spring	Rhizosphere	Plant Species	0.132
ITS2	Spring	Rhizosphere	Ploidy	0.613
ITS2	Spring	Rhizosphere	Treatment	0.108

* significant at $P < 0.05$, ** significant at $P < 0.005$.

Figures

Figure 2.1 Terrain map of ten wild wheat relative accessions used in this study in their centers of origin (*Ae. tauschii* TA 1707 is not plotted due to lack of collection data). Map courtesy of Jon Raupp, Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA.

a)


Chao1
Shannon
Alpha Diversity Measure
150
100
50
0
4
3
2
1
0
Dry
Wet
Dry
Wet
Treatment

b)

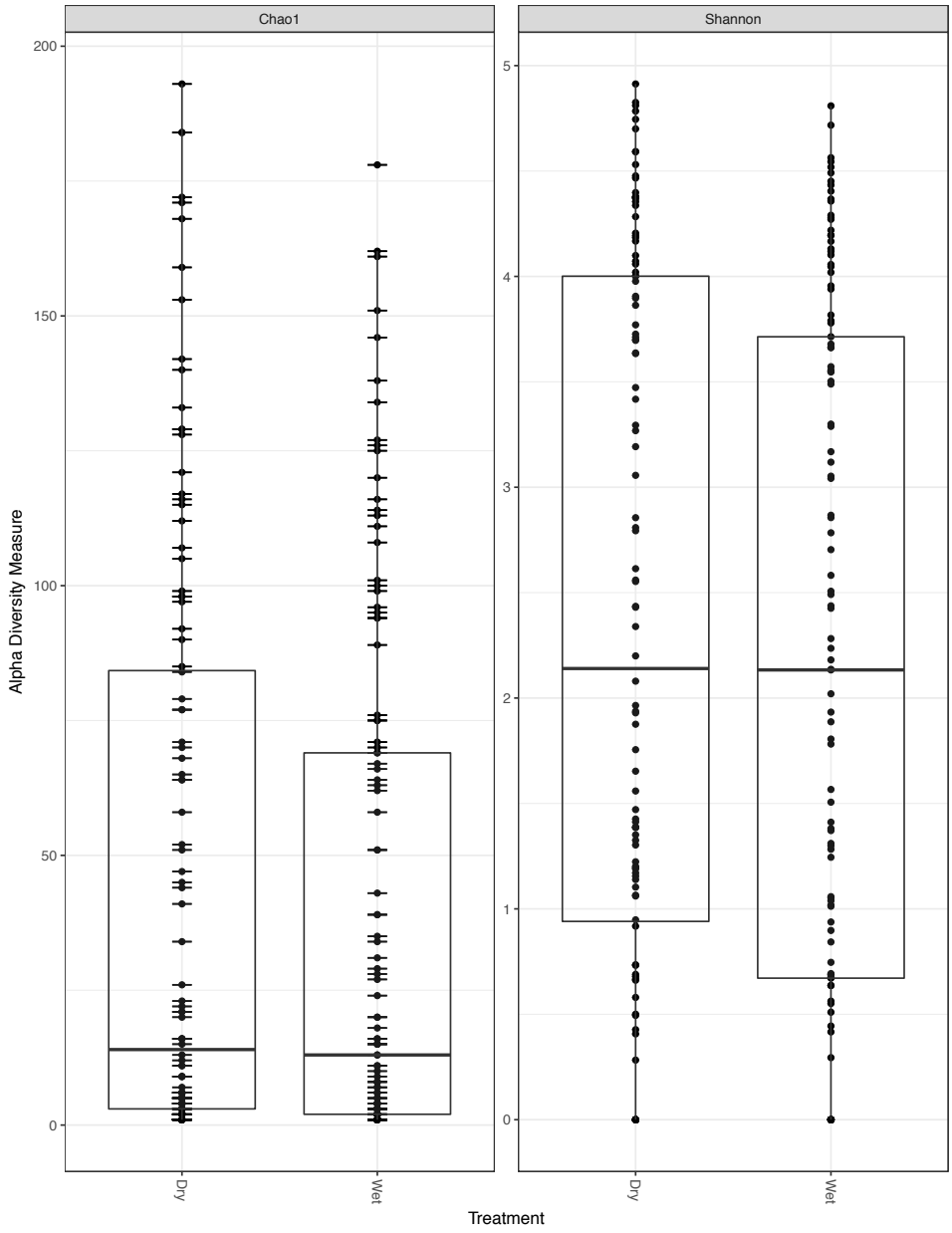
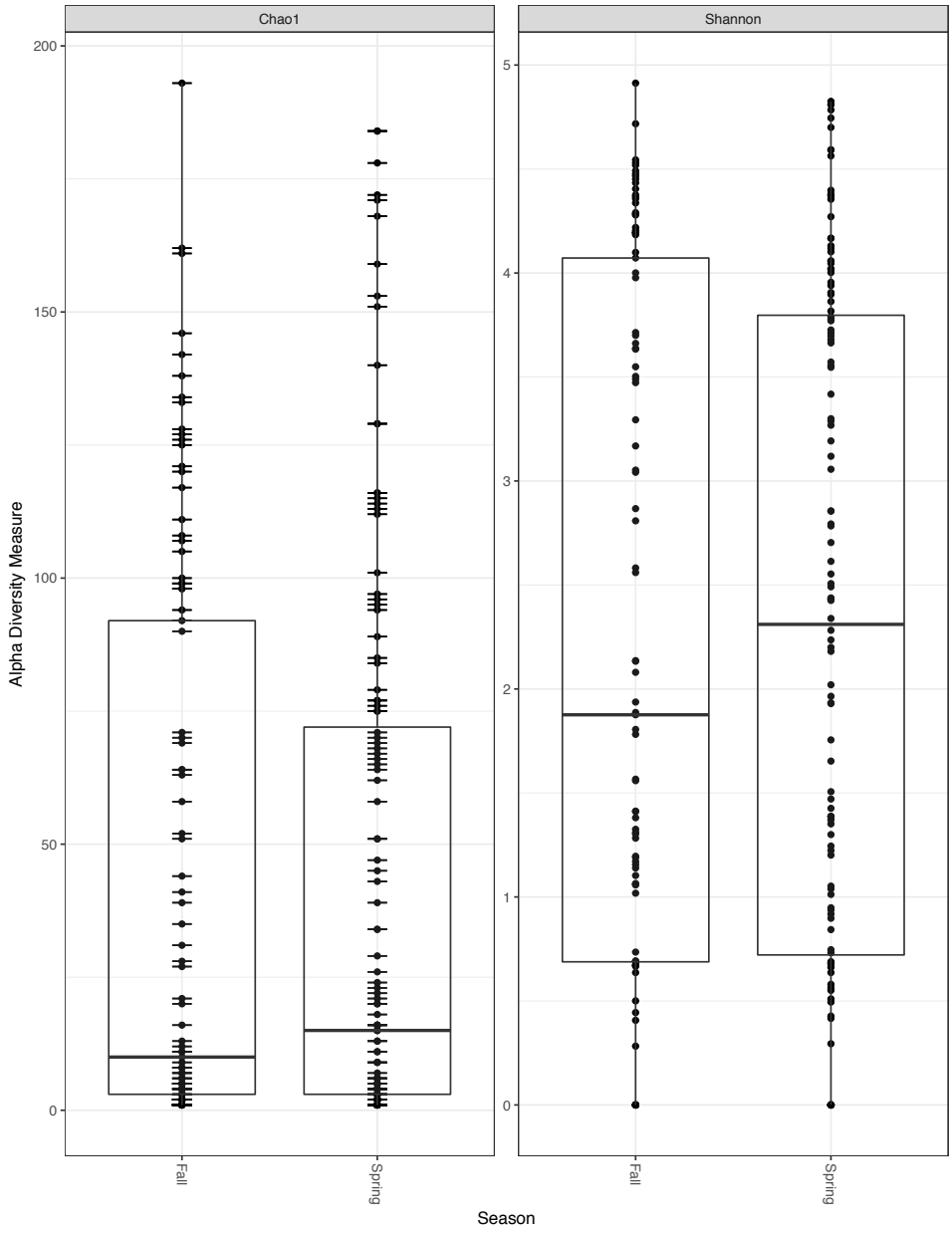


Figure 2.2 Alpha diversity panels (Chao1 and Shannon indices) for a) bacteria and b) fungi by treatment. Boxes encompass 50% of the data (interquartile range); the mid-line indicates the median value, the lower line indicates the lower quartile (25% of the data falls below this value), the upper line indicates the upper quartile (25% of the data falls above this value), and standard error bars bound the greatest and lowest values, excluding outliers.

a)


Chao1
Shannon
Alpha Diversity Measure
200
150
100
50
0
5
4
3
2
1
0
Fall
Spring
Fall
Spring
Season

b)

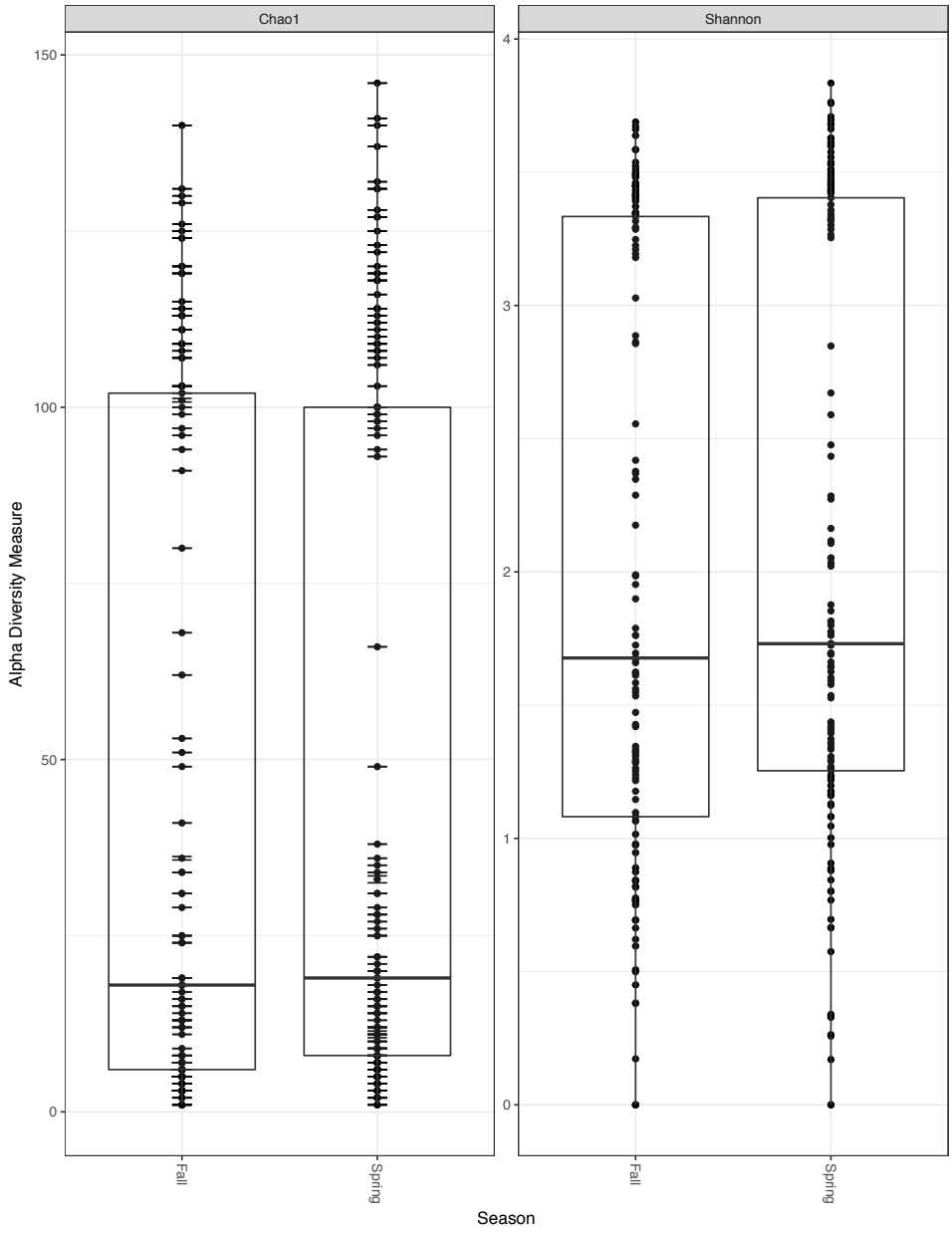
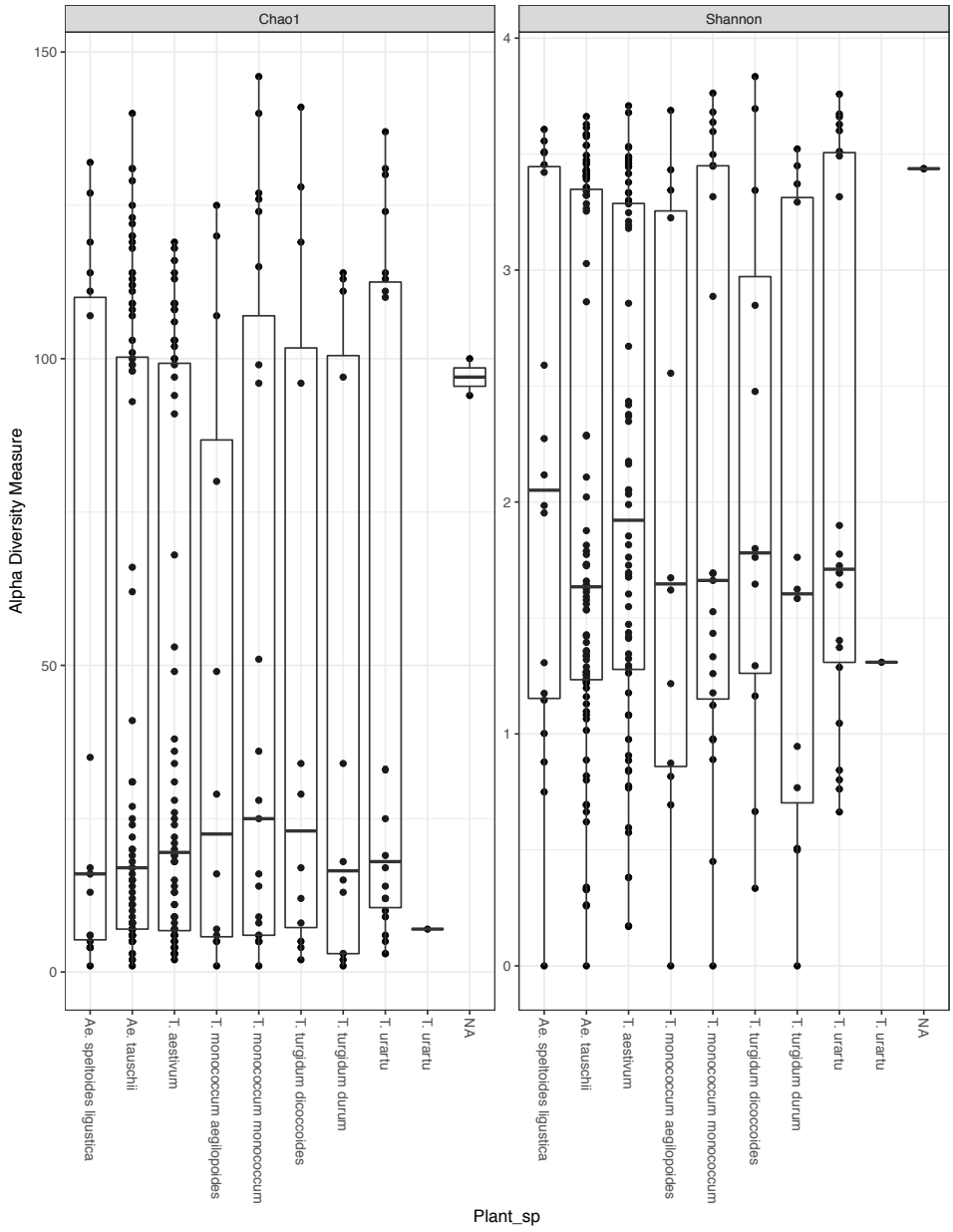


Figure 2.3 Alpha diversity panels (Chao1 and Shannon indices) for a) bacteria and b) fungi by season. Boxes encompass 50% of the data (interquartile range); the mid-line indicates the median value, the lower line indicates the lower quartile (25% of the data falls below this value), the upper line indicates the upper quartile (25% of the data falls above this value), and standard error bars bound the greatest and lowest values, excluding outliers.

a)


Chao1
Shannon
Alpha Diversity Measure
Plant_sp
Ae. speltoides ligustica
Ae. tauschii
T. aestivum
T. monococcum aegilopoides
T. monococcum monococcum
T. turgidum dicoccoides
T. turgidum durum
T. urartu
T. urartu
NA

b)

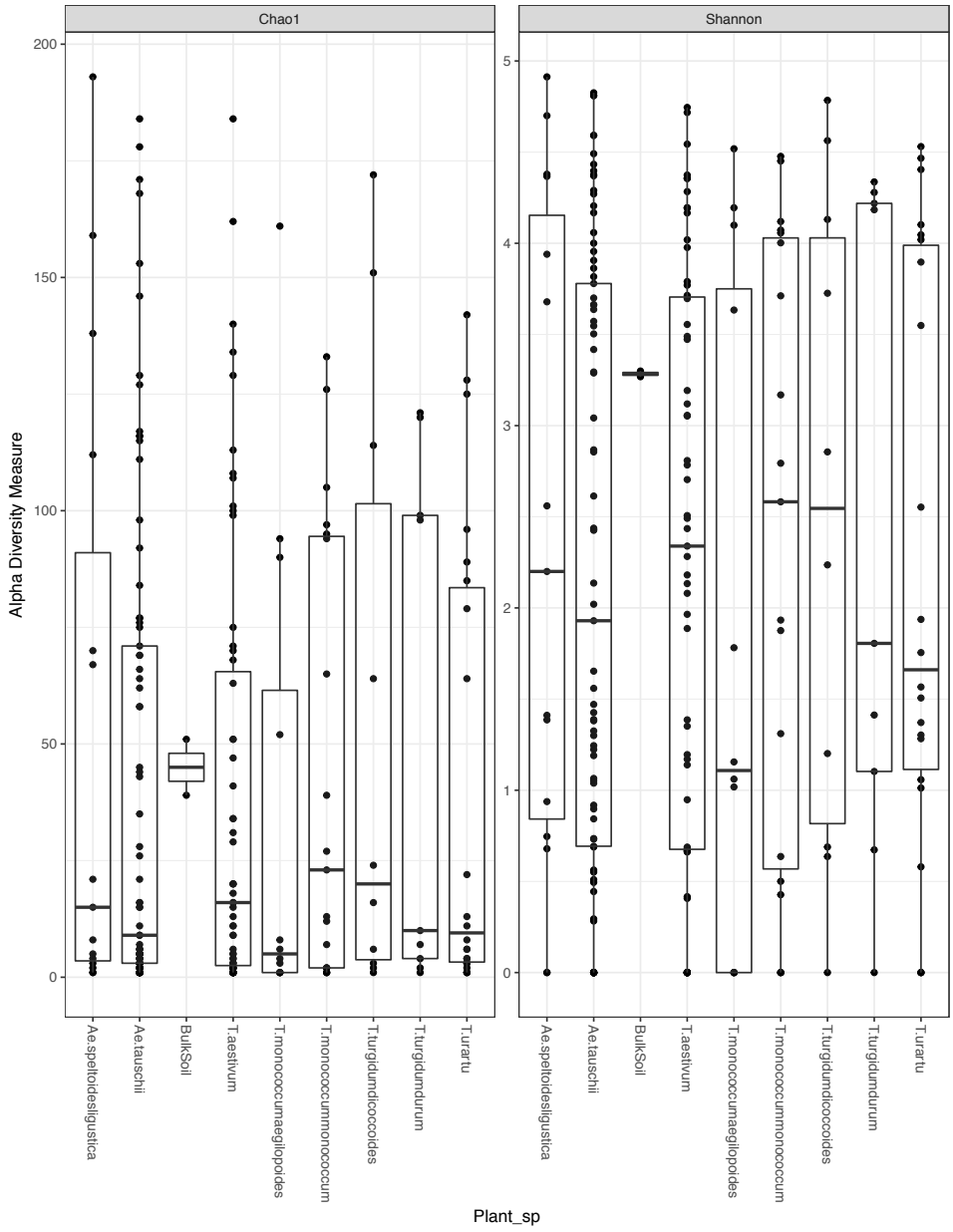
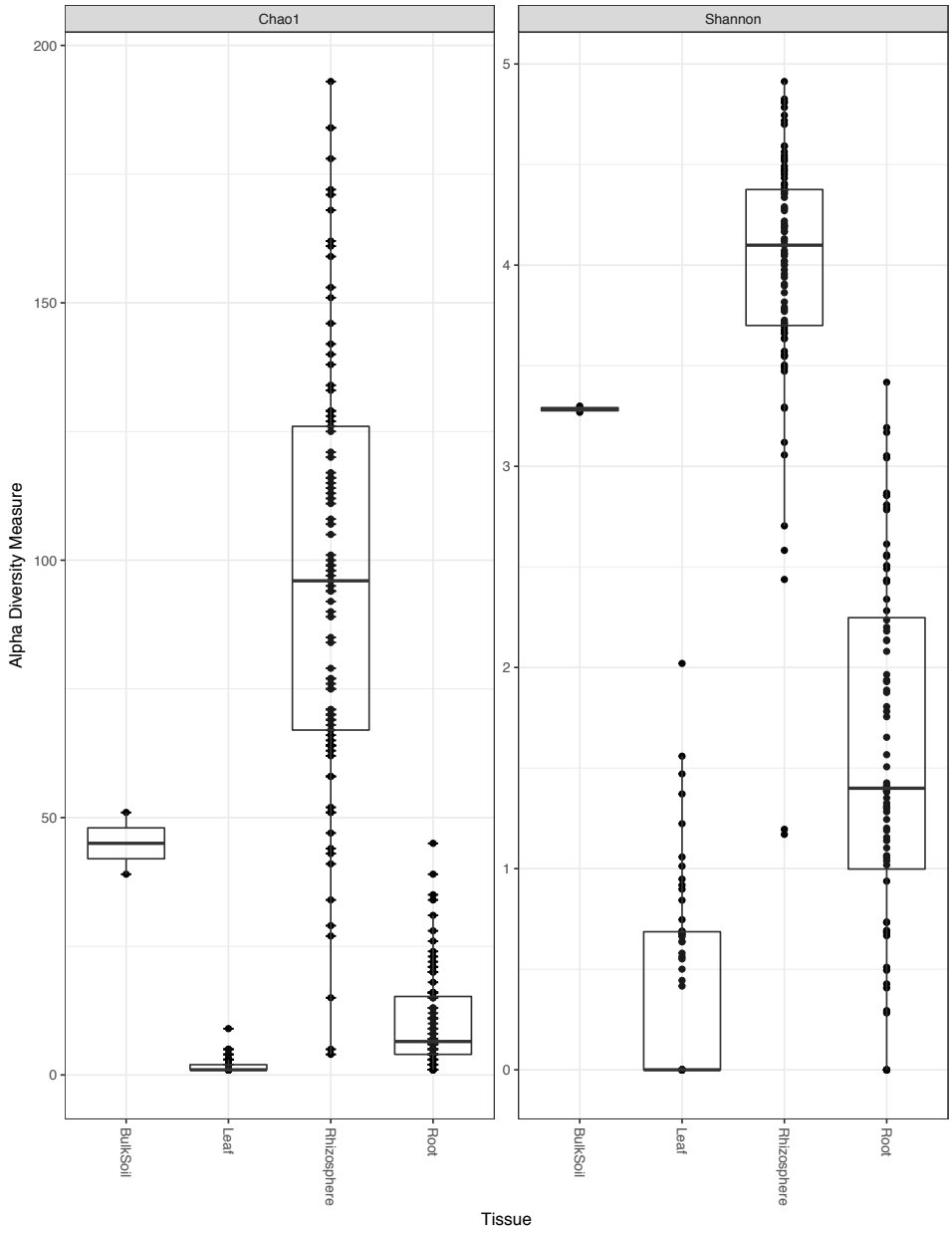


Figure 2.4 Alpha diversity panels (Chao1 and Shannon indices) for a) bacteria and b) fungi by plant species. Boxes encompass 50% of the data (interquartile range); the mid-line indicates the median value, the lower line indicates the lower quartile (25% of the data falls below this value), the upper line indicates the upper quartile (25% of the data falls above this value), and standard error bars bound the greatest and lowest values, excluding outliers. Column "NA" refers to bulk soil samples.

a)


Chao1
Shannon
Alpha Diversity Measure
Tissue
BulkSoil
Leaf
Rhizosphere
Root
0
50
100
150
200
1
2
3
4
5

b)

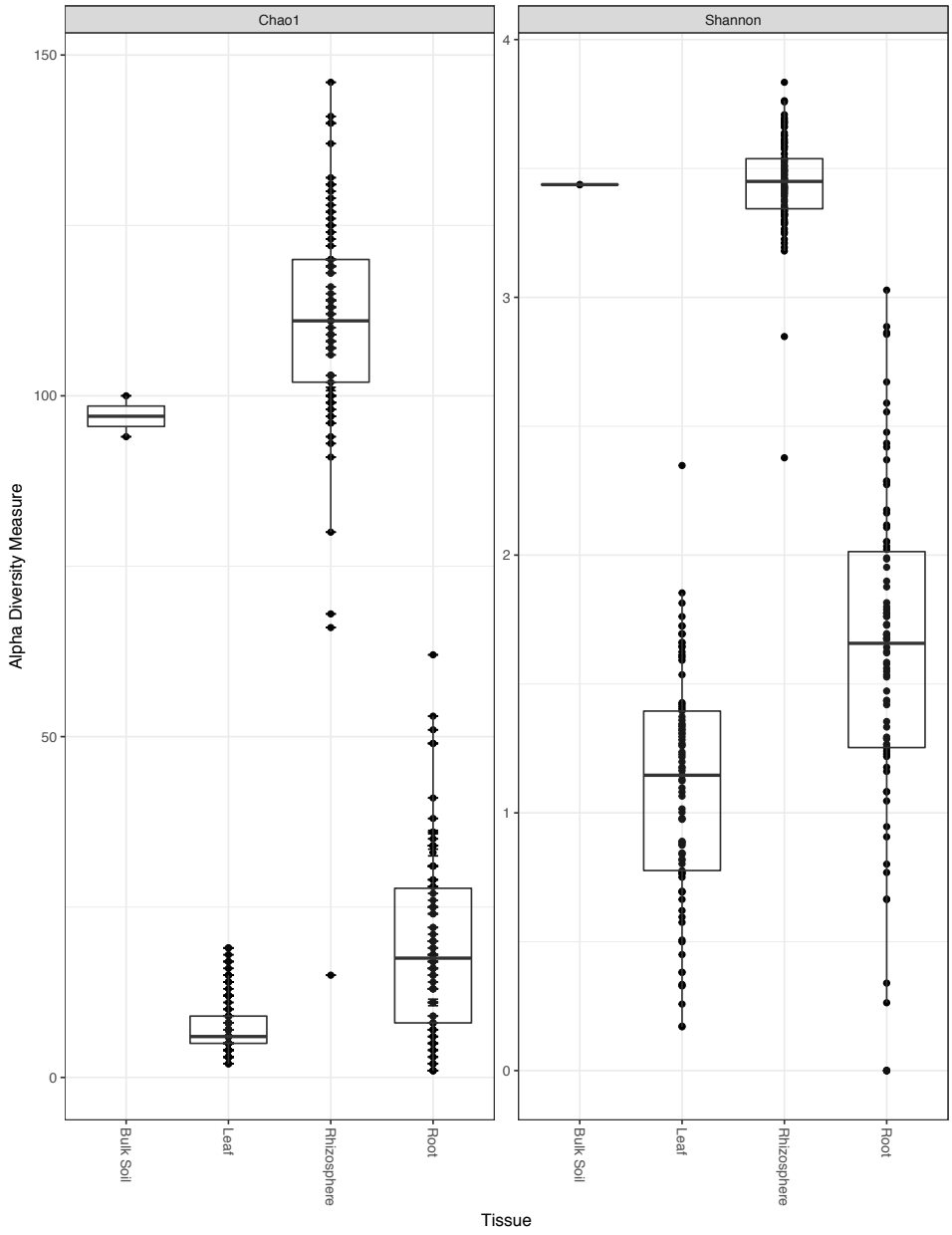
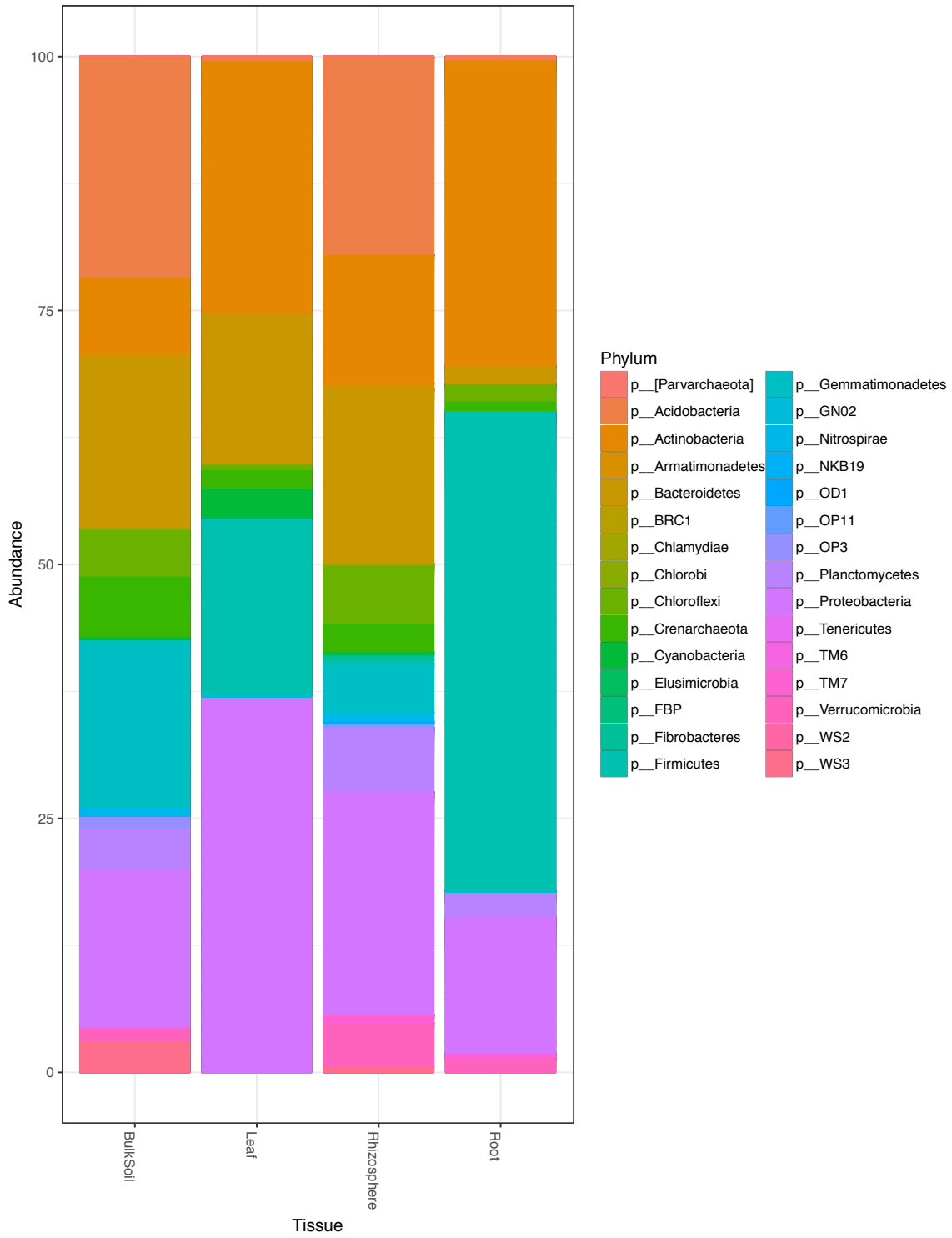


Figure 2.5 Alpha diversity panels (Chao1 and Shannon indices) for a) bacteria and b) fungi by plant tissue type. Boxes encompass 50% of the data (interquartile range); the mid-line indicates the median value, the lower line indicates the lower quartile (25% of the data falls below this value), the upper line indicates the upper quartile (25% of the data falls above this value), and standard error bars bound the greatest and lowest values, excluding outliers.

a)


100
75
50
25
0
Abundance
BulkSoil
Leaf
Rhizosphere
Root
Tissue
Phylum
p__[Parvarchaeota]
p__Acidobacteria
p__Actinobacteria
p__Armatimonadetes
p__Bacteroidetes
p__BRC1
p__Chlamydiae
p__Chlorobi
p__Chloroflexi
p__Crenarchaeota
p__Cyanobacteria
p__Elusimicrobia
p__FBP
p__Fibrobacteres
p__Firmicutes
p__Gemmatimonadetes
p__GN02
p__Nitrospirae
p__NKB19
p__OD1
p__OP11
p__OP3
p__Planctomycetes
p__Proteobacteria
p__Tenericutes
p__TM6
p__TM7
p__Verrucomicrobia
p__WS2
p__WS3

b)

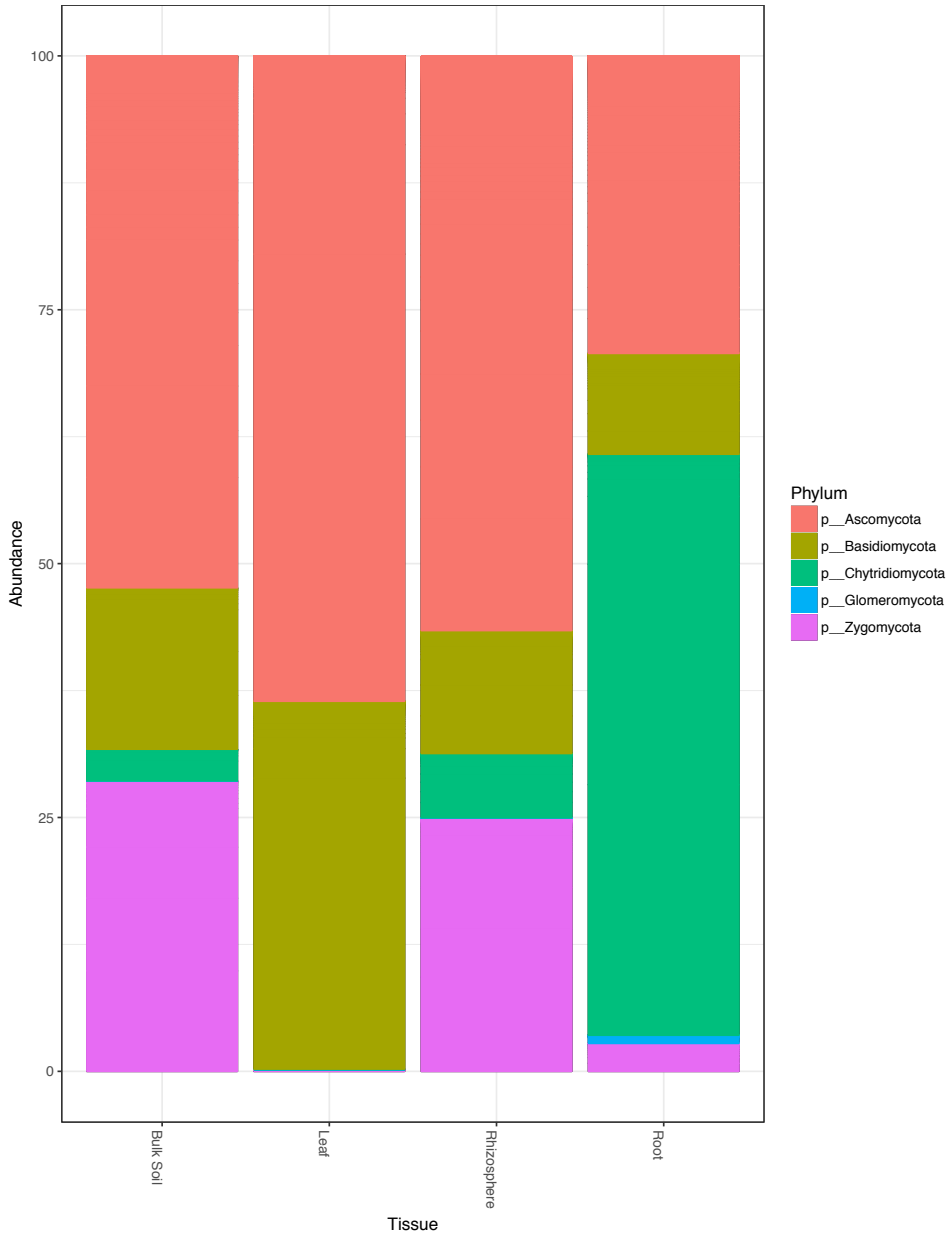
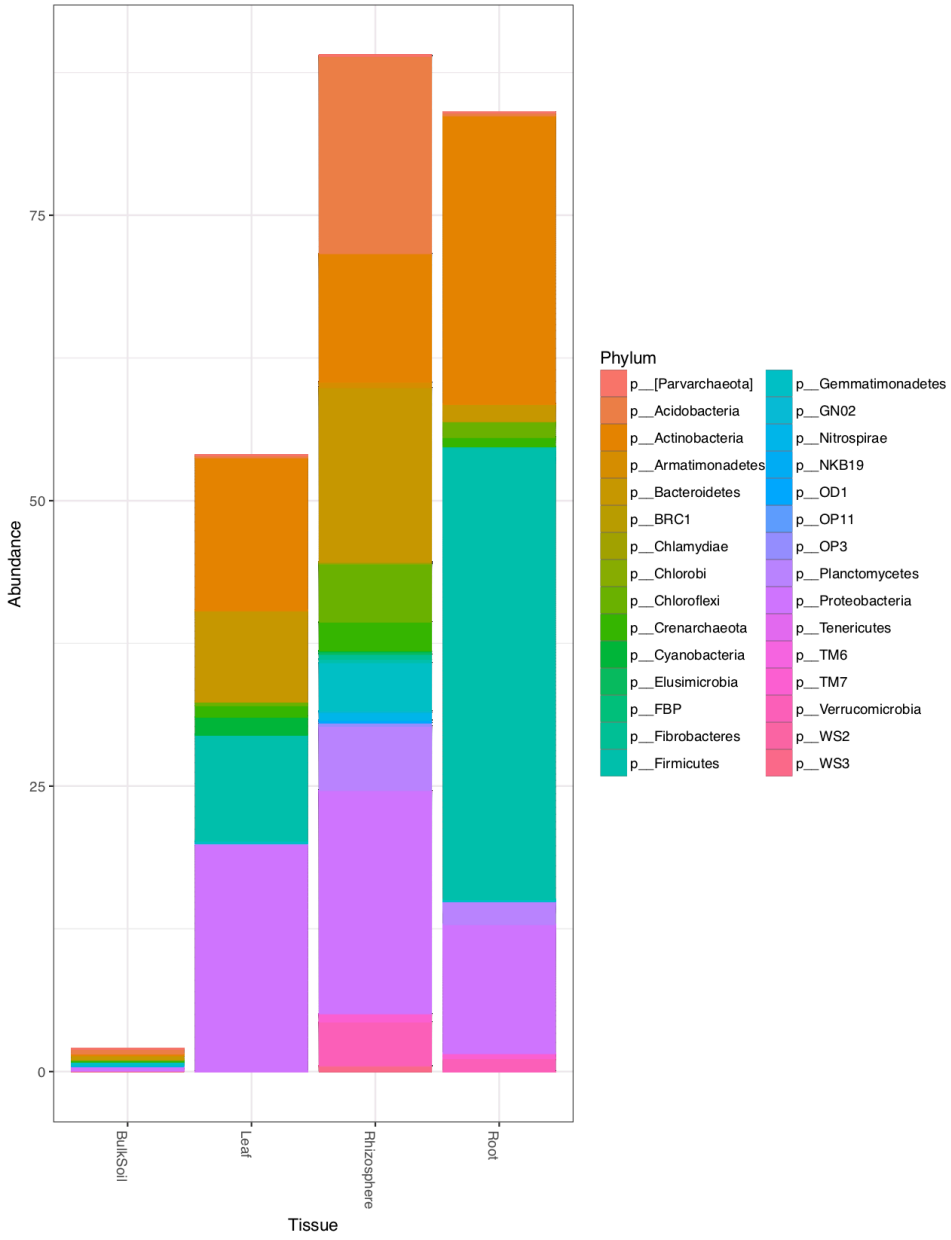


Figure 2.6 Relative abundance bar plot for a) bacterial and b) fungal phyla by plant tissue type.

a)


Abundance
75
50
25
0
BulkSoil
Leaf
Rhizosphere
Root
Tissue
Phylum
p__[Parvarchaeota]
p__Acidobacteria
p__Actinobacteria
p__Armatimonadetes
p__Bacteroidetes
p__BRC1
p__Chlamydiae
p__Chlorobi
p__Chloroflexi
p__Crenarchaeota
p__Cyanobacteria
p__Elusimicrobia
p__FBP
p__Fibrobacteres
p__Firmicutes
p__Gemmatimonadetes
p__GN02
p__Nitrospirae
p__NKB19
p__OD1
p__OP11
p__OP3
p__Planctomycetes
p__Proteobacteria
p__Tenericutes
p__TM6
p__TM7
p__Verrucomicrobia
p__WS2
p__WS3

b)

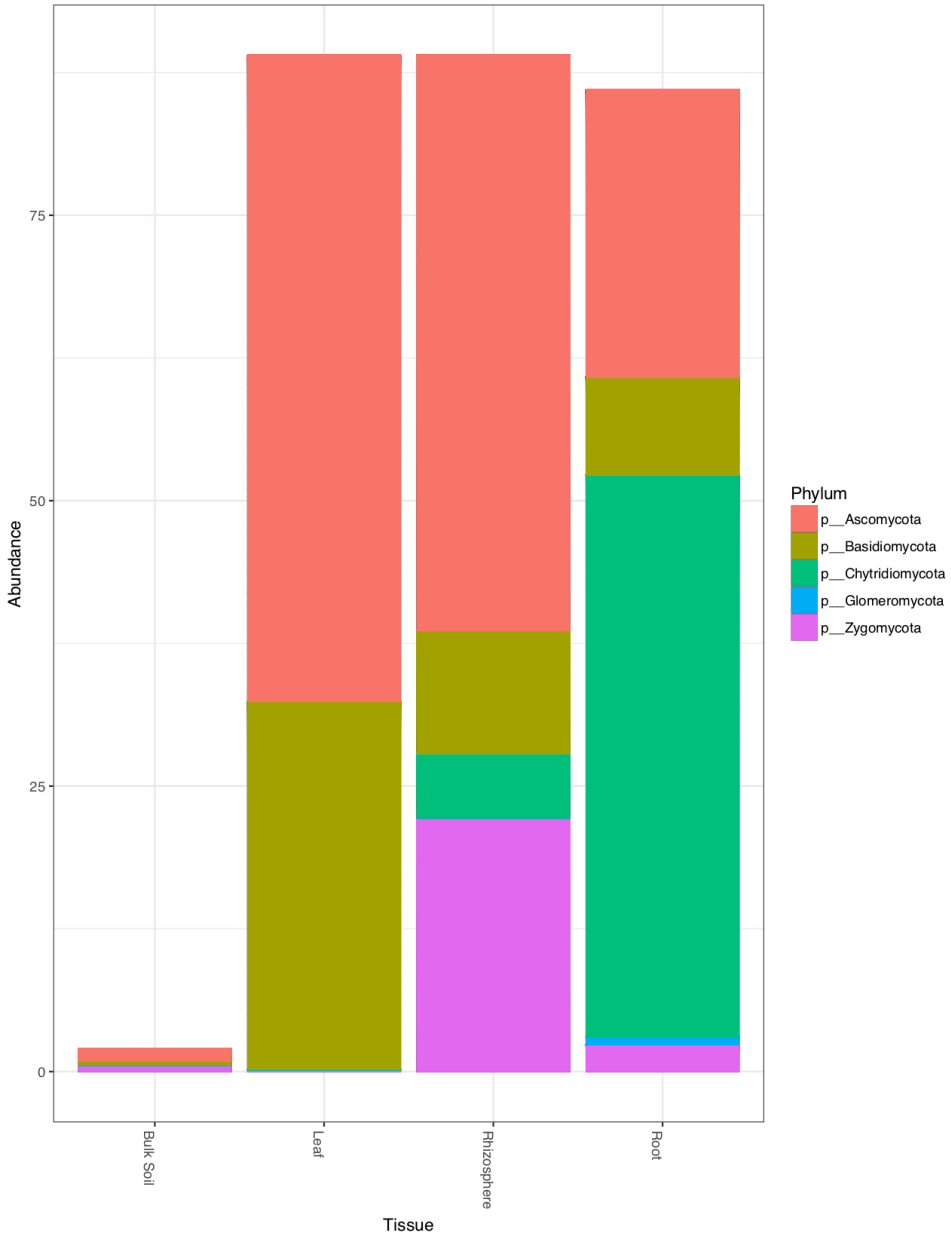


Figure 2.7 Percent abundance bar plot for a) bacterial and b) fungal phyla by plant tissue type. Bulk soil is low because only two samples were taken.

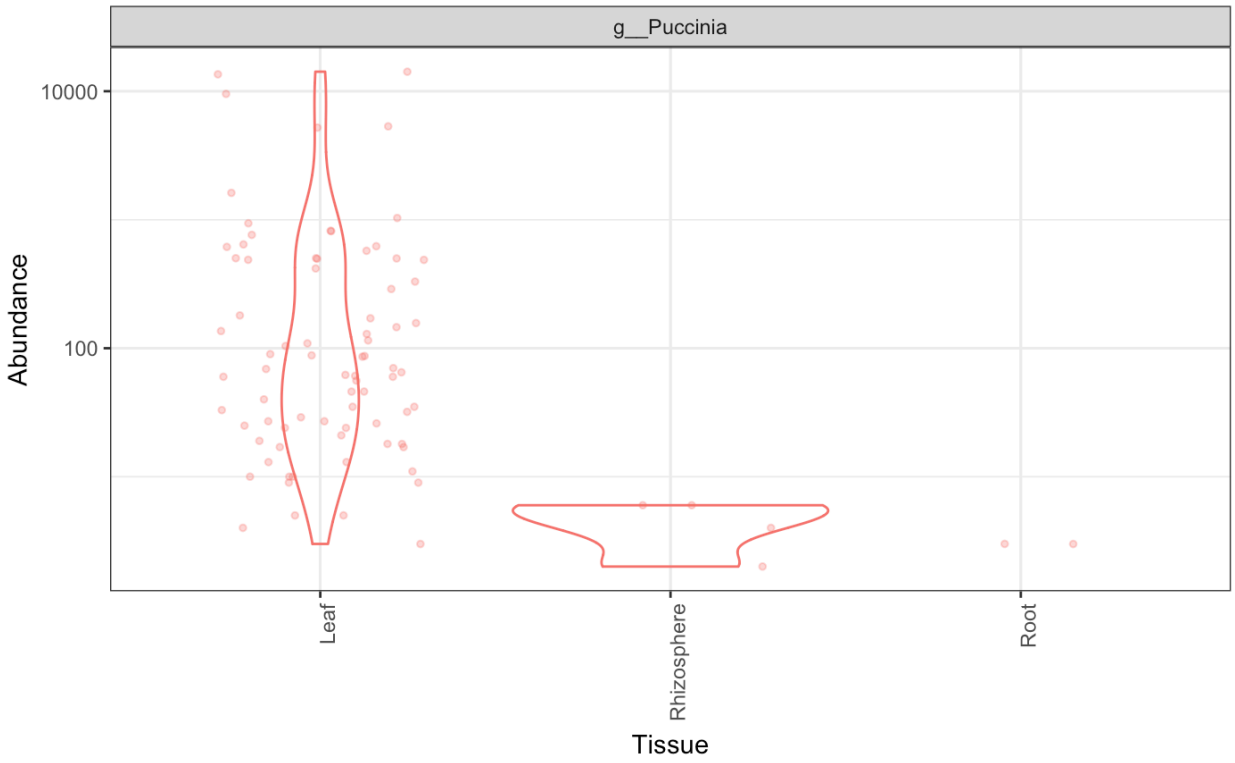


Figure 2.8 Violin plot depicting abundance of G. *Puccinia*, the stripe-rust genus, found primarily in leaf tissue.

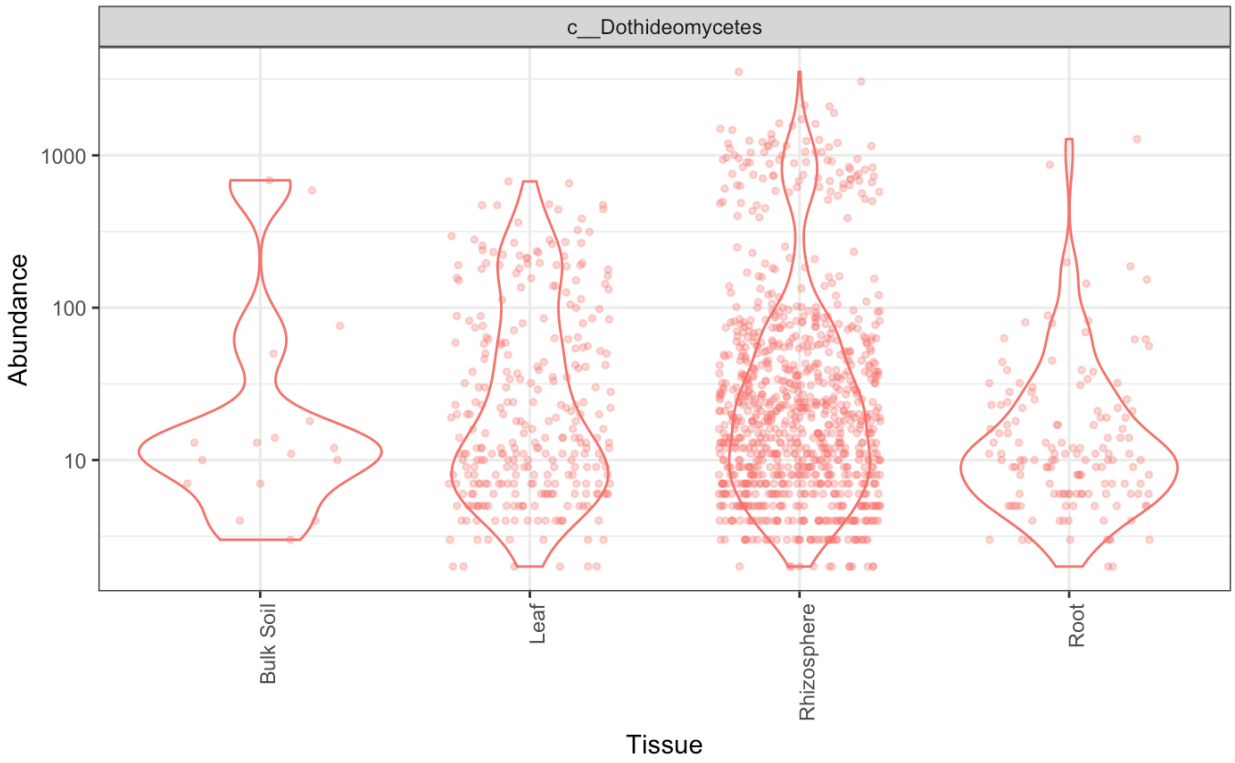


Figure 2.9 Violin plot depicting abundance of members of the class Dothidiomycetes, the largest taxonomic class of Ascomycota, across all tissue types.

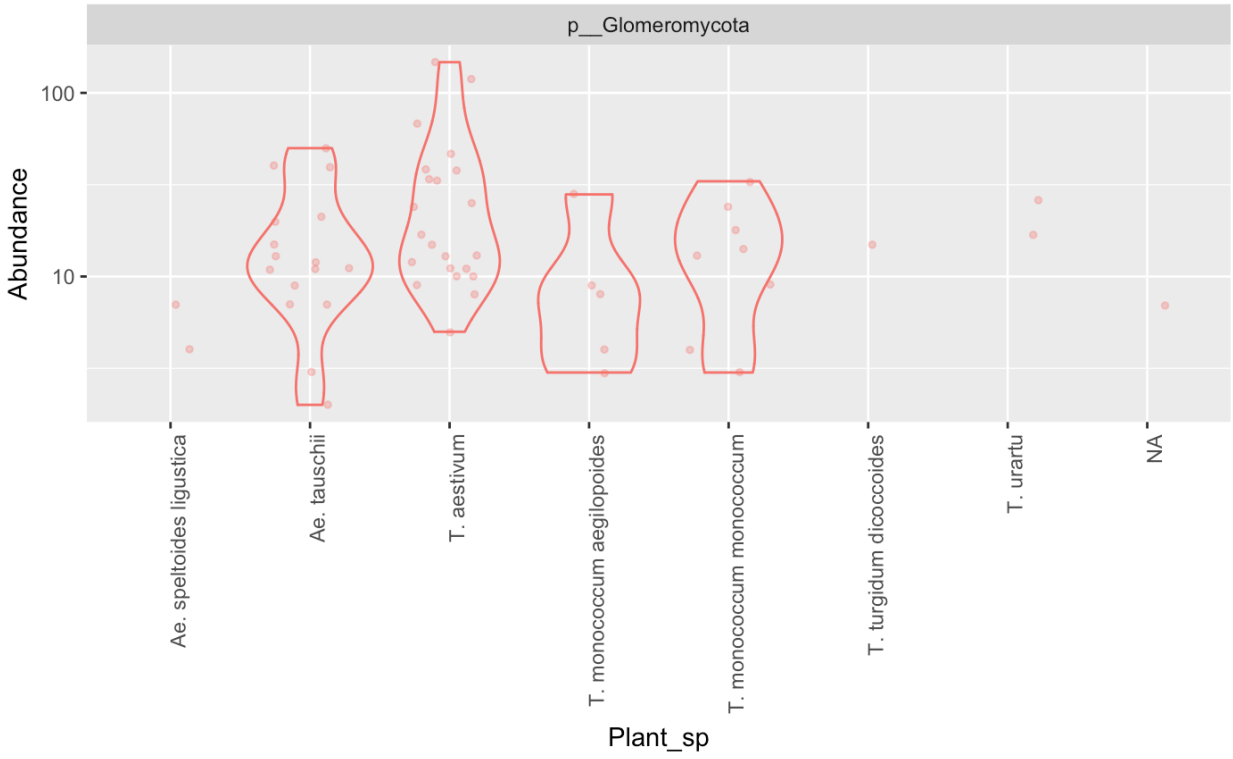
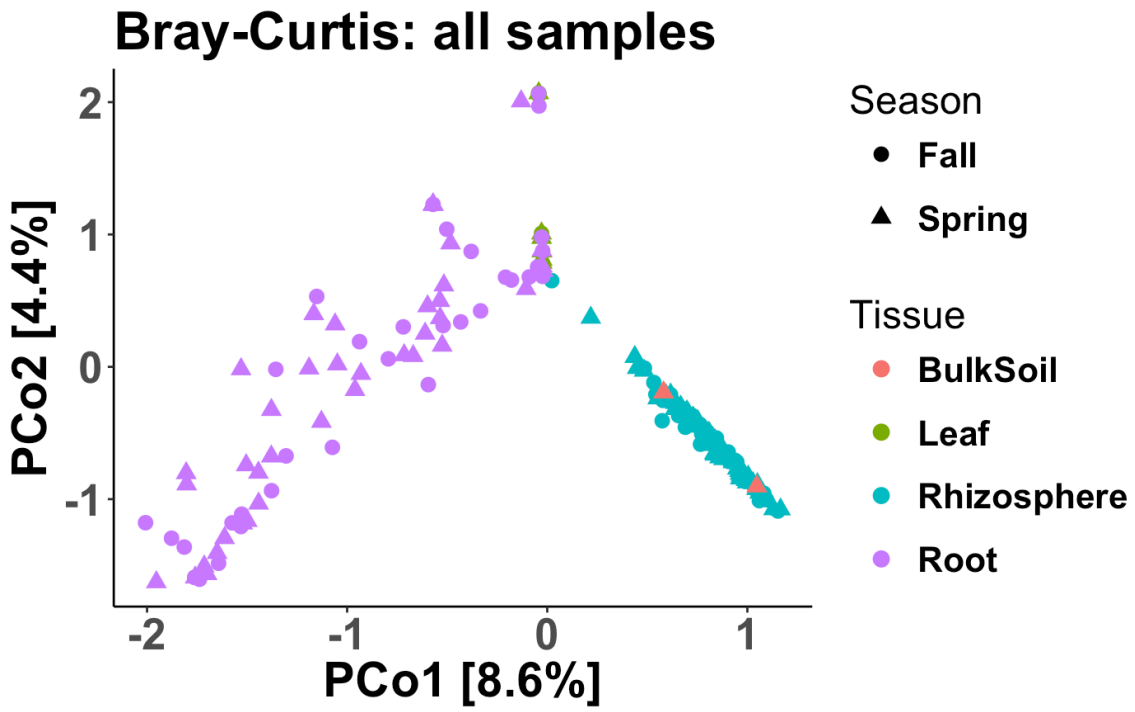


Figure 2.10 Violin plot depicting abundance of the phylum Glomeromycota, the arbuscular mycorrhizal phylum, in the roots of wheat and wild relative species.

a)


Bray-Curtis: all samples
PCo2 [4.4%]
PCo1 [8.6%]
2
1
0
-1
-2
-1
0
1
Season
Fall
Spring
Tissue
BulkSoil
Leaf
Rhizosphere
Root

b)

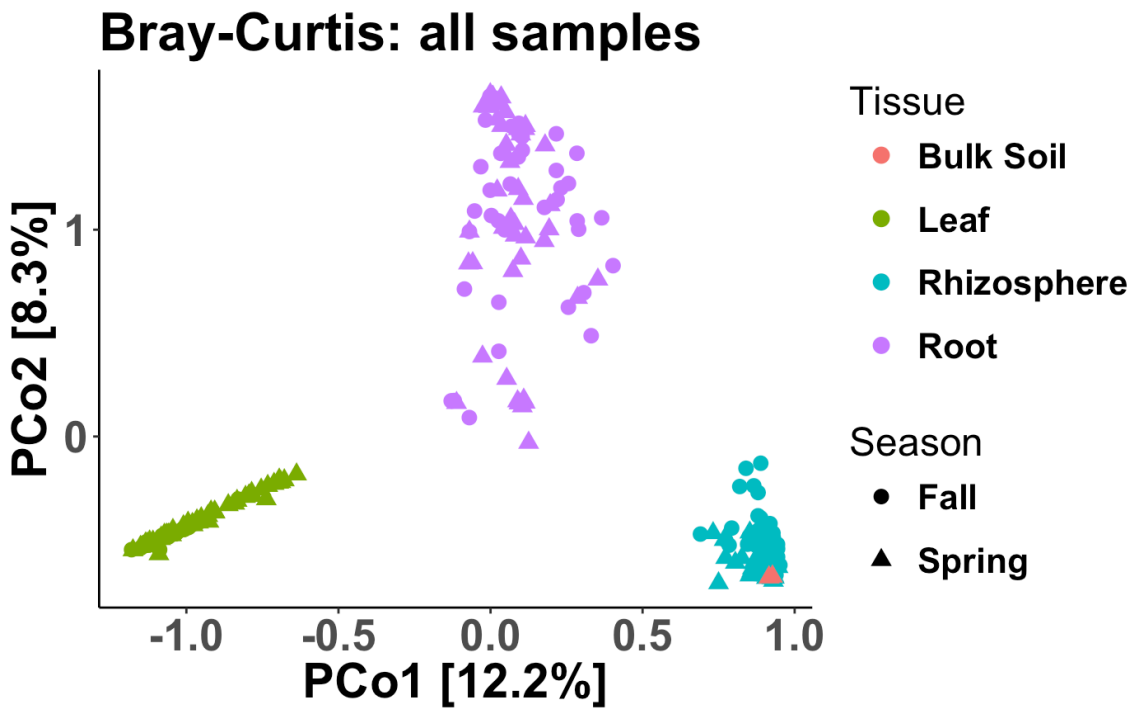
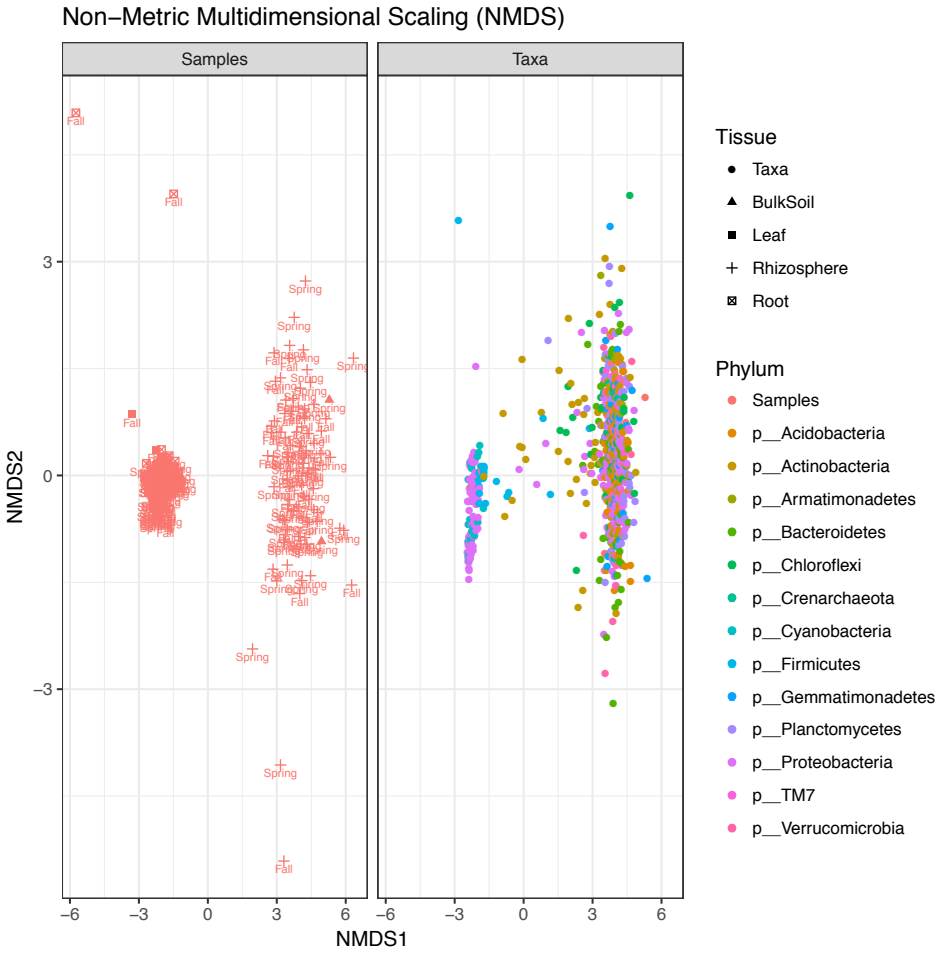


Figure 2.11 Principle coordinate analysis of plant tissue type and planting season using Bray-Curtis dissimilarity index for a) bacteria and b) fungi. Note: bacterial leaf samples are superimposed underneath root samples at apex of graph a).

a)


Non−Metric Multidimensional Scaling (NMDS)
Samples
Taxa
NMDS2
NMDS1
3
0
−3
−6
−3
0
3
6
Tissue
Taxa
BulkSoil
Leaf
Rhizosphere
Root
Phylum
Samples
p__Acidobacteria
p__Actinobacteria
p__Armatimonadetes
p__Bacteroidetes
p__Chloroflexi
p__Crenarchaeota
p__Cyanobacteria
p__Firmicutes
p__Gemmatimonadetes
p__Planctomycetes
p__Proteobacteria
p__TM7
p__Verrucomicrobia

b)

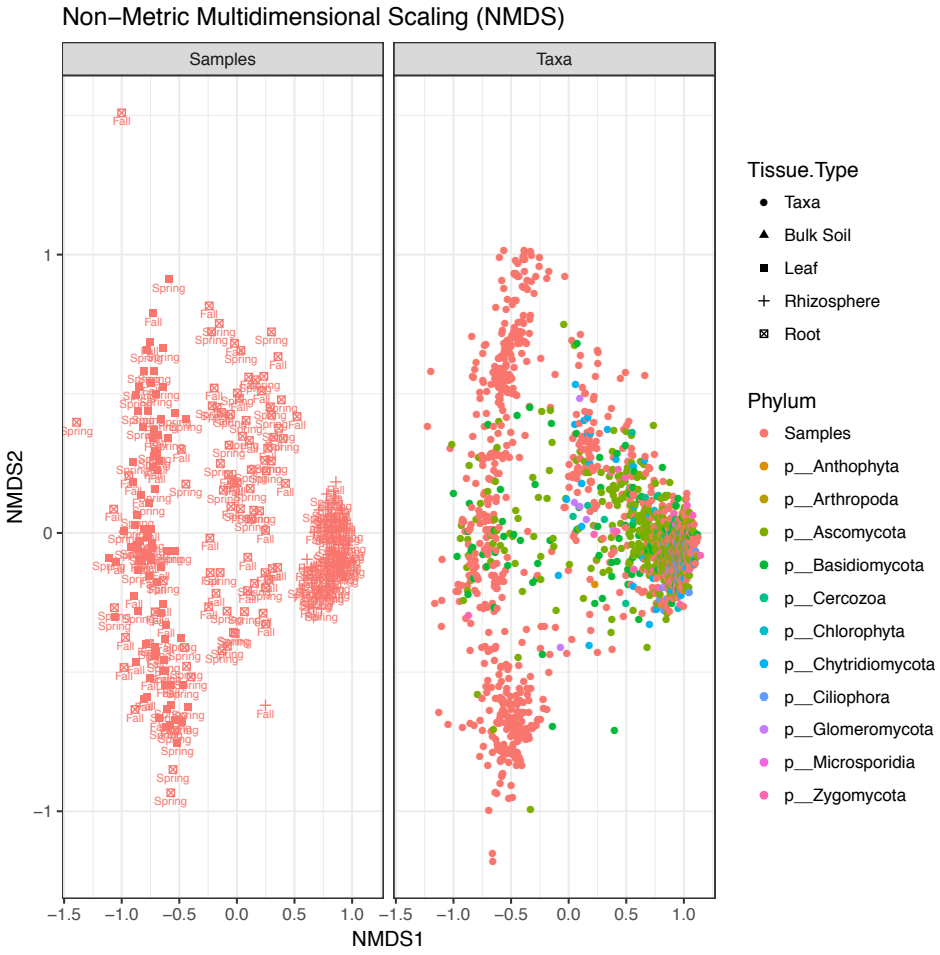
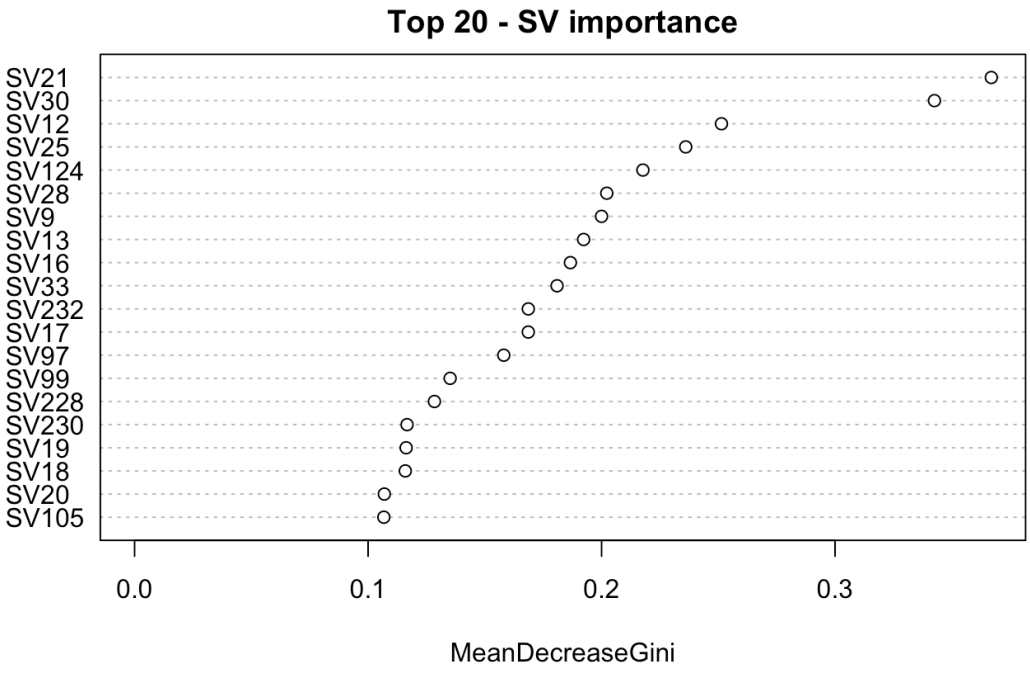


Figure 2.12 Non-metric multidimensional scaling ordination of plant tissue types and their respective microbial phyla for a) bacteria and b) fungi.

a)


Top 20 - SV importance
SV21
SV30
SV12
SV25
SV124
SV28
SV9
SV13
SV16
SV33
SV232
SV17
SV97
SV99
SV228
SV230
SV19
SV18
SV20
SV105
0.0
0.1
0.2
0.3
MeanDecreaseGini

b)

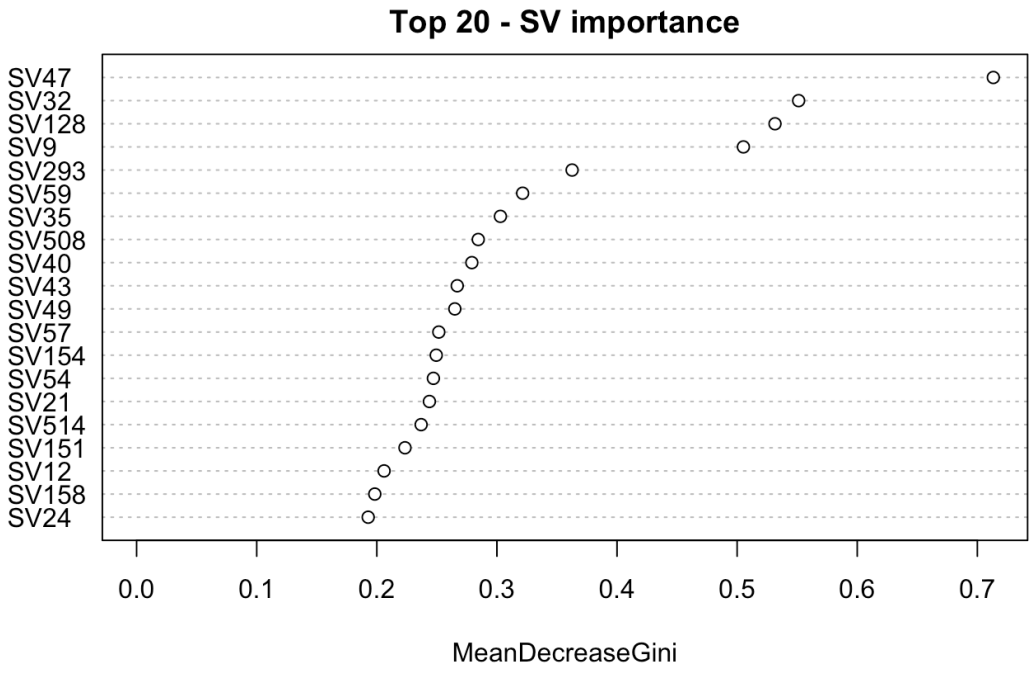
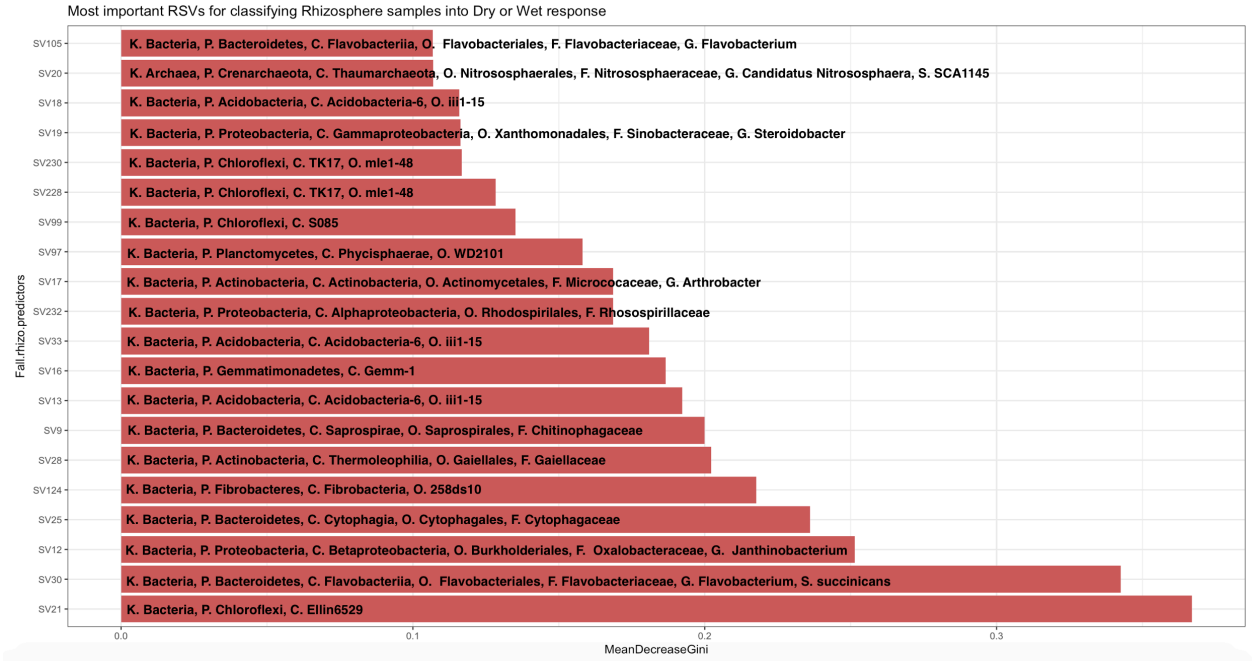


Figure 2.13 Random forest regression depicting the top 20 most important a) bacterial SV's and b) fungal SV's for discriminating rhizosphere samples between wet and dry treatments. The x-axis label "MeanDecreaseGini" refers to a decrease in the number of branches, and thus increase in homogeneity of nodes, in association trees generated by the random forest model and can be interpreted as "increase in SV importance".

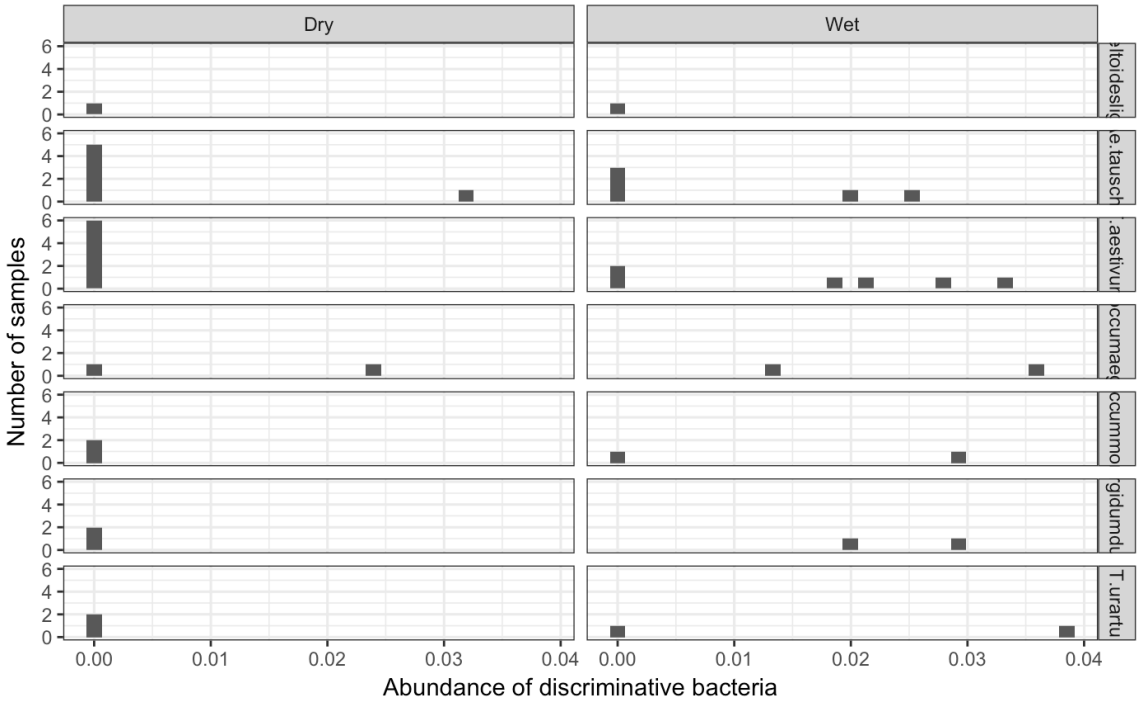
a)


Most important RSVs for classifying Rhizosphere samples into Dry or Wet response
Fall.rhizo.predictors
SV105
SV20
SV18
SV19
SV230
SV228
SV99
SV97
SV17
SV232
SV33
SV16
SV13
SV9
SV28
SV124
SV25
SV12
SV30
SV21
K. Bacteria, P. Bacteroidetes, C. Flavobacteriia, O. Flavobacteriales, F. Flavobacteriaceae, G. Flavobacterium
K. Archaea, P. Crenarchaeota, C. Thaumarchaeota, O. Nitrososphaerales, F. Nitrososphaeraceae, G. Candidatus Nitrososphaera, S. SCA1145
K. Bacteria, P. Acidobacteria, C. Acidobacteria-6, O. iii1-15
K. Bacteria, P. Proteobacteria, C. Gammaproteobacteria, O. Xanthomonadales, F. Sinobacteraceae, G. Steroidobacter
K. Bacteria, P. Chloroflexi, C. TK17, O. mle1-48
K. Bacteria, P. Chloroflexi, C. TK17, O. mle1-48
K. Bacteria, P. Chloroflexi, C. S085
K. Bacteria, P. Planctomycetes, C. Phycisphaerae, O. WD2101
K. Bacteria, P. Actinobacteria, C. Actinobacteria, O. Actinomycetales, F. Micrococcaceae, G. Arthrobacter
K. Bacteria, P. Proteobacteria, C. Alphaproteobacteria, O. Rhodospirilales, F. Rhosospirillaceae
K. Bacteria, P. Acidobacteria, C. Acidobacteria-6, O. iii1-15
K. Bacteria, P. Gemmatimonadetes, C. Gemm-1
K. Bacteria, P. Acidobacteria, C. Acidobacteria-6, O. iii1-15
K. Bacteria, P. Bacteroidetes, C. Saprospirae, O. Saprospirales, F. Chitinophagaceae
K. Bacteria, P. Actinobacteria, C. Thermoleophilia, O. Gaiellales, F. Gaiellaceae
K. Bacteria, P. Fibrobacteres, C. Fibrobacteria, O. 258ds10
K. Bacteria, P. Bacteroidetes, C. Cytophagia, O. Cytophagales, F. Cytophagaceae
K. Bacteria, P. Proteobacteria, C. Betaproteobacteria, O. Burkholderiales, F. Oxalobacteraceae, G. Janthinobacterium
K. Bacteria, P. Bacteroidetes, C. Flavobacteriia, O. Flavobacteriales, F. Flavobacteriaceae, G. Flavobacterium, S. succinicans
K. Bacteria, P. Chloroflexi, C. Ellin6529
0.0
0.1
0.2
0.3
MeanDecreaseGini

b)

Figure 2.14 Bar graphs of single variants for a) bacteria and b) fungi in order of ascending importance for discriminating rhizosphere samples into wet or dry response. Abbreviations refer to conventional taxonomic rankings.

a) SV21


Dry
Wet
Number of samples
Abundance of discriminative bacteria
0.00
0.01
0.02
0.03
0.04

b) SV30

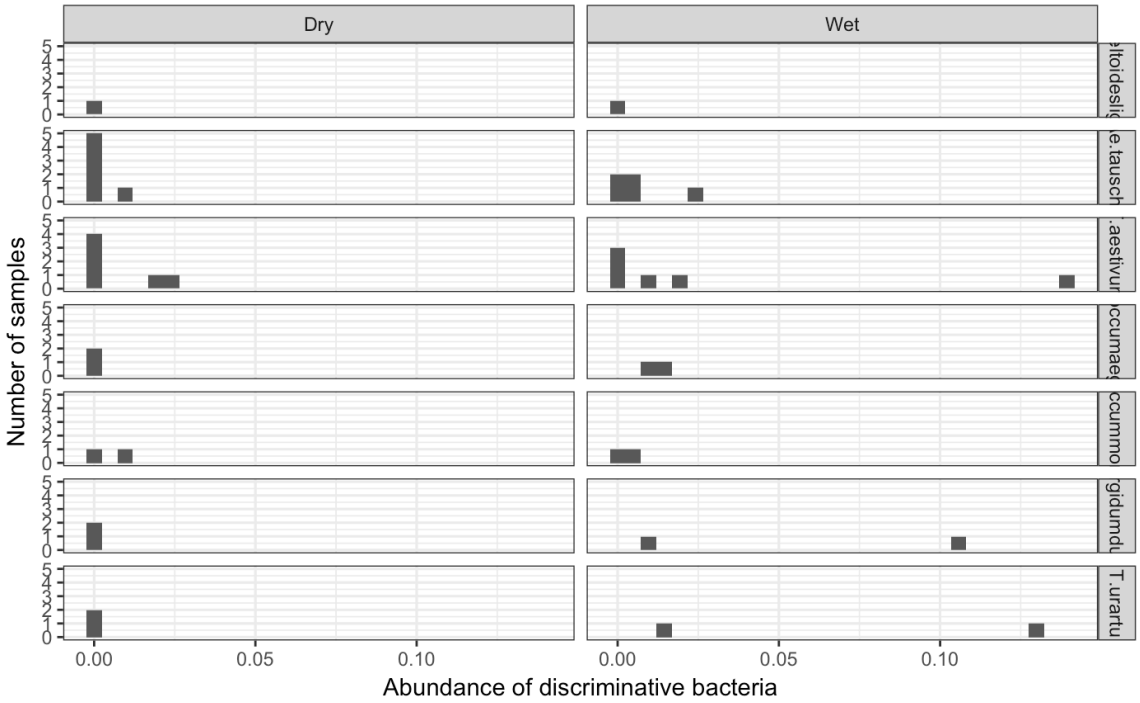
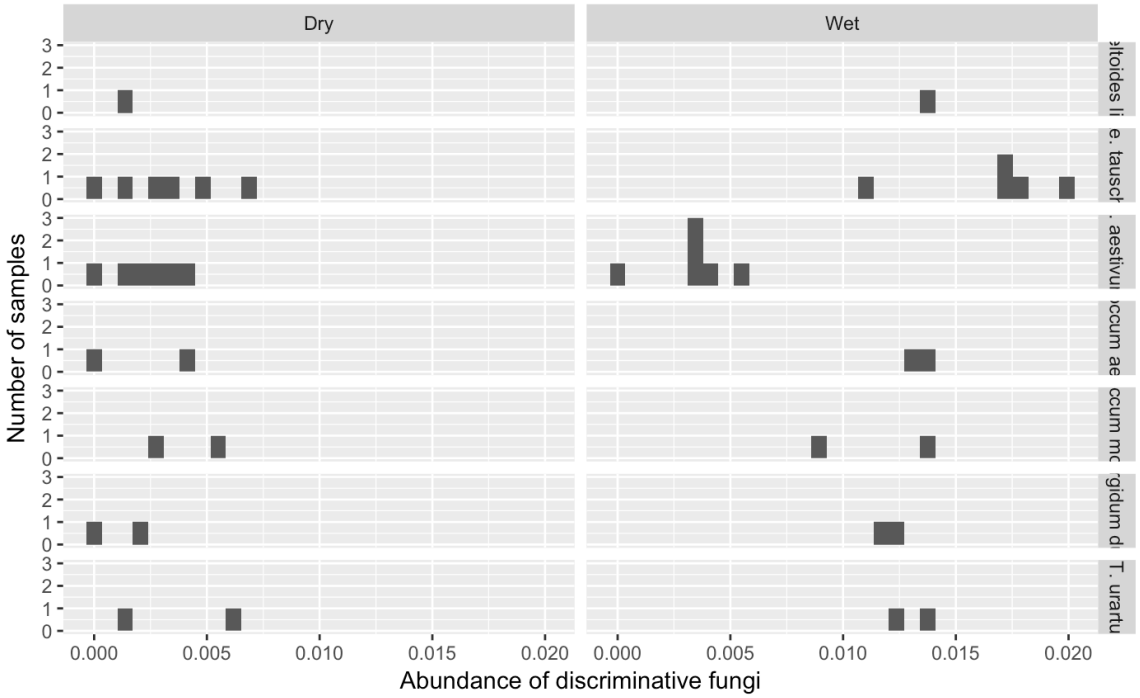


Figure 2.15 Top two most important bacterial taxa , a) SV21 and SV30, for discriminating rhizosphere samples into wet or dry response, faceted by plant species (the second most statistically significant variable after plant tissue type).

a) SV47


Dry
Wet
Number of samples
Abundance of discriminative fungi
0.000
0.005
0.010
0.015
0.020

b) SV32

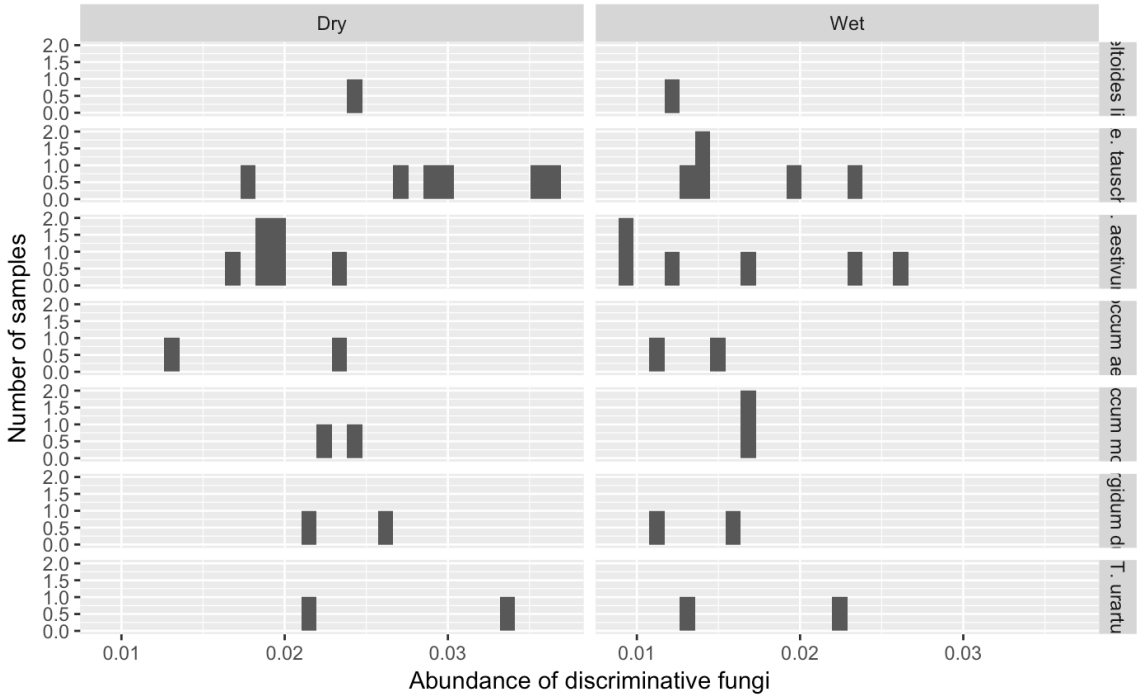


Figure 2.16 Top two most important fungal taxa , SV47 and SV32, for discriminating rhizosphere samples into wet or dry response, faceted by plant species (the second most statistically significant variable after plant tissue type).

Chapter 3

Defining the Core Microbiome of Wheat and Its Wild Relatives by Ploidy and Tissue Type

Summary

Microbiomes containing consortia of bacteria and fungi associate with plants to confer competitive advantages such as water uptake, nutrient acquisition, and stress tolerance (Andreote et al., 2014). There has been avid interest in manipulating the microbiome of crops to reduce reliance on inorganic and costly agricultural inputs (Farrar et al., 2014). Microbial community structure differs among plant species and plant tissue, and in order to leverage these differentiations for crop improvement, conserved "core" taxa must first be identified (Bulgarelli et al., 2015; Lundberg et al., 2012). Plant domestication has altered the microbial assocations in many crops, and research has turned to crop wild relatives as potential reservoirs of genetic diversity and beneficial microbial symbioses (Bulgarelli et al., 2015; Leff et al., 2016). This is the first known study to identify a core microbiome among wheat and its crop wild relatives by ploidy (or plant species identity across a domestication gradient) and tissue type.

The host genotypes in this study included three adapted wheat cultivars and 14 accessions of eight wild wheat relative species, which were planted as a common garden experiment in a randomized complete block design in Fort Collins, Colorado. Leaves, roots, and rhizosphere soil were sampled from each plant at the pre-flowering stage following winter vernalization. DNA was extracted, PCR-amplified, and sequenced on a two-lane Illumina MiSeq platform with primers designed to isolate the DNA of prokaryotes (bacteria and archaea) and fungi (Appendix Table 4.2). Raw sequence reads were processed through the bioinformatics pipelines DADA2 (for quality trimming and filtering) and Phyloseq (for community analyses) using R software.

Large phylogenetic groups of microbes serve similar functional roles in many land plants, and therefore tend to be similarly recruited; studies suggest the microbial taxa dictated by plant species identity tend to be influential but few in number (Bulgarelli et al., 2015; Fierer, 2017). The core fungi among plant tissue types contained a larger proportion of the overall fungal taxa, corroborating literature indicating the influential role of plant tissue on fungal community structure (Coleman-Derr et al., 2015). Overall, this project provides one of the first glimpses into the core microbiomes of wheat and its wild relatives that may be leveraged in the future to improve crop performance.

Introduction

Plants possess microbiomes containing bacteria, archaea, and fungi that associate across a range of symbioses. Pathogens, commensalists, and mutualists interact with plants above and below ground, within and around plant tissues, influencing the fitness of their plant hosts (Andreote et al., 2014; Lebeis, 2014). The movement from plants' centers of origin and their subsequent domestication have altered the composition of plant-microbe interactions in several crops (Bulgarelli et al., 2015; Leff et al., 2016). It is thought that the progenitors of the A, B, and D genomes in modern hexaploid wheat manage environmental stress in their native environment by establishing symbioses with a consortium of beneficial microbes (Iannucci et al., 2017). Two widely documented examples of beneficial microbes include arbuscular mycorrhizae and nitrogen-fixing bacteria (Bitterlich et al., 2018; Elkoca et al., 2008). The diverse functionalities of the plant microbiome are not well understood, nor are all of the genetic mechanisms governing the development of these symbioses that thwart sophisticated plant immune systems (Andreote et al., 2014). However, we know that microbial community structure differs somewhat

across plant species and strongly across plant tissue (Coleman-Derr et al., 2015; Lundberg et al., 2012). Many coarse taxonomic groups of microbes serve similar functional roles in the plant microbiome and are therefore conserved (Bulgarelli et al., 2015; Lundberg et al., 2012). This is one of the first studies to define the core microbiome of wheat and its wild relatives by ploidy and tissue type. The importance of wheat as a model organism for this type of comparative study is outlined in Chapter 1. By identifying core groups of prokaryotes and fungi found in wheat and its wild relatives across a domestication gradient (A, B, D, AB, and ABD genomes), as well as the core microbiome among plant tissues (leaves, roots, and rhizospheres), this study provides a foundation for future opportunities to leverage the plant microbiome for crop improvement.

Materials and Methods

Germplasm

Three wheat cultivars, Byrd, RonL, and Kharkov, represent plants with "domesticated" microbiomes in this study. Byrd is a hard red winter wheat released by the Colorado Agricultural Experiment Station in 2010, purported to have excellent yield performance in both dryland and irrigated environments, tolerance to stripe and stem rust (*Puccinia striiformis* Westend and *Puccinia graminis* Pers.:Pers. F. sp. *tritici* Eriks. E. Henn, respectively), and favorable bread making qualities (Haley et al., 2012). RonL is a hard white winter wheat released by the Kansas Agricultural Experiment Station in 2006, adapted for dryland production in Kansas, and with excellent resistance to wheat streak mosaic virus (WSMV) (Martin et al., 2007). Kharkov is a much older hard winter wheat variety with origins in western Russia, but was grown widely across the American Great Plains in the early twentieth century (Charest & Phan, 1990). The seed of 14 landraces and wild relatives of wheat (*Triticum aestivum*) was sourced from the

Wheat Genetics Resource Center at Kansas State University (https://www.k-state.edu/wgrc/) (Figure 2.1). This latter group of plants includes diploid, tetraploid, and hexaploid genotypes (Table 3.1).

Field Trial

This experiment was planted at the Agricultural Research, Development and Education Center (ARDEC) near Fort Collins, Colorado (DMS Lat: 40° 35' 6.9288" N; DMS Long: 105° 5' 3.9084" W; Elevation (m): 1,526). This arid plains region has an average temperature of 10.1°C, average annual rainfall of 40.8 cm, and alkaline sandy clay loam soil (pH 7.9)(Appendix Table 1). Seeds were sown on October 15, 2015. Two seeds of each accession were planted 12 cm apart in a randomized complete block design with two replications, each in well-watered and water-limited treatments separated by about 5 m. The water-limited treatment was planted where canola (*Brassica napus* L.) was harvested about 30 days previously, whereas the well-watered treatment area was fallow the previous year. The plants grew to the 2-3 leaf stage, went dormant by early winter, and resumed growth in early March. Because the overwintering ability of these wild accessions was unknown, a second planting was done on February 13, 2016, which is traditionally early enough in Colorado to allow a minimum of 6 weeks of cool weather for plants to vernalize. Eight seeds of each accession were planted, in the same rows as the fall-planted materials in wet and dry treatments. Five of the 17 accessions were planted in October only, seven of the 17 were planted in February only, and five of the 17 were planted in both October and February. The region was not under drought stress at any point in the growing season, per the Palmer Drought Severity Index provided by NOAA (https://www.ncdc.noaa.gov/temp-and-precip/drought/historical-palmers/). Due to abundant spring rainfall, the "wet" plot was irrigated

just once (13 mm) in June, five days prior to the sampling of October-seeded plants, and 16 days prior to the sampling of February-seeded plants. Soil moisture content analysis was not performed. The October-seeded plants were sampled on June 8 and February-seeded plants were harvested on June 19. This corresponded to the pre-heading to heading stage. Plants were not allowed to ripen to full maturity because several of the goatgrass species are considered potentially invasive to Colorado. A basic soil analysis was performed on a sample collected between the two fields at the time of harvest.

One healthy plant per plot (44 plants total) was dug up (15 cm deep) and the root ball shaken to release loose soil. Any soil that remained adhered to the root ball after vigorous shaking constituted the rhizosphere, which was collected and stored at -80°C until DNA extraction. Two bulk soil samples from the wet and dry fields were also collected for DNA sequencing. Three undamaged, mature leaf blades (non-flag leaf) were collected from each plant, along with roots from a diverse range of size classes. The roots were surface sterilized in a 0.05 M NaClO solution. Leaves were not sterilized in order to include the "phyllosphere" microbes that colonize the leaf surface. Leaves and roots were lyophilized, sealed in centrifuge tubes, and stored at room temperature until DNA extraction.

DNA Extraction

Leaf DNA was extracted with a ThermoScientific Plant DNA Kit (Thermo Fisher Scientific, Waltham, MA, USA). Root and rhizosphere DNA were extracted with a MoBio PowerMag Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). Both kits were optimized for the KingFisher™ Flex Purification System. Once extracted, the DNA concentration was calibrated using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific,

Waltham, MA, USA). The three 96-well plates (leaf, root, and rhizosphere) containing the extracted DNA were stored at -20°C until they were shipped on dry ice to the University of Minnesota Genomics Center for PCR-amplification, library preparation, and two-lane Illumina sequencing.

Next-Generation Amplicon Sequencing

The ITS2 and 16S-V4 ribosomal RNA (rRNA) operons were sequenced to identify the fungi and bacteria in each sample, respectively (Appendix Table 4.2). These primers amplify DNA fragments 260-290 bp in length and are ideal candidates for amplification because they are highly conserved, but possess sufficient variability to distinguish closely related taxa (Op De Beeck et al., 2014; Walters et al., 2015; Yao et al., 2010). Additionally, the 16S-V4 primer refines the detection of *Thaumarchaeota*, a prokaryotic phylum known to play an important role in terrestrial nitrogen and carbon cycling (Walters et al., 2015). The ITS2 operon possesses superior detection of several ectomycorrhizal fungal species, including *Sistotrema sp., Rhizopogon luteolus*, and *Wilcoxina mikolae*, as well as arbuscular mycorrhizae in the Glomeromycota (Op De Beeck et al., 2014). Since Illumina MiSeq sequencers read 300 bp in each direction, these shorter fragments allow for almost complete double-stranded overlap, increasing base call accuracy (Goodrich et al., 2014). A downside to using primers for shorter genetic elements is that the sequences generated are less informative than reads with longer single-stranded tails (Kozich et al., 2013). Longer fragment sequences can generate higher resolution OTU's, but are prone to higher error rates (Quail et al., 2012). The resultant sequences were delivered digitally as ".fastq" files for taxonomic identification and statistical analysis.

Data Analysis

The open-source software package DADA2 was used to model and correct Illumina amplicon sequencing errors for all raw .fastq reads in this study (Long et al., 2016). This high-throughput bioinformatics pipeline, built for use in R, improves the structure and quality of amplicon sequences so they can be passed downstream for taxonomic assignment and hypothesis-based analyses. The sequence cleaning pipeline for this project required five steps: filtering, dereplicating, denoising, chimera-removing, and merging. First, forward (5'→3') and reverse (3'→5') reads are trimmed where Phred quality scores fall below 20; a score of 20 or higher ensures base call accuracy of at least 99% (Bokulich et al., 2013). The tails of reads and reverse reads in general have lower quality scores (Long et al., 2016). Next, a dereplication function inputs the filtered reads and outputs a list of unique sequences and their abundances. After dereplication, the unmerged sequences are passed to a denoising algorithm that identifies total number of true sequence variants through sample inference. More abundant sequences are also checked for chimeras by identifying sequence mismatches and indels. The chimeric model flags these offending sequences, and chimeras in this project were removed to improve accuracy. Finally, the filtered forward and reverse reads are merged to create paired-end sequences poised for clustering and taxonomic assignment (Long et al., 2016).

Another common filtering tool employed in metagenomics projects is rarefaction (McMurdie & Holmes, 2014). Rarefaction creates consistent sequencing "depth" by reducing the number of sequences in every sample to the number of sequences present in the sample with the fewest unique sequences. However, a downside to this method is that it does not account for differentially abundant taxa and wastes information in smaller studies (McMurdie & Holmes,

2014). Since this experiment contained only 268 samples (few compared to animal microbiome studies), rarefaction was not used.

After performing quality control in DADA2, the cleaned sequences were classified as true sequence variants, or SV's (based on 100% sequence similarity, as opposed to OTU's that are typically clustered by 97% similarity), and assigned taxonomy using the RDP database for bacteria and the UNITE database for fungi (Koljalg et al., 2014; Wang et al., 2007). This taxonomic information was passed to the open-source R package Phyloseq for pre-processing and all further analyses (McMurdie & Holmes, 2013).

Pre-processing began by looking at the total number and distribution of reads across SV's and samples (Appendix Fig. 4.1). These graphs depicted similar distributions between bacterial and fungal reads; however, there are approximately twice as many bacterial taxa as fungal taxa. Next, the sequencing depth (or evenness) was summarized in general and category-specific histograms to evaluate the balance in this experiment (Appendix Fig. 4.2; Appendix Fig. 4.3). These graphs depicted normally distributed sequencing depth for both bacterial and fungal reads. Imbalance exists in the tissue type category because only two bulk soil samples were taken; bulk soil was only collected as a check, and the main tissues of interest (leaf, root, and rhizosphere) were sampled evenly. Imbalance also exists in the plant species category, since some species (i.e. *Aegilops tauschii* and *Triticum aestivum*) contained more accessions than other species. This imbalance was remedied by including an independent variable in the metadata spreadsheet titled "Genotype" that allowed analyses to be performed on the more evenly sampled plant accessions. Next, histograms of taxa prevalence were observed to better understand the depth of the data; specifically, how many reads of each taxon were found in each sample (Appendix Fig. 4.4). These graphs indicated that most taxa were not highly prevalent across all samples. To reduce

the length of this skewed tail and to minimize noise from taxa with extremely low prevalence, the taxa table was filtered to exclude phyla that were observed less than 10 times across the entire dataset, as well as any kingdoms besides Fungi, Bacteria, and Archaea (the last of which is low abundance, but included with bacteria for the purposes of this project). Understanding the depth and distribution of sequencing data is vital when drawing conclusions from downstream analyses.

Following pre-processing, the cleaned and assembled dataset was partitioned into subsets containing each of the five wheat/wild wheat relative genomes (A, B, D, AB, and ABD), and each of the three primary tissue types (leaf, root, and rhizosphere). Venn diagrams were created among genomes and among tissue types. Since Venn diagrams were made with four ellipses, two genome subsets were generated (A, B, D, AB, and A, B, D, ABD), and tables of the overlapping core microbial taxa were created (Appendix Tables 3-6). The core microbiomes of each subset, or the microbial taxa shared among all genomes/tissues, were subsequently described. Additionally, the non-parametic test for differences in means, the Kruskal-Wallis test, was performed to determine whether the mean number of reads per core SV were significantly different. Raw mean values were used to identify the most abundant core taxa in each of the four main factors (i.e., 16S-V4 x tissue, 16S-V4 x genome, ITS2 x tissue, and ITS x genome). All data analysis and visualization was performed in R.

Results & Discussion

After raw read processing, a total of 5,276 bacterial SV's and 1,323 fungal SV's were identified. The higher number of identified prokaryotic species is likely due to larger bacterial sequence databases. The greater ratio of bacteria:fungi biomass is indicative of agricultural soils

(Santoyo et al., 2017). While it is important to note that abundance does not always correlate directly to functional importance within microbial ecosystems, the disparately high means of the top core taxa is worth exploring (Fierer, 2017).

Of the total 5,276 prokaryotic SV's, 80 (1.5% of total prokaryotic SV's) were found to be common among all representative wheat and wild wheat relative genomes in this study (A, B, D, AB, and ABD) (Fig. 3.1-3.2). However, the means of each of these core taxa were significantly different (Kruskal-Wallis $P < 2.2e^{-16}$) (Table 3.2, Fig. 3.3). The most highly abundant bacterial taxon shared among the five genomes in this study was a member of the Family Chitinophagaceae (Phylum Bacteroidetes) (df = 79, $\mu = 86.13$). This family contains scores of diverse genera, including mostly non-motile, aerobic commensal species common in grassland and farmland soils, and some plant growth-promoting rhizobacteria (PGPR) with auxin-producing capabilities documented in the rhizospheres of tomato (*Solanumm lycopersicum*) and cowpea (*Vigna unguiculata*) (Madhaiyan et al., 2018).

The core bacterial taxa among the leaf, root, and rhizosphere tissues contained 78 SV's (1.5% of total prokaryotic SV's) and also possessed significantly different means (Kruskal-Wallis $P < 2.2e^{-16}$) (Fig. 3.4-3.5, Table 3.2). The most abundant bacterial taxon shared among the three plant tissues in this study was a member of the genus *Paenibacillus* (Phylum Firmicutes) (df = 77, $\mu = 23.94$). *Paenibacillus* contains species found in a variety of environments, including soil, water, and even insect larvae. Some *Paenibacillus* species found in rhizosphere soil are capable of conferring beneficial induced systemic resistance to plant hosts like rice (*Oryza sativa*) and *Arabidopsis*, as well as preventing *Fusarium* wilt in watermelon (*Citrullus lanatus*) (Kim et al., 2017; Yaoyao et al., 2017).

Of the total 1,323 fungal SV's, 194 (14.7% of the total fungal SV's) were present among all representative wheat and wild wheat relative genomes in this study (A, B, D, AB, and ABD) (Fig. 3.6-3.8). The higher proportion of core fungal taxa as compared to core bacterial taxa among plant genome identities corroborates existing literature suggesting that fungal communities are more strongly influenced by plant genotype (Broeckling et al., 2008; Brundrett, 2002). The most highly abundant core fungal genome taxon was *Gamsia simplex* (Phylum Ascomycota) (df = 193, μ = 798.80). The phylogeny of *Gamsia* sp. is contested and frequently included with the closely related Microascaceae genus *Wardomyces* (Christian, 2014). Saprobic fungi in these two genera are decomposers of organic matter and ubiquitous in soils worldwide (Christian, 2014).

The core fungal taxa within the three plant tissues in this study contained 38 SV's (2.9% of the total fungal SV's) (Fig. 3.9-3.10). The most abundant core fungal tissue taxon was *Gibberella intricans* (Phylum Ascomycota) (df = 37, μ = 381.62). This pathogenic species is the teleomorph, or sexual stage, of *Fusarium equiseti* (Corda) Sacc., *Fusarium falcatum* Appel & Wollenw., *Fusarium gibbosum* Appel & Wollenw., *Fusarium roseum* 'Gibbosum', and *Fusarium roseum* var. *equiseti* (CABI Compendium of Invasive Species, 2018). *Fusarium* diseases can affect the above- and below-ground parts of wheat and similar crops; therefore, it is unsurprising to find this common pathogen as an abundant core microbe of all three plant tissues in this study. The reproductive spores of *Fusarium* species can easily spread to their surrounding soils and be taken up by subsequently emergent plants (Nirenberg, 1981).

Microbiomes also display distinct compartmentalization across plant tissues. Arbuscular mycorrhizae in the Glomeromycota were only found in root tissue, as well as a large number of potentially parasitic Chytridiomycota. Leaf tissue contained several unique Proteobacteria taxa

belonging to the order Myxococcales. The rhizosphere and bulk soil samples were the only ones to possess bacteria from the phylum OD1 and several Ascomycete species, including the pathogen *Fusarium solani*.

A final set of Venn diagrams were generated to look at the number of core bacterial and fungal taxa shared among four accessions of *Aegilops tauschii* (Fig. 3.11-3.12). The accessions chosen were TA 10144, TA 10330, TA 2374, TA 2458, based on their distant geographic origins. Not all bacterial or fungal taxa identified in this study were found in this subset of *Ae. tauschii* accessions, therefore the number of taxa represented in the Venn diagrams is lower than the overall total for the study. Each of the four *Ae. tauschii* accessions contained a greater proportion of unique bacterial taxa than shared or core bacterial taxa (Fig. 3.11). While there was a greater number of unique fungal taxa within each *Ae. tauschii* accession, the proportion of shared and core taxa was also much higher (Fig. 3.12). These findings indicate that symbioses with fungal endophytes may have been more greatly conserved across differing populations of *Ae. tauschii*, whereas bacterial communities have developed distinctions through adaptation via biogeographical opportunities.

Conclusions

The overall goal of this study was to define the core microbiome of wheat and its wild relatives by ploidy and tissue type. The taxa shared among the five wheat and CWR genomes (A, B, D, AB, ABD) and their leaves, roots, and rhizospheres are referred to as “core microbiomes”. The consensus in the field of microbial ecology is that most taxa are rare, and few are abundant; however, it is estimated that approximately 2% of total soil taxa are ubiquitous and abundant (Fierer, 2017). To make matters more confounding, greater abundances of certain microbes are

not always correlated with ecological importance. Functional redundancies exist in microbial ecosystems, and an artifact of culture-independent sequencing studies is accounting for the DNA of dead (non-functional) cells (Goodrich et al., 2014).

While most of the bacterial sequence variants (SV's) in the core genome microbiome in this study belong to the Phylum Proteobacteria, the most abundant SV belongs to the Phylum Bacteroidetes in a family of Gram-negative aerobes common to farm and grassland soils. Regardless, Proteobacteria remains a vastly common bacterial phylum in soils, containing many PGPR species with promising applications for crop improvement (Hardoim et al., 2008). The majority of bacterial SV's in the core tissue microbiome belong to the Phylum Actinobacteria, but the single largest SV, *Paenibacillus,* belongs to the Phylum Firmicutes. While *Paenibacillus* is commonly found in many different soil types, the coarse phylogenetic dominance of Actinobacteria can be expected in agricultural soils like the one in this study where nutrient inputs are higher than natural ecosystems and the pH runs neutral – basic (Appendix Table 4.1) (Leff et al., 2015). The large number of microbes found in only a single plant tissue suggests strong compartmentalization of microbial communities within a plant host.

This study reinforces existing literature suggesting that plant tissue is an influential driver of microbial community structure (explaining the small number of shared core taxa)(Bulgarelli et al., 2013; Coleman-Derr et al., 2015). The higher proportion of core fungi among the five wheat and CWR genomes (14.7% of total fungal taxa) also corroborates studies indicating the more pivotal role of plant species identity on fungal communities (Broeckling et al., 2008; Brundrett, 2002). The core microbiome among bacterial genomes appears small, suggesting some plant genotype-dependent variability. There is evidence in the literature to suggest plant domestication may leave a detectable footprint among a small but influential group of microbes across closely

related plant species (Bulgarelli et al., 2015). In Chapter 2, "A Comprehensive Comparison of the Microbiomes in Wheat and Its Wild Relatives", plant species identity was not shown to be a statistically significant predictor of microbial community structure. A possible explanation for the limited core microbial taxa is the use of high resolution sequence variants (SV's), as opposed to coarser taxonomic groups that would likely reveal a large proportion of the microbiome shared amongst most plant species (Bulgarelli et al., 2015; Fierer, 2017; Lundberg et al., 2012). The comparison of four accessions of *Aegilops tauschii* indicated that many microbial symbioses, both bacterial and fungal, were conserved despite divergent breeding. The differences among microbial communities may be due to genetic adaptation by the plant hosts via biogeographical opportunities in distinct soil types. An in-situ experiment aimed at identifying microbial associations among crop wild relatives in their centers of origin could provide a more robust view of the potential interactions these different genotypes can form. Larger sampling depth and increased plant replication would also provide greater opportunities to witness plant genotype-dependent trends. Overall, this project provides one of the first comparisons of the microbiomes in wheat and its wild relatives that may be leveraged in the future to improve crop performance.

Tables

Table 3.1 Plant genotypes used in this study, by planting date.

	Planted 15 October 2015			
Entry	**Taxa**	**Acc. No.[1]**	**Ploidy[2]**	**Genome**
1	*Aegilops sharonensis*	TA 1998	2x	B
2	*Aegilops speltoides ligustica*	TA 1772	2x	B
3	*Triticum monococcum aegilopoides*	TA 177	2x	A
4	*Triticum monococcum monococcum*	TA 142	2x	A
5	*Triticum turgidum dicoccoides*	TA 61	4x	AB
6	*Triticum turgidum durum*	TA 10451	4x	AB
7	*Triticum urartu*	TA 739	2x	A
8	*Aegilops tauschii*	TA 2374	2x	D
9	*Aegilops tauschii*	TA 2536	2x	D
10	*Aegilops tauschii*	TA 10106	2x	D
	Planted 13 February 2016			
Entry	**Taxa**	**Acc. No.**	**Ploidy**	**Genome**
1	*Aegilops tauschii*	TA 1707	2x	D
2	*Aegilops tauschii*	TA 2458	2x	D
3	*Aegilops tauschii*	TA 2536	2x	D
4	*Aegilops tauschii*	TA 10144	2x	D
5	*Aegilops tauschii*	TA 10330	2x	D
6	*Triticum aestivum*	Kharkov	6x	ABD
7	*Triticum aestivum*	Byrd	6x	ABD
8	*Triticum aestivum*	RonL	6x	ABD
9	*Triticum urartu*	TA 739	2x	A
10	*Triticum monococcum monococcum*	TA 142	2x	A
11	*Aegilops speltoides ligustica*	TA 1772	2x	B
12	*Triticum turgidum dicoccoides*	TA 61	4x	AB

[1] Accession number of Wheat Genetics Resource Center, Manhattan, KS (http://www.k-state.edu/wgrc/).
[2] "x" refers to the number of chromosomes in a basic set, which is 7 for wheat and its wild relatives.

Table 3.2 Table of four most abundant core taxa, their means per sample, and Kruskal-Wallis (KW) *P*-values (non-parametric test of different means [H_A: Not all core SV means are the same]).

Primer	Factor	SV	Taxon	DF	Mean (μ)	KW *P*-value
16S-V4	Tissue	SV11	*Paenibacillus*	77	23.94	$<2.2e^{-16}$
16S-V4	Genome	SV9	F. Chitinophagaceae	79	86.13	$<2.2e^{-16}$
ITS2	Tissue	SV14	*Gibberella intricans*	37	381.62	$<2.2e^{-16}$
ITS2	Genome	SV9	*Gamsia simplex*	193	798.80	$<2.2e^{-16}$

Figures

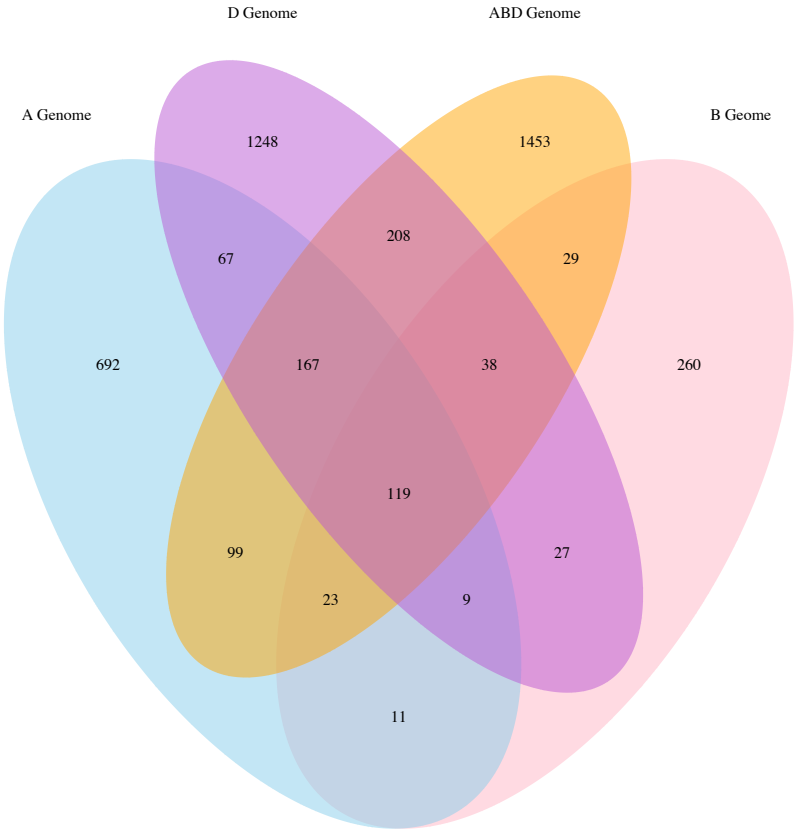


Figure 3.1 Venn diagram of core bacterial taxa among A, B, D, and ABD genome wheat species.

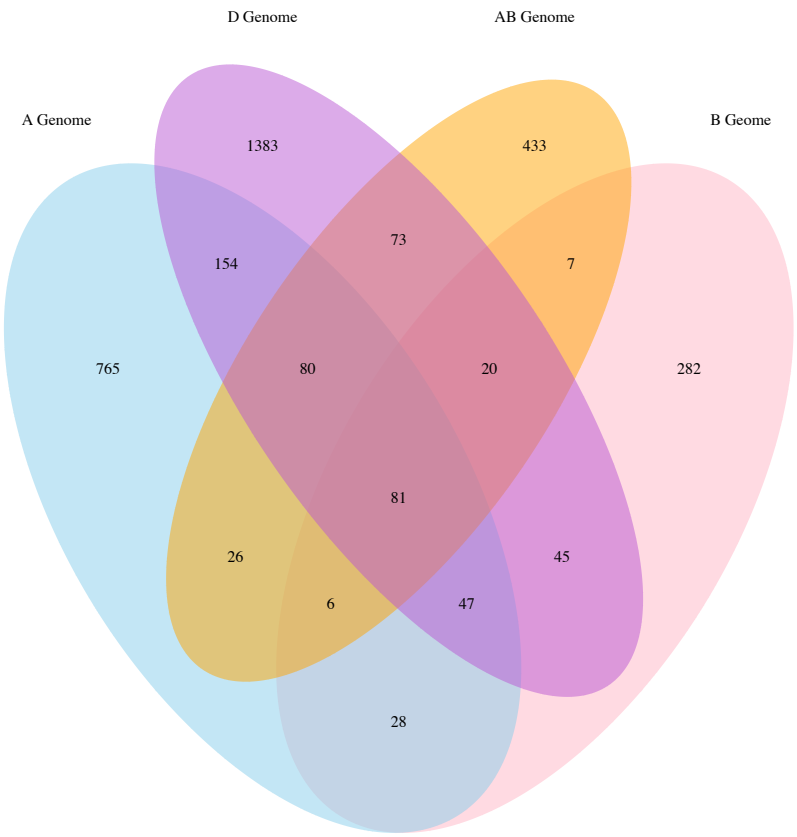


Figure 3.2 Venn diagram of core bacterial taxa among A, B, D, and AB genomes of wheat and related wild species.

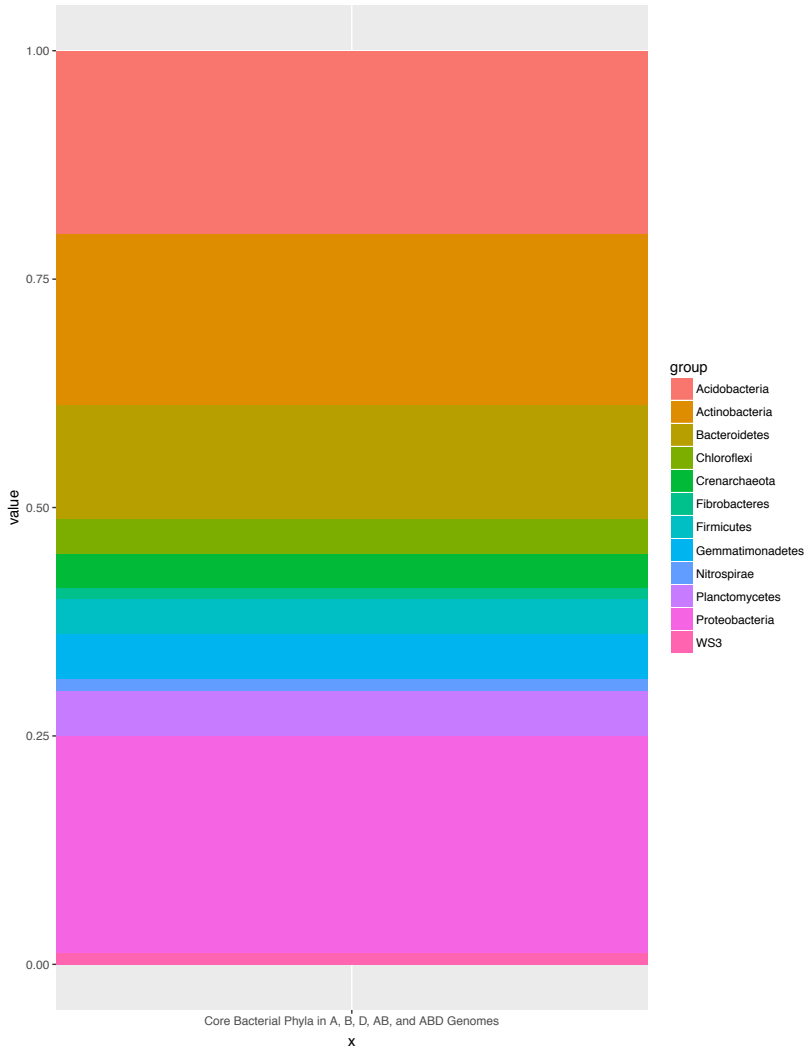


Figure 3.3 Bar plot of core bacterial taxa among A, B, D, AB, and ABD genomes of wheat and related wild species in this study.

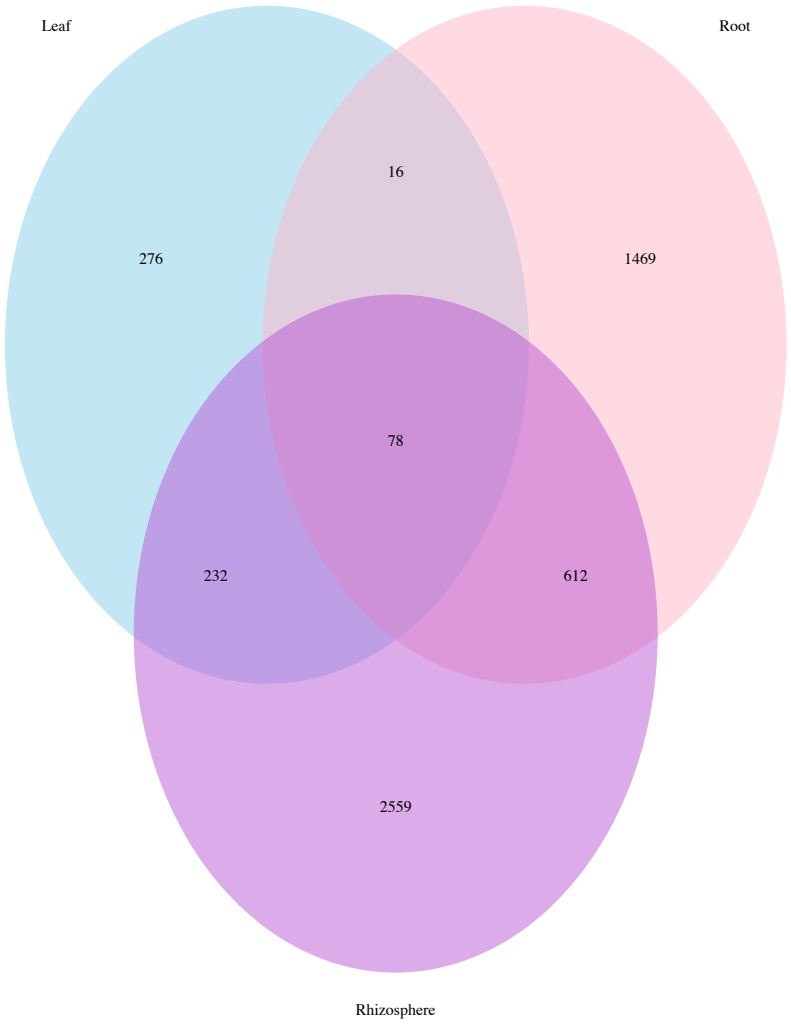


Figure 3.4 Venn diagram of core bacterial taxa among all tissue types.

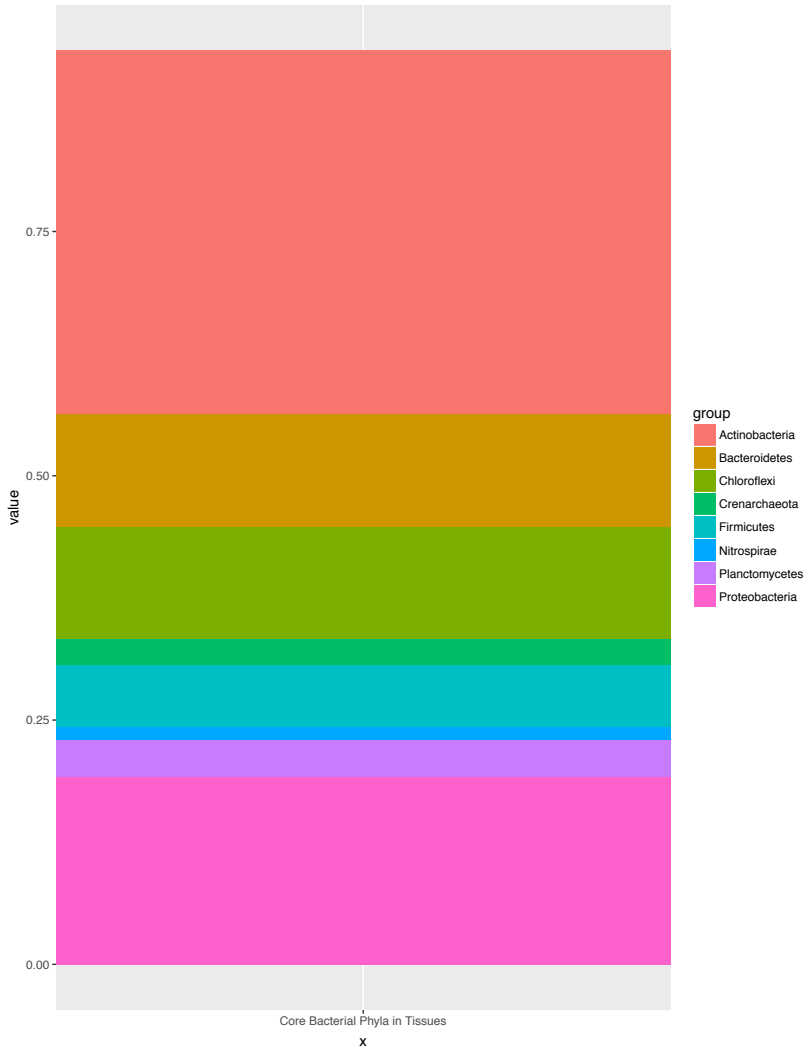


Figure 3.5 Bar plot of core bacterial taxa among all tissue types.

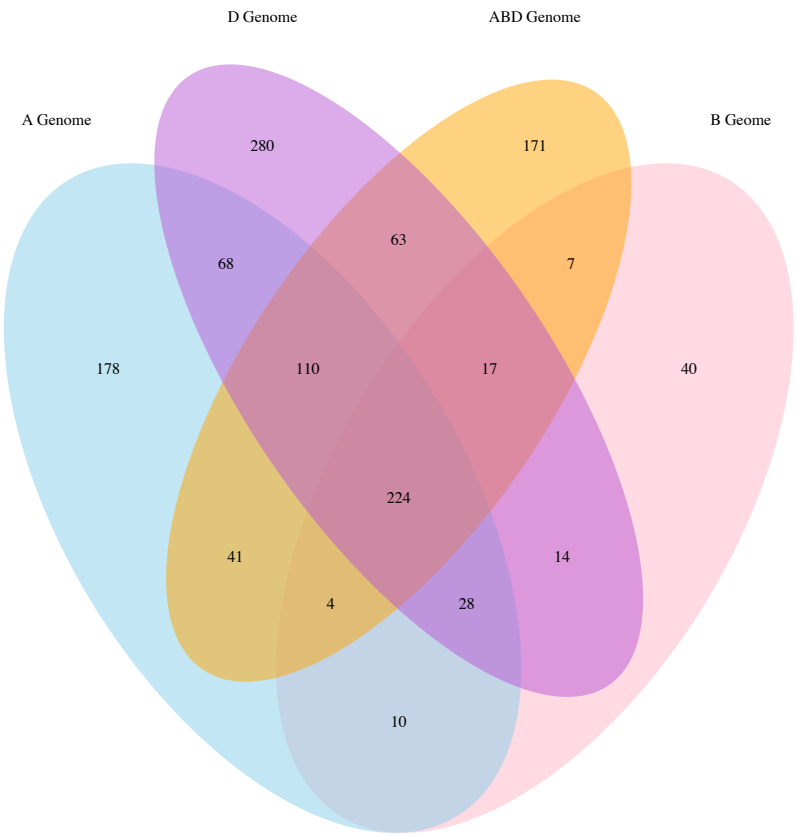


Figure 3.6 Venn diagram of core fungal taxa among A, B, D, and ABD genomes of wheat and realted wild species.

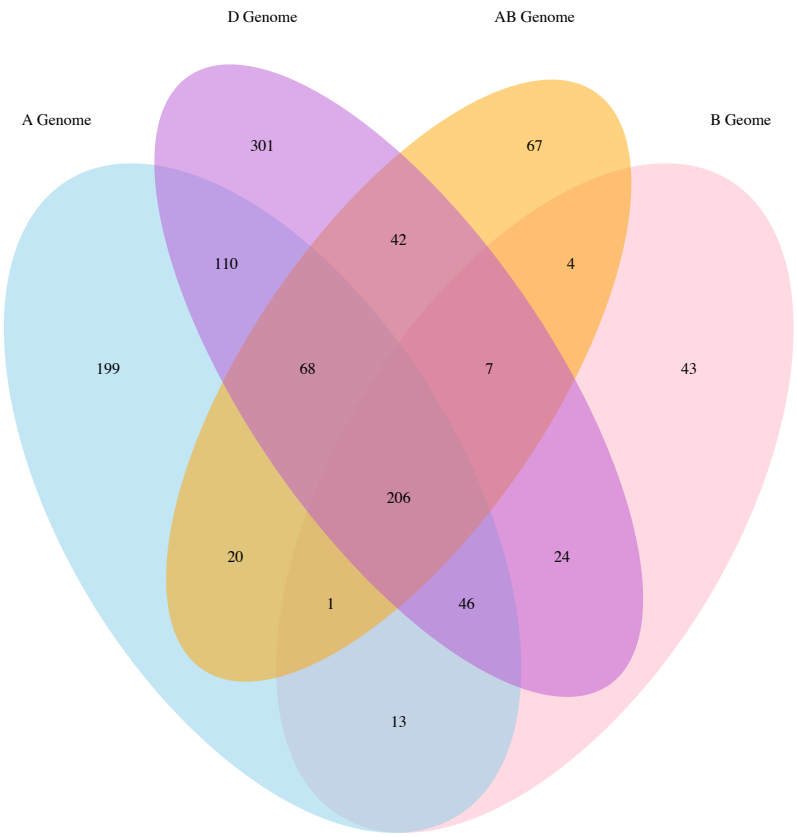


Figure 3.7 Venn diagram of core fungal taxa among A, B, D, and AB genomes of wheat and related wild species.

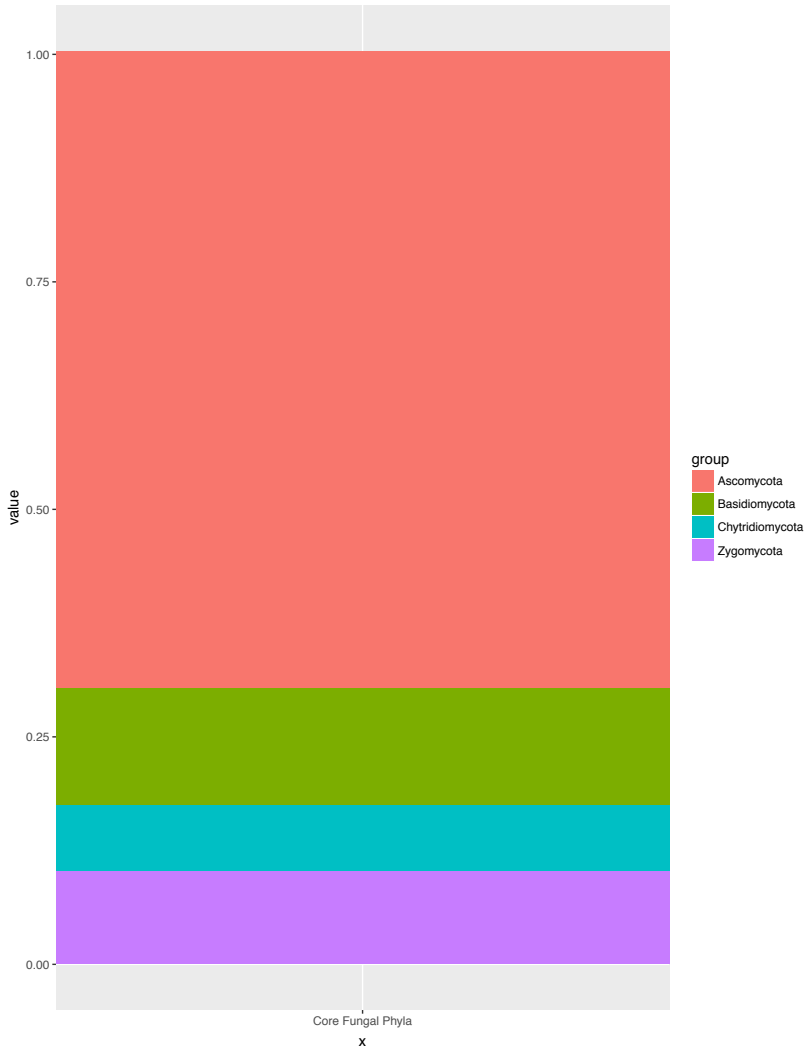


Figure 3.8 Bar plot of core fungal taxa among A, B, D, AB, and ABD genomes of wheat and related wild species in this study.

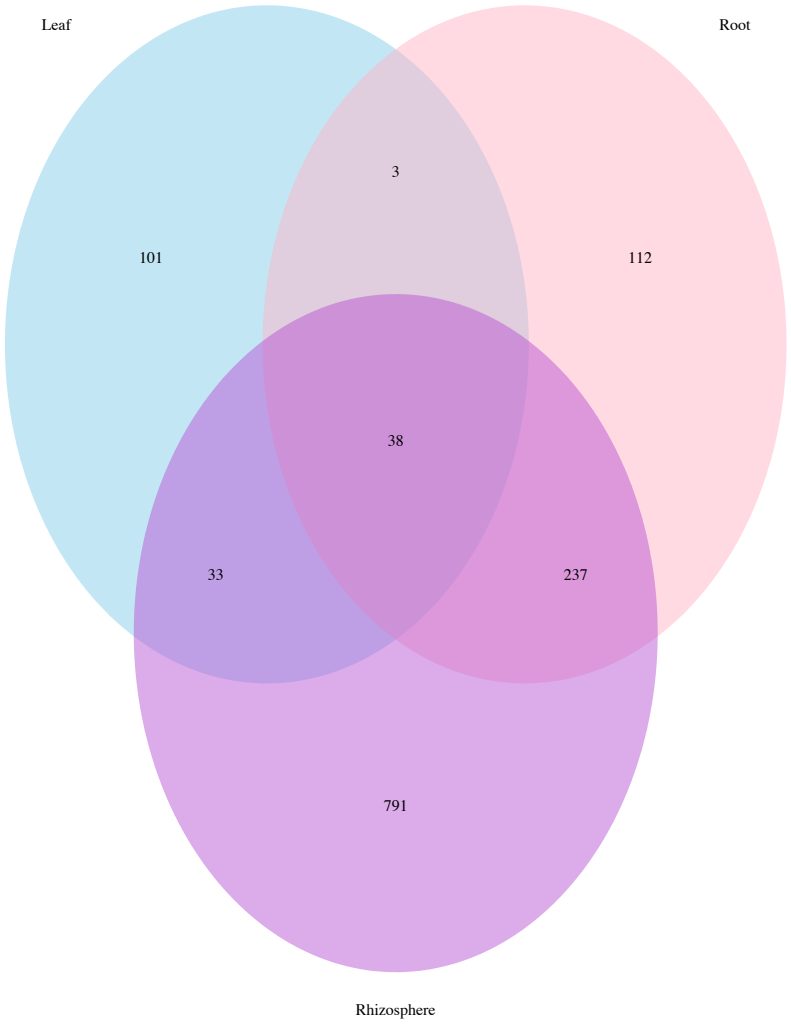


Figure 3.9 Venn diagram of core fungal taxa among all tissue types.

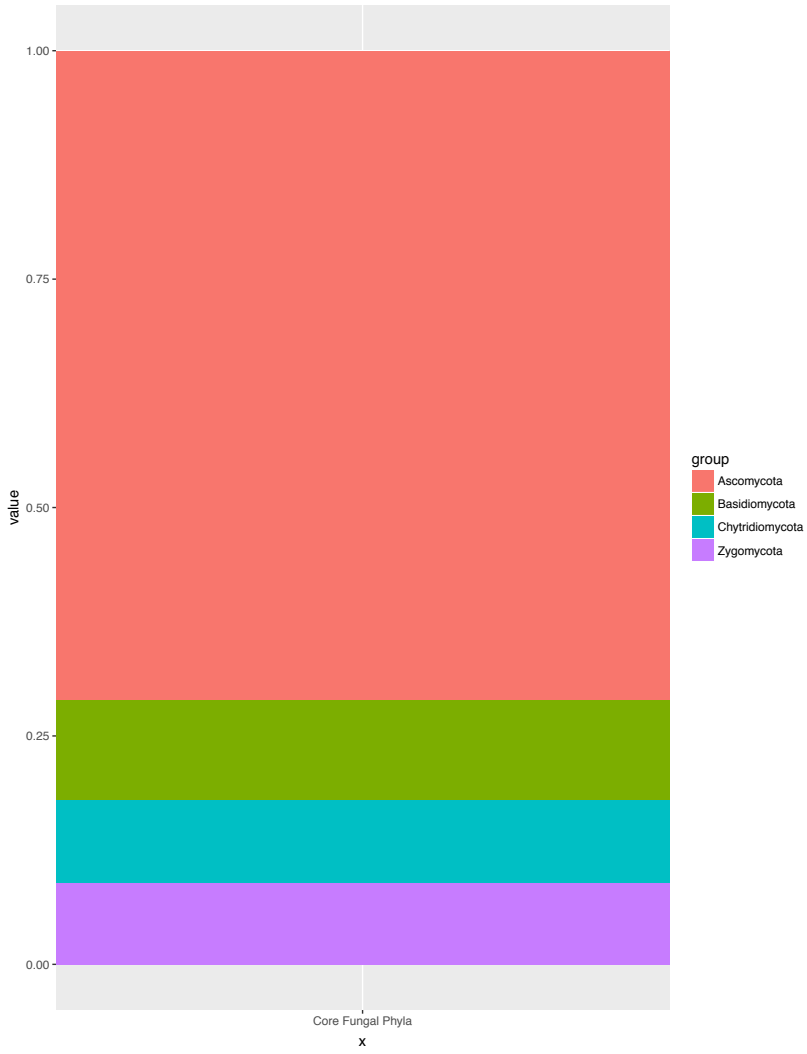


Figure 3.10 Bar plot of core fungal taxa among all tissue types.

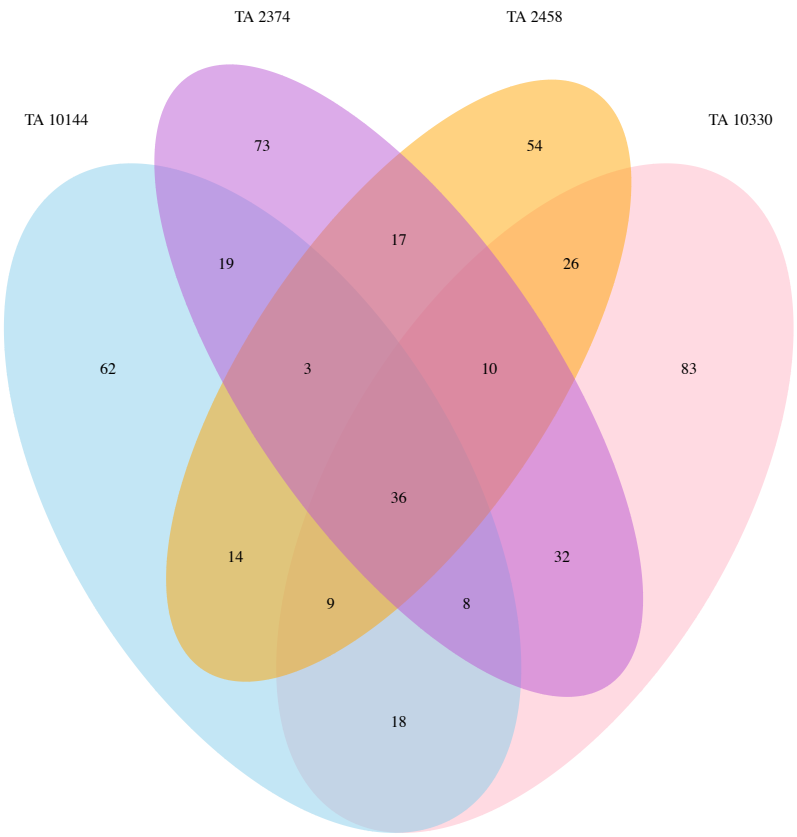


Figure 3.11 Venn diagram of core bacterial taxa among four accessions of *Aegilops tauschii* with distant geographic origins.

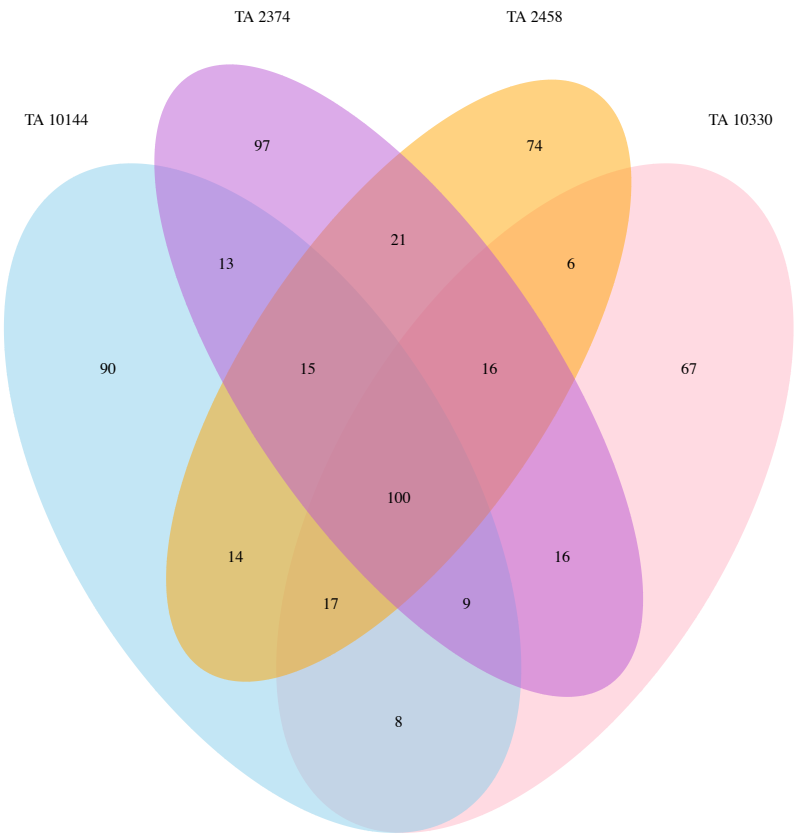


Figure 3.12 Venn diagram of core fungal taxa among four accessions of *Aegilops tauschii* with distant geographic origins.

Chapter 4

Summary and Conclusions

Summary

The plant microbiome consists of microorganisms that influence their hosts' physiology and has demonstrated importance in the health and development of plants. Some microbes confer competitive advantages, such as nutrient fixation and water uptake, and have stoked interest in the field of agriculture, where inputs like water and nitrogen are growing increasingly expensive and/or scarce (Chaparro et al., 2012). Characterizing these consortia of bacteria, fungi, and other symbiotic microbes in numerous crops is an important step in leveraging the plant microbiome for crop improvement.

Wheat is a globally important food crop with a complex evolutionary history, including polyploidy speciation events, whose wild relatives continue to grow in their centers of origin (Gustafson et al., 2009). Some studies indicate genotype-dependent microbial diversity across different wheat (*Triticum aestivum*) accessions, and suggest wild relative species might mitigate stress in their native environments through microbial interactions (Corneo et al., 2016; Iannucci et al., 2017). The narrowing genetic base of domesticated crops like wheat has led to interest in wild relatives species as reservoirs of genetic diversity, and could provide a stepping stone toward breeding for microbiomes (Warburton et al., 2006; Gopal & Gupta, 2016).

This study broadened the current knowledge of plant-microbe interactions and predictors of microbial community structure in wheat (*Triticum aestivum*) and wild wheat relatives (*Aegilops* and *Triticum* spp.) by quantifying the significance of several comprehensive variables including planting season, water treatment, plant tissue type, and plant genotype. Additionally,

the effects of domestication on the microbiome of modern hexaploid wheat and its wild relative species was explored by identifying a conserved "core" community of microbial taxa. The diversity of wheat and wild relative germplasm analyzed in a common soil makes this study unique, and the findings contribute to a larger body of work seeking to illuminate drivers of healthy microbiome acquisition.

Conclusions

Overall, no significant differences were found among the microbiomes of three hexaploid wheat cultivars and 14 landrace and wild relative accessions of different ploidy levels. This corroborates existing literature that suggests differences in the plant microbiome across closely related species is relatives minor (Leff et al., 2016). Plant tissue type (i.e. leaves, roots, and rhizosphere soil) remains a strong driver of microbial community structure. Soil moisture and pH are known predictors of microbiome diversity and may have played a role in the communities in this study, but since they were not measured conclusions cannot as yet be made (Fierer et al., 2017).

The "core" microbiome shared among five wheat and wild relative species (representing five distinct genomes: A, B, D, AB, and ABD) was generally smaller than the number of taxa unique to each genome or shared between only a few genome representatives. Plant species identity appears to play a slightly larger role in determining fungal communities than bacteria, exhibited by the greater "core" of fungal taxa. However, the small number of core taxa may be explained by the use of high resolution sequence variants (SV's), as opposed to coarser taxonomic groups that would have likely revealed a larger proportion of the microbiome shared among most plant species (Bulgarelli et al., 2015; Fierer, 2017; Lundber et al., 2012).

Interestingly, the plant species with the greatest proportion of the fungal phylum Glomeromycota were *Triticum aestivum* and *Aegilops tauschii*, suggest the D-genome may be important for recruiting arbuscular mycorrhizae.

From these results, it appears that many microbial symbioses were conserved despite divergent breeding. The differences among microbial communities may be due to genetic adaptation by the plant hosts. However, the close genetic relationships among the plant species in this study may have reduced the observable differences in microbial community structure. Additionally, common garden experiments limit the pool of potential plant-microbe interactions. Despite the domestication of modern wheat, the microbiome remains essentially the same as that of its wild relatives when grown in the same soil. Future research in these hosts' centers of origin could broaden our understanding of long-evolved plant-microbe interactions.

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Appendix Tables

Appendix Table 1 Soil analysis at field trial site at the Agricultural Research and Development Educational Center (ARDEC) in Fort Collins, Colorado, USA. Soil sample taken on day of tissue collection (June 8th, 2016).

Scott Reid/Pat Byrne/Soil & Crop Sciences										**Colorado State University**			
1170 Campus Delivery										**Soil, Water and Plant Testing Laboratory**			
Colorado State University										Natural & Environmental Sciences Bldg - A320			
										Fort Collins, CO 80523-1120			
DATE RECEIVED: 06-07-2016										(970) 491-5061 FAX: 491-2930			
DATE REPORTED: 06-10-2016													
										BILLING:			
					RESEARCH SOIL ANALYSIS								
						----------AB-DTPA----------							
Lab #	**Sample ID #**	**----------paste----------**		**Lime Estimate**	**% OM**	**----------ppm----------**							**Texture Estimate**
		pH	**EC**			**NO_3-N**	**P**	**K**	**Zn**	**Fe**	**Mn**	**Cu**	
			mmhos/cm										
R5944	2016 micro-surface	7.9	0.7	Very High	2.7	6.9	14.0	314	3.1	6.2	1.9	1.7	Sandy Clay Loam
R5944-1	2016 micro-surface					15.1							

Appendix Table 2 Selective primers for *Illumina* amplicon sequencing used in this study (UMGC Dual-Index Microbiome Amplification, 2016).

Amplification Region	Primer	Sequence
16S-V4	Meta_V4_515F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA
16S-V4	Meta_V4_806R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT
ITS2	5.8SR'	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCGATGAAGAACGCAGCG
ITS2	ITS4_Nextera	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC

Appendix Table 3 Core fungal taxa among all tissue types.

SV	Phylum	Class	Order	Family	Genus	Species
SV 14	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__intricans
SV 18	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	NA
SV 24	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__aeriuss
SV 26	p__Basidiomycota	c__Pucciniomycetes	o__Pucciniales	f__Pucciniaceae	g__Puccinia	s__striiformis
SV 27	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Guehomyces	s__pullulans
SV 28	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	NA
SV 39	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Mycosphaerellaceae	g__Mycosphaerella	s__tassiana
SV 48	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__solani
SV 60	p__Ascomycota	c__Leotiomycetes	o__Erysiphales	f__Erysiphaceae	g__Blumeria	s__graminis
SV 63	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Stachybotrys	s__chartarum
SV 66	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__oxysporum
SV 71	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Microdochium	s__bolleyi
SV 75	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__polygonia
SV 84	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__tricincta
SV 85	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Cucurbitariaceae	g__Pyrenochaetopsis	s__leptospora
SV 86	p__Basidiomycota	c__Agaricomycetes	o__Cantharellales	f__Ceratobasidiaceae	g__Thanatephorus	s__cucumeris

SV 104	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 106	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	NA
SV 134	p__Basidiomycota	c__Tremellomycetes	o__Filobasidiales	f__Filobasidiaceae	g__Filobasidium	s__magnum
SV 141	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__victoriae
SV 144	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Bipolaris	s__eleusines
SV 160	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporales_fam_Incertae_sedis	NA	NA
SV 198	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__tricincta
SV 223	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Agaricaceae	g__Coprinus	s__silvaticus
SV 251	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	s__metachromatica
SV 256	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Monographella	s__nivalis
SV 287	p__Basidiomycota	c__Ustilaginomycotina_cls_Incertae_sedis	o__Malasseziales	f__Malasseziaceae	g__Malassezia	s__restricta
SV 298	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Leptosphaeriaceae	g__Leptosphaeria	s__sclerotioides
SV 412	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	NA
SV 456	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 740	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Chalastospora	s__ellipsoidea
SV 784	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	s__alternata
SV 971	p__Basidiomycota	c__Ustilaginomycetes	o__Ustilaginales	f__Ustilaginaceae	g__Ustilago	s__hordei

SV990	p__Ascomycota	c__Dothideomycetes	o__Dothideales	f__Dothioraceae	g__Aureobasidium	s__subglaciale
SV1125	p__Ascomycota	c__Dothideomycetes	o__Dothideales	f__Dothioraceae	g__Aureobasidium	NA
SV1299	p__Ascomycota	c__Dothideomycetes	o__Dothideales	f__Dothioraceae	g__Aureobasidium	s__subglaciale
SV1301	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Bulleribasidiaceae	g__Vishniacozyma	s__carnescens
SV2068	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Leptosphaeriaceae	g__Neosetophoma	NA

Appendix Table 4 Core fungal taxa among all representative wheat and wild wheat relative genomes (A,
B, D, AB, and ABD).

SV	Phylum	Class	Order	Family	Genus	Species
SV 9	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Gamsia	s__simplex
SV 11	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__minutissima
SV 12	p__Chytridiomycota	c__Chytridiomycetes	o__Olpidiales	f__Olpidiaceae	g__Olpidium	s__brassicae
SV 14	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__intricans
SV 16	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	NA
SV 18	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	NA
SV 19	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	NA	NA
SV 20	p__Chytridiomycota	c__Chytridiomycetes	o__Olpidiales	f__Olpidiaceae	g__Olpidium	s__brassicae
SV 21	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Sporormiaceae	g__Sporormiella	NA
SV 22	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__elongata
SV 24	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__aeriuss
SV 25	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__elongata
SV 26	p__Basidiomycota	c__Pucciniomycetes	o__Pucciniales	f__Pucciniaceae	g__Puccinia	s__striiformis

SV 27	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Guehomyces	s__pullulans
SV 28	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	NA
SV 31	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__aerius
SV 32	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	NA	NA
SV 34	p__Chytridiomycota	c__Chytridiomycetes	o__Olpidiales	f__Olpidiaceae	g__Olpidium	s__brassicae
SV 35	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	NA	NA
SV 38	p__Chytridiomycota	c__Chytridiomycetes	o__Chytridiales	f__Chytridiaceae	NA	NA
SV 39	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Mycosphaerellaceae	g__Mycosphaerella	s__tassiana
SV 40	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	g__Scedosporium	s__prolificans
SV 41	p__Ascomycota	c__Sordariomycetes	o__Hypocreomycetidae_ord_Incertae_sedis	f__Plectosphaerellaceae	g__Gibellulopsis	s__nigrescens
SV 42	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Psathyrellaceae	g__Psathyrella	s__panaeoloides
SV 43	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__brasiliense
SV 44	p__Chytridiomycota	c__Chytridiomycetes	o__Rhizophlyctidales	f__Rhizophlyctidaceae	g__Rhizophlyctis	NA
SV 45	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusicolla	NA
SV 47	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Trichocladium	s__asperum
SV 48	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__solani
SV 49	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__solani

SV 52	p__Chytridiomycota	c__Chytridiomycetes	o__Chytridiales	f__Chytridiaceae	NA	NA
SV 54	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	NA	NA
SV 56	p__Chytridiomycota	c__Chytridiomycetes	o__Olpidiales	f__Olpidiaceae	g__Olpidium	s__brassicae
SV 57	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__alpina
SV 58	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Monographella	s__cucumerina
SV 59	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Nectria	s__ramulariae
SV 60	p__Ascomycota	c__Leotiomycetes	o__Erysiphales	f__Erysiphaceae	g__Blumeria	s__graminis
SV 61	p__Basidiomycota	c__Agaricomycetes	o__Trechisporales	f__Hydnodontaceae	g__Subulicystidium	s__perlongisporum
SV 62	p__Ascomycota	c__Leotiomycetes	o__Thelebolales	f__Thelebolaceae	g__Thelebolus	s__globosus
SV 63	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Stachybotrys	s__chartarum
SV 66	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__oxysporum
SV 67	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__indohii
SV 68	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	NA	NA
SV 71	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Microdochium	s__bolleyi
SV 73	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Acremoniu m	s__persicinum
SV 75	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__polygonia

SV 78	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 80	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__alpina
SV 81	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Bionectriaceae	g__Nectriopsis	s__lecanodes
SV 84	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__tricincta
SV 85	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Cucurbitariaceae	g__Pyrenochaetopsis	s__leptospora
SV 86	p__Basidiomycota	c__Agaricomycetes	o__Cantharellales	f__Ceratobasidiaceae	g__Thanatephorus	s__cucumeris
SV 87	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__exigua
SV 89	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	NA	NA
SV 91	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	g__Cheilymenia	NA
SV 92	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Podospora	s__multipilosa
SV 94	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	NA
SV 96	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__alpina
SV 102	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Monosporascus	NA
SV 104	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 106	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	NA

SV 108	p__Chytridiomycota	c__Chytridiomycetes	o__Olpidiales	f__Olpidiaceae	g__Olpidium	s__brassicae
SV 110	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__aerius
SV 114	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Gamsia	s__simplex
SV 115	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	NA	NA
SV 117	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__jensenii
SV 118	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__hyalina
SV 121	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Stachybotrys	s__elegans
SV 122	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	g__Pithoascus	NA
SV 125	p__Chytridiomycota	c__Chytridiomycetes	o__Spizellomycetales	f__Spizellomycetaceae	g__Spizellomyces	NA
SV 126	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 128	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Bisifusarium	s__dimerum
SV 130	p__Chytridiomycota	c__Chytridiomycetes	o__Spizellomycetales	f__Spizellomycetaceae	g__Spizellomyces	s__pseudodichotomus
SV 134	p__Basidiomycota	c__Tremellomycetes	o__Filobasidiales	f__Filobasidiaceae	g__Filobasidium	s__magnum
SV 135	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Acremonium	s__alternatum
SV 139	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Setophoma	s__terrestris
SV 140	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__solani

SV 141	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__victoriae
SV 144	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Bipolaris	s__eleusines
SV 148	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Podospora	s__dimorpha
SV 150	p__Ascomycota	c__Leotiomycetes	o__Helotiales	f__Helotiales_fam_Incertae_sedis	g__Tetracladium	NA
SV 151	p__Ascomycota	c__Eurotiomycetes	o__Chaetothyriales	f__Herpotrichiellaceae	g__Exophiala	s__equina
SV 152	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__aethiopicum
SV 153	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	s__grande
SV 154	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	g__Microascus	s__brevicaulis
SV 155	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	s__antarctica
SV 158	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiales_fam_Incertae_sedis	g__Mrakiella	s__aquatica
SV 160	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporales_fam_Incertae_sedis	NA	NA
SV 164	p__Ascomycota	c__Sordariomycetes	o__Magnaporthales	f__Magnaporthaceae	g__Gaeumannomyces	NA
SV 169	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Setophoma	s__terrestris
SV 171	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__chrysogenum
SV 172	p__Zygomycota	c__Mucoromycotina_cls_Incertae_sedis	o__Mucorales	f__Mucoraceae	g__Actinomucor	s__elegans
SV 176	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Gymnoascaceae	g__Gymnoascus	s__reessii

SV 178	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 180	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Cercophora	s__samala
SV 186	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Bolbitiaceae	NA	NA
SV 188	p__Ascomycota	c__Orbiliomycetes	o__Orbiliales	f__Orbiliaceae	g__Arthrobotrys	s__oligospora
SV 189	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__solani
SV 190	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 191	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Gamsia	s__simplex
SV 198	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__tricincta
SV 199	p__Basidiomycota	c__Agaricomycetes	o__Cantharellales	f__Ceratobasidiaceae	g__Ceratobasidium	NA
SV 200	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Volutella	NA
SV 209	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 211	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	g__Pithoascus	NA
SV 212	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Didymosphaeriaceae	g__Roussoella	s__intermedia
SV 214	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Psathyrellaceae	g__Psathyrella	s__panaeoloides
SV 217	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 220	p__Zygomycota	c__Mucoromycotina_cls_Incertae_sedis	o__Mucorales	f__Mucoraceae	g__Actinomucor	s__elegans
SV 221	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 224	p__Chytridiomycota	c__Chytridiomycetes	o__Rhizophlyctidales	f__Rhizophlyctidaceae	g__Rhizophlyctis	NA

SV 225	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Trichocladium	s__asperum
SV 227	p__Ascomycota	c__Leotiomycetes	o__Helotiales	f__Helotiales_fam_Incertae_sedis	g__Tetracladium	s__setigerum
SV 228	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Schizotheciu m	s__inaequale
SV 230	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	g__Geopyxis	NA
SV 237	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Bionectriaceae	g__Clonostachys	s__rosea
SV 240	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 241	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporales_fam_Incertae_sedis	NA	NA
SV 243	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	s__tricola
SV 247	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Schizotheciu m	s__inaequale
SV 250	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Onygenaceae	g__Chrysosporium	s__pseudomerdarium
SV 251	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	s__metachromatica
SV 256	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Monographella	s__nivalis
SV 261	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Ascobolaceae	NA	NA
SV 263	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 264	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 265	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Agaricaceae	g__Coprinus	s__silvaticus

SV 270	p__Chytridiomycota	c__Chytridiomycetes	o__Spizellomycetales	f__Spizellomycetaceae	g__Powellomyces	NA
SV 278	p__Ascomycota	c__Leotiomycetes	o__Helotiales	f__Helotiales_fam_Incertae_sedis	g__Tetracladium	s__setigerum
SV 283	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__aurantiogriseum
SV 287	p__Basidiomycota	c__Ustilaginomycotina_cls_Incertae_sedis	o__Malasseziales	f__Malasseziaceae	g__Malassezia	s__restricta
SV 288	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	NA
SV 293	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__chrysogenum
SV 298	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Leptosphaeriaceae	g__Leptosphaeria	s__sclerotioides
SV 311	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Onygenaceae	g__Chrysosporium	s__lobatum
SV 313	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Xylariales_fam_Incertae_sedis	g__Monosporascus	NA
SV 326	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Myrotheciu m	s__roridum
SV 332	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Phaeosphaeriaceae	g__Ophiosphaerella	NA
SV 334	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 337	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Stachybotrys	s__elegans
SV 345	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Sporormiaceae	NA	NA
SV 348	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Tremellales_fam_Incertae_sedis	g__Cryptococcus	s__aerius

SV 361	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 369	p__Chytridiomycota	c__Chytridiomycetes	o__Chytridiales	f__Chytridiaceae	NA	NA
SV 377	p__Basidiomycota	c__Agaricomycetes	o__Corticiales	f__Corticiaceae	g__Waitea	s__circinata
SV 379	p__Ascomycota	c__Leotiomycetes	o__Leotiomycetes_ord_Incertae_sedis	f__Myxotrichaceae	g__Oidiodendron	s__cerealе
SV 397	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	NA	NA
SV 402	p__Zygomycota	c__Mucoromycotina_cls_Incertae_sedis	o__Mucorales	f__Mucoraceae	g__Mucor	s__racemosus
SV 412	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporaceae	g__Alternaria	NA
SV 426	p__Chytridiomycota	c__Chytridiomycetes	o__Spizellomycetales	f__Spizellomycetaceae	g__Powellomyces	s__hirtus
SV 449	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ciliophora	NA
SV 453	p__Zygomycota	c__Mucoromycotina_cls_Incertae_sedis	o__Mucorales	f__Mucoraceae	g__Mucor	s__hiemalis
SV 455	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Guehomyces	s__pullulans
SV 456	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 457	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ochroconis	s__tshawytschae
SV 459	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	NA	NA
SV 463	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	g__Scutellinia	s__scutellata
SV 466	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Gymnoascaceae	g__Gymnoascus	NA

SV 470	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporales_fam_Incertae_sedis	g__Periconia	s__macrospinosa
SV 497	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Microascaceae	g__Microascus	s__senegalensis
SV 502	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 506	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Aspergillus	NA
SV 508	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Corynesporascaceae	g__Corynespora	NA
SV 513	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Sporormiaceae	g__Preussia	s__flanaganii
SV 514	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Onygenaceae	g__Chrysosporium	s__pseudomerdarium
SV 533	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	NA	NA
SV 536	p__Basidiomycota	c__Microbotryomycetes	o__Sporidiobolales	f__Sporidiobolales_fam_Incertae_sedis	g__Rhodotorula	s__ferulica
SV 539	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Trichocomaceae	g__Penicillium	s__aethiopicum
SV 555	p__Zygomycota	c__Mortierellomycotina_cls_Incertae_sedis	o__Mortierellales	f__Mortierellaceae	g__Mortierella	NA
SV 559	p__Zygomycota	c__Mucoromycotina_cls_Incertae_sedis	o__Mucorales	f__Mucoraceae	g__Mucor	NA
SV 564	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	g__Geopyxis	NA
SV 567	p__Ascomycota	c__Sordariomycetes	o__Sordariomycetidae_ord_Incertae_sedis	f__Sordariomycetidae_fam_Incertae_sedis	g__Savoryella	s__lignicola
SV 588	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	NA
SV 597	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Pleosporales_fam_Incertae_sedis	g__Pyrenochaeta	s__inflorescentiae

SV 616	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Acremonium	s__stromaticum
SV 619	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreaceae	g__Trichoderma	s__barbatum
SV 641	p__Basidiomycota	c__Agaricomycetes	o__Cantharellales	f__Ceratobasidiaceae	g__Thanatephorus	s__cucumeris
SV 657	p__Ascomycota	c__Eurotiomycetes	o__Arachnomycetales	f__Arachnomycetaceae	g__Arachnomyces	NA
SV 666	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Onygenaceae	g__Chrysosporium	s__lobatum
SV 688	p__Ascomycota	c__Eurotiomycetes	o__Onygenales	f__Gymnoascaceae	g__Gymnoascus	s__reessii
SV 703	p__Ascomycota	c__Pezizomycetes	o__Pezizales	f__Pyronemataceae	NA	NA
SV 724	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Clavicipitaceae	g__Metarhizium	s__marquandii
SV 726	p__Zygomycota	c__Zoopagomycotina_cls_Incertae_sedis	o__Zoopagales	f__Piptocephalidaceae	g__Syncephalis	NA
SV 739	p__Ascomycota	c__Sordariomycetes	o__Sordariomycetidae_ord_Incertae_sedis	f__Glomerellaceae	g__Colletotrichum	NA
SV 746	p__Ascomycota	c__Sordariomycetes	o__Microascales	f__Halosphaeriaceae	g__Remispora	s__stellata
SV 765	p__Basidiomycota	c__Tremellomycetes	o__Cystofilobasidiales	f__Cystofilobasidiaceae	g__Cystofilobasidium	s__macerans
SV 819	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Macroconia	s__leptosphaeriae
SV 876	p__Ascomycota	c__Leotiomycetes	o__Helotiales	f__Hyaloscyphaceae	NA	NA
SV 917	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Lophiostomataceae	g__Acrocalymma	s__fici

SV1009	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Cordycipitaceae	g__Beauveria	NA
SV1065	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Bionectriaceae	g__Hydropisphaera	s__fungicola
SV1314	p__Ascomycota	c__Sordariomycetes	o__Magnaporthales	f__Magnaporthaceae	g__Magnaporthe	NA
SV1344	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Gibberella	s__pulicaris
SV1406	p__Ascomycota	c__Pezizomycotina_cls_Incertae_sedis	o__Pezizomycotina_ord_Incertae_sedis	f__Pezizomycotina_fam_Incertae_sedis	g__Ochroconis	NA

Appendix Table 5 Core bacterial taxa among all tissue types.

SV	Kingdom	Phylum	Class	Order	Family	Genus	Species
SV 20	k__Archaea	p__Crenarchaeota	c__Thaumarchaeota	o__Nitrososphaerales	f__Nitrososphaeraceae	g__Candidatus Nitrososphaera	s__SCA1145
SV 15	k__Archaea	p__Crenarchaeota	c__Thaumarchaeota	o__Nitrososphaerales	f__Nitrososphaeraceae	g__Candidatus Nitrososphaera	s__
SV 519	k__Bacteria	p__Acidobacteria	c__Solibacteres	o__Solibacterales	f__	g__	s__
SV 864	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 68	k__Bacteria	p__Acidobacteria	c__[Chloracidobacteria]	o__RB41	f__Ellin6075	g__	s__
SV 355	k__Bacteria	p__Actinobacteria	c__Rubrobacteria	o__Rubrobacterales	f__Rubrobacteraceae	g__Rubrobacter	s__
SV 562	k__Bacteria	p__Actinobacteria	c__Rubrobacteria	o__Rubrobacterales	f__Rubrobacteraceae	g__Rubrobacter	s__
SV 304	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__	g__	s__
SV 173	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__	g__	s__
SV 425	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__	g__	s__
SV 135	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__	g__	s__
SV 45	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 56	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__

SV 42	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 126	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 28	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 257	k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__	g__	s__
SV 1920	k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__	g__	s__
SV 147	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	g__Nocardioides	s__
SV 82	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Glycomycetaceae	g__Glycomyces	NA
SV 441	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Streptomycetaceae	g__Streptomyces	s__mirabilis
SV 69	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Micrococcaceae	NA	NA
SV 17	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Micrococcaceae	g__Arthrobacter	NA
SV 136	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Microbacterium	NA
SV 48	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Agromyces	s__
SV 79	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Agromyces	s__
SV 137	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Promicromonosporaceae	g__Promicromonospora	s__
SV 509	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Promicromonosporaceae	g__Cellulosimicrobium	s__

SV 522	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Cellulomonadaceae	g__Cellulomonas	NA
SV 44	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Intrasporangiaceae	g__Phycicoccus	s__
SV 565	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardiaceae	g__Rhodococcus	s__fascians
SV 111	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Actinosynnemataceae	NA	NA
SV 712	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Dyadobacter	s__
SV 25	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__
SV 24	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__
SV 134	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans
SV 825	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans
SV 30	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans
SV 293	k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Niabella	s__
SV 1493	k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Pedobacter	NA
SV 197	k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Pedobacter	s__
SV 96	k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	f__	g__	s__
SV 344	k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	f__	g__	s__

SV 1206	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__A4b	g__	s__
SV 960	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__A4b	g__	s__
SV 228	k__Bacteria	p__Chloroflexi	c__TK17	o__mle1-48	f__	g__	s__
SV 34	k__Bacteria	p__Chloroflexi	c__Gitt-GS-136	o__	f__	g__	s__
SV 21	k__Bacteria	p__Chloroflexi	c__Ellin6529	o__	f__	g__	s__
SV 104	k__Bacteria	p__Chloroflexi	c__Ellin6529	o__	f__	g__	s__
SV 295	k__Bacteria	p__Chloroflexi	c__Ellin6529	o__	f__	g__	s__
SV 801	k__Bacteria	p__Chloroflexi	c__Ellin6529	o__	f__	g__	s__
SV 139	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Alicyclobacillaceae	g__Alicyclobacillus	s__
SV 11	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Paenibacillus	s__
SV 185	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__flexus
SV 321	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Planococcaceae	g__Paenisporosarcina	s__
SV 172	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Planococcaceae	NA	NA
SV 261	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	NA	NA
SV 66	k__Bacteria	p__Nitrospirae	c__Nitrospira	o__Nitrospirales	f__Nitrospiraceae	g__Nitrospira	s__
SV 452	k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	f__	g__	s__
SV 470	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__	s__
SV 2026	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__	s__
SV 655	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	f__Planctomycetaceae	g__Planctomyces	s__

SV 1033	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__	g__	s__
SV 72	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Polyangiaceae	g__Sorangium	s__cellulosum
SV 1117	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Nannocystaceae	g__Nannocystis	s__exedens
SV 714	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Mycoplana	s__
SV 629	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	NA	NA
SV 390	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Devosia	s__
SV 835	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Devosia	s__
SV 306	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	g__	s__
SV 75	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	NA	NA
SV 86	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Skermanella	s__
SV 232	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__	s__
SV 51	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__
SV 256	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	NA
SV 169	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	s__

SV 12	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	s__
SV 333	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	NA	NA

Appendix Table 6 Core bacterial taxa among representatives from all wheat genomes in this study (A, B, D, AB, and ABD).

SV	Kingdom	Phylum	Class	Order	Family	Genus	Speices
SV 15	k__Archaea	p__Crenarchaeota	c__Thaumarchaeota	o__Nitrososphaerales	f__Nitrososphaeraceae	g__Candidatus Nitrososphaera	s__
SV 20	k__Archaea	p__Crenarchaeota	c__Thaumarchaeota	o__Nitrososphaerales	f__Nitrososphaeraceae	g__Candidatus Nitrososphaera	s__SCA1145
SV 63	k__Archaea	p__Crenarchaeota	c__Thaumarchaeota	o__Nitrososphaerales	f__Nitrososphaeraceae	g__Candidatus Nitrososphaera	s__
SV 9	k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__	s__
SV 10	k__Bacteria	p__Acidobacteria	c__[Chloracidobacteria]	o__RB41	f__	g__	s__
SV 11	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Paenibacillus	s__
SV 12	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	s__
SV 13	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 16	k__Bacteria	p__Gemmatimonadetes	c__Gemm-1	o__	f__	g__	s__
SV 17	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Micrococcaceae	g__Arthrobacter	NA
SV 18	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 19	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__Steroidobacter	s__
SV 21	k__Bacteria	p__Chloroflexi	c__Ellin6529	o__	f__	g__	s__
SV 23	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__RB40	g__	s__
SV 24	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__
SV 25	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__

SV 27	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Streptomycetaceae	NA	NA
SV 28	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 30	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans
SV 31	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__mb2424	g__	s__
SV 33	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 35	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Adhaeribacter	s__
SV 37	k__Bacteria	p__Acidobacteria	c__[Chloracidobacteria]	o__RB41	f__Ellin6075	g__	s__
SV 38	k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__	s__
SV 39	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 40	k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__WD2101	f__	g__	s__
SV 41	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Thiotrichales	f__Piscirickettsiaceae	g__	s__
SV 42	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 43	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Geodermatophilaceae	g__	s__
SV 44	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Intrasporangiaceae	g__Phycicoccus	s__
SV 45	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	f__Gaiellaceae	g__	s__
SV 46	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	NA
SV 48	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Agromyces	s__
SV 52	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacterales	f__Syntrophobacteraceae	g__	s__
SV 53	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__

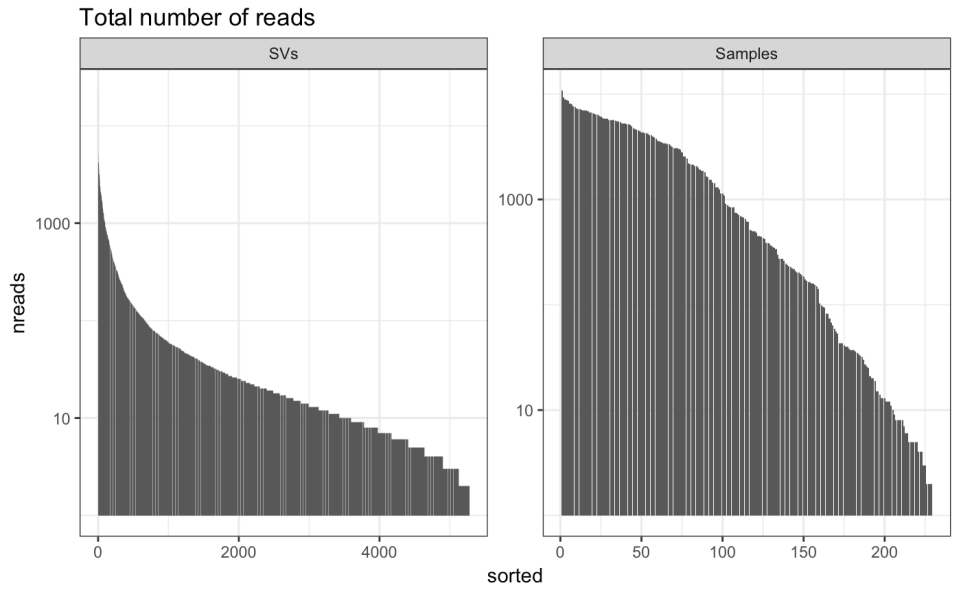
SV 54	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Arenimonas	s__
SV 59	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Thermomonas	s__
SV 60	k__Bacteria	p__Acidobacteria	c__Acidobacteria-5	o__	f__	g__	s__
SV 65	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 68	k__Bacteria	p__Acidobacteria	c__[Chloracidobacteria]	o__RB41	f__Ellin6075	g__	s__
SV 69	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Micrococcaceae	NA	NA
SV 70	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Paenibacillus	s__
SV 74	k__Bacteria	p__Acidobacteria	c__iii1-8	o__DS-18	f__	g__	s__
SV 75	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	NA	NA
SV 82	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Glycomycetaceae	g__Glycomyces	NA
SV 84	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__	g__	s__
SV 85	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__
SV 86	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Skermanella	s__
SV 87	k__Bacteria	p__WS3	c__PRR-12	o__Sediment-1	f__	g__	s__
SV 89	k__Bacteria	p__Planctomycetes	c__OM190	o__CL500-15	f__	g__	s__
SV 90	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	NA	NA
SV 91	k__Bacteria	p__Acidobacteria	c__[Chloracidobacteria]	o__RB41	f__	g__	s__
SV 93	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__

SV99	k__Bacteria	p__Chloroflexi	c__S085	o__	f__	g__	s__
SV100	k__Bacteria	p__Planctomycetes	c__OM190	o__agg27	f__	g__	s__
SV105	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	NA
SV106	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__	s__
SV124	k__Bacteria	p__Fibrobacteres	c__Fibrobacteria	o__258ds10	f__	g__	s__
SV129	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	NA
SV136	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Microbacterium	NA
SV147	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	g__Nocardioides	s__
SV155	k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__WD2101	f__	g__	s__
SV164	k__Bacteria	p__Nitrospirae	c__Nitrospira	o__Nitrospirales	f__0319-6A21	g__	s__
SV173	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__	g__	s__
SV176	k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__C111	g__	s__
SV180	k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Solirubrobacterales	f__Solirubrobacteraceae	g__	s__
SV185	k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__flexus
SV188	k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__	s__

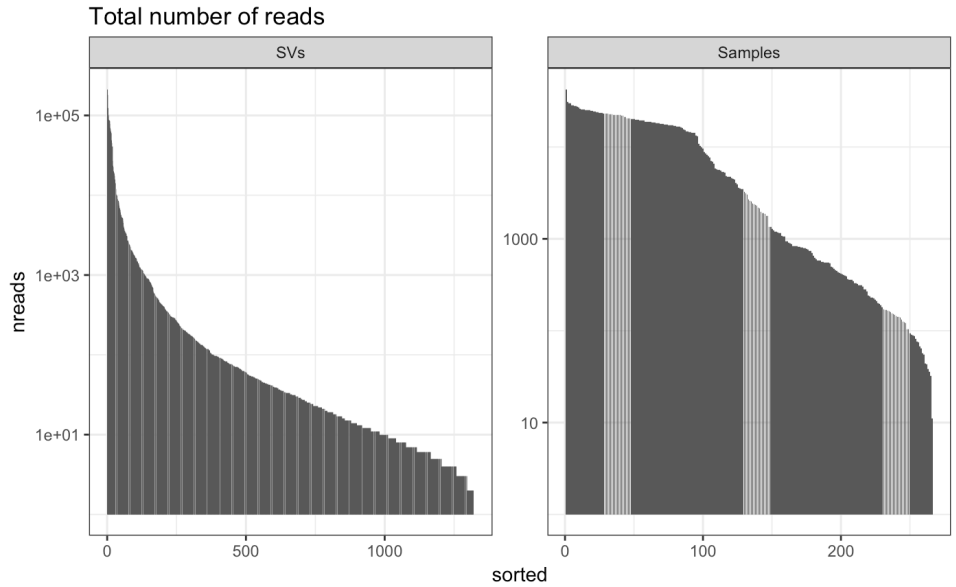
SV191	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	NA	NA
SV199	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	s__
SV203	k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__	s__
SV204	k__Bacteria	p__Gemmatimonadetes	c__	o__	f__	g__	s__
SV232	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__	s__
SV244	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Micrococcales	f__	g__	s__
SV265	k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	f__RB40	g__	s__
SV271	k__Bacteria	p__Chloroflexi	c__TK17	o__mle1-48	f__	g__	s__
SV276	k__Bacteria	p__Gemmatimonadetes	c__Gemmatimonadetes	o__	f__	g__	s__
SV370	k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__	s__
SV408	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	NA	NA
SV454	k__Bacteria	p__Gemmatimonadetes	c__Gemm-5	o__	f__	g__	s__

Appendix Figures

a)

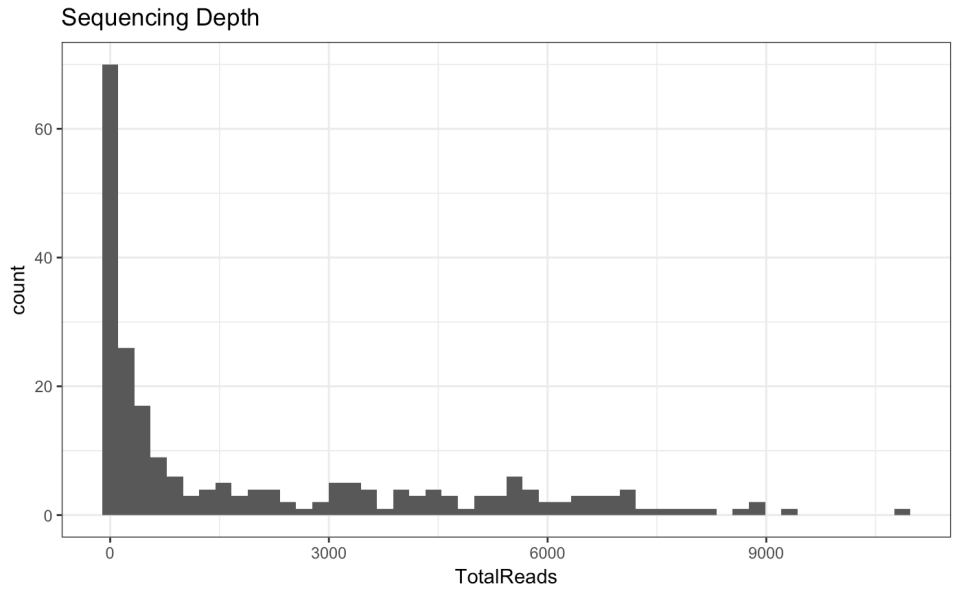


b)

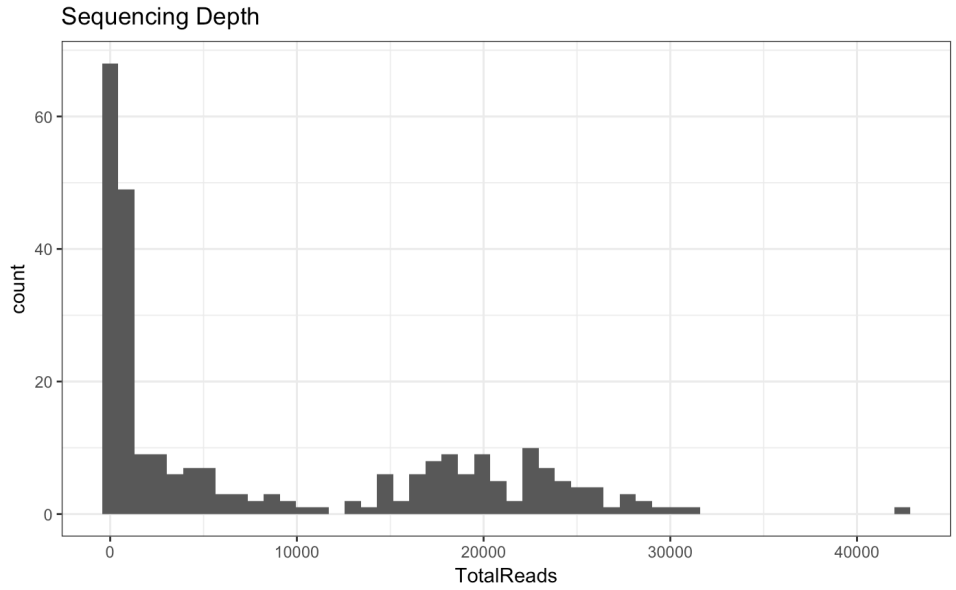


Appendix Figure 1 The distribution of a) bacterial and b) fungal reads per sequence variant (SV) and per sample before pruning.

a)

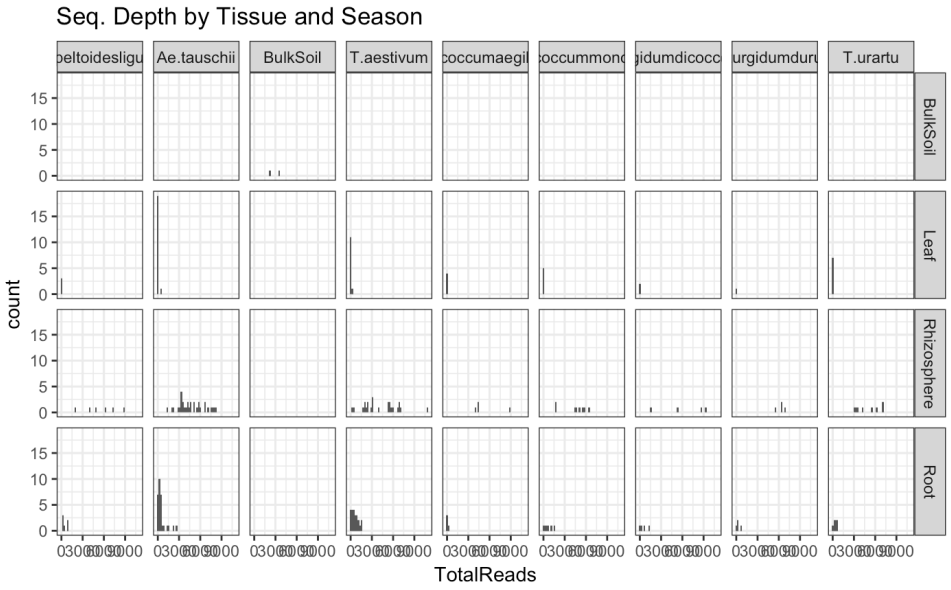


b)

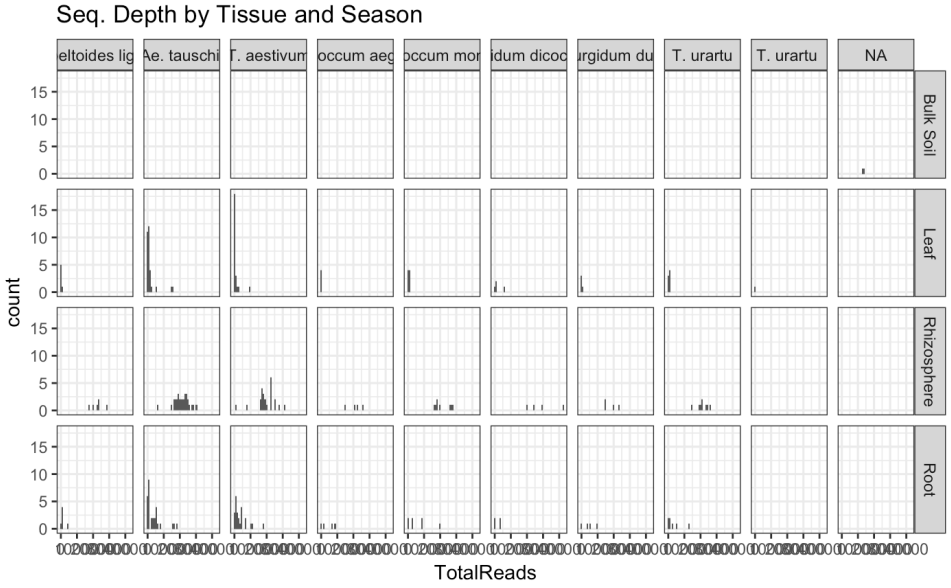


Appendix Figure 2 Overall sequencing depth prior to pruning in a) bacteria and b) fungi.

a)

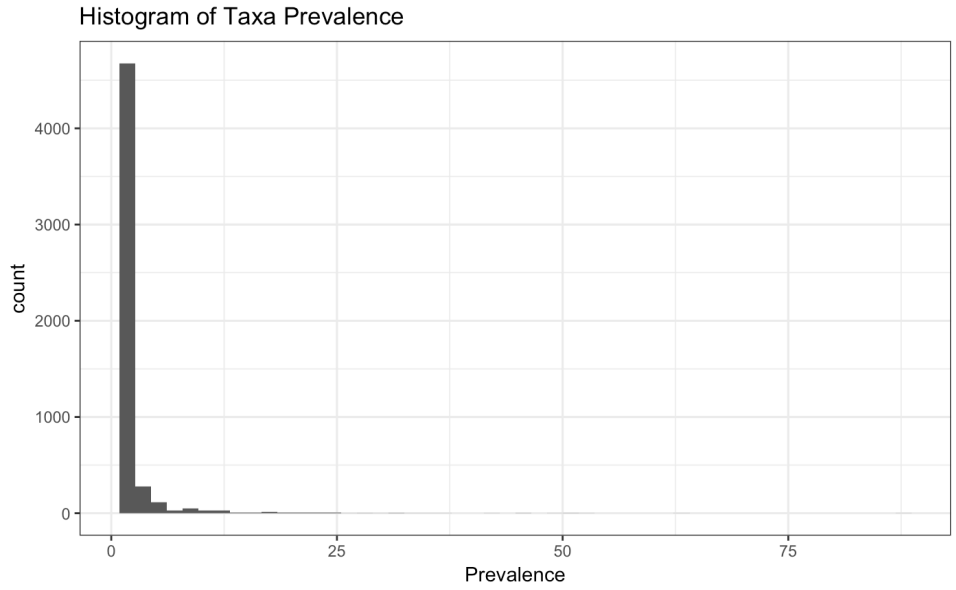


b)

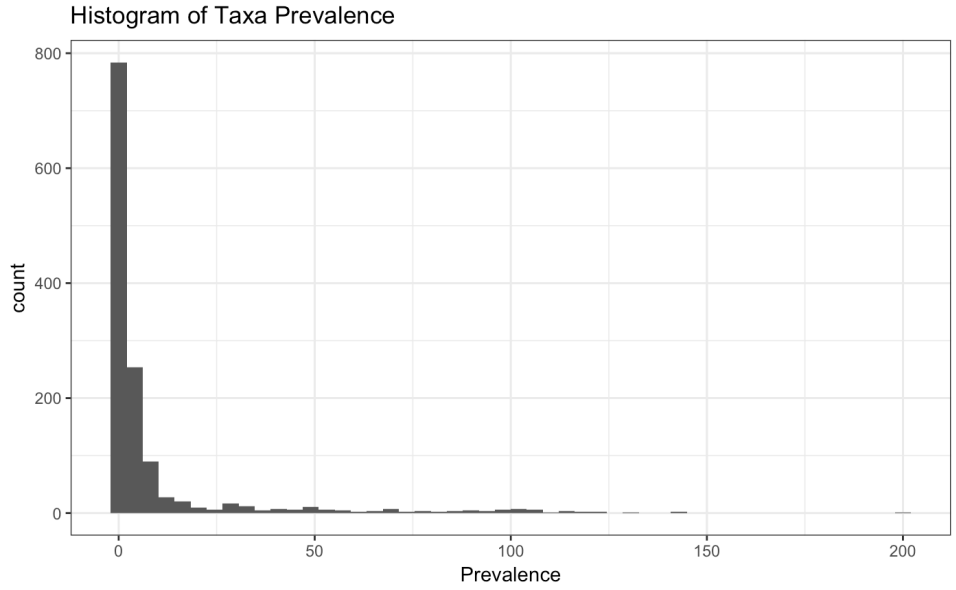


Appendix Figure 3 Sequencing depth faceted by tissue type and plant species prior to pruning in a) bacteria and b) fungi.

a)

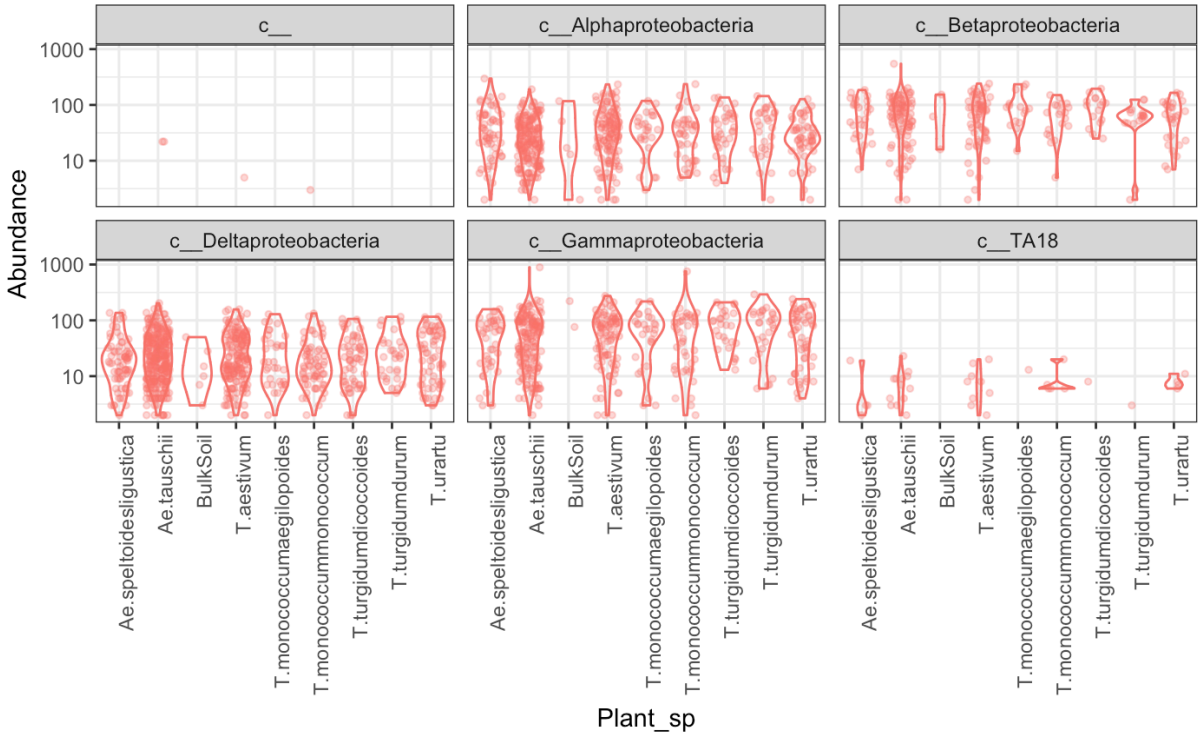


b)

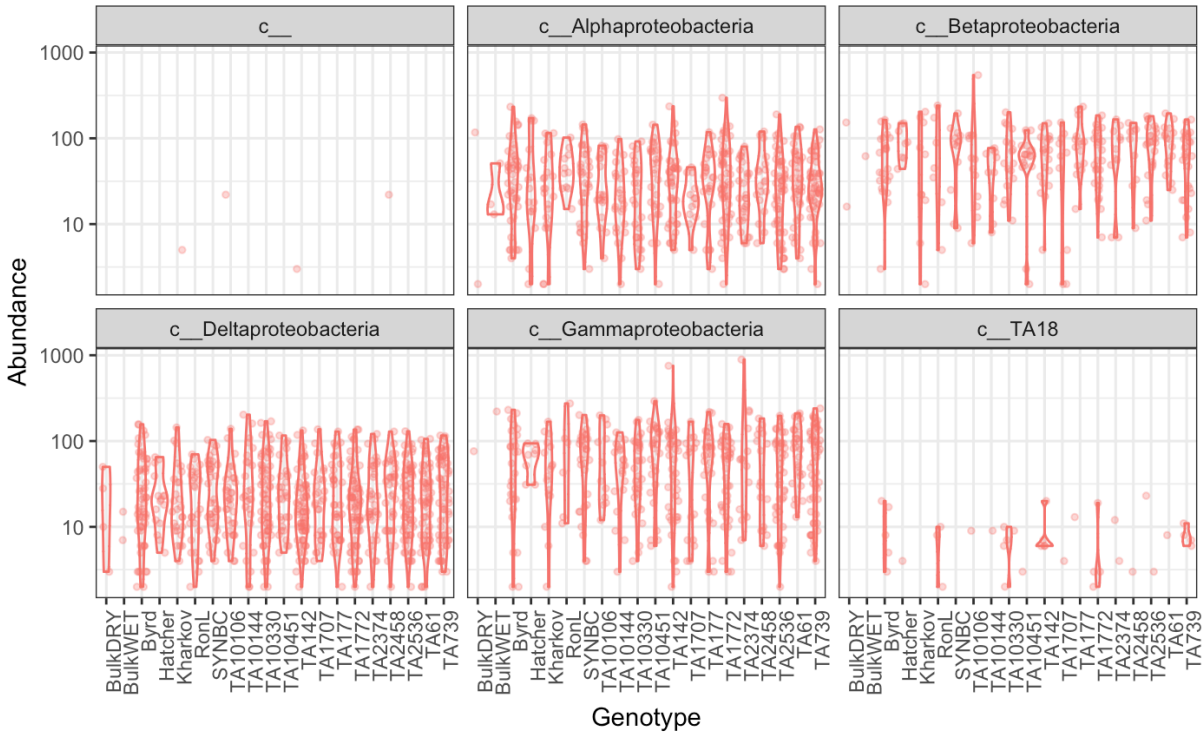


Appendix Figure 4 Histograms of taxa prevalence (number of copies) in each sample for a) bacteria and b) fungi.

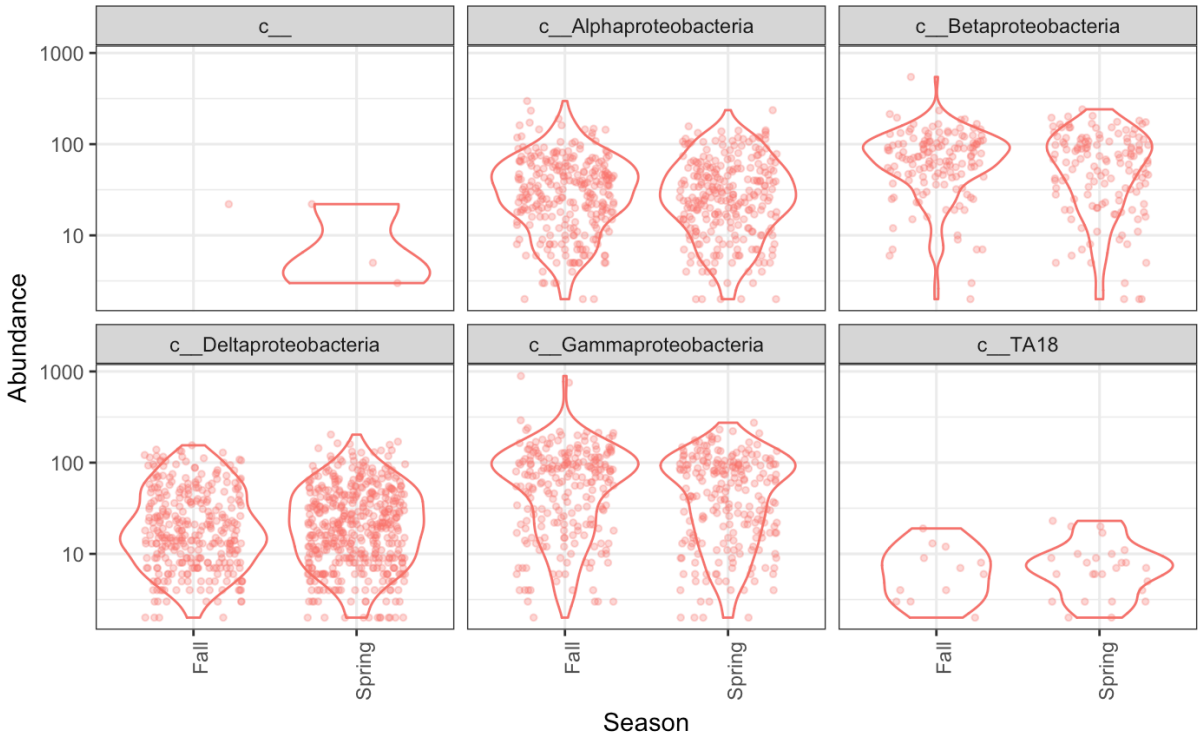
a)


c__
c__Alphaproteobacteria
c__Betaproteobacteria
c__Deltaproteobacteria
c__Gammaproteobacteria
c__TA18
Abundance
1000
100
10
Ae.speltoidesligustica
Ae.tauschii
BulkSoil
T.aestivum
T.monococcumaegilopoides
T.monococcummonococcum
T.turgidumdicoccoides
T.turgidumdurum
T.urartu
Plant_sp

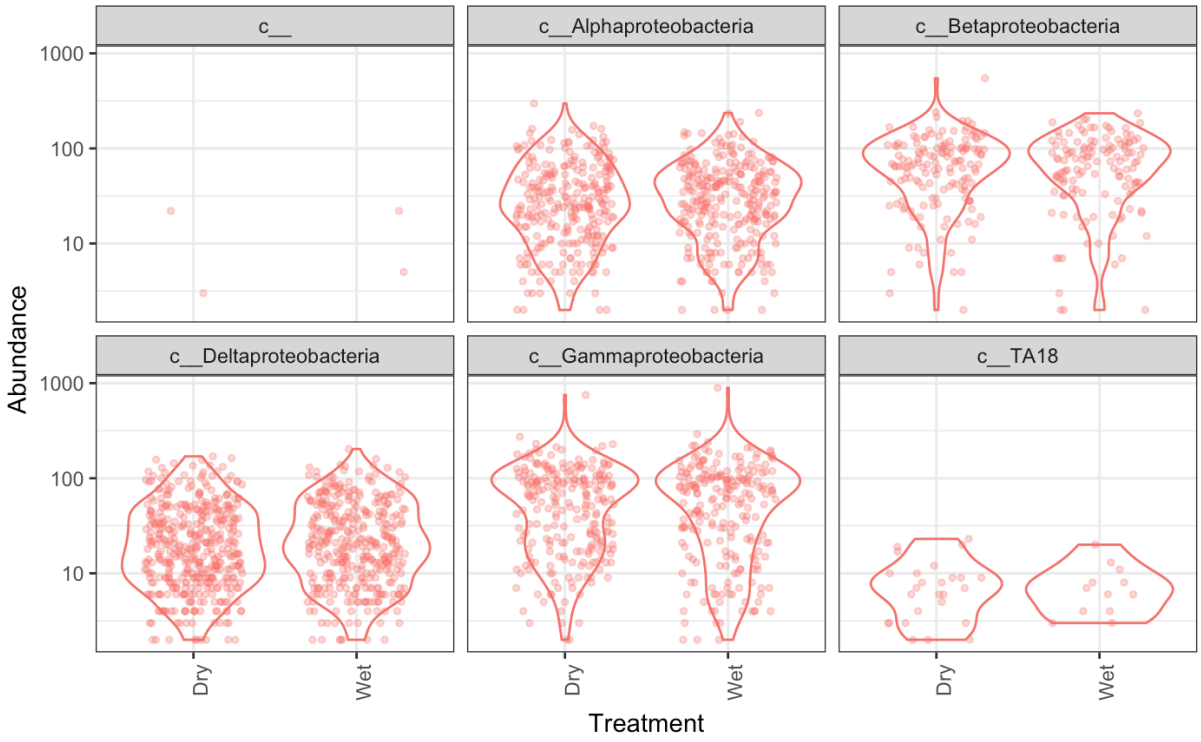
b)


c__
c__Alphaproteobacteria
c__Betaproteobacteria
c__Deltaproteobacteria
c__Gammaproteobacteria
c__TA18
Abundance
1000
100
10
BulkDRY
BulkWET
Byrd
Hatcher
Kharkov
RonL
SYNBC
TA10106
TA10144
TA10330
TA10451
TA142
TA1707
TA177
TA1772
TA2374
TA2458
TA2536
TA61
TA739
Genotype

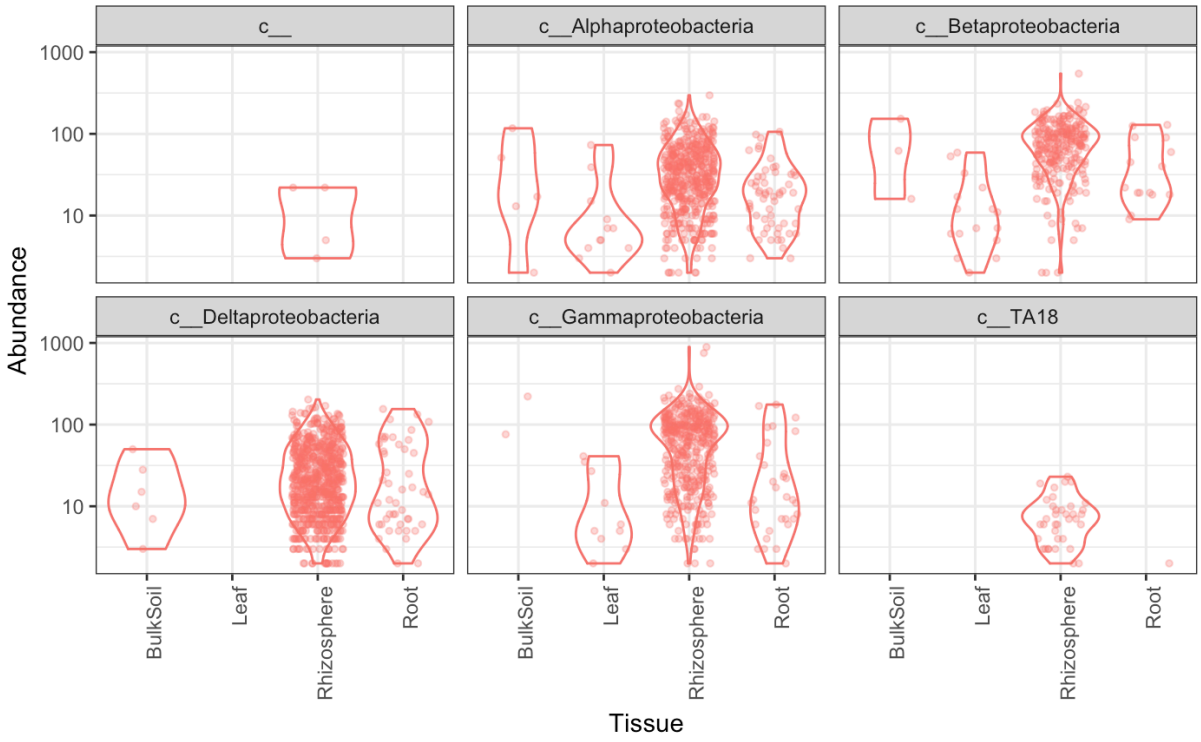
c)


c__
c__Alphaproteobacteria
c__Betaproteobacteria
c__Deltaproteobacteria
c__Gammaproteobacteria
c__TA18
Abundance
1000
100
10
Fall
Spring
Season

d)

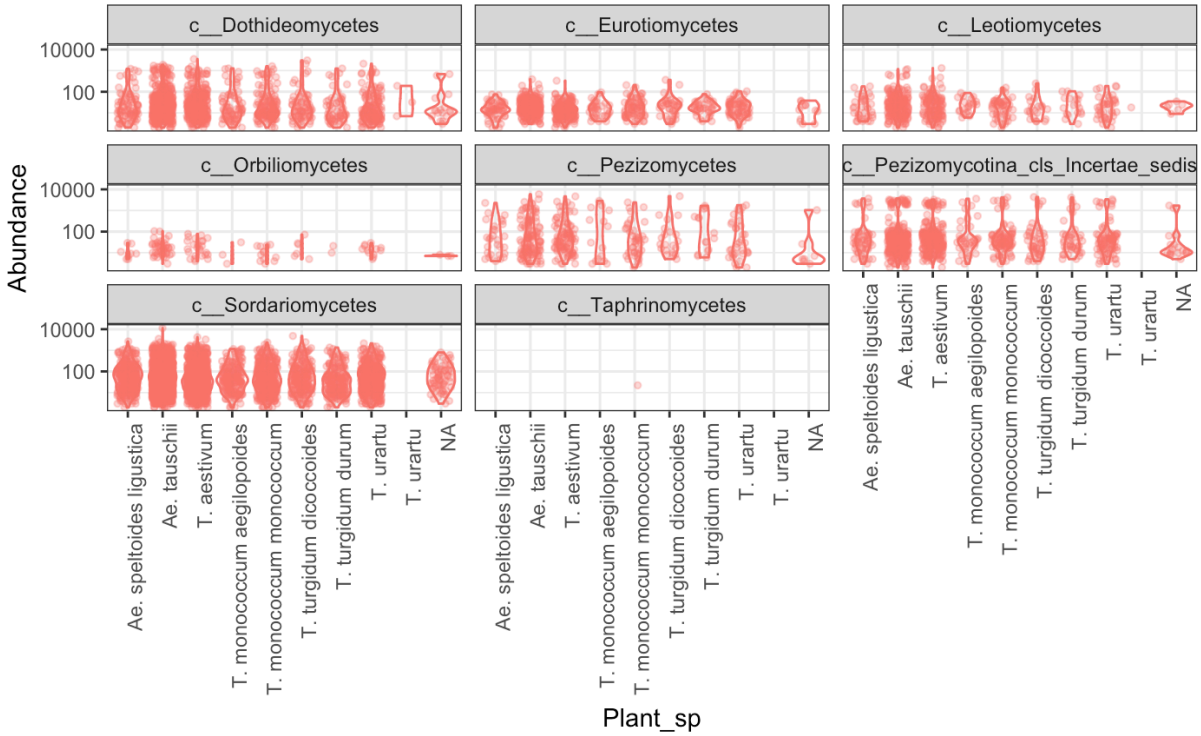

c__
c__Alphaproteobacteria
c__Betaproteobacteria
c__Deltaproteobacteria
c__Gammaproteobacteria
c__TA18
1000
100
10
Abundance
Dry
Wet
Treatment

e)

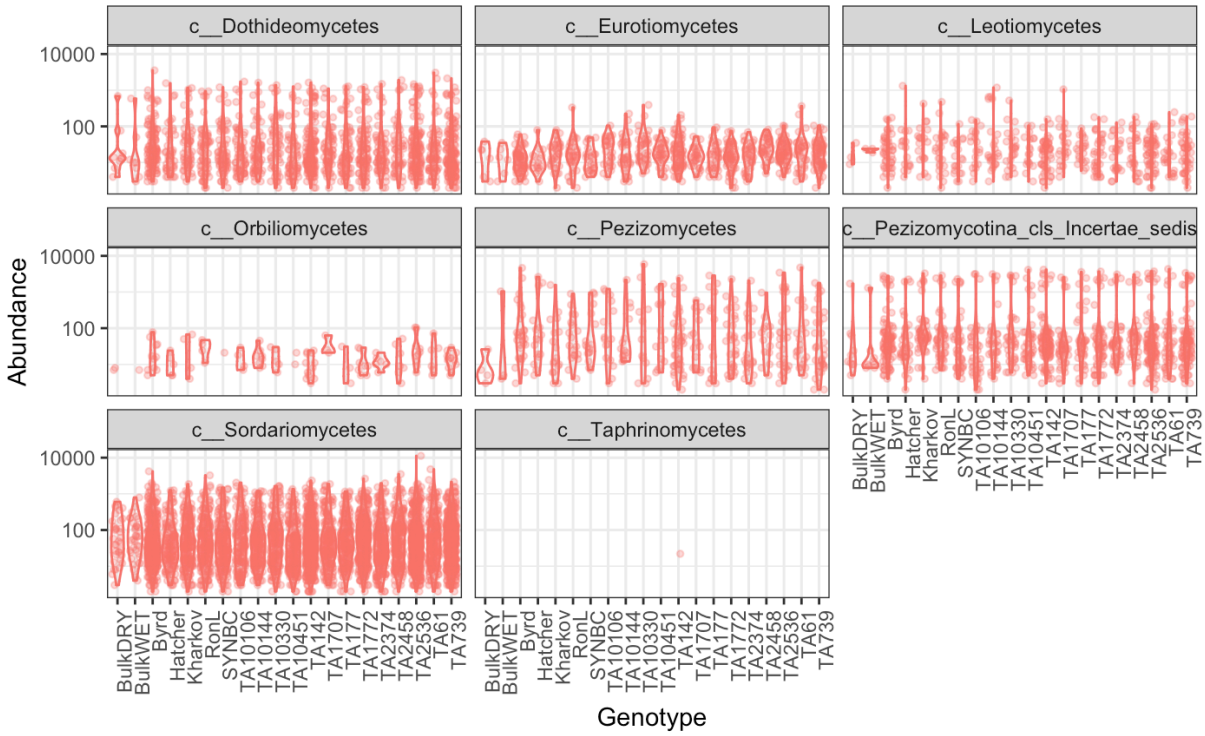


Appendix Figure 5 Violin plots of the most highly represented bacterial phylum, Proteobacteria, faceted by a) plant species, b) plant genotype, c) planting season, d) treatment, and e) tissue type (respectively). These visualizations of abundance data indicate that microbial taxa are similarly distributed among all independent variables except by tissue type.

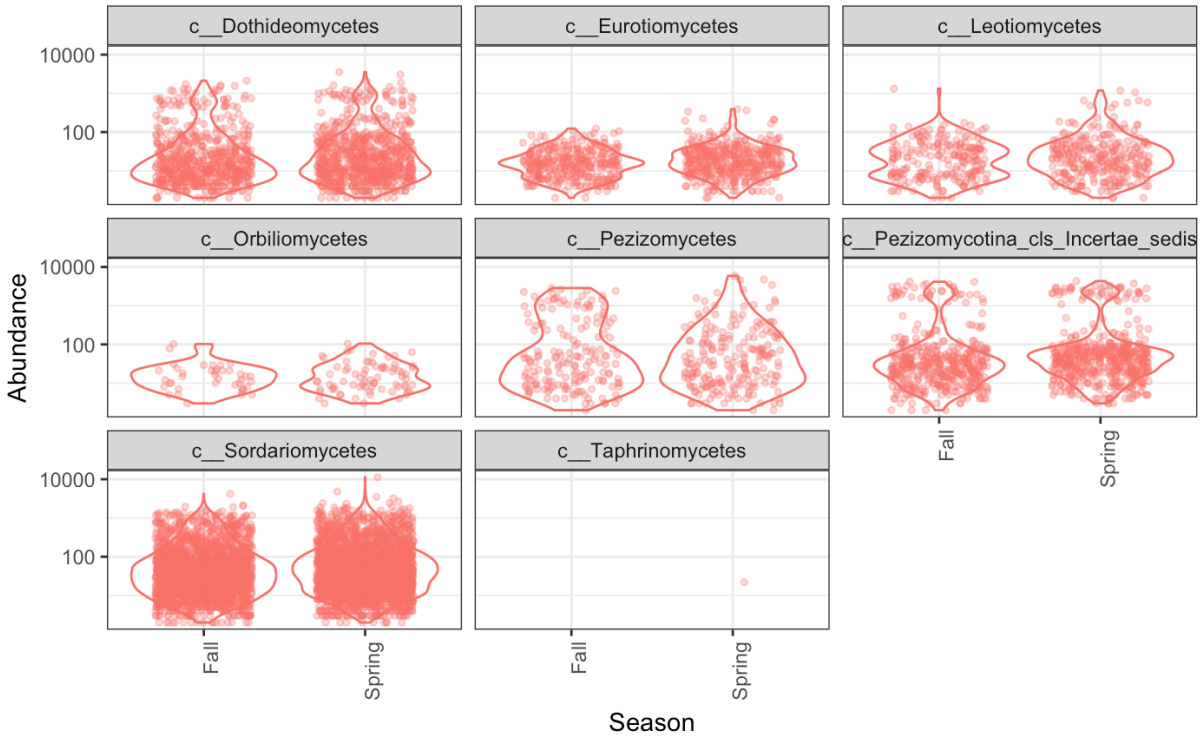
a)


c__Dothideomycetes
c__Eurotiomycetes
c__Leotiomycetes
c__Orbiliomycetes
c__Pezizomycetes
c__Pezizomycotina_cls_Incertae_sedis
c__Sordariomycetes
c__Taphrinomycetes
Abundance
10000
100
Ae. speltoides ligustica
Ae. tauschii
T. aestivum
T. monococcum aegilopoides
T. monococcum monococcum
T. turgidum dicoccoides
T. turgidum durum
T. urartu
T. urartu
NA
Plant_sp

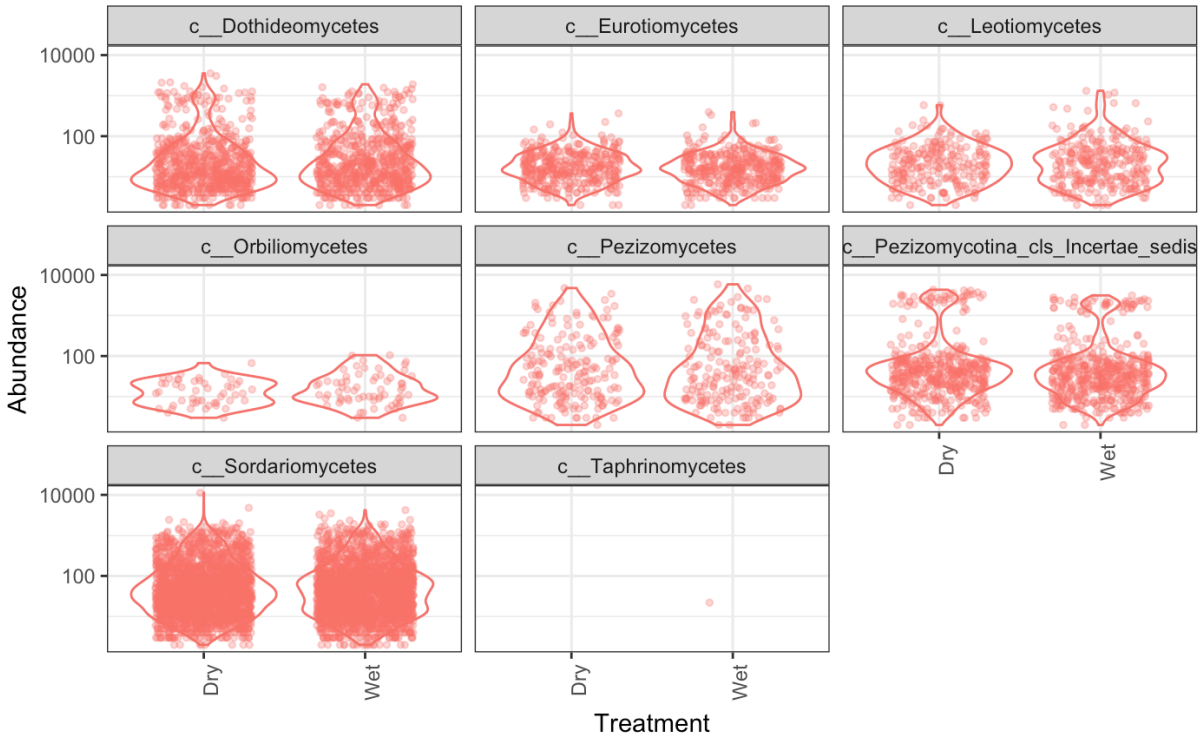
b)


c__Dothideomycetes
c__Eurotiomycetes
c__Leotiomycetes
c__Orbiliomycetes
c__Pezizomycetes
c__Pezizomycotina_cls_Incertae_sedis
c__Sordariomycetes
c__Taphrinomycetes
10000
100
Abundance
BulkDRY
BulkWET
Byrd
Hatcher
Kharkov
RonL
SYNBC
TA10106
TA10144
TA10330
TA10451
TA142
TA1707
TA177
TA1772
TA2374
TA2458
TA2536
TA61
TA739
Genotype

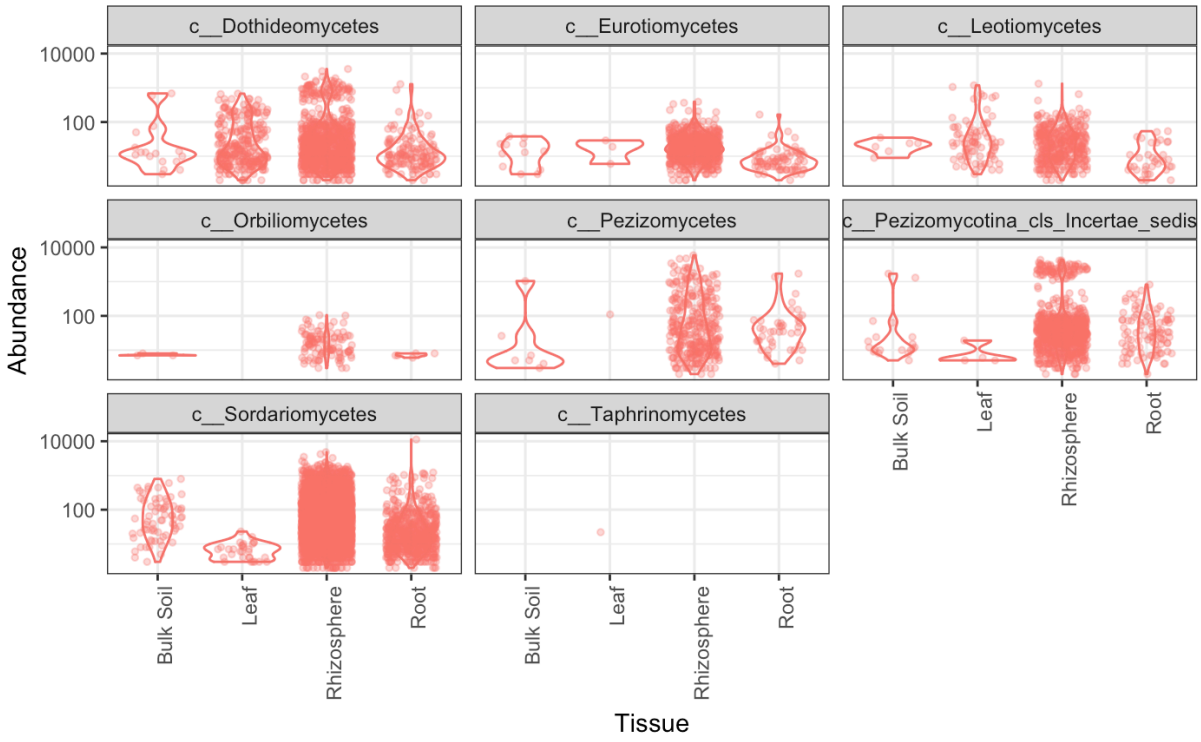
c)


c__Dothideomycetes
c__Eurotiomycetes
c__Leotiomycetes
c__Orbiliomycetes
c__Pezizomycetes
c__Pezizomycotina_cls_Incertae_sedis
c__Sordariomycetes
c__Taphrinomycetes
10000
100
Abundance
Fall
Spring
Season

d)


c__Dothideomycetes
c__Eurotiomycetes
c__Leotiomycetes
c__Orbiliomycetes
c__Pezizomycetes
c__Pezizomycotina_cls_Incertae_sedis
c__Sordariomycetes
c__Taphrinomycetes
10000
100
Abundance
Dry
Wet
Treatment

e)



Appendix Figure 6 Violin plots of the most highly represented fungal phylum, Ascomycota, faceted by a) plant species, b) plant genotype, c) planting season, d) treatment, and e) tissue type (respectively). These visualizations of abundance data indicate that microbial taxa are similarly distributed among all independent variables except by tissue type.