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Elucidating the impacts of environment and host on the assembly of plant-associated microbial communities

By

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Heidi M. L. Wipf

Abstract

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The microbial world is integrally involved with major ecological processes and affects all domains of life. With the numerous societal challenges we face today, efforts are being directed towards better utilizing microbial communities to support host health and resilience. In regards to agriculture, this includes the fortification of crop production through microbial amendments and microbiome manipulation. To better engineer microbiomes capable of promoting plant growth and ameliorating stress, additional research is needed to untangle the relative contributions of environmental and host factors in recruiting and maintaining beneficial microbes, while repelling potential pathogens. The work presented here seeks to identify and explore the importance of a set of these environmental and host forces in shaping the root and rhizosphere microbiomes of crop species. Specifically, the impacts of disturbance on plant-microbe interactions are characterized in relation to the farming practices of tillage and cover-cropping, heat and drought stress, and host evolution.

This research first explores how a set of widely employed agricultural soil management practices influence the belowground interactions between sorghum, bacteria, and fungi. Currently, it is not well characterized how cultivation systems influence microbiome assembly and activity. Utilizing next generation sequencing methods, we characterized a field system managed for close to two decades with standard tillage or no till practices in combination with either cover-cropping or letting the field lay fallow. We observed a promotion of microbial diversity by standard till and determined that fungal communities responded to a greater degree - in both composition and activity - to management practice than bacteria. Interestingly, despite distinct communities under each regime, similar plant growth outcomes were observed. This work informs understandings of how intermittent soil disturbance impacts agroecosystems and highlights the importance of cross-kingdom analyses.

This work then investigates the combined and isolated impacts of heat and drought stress on sorghum microbiome assembly. Recent studies of drought and the plant microbiome have shown a high degree of variability in bacterial enrichment under drought, particularly for the phylum

Actinobacteria. As heat often co-occurs with drought in the field, we sought to determine the relative contributions of temperature to this enrichment. Using a set of controlled growth chamber experiments, we observed that high temperatures do indeed correlate to a restructuring of sorghum-associated bacterial communities. This community differed from what was observed under drought alone, and the majority of indicator taxa within Actinobacteria were not shared between stresses. These results further our knowledge on how different abiotic stresses help modulate community interactions and lay the foundation for additional work characterizing the mechanisms involved in differential microbial enrichment.

In the final chapter of this work, the influence of host evolution on the associations between plants and their belowground microbiome is explored. Past research has shown that plant domestication and polyploidy can broadly influence plant biotic and abiotic interactions. We utilized three approaches - two field studies and one greenhouse-based experiment - to determine whether patterns in bacterial community assembly in wheat roots and rhizospheres could be partially attributable to these host factors. Collectively, we found little evidence of ploidy level and domestication status correlating with shifts in wheat bacterial communities. However, the greatest influence of the host on the microbiome appeared to occur in the rhizosphere compartment, and we suggest future work focuses on this environment to further characterize how host genomic and phenotypic changes influence plant-microbe communications. This research informs perspectives on what key driving forces may underlie microbiome structuring, as well as where future efforts may be best directed towards fortifying plant growth by microbial means.

Taken together, this work addresses fundamental gaps in our knowledge of the plant microbiome and the factors that help govern its structure and function. It demonstrates the ecological importance of agricultural soil management practices on plant-microbial interactions, uncovers the distinct roles of heat and drought stress in plant microbiome assembly, and indicates that domestication and polyploidy are minor contributors in shaping the wheat bacterial microbiome.

Dedication

Semper ad meliora

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Chapter 1

Introduction

1.1 Motivation

In the current state of our world, one of our major calls to action is improving global food security. Over a decade ago, the Food and Agriculture Organization of the United Nations recommended that in order to feed a predicted 2.3 billion more people by 2050, food production must grow by 70% (Alexandratos & Others, 2009). More recently, it has been estimated that 821 million people, or approximately 1 in 9 of the world's population, do not have enough to eat (FAO, 2019). Diets around the world rely heavily on plants, particularly grains (FAOSTAT, 2020), and efforts to boost production should consider numerous avenues for fortifying crop growth and yield.

Amidst growing demand and the need to alleviate food insecurity, climate change is a growing threat to current eco- and agrosystem functioning across the globe. The anticipated increases in extreme weather events and temperatures, paired with shifts in pest distributions, will make it increasingly difficult to grow crops in certain regions (Pachauri *et al.*, 2014). Past work has indicated that greater overall rainfall, frequencies of high intensity storms, and exposure to high temperatures and solar radiation exacerbates soil erosion rates (Nearing *et al.*, 2004). Soil salinity is also at risk of increasing and can severely impact plant growth, metabolism, and survival (Gregory, 2011). Greater water availability may allow crops in some areas to better flourish and produce yield, but other areas will find plants stricken with drought (Kang *et al.*, 2009). Environmental changes, especially increases in temperature or rainfall, can also heighten a plant's susceptibility to disease (Elad & Pertot, 2014).

From driving biogeochemical processes (Banfield & Nealson, 2018) to improving host fitness (Trivedi *et al.*, 2017), microbes are an ubiquitous and significant part of our world. Perhaps now more than ever before, it is imperative to better understand how communities, including those on the microscopic level, can come together and function for mutual aid and benefit.

1.2 Utility of microbiome-based approaches to plant fortification

To improve crop resiliency in the midst of suboptimal growing conditions, multiple approaches geared towards crop fortification should be studied and implemented. While crop varieties have been traditionally bred for improved stress tolerance and increased yields (Atkinson & Urwin, 2012), these targeted efforts to improve one plant trait can also result in the unintended weakening of other desirable ones (Coleman-Derr & Tringe, 2014). Furthermore, the genetic basis for these trait enhancements often go uncharacterized, making any realized benefit difficult to transfer to other crops (Coleman-Derr & Tringe, 2014). More recently, transgenic approaches have been employed to more rapidly improve productivity and reduce input and production expenses (James, 2003). However, there are several limitations and challenges with this process, including reductions to overall crop genetic diversity and diversification, differences in genetic tractability between plant species and varieties, disparate performances in greenhouse experiments versus later field trials, and rigorous, lengthy food and environmental safety regulations (Altieri & Others, 2000; Coleman-Derr & Tringe, 2014).

One rising area of research that offers several advantages over the aforementioned methods relates to the plant microbiome. Of the countless, diverse forms of life that are hosted in soils (Kennedy & Smith, 1995), numerous resident microbes have been shown to confer adaptive abilities to plants (Coleman-Derr & Tringe, 2014). This includes a range of plant hosts benefiting from a single isolate, as well as a shared microbial community. For instance, inoculations with *Achromobacter piechaudii* improved the salt and drought tolerance of both tomato and pepper (Mayak *et al.*, 2004). Salt-stressed maize seedlings also experienced improved root growth when inoculated with a strain of *Pseudomonas fluorescens* that was isolated from the rhizosphere of a Saharan date-palm (Zerrouk *et al.*, 2016). In addition, the presence of one microbial strain or community has been shown to increase plant tolerance to multiple stressors (Rodriguez *et al.*, 2008). For example, the microbiome of glasswort (*Salicornia* sp.) under hypersaline conditions has been correlated with improved plant growth under both salinity and drought stress (Mapelli *et al.*, 2013). This ability of microbes to stabilize plant growth across a range of stresses is likely linked, in part, to hormone-based mechanisms of influence (Coleman-Derr & Tringe, 2014). Indeed, there is considerable cross-talk that occurs in plant stress response pathways, and this could make them an attractive target in modulating responses (Atkinson & Urwin, 2012). Using a microbiome-based approach to promoting plant growth provides numerous potential benefits, including being rapidly deployed and transferable across a wide-range of crops.

1.3 Mechanisms of plant-growth promotion

There are multiple mechanisms by which microbes benefit plants. Past work has shown that plant-recruited microbes can enhance host defense, nutrient uptake, and stress tolerance in exchange for plant photosynthates (Welbaum *et al.*, 2004). Certain microbes suppress plant disease via direct antagonism against pathogens (i.e., production of antimicrobial compounds) (Mendes *et al.*, 2011), while others indirectly act to prime the plant's innate immunity for enhanced defense (Kohler *et al.*, 2008; Zamioudis & Pieterse, 2012). Furthermore, plant photosynthetic capacity has been augmented with certain microbial partners. For example, modulation of abscisic acid and sugar signaling by the soil bacterium *Bacillus subtilis* increased photosynthetic efficiency and chlorophyll content in Arabidopsis (Zhang *et al.*, 2008). In addition, the ability of a plant to acquire nutrients, including nitrogen, phosphorus, and iron, can be positively impacted by microbial associations. For example, *Azospirillum, Rhizobium,* and *Bradyrhizobium* species fix atmospheric nitrogen for plants (Lugtenberg & Kamilova, 2009), and *Bacillus megaterium* and *Penicillium bilaii* are known to solubilize phosphate (Leggett *et al.*, 2001). Microbial siderophores, such as those produced by *Bacillus* and *Pseudomonas* sp., allow for greater uptake of iron by plants (Kour *et al.*, 2019). Both nutrient uptake and stress tolerance can also be promoted with microbially mediated changes to root architecture, which can involve increased production of indole acetic acid (Kour *et al.*, 2019). Some microbes also reduce plant growth inhibition by impeding production of stress-induced ethylene (Glick *et al.*, 1998). Lastly, microbes can induce plant production of antioxidants, which may help protect against oxidative damage from abiotic stress (Sandhya *et al.*, 2010). While many microbial-derived mechanisms for promoting plant growth have been identified, challenges remain on how to translate these findings into the successful integration of desirable microbes into existing soil communities - or engineering complete, stable natural communities - so that they persist and provide a long-term benefit to the plant host.

1.4 Environmental impacts on microbiome assembly

Plant-microbe associations are highly dynamic and complex, and evidence so far suggests that the surrounding environment is one of the strongest modulators of these interactions (Siciliano *et al.*, 2001; Peiffer *et al.*, 2013; Coleman-Derr *et al.*, 2016a; Naylor *et al.*, 2017). Soils, in particular, are a major source of microbes that plants recruit from (Bulgarelli *et al.*, 2013). Soil is composed of many distinct environments, where abiotic characteristics, microbial compositions and activity of soil aggregates can greatly vary within a few micrometers (Fierer, 2017). In particular, differences in soil pH, type, structure, moisture, salinity, and organic matter significantly impact resident microbial communities (Lauber *et al.*, 2009; Griffiths *et al.*, 2011; Kuramae *et al.*, 2011, 2012; Prober *et al.*, 2015; Docherty *et al.*, 2015; Maestre *et al.*, 2015).

Some initial efforts have been directed towards characterizing how environmental factors affect agroecosystem-associated microbial communities. For example, agronomic practices play a critical role in determining short- and long-term soil quality (Turmel *et al.*, 2015), and soil management can influence fertilizer needs, carbon sequestration, soil temperature and moisture loss (Mann *et al.*, 2002; Wilhelm *et al.*, 2004; Moebius-Clune *et al.*, 2008; Busari *et al.*, 2015; Shah *et al.*, 2017). Furthermore, the widely used method of tilling soil is thought to directly impact soil microbial communities by disrupting pore space, physical community and soil structure, which can lead to increased runoff and surface erosion (Wood & Edwards, 1992; Dorr de Quadros *et al.*, 2012; Navarro-Noya *et al.*, 2013; Souza *et al.*, 2013; Carbonetto *et al.*, 2014; Rincon-Florez *et al.*, 2016). Studies on other farming practices, including cover-cropping, have shown positive effects on soil microbial diversity and abundance, including reductions in fungal pathogens (Dumontet *et al.*, 2001; Peters *et al.*, 2003; Helgason *et al.*, 2009; Dorr de Quadros *et al.*, 2012; Souza *et al.*, 2013; Murugan *et al.*, 2014). However, it is less clear what impact practices such as tillage and cover-cropping may have on plant microbiome diversity, composition, and function at large.

In addition to agronomic practices, water deficit and extreme temperatures are two other critical environmental factors that regularly and considerably restructure soil microbial communities (Bérard *et al.*, 2011) and reduce crop productivity (Lamaoui *et al.*, 2018). Hundreds of plant transcripts are known to uniquely shift with either heat or drought stress (Rizhsky *et al.*, 2002, 2004), and both stresses reduce photosynthetic rates, root nutrient uptake, and yield (Zandalinas *et al.*, 2018; Hussain *et al.*, 2019). Various plant-growth promoting microbes are capable of increasing plant biomass and growth for a variety of species experiencing these stresses (Rolli *et al.*, 2015; Kour *et al.*, 2019). For instance, seed germination improved under heat and drought stress when in the presence of root endophytes (Hubbard *et al.*, 2012). In addition, seedling heat stress tolerance can be positively impacted by bacterial seed treatment (El-Daim *et al.*, 2014). More remains to be discovered about the ways in which plant roots coordinate responses to these abiotic stresses with their associated microbiome.

1.5 Host impacts on microbiome assembly

In addition to the environmental factors that impact the microbiome, plants can directly influence the recruitment and establishment of their microbiome. Several plant characteristics are known to significantly influence microbial community assembly and succession, including host age and genotype (Micallef *et al.*, 2009a; Agler *et al.*, 2016; Sasse *et al.*, 2018). Root exudates are thought to heavily mediate these interactions (Chaparro *et al.*, 2013; Carvalhais *et al.*, 2015a) and are broadly composed of sugars, organic acids, and secondary metabolites (Sasse *et al.*, 2018). The quantity and composition of exudates varies across plant development and is impacted by the presence of pathogens or beneficial bacteria (Tang *et al.*, 1994; Mendes *et al.*, 2013; Haichar *et al.*, 2014). Additionally, of total carbon fixed by plants, it is estimated that 5-20% is solely invested in photosynthate rhizodeposits and root exudates (Marschner & Marschner, 1995). In turn, exudation can serve as a food source for soil microbial communities, attract plant-growth promoting microbes, mediate antimicrobial activity, and allow for osmoprotection (Alavi *et al.*, 2013; Sasse *et al.*, 2018). Changes in plant hormone biosynthesis, including auxin and jasmonic acid, and morphology have also been implicated in influencing the prevalence of beneficial microbes and improving plant defense (Sukumar *et al.*, 2013; Carvalhais *et al.*, 2017). However, it is unclear how broader patterns in host trait development relate to microbiome assembly.

Microbial community composition has been shown to also vary between plants, including closely related species and cultivars (Aira *et al.*, 2010; Schlemper *et al.*, 2017). These shifts are driven in part by larger evolutionary processes in the host. For instance, whole genome duplication (WGD) is thought to have extensively shaped plant evolution and diversification. In generating additional copies of the genome, a greater repertoire of genetic elements upon which natural selection can act is provided (Soltis *et al.*, 2009). This can result in novel functions and structures (Magadum *et al.*, 2013) or the production of new metabolic compounds (Griesbach & Kamo, 1996a; Levin, 2002b; Lavania *et al.*, 2012b). In addition, WGD can facilitate colonization of a wider range of habitats, including those characterized by high UV irradiation, low temperatures, nutrient-poor soils, and drought (Levin, 1983; Sugiyama, 1998; Niwa & Sasaki, 2003; Saleh *et al.*, 2008; Chandra & Dubey, 2010). While plant polyploidization has been shown to positively affect symbiotic interactions with specific fungi and rhizobia (Těšitelová *et al.*, 2013; Sudová *et al.*, 2014; Powell & Doyle, 2016; Forrester & Ashman, 2018, 2020), impacts on broader microbiome assembly are largely unknown.

Domestication is another significant feature of host evolution that may correspond to significant shifts in plant-microbial interactions. Past studies have found evidence that the process of domestication has brought about more shallow root systems in some species (Roucou *et al.*, 2018), as well as differences in root exudate profiles (Iannucci *et al.*, 2017). Furthermore, recent work has shown that growth of wild landraces and ancestors was linked to an increase in mycorrhizal dependence, relative to modern genotypes (Pérez-Jaramillo *et al.*, 2016a). Other studies indicate that domestication also impacted the microbial compositional profiles of a suite of crops, including sugar beet (*Beta vulgaris*) (Zachow *et al.*, 2014), barley (*Hordeum vulgare*) (Bulgarelli *et al.*, 2015), lettuce (*Lactuca sativa*) (Cardinale *et al.*, 2015), and common bean (*Phaseolus vulgaris*) (Pérez-Jaramillo *et al.*, 2018). However, gaps in our knowledge remain regarding how these findings relate to different species and environmental conditions, as well as in conjunction with related genomic changes.

1.6 Concluding introductory remarks

The microbial world and plants have evolved powerful, dynamic partnerships that significantly impact growth, survival, and reproduction across all kingdoms of life. My dissertation research seeks to address fundamental questions regarding microbial community ecology and host-microbiome interactions. Specifically, this work explores how agricultural soil management practices of standard and no tillage, in combination with cover-cropping, influence the composition and activity of the sorghum microbiome (Chapter 2). The identification and comparison of crop-associated bacterial and fungal community responses to soil management expands our understandings of how disturbance shapes agroecosystems. Next, responses of the sorghum microbiome to heat stress alone, and in combination with, drought stress are characterized (Chapter 3). The findings presented here further our knowledge on how abiotic stresses differentially modulate plant microbial interactions. Lastly, impacts of host evolutionary processes of polyploidization and domestication on wheat microbiome assembly are explored (Chapter 4). This research informs perspectives on what key driving forces underlie microbiome assembly in wheat. Taken together, this work helps lay the foundation for future investigations into forces structuring plant microbial recruitment and how the microbiome can be utilized for improved crop growth and food production.

Chapter 2

The Role of Agricultural Soil Management Practices in Shaping the Microbiome of *Sorghum bicolor*

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List of abbreviations

AMF	arbuscular mycorrhizal fungi
CAZy	carbohydrate active enzymes
CC	cover-cropping
COG	clusters of orthologous groups of proteins
NO	leaving fields fallow
NT	no tillage
ST	standard tillage

2.1 Abstract

Soils play important roles in biological productivity. While past work suggests that microbes affect soil health and respond to agricultural practices, it is not well known how soil management shapes crop host microbiomes. To elucidate the impact of management on microbial composition and function in the sorghum microbiome, we performed 16S rDNA and ITS2 amplicon sequencing and metatranscriptomics on soil and root samples collected from a site in California's San Joaquin Valley that is under long-term cultivation with 1) standard (ST) or no tilling (NT) and 2) cover-cropping (CC) or leaving the field fallow (NO). Our results revealed that microbial diversity, composition, and function change across till and cover type, with a heightened response in fungal communities, versus bacterial. Surprisingly, ST harbored greater microbial alpha diversity than NT, indicating that tillage may open niche spaces for broad colonization. Across management regimes, we observed class-level taxonomic level shifts. Additionally, we found significant functional restructuring across treatments, including enrichment for microbial lipid and carbohydrate transport and metabolism and cell motility with NT. Differences in carbon cycling were also observed, with increased prevalence of glycosyltransferase and glycoside hydrolase carbohydrate active enzyme families with CC. Lastly, treatment significantly influenced arbuscular mycorrhizal fungi, who had the greatest prevalence and activity under ST, suggesting that soil practices mediate known beneficial plant-microbe relationships. Collectively, our results demonstrate how agronomic practices impact critical interactions within the plant microbiome and inform future efforts to configure trait-associated microbiomes in crops.

2.2. Introduction

The soil underfoot is intimately tied to the wealth and wellbeing of our nations, where numerous, vital ecosystem services are provided by the approximate one fourth of the world's biodiversity that is hosted by soil (Schloss & Handelsman, 2006). Soil health is defined as "the capacity of soil to function as a living system" (FAO, 2011) and is characterized by the ability to sustain biological productivity, promote plant and animal health, and preserve air and water quality (Koch *et al.*, 2013). In particular, healthy soils can improve crop yields by promoting nutrient

cycling, water retention, pest and disease control, and the storing, filtering, and transformation of a wide array of compounds (Stavi *et al.*, 2016). Despite this key involvement in the degree and maintenance of agricultural productivity, soil health is on the decline and about one third of land globally is impacted by soil degradation (Panel, 2014).

Agronomic soil management practices are a critical factor in determining short- and long-term soil health (Turmel *et al.*, 2015). Tillage is one long-standing method that has been conventionally used to control weeds and loosen soil in preparation for planting. With intensive, mechanical agitation of soil to depths up to 45 centimeters, tillage typically leaves less than 15% of the previous year's crop residue on the soil surface (Derpsch, 2003). While standard tillage (ST) can more evenly distribute organic matter and nitrogen, remove unwanted plants and potential pathogens, and aerate the soil, it can also lead to soil compaction below the depth of tillage, erosion, an increased need for fertilizer application, decreased carbon sequestration, and increased rates of soil moisture loss (Mann *et al.*, 2002; Wilhelm *et al.*, 2004; Moebius-Clune *et al.*, 2008; Shah *et al.*, 2017). One alternative is reduced or no tillage (NT), which leaves 15-30% (reduced tillage) or more of the previous crop residue on the soil surface. This method can abate runoff and erosion by facilitating water and fertilizer infiltration, improve carbon sequestration, decrease soil temperature fluctuations, and requires fewer fuel and labor inputs than ST (Derpsch, 2003; Hobbs *et al.*, 2008; Busari *et al.*, 2015). Potential disadvantages of NT, however, can include increased herbicide dependence for weed control and soil health benefits that manifest over multiple growing seasons (Derpsch, 2003). An additional practice that can benefit soil health, and is often used in conjunction with both ST and NT, is cover-cropping (CC), where one or more crops are grown in off-season months as an alternative to letting fields lie fallow (NO). CC can reduce soil erosion, hinder weeds, and enrich soils with nitrates and organic material, as well as augment biological diversity, increase crop yields, improve water availability, and propagate arbuscular mycorrhizal fungi (AMF) (Dodd & Jeffries, 1986; Lal *et al.*, 1991; Campbell *et al.*, 1996; Shirley *et al.*, 1998; Kabir & Koide, 2002; Strock *et al.*, 2004; Wortman *et al.*, 2012). Moreover, management practices employed in combination can have synergistic impacts on increasing crop yield over time (Miguez & Bollero, 2005; Calegari *et al.*, 2008; Chávez-Romero *et al.*, 2016).

One research direction that may yield important insights into the mechanisms that support soil functioning and improve crop performance is investigating the impact of agricultural practices on the microbiomes of plant hosts and the surrounding soil. It is well-known that plant root-associated microbiomes can alter plant fitness and that plant root microbiomes are largely derived from the surrounding soil microbiome (Chaparro *et al.*, 2012; Lareen *et al.*, 2016; Stringlis *et al.*, 2018; Bergelson *et al.*, 2019). What the combined effects of these soil practices are on agroecosystem-associated microbial communities has been less studied. Past work has shown that NT and CC can broadly increase soil microbial diversity and abundance, as well as reduce amounts of fungal pathogens, as compared to conventional practices of ST and NO (Dumontet *et al.*, 2001; Peters *et al.*, 2003; Helgason *et al.*, 2009; Dorr de Quadros *et al.*, 2012; Souza *et al.*, 2013; Murugan *et al.*, 2014). In comparison to soils, it is even less clear what impact tillage and CC have on plant microbiome diversity, composition, and function. In the last few years, it has been demonstrated that wheat rhizosphere bacterial communities are indeed influenced by tillage (Yin *et al.*, 2017). There is also some evidence suggesting that AMF are

thought to be negatively impacted by intense tillage and monocultures, with higher spore density and active hyphal length reported in reduced tillage systems (Kabir *et al.*, 1997a; Gosling *et al.*, 2006; Borie *et al.*, 2006), and Rosner *et al.* observed that reduced tillage with CC may increase AMF root colonization in some plant hosts (*Helianthus annuus* L.), but not others (*Triticum aestivum* L.) (Rosner *et al.*, 2018).

Furthermore, relatively few studies have compared the responses between bacterial and fungal communities across agricultural soil management regimes, despite evidence of important functional complementation and antagonistic interactions existing between kingdoms (Getzke *et al.*, 2019). Co-inoculations of multiple bacteria and fungi have been shown to improve plant growth, survival, and productivity over single inoculations (van der Heijden *et al.*, 2016; Durán *et al.*, 2018), and changes in community balances can be linked to disease in associated hosts (Kim *et al.*, 2014; Durán *et al.*, 2018). In addition, tillage has been shown to decrease the soil biomass of fungi while that of bacteria increased (Cookson *et al.*, 2008), suggesting that bacteria and fungi may respond differently to soil management. Indeed, when comparing varying tillage intensities in combination with conventional and organic management systems, soil bacterial communities were largely structured by tillage, while fungi were primarily impacted by system type (Hartman *et al.*, 2018). While Anderson *et al.* also found that soil bacterial community richness and composition were more impacted by tillage than fungi (Anderson *et al.*, 2017a), Verónica Acosta-Martínez *et al.* suggests that bacteria may be more impacted by practices other than tillage, such as crop rotation (Acosta-Martínez *et al.*, 2010b). Lastly, within wheat roots, fungi may be more impacted by tillage than bacterial communities (Hartman *et al.*, 2018). Collectively, this work demonstrates that agricultural practices can significantly impact agricultural-associated microbiomes and elicit different responses in the bacterial and fungal fractions of these communities. However, more research is needed to understand how combinatorial agricultural soil management practices impact the assembly and activity of crop microbiomes, and their relation to plant fitness.

To address these knowledge gaps, we utilized a field site in California's San Joaquin Valley. It has been managed for close to two decades with a combination of 1) NT or ST practices and 2) CC or NO to test how these management practices impact the composition and function of sorghum root microbial communities. Using 16S rRNA and ITS2 amplicon sequencing, in addition to metatranscriptomics, we test four specific hypotheses regarding the impact of these practices on the crop microbiome. **1)** fungal communities are influenced by management regime to a greater extent than bacteria, due to the vulnerability of hyphal networks to damage from tillage. **2)** NT differentially impacts composition and function in microbial communities, as compared to ST, and will reflect greater niche differentiation due to reduced disturbance. **3)** more carbon cycling activity occurs with ST than NT, as past crop residue is shredded and buried for degradation. **4)** CC promotes increased microbial diversity and enriches for AMF, over NO, due to increased resources provided by plants grown in the off season.

2.3 Results

2.3.1 Minimal variation in measured plant and soil chemical characteristics across soil management types

To address our hypotheses on how soil management practices impact crop plant-microbe interactions, we surveyed the bacterial and fungal communities of soil and the rhizospheres and roots of *Sorghum bicolor* grown at the West Side Agricultural Research Station in Five Points, California, where plots are under long-term management with NT or ST and CC or NO. To allow for comparisons between the effects of agronomic practice on soil and plant-associated microbiomes, as well as investigate developmental and temporal variability, samples were collected before and after flowering in the summer of 2016, as well as before flowering in 2017, from three replicate plots (Figure 2-1). At the time of each sampling, plants from which roots and rhizospheres were harvested were phenotyped. Interestingly, we observed that management practices impacted aboveground phenotypes for time point 3 only (Figure 2-2); plant height was significantly explained by till type (p-value=0.008) and cover type (p-value<0.001), while both treatment types were non-significant for fresh shoot biomass and yield variation (Figure 2-3).

To characterize a range of soil environmental factors that could contribute to explaining our results, soils sampled in 2016 were chemically profiled. Soils were found to vary minimally across treatment (Figure 2-4, 2-5), where statistically significant differences were found across treatments for only two measured parameters: aluminum concentration (till type: F=10.67, p-value=.031; cover type: F=10.67, p-value=.031) and calcium base saturation (till type: F=22.857, p-value=0.009). However, this range in calcium base saturation (77.67-82.00%) is not expected to differentially impact plant growth (Singh *et al.*, 2013). Additionally, these levels of aluminum (8.33-10.00 ppm, respectively) are not expected to impact plant performance in pH levels above 5 (Delhaize & Ryan, 1995).

2.3.2 Microbial community diversity and composition are significantly altered by management practice, with greater impacts for fungi

To survey the impacts of till and cover type on plant-associated bacterial and fungal microbiomes, community composition was investigated for each sample type (soil, rhizosphere, and root) using Illumina MiSeq sequencing of the V3-V4 region of the 16S rRNA gene and 5.85Fun-ITS4Fun region of the internal transcribed spacer 2 (ITS2) gene. We predicted that less mechanical soil disturbance would promote niche differentiation over time, and this would be reflected with higher diversity in samples collected from NT managed fields. Surprisingly, our results indicated that Shannon's diversity is greater in fields managed by ST, versus NT (p-value<0.001; means, bacteria: 5.61 vs. 5.48 and fungi: 2.61 vs. 2.26; Figures 2-6a,d; 2-7a,d) for soil (both kingdoms) and rhizosphere (fungi only) samples . We also observed that till type structured the alpha diversity of fungal communities to a greater extent than bacteria (F=31, p-value<0.001; F=13, p-value<0.001, respectively), while the factor of cover type was significant for bacteria only (F=6, p-value=0.02) (Figures 2-8a;2-9a). As predicted, bacterial and fungal Shannon's diversity was higher on average with CC for rhizosphere and root-associated communities as well (Figure 2-6a,d). Interestingly, we also found that outside of time point, till type is highly statistically significant in structuring Shannon's diversity in soils (bacteria: F=13, p-value<0.001; fungi: F=54, p-value<0.001) and in rhizospheres to a lesser extent (bacteria: F=3, p-value=0.07, fungi: F=53, p-value<0.001) (Figure 2-9a). Within roots, till type is only statistically significant at the after flowering time point (TP2) for fungi (F=12, p=.003), while

bacterial root communities are significantly structured by cover type only (F=4, p-value=0.04). Taken together, these results demonstrate that till and cover type influence the alpha diversity of sorghum-associated microbiomes, with increased Shannon's diversity under ST and CC, and suggest that till type shapes fungal soil communities in particular to a much greater degree than bacteria.

Till and cover type are expected to influence a number of soil characteristics that can impact microbial fitness (Hacquard, 2016), including soil pore size, nutrient bioavailability, and moisture. We therefore hypothesized that treatment would significantly structure the beta diversity of hosted microbial communities. In addition, we predicted that till type would impact fungi to a greater extent than bacteria, due, in part, to their formation of extensive hyphae networks. To visualize and quantify the differences between microbial communities (beta diversity), we used unconstrained principal coordinate analysis (PCoA), canonical analysis of principal coordinates (CAPs), and permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis dissimilarity distances. Beta diversity is significantly explained by till (p-value<0.001) and cover type (p-value<0.001) and greater variation is attributed to till type for fungal communities, versus bacterial (Figures 2-6b,e; 2-7b,e; 2-8b; 2-9b). Furthermore, management treatment appears to have a greater impact during initial stages of rhizosphere colonization, as the variation in beta diversity attributed to till and cover type is generally higher for samples collected at the pre-flowering time point, as compared to the post-flowering samples; this pattern is reversed in the root, where microbial beta diversity variation for till and cover type is greater post flowering. These analyses further indicate that **1)** bacterial and fungal communities are distinctly shaped by soil management type, with a greater shift across till type occurring in fungi, and that **2)** plants likely buffer community shifts, with the strongest response to soil management type found in soils, then rhizosphere and roots, respectively.

We also analyzed community composition to determine whether certain taxonomic lineages would show preference for any of the four management practices. In addition to detectable shifts in class-level relative abundances for both bacteria and fungi between soil management types within each sample type, we found that fungal classes shifted in relative abundance to a greater extent than bacteria (Figures 2-6c,f; 2-7c,f). These observable shifts across treatment included a greater prevalence of Bacilli with ST and Gammaproteobacteria with NT, in addition to a greater relative abundance with ST of Pezizomycetes, as well as Agaricomycetes in pre-flowering root fungal communities. In addition, Eurotiomycetes and Dothideomycetes were generally present at higher relative abundances in samples from NT plots. To further explore taxonomic patterns, we performed an indicator species analysis ($p \leq .001$) across all sample types to determine whether certain OTUs correlated to a specific treatment (NTCC, NTNO, STCC, and STNO), till (NT and ST), and cover (CC and NO) type (Figure 2-9, from inner ring outwards, respectively). We observed that there is a greater number of indicators for: ST over NT (bacteria: 539 vs. 350; fungi: 50 vs. 36) and CC over NO (bacteria: 77 vs. 34; fungi: 21 vs. 7) for both kingdoms (Figure 2-10). Interestingly, several bacterial phyla were largely specific to treatment: Gemmatimonadetes, Firmicutes, Verrucomicrobia, and Chloroflexi mainly consisted of indicators for STCC and STNO (Figure 2-10a). For fungi, the majority of indicator OTUs were in the phylum Ascomycota and treatment indicators similarly appeared to phylogenetically cluster (Figure 2-10b). Taken together, these results indicate that: 1) management impacts

plant-associated bacterial and fungal communities - with greater community shifts attributed to changes in till versus cover type, 2) ST may support greater diversification than NT, and 3) fungal communities demonstrate a heightened sensitivity to soil management practice than bacteria.

2.3.3 Microbial activity varies across soil practices, with the greatest changes observed between till types and within fungal communities

Changes in community composition determined by amplicon sequencing may, or may not, reflect changes in the active fraction of the microbiome. In order to evaluate treatment effects on the portion of the microbial community that is active, we sequenced and annotated via JGI's IMG/M system the metatranscriptomes of soil and rhizosphere communities at time point 3, corresponding to a vegetative stage of rapid growth in sorghum development. As NT retains past crop residues and ST is a major soil disturbance, we hypothesized that management practice would correspond to distinct functional changes between samples. To first broadly survey impacts on the active microbial community, we investigated bacterial and fungal beta diversity, as determined by Bray-Curtis dissimilarity (Figure 2-11). We found that till type is a significant factor for both (p-value$\leq$0.012) and contributed to 14.3% (bacteria) and 17.6% (fungi) of beta diversity variation in soils and 30.3% (bacteria) and 36.2% (fungi) in rhizospheres (Figures 2-11; 2-12). Cover type is also a significant factor in soils (bacteria: p-value=0.041; fungi: p-value=0.068), but not rhizospheres, explaining 13.4% (bacteria) and 12.7% (fungi) of variation (Figures 2-11; 2-12). These analyses indicate that treatment corresponds to distinct soil and rhizosphere communities and till type influences diversity to a greater extent for fungi than for bacteria, as was observed in our amplicon sequencing results.

Within both soil and the rhizosphere, we observed treatment responses in the class-level relative abundances of active microbes, as well as in relative functional activity, as described using the Clusters of Orthologous Groups of proteins (COG) database (Tatusov *et al.*, 2000) (Figure 2-13). Bacterial community activity is largely attributed to the classes Alphaproteobacteria and Actinobacteria, and we detected several classes in the rhizosphere that vary across treatment, including STCC hosting the highest relative levels of Rubrobacterial and Clostridial activity (Figure 2-13a). We also found evidence of a greater response to treatment in fungal relative activity, over bacterial (Figure 2-14), with NT hosting greater relative activity by active Agaricomycetes, Pezizomycetes, and Basidiobolomycetes than ST (Figure 2-13c). While bacterial activity on the level of COG category did not generally vary across treatments (Figure 2-13b), we observed that ST fungal communities harbored relatively higher levels of energy production and conversion and coenzyme transport and metabolism, while NT activity profiles were characterized by relatively greater transcription, amino acid transport and metabolism, and post-translational modification, protein turnover, and chaperones (Figure 2-13d). CC also correlated with relatively higher levels of fungal inorganic ion transport and metabolism in the rhizosphere (Figure 2-13d). Unsurprisingly, the greatest shifts in activity were between sample types, with the following COG categories more represented in the rhizosphere: energy production and conversion (for bacteria), translation, ribosomal structure, and biogenesis (bacteria), inorganic ion transport and metabolism (fungi), and signal transduction mechanisms (both) (Figure 2-13b,d). These analyses reveal that soil management has distinct impacts on what taxonomic groups are active in the soil and rhizosphere, and treatment effects appear more

pronounced in what types of activity occurs in associated bacterial and fungal communities; additionally, metatranscriptome analysis further demonstrates heightened responses to treatment within the fungal microbiome, as compared to bacteria.

To further characterize treatment impacts on microbial communities and determine whether treatment significantly selects for specific functions, we analyzed the fold enrichment (Figure 2-15) and depletion (Figure 2-16) of genes from NT versus ST, as well as CC versus NO, soil and rhizosphere samples. As anticipated, till type impacted a broader range of functions than cover type; however, the functions that are impacted by cover type are generally enriched and depleted to a higher fold degree than those for till type (Figures 2-15; 2-16, respectively). Bacterial and fungal communities responded to treatment with significant fold changes in largely the same COG categories; for instance, both kingdoms are enriched under NT for energy production and conversion in soils and carbohydrate and lipid transport and metabolism in rhizospheres (Figure 2-15). Additionally, several COG categories had a set of genes that were significantly enriched, while different sets were significantly depleted (Figures 2-15; 2-16), including cell motility. These findings indicate that NT - and to a lesser degree, CC - significantly impact a range of functions within both bacterial and fungal communities, and, interestingly, gene activity may fluctuate more dramatically for bacteria than fungi, where a greater number of statistically significant fold changes occur under NT and CC management types.

Furthermore, we performed an indicator species analysis ($p \leq .001$) on metatranscriptomes to determine whether particular microbial functions uniquely characterize the soil and rhizosphere from plots managed by NT, ST, CC, and/or NO, (Figure 2-17). Similar to our amplicon data analyses, we found more indicators for ST (STCC and STNO), versus NT (NTCC and NTNO), as well as for NO over CC, treatments (Figure 2-17a,b). Interestingly, the majority of indicators did not have a COG annotation, and no clear patterns appeared in function categories (Figure 2-17a,b). When we explored which COG-annotated indicators arise in cover type, however, NO indicators were present to a greater degree than those for CC and comprise a range of activity, including genes in the COG categories: carbohydrate and amino acid transport and metabolism (Figure 2-17c,d). Together, these analyses further demonstrated that gene activity may fluctuate more distinctly with ST and NO practices, as well as for a broader range of activities in bacteria, versus fungi, where a larger number of indicator genes in a greater number of COG categories are present in ST, NO, and bacterial communities.

2.3.4 Patterns of microbially driven carbon cycling vary across soil management types

In order to test our hypothesis that ST practices of shredding crop residue to incorporate into soils would significantly alter microbial community dynamics in relation to carbon cycling, we performed an analysis of carbohydrate active enzyme (CAZy) gene activity (Figure 2-18). While it was unsurprising to find that rhizospheres promoted a relatively greater amount of detected CAZy gene transcripts than soils, we curiously discovered that NO systems corresponded to more CAZy transcriptional activity than CC in soils and less in rhizospheres (Figure 2-18). Interestingly, we also detected relatively similar amounts of CAZy transcriptional activity for bacteria and fungi within treatments and sample types (Figure 2-18), though more sample reads were of bacterial origin than fungal. The largest CAZy transcript-based responses to treatment

were found in two families, glycosyltransferases and glycoside hydrolases (Figure 2-18), which are involved in the biosynthesis of sugars (disaccharides, oligosaccharides and polysaccharides) and the hydrolysis of a glycosidic bond between between carbohydrates or between a carbohydrate and a different substrate, respectively(Cantarel *et al.*, 2009). In our analysis of the putative substrates for active CAZy genes - based off of (Nuccio *et al.*) - we saw similar patterns across treatments and sample types, as well as observed that NTNO soils appeared particularly active in degrading a wide variety of carbon sources, including xylan, oligosaccharides, glycogen, lignin, and cellulose (Figure 2-19). These analyses suggest that microbes in fields managed with CC are primed for plant-based carbon usage, while NO supports microbial communities that can utilize a greater diversity of carbon substrates in both rhizospheres and soils alone, which may have implications for soil carbon sequestration.

Collectively, these results demonstrate that agricultural practices of NT, ST, CC, and NO impact bacterial and fungal community activity, and these shifts are demonstrated not only in the soil communities, but in sorghum rhizospheres as well. Our data demonstrates shifts in taxonomic groups and a suite of COG categories and CAZy genes across soil management types. Furthermore, till type significantly impacts a greater number of functions than cover type, and bacterial and fungal communities have slightly different enriched and depleted COG categories with NT, versus ST, as well as with CC versus NO. Lastly, microbial CAZy activity varies across soil management treatment, with opposite patterns occurring in rhizospheres versus soils for overall activity amounts associated with till and cover type, which may be indicative of further differences in microbial community specialization to the varying soil environment.

2.3.5 Arbuscular mycorrhizal fungi differentially respond to soil management type

Some soil practices can prime soils for plant-microbe mutualisms (Hallama *et al.*, 2019). In order to determine how soil management regime may impact plant-microbe interactions known to promote plant health, we compared the amount and activity of AMF across management treatments from amplicon and metatranscriptomic data (Figure 2-20). We observed a greater abundance of AMF with CC, and we also surprisingly found that AMF were most prevalent in samples collected from plots managed with STCC (Figure 2-20a). Furthermore, while AMF were least prevalent in STNO samples, STNO corresponded with the most overall AMF activity (Figure 2-20a,b). Functional profiles also varied widely across treatments and sample types, where AMF activity under NT and CC was characterized by greater relative levels of lipid and nucleotide transport and metabolism, as well as signal transduction mechanisms (Figure 2-20c). Taken together, these results show evidence that soil management practices greatly influence abundance and activity of one of the most well-known fungal symbionts of agricultural importance (Basu *et al.*, 2018).

2.4 Discussion

Our study provides an initial look at how the agricultural soil management practices of standard and no tillage, in combination with cover-cropping and leaving fields fallow, shape the assembly and activity of crop-associated bacterial and fungal communities during sorghum development. Past work suggests that microbial communities are influenced by soil management, where microbial diversity can increase with reduced tillage (Lupwayi *et al.*, 1998; Nivelle *et al.*, 2016;

Schmidt *et al.*, 2018) and CC (Nivelle *et al.*, 2016; Schmidt *et al.*, 2019), and CC can promote microbial abundance (Chavarría *et al.*, 2016; Schmidt *et al.*, 2018), but combined practice impacts on the microbiome are not well characterized and understood. We hypothesized that we would find reduced fungal and bacteria diversity with ST, reasoning that NT could allow for the construction of distinct niches for colonization by distinct groups of microorganisms over time. Surprisingly, we found that ST was associated with greater diversity for the majority of sample types and time points, as well as larger numbers of indicator OTUs. To our knowledge, only one other study has reported that intensive tillage is associated with increased bacterial and fungal diversity, as compared to reduced and no till regimes (Hartman *et al.*, 2018). A possible explanation for this increased diversity with ST may involve the impacts of disturbance, where tillage may increase nutrient availability and open niches for colonization that may otherwise have been inaccessible due to competitive exclusion (Cadotte, 2007; Choi *et al.*, 2017). Furthermore, disturbance has been shown to be an important driver in endophyte community variation (Griffin *et al.*, 2019) and influence microbial diversity (Christian *et al.*, 2015). As such, ST here may present an intermediate level of disturbance that may support a greater variety of specialist and generalist microbes (Andrews & Harris, 1986; Cadotte, 2007). Past studies do suggest that disturbance can lead to fungal or bacterial community shifts in host niches - where antibiotic use has been associated with overgrowth of the fungus *Candida albicans* in animal guts and root-associated fungi were detrimental to plant hosts in the absence of commensal bacterial (reviewed in (Getzke *et al.*, 2019)). Similar results have been observed in other systems as well: (Galand *et al.*, 2016). found increased microbial diversity with disturbance in marine sediments (Galand *et al.*, 2016), and combinations of temperature and physical disturbance have been correlated with increased coral microbiome diversity (McDevitt-Irwin *et al.*, 2019). Others have observed that the adaptive diversification of microbial communities was significantly greater in the absence of an established community, which NT systems may engender (Gómez & Buckling, 2013). We recommend future studies sample a range of time points post-tillage to help further our understanding of how ST systems impact diversity and what factors microbial diversification is dependent on. Additionally, as increased diversity has been shown to promote community recovery post environmental disturbance (Feng *et al.*, 2017), we recommend more studies to test whether this increased diversity in managed soil is beneficial to plant yield and growth under varying climate and nutrient conditions, as well as explore what costs to host fitness may occur with increased diversity, in order to better understand how resident diversity may inhibit or promote resilience in the face of changing environmental conditions.

Several other factors may explain, in part, this response in microbial diversity. One factor is sampling depth. Recent work on the soil microbiome shows diversity increases with increasing soil depths with NT and different depths and management types favor distinct microbial life strategies (Schmidt *et al.*, 2018). Future efforts in this area should consider including depth as an additional experimental parameter. Another factor that may influence patterns drawn from community characterization is the taxonomic level at which analyses are performed. One study that explicitly explored this found increased microbial diversity in no-till systems only when looking beyond the phylum level (Dorr de Quadros *et al.*, 2012), and future studies may consider finer scale and a wider range of analyses (Wakita *et al.*, 2018), including indicator species and/or functional guild analyses. Lastly, one additional factor that may potentially contribute to reduced microbial diversity in NT systems may relate to how soils managed by NT are often wetter than

those managed by ST (Hill *et al.*, 1985), which can reduce the number of microaggregates, and therefore microhabitats, that are conducive to the establishment and maintenance of microbial diversity (Bach *et al.*, 2018).

There is evidence that reduced and no till can improve carbon sequestration and reduce atmospheric carbon release, as compared to ST (de M. Sá *et al.*, 2001; McConkey, 2003; Six *et al.*, 2006; Sundermeier *et al.*, 2011). We thus hypothesized that samples from plots under NT management would support a distinct functional profile that would include lower amounts of carbon cycling than with ST, due, in part, to past crop residue being more intact and therefore taking longer to break down. We further hypothesized that CC would also correspond to a functional profile that differs from NO, due to an enrichment of plant-associated microbes and evidence that CC favors moderately-fast growing microbes with a relatively high metabolic range (Schmidt *et al.*, 2018). Cover-crops have been found to increase plant and microbial biomass and alter soil microbiomes (De Graaff *et al.*, 2010; Navarro-Noya *et al.*, 2013; Ramirez-Villanueva *et al.*, 2015; Finney *et al.*, 2017; Drummelsmith, 2020), due, in part, to increased inputs of organic carbon from exudates and litter, as well as influences on soil aggregation and moisture (Bertin *et al.*, 2003; Vukicevich *et al.*, 2016). Indeed, our results demonstrate that each treatment type corresponds to a distinct functional profile, which corroborates prior work (Schmidt *et al.*, 2019), in which agricultural soil management practices shifted fungal functional composition, with no-till increasing the relative proportions of symbiotrophs while decreasing saprotrophs. However, another study found similar microbial activity in no-till and conventional till soils (Carpenter-Boggs *et al.*, 2003). We recommend that additional studies in different fields and across time points also consider collecting metatranscriptomic analysis to better understand how microbial activity is influenced by soil management. Furthermore, we found treatment correlates with much greater changes in function than composition, highlighting both the importance of metatranscriptomic analysis in microbiome responses to the environment and that amplicon data alone is not sufficient in determining whether, and to what degree, a microbiome is changing in response to a disturbance.

With NT often employed with the aims of improving soil health, we further investigated how soil management impacted carbon cycling dynamics using the CAZy database. Carbohydrate active enzyme classes glycosyltransferase and glycoside hydrolase - involved with the making and breaking of glycosidic bonds, respectively - were present at high abundances for all treatments, and are important for energy mobilization, defense, signaling, symbiosis, and secondary plant metabolism (Minic, 2008). While minimal activity was detected in soils with CC, we surprisingly found high CAZy gene levels characterize NO treatments, with the highest levels from NTNO samples. This may be due to selective pressures in NTNO soils that favor the prolonged breakdown of complex carbohydrates in soil residue as a primary energy source, and we suggest more studies to determine the implications on overall carbon sequestration ability. In rhizospheres, CC corresponded to higher levels of carbohydrate-related activity than NO, with the highest in STCC, suggesting that CC selects for microbes that readily utilize plant-based photosynthates and STCC systems may be sourced with more available carbon from both the living plant and the shredded past crop residue. Future work may include metabolomics and radiolabeled substrate to determine how carbon break down and usage may differ across management treatments.

Evidence from recent studies suggests that bacterial and fungal communities differ in sensitivity to environmental parameters (Peay *et al.*, 2016; Likar *et al.*, 2017; Hartman *et al.*, 2018). Given that 1) tillage is a major physical disturbance, 2) many soil fungi grow in extended hyphal networks across considerable distances (Ritz & Young, 2004), and 3) fungi are considered dominant decomposers of crop residue (Neely *et al.*, 1991) - which is more present in no till and cover-cropping systems - we hypothesized that fungal communities would be altered to a greater extent than bacteria by implemented soil management practices. As predicted, we found that fungal communities did shift across soil management to a greater extent than bacteria. Specifically, till type was more highly attributed to impacting beta diversity, and management corresponded to greater shifts in the relative abundances of fungal classes, including Pezizomycetes and Eurotimycetes, and functional categories, such as coenzyme and inorganic ion transport and metabolism. Furthermore, many fungal community members at high taxonomic levels respond similarly to till type, suggesting that tilling structures communities by acting on traits broadly shared within the kingdom, while bacteria are less sensitive, more resilient, and/or inhabit more diverse niche spaces. Our findings contradict other studies that compare bacterial and fungal responses to agricultural soil management using amplicon sequencing, where it was observed that the bacterial communities of soil and wheat roots are more sensitive than fungi to till type (Hartman *et al.*, 2018; Schmidt *et al.*, 2018), and that tillage impacted soil bacterial communities to a greater extent (Anderson *et al.*, 2017b). Many more studies have explored soil management responses in soil bacteria and fungi with regards to biomass, but reported conflicting results; some observed increased ratios of fungal to bacterial biomass in no-till systems (Frey *et al.*, 1999; Schutter *et al.*, 2001; Acosta-Martínez *et al.*, 2010a), while others lower to no changes in fungal to bacterial biomass (Feng et al. 2003; Helgason et al. 2009; Mathew et al. 2012; Mbuthia et al. 2015). In addition, other studies have found an increase or no change in fungal (Wang *et al.*, 2010; Dong *et al.*, 2017) and bacterial (Navarro-Noya *et al.*, 2013) diversity with no tillage. These differences could partly be explained by a range in sampling locations and times throughout the year, which can correspond to varying climatic factors, soil moisture levels, and chemical and physical soil characteristics, all of which may influence associated community dynamics (Feng *et al.*, 2003; Fierer, 2017). There is also evidence that length of time from when the practice of no till is first adopted to date of sampling can also influence results, where it can take several years to see a stabilization in soil parameters and improvement in plant yield (Busari *et al.*, 2015). When analyzing the active community, we similarly discovered that the activity and composition of fungal communities shifts to a greater extent than bacterial communities across treatments. Fungi have been shown to have higher carbon-to-nitrogen ratios (Wallenstein *et al.*, 2006), slower biomass turnover (Rousk & Bååth, 2011), and broader enzymatic capabilities at large (Boer *et al.*, 2005) than bacteria, and there is some evidence showing that differences between bacterial and fungal physiology may result in large scale impacts on carbon cycling (Waring *et al.*, 2013). Despite broad-level differences between bacterial and fungal activity across treatment, we detected a similar number of CAZy transcripts for fungi and bacteria, suggesting that both kingdoms may have a similar level of carbon usage in soils and rhizospheres at the sampled time point. This joins other evidence of some functional redundancy across kingdoms regarding the breakdown of organic matter (Banerjee *et al.*, 2016). We recommend future studies take into account multi-kingdom responses to agricultural soil management practices, and consider multiple time points to better understand

how microbial dynamics may vary over time and in accordance with important stages of plant development with these management practices, in order to advance our understandings of how crop-associated microbial communities can be modulated by soil management for climate-smart agriculture (Cordovil *et al.*, 2020) and how they relate to plant growth and yield.

We predicted an enrichment for AMF with CC, as compared to fallow treatments, due to CC increasing selection pressure for plant-associated microbes and providing continuous photosynthate energy resources in the off season, where cover crops have been shown to have species-specific effects on microbial communities (Finney *et al.*, 2017) and can promote AMF associations (Kabir *et al.*, 1997a,b, 2008; Neelam *et al.*, 2010; Hontoria *et al.*, 2019). CC appeared to make the most difference in overall AMF abundance when fields were also managed with STCC, suggesting that AMF associations are available and more desirable for plants growing in fields managed with STCC. This may be due to less competition with other mutualists, where AMF species have been shown to compete for root colonization, with inter-species impacts on plant growth (Thonar *et al.*, 2014; Engelmoer *et al.*, 2014; Knegt *et al.*, 2016). Interestingly, relative activity of AMF varied significantly across treatments in both the soil and rhizosphere, suggesting different roles are being played for the host, depending on proximity (from soil to rhizosphere). Future work exploring how soil treatment impacts the performance and proliferation of other plant-growth promoting microbes, including those introduced by a microbial biostimulant, is suggested, in order to determine whether certain soil practices and communities can promote mutualisms.

Lastly, all four soil management treatments supported similar plant growth and yield phenotypes, while their associated microbial communities varied. We recommend further studies to explore what mechanisms are at play in providing plant benefits in lower diversity systems, as compared to higher diversity systems, via means such as transcriptomics in plants and metabolomics. In addition, determining the rate of host colonization in different management regimes, by utilizing a series of time points earlier in and across plant development, would add to our understanding of microbial agroecology and how farming practices influence plant-microbe interactions. This work would also help determine whether host colonization is slower in NT systems, particularly with CC, with the potentially increased presence of more competitors in the microbial community.

In summation, utilizing the ability of microbial assemblages to promote plant growth is one promising means of improving crop performance and has attracted interest in both academia and industry. Despite the importance of the plant microbiome in promoting plant yield and numerous studies demonstrating that the plant microbiome is influenced by the local soil community, not much is known how current agricultural soil management practices influence the assembly and activity of plant-associated microbial communities. Here, we employed 16S rDNA and ITS2 amplicon sequencing and metatranscriptomics to characterize bacterial and fungal communities in sorghum roots and rhizospheres and soils managed by standard and no tilling, in conjunction with cover cropping and leaving a field fallow during the off-season months. We observed that standard tilling and cover-cropping correlated with increased microbial diversity, fungi are more sensitive than bacteria to till type, as evidenced by shifts in composition and activity, and the activity and association of arbuscular mycorrhizal fungi vary with treatment type. This work

furthers our understandings of how microbial communities respond to soil management practices and provides direction for how we might better optimize soil environments for beneficial plant-microbe interactions.

2.5 Materials and Methods

2.5.1 Field experimental design

This study was conducted at a 3.6 hectare field site maintained by the University of California West Side Research and Extension Center in Five Points, California (36°20′29″N, 120°7′14″W), in which plots have been managed by standard (ST) and no tilling (NT) practices from 1999 to the present day. Prior to the start of tilling treatments, a barley (*Hordeum vulgare* L.) crop was grown and removed in 1998 to reduce any potential differences in soil fertility and moisture from previous land usage. A yearly tomato-cotton rotation was then planted across 4 replicate blocks up until 2014, when a rotation of sorghum (*Sorghum bicolor*) and garbanzo beans (*Cicer arietinum*) was adopted.

The site consists of 32 plots that are each 9.1 meters wide by 30.5 meters long, with either a 9.1 meter buffer or border plot between treatment plots. Each treatment plot consists of six planting beds. Plots are divided into four soil management treatments (Figure 2-1). ST treatment has been described in detail previously(Mitchell *et al.*, 2015), and it entails past crop residue shredding, multiple soil diskings to mix residues to a depth of 20 centimeters (cm), use of a subsoil shank at about 30.5-45.7 cm, additional disking to 20 cm to soil clods, and pulverization of the surface 20 cm of soil. In addition, planting beds were broken down and remade following each harvest. During the first eight years, the NT treatment limited soil disturbances to shallow weed removal and tractor traffic was restricted to certain furrows. In 2012, NT fields became true no-tillage systems with the only soil disturbance occurring at the time of seeding or transplanting. During the entire length of this study, the location of NT planting beds were preserved. Rows within the ST and NT treatments are either left fallow (NO) or planted with a cover-crop mixture (CC) in October, following the year's harvest, that was originally made up of Juan triticale (Triticosecale Wittm.), Merced rye (Secale cereale L.), and common vetch (Vicia sativa L.); in 2010, pea (*Pisum sativum* L.), faba bean (*Vicia faba* l.), radish (*Raphanus sativus*), and Phacelia (*Phacelia tanacetifolia*) were added to the mix. Cover crops were cut in mid-March of the following years using a Buffalo Rolling Stalk Chopper (Buffalo Equipment, Columbus, NE). In the STCC system, the chopped cover crop was disked into the soil to a depth of about 20 cm. The cut cover crop in the NTCC system was sprayed with a 2% solution of glyphosate [N-(phosphonomethyl)glycine] and left on the surface as a mulch. Dry fertilizer (11-52-0 N-P-K) was applied in equal amounts to each plot pre-planting of sorghum at 89.2 kg ha^{-1} (9.8 kg ha^{-1} N and 46.4 kg ha^{-1} P) using a standard straight fertilizer shank at depths of 15 cm. Additional N (urea) was side dress applied at 111.5 kg ha^{-1} for a total of 51.3 kg N ha^{-1} in two lines about 18 cm from the transplants and about 15-cm deep about 4 weeks after transplanting.

The average surface residue that typically remains following the combination of these four treatment types follows a decreasing gradient, with over 90% for NTCC, between 40% and 70% for NTNO, between 10% and 20% for STCC, and below 5% for STNO (Mitchell *et al.*, 2015).

2.5.2 Sample collection and processing

In the summer of 2016, we sampled at two time points corresponding to before and after flowering - 40 and 80 days after planting, respectively - for sorghum, where the rhizosphere and roots were collected for 3 plants positioned 20.4 meters apart and 3 beds into each plot, and soil 20 cm away from harvested plants in furrows, with a soil auger to sample the soil layer 15 cm from the surface, per treatment (NTCC, NTNO, STCC, STNO) per 3 replicate blocks. In addition, at each position we collected topsoil for each treatment per 3 replicate blocks (12 samples in all) to total approximately 500 grams, in order to determine soil organic matter, pH, cation exchange capacity, and macro- and micronutrient levels, as analyzed by the Soil and Plant Nutrient Testing Laboratory at the University of Massachusetts, Amherst. Plant height and fresh shoot weight were measured for each plant harvested.

The following summer, we similarly sampled soils and sorghum roots and rhizospheres at a before flowering time point - 38 days after planting. We collected from 2 plants that were located 20.4 meters into the row and 3 beds into each plot, sampling per treatment per 3 replicate blocks. One plant was sampled for amplicon sequencing, while the other for metatranscriptomics, in order to better correlate community composition with activity, as well as help validate year one results. Plant height and fresh shoot weight were again measured for each plant harvested. Plant yield was measured when sorghum was harvested in October of those years (10/12/2016 and 10/16/2017).

2.5.3 DNA extraction and library preparation

Samples (108 per time points 1 and 2, 36 for time point 3, 252 total) were transported from the field to the laboratory on dry ice and DNA extracted using the MoBio Power Soil DNA isolation kit (Catalog No. 12888-100; MoBio, Carlsbad, CA, USA). Concentrations were measured with a Qubit 3 Fluorometer, and dilutions then made to 5ng/µl. To construct 16S rDNA and internal transcribed spacer 2 (ITS2) amplicon libraries, DNA from each sample was amplified using a dual-indexed 16S rRNA and ITS2 Illumina iTags primer set specific to the V3-V4 16S rRNA (Takahashi *et al.*, 2014) and 5.85Fun-ITS4Fun ITS2 region (Taylor *et al.*, 2016), respectively, using 5-Prime Hot Master Mix (catalog No. 2200410), according to protocols and conditions detailed in (Simmons *et al.*, 2018) and (Gao *et al.*, 2019). Replicates were pooled and the DNA concentration for each sample was then quantified using a Qubit 3 Fluorometer. Using 100 ng of each PCR product, amplicons were next pooled. Before submitting for sequencing, pooled samples were cleaned with 1.0 X volume Agencourt AMPureXP (Beckman-Coulter, West Sacramento, CA) beads, according to the manufacturer's directions, except for the modifications of using 1.0 X rather than 1.6 X volume beads per sample, dispensing 1500 µl 70% EtOH rather than 200 µl, and eluting in 100 µl DNase-free H2O rather than 40 µl. An aliquot of the pooled amplicons was diluted to 10 nM in 30 µl total volume and then submitted to the QB3 Vincent J. Coates Genomics Sequencing Laboratory for sequencing, using the Illumina MiSeq platform's 300bp paired-end reads with v3 chemistry.

2.5.4 Amplicon sequence data processing

The resulting 16S amplicon libraries produced on average approximately 38,135, 44,906, and 37,852 reads per sample for soils, rhizospheres, and roots, respectively. The resulting ITS2 amplicon libraries produced on average approximately 44,848, 26,598, and 23,158 reads per

sample for soils, rhizospheres, and roots, respectively. The resulting read data was processed with the custom pipeline iTagger from the Joint Genome Institute in Qiime2, detailed in (Deng *et al.*, 2019). To remove low abundance operational taxonomic units (OTUs) that are, in many cases, artifacts generated through the sequencing process, we removed OTUs without at least 4 reads in at least 3 samples. We also removed samples that had fewer than 10,000 fungal reads, which yielded 3,155 bacterial and 454 fungal high-abundance OTUs for downstream analyses. To account for differences in sequencing read depth across samples, all samples were rarefied to 10,000 reads per sample for specific analyses.

2.5.5 RNA extraction and library preparation

For the summer 2017 field season, we collected soil and rhizosphere samples from one plant per treatment per block (3 plants per treatment, 12 rhizosphere and 12 soil samples in all) for metatranscriptomic analysis. Samples were harvested just before flowering (the second growth stage with panicle formation, 38 days after transplanting), to determine what microbial processes are ongoing close to a critical period for grain production (Lupwayi *et al.*, 1998). Soil and rhizosphere samples were flash frozen with liquid nitrogen and stored on dry ice.

RNA from samples was extracted and cDNA synthesized using the PowerMax soil DNA isolation kit for RNA extraction, as the MoBio PowerMax (Catalog No. 12988-10) and PowerSoil kit use the same silica membrane, with a modified protocol provided by MoBio that is detailed in(Xu *et al.*, 2018). The resultant RNA was washed with 70% ethanol and resuspended in 100 µl RNase-free H2O, and the remaining DNA digested using a DNase Max Kit (Qiagen, 171 Catalog No. 15200-50), according to the manufacturer's protocol. RNA was purified using an RNeasy PowerClean Pro Cleanup Kit (MoBio, Carlsbad, CA, USA) and ribosomal RNA from bacteria removed with the Ribo-Zero rRNA Removal Kit (Bacteria, Illumina, Catalog No. MRZB12424), as per manufacturer's instructions. Concentration and quality were then assessed using a Qubit 3 Fluorometer (Invitrogen, Carlsbad, CA, USA) and Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA), respectively. Subsequently, the TruSeq Stranded Total RNA Library Prep Plant SetA Kit (Illumina, Catalog No. 20020610) was used, according to the manufacturer's instructions, to make 300-500bp fragment libraries for sequencing on the Illumina HiSeq4000 platform with 150bp paired end reads at the QB3 Vincent J. Coates Genomics Sequencing Laboratory. Returned reads were assembled with Trinity and then submitted to Integrated Microbial Genomes & Microbiomes at the Joint Genome Institute for annotation and taxonomic assignment (pipeline version 4.16.5; Taylor et al. 2016).

2.5.6 Metatranscriptome sequence data processing

The metatranscriptome data analysis pipeline is detailed in (Xu *et al.*, 2018) and read statistics, sequencing depth, rRNA and tRNA contamination levels from archaea, bacteria and eukaryote are reported in Figure 2-21. In brief, we performed a quality control of raw fastq data with the software FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and then cut sequencing adaptors and removed short reads that were less than 35 bp, as well as reads with more than three Ns (flags: -q 15,15 -m 35 --max-n 3), with CUTADAPT version 1.9 (Martin, 2011). Remaining reads were compared with the Silva and Rfam database for identification of ribosomal RNA (18S, 28S, 16S, 23S, 5.8S, 12S, and 5S) and transfer RNA, which were removed with BBmap (Version 35.34, sourceforge.net/projects/bbmap/). All remaining clean mRNA reads

from all of our datasets were combined with MEGAHIT (version 1.1.1) with odd numbered k-mers from length 21 to 99 (Li et al., 2015). Contig coverage was determined by mapping the initial unassembled reads to the combined assembled contigs using BBMap, where the pileup.sh script (BBMap) was used to calculate contig-wise average coverage. The assembled contigs with lengths >= 200 bp and covered percentage >= 50% were submitted to JGI IMG (https://img.jgi.doe.gov) for gene calling, functional annotation and taxonomy assignment as described by(Huntemann *et al.*, 2016). In brief, the IMG pipeline performs an additional quality control and protein-coding genes are identified using a consensus of four different *ab initio* gene prediction tools: prokaryotic GeneMark.hmm 265 (v. 2.8) (Lukashin, 1998), MetaGeneAnnotator (v. Aug 2008) (Noguchi *et al.*, 2008), Prodigal (v. 2.6.2) (Hyatt *et al.*, 2010) and FragGeneScan (v. 1.16) (Rho *et al.*, 2010). The predictions from all tools are combined and protein-coding genes with translations shorter than 32 amino acids are deleted. Functional annotation of associated protein-coding genes is performed with COGs, Pfams, KO terms, EC numbers and phylogenetic lineage for contigs (Bray *et al.*, 2016). For phylogenetic lineage analysis, the best hit for genes with greater than 30 percent identity to a defined taxonomic lineage was picked for further analysis (this threshold permitted classification of ~63.7% of all genes). Genes belonging to bacteria and fungi were picked manually and used for relative abundance calculations in R. Of the assembled reads assigned taxonomy (51%) in our dataset, 48.47% are from bacteria, 48.33% from eukaryota (25% from Fungi alone), 2.30% from archaea, and 0.89% from viruses. Relative gene expression levels for each taxa were determined by counting the number of reads that were assigned to a particular protein-encoding gene. Normalization was obtained by dividing each gene count by the total mRNA read count of each dataset. For taxonomy assignment based on the core gene set, we calculated relative abundances based only on the abundances of nine core genes (*gyrA*, *recA*, *rpoB*, *rpoA*, *gyrB*, *gap*, *rho*, *ftsZ*, and *secA*) commonly represented across a large percentage of bacterial lineages from the IMG Bacterial Database. As MEGAHIT does not report the specific reads incorporated into the assembled contigs, we used BBMap to map the mRNA reads back to contigs to calculate the percentage of reads assembled per sample (bbwrap.sh with flags: kfilter=22 subfilter=15 maxindel=80). Kallisto (https://pachterlab.github.io/kallisto/) was used to map the high-quality mRNA reads per sample to these IMG-derived gene sequences to provide a per-gene coverage estimate with 'sequence-based bias correction' and 'strand specific reads, first read reverse' flags. The raw gene counts table was passed to edgeR(Robinson & Oshlack, 2010) to perform the normalization by calcNormFactors function with the TMM method (Nakayasu *et al.*, 2016) and to calculate which genes were differentially expressed with quasi-likelihood F-tests (P<=0.05). Hypergeometric tests were used to calculate the enrichment of functional categories and subcategories (P<=0.05).

2.5.7 Data Availability

All raw sequences are deposited in Sequence Read Archive with the accession code: SUB7328543, and all scripts used for the statistical analysis of data in this manuscript are included as part of a public repository on github (https://github.com/dcolemanderr/Till-Soil-Management-Study). In addition, an assembly of the metatranscriptome can be accessed and downloaded via IMG/MER (https://www.img.jgi.doe.gov) using the IMG ID 3300029287.

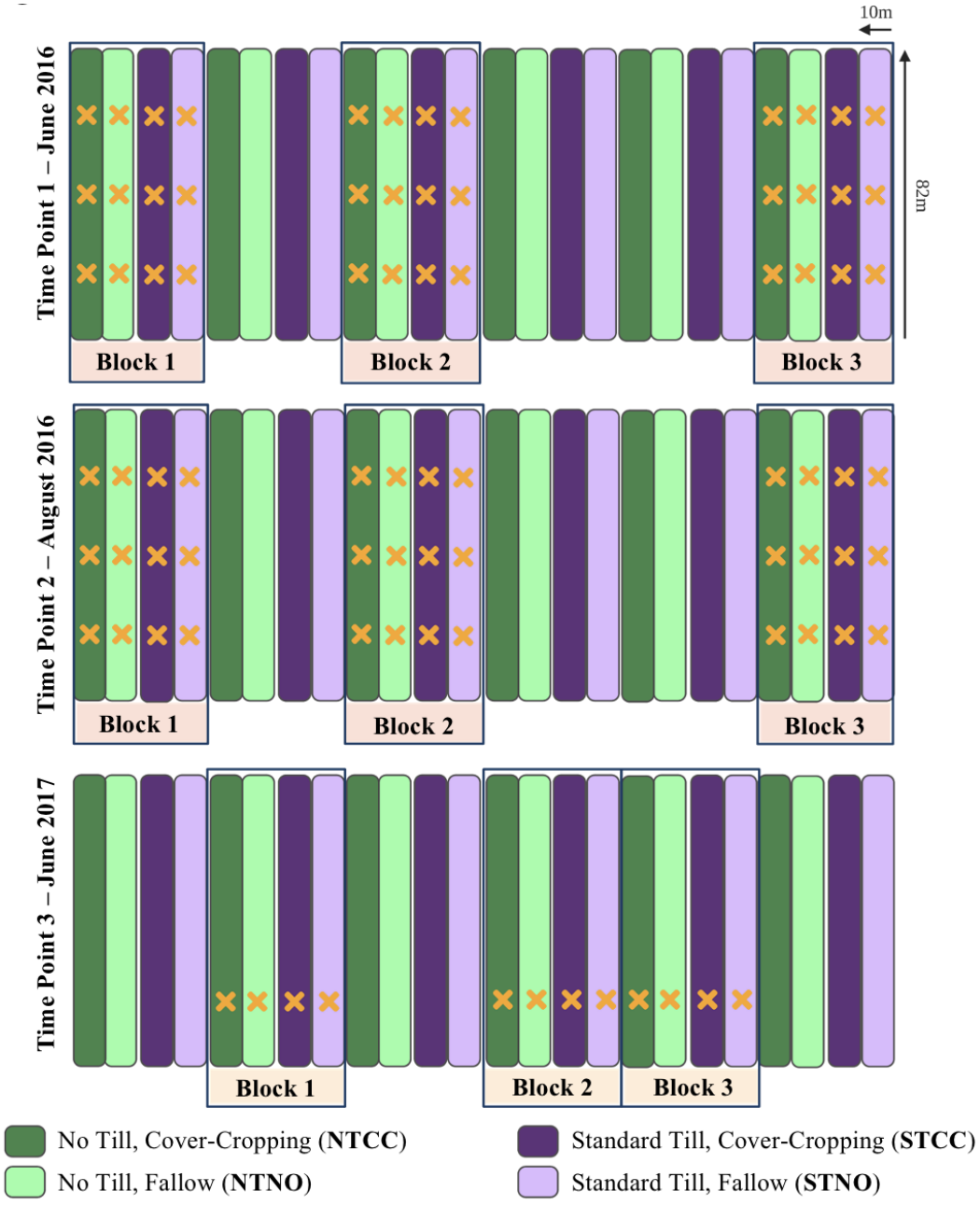


Figure 2-1.
Diagram of field layout and sampling scheme. On a 3.6 hectare field site located at the University of California West Side Research and Extension Center in Five Points, California, 82 meters long by 10 meters wide plots are managed by the following treatment types: no till with cover-cropping, no till with leaving the field fallow during the off-season months, standard till with cover-cropping, or standard tillage with leaving the field fallow, as is represented with dark green, light green, dark purple, and light purple, respectively. Each treatment plot consists of 6 beds, and a 6-bed buffer area separates treatments to provide for tractor operations. The sorghum cultivar *Sorghum bicolor* hybrid KS585 was planted in plots sampled, and three blocks per treatment type were sampled per time point (before and after flowering in 2016 - 40 and 80 days

after planting, respectively - and before flowering in 2017 - 38 days after planting). In 2016, three positions - marked by the orange X - were sampled per treatment plot per block per time point, and one position per treatment plot per block per time point was sampled in 2017; positions are spaced 20.4 meters apart and were collected 3 beds into each plot. Soil samples were collected 20 centimeters away from harvested plants, in furrows, and with a soil auger to sample the soil layer approximately 15 centimeters from the surface. Figure was created with BioRender.com.

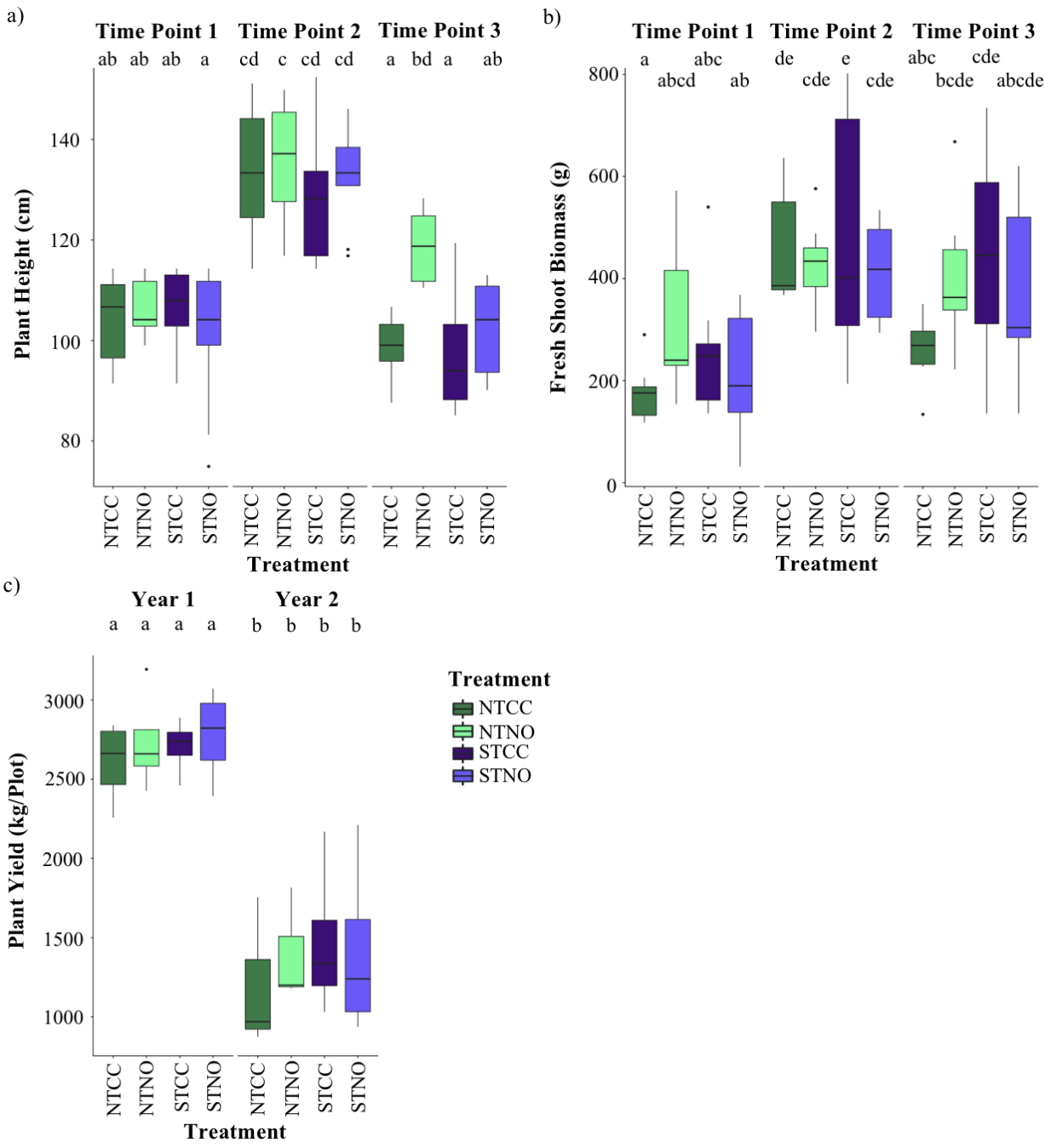


Figure 2-2.
Management type corresponds to slight variation in plant height, fresh shoot biomass, and plant yield. Boxplots of phenotypic data measured from plants that were also sampled for microbial community profiling (a, b) or from the blocks sampled from (c). Median plant height in centimeters (a), fresh shoot biomass in grams (b), and plant yield in kilograms per plot (c) are presented, with treatments: no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season (NTNO), standard till with cover-cropping (STCC), or standard till leaving the field fallow (STNO), as is denoted with dark green, light green, dark purple, and light purple, respectively.

Figure 2-3.
Plant phenotypic variation largely unattributed to till and cover type. ANOVA results for a) plant height and shoot biomass - as subset by time point (1, 2, and 3) - and b) yield - as subset by year (2016 and 2017). Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

a)

		Plant Height						Shoot Biomass					
	Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**	
TP1	Till_Type	1	42.4	42.4	0.626	0.44026		1	7159	7159	0.695	0.4168	
	Cover_Type	1	18	18	0.266	0.61297		1	12411	12411	1.205	0.2886	
	Block	2	1007.4	503.7	7.435	0.00521	**	2	42862	21431	2.08	0.1574	
	Till_Type:Cover_Type	1	167.1	167.1	2.467	0.13585		1	59104	59104	5.737	0.0292	*
	Till_Type:Block	2	214.6	107.3	1.584	0.2357		2	26710	13355	1.296	0.3007	
	Cover_Type:Block	2	36.1	18	0.266	0.76952		2	86108	43054	4.179	0.0347	*
	Till_Type:Cover_Type:Block	2	232.1	116	1.713	0.2118		2	56767	28384	2.755	0.0937	.
	Residuals	16	1083.9	67.7				16	164824	10302			
TP2	Till_Type	1	2019	2019	0.037	0.849		1	241	240.7	1.987	0.1684	
	Cover_Type	1	17415	17415	0.316	0.578		1	121	120.7	0.996	0.3258	
	Block	2	100192	50096	0.91	0.413		2	962	481.1	3.97	0.0288	*
	Till_Type:Cover_Type	1	47393	47393	0.861	0.361		1	7	6.9	0.057	0.8125	
	Till_Type:Block	2	12291	6145	0.112	0.895		2	192	95.9	0.792	0.4618	
	Cover_Type:Block	2	3919	1959	0.036	0.965		2	208	104.1	0.859	0.4331	
	Till_Type:Cover_Type:Block	2	88411	44206	0.803	0.457		2	183	91.7	0.757	0.4775	
	Residuals	32	1762303	55072				32	3878	121.2			
TP3	Till_Type	1	34961	34961	1.824	0.2017		1	456.3	456.3	10.065	0.008031	**
	Cover_Type	1	8664	8664	0.452	0.5141		1	942.1	942.1	20.782	0.000656	***
	Block	2	56784	28392	1.482	0.266		2	366.3	183.1	4.04	0.045562	*
	Till_Type:Cover_Type	1	72161	72161	3.766	0.0762	.	1	344.5	344.5	7.6	0.017382	*
	Till_Type:Block	2	94249	47125	2.459	0.1273		2	446.5	223.2	4.924	0.02745	*
	Cover_Type:Block	2	104916	52458	2.737	0.1049		2	445.2	222.6	4.91	0.027665	*
	Till_Type:Cover_Type:Block	2	88085	44043	2.298	0.1429		2	71.3	35.7	0.787	0.477553	
	Residuals	12	229956	19163				12	544	45.3			

b)

	2016 Yield						2017 Yield					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**	
Till_Type	1	12.3	12.25	0.452	0.514		1	39.06	39.06	1.728	0.213	
Cover_Type	1	2.2	2.25	0.083	0.778		1	1.56	1.56	0.069	0.797	
Till_Type:Cover_Type	1	0	0	0	1		1	14.06	14.06	0.622	0.446	
Residuals	12	325.5	27.12				12	271.25	22.6			

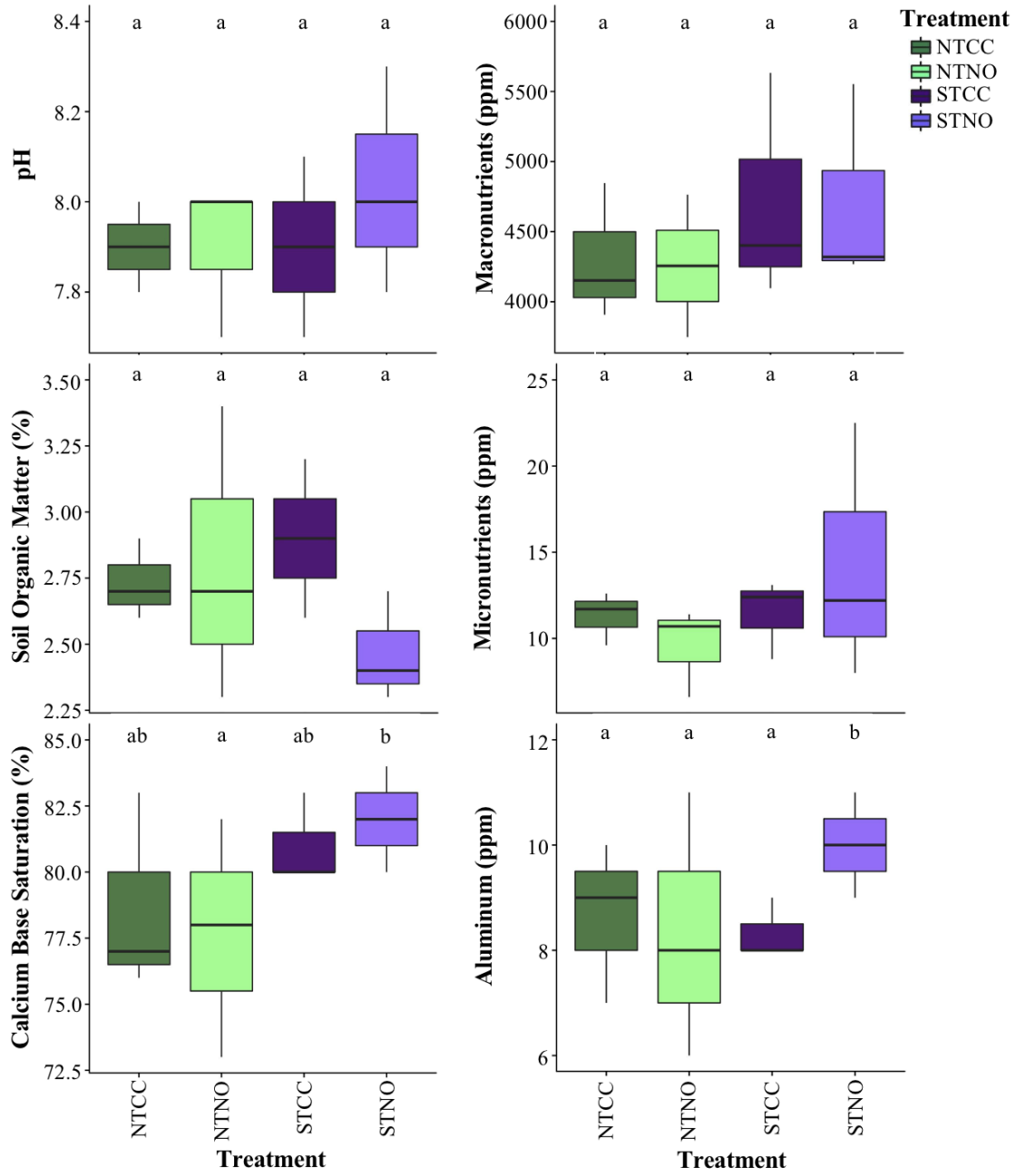


Figure 2-4.
Soil chemistry varies minimally across treatment plots. Boxplots of soil pH, macronutrients (ppm; P, K, Ca, Mg, S), micronutrients (ppm; B, Mn, Zn, Cu, Fe), soil organic matter (%), calcium base saturation (%), and aluminum (ppm) levels. Soil samples were collected during the first sampling time point in 2016 from each block per treatment: no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), or standard till leaving the field fallow (STNO), as is represented with dark green, light green, dark purple, and light purple, respectively.

Figure 2-5.
Soil chemical analysis results. A soil sample was collected at the first sampling time point in 2016 from all three blocks from each treatment plot: no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), or standard till leaving the field fallow (STNO). The following parameters are reported on: pH, levels of phosphorus (P ppm), potassium (K ppm), calcium (Ca ppm), magnesium (Mg ppm), sulfur (S ppm), boron (B ppm), manganese (Mn ppm), zinc (Zn ppm), copper (Cu ppm), iron (Fe ppm), aluminum (Al ppm), lead (Pb ppm), and cation exchange capacity (CEC ppm), percentage base saturation of calcium (% Ca Base Saturation), magnesium (% Mg Base Saturation), and potassium (% K Base Saturation), scoop density (g/cm3), percentage organic matter, and levels of nitrate (NO3-N ppm) and ammonium (NH4+ ppm).

ID	1.sc	2.sn	3.cc	4.cn	5.sc	6.sn	7.cc	8.cn	9.sc	10.sn	11.cc	12.cn
Block	1	1	1	1	2	2	2	2	3	3	3	3
Treatment	STCC	STNO	NTCC	NTNO	STCC	STNO	NTCC	NTNO	STCC	STNO	NTCC	NTNO
pH	7.9	7.8	7.9	8	7.7	8.3	7.8	8	8.1	8	8	7.7
P (ppm)	59.9	62.7	62.1	65.3	46.9	39.4	42.4	45.2	40.8	47.2	90.3	67.3
K (ppm)	512	462	420	380	485	334	421	406	345	453	628	780
Ca (ppm)	4580	4555	3923	3850	3439	3520	2996	2882	3258	3353	3005	2882
Mg (ppm)	415	378	373	393	368	381	406	378	404	369	387	409
S (ppm)	65.3	95	67.9	75.3	62.6	44.8	42.3	36.4	48.9	46.4	41.3	41.6
Macronutrients (ppm)	5632.2	5552.7	4846	4763.6	4401.5	4319.2	3907.7	3747.6	4096.7	4268.6	4151.6	4179.9
B (ppm)	3.5	3.5	2.7	3	3.1	2.5	2.5	2.1	2.8	2.7	3.4	3.3
Mn (ppm)	7.8	17.9	8.2	6.9	9.2	4.9	6.3	4	5.5	8.6	8.3	7.3
Zn (ppm)	0.4	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.2
Cu (ppm)	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Fe (ppm)	0.5	0.6	0.4	0.4	0.4	0.3	0.4	0.2	0.2	0.6	0.5	0.4
Micronutrients (ppm)	12.4	22.5	11.7	10.7	13.1	8	9.6	6.6	8.8	12.2	12.6	11.4
Al (ppm)	9	11	10	11	8	10	9	8	8	9	7	6
Pb (ppm)	0.4	0	0	0	0	0	0	0	0	0	0	0
CEC (ppm)	27.6	27.1	23.7	23.4	21.5	21.6	19.4	18.5	20.5	20.9	19.8	19.5
% Ca Base Saturation	83	84	83	82	80	82	77	78	80	80	76	73
% Mg Base Saturation	12	11	13	14	14	14	17	17	16	14	16	17
% K Base Saturation	5	4	5	4	6	4	6	6	4	6	8	10
Scoop Density (g/cm3)	1.13	1.22	1.23	1.19	1.26	1.22	1.15	1.23	1.18	1.27	1.23	1.15
% Organic Matter	3.2	2.7	2.9	2.7	2.9	2.4	2.7	2.3	2.6	2.3	2.6	3.4
NO3-N (ppm)	32	44	21	20	36	6	17	15	16	24	18	36
NH4+ (ppm)	2	2	2	2	2	1	2	1	1	2	2	2

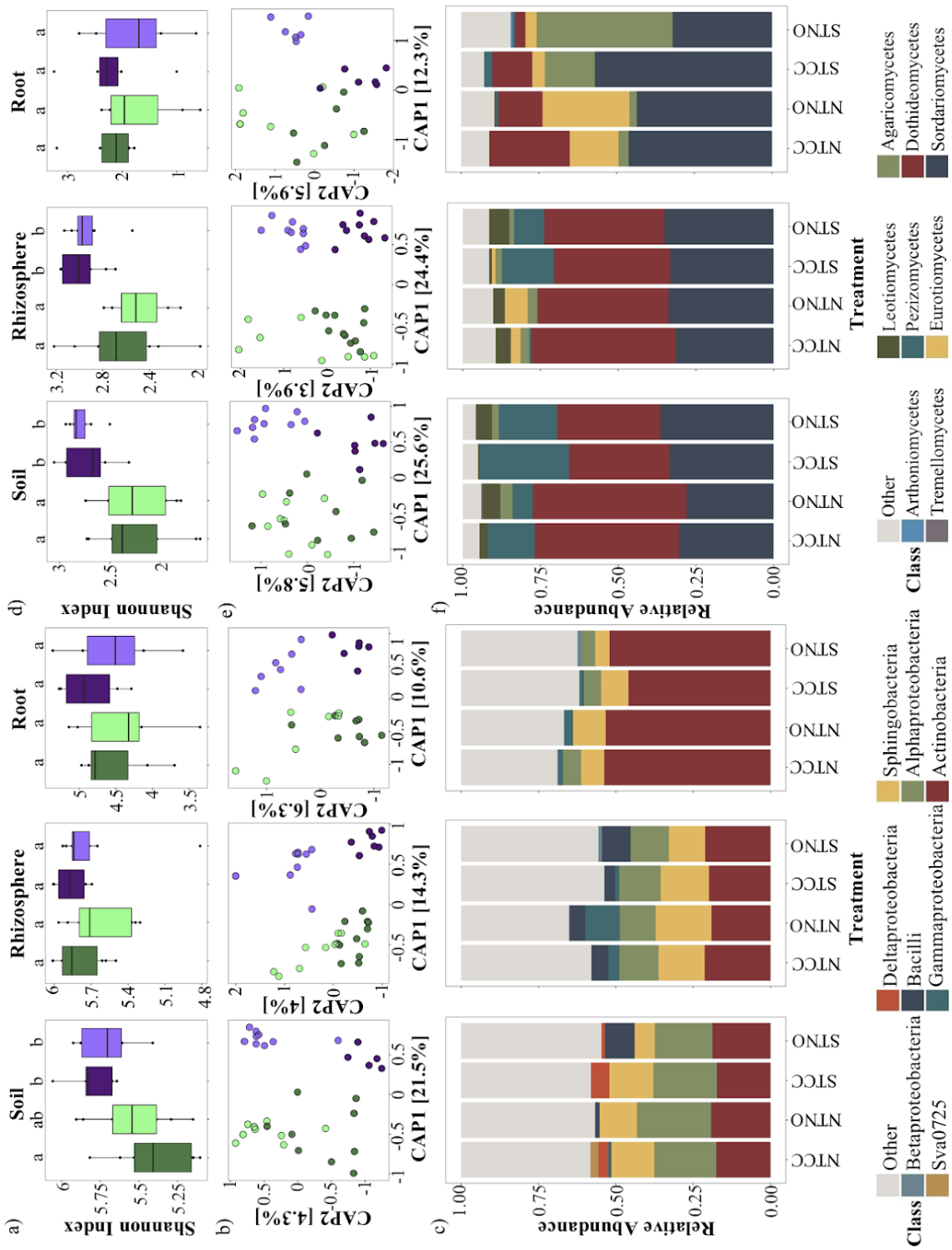


Figure 2-6.
Microbial communities are differentially impacted by soil management practice. Amplicon data from time point 1 demonstrating impacts of soil management on bacterial (a,b,c) and fungal (d,e,f) communities in the soil, rhizosphere, and roots for alpha diversity with Shannon index (a,d), beta diversity with Bray-Curtis dissimilarity (b,e), and class-level relative abundances (c,f).

Boxplots show median Shannon diversity (a,d) with letters representing statistical significance (analysis of variance, $p<0.05$) between treatments within each sample type, constrained ordinations for canonical analysis of principal coordinates till (x axis, CAP1) and cover type (y axis, CAP2) with Bray-Curtis dissimilarity (b,e), and stacked bar plots (c,f) of the 10 most abundant classes for bacteria and fungi, respectively. Treatments no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), and standard till leaving the field fallow (STNO) are represented with dark green, light green, dark purple, and light purple, respectively, for a, b, d, and e.

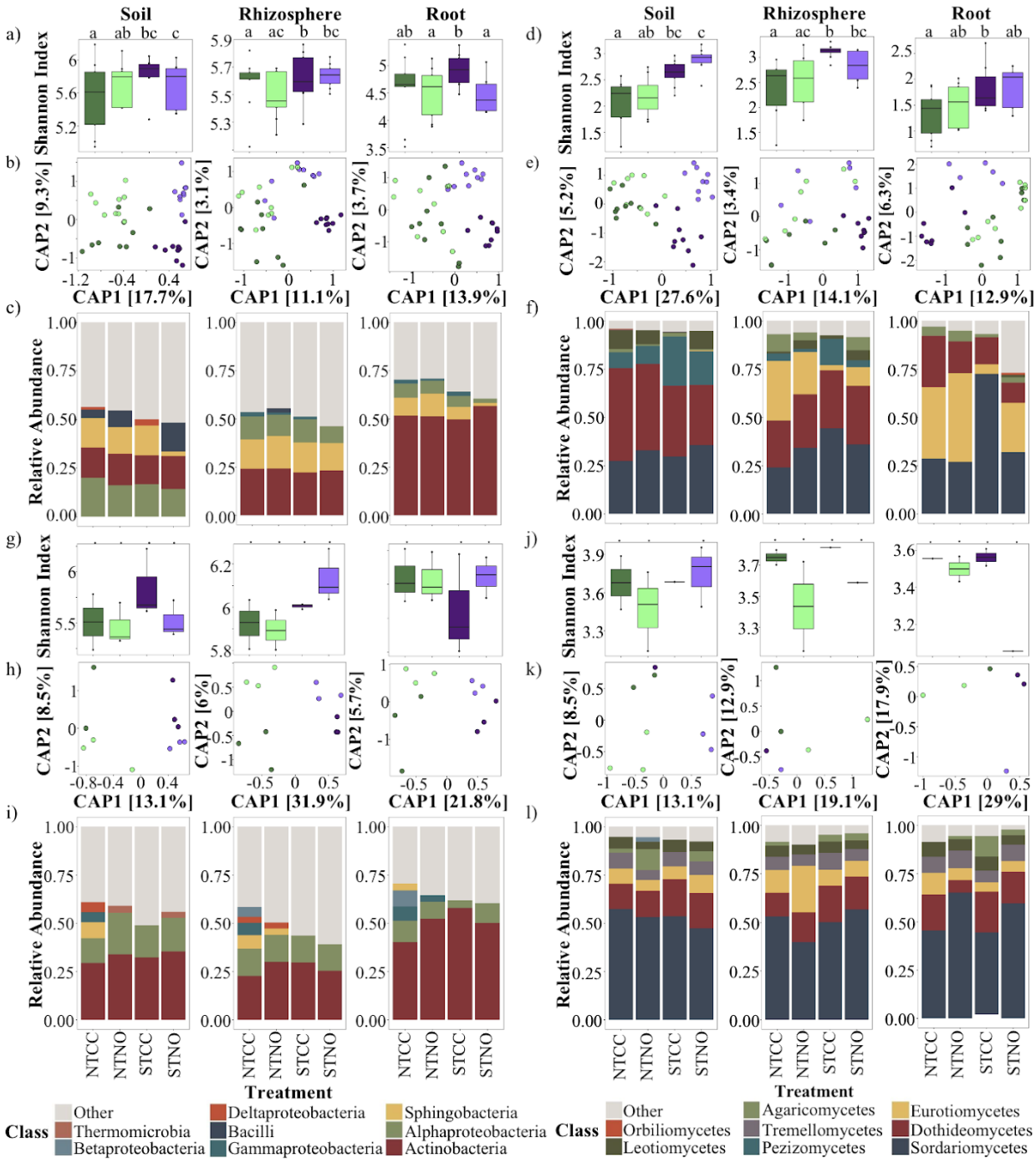


Figure 2-7.
Microbial communities are differentially impacted by soil management practice. Amplicon data from time points 2 (a-f) and 3 (g-l) demonstrating impacts of soil management on bacterial (a-c,g-i) and fungal (d-f,j-l) communities in the soil, rhizosphere, and roots for alpha diversity with Shannon index (a,d,g,j), beta diversity with Bray-Curtis dissimilarity (b,e,h,k), and class-level relative abundances (c,f,i,l). Boxplots show median Shannon diversity (a,d,g,j) with letters representing statistical significance (analysis of variance, $p<0.05$) between treatments within each sample type, constrained ordinations for canonical analysis of principal coordinates

till (x axis, CAP1) and cover type (y axis, CAP2) with Bray-Curtis dissimilarity (b,e,h,k), and stacked bar plots (c,f,i,l) of the 9 most abundant classes for bacteria and fungi, respectively. Treatments no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), and standard till leaving the field fallow (STNO) are represented with dark green, light green, dark purple, and light purple, respectively, for a, b, d, e, g, h, j, and k.

Figure 2-8.
Till and cover type significantly structure alpha and beta diversity for a number of compartments (soil, rhizosphere, root). PERMANOVA results conducted for a) Shannon index and b) Bray-Curtis dissimilarity with bacterial (16S) and fungal (ITS2) amplicon sequencing data for time point 1. Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

a)

		16S						ITS2					
Data	**Factor**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**	
Soil	Till_Type	1	0.622	0.622	12.227	0.002	**	1	2.324	2.324	23.946	0.000	***
	Cover_Type	1	0.014	0.014	0.278	0.604		1	0.013	0.013	0.133	0.718	
	Block	2	0.023	0.012	0.226	0.800		1	0.038	0.038	0.394	0.536	
	Till_Type:Cover_Type	1	0.097	0.097	1.906	0.183		1	0.009	0.009	0.094	0.762	
	Till_Type:Block	2	0.086	0.043	0.841	0.446		1	0.061	0.061	0.632	0.434	
	Cover_Type:Block	2	0.035	0.018	0.347	0.711		1	0.189	0.189	1.943	0.175	
	Till_Type:Cover_Type:Block	2	0.116	0.058	1.136	0.341		1	0.002	0.002	0.019	0.892	
	Residuals	20	1.018	0.051				26	2.523	0.097			
Rhizosphere	Till_Type	1	0.011	0.011	0.205	0.655		1	1.348	1.348	20.490	0.000	***
	Cover_Type	1	0.219	0.218	4.092	0.054		1	0.135	0.135	2.056	0.162	
	Block	2	0.227	0.114	2.126	0.141		1	0.036	0.036	0.539	0.469	
	Till_Type:Cover_Type	1	0.006	0.006	0.110	0.743		1	0.024	0.024	0.364	0.551	
	Till_Type:Block	2	0.023	0.011	0.212	0.810		1	0.025	0.025	0.375	0.545	
	Cover_Type:Block	2	0.311	0.155	2.910	0.074		1	0.002	0.002	0.031	0.862	
	Till_Type:Cover_Type:Block	2	0.011	0.006	0.106	0.900		1	0.059	0.059	0.903	0.350	
	Residuals	24	1.281	0.053				29	1.908	0.066			
Root	Till_Type	1	0.273	0.273	1.486	0.241		1	0.005	0.005	0.011	0.919	
	Cover_Type	1	0.371	0.371	2.015	0.175		1	1.502	1.502	2.999	0.101	
	Block	2	1.883	0.941	5.120	0.019	*	1	0.009	0.009	0.018	0.894	
	Till_Type:Cover_Type	1	0.013	0.013	0.068	0.797		1	0.007	0.007	0.013	0.910	
	Till_Type:Block	2	0.594	0.297	1.616	0.230		1	0.015	0.015	0.031	0.863	
	Cover_Type:Block	2	0.456	0.228	1.241	0.315		1	0.257	0.257	0.513	0.484	
	Till_Type:Cover_Type:Block	2	0.662	0.331	1.801	0.197		1	1.275	1.275	2.546	0.129	
	Residuals	16	2.942	0.184				17	8.513	0.501			

b)

		16S							ITS2						
Data	**Factor**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**P value**	
Soil	Till_Type	1	0.643	0.643	10.139	0.213	0.001	***	1	1.229	1.229	14.040	0.262	0.001	***
	Cover_Type	1	0.136	0.136	2.142	0.045	0.016	*	1	0.275	0.275	3.144	0.059	0.007	**
	Block	2	0.248	0.124	1.952	0.082	0.009	**	1	0.284	0.284	3.241	0.061	0.002	**

	Till_Type:Cover_Type	1	0.118	0.118	1.856	0.039	0.032	*	1	0.218	0.218	2.493	0.047	0.014	*
	Till_Type:Block	2	0.175	0.088	1.382	0.058	0.084	.	1	0.143	0.143	1.631	0.030	0.123	
	Cover_Type:Block	2	0.198	0.099	1.557	0.066	0.036	*	1	0.133	0.133	1.522	0.028	0.134	
	Till_Type:Cover_Type:Block	2	0.229	0.114	1.802	0.076	0.024	*	1	0.130	0.130	1.486	0.028	0.136	
	Residuals	20	1.269	0.063		0.421			26	2.276	0.088		0.485		
	Total	31	3.015			1.000			33	4.689			1.000		
Rhizosphere	Till_Type	1	0.511	0.511	6.672	0.138	0.001	***	1	1.265	1.265	12.663	0.245	0.001	***
	Cover_Type	1	0.162	0.162	2.120	0.044	0.003	**	1	0.216	0.216	2.160	0.042	0.036	*
	Block	2	0.307	0.153	2.002	0.083	0.001	***	1	0.127	0.127	1.274	0.025	0.226	
	Till_Type:Cover_Type	1	0.171	0.171	2.233	0.046	0.002	**	1	0.186	0.186	1.862	0.036	0.056	.
	Till_Type:Block	2	0.208	0.104	1.356	0.056	0.084	.	1	0.096	0.096	0.962	0.019	0.431	
	Cover_Type:Block	2	0.270	0.135	1.763	0.073	0.003	**	1	0.208	0.208	2.079	0.040	0.041	*
	Till_Type:Cover_Type:Block	2	0.230	0.115	1.498	0.062	0.037	*	1	0.172	0.172	1.725	0.033	0.096	.
	Residuals	24	1.839	0.077		0.497			29	2.896	0.100		0.561		
	Total	35	3.697			1.000			36	5.166			1.000		
Root	Till_Type	1	0.311	0.311	4.086	0.104	0.001	***	1	0.968	0.968	3.488	0.116	0.001	***
	Cover_Type	1	0.193	0.193	2.538	0.065	0.005	**	1	0.575	0.575	2.071	0.069	0.006	**
	Block	2	0.507	0.254	3.334	0.170	0.001	***	1	0.389	0.389	1.403	0.046	0.126	
	Till_Type:Cover_Type	1	0.133	0.133	1.747	0.045	0.048	*	1	0.423	0.422	1.523	0.050	0.094	.
	Till_Type:Block	2	0.168	0.084	1.106	0.057	0.290		1	0.343	0.343	1.237	0.041	0.214	
	Cover_Type:Block	2	0.275	0.137	1.807	0.092	0.013	*	1	0.379	0.379	1.366	0.045	0.120	
	Till_Type:Cover_Type:Block	2	0.173	0.087	1.138	0.058	0.269		1	0.581	0.581	2.095	0.069	0.007	**
	Residuals	16	1.217	0.076		0.409			17	4.717	0.277		0.563		
	Total	27	2.976			1.000			24	8.375			1.000		

Figure 2-9.
Variation in alpha and beta diversity is most explained by sample type (soil, rhizosphere, root), then time point and till type. PERMANOVA results conducted for a) Shannon index and b) Bray-Curtis dissimilarity with bacterial (16S) and fungal (ITS2) amplicon sequencing data for all data. Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

a)

All data	**16S**						**ITS2**					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P value**	
Sample_Type	2	59.76	29.879	399.385	< 2e-16	***	2	27.95	13.973	50.194	< 2e-16	***
TimePoint	2	1.19	0.597	7.974	0.000538	***	1	8.7	8.7	31.251	9.42E-08	***
Till_Type	1	0.99	0.992	13.266	0.000387	***	1	8.63	8.635	31.017	1.04E-07	***
Cover_Type	1	0.41	0.415	5.546	0.019997	*	1	0.01	0.012	0.042	0.83774	
Block	2	0.06	0.032	0.431	0.650895		1	0.12	0.123	0.441	0.50761	
Sample_Type:TimePoint	4	1.02	0.255	3.415	0.010825	*	2	0.32	0.159	0.57	0.56678	
Sample_Type:Till_Type	2	0.19	0.097	1.296	0.277033		2	0.46	0.232	0.835	0.43567	
TimePoint:Till_Type	2	0.11	0.055	0.739	0.479663		1	0.01	0.014	0.049	0.82537	
Sample_Type:Cover_Type	2	0.29	0.143	1.905	0.152862		2	0.86	0.43	1.543	0.2168	
TimePoint:Cover_Type	2	0.16	0.082	1.091	0.338953		1	0.36	0.356	1.28	0.25949	
Till_Type:Cover_Type	1	0.21	0.205	2.741	0.100168		1	0.03	0.027	0.098	0.75455	
Sample_Type:Block	4	1.67	0.416	5.567	0.000358	***	2	0.34	0.17	0.612	0.5434	
TimePoint:Block	4	1.08	0.27	3.606	0.007993	**	1	0.76	0.758	2.724	0.1008	
Till_Type:Block	2	0.35	0.177	2.361	0.098255	.	1	0.02	0.022	0.077	0.78135	
Cover_Type:Block	2	0.11	0.055	0.735	0.481253		1	0	0.001	0.002	0.96328	
Sample_Type:TimePoint:Till_Type	4	0.43	0.107	1.426	0.228911		2	0.51	0.253	0.908	0.40526	
Sample_Type:TimePoint:Cover_Type	4	0.68	0.171	2.279	0.064117	.	2	0.47	0.233	0.836	0.4353	
Sample_Type:Till_Type:Cover_Type	2	0.27	0.136	1.82	0.166132		2	0.26	0.129	0.465	0.62923	
TimePoint:Till_Type:Cover_Type	2	0.2	0.098	1.315	0.272058		1	0.24	0.239	0.859	0.35548	
Sample_Type:TimePoint:Block	8	0.99	0.124	1.657	0.114817		2	1.11	0.555	1.992	0.13975	
Sample_Type:Till_Type:Block	4	0.97	0.242	3.232	0.014443	*	2	0.32	0.159	0.571	0.56586	
TimePoint:Till_Type:Block	4	0.27	0.068	0.904	0.463816		1	0.01	0.009	0.032	0.85772	
Sample_Type:Cover_Type:Block	4	0.16	0.039	0.525	0.717676		2	0.44	0.22	0.79	0.45557	
TimePoint:Cover_Type:Block	4	1.73	0.433	5.781	0.000256	***	1	2.5	2.499	8.976	0.00317	**
Till_Type:Cover_Type:Block	2	0.82	0.41	5.479	0.005175	**	1	0.01	0.012	0.042	0.8371	
Sample_Type:TimePoint:Till_Type: Cover_Type	4	0.31	0.078	1.045	0.386449		2	0.97	0.485	1.741	0.17854	
Sample_Type:TimePoint:Till_Type:Block	8	0.23	0.029	0.389	0.924999		2	0.25	0.125	0.449	0.63879	
Sample_Type:TimePoint:Cover_Type:Block	8	1.25	0.156	2.09	0.041032	*	2	0.18	0.091	0.326	0.72221	
Sample_Type:Till_Type:Cover_Type:Block	4	0.83	0.207	2.769	0.02997	*	2	0.02	0.008	0.028	0.9722	
TimePoint:Till_Type:Cover_Type:Block	4	0.62	0.154	2.061	0.089573	.	1	0.52	0.517	1.859	0.17465	
Sample_Type:TimePoint:Till_Type: Cover_Type:Block	7	0.92	0.132	1.76	0.100566		2	1.74	0.871	3.128	0.04647	*

Residuals	132	9.88	0.075				162	45.1	0.278			

b)

All data	**16S**							**ITS2**						
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**P value**		**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**P value**	
Sample_Type	2	14.586	7.2929	101.887	0.3344	0.001	***	2	12.592	6.296	35.7	0.2028	0.001	***
TimePoint	2	3.591	1.7953	25.082	0.0823	0.001	***	1	3.96	3.9596	22.5	0.0638	0.001	***
Till_Type	1	2.452	2.452	34.256	0.0562	0.001	***	1	3.641	3.6406	20.664	0.0586	0.001	***
Cover_Type	1	0.529	0.5285	7.384	0.0121	0.001	***	1	0.838	0.8382	4.758	0.0135	0.001	***
Block	2	0.387	0.1937	2.706	0.0089	0.006	**	1	0.281	0.2814	1.597	0.0045	0.103	
Sample_Type:TimePoint	4	1.541	0.3852	5.382	0.0353	0.001	***	2	0.989	0.4944	2.806	0.0159	0.003	**
Sample_Type:Till_Type	2	0.717	0.3583	5.006	0.0164	0.001	***	2	1.726	0.8631	4.899	0.0278	0.001	***
TimePoint:Till_Type	2	0.641	0.3207	4.481	0.0147	0.001	***	1	0.529	0.5294	3.005	0.0085	0.004	**
Sample_Type:Cover_Type	2	0.292	0.1458	2.037	0.0067	0.015	*	2	0.712	0.3561	2.021	0.0115	0.022	*
TimePoint:Cover_Type	2	0.192	0.0961	1.342	0.0044	0.162		1	0.229	0.2294	1.302	0.0037	0.188	
Till_Type:Cover_Type	1	0.351	0.3505	4.897	0.008	0.001	***	1	0.517	0.5167	2.933	0.0083	0.006	**
Sample_Type:Block	4	0.433	0.1083	1.513	0.0099	0.042	*	2	0.364	0.182	1.033	0.0059	0.381	
TimePoint:Block	4	0.803	0.2008	2.805	0.0184	0.001	***	1	0.512	0.512	2.906	0.0083	0.008	**
Till_Type:Block	2	0.254	0.1271	1.776	0.0058	0.035	*	1	0.177	0.1767	1.003	0.0029	0.41	
Cover_Type:Block	2	0.324	0.162	2.263	0.0074	0.006	**	1	0.294	0.294	1.669	0.0047	0.094	.
Sample_Type:TimePoint:Till_Type	4	0.385	0.0963	1.346	0.0088	0.083	.	2	0.332	0.1662	0.943	0.0054	0.525	
Sample_Type:TimePoint:Cover_Type	4	0.334	0.0835	1.166	0.0077	0.2		2	0.325	0.1625	0.923	0.0052	0.562	
Sample_Type:Till_Type:Cover_Type	2	0.209	0.1043	1.457	0.0048	0.106		2	0.513	0.2564	1.455	0.0083	0.1	.
TimePoint:Till_Type:Cover_Type	2	0.237	0.1184	1.654	0.0054	0.053	.	1	0.267	0.2668	1.515	0.0043	0.132	
Sample_Type:TimePoint:Block	8	0.869	0.1087	1.518	0.0199	0.011	*	2	0.461	0.2306	1.309	0.0074	0.152	
Sample_Type:Till_Type:Block	4	0.264	0.066	0.922	0.0061	0.569		2	0.343	0.1715	0.973	0.0055	0.465	
TimePoint:Till_Type:Block	4	0.585	0.1462	2.042	0.0134	0.002	**	1	0.178	0.1782	1.011	0.0029	0.378	
Sample_Type:Cover_Type:Block	4	0.373	0.0931	1.301	0.0085	0.119		2	0.446	0.2231	1.267	0.0072	0.178	
TimePoint:Cover_Type:Block	4	0.65	0.1625	2.27	0.0149	0.001	***	1	0.829	0.829	4.706	0.0134	0.001	***
Till_Type:Cover_Type:Block	2	0.323	0.1617	2.259	0.0074	0.01	**	1	0.187	0.1874	1.064	0.003	0.337	
Sample_Type:TimePoint:Till_Type:Cover_Type	4	0.285	0.0713	0.996	0.0065	0.433		2	0.358	0.179	1.016	0.0058	0.4	
Sample_Type:TimePoint:Till_Type:Block	8	0.531	0.0664	0.927	0.0122	0.661		2	0.321	0.1606	0.911	0.0052	0.527	
Sample_Type:TimePoint:Cover_Type:Block	8	0.644	0.0805	1.124	0.0148	0.219		2	0.739	0.3694	2.096	0.0119	0.012	*
Sample_Type:Till_Type:Cover_Type:Block	4	0.284	0.071	0.992	0.0065	0.451		2	0.269	0.1345	0.764	0.0043	0.741	
TimePoint:Till_Type:Cover_Type:Block	4	0.629	0.1572	2.196	0.0144	0.002	**	1	0.304	0.3039	1.725	0.0049	0.068	.
Sample_Type:TimePoint:Till_Type:Cover_Type:Block	7	0.481	0.0687	0.959	0.011	0.534		2	0.314	0.157	0.891	0.0051	0.561	

Residuals	132	9.448	0.0716		0.2166			162	28.541	0.1762		0.4597		
Total	238	43.622			1			209	62.089			1		

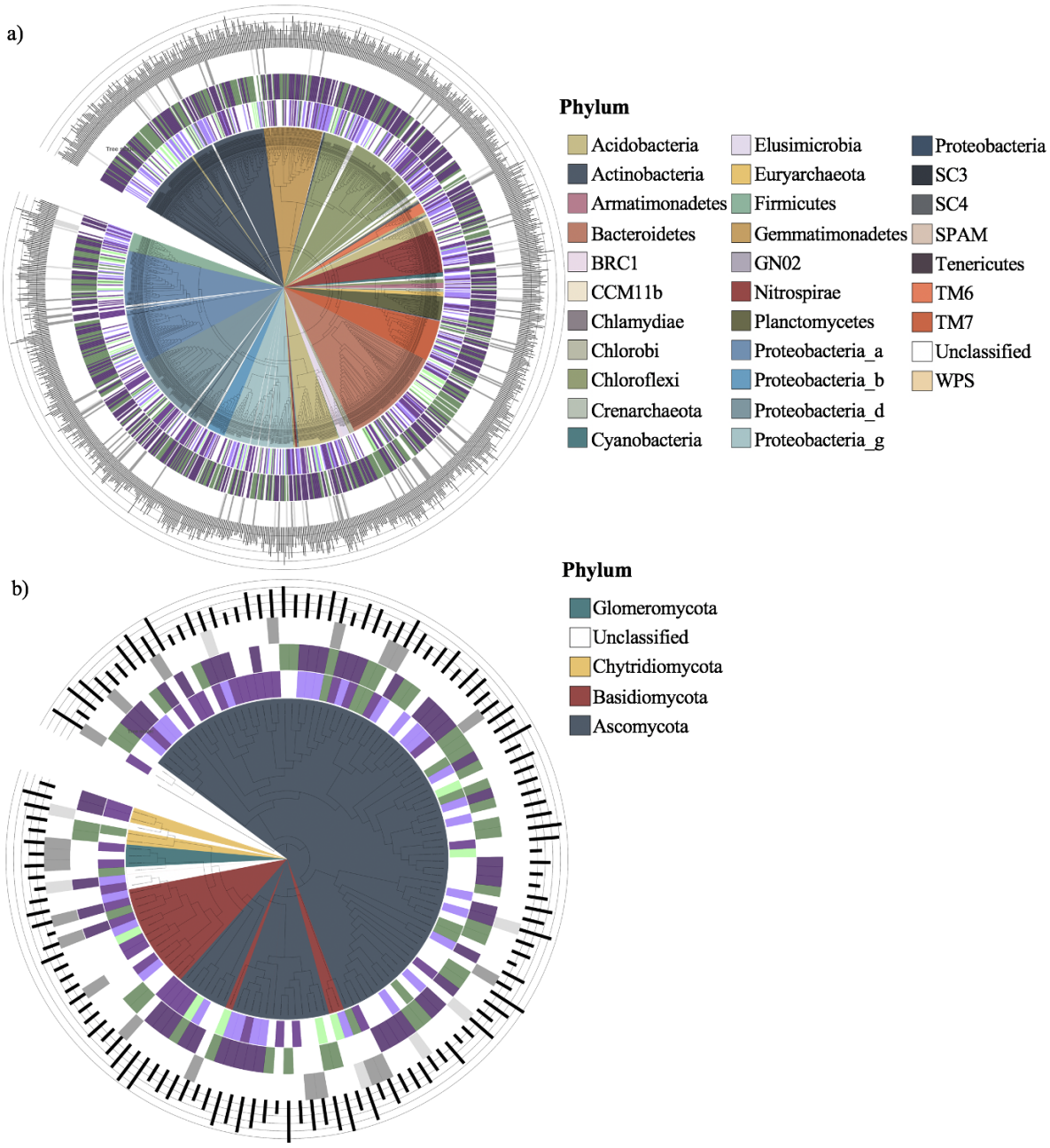


Figure 2-10.
Soil management indicators partially cluster by phylogeny and correspond most to standard till treatments. Phylogenetic tree of bacterial (a) and fungal (b) OTUs that are indicator species ($p \leq 0.001$) for treatment type (innermost ring) of no till with cover-cropping (NTCC, dark green), no till leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), or standard till leaving the field fallow (STNO, light purple), till type (2nd innermost ring) of no or standard till, as represented with green or purple, respectively, and cover type (3rd innermost ring) of cover crops or leaving the field fallow, as represented by dark or light grey, respectively. Outermost bars represent the total abundance of each genus on a log 10 scale. Inner sections are colored by phylum. Figure generated with interactive Tree of Life.

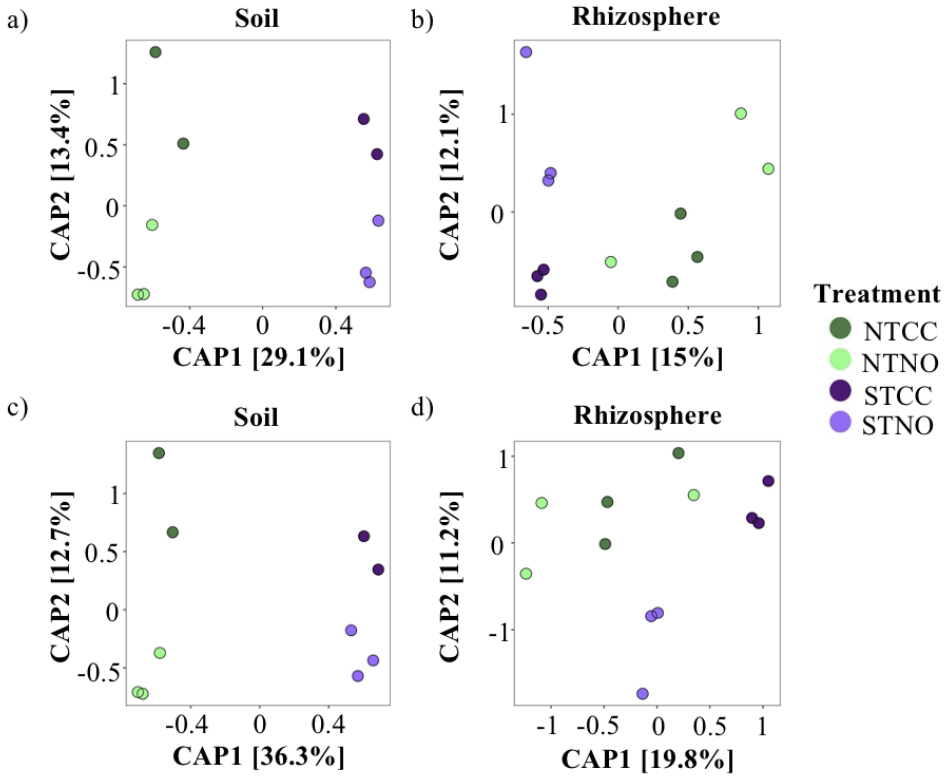


Figure 2-11.
Active microbial communities group by soil management type. Beta diversity, as determined by Bray-Curtis dissimilarity, for active bacteria (a, b) and fungi (c, d), represented with constrained ordinations for canonical analysis of principal coordinates till type (x axis, CAP1) and cover type (y axis, CAP2). Samples are colored by treatment type: no till with cover-cropping (NTCC, dark green), no till with leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), and standard till with leaving the field fallow (STNO, light purple).

Figure 2-12.
Till type significantly contributes to beta diversity variation. PERMANOVA results conducted on Bray-Curtis dissimilarity for active bacterial and fungal communities in the soil and sorghum rhizosphere. Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

		Active Bacteria							Active Fungi						
Data	**Factor**	**Df**	**Sums Sqs**	**Mean Sqs**	**F value**	**R2**	**P value**		**Df**	**Sums Sqs**	**Mean Sqs**	**F value**	**R2**	**P value**	
Soil	Till_Type	1	0.4132	0.4132	3.4635	0.2824	0.002	**	1	0.5856	0.5856	5.1468	0.3551	0.001	***
	Cover_Type	1	0.19502	0.19502	1.6347	0.1333	0.061	.	1	0.20258	0.20258	1.7805	0.1229	0.072	.
	Block	2	0.24234	0.12117	1.0157	0.1656	0.456		2	0.26886	0.13443	1.1815	0.1631	0.299	
	Till_Type: Cover_Type	1	0.13545	0.13545	1.1354	0.0926	0.294		1	0.13676	0.13676	1.202	0.0829	0.257	
	Residuals	4	0.4772	0.1193		0.3261			4	0.45512	0.11378		0.2760		
	Total	9	1.46322			1			9	1.64893			1		
Rhizosphere	Till_Type	1	0.19936	0.199363	1.81395	0.1543	0.045	*	1	0.24284	0.242845	2.16051	0.1723	0.031	*
	Cover_Type	1	0.15969	0.159694	1.45301	0.1236	0.14		1	0.1882	0.188198	1.67434	0.1335	0.108	
	Block	2	0.15624	0.078119	0.71078	0.1209	0.874		2	0.15725	0.078626	0.69951	0.1115	0.844	
	Till_Type: Cover_Type	1	0.1171	0.1171	1.06546	0.0907	0.339		1	0.14708	0.147078	1.30851	0.1043	0.21	
	Residuals	6	0.65943	0.109906		0.5105			6	0.67441	0.112401		0.4784		
	Total	11	1.29183			1			11	1.40978			1		

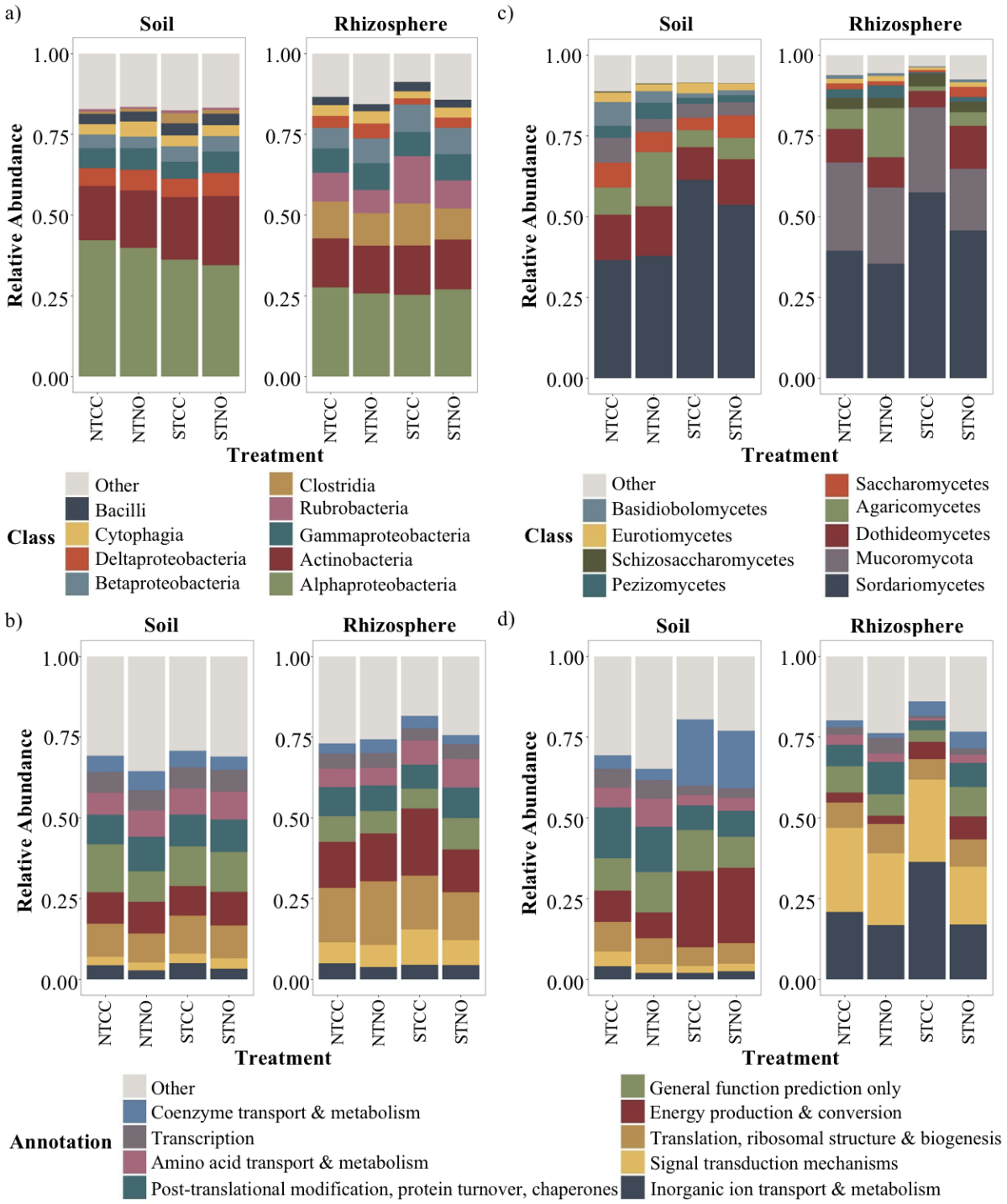


Figure 2-13.
Microbial activity varies with soil management practice. Stacked bar plots of the relative abundances of the top 9 most abundant classes in active bacteria (a) and fungi (c), and of functions, as characterized by COG category, across treatments: no till with cover-cropping (NTCC), no till with leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), and standard till with leaving the field fallow (STNO), for soil and rhizosphere samples collected during time point 3.

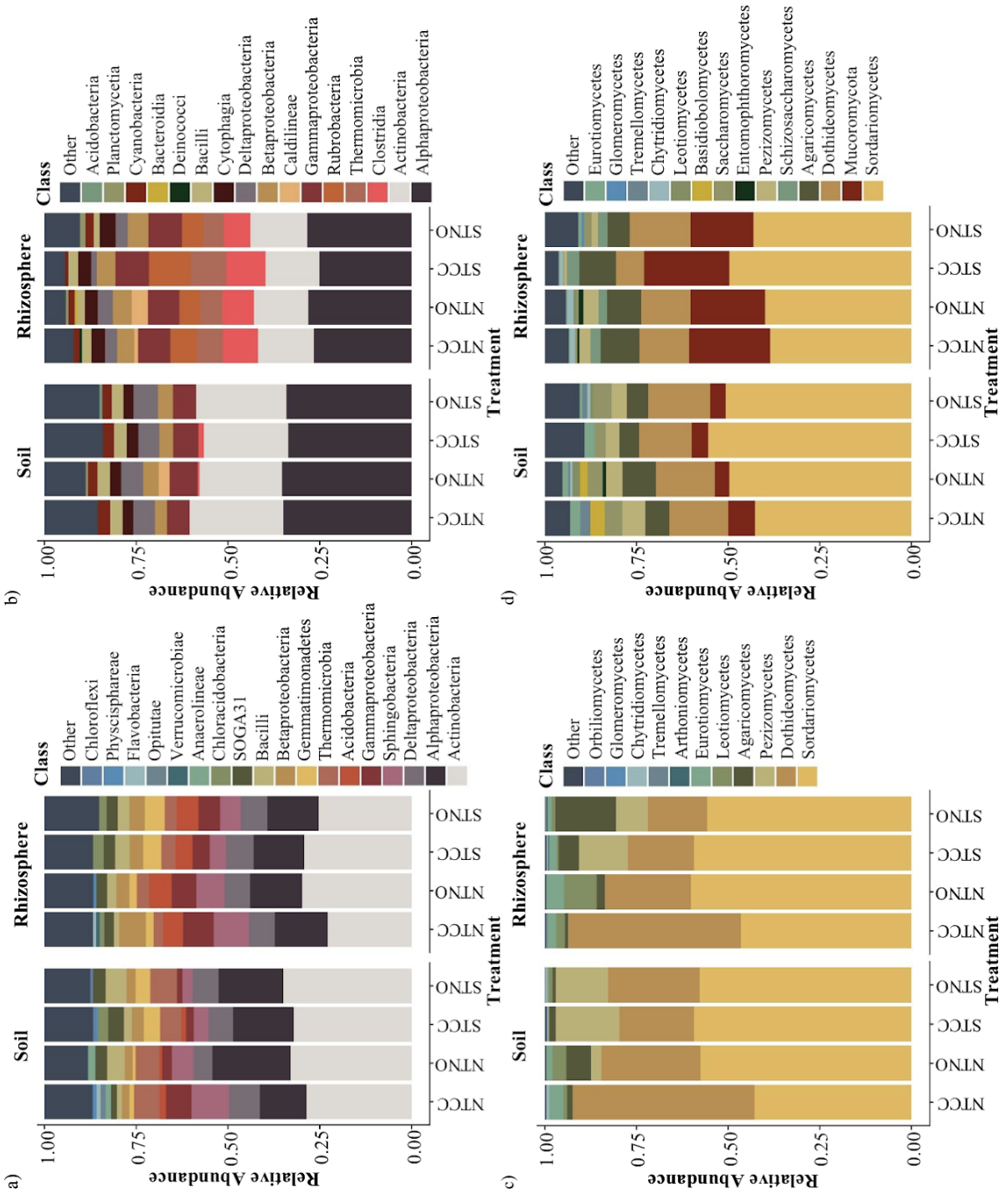


Figure 2-14.
Differences across soil management type in what microbial classes are active, versus detected at large. Stacked bar plots of (a) bacterial class-level relative abundances from time point 3 soil and rhizosphere 16S amplicon data, (b) active bacterial class-level relative abundances from time point 3 soil and rhizosphere metatranscriptomic data, (c) fungal class-level relative abundances from time point 3 soil and rhizosphere ITS2 amplicon data, (d) active fungal class-level relative abundances from time point 3 soil and rhizosphere metatranscriptomic data, across treatments:

no till with cover-cropping (NTCC), no till with leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), and standard till with leaving the field fallow (STNO).

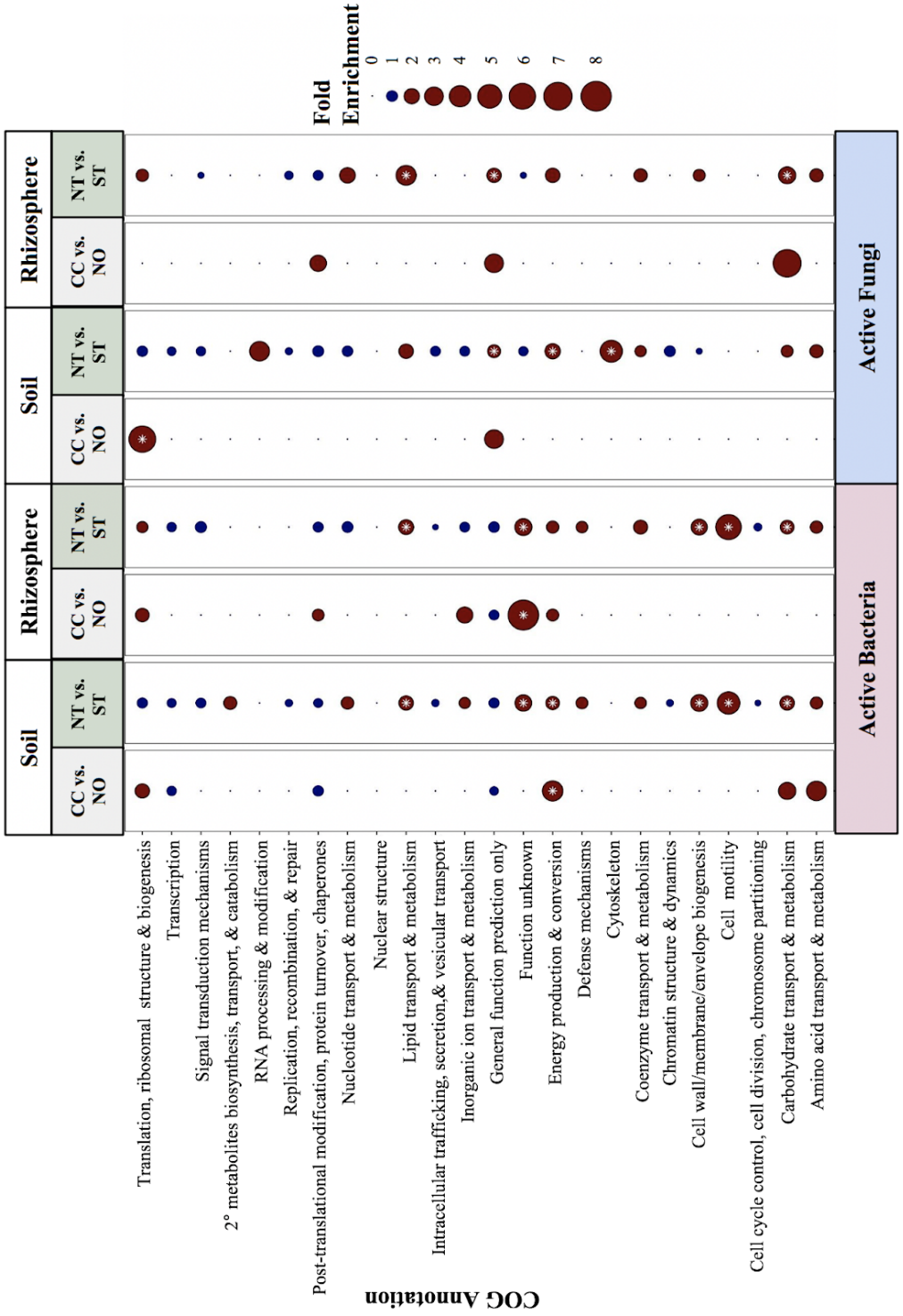


Figure 2-15.
Soil management type enriches for numerous functions in soils versus the rhizosphere and for bacteria versus fungi. Clusters of Orthologous Groups (COG) category enrichment analysis of differentially expressed a) bacterial and b) fungal genes individually performed for soil and rhizosphere samples to determine what genes are enriched with cover-cropping, in comparison to when the field is left fallow (CC vs. NO) and with no till, in comparison to standard till (NT vs.

ST). Size of the circle represents degree of fold enrichment, which is calculated as the percent of total differentially expressed genes (DEG) that have a significant ($p \leq .01$) positive log2foldchange in a given COG category out of all DEGS, divided by the percent of total genes in that COG category. Color of the circle represents whether the fold enrichment is less than or equal to 1 (blue) or greater than 1 (red), and a white asterisk within the circle represents a statistically significant ($p \leq .05$) fold enrichment, as determined by a hypergeometric test.

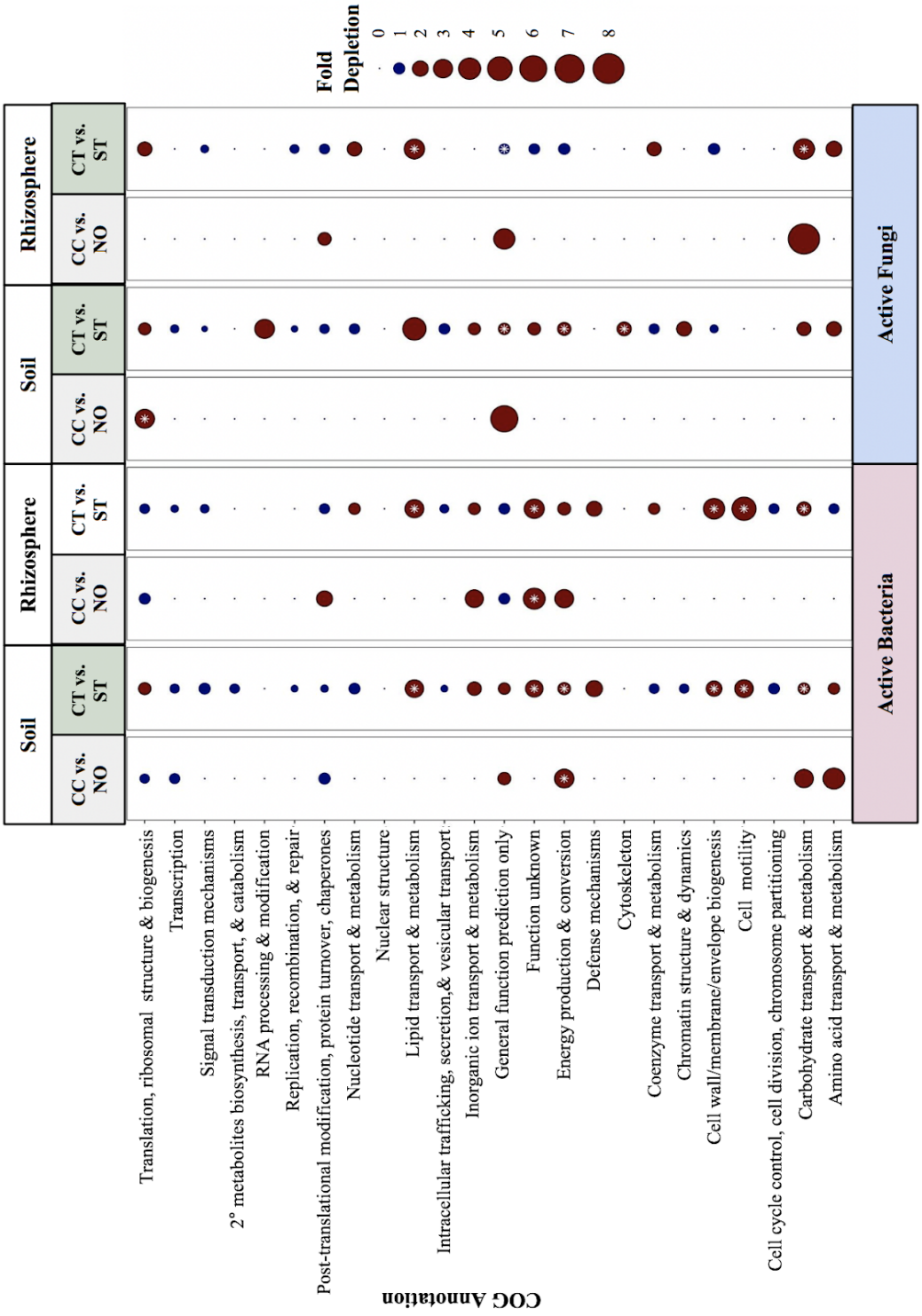


Figure 2-16.
Soil management type differentially depletes numerous functions in soils versus the rhizosphere and for bacteria versus fungi. Clusters of Orthologous Groups (COG) category depletion analysis of differentially expressed a) bacterial and b) fungal genes individually performed for soil and rhizosphere samples to determine what genes are enriched with cover-cropping, in comparison to when the field is left fallow, (CC vs. NO) and with no till, in comparison to standard till, (CT vs.

ST). Size of the circle represents degree of fold depletion, which is calculated as the percent of total differentially expressed genes (DEG) that have a significant ($p \leq .01$) negative log2foldchange in a given COG category out of all DEGS, divided by the percent of total genes in that COG category. Color of the circle represents whether the fold enrichment is less than or equal to 1 (blue) or greater than 1 (red), and a white asterisk within the circle represents a statistically significant ($p \leq .05$) fold enrichment, as determined by a hypergeometric test.

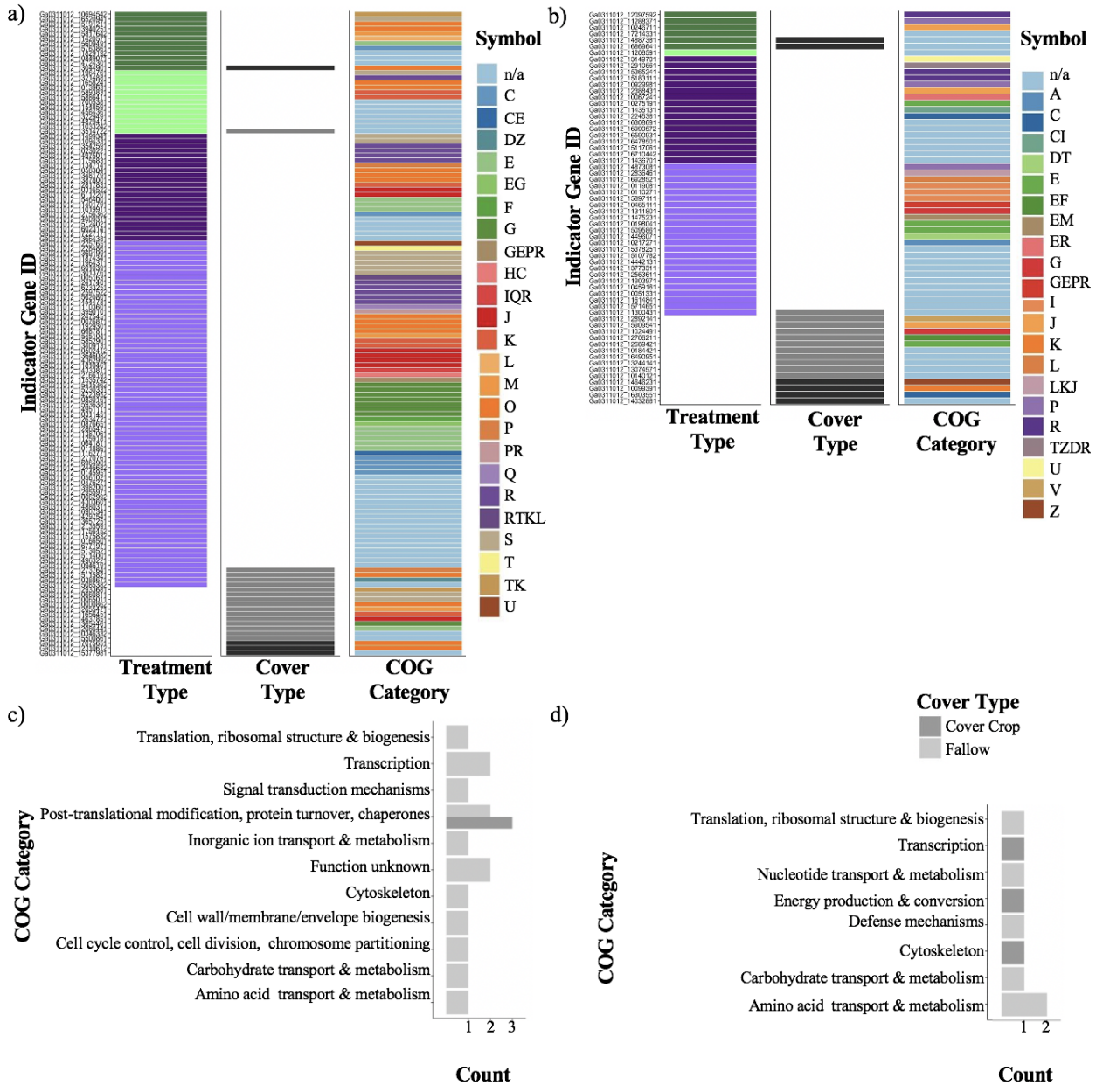


Figure 2-17.
Soil management indicators span many functions and are most representative of standard till and fallow treatments. Plots of active (a) bacterial and (b) fungal genes that are indicators ($p \leq .001$) for treatment treatment type (first column) of no till with cover-cropping (NTCC, dark green), no till leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), or standard till leaving the field fallow (STNO, light purple); cover type (second column) of cover crops or leaving the field fallow, as represented by dark or light grey, respectively; and the COG category (third column) for each indicator gene. Counts represented in bar graphs for each indicator of cover type for (c) bacteria and (d) fungi, with dark grey for cover cropping and light grey for fallow treatment.

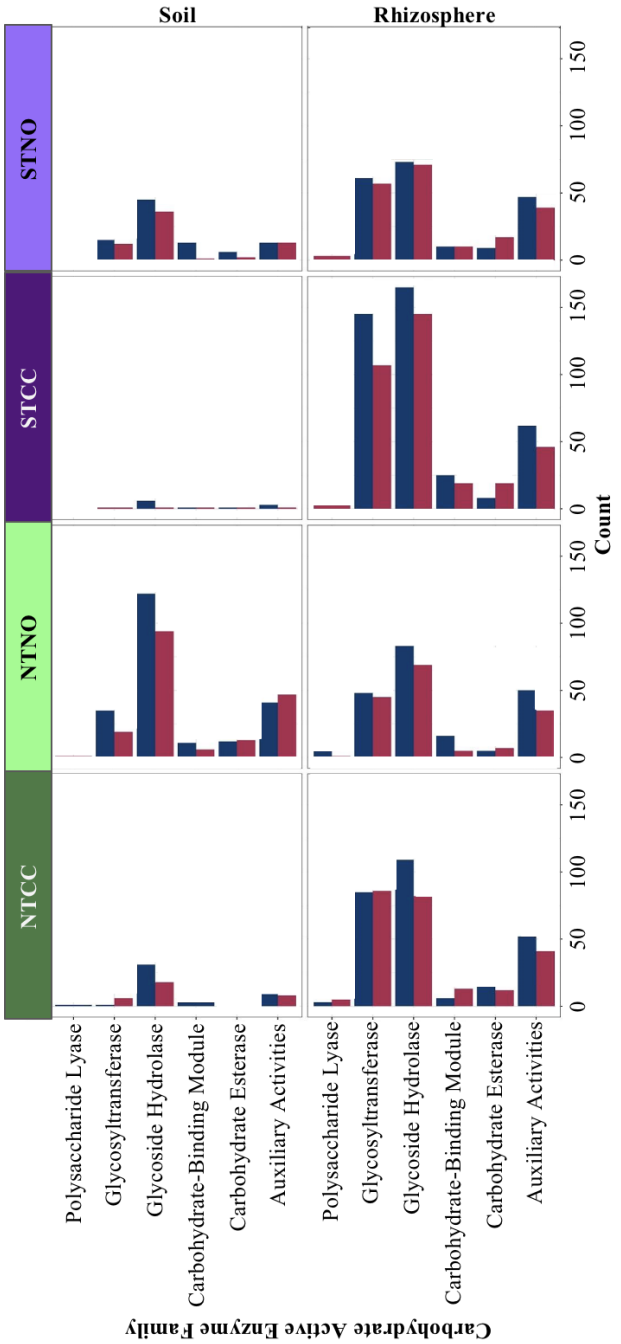


Figure 2-18.
Soil management regimes differentially alter carbon cycling dynamics. Bar plots depicting the number of carbohydrate active enzymes attributed to bacteria (pink) and fungi (blue) in the soil and rhizosphere across treatments - no till with cover-cropping (NTCC, dark green), no till leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), or standard till leaving the field fallow (STNO, light purple) - in the family classifications: polysaccharide lyases, glycosyltransferases, glycoside hydrolases, carbohydrate-binding modules, carbohydrate esterases, and auxiliary activities.

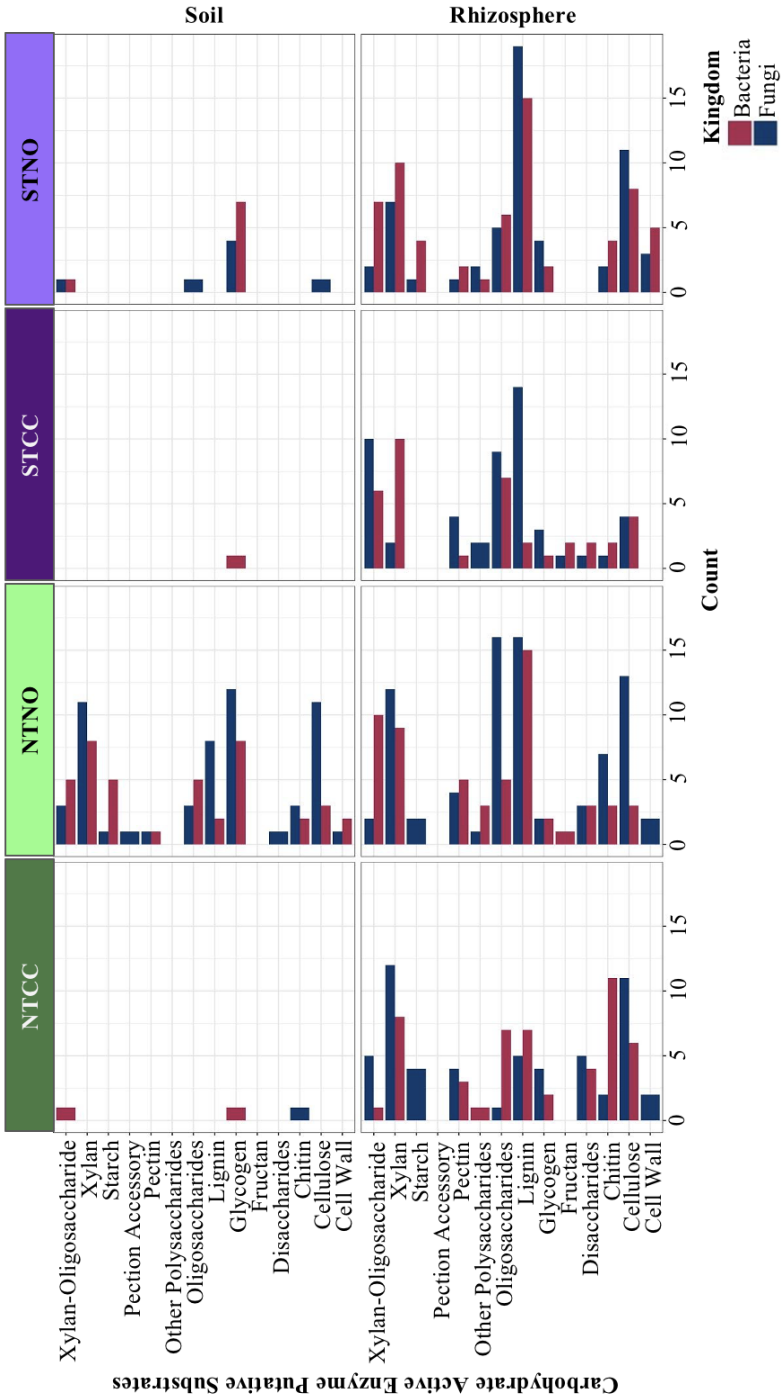


Figure 2-19.
A range of substrates are differentially metabolized across soil management treatment. Bar plots show counts of putative substrates to carbohydrate active enzymes (CAZy) attributed to bacteria (pink) and fungi (blue) in the soil and rhizosphere across treatments - no till with cover-cropping (NTCC, dark green), no till leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), or standard till leaving the field fallow (STNO, light purple), as based off the analysis performed by (Nuccio *et al.*).

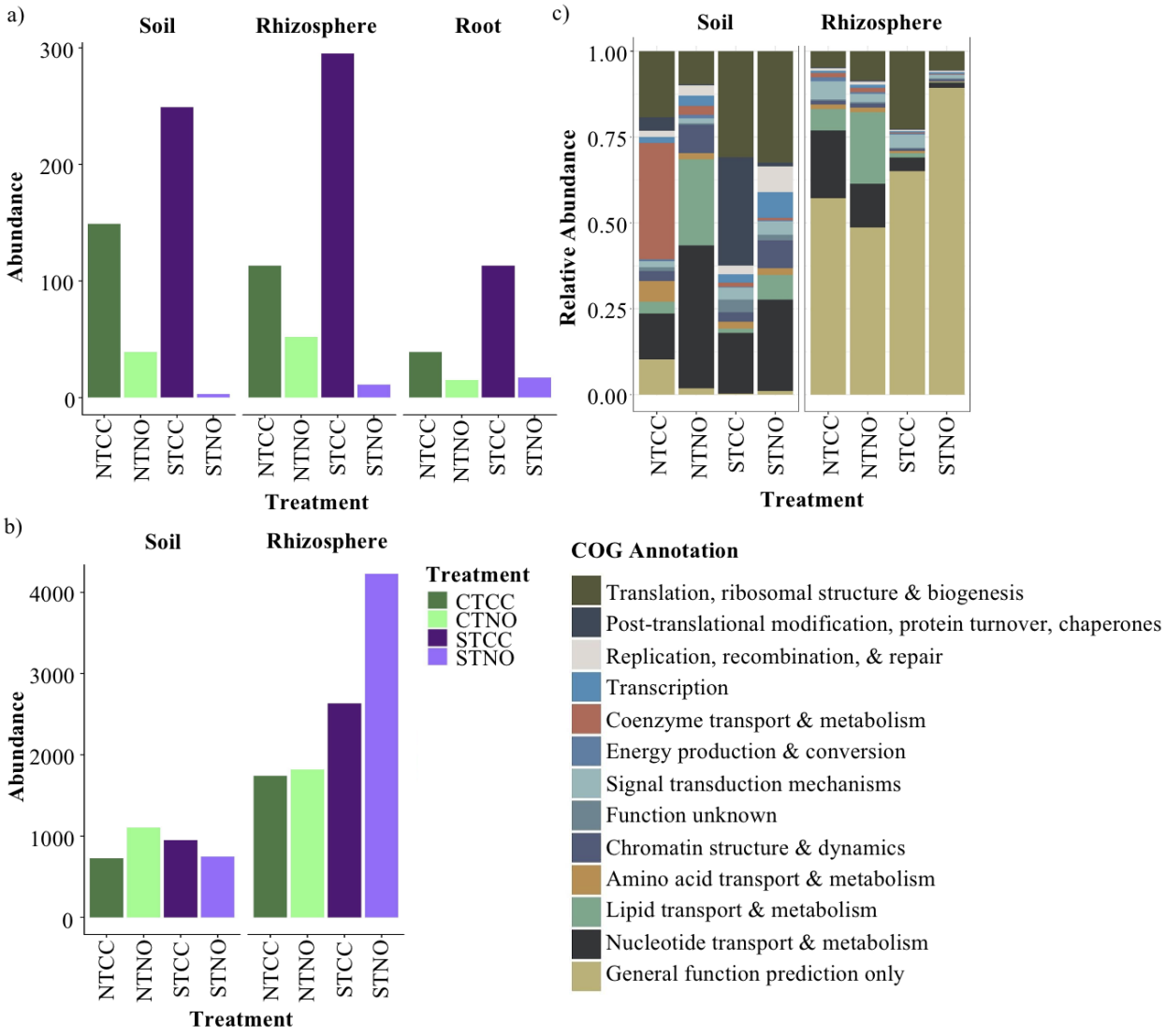


Figure 2-20.
Abundances and activity of agriculturally important fungal symbionts vary across soil management regimes. Bar plots showing counts of arbuscular mycorrhizal fungi (phylum Glomeromycota) at each time point with amplicon data (a) and metatranscriptomic data (b), as well as relative abundances of COG categories attributed to activity (c) across treatments of: no till with cover-cropping (NTCC, dark green), no till with leaving the field fallow during the off-season months (NTNO, light green), standard till with cover-cropping (STCC, dark purple), and standard till leaving the field fallow (STNO, light purple).

Figure 2-21.
Overview of metatranscriptome libraries generated. Read statistics, sequencing depth, rRNA and tRNA contamination levels, mRNA read counts and percent assembled reads are reported for each metatranscriptome library. The assembled reads in our dataset are 2.3% from Archaea, 48.47% from Bacteria, 48.33% from Eukaryota (25% from Fungi only), and 0.89% from Viruses. Treatment includes no till with cover-cropping (NTCC), no till leaving the field fallow during the off-season months (NTNO), standard till with cover-cropping (STCC), or standard till leaving the field fallow (STNO).

Total Number of Genes	% RNA Genes	% Protein Coding Genes	Number of Protein Coding Genes	% Genes with Function Prediction	Number of Reads with Taxonomic Assignment	% Archeal Reads	% Bacterial Reads	% Total Eukaryotic Reads	% Fungal Only Reads	% Viral Reads
1,090,819	14.14%	85.86%	936,623	56.57%	531,482	2.30%	48.47%	48.33%	25.00%	0.89%

Treatment	Sample Type	Block	GOLD Analysis Project ID	IMG Taxon ID	Number of Sequences	Number of Bases	% rRNA and tRNA Reads	Number of Protein Coding Reads	% of Reads Assembled
NTCC	Rhizosphere	B1	Ga0311004	3300029276	61,980	36,116,671	8.80%	59,566	91.20%
NTCC	Rhizosphere	B2	Ga0256744	3300023309	53,032	28,193,070	18.24%	45,754	81.76%
NTCC	Rhizosphere	B3	Ga0311001	3300029277	103,882	54,507,166	5.61%	105,343	94.39%
NTCC	Soil	B1	Ga0311009	3300029278	29,269	14,475,400	20.62%	23,818	79.38%
NTCC	Soil	B3	Ga0311006	3300029270	14,735	7,560,502	42.76%	7,915	42.76%
NTNO	Rhizosphere	B1	Ga0311000	3300029272	37,273	18,984,266	26.83%	27,413	73.17%
NTNO	Rhizosphere	B2	Ga0310982	3300029283	51,107	26,232,484	10.77%	47,720	89.23%
NTNO	Rhizosphere	B3	Ga0256714	3300023304	32,210	16,744,710	14.08%	28,175	85.92%
NTNO	Soil	B1	Ga0256741	3300023306	44,370	22,720,376	8.43%	41,998	91.57%
NTNO	Soil	B2	Ga0310983	3300029275	51,310	24,555,821	8.48%	50,787	91.52%
NTNO	Soil	B3	Ga0311005	3300029281	65,591	33,823,734	8.67%	64,571	91.33%
STCC	Rhizosphere	B1	Ga0311003	3300029282	80,084	50,708,870	5.54%	81,223	94.46%
STCC	Rhizosphere	B2	Ga0256720	3300023438	61,997	37,774,483	9.59%	59,106	90.41%
STCC	Rhizosphere	B3	Ga0311011	3300029280	49,992	31,887,832	11.44%	46,886	88.56%
STCC	Soil	B2	Ga0311002	3300029279	35,806	18,059,170	19.81%	28,721	80.19%
STCC	Soil	B3	Ga0263217	3300024457	17,049	8,845,536	48.41%	8,574	51.59%
STNO	Rhizosphere	B1	Ga0311008	3300029273	32,269	17,665,579	33.94%	21,596	66.06%
STNO	Rhizosphere	B2	Ga0256743	3300023436	30,583	15,805,537	27.54%	22,716	72.46%
STNO	Rhizosphere	B3	Ga0310981	3300029285	100,237	49,904,772	11.44%	93,408	88.56%
STNO	Soil	B1	Ga0311007	3300029271	22,525	11,661,077	34.18%	14,630	65.82%
STNO	Soil	B2	Ga0256742	3300023305	24,239	11,720,513	19.41%	20,002	80.59%
STNO	Soil	B3	Ga0311010	3300029274	43,411	22,503,338	18.47%	36,701	81.53%

Chapter 3

Distinguishing Between the Impacts of Heat and Drought Stress on the Root Microbiome of *Sorghum bicolor*

Parts of this chapter have been adapted from the following with permission:
Heidi M-L. Wipf, Thảo-Nguyên Bùi, and Devin Coleman-Derr, Distinguishing between the impacts of heat and drought stress on the root microbiome of *Sorghum bicolor*. Phytobiomes Journal. Submitted July 17, 2020; in review.

3.1 Abstract

Water deficit and extreme temperatures regularly and considerably reduce crop productivity. While several studies have explored the role of the plant microbiome in drought tolerance, it remains to be determined how the often co-occurring factor of heat influences plant-microbe interactions. In order to compare the roles temperature and drought stress play in plant microbial recruitment, we conducted a growth chamber experiment with a series of temperatures (22°C, 30°C, and 38°C) and watering (drought versus watered) conditions in *Sorghum bicolor*, sampling soil mixtures and seedling roots at 7 and 21 days post initial stress initiation. We found that bacterial root communities had the lowest alpha diversity when under drought and at 38°C, and that temperature influenced the beta diversity of soil mixture and root microbiomes to a greater extent than watering treatment. Additionally, we observed that the relative abundances of Actinobacteria increased both under drought and incrementally with higher temperatures. Further, unique Actinobacteria were indicator species of either temperature or watered conditions and were one of the top phyla whose indicators are predominantly of high temperatures in watered conditions. Together, these data suggest that heat and drought stress differentially impact microbiome assembly in significant ways and unique Actinobacteria are recruited under either stress. This work informs our understandings of how abiotic stresses shape crop microbiomes, as well as highlights the need for additional work to describe what mechanisms are involved in host-mediated microbial recruitment and adaptation under various stresses in field-based studies.

3.2 Introduction

In the predictions of what the future holds for our global community, numerous questions arise surrounding how to fortify food production in light of heightening impacts of climate change and rapid population growth. An important component of solution-oriented efforts for improved food security involves understanding the mechanics and interplay of the microbial world with that of crop plants. Recent work has highlighted the significance of plant-microbe interactions in supporting plant health and yield (Santoyo *et al.*, 2017), and evidence suggests microbes can promote plant drought tolerance (Xu & Coleman-Derr, 2019). Further, microbes in roots (i.e. endophytes) are known to impact an extensive range of drought traits in host plants (Malinowski & Belesky, 2000; Dastogeer *et al.*, 2018; Dhanyalakshmi *et al.*, 2019; Qin *et al.*, 2019). While several studies have been conducted in the field investigating drought stress impacts on the microbiome, high temperatures often co-occur with water deficit and may potentially influence microbial recruitment to a significant degree. Indeed, past work indicates that drought and heat waves can restructure soil microbial communities at large to a greater extent when in combination, over soil drying alone (Bérard *et al.*, 2011). More research is needed to tease apart the contributions of heat and drought stress on the structuring of the plant microbiome, however.

In plants, a significant number of studies have noted separate and interactive effects of drought and heat stress on growth and yield. A number of genetic, biochemical, and physiological factors influence stress responses (Lamaoui *et al.*, 2018), and hundreds of transcripts shift with either heat and drought stress, in addition to a unique response when both stresses are combined (Rizhsky *et al.*, 2002, 2004). Furthermore, when alone, and to a greater extent when combined, both stresses reduce photosynthetic rates, root nutrient uptake, and yield (Zandalinas *et al.*, 2018; Hussain *et al.*, 2019). Root morphological traits can significantly vary across drought, heat, and both combined stresses, with increasing reductions in root biomass and length, respectively (Wu *et al.*, 2017). Given this variation, and that root system architecture is known to influence root and rhizosphere microbiome establishment (Saleem *et al.*, 2018), these factors may differentially influence microbiome assembly. However, we know of no prior studies that have distinguished between heat and drought stress impacts on the sorghum root microbiome.

Host recruitment of microbes can significantly impact the extent to which abiotic stress influences plant growth. Seed germination has been shown to improve under heat and drought stress when endophytes are present (Hubbard *et al.*, 2012), and bacterial seed treatment positively impacts seedling heat stress tolerance (El-Daim *et al.*, 2014). Past work has also shown that plant biomass can be bolstered under drought stress with the addition of various plant-growth promoting microbes (Rolli *et al.*, 2015). One pattern of particular interest is that plants under drought enrich for monoderm bacteria, particularly Actinobacteria (Santos-Medellín *et al.*, 2017; Fitzpatrick *et al.*, 2018; Xu *et al.*, 2018), which have been shown to be more prevalent across a variety of drought-treated soils and rhizospheres (Bouskill *et al.*, 2013, 2016; Nessner Kavamura *et al.*, 2013). Interestingly, Actinobacteria have been shown to promote plant growth via a variety of mechanisms (Sathya *et al.*, 2017) and are also thought to be highly tolerant of arid environments (Stevenson & Hallsworth, 2014; Cherif *et al.*, 2015). Although this robustness is due, in part, to their ability to sporulate, recent work suggests Actinobacterial enrichment under drought in the plant microbiome is not correlated with this ability (Naylor *et al.*, 2017).

Although the size of Actinobacterial enrichment under drought is known to vary across different host systems and studies, as well as study sites, the factors explaining this variation remain to be fully characterized. Naylor et al. observed slight increases - of 3-20% - in the relative abundance of the phylum Actinobacteria in a variety of grass species roots under drought, in a field site ranging between 6°C and 25°C in temperature (Naylor *et al.*, 2017). Conversely, in a field site that reaches high daytime temperatures of 32°C to 38°C, the relative abundance Actinobacteria in droughted plant roots varied between 35-80% (Xu *et al.*, 2018). While the effects of drought on plants have been extensively reported on, it can be difficult to tease apart impacts of drought and co-occurring heat in field studies, including the degree of involvement of either in helping explain differences in the magnitude of Actinobacterial enrichment. Other edaphic factors - including variation in soil aggregate size and pore space, as well as chemical composition - in addition to differences in drought inducement, may also contribute to these reported differences, and more work is needed to better characterize the impacts of heat stress, both in combination with and isolated from drought stress, on microbial assembly in plant roots.

In this study, we sought to disentangle temperature and drought impacts on plant-associated microbial communities. We hypothesized that Actinobacteria would be enriched to the greatest extent in roots when both heat and drought stress were applied, versus either alone. In growth chambers, we utilized three temperature conditions (22°C, 30°C, 38°C) and a drought and control watering regime to induce a combination of heat and drought stress on sorghum seedlings. We sampled at two time points - 16 days old and 7 days stressed, and 30 days old, 21 days stressed - in order to characterize the associated microbiome over a period of prolonged stress exposure. Our results demonstrate that temperature and watering treatments distinctly impact the diversity and composition of sorghum root bacterial communities, particularly in relation to degree of Actinobacterial enrichment. Further, we show that time point influences the relative impact of heat versus drought on the amount and type of indicator species present. These findings contribute to our understandings of how abiotic stress factors help coordinate host recruitment of microbial communities, specifically in a drought-tolerant crop, and inform efforts geared towards utilizing the microbiome for improved plant growth in adverse growing conditions.

3.3 Results

3.3.1 Phenotypic analysis of heat and drought stress

To determine how heat stress may contribute to an enrichment of Actinobacteria, as characterized prior in roots under drought (Naylor *et al.*, 2017; Xu *et al.*, 2018; Simmons *et al.*, 2020), we utilized a set of growth chambers to grow sorghum in a mixture of field soil, calcine clay, and vermiculite (1:17:2) and initiated temperature and drought stresses when seedlings were 9 days old. To establish treatment effect sizes, we measured percent moisture in soil mixtures, plant height, fresh shoot biomass, and leaf relative water content at both times of sampling the soil and root microbiomes, with 16 day old seedlings having undergone 7 days of stress conditions (TP1), and 2 weeks later with 30 day old plants that were 21 days stressed (TP2) (Figure 3-1).

Within each temperature treatment, we observed that drought markedly impacts moisture content of soil mixtures, as most apparent by the second time point ($p\text{-value} < 0.001$) (Figure 3-2). Importantly, however, within the watered treatments, no significant effect of temperature was observed for mixture moisture levels, as well as leaf relative water content (Figure 3-3a). This suggested that downstream effects on microbiome composition could be primarily attributed to either temperature alone or temperature by watering treatment. We also observed that plant height and shoot biomass were generally reduced with increasing temperature and drought (Figure 3-3b-d). Conducting analyses of variance (ANOVA) for all attributes measured, temperature and watering treatment were statistically significant factors in all tests (Figure 3-4). These results indicated that our temperature and drought treatments did induce significant physiological changes in the plant host and soil mix moisture levels, providing us with a platform to better distinguish heat and drought impacts on the plant microbiome.

3.3.2 Bacterial diversity shifts most significantly in response to combined temperature and drought stress

In order to characterize the separate and combined impacts of heat and drought stress on the sorghum microbiome, we performed amplicon sequencing of the V3-V4 region of the 16S rRNA gene for soil mixture and root bacterial microbiomes across both time points. Using Shannon diversity indices, we determined that alpha diversity at large decreases under drought, with the only exception being roots from the 22°C temperature treatment (Figure 3-5). We also found that higher temperatures (30°C and 38°C versus 22°C) corresponded to higher alpha diversity levels in both soil mixture and root fractions (Figure 3-5). Distinctions based on time point, particularly in roots, were apparent, where diversity was highest in the watered 30°C treatment in the first round of sampling, and by TP2, was highest in the watered 38°C treatment (Figure 3-5). With ANOVA, we determined that watering treatment is a significant factor across all time points and sample types (p-values<0.05), while temperature treatment is a statistically significant factor in explaining alpha diversity variation only for TP1 roots (p-value<0.01) and TP2 soil mixtures (p-value<0.001) (Figure 3-6). We found similar patterns when looking at phylogenetic diversity (Faith, 1992).

To further explore the impacts of temperature and watering treatment on bacterial composition, as compared with more well-characterized factors of sample type and time point, we utilized Principle Coordinate Analysis (PCoA) and Canonical Analysis of Principal (CAP) Coordinates of Bray-Curtis dissimilarity. As expected, we found that samples clustered primarily by sample type (Figure 3-7), and variation in beta diversity is explained to a greater degree by temperature ($F=9.3$, p-value<0.001) than watering treatment ($F=6.6$, p-value<0.001), as determined by permutational multivariate analysis of variance (PERMANOVA) (Figure 3-6). Further, these factors combined explained between 36% to 39.5% of the variation in roots, and 29.4% to 42.8% in the soil mixture (Figure 3-8). Additionally, the variation in beta diversity attributed to temperature treatment is greater in roots (29.7-31.4%) than soil mixture (21-30.9%), but the opposite pattern occurs with watering treatment (6.3% to 8.1% for roots versus 8.4% to 11.9% for soil mixture) (Figure 3-8). Unsurprisingly, the later time point also showed an accentuation of the variation attributed to both factors. Our analyses further indicated that temperature and watering treatments, separately, are statistically significant factors explaining beta diversity variation within all sample types and time points (p-value<0.01), with the important exception of watering treatment in TP1 roots (Figure 3-9). These results suggest that the bacterial diversity associated with both plant roots and the surrounding substrate is significantly influenced by heat and drought stress, and temperature treatment may exert a greater force in shaping community dynamics than drought.

3.3.3 Relative abundance of Actinobacteria dynamically responds to both heat and drought treatment

To further explore the impacts of temperature and watering treatment on bacterial composition, as compared to more well-studied factors of sample type and time point, we plotted the relative abundances of the top 11 dominant phyla (Figure 3-10). For soil mixtures, we observed that several patterns emerged across treatments, including a relatively reduced abundance of Gemmatimonadetes, Acidobacteria, and Bacteroidetes under drought and greater abundance of Actinobacteria and Firmicutes with drought (Figure 3-10a,b). Additionally, increasing

temperature promoted Verrucomicrobia abundance, while drought repressed it in soil mixtures (Figure 3-10a,b). Root fractions showed more distinct changes in composition with treatment than soil mixtures, where we detected decreasing relative abundances of Proteobacteria with heat treatment, while Bacteroidetes increased (Figure 3-10c,d). For Actinobacteria specifically, increasing temperature and drought corresponded with greater relative abundances in both plant roots and the surrounding soil mixture (Figure 3-10). To further characterize shifts in Actinobacteria due to treatment, we plotted the top 9 most abundant genera in the phylum Actinobacteria by sample type (Figure 3-11). We found greater counts in roots, as compared to the soil mix, and observed that *Streptomyces* largely dominated the root fraction for both time points, particularly when higher temperature was combined with drought (Figure 3-11). These results indicate that drought and temperature correlate with distinct restructuring of bacterial communities, as observable on the phylum level, with temperature having a significant, isolated impact on Actinobacteria in roots, that corresponds to greater Actinobacterial enrichment when higher temperatures and drought are combined.

3.3.4 Temperature is associated with a greater number of indicator taxa than drought in roots

To identify which bacterial lineages may be differentially enriched relative to temperature and watering conditions, we performed a series of indicator species analyses (p-value<0.05) in TP 1 and 2 root fractions (Figures 3-12; 3-13; 3-14). We observed that a majority of indicator taxa corresponded to watered treatment conditions in both time points (TP1: 9 watered indicators, 100% of all indicators; TP2: 77, 83%) (Figures 3-13a; 3-12a), and Actinobacteria were wholly indicative of drought in TP2 (13 OTUs) (Figure 3-12a,c). In order to determine which indicators uniquely corresponded to temperature, versus drought and temperature combined, we subset our data into watered and drought samples for separate analyses. We also eliminated the middle temperature range (30°C) for this analysis and focused on the 22°C and 38°C temperature treatments in attempts to analyze the greatest distinction between treatments. For TP1 watered roots, there were more indicators for high temperature (28), as compared to 22°C (16), and interestingly, a majority of Actinobacteria were indicators for 38°C (83%, 5 out of 6 total) and all indicator Cyanobacteria grouped with 38°C (Figure 3-13b,d). In the watered subset for TP2 roots, we similarly found a greater number of indicators for 38°C than 22°C treatments (64 versus 57, respectively), and both types were generally well-parsed across major phyla, with the exception of Actinobacteria, which was primarily comprised by high temperature indicators (82% for 38°C, 9 out of 11 total) (Figure 3-12b,c). Additionally, Chloroflexi and Cyanobacteria only contained high temperature indicators in TP2 watered roots (Figure 3-12b).

In the drought subset for TP1 roots, where there were overall less temperature indicators for drought, as compared to watered subset (21 versus 41, respectively), and a minority of Actinobacteria were indicative of 38°C (1 out of 3 total) (Figure 3-13c). Analyzing the drought subset of TP2 roots, 58 of 63 indicators corresponded to 22°C and approximately one fourth of Actinobacteria (3 out of 11 total) were indicators corresponding to high temperatures (Figure 3-14). Interestingly, of all Actinobacteria indicators from these analyses (32), only 4 were a shared indicator of both watering treatment and temperature treatment (Figure 3-15). Taken together, these results demonstrate that not only does temperature alone have a significant impact on structuring the bacterial root microbiome, but high temperature corresponds to unique indicator taxa that overlap little with those corresponding to drought. Additionally, time point

comparisons suggest that heat exerts a greater influence than drought on root microbiome assembly more rapidly after initial stress induction.

3.4 Discussion

This study provides one of the first looks into how heat and drought stress, separately and when combined, contribute to assembly patterns in the root bacterial microbiome, particularly with regards to enrichment of the phylum Actinobacteria. We hypothesized that heat and drought stress together would correspond to distinct microbial community profiles, including a greater enrichment of Actinobacteria, than with either stress alone. Using growth chambers to test how a series of temperatures and drought impact bacterial root endophytes, we found that increasing temperature did indeed increase the relative abundance of Actinobacteria, independent of drought, in the root. In heated soils, Actinobacteria have been observed as a dominant taxa in soil communities (Riah-Anglet *et al.*, 2015), as well in sugar beet (*Beta vulgaris* L.) rhizospheres (van der Voort *et al.*, 2016). Actinobacterial metabolism is also known to shift in response to heat stress (Bursy *et al.*, 2008), and the ability of Actinobacteria to withstand high temperatures has been used in selective isolation (Hayakawa *et al.*, 2010; Guo & Zhang, 2014). Some factors that are thought to contribute these characteristics include how many Actinobacterial taxa are Gram-positive and monderms - where a thick peptidoglycan cell wall and ability to accumulate osmolytes may improve desiccation resistance (Hartman & Tringe, 2019), can sporulate to enter a stable and quiescent state to better persist through periods of environmental stress (Holmes *et al.*, 2000), and can produce various siderophores and other secondary metabolites that prove advantageous for survival and recruitment by plants hosts (Lewin *et al.*, 2016). Tolerance to high temperatures is also commonly associated with changes in membrane lipids, increases in disulfide bonds, and genome reductions (Wang *et al.*, 2015). We recommend that future work describes the specific molecular mechanisms involved in differential microbial community assembly for host plants experiencing heat and drought stress - when stressors are in isolation, as well when combined. In further characterizing how heat impacts bacterial root communities, particularly for the phyla Actinobacteria, there is potentially greater potential for targeted and improved fortification of crops grown under more extreme climatic conditions.

In analyzing the distinct impact of heat on root bacterial communities, we found differentiation between drought and water in terms of specific indicator taxa, where Actinobacterial enrichment predominantly corresponded to higher temperatures in watered conditions, rather than under drought conditions. This suggests that drought may be driving specific Actinobacterial enrichment that may overpower heat stress-driven responses when together, and a different subset of Actinobacteria may be specialized for heat responses. This may be partially explained by how plants respond to these two different abiotic stressors, which has been shown to influence microbial recruitment (Fitzpatrick *et al.*, 2018). Past work indicates that heat stress may be more difficult to buffer against than drought, where plants can employ a number of physiological changes to conserve water and help bolster turgidity, but high temperatures can damage protein synthesis and structures, membranes, and inactivate major enzymes (Fahad *et al.*, 2017). Further, plant growth is reduced during drought (Anjum *et al.*, 2011), which corresponds with a slowing of root respiration (Bryla *et al.*, 1997), and drought may be impacting the degree of heat stress responses in root communities. Past work has also similarly found drought indicators wholly

comprise (Naylor *et al.*, 2017) or are largely dominated by the phylum Actinobacteria (Xu *et al.*, 2018), whereas Proteobacteria - particularly the classes Alpha- and Betaproteobacteria - are dominant within watered indicators (Naylor *et al.*, 2017; Xu *et al.*, 2018). We recommend further studies to characterize what genomic and metabolic differences may exist in Actinobacterial indicators of heat versus drought stress in plant roots, to better understand how each abiotic stress contributes to microbial recruitment and the implications of those individual taxa on plant growth.

Our results also demonstrate a difference in timing with the degree watering treatment, versus temperature, impacts root bacterial communities, including the number of indicator taxa for each respective factor. We found that heat rapidly promotes Actinobacterial enrichment, and this can occur in the absence of drought, suggesting that heat can rapidly restructure bacterial communities and drive recruitment of heat-specific indicators, while drought stress impacts - particularly with regards to Actinobacterial enrichment - are more apparent in the second time point, after two additional weeks of stress exposure. Indeed, drought has been shown to delay the early development of the sorghum microbiome (Xu *et al.*, 2018), as compared to watered controls, and we recommend more studies to determine if high temperatures prompt a more rapid development of the microbiome. In addition, not only have various phyla been shown to respond to distinct patterns of root exudation across plant growth (Chaparro *et al.*, 2014; Vives-Peris *et al.*, 2018), but both stressors are also known to influence root exudation rates and composition, where a higher proportion of carbon is allocated to exudation and greater numbers of secondary metabolites can be present (Mainali *et al.*, 2014; Gargallo-Garriga *et al.*, 2018; Vives-Peris *et al.*, 2018; Williams & de Vries, 2020). Some evidence also suggests that increased temperature can increase exudation rates (Uselman *et al.*, 2000), while drought reduces overall exudation (Williams & de Vries, 2020). To better understand how bacterial communities may uniquely collaborate with plants to survive environmental challenges, we recommend future studies look at long-term impacts of both stresses alone and combined, where some evidence suggests crops are more sensitive to stress in reproductive stages of development (Hussain *et al.*, 2019) and developmental stage is known to impact microbial community dynamics (Chaparro *et al.*, 2014).

Here, we demonstrate that elevated temperature is sufficient to significantly impact root bacterial communities and is distinct from the impact of drought, as well as show that Actinobacterial enrichment under drought increased with higher temperatures. We recommend future work considers the unique roles these separate abiotic factors may play in microbiome assembly and activity, particularly in relationship to plant growth and yield. Such efforts inform the aim of using endophytes for targeted manipulation of specific traits in crop plants, such as heat and drought tolerance and increased yield.

3.5 Materials and Methods

3.5.1 Experimental set-up.

To test the individual and combined impacts of drought and temperature stress on the root bacterial microbiome of sorghum, we conducted a set of growth chambers experiments in the Fall of 2019 to modulate temperature and other growing conditions. Relative humidity was set at 30% and a 16/8 hour period of light/dark was employed for the duration of the experiment,

where photosynthetic photon flux density ranged between 80 and 120 μmole/m²/s. In order for greater ease in extracting roots from the growing substrate, a mixture of vermiculite, calcine clay, and field soil collected from the UC Gill Tract (10:85:5) was utilized. At the start of the experiment, samples of the field soil and soil mixture batches were collected for DNA extraction and 16S rRNA gene sequencing and stored at -80°C until processing.

With a ten percent bleach solution, seeds of *Sorghum bicolor* (L.) Moench variety RTx430 were surface sterilized and rinsed with autoclaved water, and 3 seeds per 3.8 liters pot were then planted approximately 2 cm below the soil mixture surface. Sixty pots were seeded in total, to allow for 5 replicates per harvesting time point (2) per watering treatment (2) per temperature treatment (3). For the first 8 days after planting, temperature was set at a constant 30°C and seedlings were thinned to one plant per pot. On day 9, temperature and drought treatments were begun, where plants were randomly selected to be grown under one the following temperatures: 22°C day/16°C night, 30°C day/24°C night, or 38°C day/32°C night - with a total of 20 plants in each temperature treatment, 10 of which also were subjected to drought by the cessation of all watering. Control plants were checked daily and watered as needed to keep soil mixtures fully saturated, as determined by water draining into the base pan, and this excess water was removed. Twice weekly, plants were rotated within growth chambers to minimize corner and center effects due to lighting and potential differences in water evaporation rates.

3.5.2 Sample collection and processing.

In addition to harvesting plant roots and soil, measurements to determine soil moisture and plant height, shoot biomass, and leaf relative water content were collected at a first time point, when plants were 16 days old and 7 days stressed, and a second time point when plants were 30 days old and 21 days stressed (Supplementary Figure 1). Five plants were harvested, as replicates, per treatment per time point, along with a sample of the soil mixture approximately 7 to 8 centimeters away from plant stems, at depths 0-20 cm below the mixture surface with a 10% bleach sterilized small hand auger. Whole roots were collected, and roots and soil mixture samples were stored on dry ice until transferred to a -80°C freezer to hold until processing.

Root fractions were cleaned as described in detail previously (Simmons *et al.*, 2018). In brief, whole roots were placed in an epiphyte removal buffer (0.75% KH2PO4, 0.95% K2HPO4, 1% Triton X-100 in ddH2O; filter sterilized at 0.2 μM) and then sonicated (pulses at 160 W for 30 seconds, separated by a 30 second pause for 10 minutes at 4 °C), followed by two subsequent steps of rinsing with sterile water, in order to fully remove the rhizosphere fraction. After drying with absorbent tissue, roots were homogenized and ground to a fine powder using liquid nitrogen and a mortar and pestle, before being returned to -80°C.

3.5.3 DNA extraction and library preparation

Soil mixture and powdered root DNA was isolated using extraction kits (DNeasy PowerSoil Kit, Qiagen Inc., Carlsbad, CA) following the manufacturer's protocol. We then amplified the V3-V4 region of 16S ribosomal gene using a dual-indexed 16s rRNA Illumina iTags primer (341 F (5′-CCTACGGGNBGCASCAG-3′) and 785 R (5′-GACTACNVGGGTATCTAATCC-3′) as described previously (Takahashi *et al.*, 2014) using 5-Prime Hot Master Mix (catalog No. 2200410). After DNA extraction, DNA was diluted to 5 ng/μl and randomized in 96-well plates.

Water blanks were included on each 96-well plate as negative controls. PNA clamps were used to minimize host-derived amplicons from both chloroplast and mitochondrial 16S rRNA gene sequences (Lundberg *et al.*, 2013). Reactions included 11.12 µL DNase-free sterile H20, 0.4 µg BSA, 10.0 µL 5-Prime Hot Master Mix, and 2 µL template, and 0.75 µM of chloroplast and mitochondria PNAs. PCR reactions were performed in triplicate in three thermocyclers (to account for possible thermocycler bias) with the following conditions: initial 3 min cycle at 94 °C, then 30 cycles of 45 seconds at 94 °C, 10 sec at 78 °C, 1 min at 50 °C, and 1.5 min at 72 °C, followed by a final cycle of 10 min at 72 °C. Triplicates were then pooled and the DNA concentration of each sample was quantified using a Qubit 3 Fluorometer (Invitrogen, Carlsbad, CA, USA). Pools of amplicons were constructed using 100 ng for each PCR product. Before submitting for sequencing, pooled samples were cleaned with 1.0X volume Agencourt AMPureXP beads (Beckman-Coulter, West Sacramento, CA), according to the manufacturer's directions, except for the modifications of using 1.0X, rather than 1.6X, volume beads per sample, dispensing 1500 µL 70% EtOH to each well rather, than 200 µL, and eluting in 100 µL DNase-free H20, rather than 40 µL. An aliquot of the pooled amplicons was diluted to 10 nM in 30 µL total volume before submitting to the QB3 Vincent J. Coates Genomics Sequencing Laboratory facility at the University of California, Berkeley for sequencing using Illumina Miseq. 300 bp pair-end with v3 chemistry. Sequences were returned demultiplexed and with adaptors removed.

3.5.4 Amplicon sequence data processing, OTU classification and taxonomic assignment

Our sequencing data was analyzed using the iTagger pipeline developed by the U.S. Department of Energy's Joint Genome Institute (Tremblay *et al.*, 2015). This pipeline wraps several packages for the filtering, merging, clustering and taxonomy assignment, including CUTADAPT, FLASH, USEARCH, and RDP (Wang *et al.*, 2007a; Magoc & Salzberg, 2011; Martin, 2011; Alloui *et al.*, 2015). In brief, after filtering 16S rRNA raw reads for known contaminants (Illumina adapter sequence and PhiX), primer sequences were trimmed from the 5′ ends of both forward and reverse reads. Low-quality bases were trimmed from the 3′ ends prior to assembly of forward and reverse reads with FLASH (Magoc & Salzberg, 2011). The remaining merged reads were clustered with simultaneous chimera removal using UPARSE (Edgar, 2013). After clustering, 3,080,184 read counts mapped to 4,369 operational taxonomic units (OTUs) at 97% identity. The resulting reads produced on average approximately 998,475 and 1,431,892 reads per sample for soil mixtures and roots, respectively. Taxonomies were assigned to each OTU using the RDP Naïve Bayesian Classifier with custom reference databases (Wang *et al.*, 2007b). For the 16S rRNA V3-V4 data, this database was compiled from the May 2013 version of the GreenGenes 16S database (DeSantis *et al.*, 2006), the Silva 16S database (Quast *et al.*, 2013), and additional manually curated 16S rRNA sequences, trimmed to the V3-V4 region. After taxonomies were assigned to each OTU, we discarded all OTUs that were not assigned a Kingdom level RDP classification score of at least 0.5. To remove low abundance OTUs that are in many cases artifacts generated through the sequencing process, we removed OTUs without at least 2 reads in at least 2 samples. We also removed samples that had less than 10,000 reads, which yielded 1,997 high-abundance OTUs (respectively) for downstream analyses. These thresholds were found to be suitable using technical replicates in a dataset published previously (Coleman-Derr *et al.*, 2016b). To account for differences in sequencing read depth across samples, all samples were

rarefied to 10,000 reads per sample for specific analyses to yield 1,140,000 measurable, rarefied reads for downstream analysis.

3.5.5 Statistical analyses

RStudio (version 1.0.136; RStudio Team) was utilized for all statistical analyses with the packages phyloseq (McMurdie & Holmes, 2013) and vegan (Dixon, 2003). For plant phenotype data, scatter plots were generated using ggplot2, and Analysis of Variance (ANOVA) was performed with function aov. For the Alpha diversity measurement, Shannon Index of diversity and observed OTUs were calculated with the estimate_richness function in the R package phyloseq. ANOVAs were performed with function aov for Sample Type, Temperature Treatment, Watering Treatment, and Time Point. A Tukey's Post Hoc test was performed using the function TukeyHSD in the stats package and with HSD.test in the package agricolae to test which levels were significantly different from one another. Beta diversity was measured using Bray-Curtis distances and UniFrac distance with function ordinate in the R package phyloseq. Trees were built with default parameters using FastTree (Price *et al.*, 2010) with an alignment constructed in Muscle (Edgar, 2004), and figures produced with the Integrative Tree of Life (Letunic & Bork, 2007). Permutation multivariate analysis of variance analyses (PERMANOVA) were performed with the Adonis function in the R package Vegan using 999 permutations and the Bray-Curtis distances as inputs. Canonical Analysis of Principal Coordinates (CAPs) was performed for subsets of the data with each sample type and time point to determine the percent variance explained by treatment, time point and replicate, or treatment, sample type, and replicate, respectively, using the capscale function in the R package vegan (Dixon, 2003). The non-parametric Kruskal-Wallis test in R was used to compare Shannon indices and class-level relative abundances between treated and untreated within each time point and sample type. Indicator species analyses run on root samples to determine genera that were enriched for either control or amendment treatments were performed using R package indicspecies (De Cáceres & Legendre, 2009), with p-values < 0.05 based on permutation tests run with 999 permutations.

3.5.6 Data Availability

The raw sequencing reads for this project are deposited in the NCBI Short Read Archive and can be accessed through BioProject SUB7734635. All scripts used can be found at a public repository on Github: (https://github.com/colemanderr-lab/Wipf-2020).

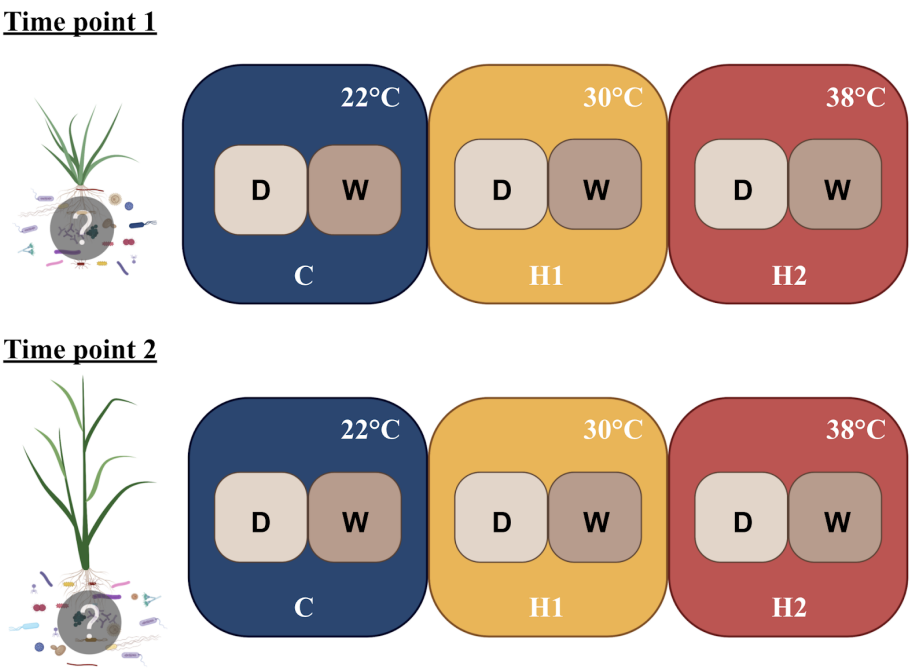


Figure 3-1.
Diagram of experimental set up and sampling. Abbreviations are as follows: ‘C’ denotes 22°C temperature treatment, ‘H1’ 30°C, and ‘H2’ 38°C; ‘D’ denotes drought, and ‘W’ watered conditions. Figure was created with BioRender.com.

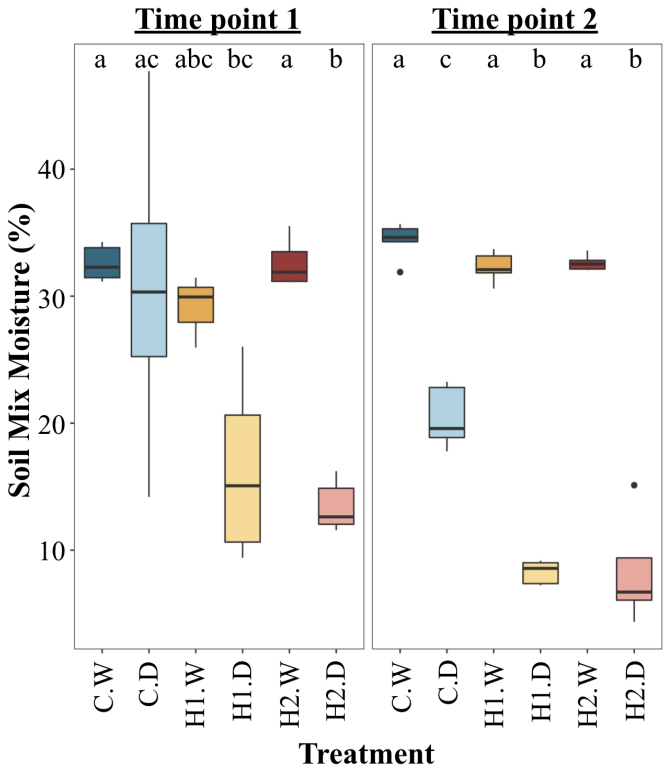


Figure 3-2.
Soil moisture is significantly impacted by drought and temperature. Boxplot of soil mixture moisture level (percent) across all treatments: C.W (22°C and watered), C.D (22°C and drought), H1.W (30°C and watered), H1.D (30°C and drought), H2.W (38°C and watered), and H2.D (38°C and drought).

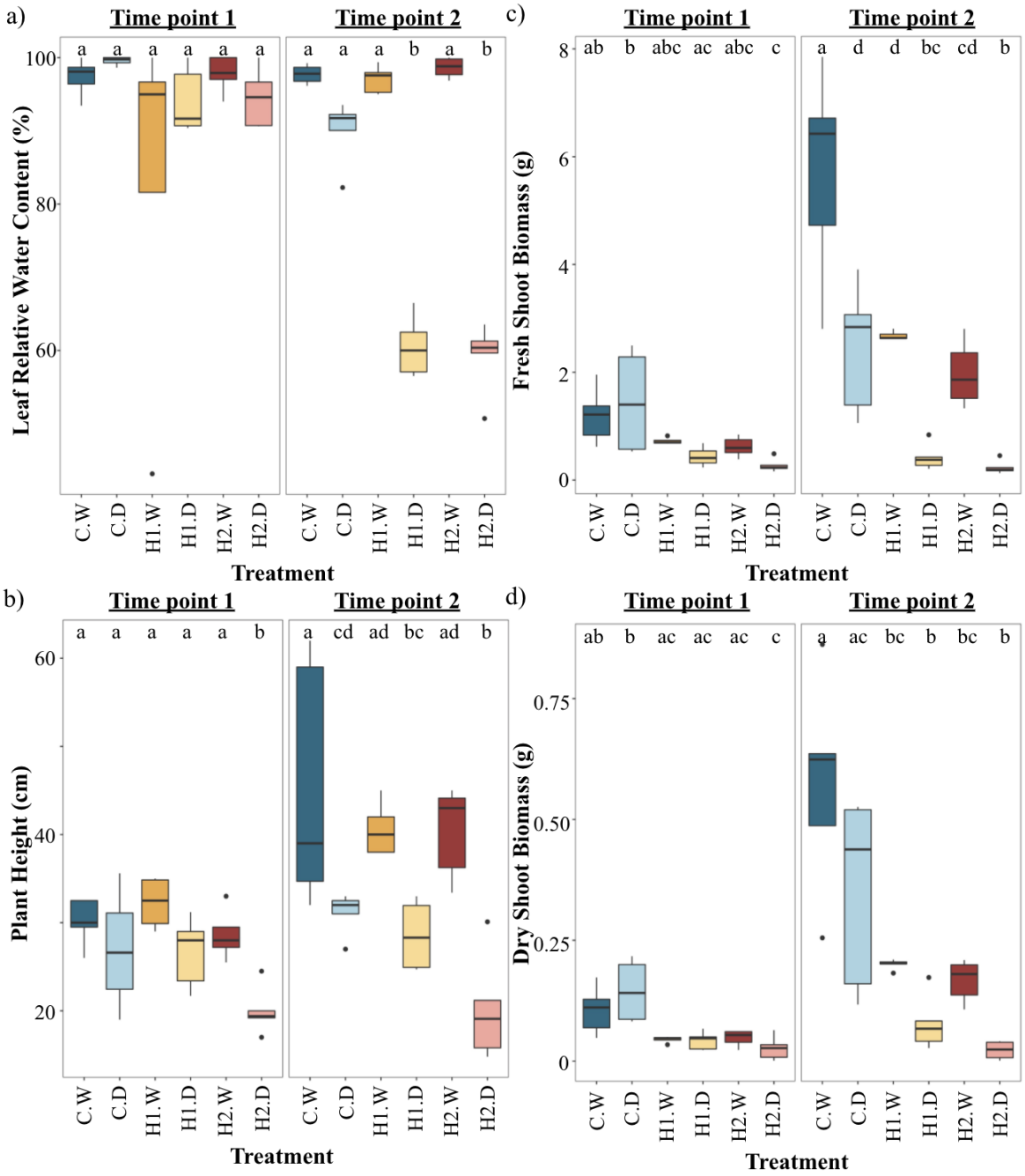


Figure 3-3.
Plant phenotype is significantly impacted by temperature and watering treatment. Boxplots of phenotypic data collected at both time points for of phenotypic data collected at both time points for a) plant height (in centimeters), b) leaf relative water content (percent), a) fresh shoot biomass (in grams) and b) dry shoot biomass (in grams) across all treatments: C.W (22°C and watered), C.D (22°C and drought), H1.W (30°C and watered), H1.D (30°C and drought), H2.W (38°C and watered), and H2.D (38°C and drought).

Figure 3-4.
Temperature and watering treatment significantly impact measured phenotypic parameters. Analysis of variance (ANOVA) results for soil mixture moisture content, plant height, leaf relative water content, and fresh and dry shoot biomass. Factors tested include time point, temperature and water treatment, and original mixture batch. Abbreviations are as follows: 'Trt' for treatment, ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

Data	Factor	Df	Sum Sq	Mean Sq	F value	P value	
Soil Mixture Moisture	TimePoint	1	79	79	3.062	0.09626	.
	Temp_Trt	2	820	410	15.858	8.90E-05	***
	Water_Trt	1	3956	3956	153.053	1.55E-10	***
	MixtureBatch	27	724	27	1.038	0.47507	
	TimePoint:Temp_Trt	2	8	4	0.162	0.85187	
	TimePoint:Water_Trt	1	284	284	11.005	0.00362	**
	Temp_Trt:Water_Trt	2	252	126	4.875	0.01955	*
	TimePoint:Temp_Trt:Water_Trt	2	9	5	0.183	0.83398	
	Residuals	19	491	26			
Plant Height	TimePoint	1	814.2	814.2	21.255	0.000191	***
	Temp_Trt	2	363.2	181.6	4.74	0.021376	*
	Water_Trt	1	1709.9	1709.9	44.639	2.18E-06	***
	MixtureBatch	27	966.5	35.8	0.934	0.5727	
	TimePoint:Temp_Trt	2	62.2	31.1	0.812	0.458613	
	TimePoint:Water_Trt	1	282.5	282.5	7.376	0.013713	*
	Temp_Trt:Water_Trt	2	47	23.5	0.614	0.551616	
	TimePoint:Temp_Trt:Water_Trt	2	1.1	0.5	0.014	0.986204	
	Residuals	19	727.8	38.3			
Leaf Relative Water Content	TimePoint	1	1569	1568.6	22.444	0.000126	***
	Temp_Trt	2	1460	730.2	10.448	0.000782	***
	Water_Trt	1	2584	2583.6	36.967	6.08E-06	***
	MixtureBatch	27	4276	158.4	2.266	0.031648	*
	TimePoint:Temp_Trt	2	158	78.9	1.128	0.343334	
	TimePoint:Water_Trt	1	2059	2059.4	29.467	2.59E-05	***
	Temp_Trt:Water_Trt	2	133	66.4	0.95	0.403472	
	TimePoint:Temp_Trt:Water_Trt	2	376	187.8	2.688	0.092496	.
	Residuals	20	1398	69.9			
Fresh Shoot Biomass	TimePoint	1	31.89	31.89	41.702	2.69E-06	***
	Temp_Trt	2	43.12	21.56	28.19	1.52E-06	***
	Water_Trt	1	23.94	23.94	31.303	1.78E-05	***
	MixtureBatch	27	27.05	1	1.31	0.269403	
	TimePoint:Temp_Trt	2	8.43	4.21	5.508	0.012426	*

	TimePoint:Water_Trt	1	12.3	12.3	16.088	0.000686	***
	Temp_Trt:Water_Trt	2	0.31	0.15	0.201	0.81938	
	TimePoint:Temp_Trt:Water_Trt	2	0.5	0.25	0.326	0.725625	
	Residuals	20	15.3	0.76			
Dry Shoot Biomass	TimePoint	1	0.3917	0.3917	40.803	3.12E-06	***
	Temp_Trt	2	0.6278	0.3139	32.699	4.96E-07	***
	Water_Trt	1	0.0873	0.0873	9.096	0.00683	**
	MixtureBatch	27	0.3266	0.0121	1.26	0.30018	
	TimePoint:Temp_Trt	2	0.1723	0.0861	8.974	0.00165	**
	TimePoint:Water_Trt	1	0.0667	0.0667	6.95	0.01583	*
	Temp_Trt:Water_Trt	2	0.0036	0.0018	0.189	0.82921	
	TimePoint:Temp_Trt:Water_Trt	2	0.0015	0.0008	0.08	0.92349	
	Residuals	20	0.192	0.0096			

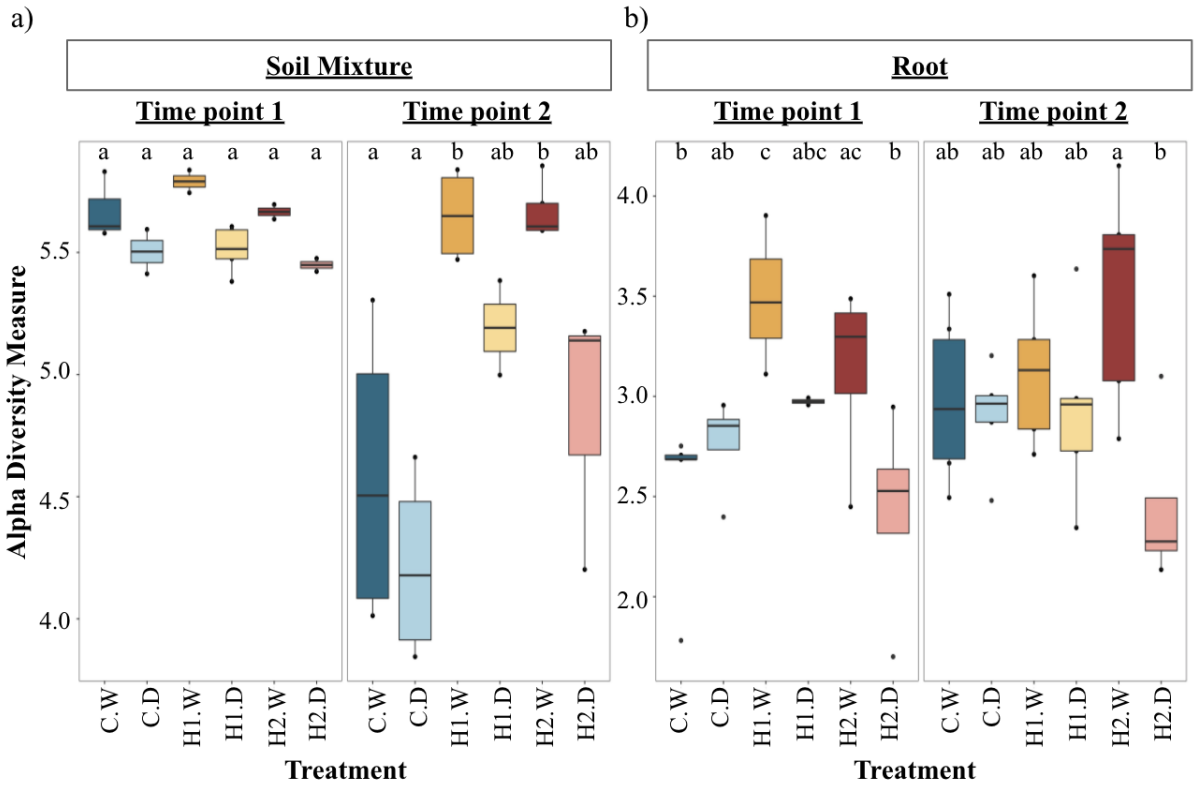


Figure 3-5.
Temperature and watering treatments differentially impact bacterial alpha diversity. Boxplots of alpha diversity, as based on Shannon diversity index, for the a) soil mixture and b) roots across both time points and all treatments: C.W (22°C and watered), C.D (22°C and drought), H1.W (30°C and watered), H1.D (30°C and drought), H2.W (38°C and watered), and H2.D (38°C and drought).

Figure 3-6.
Temperature and watering treatments significantly structure bacterial alpha diversity. Analysis of variance (ANOVA) results of Shannon Diversity indices for all data, as well as subsets by time point and sample type. Abbreviations are as follows: 'Trt' for treatment, ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

	Factor	Df	Sum Sqs	Mean Sqs	F value	P value	
All Data	SampleType	1	120.81	120.81	802.452	< 2e-16	***
	TimePoint	1	0.38	0.38	2.552	0.115299	
	Temp_Trt	2	3.38	1.69	11.224	7.07E-05	***
	Water_Trt	1	3.45	3.45	22.914	1.12E-05	***
	Replicate	5	0.73	0.15	0.967	0.445387	
	SampleType:TimePoint	1	2.56	2.56	17.02	0.000114	***
	SampleType:Temp_Trt	2	0.87	0.44	2.905	0.062376	.
	TimePoint:Temp_Trt	2	0.33	0.17	1.101	0.33893	
	SampleType:Water_Trt	1	0.11	0.11	0.753	0.389076	
	TimePoint:Water_Trt	1	0.16	0.16	1.045	0.310702	
	Temp_Trt:Water_Trt	2	1.68	0.84	5.566	0.006022	**
	SampleType:TimePoint:Temp_Trt	2	2.02	1.01	6.705	0.002333	**
	SampleType:TimePoint:Water_Trt	1	0.02	0.02	0.162	0.68885	
	SampleType:Temp_Trt:Water_Trt	2	0.33	0.17	1.107	0.337063	
	TimePoint:Temp_Trt:Water_Trt	2	0.32	0.16	1.059	0.353092	
	SampleType:TimePoint:Temp_Trt:Water_Trt	2	0.04	0.02	0.124	0.883374	
	Residuals	61	9.18	0.15			
TP1_Mix	Temp_Trt	2	0.00212	0.00106	0.086	0.9192	
	Water_Trt	1	0.18895	0.18895	15.281	0.0113	*
	Replicate	5	0.0177	0.00354	0.286	0.902	
	Temp_Trt:Water_Trt	2	0.01777	0.00889	0.719	0.5317	
	Residuals	5	0.06183	0.01237			
TP2_Mix	Temp_Trt	2	5.256	2.6279	20.161	0.000209	***
	Water_Trt	1	1.49	1.4899	11.431	0.006133	**
	Replicate	5	0.966	0.1932	1.482	0.271978	
	Temp_Trt:Water_Trt	2	0.225	0.1123	0.861	0.449296	
	Residuals	11	1.434	0.1303			
TP1_Root	Temp_Trt	2	1.4181	0.7091	10.252	0.00253	**
	Water_Trt	1	0.431	0.431	6.232	0.0281	*
	Replicate	4	1.9526	0.4881	7.058	0.00367	**
	Temp_Trt:Water_Trt	2	0.9836	0.4918	7.111	0.00919	**
	Residuals	12	0.8299	0.0692			
TP2_Root	Temp_Trt	2	0.0568	0.0284	0.18	0.8368	
	Water_Trt	1	1.2004	1.2004	7.604	0.0125	*
	Replicate	5	1.6923	0.3385	2.144	0.1041	

	Temp_Trt:Water_Trt	2	0.9033	0.4516	2.861	0.082	.
	Residuals	19	2.9995	0.1579			

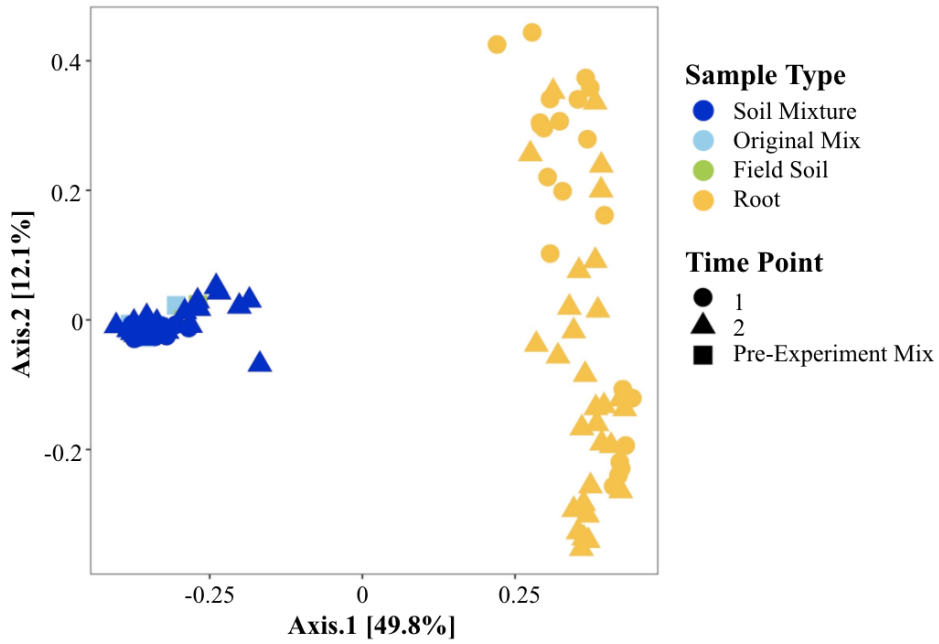


Figure 3-7.
Sample beta diversity is structured by sample type. Principal coordinates analysis of all samples using Bray-Curtis dissimilarity, with sample type indicated by color (soil mixture at time of sampling at time points 1 and 2, dark blue; original soil mix at the start of the experiment, cyan; field soil included in soil mixture, light green; root samples at time of sampling at time points 1 and 2, yellow) and time point by shape (time point 1, circle; time point 2, triangle; pre-experiment sampling, square. Field soil samples are tightly clustered with and located behind soil mixture samples.

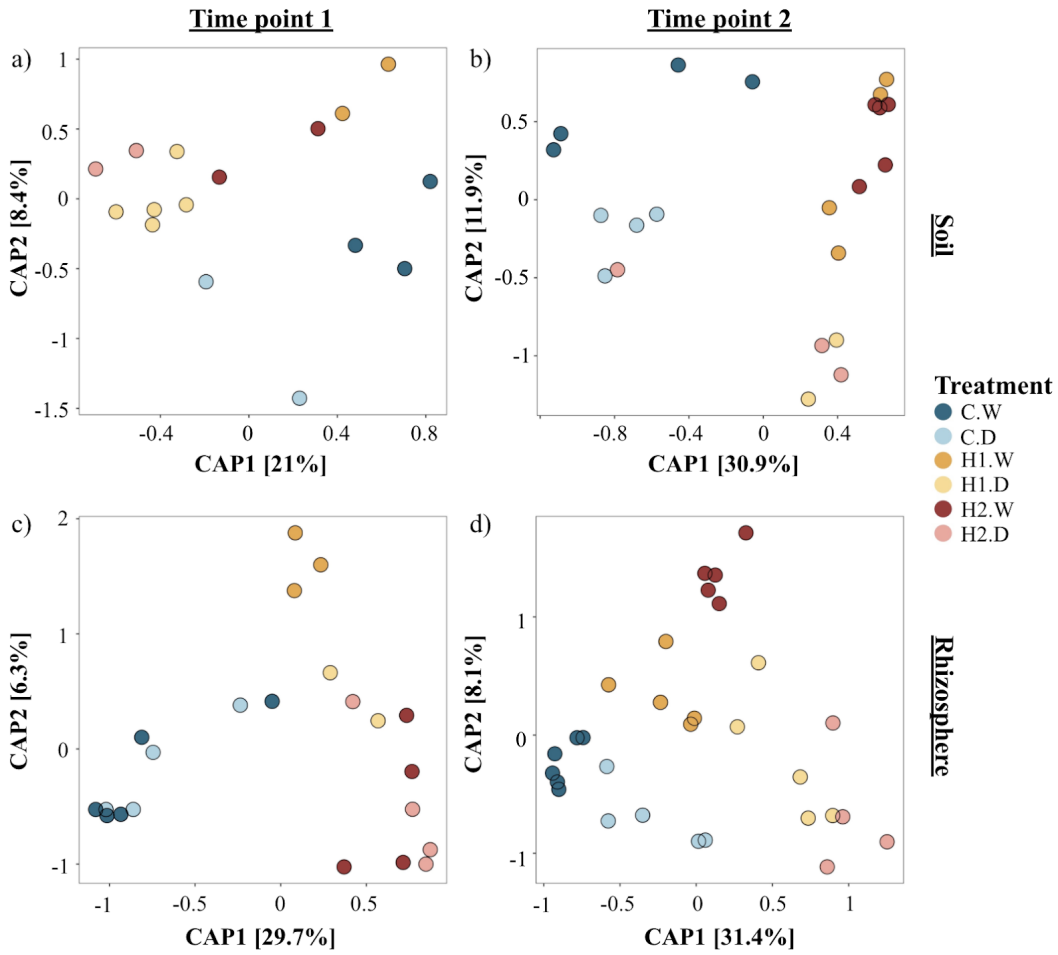


Figure 3-8.
Samples cluster by temperature and watering treatments. Constrained analysis of principal coordinates (CAP) of beta diversity, as based on Bray-Curtis dissimilarity, with the model of CAP1 attributed to temperature treatment, and CAP2 attributed to watering treatment for the a,b) soil mixture and c,d) roots for each time points and treatment: C.W (22°C and watered; blue), C.D (22°C and drought; light blue), H1.W (30°C and watered; orange), H1.D (30°C and drought; yellow), H2.W (38°C and watered; red), and H2.D (38°C and drought; light red).

Figure 3-9.
Temperature and watering treatments significantly structure bacterial beta diversity. Permutational multivariate analysis of variance (PERMANOVA) results of Bray-Curtis dissimilarity for all data, as well as subsets by time point and sample type. Abbreviations are as follows: 'Trt' for treatment, ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

	Factor	Df	Sum Sqs	Mean Sqs	F value	R2	P value	
All Data	SampleType	1	10.6147	10.6147	125.229	0.46089	0.001	***
	TimePoint	1	0.647	0.647	7.634	0.02809	0.002	**
	Temp_Trt	2	1.5804	0.7902	9.322	0.06862	0.001	***
	Water_Trt	1	0.5604	0.5604	6.612	0.02433	0.001	***
	SampleType:TimePoint	1	0.6137	0.6137	7.24	0.02665	0.002	**
	SampleType:Temp_Trt	2	1.073	0.5365	6.329	0.04659	0.001	***
	TimePoint:Temp_Trt	2	0.365	0.1825	2.153	0.01585	0.029	*
	SampleType:Water_Trt	1	0.3987	0.3987	4.703	0.01731	0.001	***
	TimePoint:Water_Trt	1	0.1622	0.1622	1.913	0.00704	0.087	.
	Temp_Trt:Water_Trt	2	0.2938	0.1469	1.733	0.01276	0.093	.
	SampleType:TimePoint:Temp_Trt	2	0.331	0.1655	1.953	0.01437	0.058	.
	SampleType:TimePoint:Water_Trt	1	0.1228	0.1228	1.449	0.00533	0.181	
	SampleType:Temp_Trt:Water_Trt	2	0.2242	0.1121	1.323	0.00973	0.185	
	TimePoint:Temp_Trt:Water_Trt	2	0.2522	0.1261	1.488	0.01095	0.149	
	SampleType:TimePoint:Temp_Trt:Water_Trt	2	0.1976	0.0988	1.165	0.00858	0.287	
	Residuals	66	5.5943	0.0848		0.2429		
	Total	89	23.0311			1		
TP1_Mix	Temp_Trt	2	0.14243	0.071217	1.9531	0.17904	0.007	**
	Water_Trt	1	0.13122	0.131221	3.5987	0.16494	0.001	***
	Replicate	5	0.24794	0.049588	1.3599	0.31166	0.07	.
	Temp_Trt:Water_Trt	2	0.09165	0.045823	1.2567	0.1152	0.152	
	Residuals	5	0.18232	0.036463		0.22917		
	Total	15	0.79556			1		
TP2_Mix	Temp_Trt	2	0.71336	0.35668	5.7481	0.31689	0.001	***
	Water_Trt	1	0.29403	0.29403	4.7385	0.13062	0.002	**
	Replicate	5	0.34661	0.06932	1.1172	0.15397	0.34	
	Temp_Trt:Water_Trt	2	0.21454	0.10727	1.7287	0.0953	0.076	.
	Residuals	11	0.68257	0.06205		0.30321		
	Total	21	2.25112			1		
TP1_Root	Temp_Trt	2	1.478	0.73901	6.9036	0.36504	0.001	***
	Water_Trt	1	0.107	0.10701	0.9997	0.02643	0.416	
	Replicate	4	0.9068	0.2267	2.1178	0.22397	0.012	*
	Temp_Trt:Water_Trt	2	0.2725	0.13623	1.2727	0.0673	0.244	
	Residuals	12	1.2845	0.10705		0.31726		

	Total	21	4.0488			1		
TP2_Root	Temp_Trt	2	1.0779	0.53896	6.7327	0.26492	0.001	***
	Water_Trt	1	0.6934	0.69342	8.6623	0.17042	0.001	***
	Replicate	5	0.4464	0.08928	1.1153	0.10971	0.301	
	Temp_Trt:Water_Trt	2	0.3301	0.16504	2.0617	0.08113	0.032	*
	Residuals	19	1.521	0.08005		0.37381		
	Total	29	4.0688			1		

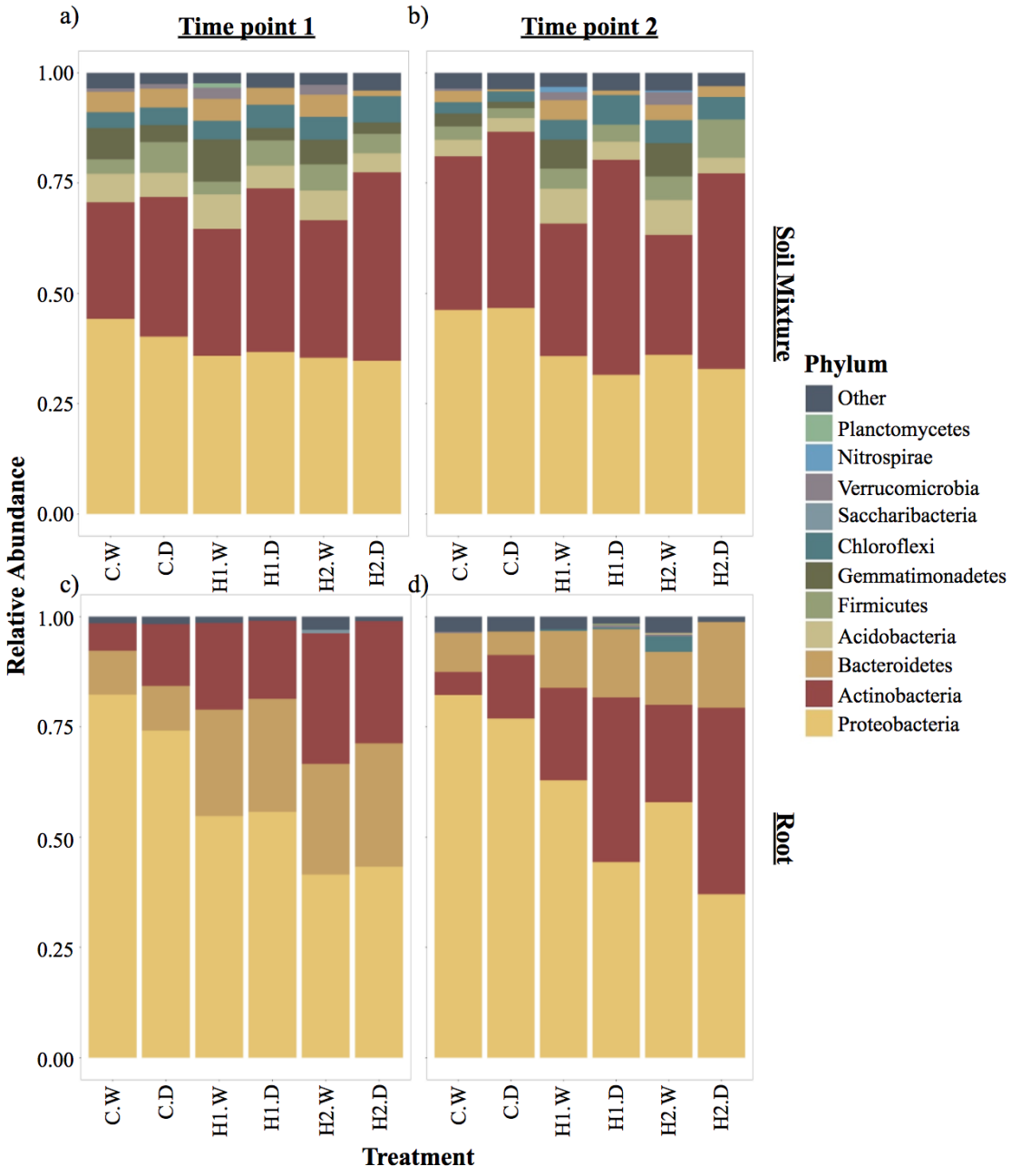


Figure 3-10.
Relative abundances of Actinobacteria increase with heat and drought over time. Bar graphs of the relative abundances of top 11 phylum across time point and sample type for treatments C.W (22°C and watered), C.D (22°C and drought), H1.W (30°C and watered), H1.D (30°C and drought), H2.W (38°C and watered), and H2.D (38°C and drought).

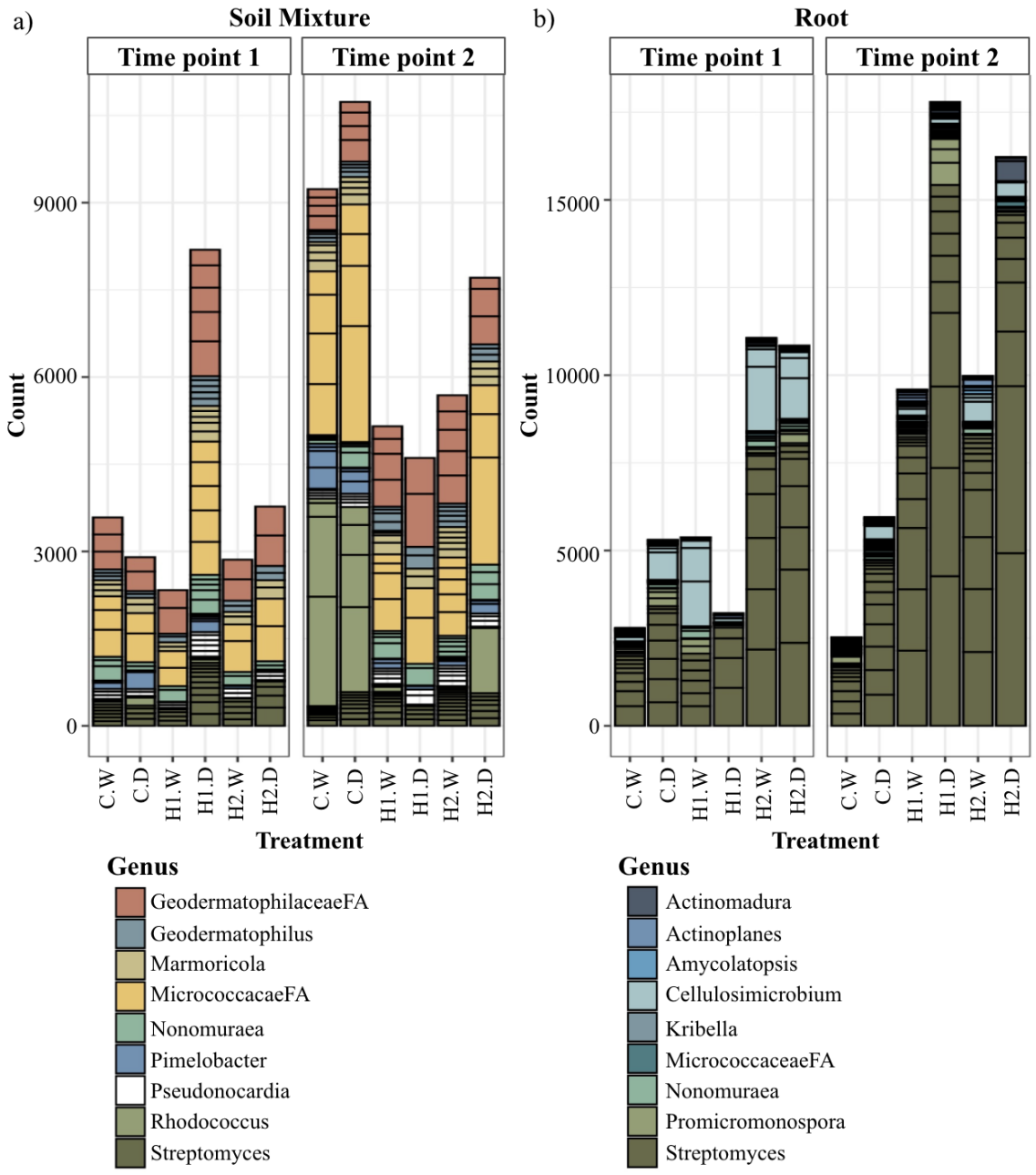


Figure 3-11.
Heat and drought correspond to increased counts of dominant Actinobacterial genera in the root. Bar graphs displaying the abundance of the top 10 most abundant genera in the phylum Actinobacteria for a) the soil mixture fraction, and b) roots, as occurs across time points and treatments, where 'C.W' corresponds to 22°C and watered, 'C.D' to 22°C and drought, 'H1.W' to 30°C and watered, 'H1.D' to 30°C and drought, 'H2.W' to 38°C and watered, and 'H2.D' to 38°C and drought.

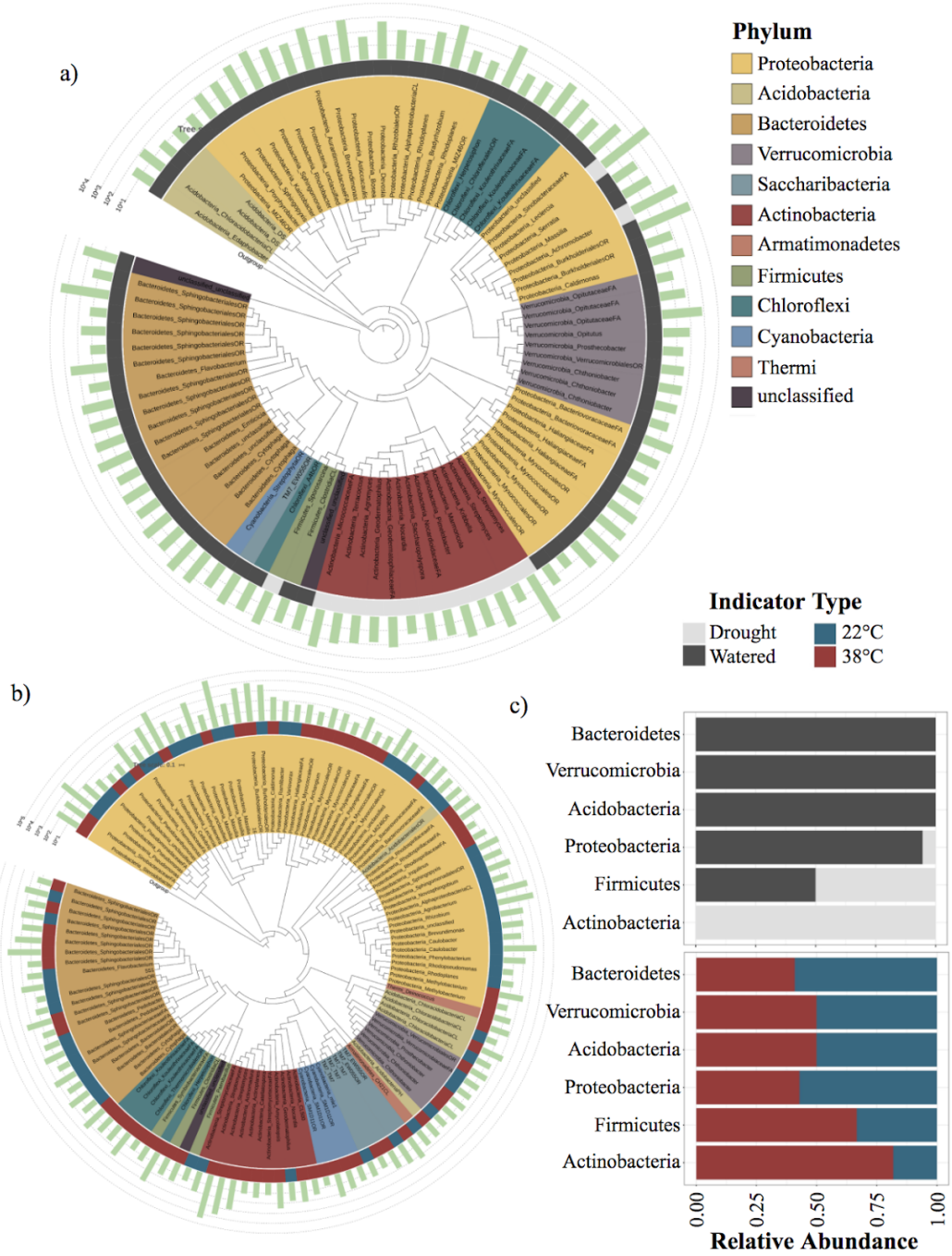


Figure 3-12.
Actinobacteria are largely indicators of drought at time point 2. Indicator specie analysis ($p<0.05$) results with time point 2 roots for a) watered (dark grey) and drought (light grey) conditions, as indicated with the outer ring, b) when data is subset to watered conditions, 22°C (blue) and 38°C (red) indicators, as is indicated with the outer ring, with the outer bar graph showing log10 abundance for each OTU, and c) bar graphs showing the relative abundance of the top 6 phyla represented of either indicator type.

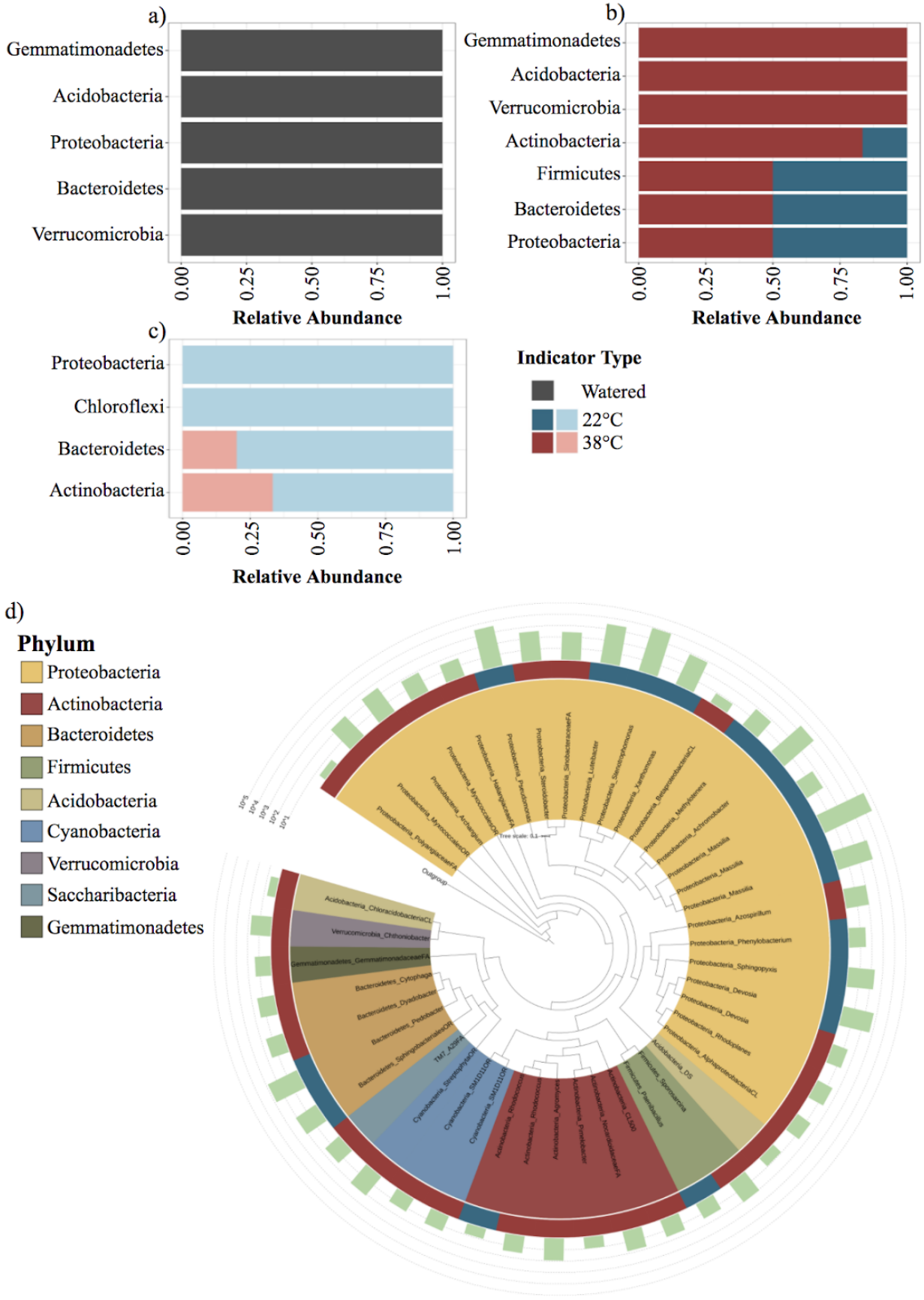

a)
Gemmatimonadetes
Acidobacteria
Proteobacteria
Bacteroidetes
Verrucomicrobia
0.00
0.25
0.50
0.75
1.00
Relative Abundance
b)
Gemmatimonadetes
Acidobacteria
Verrucomicrobia
Actinobacteria
Firmicutes
Bacteroidetes
Proteobacteria
0.00
0.25
0.50
0.75
1.00
Relative Abundance
c)
Proteobacteria
Chloroflexi
Bacteroidetes
Actinobacteria
0.00
0.25
0.50
0.75
1.00
Relative Abundance
Indicator Type
Watered
22°C
38°C
d)
Phylum
Proteobacteria
Actinobacteria
Bacteroidetes
Firmicutes
Acidobacteria
Cyanobacteria
Verrucomicrobia
Saccharibacteria
Gemmatimonadetes

Figure 3-13.
Actinobacteria are largely indicators of heat in watered samples at time point 1. Indicator species analysis results with time point 1 roots for a) 22°C (blue) and 38°C (red) when subset to watered conditions, as indicated with the outer banded ring, with the outer bar graph showing log10 abundance for each OTU; bar graph showing the relative abundance of either indicator type by phylum for b) TP1 roots comparing watered versus drought, c) 22°C (blue) and 38°C (red) when subset to watered conditions, and d) 22°C (blue) and 38°C (red) when subset to drought conditions.

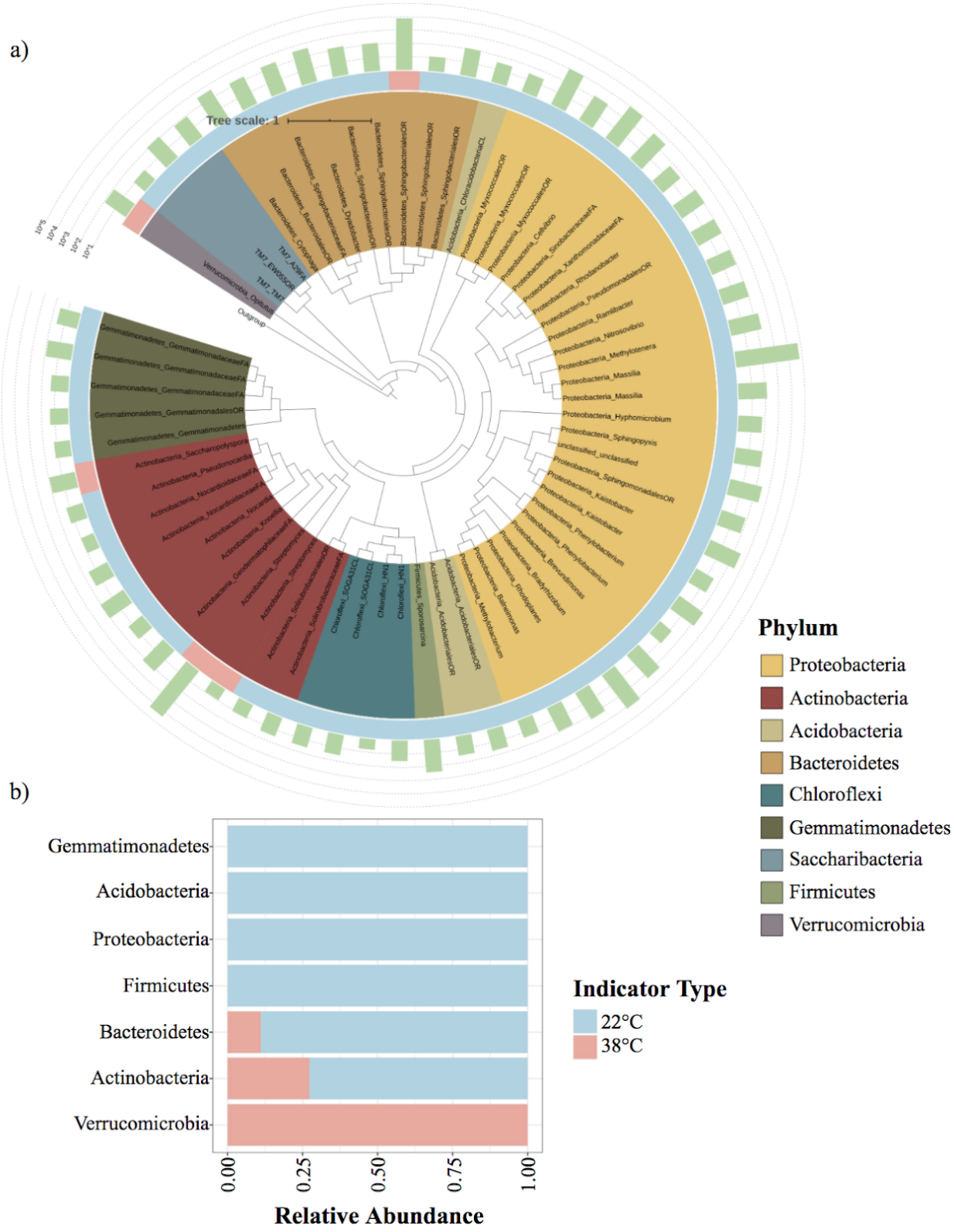


Figure 3-14.
Actinobacteria are predominantly indicators for cool temperatures in droughted samples at time point 2. Indicator specie analysis results with time point 2 roots for a) 22°C (blue) and 38°C (red)

when subset to drought conditions, as indicated with the outer banded ring, with the outer bar graph showing log10 abundance for each OTU; b) bar graph showing the relative abundance of indicators by phylum.

Figure 3-15.
Different Actinobacteria are indicators of heat and drought stress. Table of unique and shared Actinobacteria indicator species across root data subsets, where 'TP' denotes 'time point', 'W' for watered samples that were analyzed for temperature indicators, and 'D' for drought samples that were analyzed for temperature indicators.

Data Set	Test	Number of Actinobacteria Indicator OTUs	Indicator OTUs
TP1	Watering Treatment Indicators (Drought vs. Watered)	1	5
TP2	Watering Treatment Indicators (Drought vs. Watered)	13	5,6,9,28,33,35,46,47,59,70,115,401,502
TP1_W	Temperature Treatment Indicators (22°C vs. 38°C)	8	26,33,118,283,541,572,573,616
TP2_W	Temperature Treatment Indicators (22°C vs. 38°C)	2	143,572
TP1_D	Temperature Treatment Indicators (22°C vs. 38°C)	3	59,66,76
TP2_D	Temperature Treatment Indicators (22°C vs. 38°C)	11	1,9,36,39,50,100,115,225,262,354,372
TP1 ~ TP2	Comparison of Indicators	1 shared	5
TP1_W ~ TP2	Comparison of Indicators	1 shared	33
TP1_D ~ TP2	Comparison of Indicators	1 shared	59
TP1_W ~ TP2_W	Comparison of Indicators	1 shared	572
TP2_D ~ TP2	Comparison of Indicators	2 shared	9,115
TP2_W ~ TP2	Comparison of Indicators	0 shared	.
TP2_W ~ TP2_D	Comparison of Indicators	0 shared	.
TP1_W ~ TP1_D	Comparison of Indicators	0 shared	.
TP1_W ~ TP1	Comparison of Indicators	0 shared	.
TP1_D ~ TP1	Comparison of Indicators	0 shared	.
TP1_D ~ TP2_D	Comparison of Indicators	0 shared	.
TP2_W ~ TP1	Comparison of Indicators	0 shared	.
TP2_D ~ TP1	Comparison of Indicators	0 shared	.

Chapter 4

Domestication and Ploidy Influence Assembly of the Wheat Bacterial Microbiome

4.1 Abstract

While numerous studies implicate the microbiome in host fitness, contributions of host evolution to microbial recruitment remain largely uncharacterized. In this study, we analyzed 16S rRNA gene amplicon sequencing data from two field studies and a greenhouse experiment to determine how ploidy level (2n, 4n, 6n) and domestication status (cultivated vs. wild) correspond to the bacterial belowground microbiome of wheat (*Triticum* sp.). In our pilot study, we observed that host ploidy level was statistically significant in explaining variation in alpha and beta diversity for rhizosphere microbiomes, as well as correlated with distinct phylum-level shifts in composition. Using a reduced complexity field soil inoculum and controlled greenhouse conditions, we again found evidence of genomic lineage and ploidy level influencing root alpha and beta diversity (p-value<0.05). However, in a follow-up field experiment using an expanded set of *Triticum* genomes that included both wild and domesticated varieties, we did not find a strong signal for either diploid genome lineages, domestication status, or ploidy level in shaping rhizosphere bacterial communities. Taken together, these results suggest that while host ploidy and domestication may have some minor influence on microbial assembly, these impacts are subtle and difficult to assess in belowground compartments for wheat. By improving our understanding of the degree to which host ploidy and cultivation factors shape the plant microbiome, this work highlights the need for future studies to explore how other patterns of host genetics influence microbiome recruitment and may allow for expanded collaborative potential between plants and microbes.

4.2 Introduction

Over a relatively brief period of evolutionary time, crop domestication and polyploidy have significantly altered plant phenotype (Renny-Byfield & Wendel, 2014). This includes changes in fruit size, grain quality, and flowering time (Renny-Byfield & Wendel, 2014; Panchy *et al.*, 2016). Although geared towards crop improvement, selection for these aboveground traits may have resulted in large, unintended, and negative impacts on belowground characteristics, including those involved in forming beneficial associations with soil microbes.

Recent studies have shown that host factors, such as plant age and genotype, can affect microbial community assembly and succession (Micallef *et al.*, 2009b; Agler *et al.*, 2016). Root exudates are thought to play a pivotal role in such host modulation of microbiomes (Chaparro *et al.*, 2013; Carvalhais *et al.*, 2015b), where exudation profiles vary across plant development, environments, and with the presence of pathogens and beneficial bacteria (Tang *et al.*, 1994; Mendes *et al.*, 2013; Haichar *et al.*, 2014). Much remains to be understood, however, about how larger evolutionary processes in the host, such as whole genome duplication (WGD) and domestication, impact microbial recruitment and community assembly.

WGD involves a rapid increase in genome size and total gene set (Panchy *et al.*, 2016), and past work suggests that it has extensively shaped plant evolution and diversification (Soltis *et al.*, 2009; Jackson & Chen, 2010). The major genomic, epigenetic, and transcriptomic changes that occur after a WGD event are often coupled with phenotypic alterations that influence biotic interactions (Adams & Wendel, 2005; te Beest *et al.*, 2012). When WGD occurs following a

hybridization event, an allopolyploid can result (Matsuoka *et al.*, 2014). The resultant state of polyploidy can lead to shifts in cell architecture (Levin, 2002a; Lavania *et al.*, 2012a), production of novel compounds (Griesbach & Kamo, 1996b; Levin, 2002a; Lavania *et al.*, 2012a), and the colonization of a wider range of habitats, including those characterized by high UV irradiation, low temperatures, nutrient-poor soils, and drought (Levin *et al.*, 1990; Niwa & Sasaki, 2003; Sugiyama, 2006; Saleh *et al.*, 2008; Chandra & Dubey, 2010; Deng *et al.*, 2012).

Many crops, including wheat, maize, coffee, and cotton, are allopolyploids, and some have put forward the hypothesis that the phenotypic alterations following WGD have played an integral role in the domestication and improvement process (Renny-Byfield & Wendel, 2014). Allopolyploids have been shown to grow larger, more quickly, and produce higher yields as compared to their diploid progenitors (Hofberger *et al.*, 2013). Additional studies have described changes in multiple plant traits associated with domestication, including a shallower root system (Roucou *et al.*, 2018) and altered root exudate profile (Iannucci *et al.*, 2017). Increasing gene dosage via WGD can also lead to enhanced levels of metabolites involved in defense, competition, and stress tolerance (Renny-Byfield & Wendel, 2014). For instance, WGD and local tandem duplications have been implicated in the utilization and diversification of anti-herbivory glucosinolates in the Brassicaceae family (Hofberger *et al.*, 2013). In addition, ploidy level has been implicated in microbial symbiont selection, as well as the degree of association with arbuscular mycorrhizal fungi (Těšitelová *et al.*, 2013; Sudová *et al.*, 2014; Forrester & Ashman, 2018). In particular, a polyploid legume was found to have a greater capacity to interact with rhizobia via enhanced nodulation formation and attained greater biomass in response to nitrogen than its two diploid progenitors (Powell & Doyle, 2016). However, the consequences of WGD on plant recruitment of the broader microbiome remain unclear.

The effects of domestication on the plant microbiome are in the initial stages of being characterized. Recent work has shown an increased mycorrhizal dependence in wild landraces and ancestors, as compared to modern genotypes, implicated in achieving maximum plant growth and yield (Pérez-Jaramillo *et al.*, 2016b). Similarly, domestication has been shown to impact the microbial compositional profiles of a suite of crops, including sugar beet (*Beta vulgaris*) (Zachow *et al.*, 2014), barley (*Hordeum vulgare*) (Bulgarelli *et al.*, 2015), lettuce (*Lactuca sativa*) (Cardinale *et al.*, 2015), and common bean (*Phaseolus vulgaris*) (Pérez-Jaramillo *et al.*, 2018). Determining the principles of microbiome assembly with polyploidization and domestication will help improve our understanding of the processes that shape the evolution of microbial recruitment in the plant microbiome.

Triticum aestivum (bread wheat, hexaploid genome: AABBDD) and its relatives offer an ideal system for studying domestication and allopolyploid evolution, due to a near-complete sequenced genome of *T. aestivum (Zimin et al., 2017)*, the existence of several recently evolved polyploid relatives (including *T. turgidum*, emmer wheat, AABB), the fact that most diploid progenitors are known (including *T. monoccocum*, einkorn wheat, AA, and *Aegilops tauschii*, DD), novel hybridizations can be developed to generate synthetic allopolyploids with beneficial traits not present in current domesticated wheat species (He *et al.*, 2003), and the potential for translating findings directly into agriculturally impactful outcomes. Notably, the speciation process for wheat coincides with eco-geographical expansion and is thought to have contributed

to the ability of modern wheats to grow in diverse climates and elevations (Dubcovsky & Dvorak, 2007).

In this study, we aimed to characterize ploidy level and domestication effects on the microbiome hosted by the major cereal crop, wheat. In one out of two field studies and a greenhouse experiment, we observed evidence of ploidy and domestication impacts on wheat microbiome assembly, particularly with regards to rhizosphere compartments, where polyploids and wild species correlate with increased bacterial diversity and greater relative abundances of the phyla Bacteroidetes and Actinobacteria. These results suggest that host genetics, as impacted by WGD and artificial selection, minorly contribute to the shaping of wheat microbiomes, and inform future studies aimed at untangling the contributions of various host factors on microbial recruitment and community assembly.

4.3 Results

4.3.1 Polyploids in the Triticeae tribe harbor greater rhizosphere bacterial diversity than diploids

Host genetics are known to contribute to microbiome assembly (Peiffer *et al.*, 2013; Edwards *et al.*, 2015; Naylor *et al.*, 2017). However, their contribution is often more subtle than environmental factors and is less understood (Compant *et al.*, 2019). In this study, we took three approaches to determine the effects of ploidy and domestication on the assembly of the wheat root-associated bacterial microbiome (Figure 4-1). Using two field studies and one greenhouse experiment, we sought to characterize the bacterial communities associated with roots and rhizospheres across a set of environments, plant ages, and genotypes.

In a previous study by our group (Naylor *et al.*, 2017), we demonstrated that host species significantly impacts establishment of the root microbiome. In this study, we reanalyzed a subset of this 16S rRNA data, focusing on the soil, rhizosphere, and roots of cultivated *T. monococcum* (2n, AA), *T. turgidum* (4n, AABB), and two varieties of *T. aestivum* (6n, AABBDD). These plants, which were grown in a field during the summer of 2015, were harvested at early (pre-flowering, 5 weeks post-transplantation) and late (post-flowering, 12 weeks post-transplantation) time points, with three randomized, replicate blocks per time point. We found that alpha diversity, as measured by the Shannon Diversity metric, was most explained by sample type (root, rhizosphere, soil) ($F=91.4$, p-value<0.001), then ploidy ($F=4.4$, p-value<0.05) (Figures 4-2; 4-3a). In addition, alpha diversity was highest in rhizosphere compartments, as compared to roots (mean 5.8 versus 4.7), and between early and late time points, rhizosphere alpha diversity was slightly higher for polyploids, as compared to diploids (5.9 versus 5.6, respectively) (Figure 4-4a). Within rhizospheres, ploidy was also a highly significant factor in explaining alpha diversity variation ($F=23.6$, p-value<0.001) (Figure 4-3). As measured by Bray-Curtis distances, beta diversity variation was most explained by sample type ($F=22.2$), followed by time point ($F=6.9$), block ($F=3.0$), and ploidy ($F=2.7$) (p-values<0.01) (Figures 4-4b; 4-3b). Lastly, in both roots and rhizospheres, we found that ploidy was a significant factor (p-value<0.001) explaining between 15.6-16.1% of variation in beta diversity (Figure 4-3b). Together, these data give encouraging evidence that ploidy level may influence wheat-microbial interactions, particularly in the rhizosphere.

To distinguish the impacts of wheat ploidy and domestication on the root-associated bacterial microbiome, we performed a follow-up field experiment, conducted in the summer of 2017, in which an expanded number of wheat genotypes were grown. These included both wild and cultivated wheat varieties of each ploidy, as well as wild and cultivated barley varieties (*H. vulgare*, 2n), and diploid (*Gossypium arboreum* and *G. raimondii*) and tetraploid (*G. hirsutum*) cotton species, serving as outgroups (Figures 4-1; 4-5). We observed that the rhizospheres of polyploids had a slight mean increase, as compared to cultivated diploids, on the level of Shannon diversity (for wheat alone, 6.0 versus 5.8, respectively), and, interestingly, wild wheat diploids had the greatest mean alpha diversity (6.1) (Figures 4-6a; 4-7a). We further found beta diversity was not significantly structured by ploidy level, to the degree to which we were able to determine in this later time point (Figures 4-6b; 4-7b). Collectively, our 2017 field results were unable to recapitulate the significant shifts in rhizosphere observed in our 2015 field season. This may be due to how environmental conditions in the field can mask subtle shifts of host genotype on root microbiomes (Wagner *et al.*, 2016; Compant *et al.*, 2019), and the experiment was marked by a greater prevalence of overcast days and rainfall. Alternatively, with the inclusion of a greater number of wheat varietals in this approach, our results suggest that other host genetic factors, rather than host ploidy specifically, may have contributed to differences observed in the small study.

To complement our field study, we concurrently performed a greenhouse study, where environmental conditions could be better controlled. Field soil inoculum was added to autoclaved potting soil with germinated seeds of: *A. searsii* (2n), *T. monococcum* (2n), *T. turgidum* (4n), *T. aestivum* (6n), and the outgroups *H. vulgar* (2n), *G. arboreum* (2n) and *G. hirsutum* (4n) (Figures 4-1; 4-8). In profiling the bacterial community of our inoculum, we determined that it retained similar levels of alpha diversity and overall composition as its field soil source, though was unsurprisingly distinct with Bray-Curtis beta diversity metrics (Figure 4-9). We sampled the bacterial communities of plant roots and rhizosphere fractions, along with soil, when plants were approximately five weeks old. Interestingly, we observed a pattern of increased alpha diversity in rhizospheres, as well as the roots, particularly for 6n wheat varieties, but this was not statistically significant at large (Figures 4-10;4-11a). No distinct patterns in beta diversity, as shaped by ploidy level, were observed as well (Figure 4-11b,c). Taken together, these findings indicate that ploidy influences on the microbiome are small, but most pronounced in the rhizosphere. Additionally, plant age and soil type, as well as greenhouse versus field study, appear to contribute to the degree in which patterns of bacterial recruitment for wheat are detectable by amplicon sequencing.

4.3.2 Ploidy corresponds to distinct shifts in bacterial community composition

To determine whether there are high-level taxonomic patterns across ploidy levels, we plotted the relative abundances for each approach for both rhizosphere and root compartments (Figures 4-12; 4-13). From our initial pilot study, it was apparent that high taxonomic level shifts were evident across ploidy levels (Figure 4-13). While we determined that Actinobacteria were present in greater relative amounts in polyploid roots, as compared to diploid counterparts, for both the pilot field study and greenhouse experiment, there was a lot of variance in what classes were dominant (Figure 4-12). This variation may be indicative of microbiome fluctuations mediated by plant development (Figure 4-12a), as well as the impact of different environmental

conditions (Figure 4-12c). Interestingly, we also observed that several lower abundant classes were only present in polyploid fractions, particularly in the rhizosphere, including Solibacteres, Acidobacteria, Bacilli, and Chloracidobacteria (Figure 4-12a,b). Lastly, the higher prevalence of Actinobacteria in the rhizosphere microbiomes from the 2017 field study, relative to the other two approaches, may be a sign of greater stress experienced (Hartman & Tringe, 2019), and therefore possibly greater masking of genotype effects by environmental conditions (Figure 4-12b). Taken together, these findings indicate that increased ploidy correlates with small changes in bacterial community composition.

4.3.3 Domestication reduces bacterial community diversity and alters its composition

To investigate how cultivated and wild species differ in the bacterial communities they host, we compared alpha and beta diversity, as well as class-level relative abundances, within our summer 2017 field and reduced complexity community greenhouse experiments. Domestication status was borderline significant in explaining variation in alpha (p-value=0.05) and beta diversity (p-value=0.06) of rhizosphere bacterial communities from the field (Figures 4-7; 4-14a), and we observed higher alpha diversity on average in wild, versus cultivated, wheat varieties in the rhizosphere from diploid plants only (Figure 4-6a). However, only subtle shifts in rhizosphere bacterial community composition were observed between wild and cultivated varieties within each ploidy; for instance, relative abundances of Actinobacteria were only slightly higher in wild diploid plants, and there were greater relative levels of Gammaproteobacteria and Sphingobacteria in cultivated diploids compared with wild diploids. However, this pattern was not observed between polyploid varieties (Figure 4-12b). While we did find that wild varieties, as compared to cultivated species, had higher means of alpha diversity within each ploidy level in our greenhouse experiment (Figure 4-11a), no statistically significant effect on beta diversity could be attributed to domestication status (Figure 4-14b,c). Additionally, when we compared the bacterial community compositions of wild species versus cultivated ones, we found more distinct shifts in our greenhouse-based approach, than those in our field study results. In particular, Gammaproteobacteria were present to greater extents in wild rhizospheres and roots (Figure 4-12c), and Beta- and Deltaproteobacteria for cultivated counterparts, with the exception of 4n roots and 6n rhizospheres for each respective class. These analyses suggest that domestication status may have a smaller impact on wheat microbiome assembly than altered ploidy level - though these factors are thought to often be conflated - and provide evidence that degree of impact of host genotypic features on associated bacterial communities may indeed depend on environmental conditions.

4.3.4 Genomic lineage and domestication contribute to shifts in the bacterial microbiome

To determine whether a subset of genomes that contribute to polyploid wheat show differences in microbiome assembly, we looked at how genome lineage (“AA”, “DD”, and “SS”, a species within the same *Aegilops* genus as the DD contributor) within diploids correlates to shifts in bacterial community diversity and composition (Figures 4-15; 4-16). We observed the greatest Shannon’s diversity for the “SS” genome in both field and greenhouse studies, as well as for wild species, versus cultivated (Figures 4-15a,4-16a). However, domestication status was the only significant factor tested in explaining rhizosphere beta diversity variation in the field study (p-value=0.03) (Figures 4-7b; 4-15b; 4-16b). Furthermore, genome lineages correlated with distinct class-level relative abundance profiles, where we observed that “AA” genomes had

greater relative amounts of Gammaproteobacteria, Sphingobacteria, and the presence of Deltaproteobacteria, with decreased levels of Gemmatimonadetes, as compared to “SS” and “DD” genomes, in the field study (Figure 4-15c). In our greenhouse experiment, Betaproteobacteria dominated “AA” genome-associated bacterial communities, and had fewer relative amounts of the classes Deltaproteobacteria and Sphingobacteria (Figure 4-16c). These analyses suggest that genome lineages could differentially contribute to host-mediated bacterial recruitment, particularly in the rhizosphere.

We then compared whether distinct compositional shifts correlated with ploidy and domestication status could also be observed in our outgroups of cotton (2n and 4n varieties, wild and cultivated) and barley (wild and cultivated diploid species). Analyzing rhizosphere and root bacterial communities from both the field and greenhouse study, we found discernable phylum-level shifts that correlated with changing ploidy and domestication status, with the most evident change witnessed in rhizosphere communities and with the greatest differences generally between domestication status (Figure 4-17). These analyses suggest that belowground bacterial community assembly varies in sensitivity to host factors of ploidy and domestication status across crop species.

4.4 Discussion

Our experiments indicate that host factors of domestication and polyploidy influence wheat bacterial microbiome assembly to a smaller extent than those of the environment and, under certain circumstances, correlate to no distinct community shifts. At large, this characterization of the contributions of host evolution to microbial recruitment better informs efforts to prime plant-microbe associations for greater benefit to host health and fitness. In particular, we found some evidence in support of our hypothesis that increased ploidy would correlate to greater microbial diversity, which was particularly prominent in our initial field study and greenhouse study using a community of reduced complexity. The disparate pattern in our second field study, where host influence was not apparent in microbiome structuring, may be indicative of how certain environmental factors reduce discernable host-based influences (Compant *et al.*, 2019). These different findings also highlight some of the difficulty in translating greenhouse findings to phenotypic changes observed in fields. Furthermore, our data indicates that the rhizosphere fraction shows the most prominent patterns of altered bacterial community processes, as reflected in diversity and composition, in relationship to ploidy level and domestication status.

These results regarding ploidy effects on bacterial recruitment loosely corroborate past work in which a cordgrass allopolyploid (genus *Spartina*) was found to harbor a more diverse rhizosphere bacterial community, as compared to diploid counterparts (Cavé-Radet *et al.*). Another study using *Arabidopsis* reported that rhizosphere community composition - but not alpha diversity - shifted with whole genome duplication and differences in host genotype (Ponsford *et al.*). Indeed, degree of impact from host genetic factors, including ploidy level, on the plant microbiome seems to vary across species, as observed in our cotton outgroup compared to tested wheat species, as well as demonstrated in past work (Peiffer *et al.*, 2013; Fitzpatrick *et al.*, 2018; Wei *et al.*, 2019). This likely is attributable, in part, to varying diversity and rate of plant root exudation across different accessions, species, and growing conditions (Micallef *et al.*,

2009b; Badri & Vivanco, 2009). In addition, other studies provide additional evidence that rhizospheres - which host a more diverse and greater pool of microbes than the root itself to exert influence on - may be ideal for better understanding host contributions to microbiome assembly (Berendsen *et al.*, 2012), including microbiome-based genome-wide association studies (Deng *et al.*). In order to better understand contributions of ploidy level to microbiome assembly, versus what may be attributable to other genotype dependent differences, we recommend that future work investigate how finer genetic differences across ploidy levels may relate to differences in microbiome composition and activity (e.g. using highly inbred lines and first generation hybrids).

Our findings also minimally support our hypothesis that cultivated species would host reduced levels of microbial diversity, as compared to wild varieties. Though this effect was smaller than that which was observed in correlation to changing ploidy level, it corroborates past findings comparing the rhizosphere bacterial communities of wild legumes, beets, and maize and their modern cultivar counterparts (Pérez-Jaramillo *et al.*, 2016b). Past work has also suggested that the small differences from host genotype in associated bacterial communities may be signatures form domestication (Bulgarelli *et al.*, 2015). For example, the loss of Bacteroidetes has been suggested as a signature of domestication (Pérez-Jaramillo *et al.*, 2018); in this study, we did not observe greater relative abundance of Bacteroidetes in wild species, but wild wheat did harbor certain classes of Proteobacteria to a greater extent than cultivated counterparts. In order to determine what may be driving these compositional differences, we suggest investigations into how nutrient availability and starting soil community influence microbiome assembly of a greater assortment of wild and cultivated plant varieties. Furthermore, as wheat makes important associations with fungi, we suggest future work addresses how host factors influence fungal recruitment.

In summation, there is evidence from past work to suggest that host factors involved in plant evolution, including polyploidization and artificial selection, could have important implications for microbiome assembly. Here, we observe minimal impacts of genome lineage, ploidy level, and domestication on the diversity and composition of wheat root and rhizosphere bacterial communities. Although differences found were not consistently observed, there are environment specific results that suggest ploidy and domestication may play a minor role in certain instances. We recommend that future studies narrow in on recent, specific gene duplications to better understand the genetic interplay behind plant-microbe signaling potential and networking. In addition, further investigations into how host evolution can drive plant-microbial interactions may consider how the plant fungal microbiome responds to these host factors. Additional work will help inform where to place future efforts in better modulating the microbiome, particularly in light of addressing the needs of food security and plant growth under harsher climatic conditions.

4.5 Materials and Methods

4.5.1 Experimental set-up

The experimental field site used in our study is an agricultural field located in Albany, California (37.8864°N, 12 2.2982°W) and is characterized by a silty loam soil with low pH (5.2) (Naylor *et*

al., 2017). We utilized previously published data from (Naylor *et al.*, 2017), in which the bacterial community associated with *Triticum monococcum* (2n), *T. turgidum* (4n), and two varieties of *T. aestivum* (6n; Bountiful Garden and GH101) were grown during the summer of 2015 and profiled at two time points: early (pre-flowering, 5 weeks post-transplantation) and late (post-flowering, 12 weeks post-transplantation). Three replicate bulk soil, rhizosphere and root samples collected from randomized blocks at each time point, as previously described in (Naylor *et al.*, 2017).

For our second approach, in the summer of 2017 we grew an expanded number of genotypes (27 in total) in each ploidy level that included species under varying domestication status in a randomized block design in the same field as mentioned above, with four replicate plant per species (Supplemental Table 2). Seeds from each genotype were surface sterilized with a 50% bleach solution for 10 minutes, rinsed three to four times with autoclaved water, and planted in plug trays of field soil in the greenhouse. Cotton seeds were first delinted with concentrated hydrochloric acid for a few minutes. At 2 weeks old, seedlings were transferred to the field, where they were planted one foot away from a surface drip line and weeds hand-removed, and plant roots with rhizosphere were harvested at 14 weeks old using shovels and shears that were sterilized with 70% ethanol in between plants. All samples were stored on dry ice before transferring to a -80°C freezer in the lab, in which they remained until further processing.

Our greenhouse experiment was undertaken in the summer of 2017, where seeds from cultivated and wild genotypes (13) were surface sterilized by soaking in a 50% bleach solution for 10 minutes, then rinsed three to four times with autoclaved watered, and germinated on sterile water agar plates at 28°C in the dark (Supplemental Table 4). Cotton seeds were first delinted with concentrated hydrochloric acid for a few minutes. Potting soil (Sunshine MVP, Sun Gro Horticulture, Agawam, MA) was autoclaved before filling 70% ethanol sterilized 3.8 liter pots. Germinated seeds were planted 2.5 centimeters below the soil surface and 30 mL of a field soil inoculum was added per plant. Adapting(Liu *et al.*, 2010), the inoculum was prepared as follows: 1050 mL of autoclaved, double-distilled water (ddH_2O) was added to 1200 grams of collected field soil. This mixture was stirred regularly for 20 minutes before being centrifuged at 1000 rpm for 4 minutes at 21°C. The supernatant was collected and diluted up to 2000 mL with autoclaved ddH_2O. Samples of original field soil, pelleted soil, and inoculum were collected and stored at -80°C for bacterial community profiling. Control pots of autoclaved soil alone, as well as autoclaved soil with the inoculum added, were also included with potted seeds, and all were placed under a 16 hour light/8 hour dark regime with natural sunlight and supplemental light as needed, at 27°C with ~50% relative humidity. We rotated pots weekly and watered as needed with autoclaved ddH2O to keep soil well-saturated. When plants were 5 weeks old, we harvested root, rhizosphere, and edge soil with l0% bleach sterilized equipment. All samples were placed on dry ice before transporting to the lab's -80°C freezer for storage until further processing.

Root fractions were cleaned as described in detail previously(Simmons *et al.*, 2018). In brief, whole roots were placed in an epiphyte removal buffer (0.75% KH2PO4, 0.95% K2HPO4, 1% Triton X-100 in ddH2O; filter sterilized at 0.2 μM) and then sonicated (pulses at 160 W for 30 seconds, separated by a 30 second pause for 10 minutes at 4 °C). Root were then removed and placed into a separate sterile tube and rinsed two to four times with sterile water, in order to fully

separate the rhizosphere fraction. After drying with absorbent tissue, roots were homogenized and ground to a fine powder using liquid nitrogen and a mortar and pestle, before being returned to -80°C. The rhizosphere fraction was collected by centrifugation of the root wash mixture at 3500 rpm for 5 minutes after removal of the root tissue, and stored at -80°C.

4.5.2 DNA extraction and library preparation

Soil, inoculum, rhizosphere, and root DNA were isolated using extraction kits (DNeasy PowerSoil Kit, Qiagen Inc., Carlsbad, CA) following the manufacturer's protocol. Due to low DNA concentrations, we combined three replicates of the inoculum, centrifuged at 450 rcf for 4 minutes at 21°C, and analyzed the pellet. We then amplified the V3-V4 region of 16S ribosomal gene using a dual-indexed 16s rRNA Illumina iTags primer (341 F (5′-CCTACGGGNBGCASCAG-3′) and 785 R (5′-GACTACNVGGGTATCTAATCC-3′) as described previously(Takahashi *et al.*, 2014) using 5-Prime Hot Master Mix (catalog No. 2200410). After DNA extraction, DNA was diluted to 5 ng/µl and randomized in 96-well plates. Water blanks were included on each 96-well plate as negative controls. PNA clamps were used to minimize host-derived amplicons from both chloroplast and mitochondrial 16S rRNA gene sequences(Lundberg *et al.*, 2013). Reactions included 11.12 µL DNase-free sterile H20, 0.4 µg BSA, 10.0 µL 5-Prime Hot Master Mix, and 2 µL template, and 0.75 µM of chloroplast and mitochondria PNAs. PCR reactions were performed in triplicate in three thermocyclers (to account for possible thermocycler bias) with the following conditions: initial 3 min cycle at 94 °C, then 30 cycles of 45 seconds at 94 °C, 10 sec at 78 °C, 1 min at 50 °C, and 1.5 min at 72 °C, followed by a final cycle of 10 min at 72 °C. Triplicates were then pooled and the DNA concentration of each sample was quantified using a Qubit 3 Fluorometer (Invitrogen, Carlsbad, CA). Pools of amplicons were constructed using 100 ng for each PCR product. Before submitting for sequencing, pooled samples were cleaned with 1.0X volume Agencourt AMPureXP beads (Beckman-Coulter, West Sacramento, CA), according to the manufacturer's directions, except for the modifications of using 1.0X, rather than 1.6X, volume beads per sample, dispensing 1500 µL 70% EtOH to each well rather, than 200 µL, and eluting in 100 µL DNase-free H20, rather than 40 µL. An aliquot of the pooled amplicons was diluted to 10 nM in 30 µL total volume before submitting to the QB3 Vincent J. Coates Genomics Sequencing Laboratory facility at the University of California, Berkeley for sequencing using Illumina Miseq. 300 bp pair-end with v3 chemistry. Sequences were returned demultiplexed and with adaptors removed.

4.5.3 Amplicon sequence data processing, OTU classification and taxonomic assignment

Our sequencing data was analyzed using the iTagger pipeline developed by the U.S. Department of Energy's Joint Genome Institute (Tremblay *et al.*, 2015). This pipeline wraps several packages for the filtering, merging, clustering and taxonomy assignment, including CUTADAPT, FLASH, USEARCH, and RDP (Wang *et al.*, 2007a; Magoc & Salzberg, 2011; Martin, 2011; Alloui *et al.*, 2015). In brief, after filtering 16S rRNA raw reads for known contaminants (Illumina adapter sequence and PhiX), primer sequences were trimmed from the 5′ ends of both forward and reverse reads. Low-quality bases were trimmed from the 3′ ends prior to assembly of forward and reverse reads with FLASH (Magoc & Salzberg, 2011). The remaining merged reads were clustered with simultaneous chimera removal using UPARSE (Edgar, 2013). After clustering, 5,073,947, 7,451,220, and 6,140,220 read counts mapped to 4,999, 7,612, and 3,220 operational

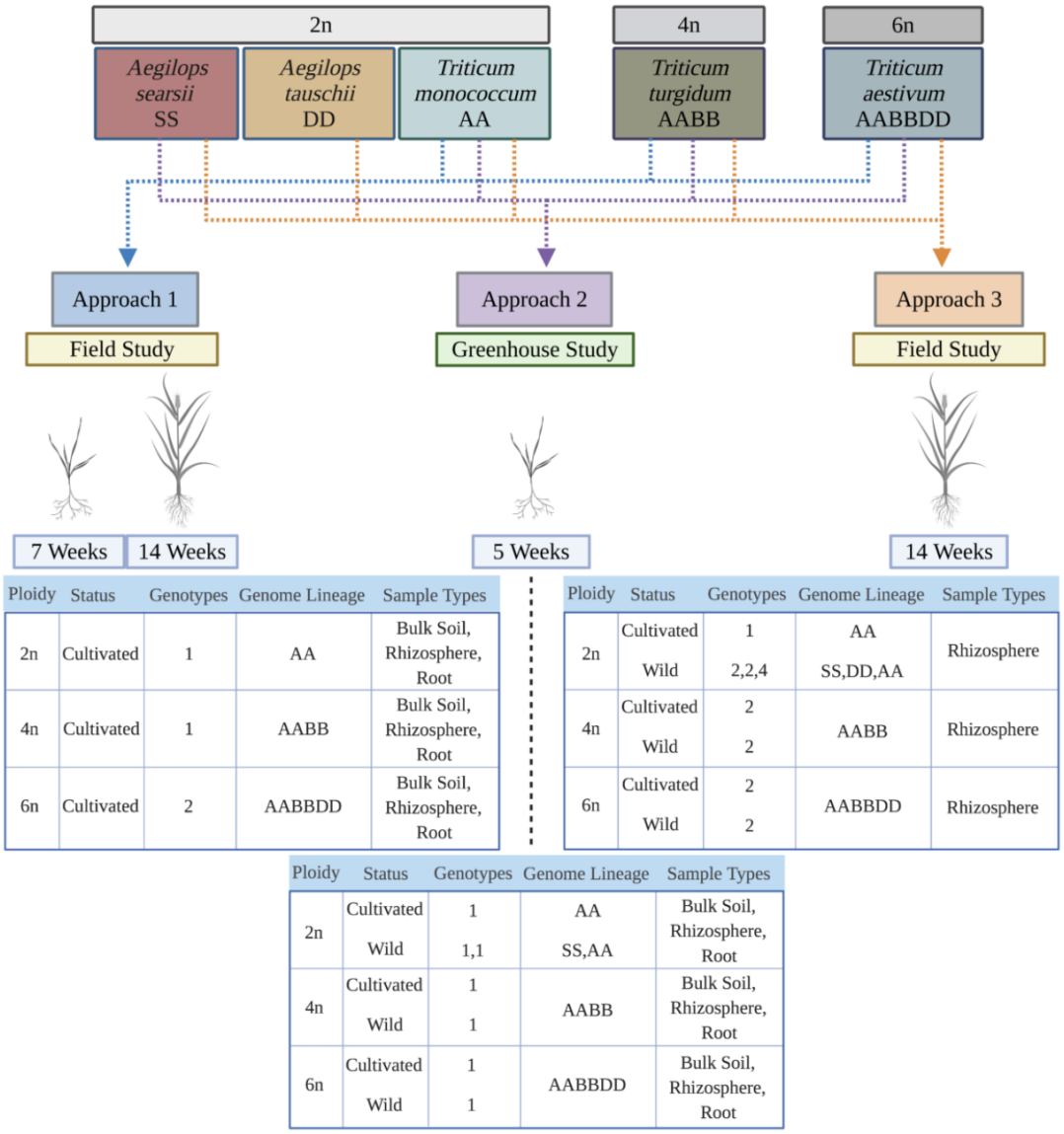
taxonomic units (OTUs) at 97% identity for the 2015 field study, greenhouse experiment, and the 2017 field study, respectively. The resulting reads produced on average approximately 44,369, 46,720, and 34,544 reads for the 2015 field study, and 41,221, 34,889, and 26,297 reads for the greenhouse experiment, per sample for soil, rhizosphere, and roots, respectively. The resulting reads produced on average approximately 37,071 reads per rhizosphere sample for the 2017 field study. Taxonomies were assigned to each OTU using the RDP Naïve Bayesian Classifier with custom reference databases (Wang *et al.*, 2007b). For the 16S rRNA V3-V4 data, this database was compiled from the May 2013 version of the GreenGenes 16S database (DeSantis *et al.*, 2006), the Silva 16S database (Quast *et al.*, 2013), and additional manually curated 16S rRNA sequences, trimmed to the V3-V4 region. After taxonomies were assigned to each OTU, we discarded all OTUs that were not assigned a Kingdom level RDP classification score of at least 0.5. To remove low abundance OTUs that are in many cases artifacts generated through the sequencing process, we removed OTUs without at least 2 reads in at least 2 samples. We also removed samples that had less than 10,000 reads, which yielded 4,309, 2,598 and 3,220 high-abundance OTUs for the 2015 field study, greenhouse experiment, and the 2017 field study, respectively, for downstream analyses. These thresholds were found to be suitable using technical replicates in a dataset published previously (Coleman-Derr *et al.*, 2016b). To account for differences in sequencing read depth across samples, all samples were rarefied to 10,000 reads per sample for specific analyses to yield 1,160,000, 1,620,000 , and 2,000,000 measurable, rarefied reads, for the 2015 field study, greenhouse experiment, and the 2017 field study, respectively, for downstream analysis.

4.5.4 Statistical analyses

RStudio (version 1.0.136; RStudio Team) was utilized for all statistical analyses with the packages phyloseq(McMurdie & Holmes, 2013) and vegan(Dixon, 2003). For plant phenotype data, scatter plots were generated using ggplot2, and Analysis of Variance (ANOVA) was performed with function aov. For the Alpha diversity measurement, Shannon Index of diversity and observed OTUs were calculated with the estimate_richness function in the R package phyloseq. ANOVAs were performed with function aov for Sample Type, Temperature Treatment, Watering Treatment, and Time Point. A Tukey's Post Hoc test was performed using the function TukeyHSD in the stats package and with HSD.test in the package agricolae to test which levels were significantly different from one another. Beta diversity was measured using Bray-Curtis distances with function ordinate in the R package phyloseq. Permutation multivariate analysis of variance analyses (PERMANOVA) were performed with the Adonis function in the R package Vegan using 999 permutations and the Bray-Curtis distances as inputs. The non-parametric Kruskal-Wallis test in R was used to compare Shannon indices and class-level relative abundances between treated and untreated within each time point and sample type.

4.5.5 Data Availability

The raw sequencing reads for this project are deposited in the NCBI Short Read Archive and can be accessed through BioProject SUB7801202. All scripts used can be found at a public repository on Github: (https://github.com/colemanderr-lab/Wipf-2020).



Ploidy	Status	Genotypes	Genome Lineage	Sample Types
2n	Cultivated	1	AA	Bulk Soil, Rhizosphere, Root
4n	Cultivated	1	AABB	Bulk Soil, Rhizosphere, Root
6n	Cultivated	2	AABBDD	Bulk Soil, Rhizosphere, Root

Ploidy	Status	Genotypes	Genome Lineage	Sample Types
2n	Cultivated	1	AA	Rhizosphere
	Wild	2,2,4	SS,DD,AA	
4n	Cultivated	2	AABB	Rhizosphere
	Wild	2		
6n	Cultivated	2	AABBDD	Rhizosphere
	Wild	2		

Ploidy	Status	Genotypes	Genome Lineage	Sample Types
2n	Cultivated	1	AA	Bulk Soil, Rhizosphere, Root
	Wild	1,1	SS,AA	
4n	Cultivated	1	AABB	Bulk Soil, Rhizosphere, Root
	Wild	1		
6n	Cultivated	1	AABBDD	Bulk Soil, Rhizosphere, Root
	Wild	1		

Figure 4-1.
Overview of experimental approaches used to study the contributions of domestication and ploidy level on wheat bacterial microbiome assembly. Approach 1) analysis of data from (Naylor *et al.*, 2017) with 4 genotypes (*Triticum monococcum,* 2n; *T. turgidum*, 4n; 2 varieties *T. aestivum*, 6n) grown in the field the summer of 2015, sampled at 7 weeks old and 14 weeks. Approach 2) a greenhouse study in which 13 genotypes were sampled at 5 weeks old (wild *Aegilops searsii* (2n), wild and cultivated *Gossypium hirsutum* (4n) and wild *G. arboreum* (2n), and a cultivated and wild line of: *T. monococcum and Hordeum vulgare* (2n), *T. turgidum* (4n), and *T. aestivum* (6n)). Approach 3) a field study conducted in the summer of 2017 in which 27 genotypes were sampled at 14 weeks old (2 wild lines of *A. searsii and T. tauschii* (2n), wild and cultivated *Gossypium hirsutum* (4n) and wild *G. arboreum* and *G. raimondii* (2n), two cultivated and one wild line of: *Hordeum vulgare* (2n), *T. monocuccum* and *T. urartu* (2n), 2 cultivated and 2 wild lines of both *T. turgidum* (4n) and *T. aestivum*). Three replicates per genotype for approach 1, 4 per genotype for approaches 2 and 3. Figure created with BioRender.com.

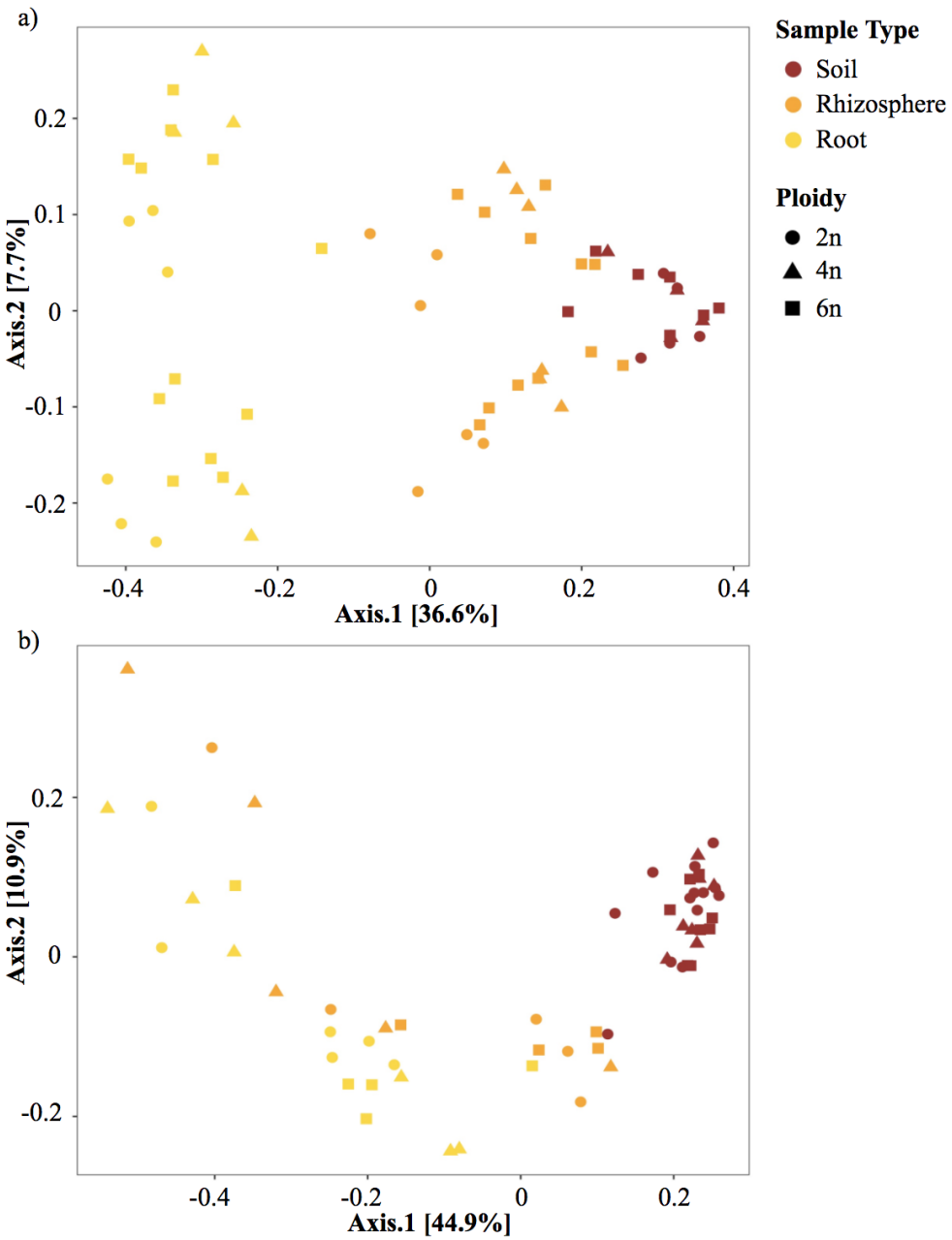


Figure 4-2.
Samples cluster predominately by sample type. Principal coordinates analyses using Bray-Curtis dissimilarity for wheat bacterial communities from the a) 2015 field study, and b) greenhouse study. Sample type is distinguished by color (brown for soil, orange for rhizosphere, yellow for root) and ploidy level by shape (circles denoting 2n, triangles denoting 4n, and squares denoting 6n).

Figure 4-3.
Ploidy is a statistically significant factor in explaining alpha and beta diversity variation in wheat bacterial communities. Analysis of variance (ANOVA) results for a) Shannon Diversity and b) Bray-Curtis dissimilarity for all data, as well as subsets by sample type, for the bacterial communities sampled from wheat during the 2015 field study. Abbreviations are as follows: 'Df' for degrees of freedom in the factor, 'Sum Sq' for the sum of squares due to the factor, 'Mean Sq' for the mean sum of squares due to the factor, and 'F value' for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

a)

Shannon	**Factor**	**Df**	**Sum Sqs**	**Mean Sqs**	**F value**	**P value**	
All data	Sample_Type	2	15.84	7.92	91.474	<2e-16	***
	Ploidy	2	0.77	0.385	4.444	0.0175	*
	TimePoint	1	0.019	0.019	0.224	0.6381	
	Block	1	0.097	0.097	1.118	0.2961	
	Sample_Type:Ploidy	4	0.665	0.166	1.919	0.1241	
	Sample_Type:TimePoint	2	0.507	0.253	2.927	0.064	.
	Ploidy:TimePoint	2	0.086	0.043	0.499	0.6103	
	SampleType:Ploidy:TimePoint	4	0.342	0.085	0.987	0.4246	
	Residuals	44	3.81	0.087			
	Total		22.136				
Root	Ploidy	2	0.604	0.302	1.441	0.266	
	TimePoint	1	0.266	0.26556	1.267	0.277	
	Block	1	0.164	0.16376	0.782	0.39	
	Ploidy:TimePoint	2	0.16	0.08018	0.383	0.688	
	Residuals	16	3.353	0.20954			
	Total		*4.547*				
Rhizosphere	Ploidy	2	0.53	0.265	23.567	1.25E-05	***
	TimePoint	1	0.0111	0.01108	0.985	0.335	
	Block	1	0.0187	0.01867	1.661	0.215	
	Ploidy:TimePoint	2	0.0374	0.0187	1.663	0.219	
	Residuals	17	0.1912	0.01124			
	Total		*0.7884*				
Soil	Ploidy	2	0.29533	0.14767	7.645	0.01147	*
	TimePoint	1	0.21805	0.21805	11.29	0.00839	**
	Block	1	0.00457	0.00457	0.237	0.63835	
	Ploidy:TimePoint	2	0.26916	0.13458	6.968	0.01485	*
	Residuals	9	0.17383	0.01931			
	Total		*0.96094*				

b)

Bray	**Factor**	**Df**	**Sums Sqs**	**Mean Sqs**	**F value**	**R2**	**P value**	
All data	SampleType	2	4.6849	2.34246	22.1991	0.37153	0.001	***
	Ploidy	2	0.5735	0.28676	2.7176	0.04548	0.004	**
	TimePoint	1	0.7303	0.73031	6.9211	0.05792	0.001	***

	Block	1	0.3142	0.31419	2.9775	0.02492	0.005	**
	SampleType:Ploidy	4	0.5622	0.14054	1.3319	0.04458	0.134	
	SampleType:TimePoint	2	0.4539	0.22697	2.1509	0.036	0.015	*
	Ploidy:TimePoint	2	0.2863	0.14317	1.3568	0.02271	0.147	
	SampleType:Ploidy:TimePoint	4	0.3616	0.0904	0.8567	0.02868	0.665	
	Residuals	44	4.6429	0.10552		0.36819		
	Total	62	12.6099			1		
Root	Ploidy	2	0.555	0.2775	2.503	0.16095	0.001	***
	TimePoint	1	0.653	0.65296	5.8896	0.18936	0.001	***
	Block	1	0.2137	0.21365	1.9271	0.06196	0.016	*
	Ploidy:TimePoint	2	0.2527	0.12635	1.1397	0.07329	0.273	
	Residuals	16	1.7739	0.11087		0.51444		
	Total	22	3.4482			1		
Rhizosphere	Ploidy	2	0.39328	0.19664	2.2714	0.15556	0.001	***
	TimePoint	1	0.31712	0.31712	3.663	0.12543	0.001	***
	Block	1	0.14834	0.14834	1.7135	0.05867	0.022	*
	Ploidy:TimePoint	2	0.19775	0.09887	1.1421	0.07822	0.225	
	Residuals	17	1.47174	0.08657		0.58212		
	Total	23	2.52824			1		
Soil	Ploidy	2	0.18318	0.091589	0.72313	0.09605	0.911	
	TimePoint	1	0.21564	0.215644	1.70259	0.11307	0.055	.
	Block	1	0.21281	0.212812	1.68023	0.11158	0.036	*
	Ploidy:TimePoint	2	0.15565	0.077824	0.61445	0.08161	0.985	
	Residuals	9	1.13991	0.126657		0.59769		
	Total	15	1.90719			1		

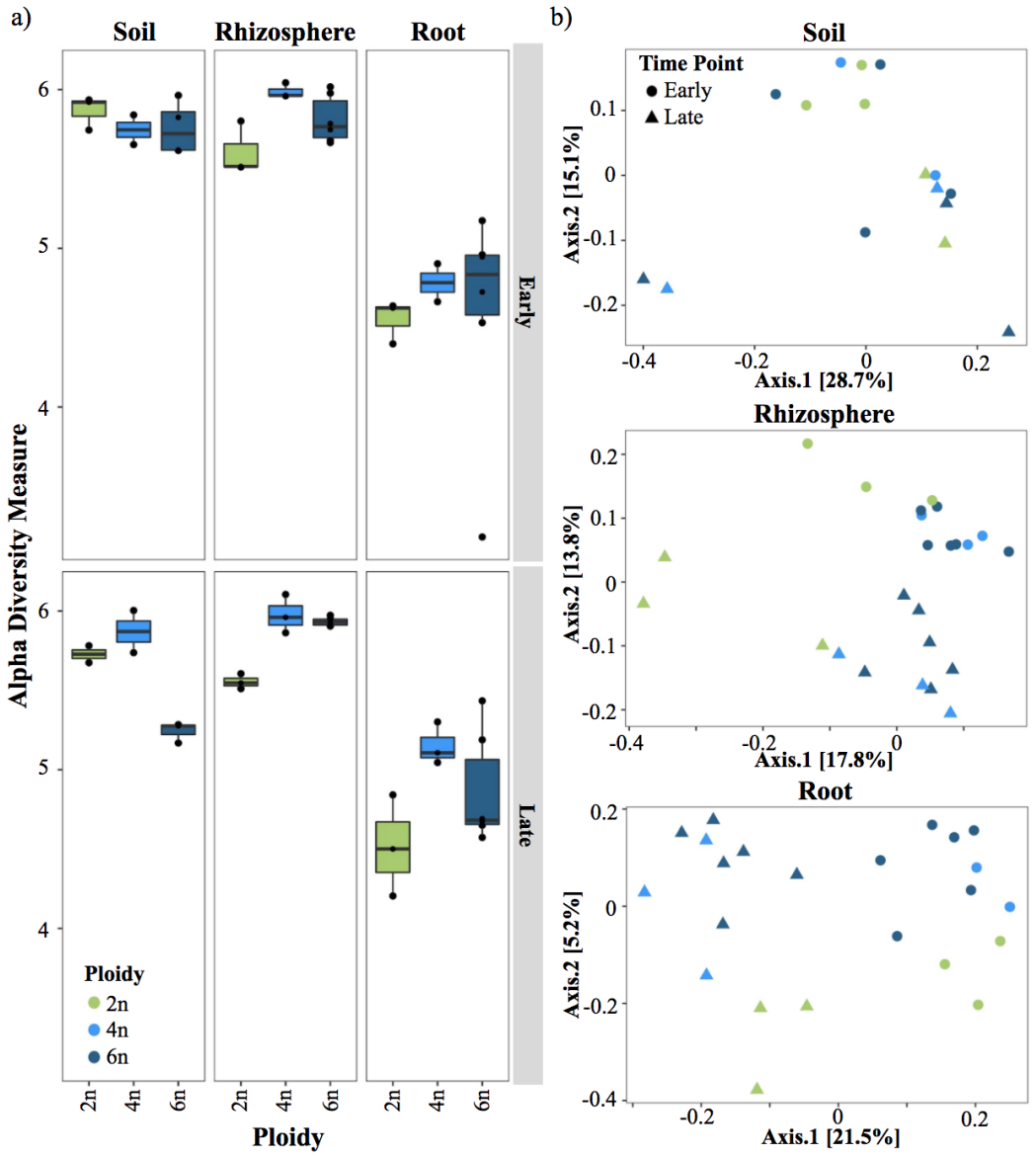


Figure 4-4.
Polyploidy corresponds to higher alpha diversity, as compared to diploids. Analyses of the 2015 field study data presented as a) boxplots of Shannon's Diversity and b) principal coordinates analysis plots based on Bray-Curtis dissimilarity, faceted by sample type (soil, rhizosphere, and root) and time point (early and late; indicated by circles and triangles, respectively, in b). Ploidy level represented by light green (2n), blue (4n), and dark blue (6n).

Figure 4-5.
Genetic and source information on lines used for 2017 field study. ‘CIMMYT’ denotes sourcing from the International Maize and Wheat Improvement Center.

Ploidy	Taxonomy	Origin	Status	Genome Lineage
2n	*Aegilops tauschii* Coss.	Xinjiang, China	Wild	DD
2n	*Aegilops searsii* Feldman & Kislev ex K. Hammer	Jordan	Wild	SS
2n	*Aegilops searsii* Feldman & Kislev ex K. Hammer	Southern Israel	Wild	SS
2n	*Aegilops tauschii* Coss.	Azerbaijan	Wild	DD
2n	*Hordeum vulgare* L. subsp. vulgare	Seine-et-Marne, France	Cultivated	Outgroup
2n	*Hordeum vulgare* L. subsp. vulgare	Arkansas, United States	Cultivated	Outgroup
2n	*Hordeum vulgare* L. subsp. spontaneum (K. Koch) Thell.	Central, Israel	Wild	Outgroup
4n	*Gossypium hirsutum* L.	Mississippi, United States	Cultivated	Outgroup
4n	*Gossypium hirsutum* L.	Oaxaca México	Wild	Outgroup
2n	*Gossypium arboreum* L.	India	Wild	Outgroup
2n	*Gossypium raimondii* Ulbr.	Cajamarca Perú	Wild	Outgroup
6n	GAN/AE.SQUARROSA (180)	CIMMYT	Synthetic	AABBDD
6n	CROC_1/AE.SQUARROSA (507)	CIMMYT	Synthetic	AABBDD
4n	*Triticum turgidum* L. subsp. dicoccon (Schrank) Thell.	Madhya Pradesh, India	Cultivated	AABB
2n	*Triticum monococcum* L. subsp. monococcum	Thuringia, Germany	Cultivated	AmAm
6n	*Triticum aestivum* L. subsp. macha (Dekapr. & Menabde) Mackey	Mazandaran, Iran	Wild	AABBDD
4n	*Triticum turgidum* L. subsp. paleocolchicum A. Love & D. Love	England United Kingdom	Wild	AABB
4n	*Triticum turgidum* L. subsp. dicoccoides (Korn. ex Asch. & Graebn.) Thell.	Cluj, Romania	Wild	AABB
2n	*Triticum monococcum* L. subsp. monococcum	Georgia	Landrace	AmAm
2n	*Triticum monococcum* L. subsp. aegilopoides (Link) Thell.	England, United Kingdom	Wild	AmAm
2n	*Triticum monococcum* L. subsp. aegilopoides (Link) Thell.	Naxcivan, Azerbaijan	Wild	AmAm
6n	*Triticum aestivum* L. subsp. macha (Dekapr. & Menabde) Mackey	Latium, Italy	Wild	AABBDD
2n	*Triticum urartu* Tumanian ex Gandilyan	Mardin, Turkey	Wild	AA
2n	*Triticum urartu* Tumanian ex Gandilyan	El Beqaa, Lebanon	Wild	AA
4n	*Triticum turgidum* L. subsp. durum (Desf.) Husn.	Cape Province, South Africa	Cultivated	AABB
6n	*Triticum aestivum* L. subsp. aestivum	Free State, South Africa	Cultivated	AABBDD
6n	*Triticum aestivum* L. subsp. aestivum	Delhi, India	Cultivated	AABBDD

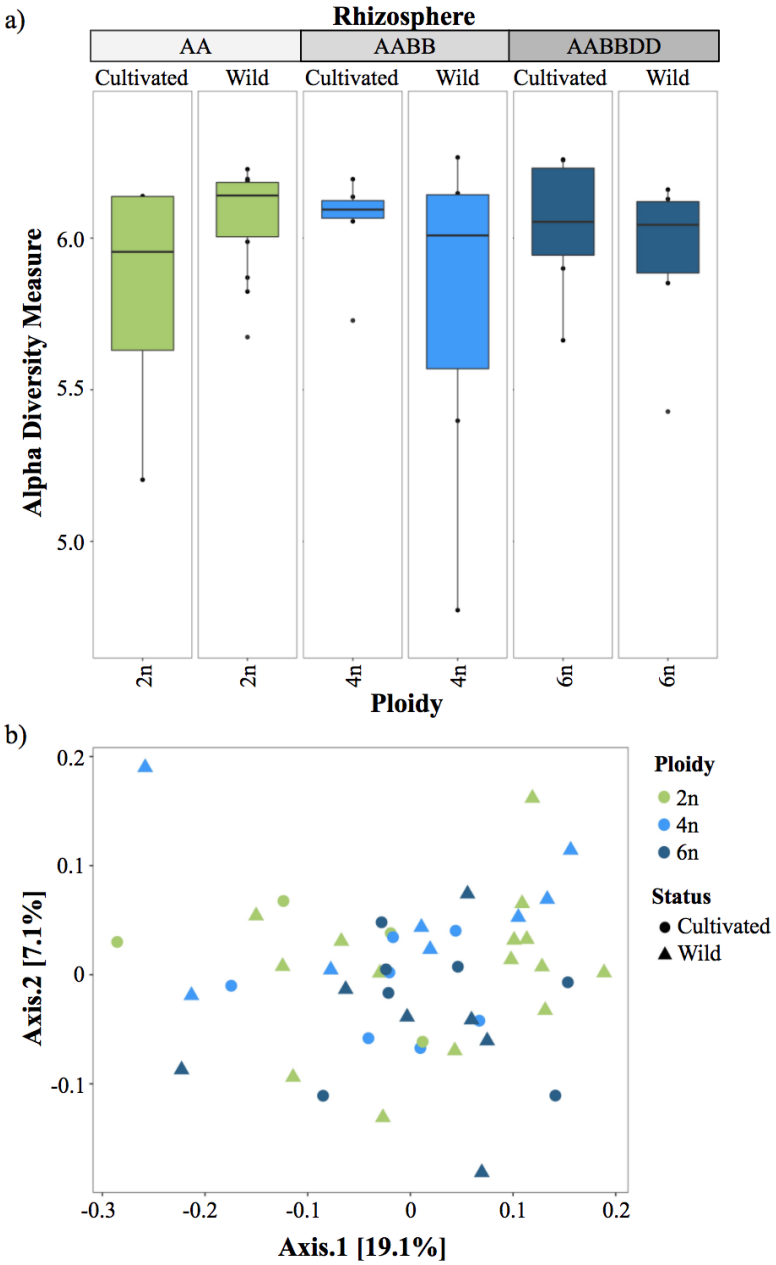


Figure 4-6.
Polyploidy and domestication status do not correspond to distinct shifts in bacterial diversity in the 2017 field study. Alpha and beta diversity analyses of wheat rhizosphere bacterial communities, presented as a) boxplots of Shannon's Diversity and b) principal coordinates analysis plots using Bray-Curtis dissimilarity with ploidy indicated by color (light green, 2n; blue, 4n; dark blue 6n) and domestication status (circle, cultivated genotype; triangle, wild genotype). Genome lineage is denoted by 'AA', 'AABB', and 'AABBDD.'

Figure 4-7.
Ploidy and domestication status minimally explain variation in bacterial alpha and beta diversity. Analysis of variance (ANOVA) results from 2017 field experiment rhizosphere bacterial microbiome data of a) Shannon Diversity and b) Bray-Curtis dissimilarity. Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.

a)

Analysis	Factor	Df	Sums Sqs	Mean Sqs	F value	P value	
Genome Lineage	GenomeLineage	2	0.0416	0.02078	0.293	0.7486	
	Status	2	0.29	0.145	2.042	0.1487	
	Block	1	0.0814	0.0814	1.146	0.2935	
	GenomeLineage:Block	2	0.167	0.08349	1.175	0.3235	
	Status:Block	2	0.3757	0.18786	2.645	0.0887	.
	Residuals	28	1.9888	0.07103			
Ploidy	Ploidy	2	0.0819	0.041	0.566	0.5727	
	Status	1	0.0255	0.0255	0.353	0.5563	
	Block	1	0.2851	0.2851	3.939	0.0549	.
	Ploidy:Status	2	0.4531	0.2265	3.13	0.0558	.
	Ploidy:Block	2	0.0238	0.0119	0.164	0.8491	
	Status:Block	1	0.0161	0.0161	0.222	0.6401	
	Ploidy:Status:Block	2	0.6679	0.3339	4.613	0.0165	*
	Residuals	36	2.606	0.0724			
Domestication Status	GenomeLineage	5	0.644	0.1289	0.99	0.4316	
	Status	1	0.059	0.0591	0.454	0.503	
	Block	1	0.224	0.2238	1.72	0.1949	
	GenomeLineage:Status	3	1.07	0.3566	2.74	0.0514	.
	GenomeLineage:Block	5	0.167	0.0334	0.256	0.9349	
	Status:Block	1	0.009	0.0088	0.068	0.7953	
	GenomeLineage:Status:Block	3	0.676	0.2255	1.732	0.1704	
	Residuals	58	7.549	0.1301			

b)

Analysis	Factor	Df	Sums Sqs	Mean Sqs	F value	R2	P value	
Genome Lineage	GenomeLineage	2	0.14006	0.070032	1.0327	0.05225	0.366	
	Status	2	0.21657	0.108286	1.5968	0.0808	0.032	*
	Block	1	0.12929	0.129287	1.9065	0.04823	0.009	**
	GenomeLineage:Block	2	0.14126	0.070631	1.0416	0.0527	0.358	
	Status:Block	2	0.15451	0.077256	1.1393	0.05764	0.234	
	Residuals	28	1.89875	0.067813		0.70837		
	Total	37	2.68045			1		
Ploidy	Ploidy	2	0.1417	0.070865	1.07524	0.0435	0.296	
	Status	1	0.0767	0.076677	1.16342	0.02353	0.204	

	Block	1	0.1487	0.148742	2.25688	0.04565	0.002	**
	Ploidy:Status	2	0.1657	0.08285	1.25709	0.05085	0.103	
	Ploidy:Block	2	0.1139	0.056974	0.86447	0.03497	0.758	
	Status:Block	1	0.0712	0.071237	1.08088	0.02186	0.297	
	Ploidy:Status:Block	2	0.1679	0.083939	1.27361	0.05152	0.1	.
	Residuals	36	2.3726	0.065906		0.72813		
	Total	47	3.2585			1		
Domestication Status	GenomeLineage	5	0.4149	0.082974	1.12172	0.0704	0.182	
	Status	1	0.1122	0.112209	1.51694	0.01904	0.06	.
	Block	1	0.2125	0.21245	2.8721	0.03605	0.001	***
	GenomeLineage:Status	3	0.2482	0.082748	1.11866	0.04213	0.212	
	GenomeLineage:Block	5	0.3286	0.065728	0.88857	0.05577	0.773	
	Status:Block	1	0.0552	0.055179	0.74596	0.00936	0.825	
	GenomeLineage:Status:Block	3	0.2311	0.077041	1.04151	0.03922	0.376	
	Residuals	58	4.2903	0.07397		0.72803		
	Total	77	5.893			1		

Figure 4-8.
Genetic and source information on lines used for the greenhouse experiment (Approach 2). ‘CIMMYT’ denotes the International Maize and Wheat Improvement Center.

Ploidy	Taxonomy	Origin	Status	Genome Lineage
2n	*Aegilops searsii* Feldman & Kislev ex K. Hammer	Southern Israel	Wild	SS
2n	*Hordeum vulgare* L. subsp. vulgare	Seine-et-Marne, France	Cultivated	Outgroup
2n	*Hordeum vulgare* L. subsp. spontaneum (K. Koch) Thell.	Central Israel	Wild	Outgroup
4n	*Gossypium hirsutum* L.	Mississippi, United States	Cultivated	Outgroup
4n	*Gossypium hirsutum* L.	Oaxaca, México	Wild	Outgroup
2n	*Gossypium arboreum* L.	India	Wild	Outgroup
2n	*Triticum monococcum* L. subsp. monococcum	Thuringia, Germany	Cultivated	AmAm
2n	*Triticum monococcum* L. subsp. aegilopoides (Link) Thell.	England, United Kingdom	Wild	AmAm
4n	*Triticum turgidum* L. subsp. durum (Desf.) Husn.	Cape Province, South Africa	Cultivated	AABB
4n	*Triticum turgidum* L. subsp. paleocolchicum A. Love & D. Love	England, United Kingdom	Wild	AABB
6n	*Triticum aestivum* L. subsp. aestivum	Brazil	Cultivated	AABBDD
6n	GAN/AE.SQUARROSA (180)	CIMMYT	Synthetic	AABBDD
6n	*Triticum aestivum* L. subsp macha Dekapr & Menabde Mackey	Switzerland	Wild	AABBDD

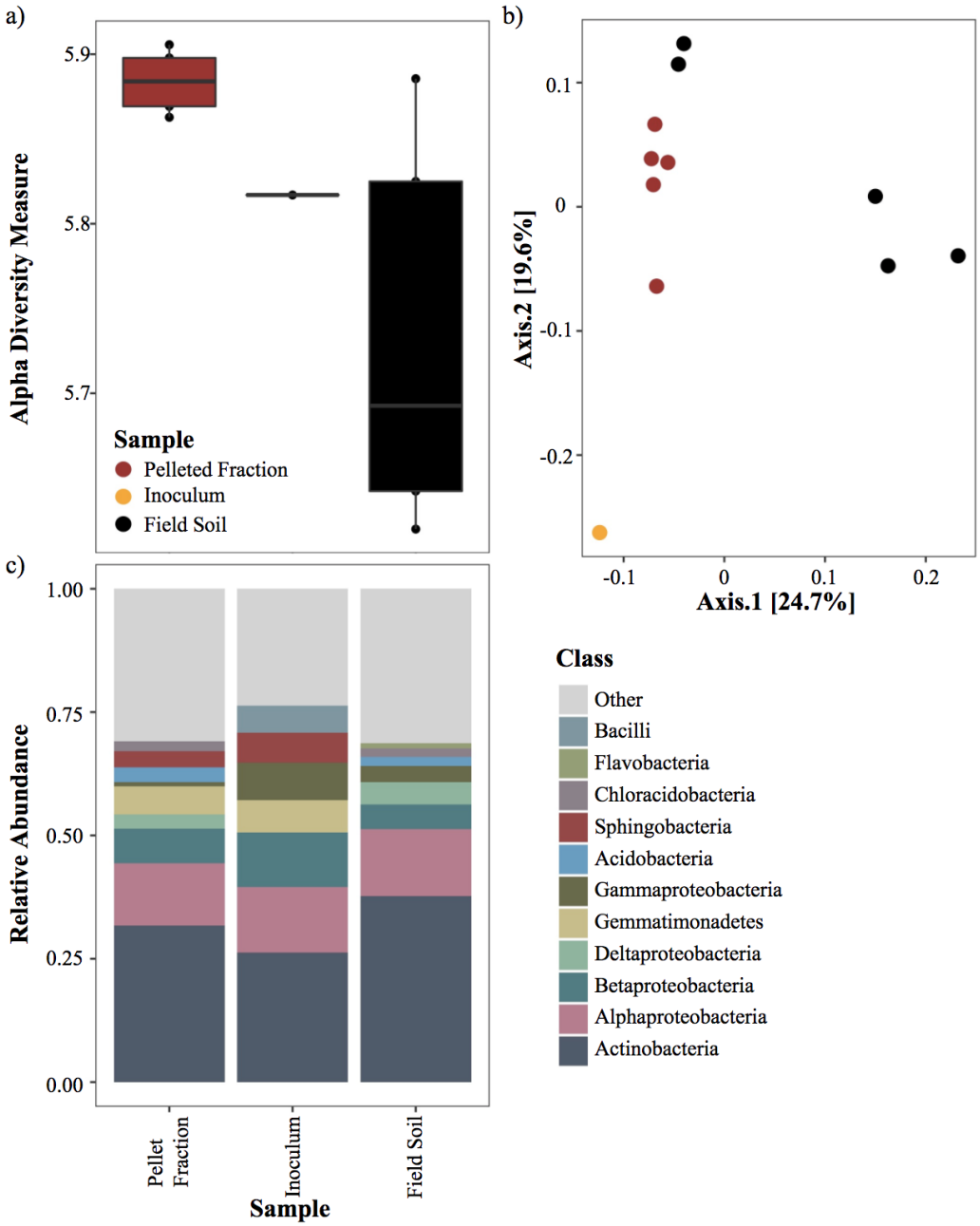


Figure 4-9.
Characterization of the soil inoculum's bacterial community. Analysis of inoculum used in the greenhouse study, with a) boxplots of Shannon's Diversity, b) principle coordinate analysis using Bray-Curtis dissimilarity, and c) class-level relative abundances of the bacterial community comprising the soil inoculum used, source field soil, and the centrifuged fraction separated from the inoculum.

Figure 4-10.
Analysis of variance results from greenhouse study data of the a) Shannon Diversity and b) Bray-Curtis dissimilarity of wheat bacterial microbiomes. Abbreviations are as follows: ‘Df’ for degrees of freedom in the factor, ‘Sum Sq’ for the sum of squares due to the factor, ‘Mean Sq’ for the mean sum of squares due to the factor, and ‘F value’ for the F-statistic. A p-value less than 0.001 is denoted by '***', less than 0.01 '**', less than 0.05 '*', and less than 0.10 '.'.
a)

Analysis	Factor	Df	Sums Sqs	Mean Sqs	F value	P value	
All data	Sample_Type	2	6.7	3.348	6.896	0.00144	**
	GenomeLineage	5	3.45	0.69	1.421	0.22124	
	Status	2	0.26	0.13	0.267	0.76585	
	Ploidy	1	0.18	0.18	0.37	0.54408	
	Sample_Type:GenomeLineage	8	5.19	0.649	1.337	0.23119	
	Sample_Type:Status	4	1.4	0.35	0.72	0.57963	
	GenomeLineage:Status	3	0.11	0.036	0.075	0.97358	
	Sample_Type:Ploidy	2	2.78	1.39	2.864	0.0608	.
	Status:Ploidy	1	0.23	0.227	0.468	0.4952	
	Sample_Type:GenomeLineage:Status	6	1.84	0.306	0.631	0.70546	
	Sample_Type:Status:Ploidy	2	0.75	0.377	0.776	0.46242	
	Residuals	125	60.69	0.486			
Genome Lineage, Soil	GenomeLineage	4	2.51	0.6279	1.435	0.228	
	Status	1	0.34	0.3436	0.785	0.378	
	GenomeLineage:Status	3	0.11	0.0353	0.081	0.97	
	Residuals	96	42	0.4375			
Genome Lineage, Rhizosphere	GenomeLineage	4	5.173	1.2933	1.556	0.231	
	Status	1	1.4	1.4	1.685	0.212	
	GenomeLineage:Status	3	0.478	0.1593	0.192	0.901	
	Residuals	17	14.128	0.8311			
Genome Lineage, Root	GenomeLineage	4	1.573	0.3933	0.79	0.545	
	Status	1	0.002	0.0019	0.004	0.951	
	GenomeLineage:Status	3	1.402	0.4672	0.939	0.44	
	Residuals	20	9.953	0.4976			
Ploidy, Soil	Ploidy	2	0.0769	0.03845	1.524	0.245	
	Status	1	0.0077	0.00765	0.303	0.589	
	Ploidy:Status	2	0.0681	0.03403	1.349	0.284	
	Residuals	18	0.4541	0.02523			
Ploidy, Rhizosphere	Ploidy	2	3.843	1.9213	1.272	0.346	
	Status	1	0.949	0.949	0.628	0.458	
	Ploidy:Status	2	0.438	0.2192	0.145	0.868	
	Residuals	6	9.065	1.5109			
Ploidy, Root	Ploidy	2	1.457	0.7286	0.812	0.474	
	Status	1	0.017	0.0165	0.018	0.895	
	Ploidy:Status	2	1.381	0.6904	0.769	0.492	

	Residuals	9	8.077	0.8975			
Domestication Status, Soil	GenomeLineage	4	0.3611	0.09028	1.605	0.191	
	Status	1	0.0396	0.03964	0.705	0.406	
	GenomeLineage:Status	3	0.0764	0.02545	0.452	0.717	
	Residuals	41	2.3063	0.05625			
Domestication Status, Rhizosphere	GenomeLineage	4	5.173	1.2933	1.556	0.231	
	Status	1	1.4	1.4	1.685	0.212	
	GenomeLineage:Status	3	0.478	0.1593	0.192	0.901	
	Residuals	17	14.128	0.8311			
Domestication Status, Root	GenomeLineage	4	1.573	0.3933	0.79	0.545	
	Status	1	0.002	0.0019	0.004	0.951	
	GenomeLineage:Status	3	1.402	0.4672	0.939	0.44	
	Residuals	20	9.953	0.4976			

b)

Analysis	**Factor**	**Df**	**Sums Sqs**	**Mean Sqs**	**F value**	**R2**	**P value**	
All plant	Sample_Type	2	5.9936	2.99679	20.0987	0.19126	0.001	***
	GenomeLineage	5	3.4966	0.69931	4.6901	0.11158	0.001	***
	Status	2	0.1417	0.07087	0.4753	0.00452	0.986	
	Sample_Type:GenomeLineage	8	1.1922	0.14903	0.9995	0.03805	0.498	
	Sample_Type:Status	4	0.2354	0.05885	0.3947	0.00751	1	
	GenomeLineage:Status	3	0.3179	0.10597	0.7107	0.01015	0.852	
	Sample_Type:GenomeLineage:Status	6	0.4267	0.07112	0.477	0.01362	1	
	Residuals	131	19.5326	0.1491		0.62331		
	Total	161	31.3367			1		
Genome Lineage, Soil	GenomeLineage	1	0.17941	0.179409	2.2601	0.17603	0.008	**
	Status	1	0.046	0.046001	0.5795	0.04513	0.94	
	Residuals	10	0.7938	0.07938		0.77884		
	Total	12	1.01921			1		
Genome Lineage, Rhizosphere	GenomeLineage	1	0.19184	0.19184	1.58905	0.37513	0.2	
	Status	1	0.07811	0.078106	0.64697	0.15273	0.6667	
	Residuals	2	0.24145	0.120726		0.47214		
	Total	4	0.5114			1		
Genome Lineage, Root	GenomeLineage	1	0.13722	0.137223	1.53625	0.29798	0.1556	
	Status	1	0.05531	0.055313	0.61925	0.12011	0.8667	
	Residuals	3	0.26797	0.089323		0.5819		
	Total	5	0.46051			1		
Ploidy, Soil	Ploidy	2	0.14678	0.073388	1.05872	0.09074	0.359	
	Status	1	0.048	0.047998	0.69243	0.02967	0.778	
	Ploidy:Status	2	0.17507	0.087533	1.26278	0.10823	0.183	
	Residuals	18	1.24772	0.069318		0.77136		
	Total	23	1.61756			1		
Ploidy, Rhizosphere	Ploidy	2	0.33147	0.165737	1.31234	0.23758	0.25	

	Status	1	0.12984	0.129841	1.0281	0.09306	0.339	
	Ploidy:Status	2	0.17616	0.088079	0.69743	0.12626	0.649	
	Residuals	6	0.75775	0.126292		0.5431		
	Total	11	1.39522			1		
Ploidy, Root	Ploidy	2	0.19499	0.097497	1.00529	0.14731	0.39	
	Status	1	0.0433	0.043302	0.44648	0.03271	0.832	
	Ploidy:Status	2	0.21258	0.106289	1.09595	0.16059	0.301	
	Residuals	9	0.87285	0.096984		0.65939		
	Total	14	1.32373			1		
Domestication Status, Soil	Ploidy	2	0.1804	0.090195	1.20728	0.04844	0.213	
	Status	1	0.1105	0.110468	1.47864	0.02967	0.123	
	Ploidy:Status	2	0.1456	0.072823	0.97475	0.03911	0.461	
	Residuals	44	3.2872	0.074709		0.88278		
	Total	49	3.7237			1		
Domestication Status, Rhizosphere	Ploidy	2	0.2305	0.11523	0.92081	0.072	0.477	
	Status	1	0.2304	0.23042	1.84132	0.07198	0.117	
	Ploidy:Status	2	0.2373	0.11867	0.94833	0.07415	0.465	
	Residuals	20	2.5027	0.12514		0.78187		
	Total	25	3.2009			1		
Domestication Status, Root	Ploidy	2	0.5997	0.299829	2.28263	0.15348	0.025	*
	Status	1	0.0413	0.041278	0.31426	0.01057	0.985	
	Ploidy:Status	2	0.245	0.122511	0.93269	0.06271	0.482	
	Residuals	23	3.0211	0.131352		0.77324		
	Total	28	3.9071			1		

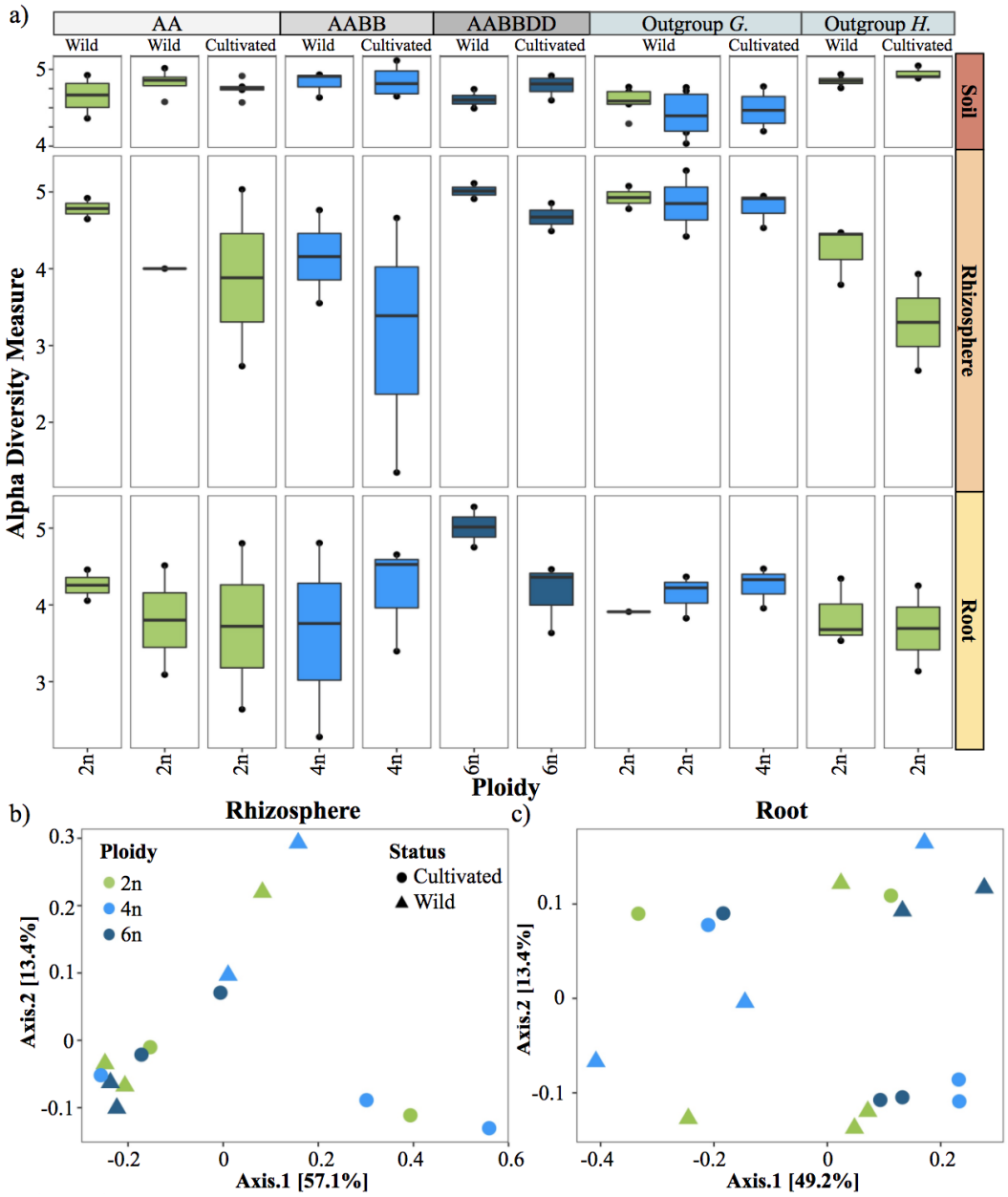


Figure 4-11.
Alpha diversity shifts with ploidy level and domestication status. Alpha and beta diversity analyses of greenhouse study data, presented as a) boxplots of Shannon Diversity and principal coordinates analyses using Bray-Curtis dissimilarity of wheat b) rhizosphere and c) root samples, with ploidy indicated by color (light green for 2n, blue for 4n, dark blue for 6n) and domestication status by shape in b) and c) (circle for cultivated and triangle for wild species). Genome lineage is denoted by 'AA', 'AABB', and 'AABBDD.' Outgroup *G.* and *H.* denote genotypes in the genera *Gossypium* and *Hordeum*, respectively.

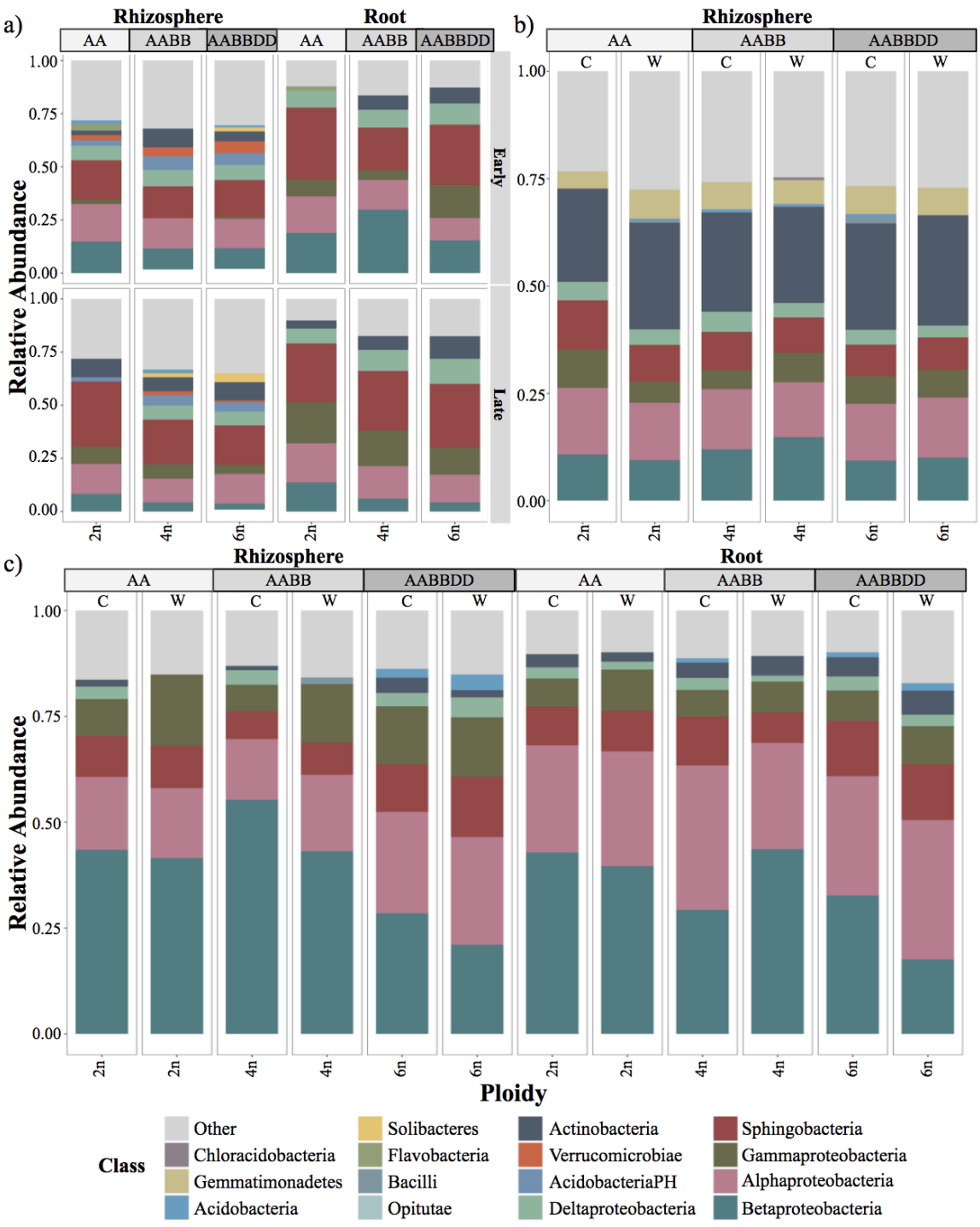


Figure 4-12.
Ploidy level and domestication status correspond to compositional shifts in the 2015 field and 2017 greenhouse studies. Stacked bar plots of class-level relative abundances for wheat bacterial communities sampled in the a) 2015 field study from rhizospheres and roots, b) 2017 field study from rhizospheres, and c) greenhouse study from rhizosphere and roots. Genome lineage is denoted by 'AA', 'AABB', and 'AABBDD'; 'C' denotes the bacterial community is from a cultivated genotype, 'W' from a wild genotype.

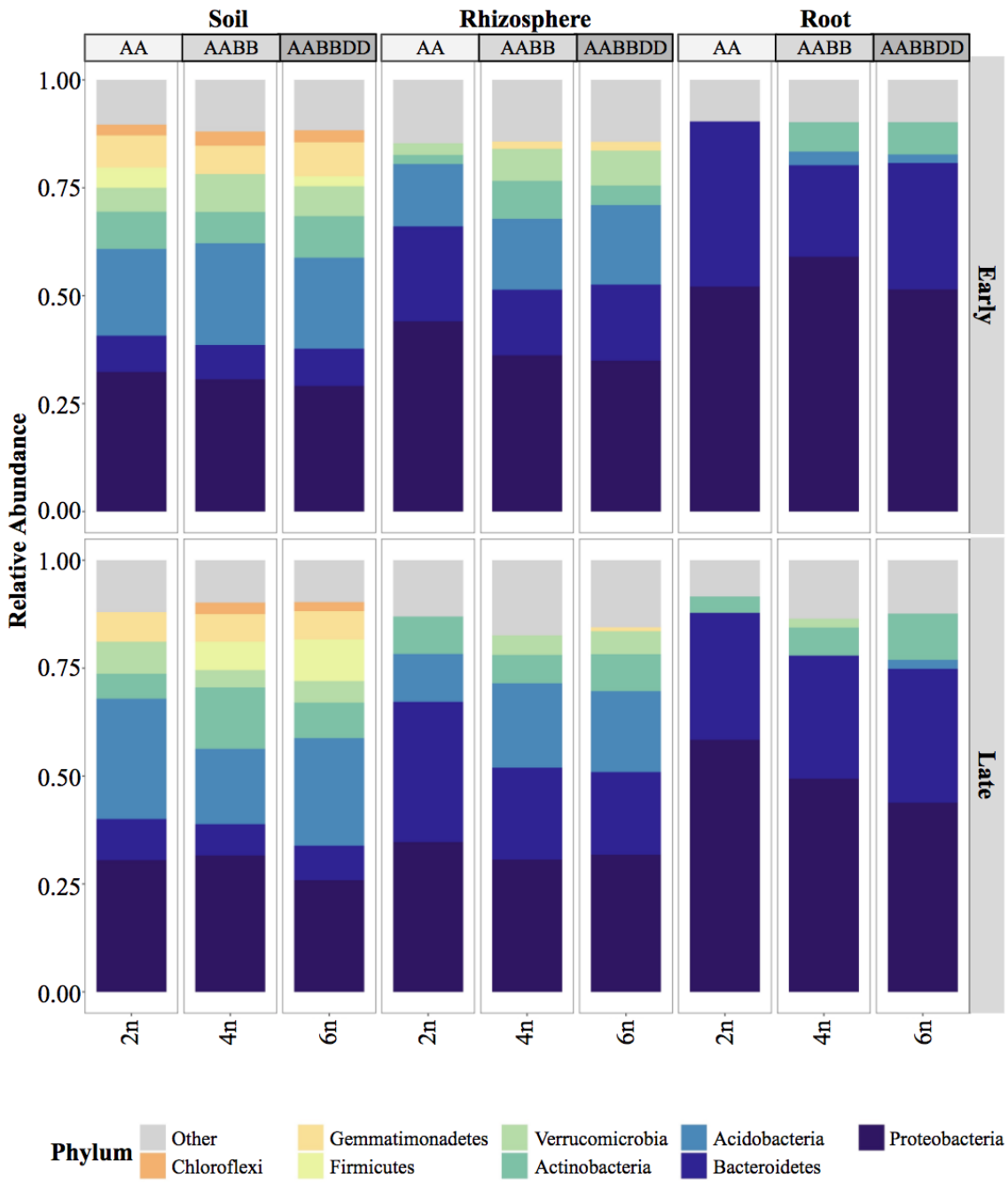


Figure 4-13.
Ploidy level corresponds to distinct bacterial community compositional shifts. Stacked bar graphs of phylum-level relative abundances of wheat bacterial community data from the 2015 field study, as faceted by sample type (soil, rhizosphere, and root) and time point (early and late). Genome lineage is denoted by 'AA', 'AABB', and 'AABBDD.'

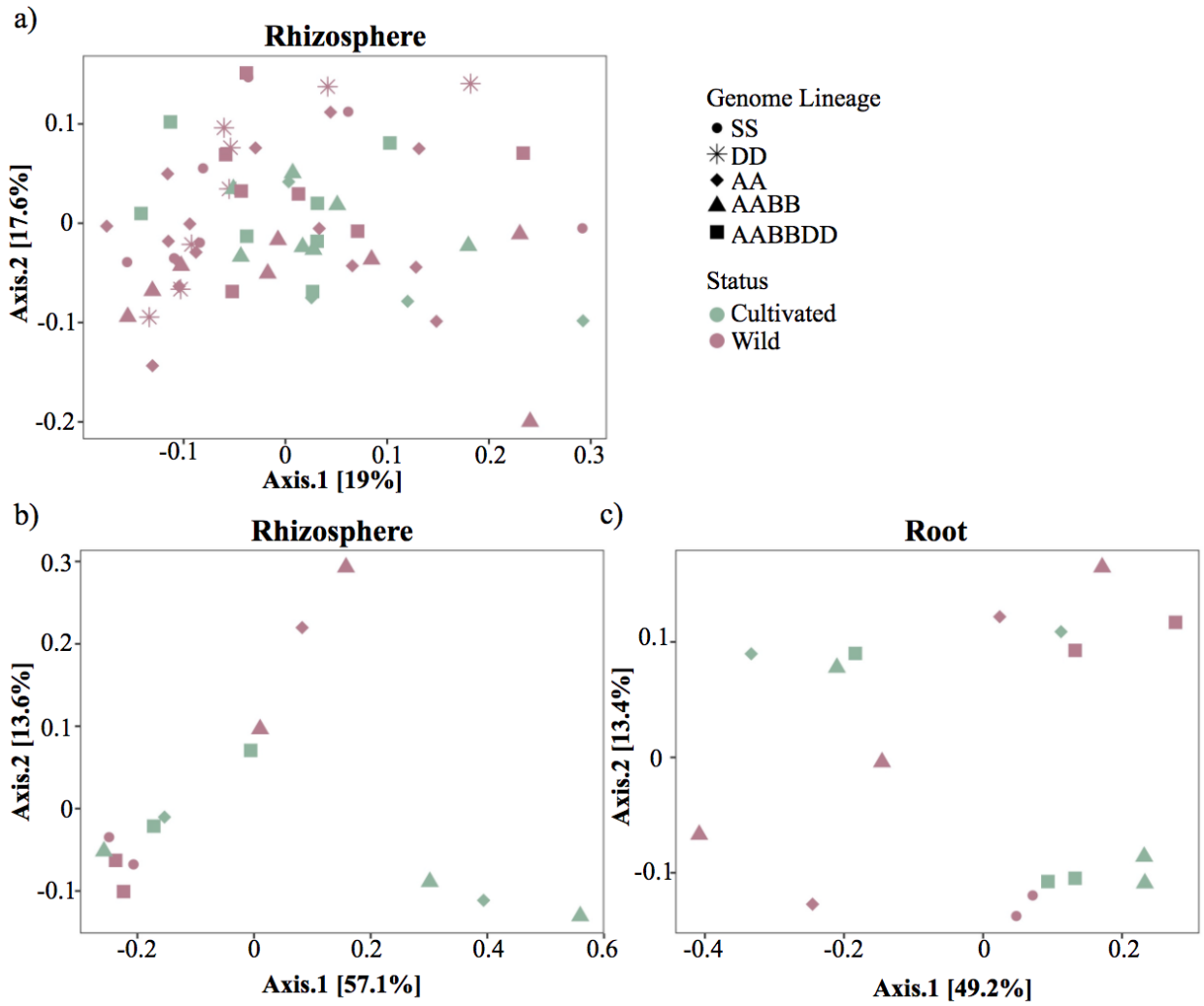


Figure 4-14.
Domestication status does not distinctly structure wheat bacterial communities. Principal coordinates analysis of samples using Bray-Curtis dissimilarity for a) rhizosphere bacterial microbiome data from the 2017 field study, and b) rhizosphere and c) root bacterial microbiome data from our greenhouse study, as demarcated by genome lineage ('SS' is denoted by a circle, 'DD' by an asterix, 'AA' a diamond, 'AABB' a triangle, and 'AABBDD' a square) and domestication status (green for cultivated genotypes, pink for wild).

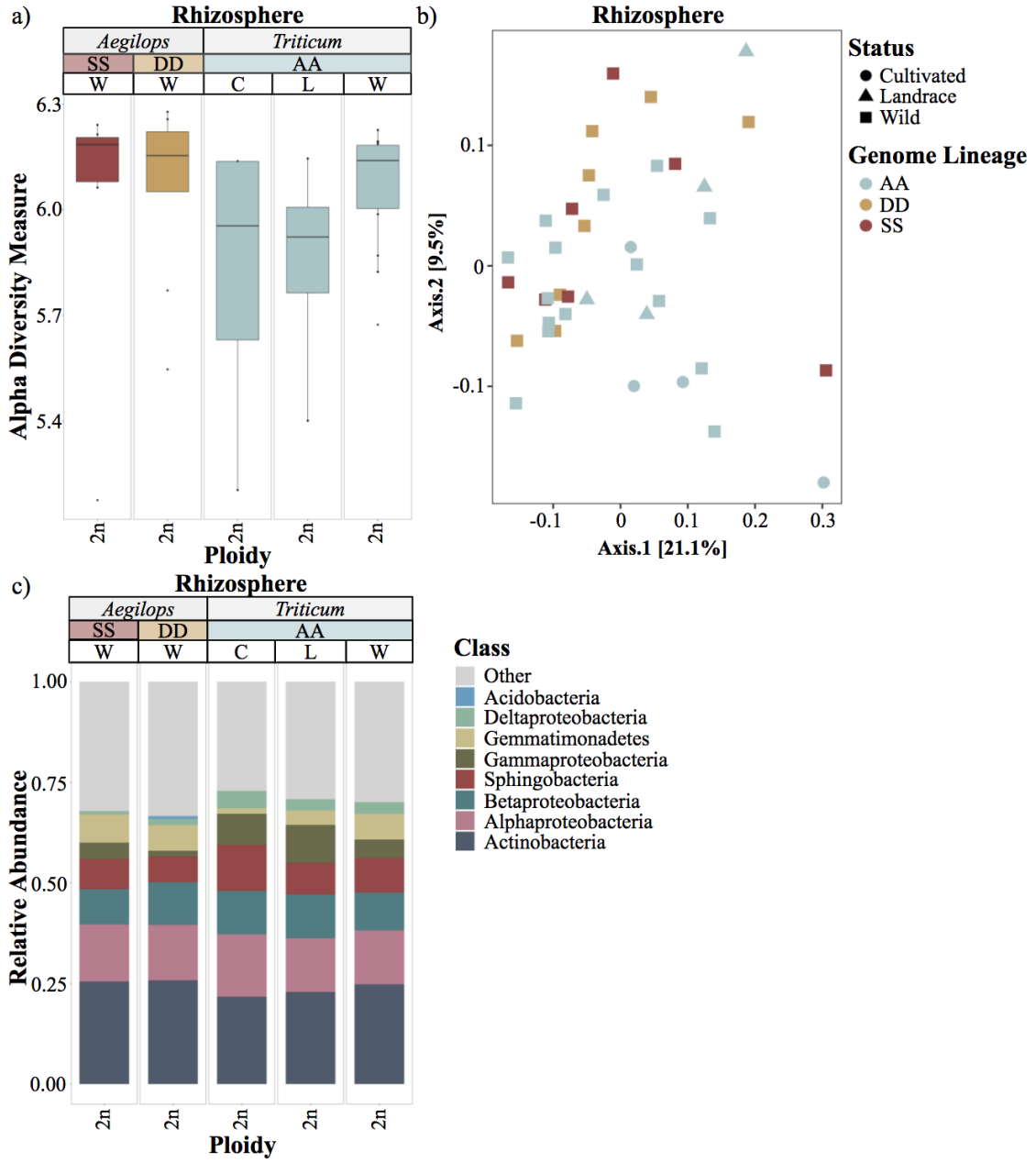


Figure 4-15.
Genome lineage and domestication status slightly structure rhizosphere bacterial communities. Diversity and composition analysis of diploid genome lineages from wheat rhizosphere bacterial communities sampled in the 2017 field study, with a) boxplots of Shannon diversity, b) principal coordinates analysis plot using Bray-Curtis dissimilarity, and c) stacked bar graphs of class-level relative abundances, across ploidy level and parsed by genome lineage ('SS'- red, 'DD'- orange, and 'AA'- light blue) and domestication status ('C' for cultivated, 'W' for wild, and 'L' for landrace genotypes).

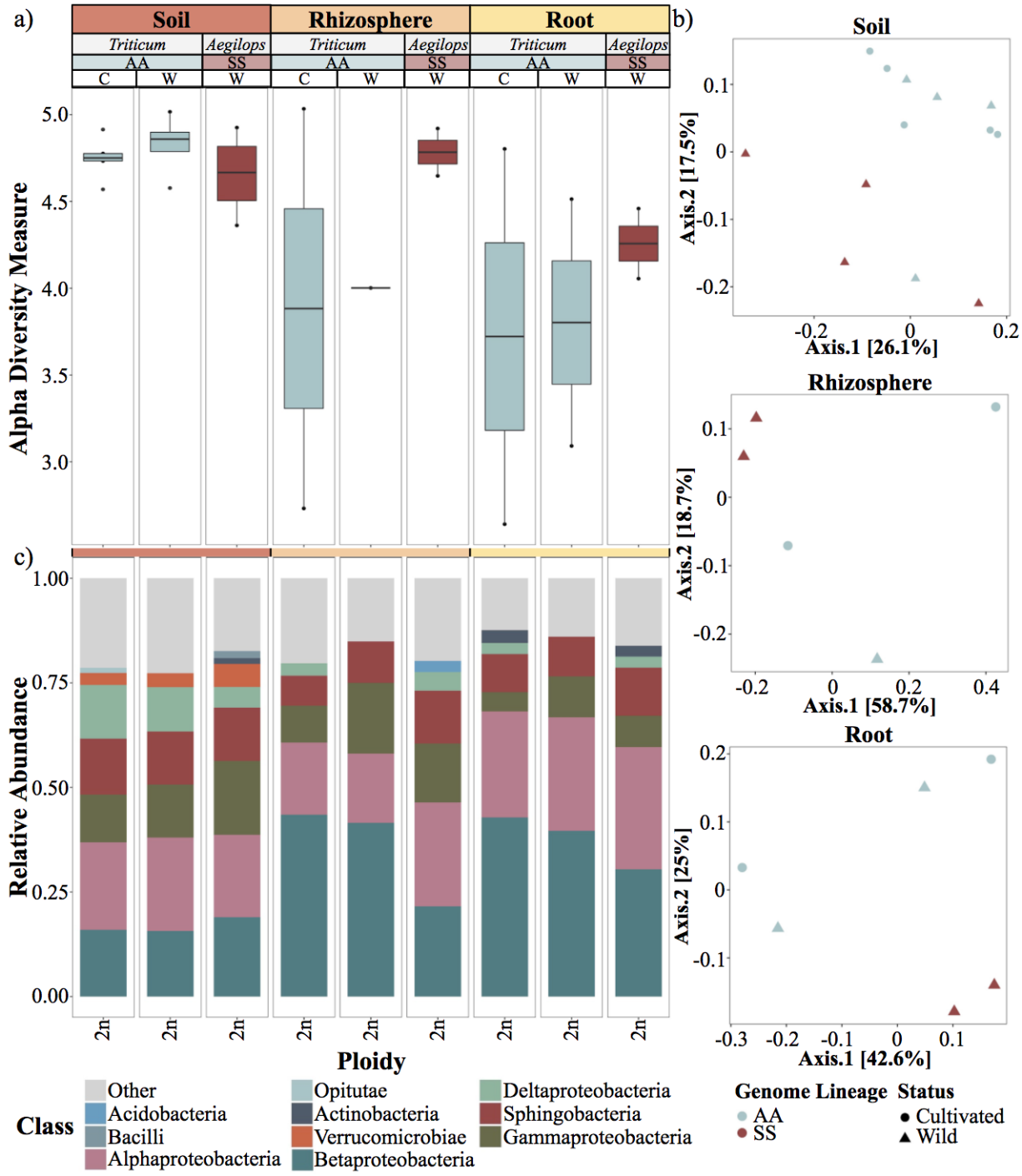


Figure 4-16.
Wheat bacterial communities show slight variation across genome lineage and domestication status. Diversity and composition analysis of diploid genome lineages from root and rhizosphere bacterial data from the greenhouse study, with a) boxplots of Shannon diversity, b) principal coordinates analysis plot using Bray-Curtis dissimilarity, and c) stacked bar graphs of class-level relative abundances, across ploidy level and parsed by genome lineage ('SS'- red and 'AA'- light blue), domestication status ('C' and circles for cultivated; 'W' and triangles for wild genotypes).

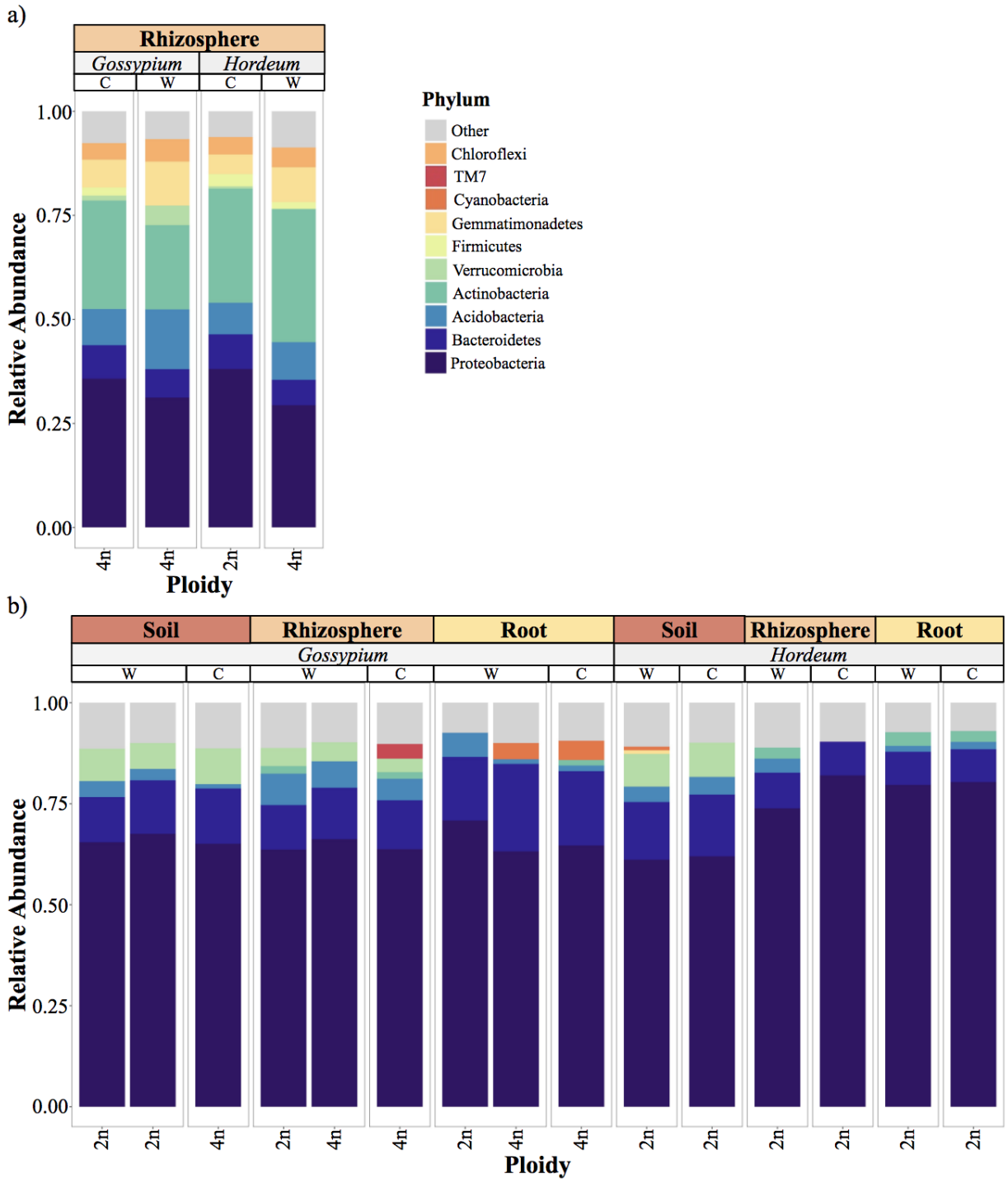


Figure 4-17.
Outgroup species show distinct shifts across ploidy and domestication status. Stacked bar graphs of phylum-level relative abundances across outgroups cotton (*Gossypium* sp.) and barley (*Hordeum vulgare*), from a) the 2017 field study and b) the greenhouse study, as faceted by sample type (soil, rhizosphere, and root) and domestication status ('W' for wild, 'C' for cultivated genotypes).

References

Acosta-Martínez V, Burow G, Zobeck TM, Allen VG. **2010a**. Soil Microbial Communities and Function in Alternative Systems to Continuous Cotton. *Soil Science Society of America journal. Soil Science Society of America* **74**: 1181–1192.

Acosta-Martínez V, Dowd SE, Bell CW, Lascano R, Booker JD, Zobeck TM, Upchurch DR. **2010b**. Microbial Community Composition as Affected by Dryland Cropping Systems and Tillage in a Semiarid Sandy Soil. *Diversity* **2**: 910–931.

Adams KL, Wendel JF. **2005**. Polyploidy and genome evolution in plants. *Current opinion in plant biology* **8**: 135–141.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, Kemen EM. **2016**. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS biology* **14**: e1002352.

Aira M, Gómez-Brandón M, Lazcano C, Bååth E, Domínguez J. **2010**. Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil biology & biochemistry* **42**: 2276–2281.

Alavi P, Starcher M, Zachow C, Mueller H, Berg G. **2013**. Root-microbe systems: the effect and mode of interaction of Stress Protecting Agent (SPA) Stenotrophomonas rhizophila DSM14405T. *Frontiers in plant science* **4**: 141.

Alexandratos N, Others. **2009**. World food and agriculture to 2030/50: highlights and views from mid-2009. In: How to feed the World in 2050. Proceedings of a technical meeting of experts, Rome, Italy, 24-26 June 2009. Food and Agriculture Organization of the United Nations (FAO), 1–32.

Alloui T, Bousseboughi I, Chaoui A, Nouar AZ, Chettah MC. **2015**. Usearch: A Meta Search Engine based on a New Result Merging Strategy. *Proceedings of the 7th International Joint Conference on Knowledge Discovery, Knowledge Engineering and Knowledge Management*.

Altieri MA, Others. **2000**. The ecological impacts of transgenic crops on agroecosystem health. *Ecosystem Health* **6**: 13–23.

Anderson C, Beare M, Buckley HL, Lear G. **2017a**. Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils. *PeerJ* **5**: e3930.

Anderson C, Beare M, Buckley HL, Lear G. **2017b**. Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils. *PeerJ* **5**: e3930.

Andrews JH, Harris RF. **1986**. r- and K-Selection and Microbial Ecology. *Advances in Microbial Ecology*: 99–147.

Anjum SA, Xie X-Y, Wang L-C, Saleem MF, Man C, Lei W. **2011**. Morphological, physiological and biochemical responses of plants to drought stress. *African journal of*

agricultural research **6**: 2026–2032.

Atkinson NJ, Urwin PE. **2012**. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of experimental botany* **63**: 3523–3543.

Bach EM, Williams RJ, Hargreaves SK, Yang F, Hofmockel KS. **2018**. Greatest soil microbial diversity found in micro-habitats. *Soil Biology and Biochemistry* **118**: 217–226.

Badri DV, Vivanco JM. **2009**. Regulation and function of root exudates. *Plant, cell & environment* **32**: 666–681.

Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, Richardson AE. **2016**. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biology and Biochemistry* **97**: 188–198.

Banfield JF, Nealson KH. **2018**. *Geomicrobiology: Interactions between Microbes and Minerals*. Walter de Gruyter GmbH & Co KG.

Basu S, Rabara RC, Negi S. **2018**. AMF: The future prospect for sustainable agriculture. *Physiological and Molecular Plant Pathology* **102**: 36–45.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesová M, Pysek P. **2012**. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of botany* **109**: 19–45.

Bérard A, Bouchet T, Sévenier G, Pablo AL, Gros R. **2011**. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. *European journal of soil biology* **47**: 333–342.

Berendsen RL, Pieterse CMJ, Peter A H. **2012**. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

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

Bertin C, Yang X, Weston LA. **2003**. The role of root exudates and allelochemicals in the rhizosphere. *Plant and soil* **256**: 67–83.

Boer W de, de Boer W, Folman LB, Summerbell RC, Boddy L. **2005**. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS microbiology reviews* **29**: 795–811.

Borie F, Rubio R, Rouanet JL, Morales A, Borie G, Rojas C. **2006**. Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chilean Ultisol. *Soil and Tillage Research* **88**: 253–261.

Bouskill NJ, Lim HC, Borglin S, Salve R, Wood TE, Silver WL, Brodie EL. **2013**. Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. *The ISME journal* **7**: 384–394.

Bouskill NJ, Wood TE, Baran R, Ye Z, Bowen BP, Lim H, Zhou J, Van Nostrand JD, Nico P, Northen TR, *et al.* 2016. Belowground Response to Drought in a Tropical Forest Soil. I. Changes in Microbial Functional Potential and Metabolism. *Frontiers in microbiology* **7**.

Bray NL, Pimentel H, Melsted P, Pachter L. **2016**. Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology* **34**: 525–527.

Bryla DR, Bouma TJ, Eissenstat DM. **1997**. Root respiration in citrus acclimates to temperature and slows during drought. *Plant, cell & environment* **20**: 1411–1420.

Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC, Schulze-Lefert P. **2015**. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell host & microbe* **17**: 392–403.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and Functions of the Bacterial Microbiota of Plants. *Annual review of plant biology* **64**: 807–838.

Bursy J, Kuhlmann AU, Pittelkow M, Hartmann H, Jebbar M, Pierik AJ, Bremer E. **2008**. Synthesis and uptake of the compatible solutes ectoine and 5-hydroxyectoine by Streptomyces coelicolor A3 (2) in response to salt and heat stresses. *Applied and environmental microbiology* **74**: 7286–7296.

Busari MA, Kukal SS, Kaur A, Bhatt R, Dulazi AA. **2015**. Conservation tillage impacts on soil, crop and the environment. *International Soil and Water Conservation Research* **3**: 119–129.

Cadotte MW. **2007**. Competition-colonization trade-offs and disturbance effects at multiple scales. *Ecology* **88**: 823–829.

Calegari A, Hargrove WL, Rheinheimer DDS, Ralisch R, Tessier D, de Tourdonnet S, de Fatima Guimarães M. **2008**. Impact of Long-Term No-Tillage and Cropping System Management on Soil Organic Carbon in an Oxisol: A Model for Sustainability. *Agronomy journal* **100**: 1013–1019.

Campbell CA, McConkey BG, Zentner RP, Selles F, Curtin D. **1996**. Tillage and crop rotation effects on soil organic C and N in a coarse-textured Typic Haploboroll in southwestern Saskatchewan. *Soil and Tillage Research* **37**: 3–14.

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. **2009**. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. *Nucleic acids research* **37**: D233–8.

Carbonetto B, Rascovan N, Álvarez R, Mentaberry A, Vázquez MP. **2014**. Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and

tillage systems in Argentine Pampas. *PloS one* **9**: e99949.

Cardinale M, Grube M, Erlacher A, Quehenberger J, Berg G. **2015**. Bacterial networks and co-occurrence relationships in the lettuce root microbiota. *Environmental microbiology* **17**: 239–252.

Carpenter-Boggs L, Stahl PD, Lindstrom MJ, Schumacher TE. **2003**. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. *Soil and Tillage Research* **71**: 15–23.

Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM. **2015a**. Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Molecular Plant-Microbe Interactions®* **28**: 1049–1058.

Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM. **2015b**. Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Molecular plant-microbe interactions: MPMI* **28**: 1049–1058.

Carvalhais LC, Schenk PM, Dennis PG. **2017**. Jasmonic acid signalling and the plant holobiont. *Current opinion in microbiology* **37**: 42–47.

Cavé-Radet A, Monard C, El-Amrani A, Salmon A, Ainouche M, Yergeau É. Phenanthrene contamination and ploidy level influence the rhizosphere microbiome of Spartina.

Chandra A, Dubey A. **2010**. Effect of ploidy levels on the activities of Δ1-pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in Cenchrus species grown under water stress. *Plant physiology and biochemistry: PPB / Societe francaise de physiologie vegetale* **48**: 27–34.

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM. **2013**. Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PloS one* **8**: e55731.

Chaparro JM, Badri DV, Vivanco JM. **2014**. Rhizosphere microbiome assemblage is affected by plant development. *The ISME journal* **8**: 790–803.

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. **2012**. Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils* **48**: 489–499.

Chavarría DN, Verdenelli RA, Serri DL, Restovich SB, Andriulo AE, Meriles JM, Vargas-Gil S. **2016**. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. *European journal of soil biology* **76**: 74–82.

Chávez-Romero Y, Navarro-Noya YE, Reynoso-Martínez SC, Sarria-Guzmán Y, Govaerts B, Verhulst N, Dendooven L, Luna-Guido M. **2016**. 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue

management. *Soil and Tillage Research* **159**: 1–8.

Cherif H, Marasco R, Rolli E, Ferjani R, Fusi M, Soussi A, Mapelli F, Blilou I, Borin S, Boudabous A, *et al.* 2015. Oasis desert farming selects environment-specific date palm root endophytic communities and cultivable bacteria that promote resistance to drought. *Environmental microbiology reports* **7**: 668–678.

Choi S, Song H, Tripathi BM, Kerfahi D, Kim H, Adams JM. **2017**. Effect of experimental soil disturbance and recovery on structure and function of soil community: a metagenomic and metagenetic approach. *Scientific reports* **7**: 2260.

Christian N, Whitaker BK, Clay K. **2015**. Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Frontiers in microbiology* **6**: 869.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016a**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *The New phytologist* **209**: 798–811.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016b**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *The New phytologist* **209**: 798–811.

Coleman-Derr D, Tringe SG. **2014**. Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Frontiers in microbiology* **5**: 283.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of advertising research* **19**: 29–37.

Cookson WR, Murphy DV, Roper MM. **2008**. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. *Soil biology & biochemistry* **40**: 763–777.

Cordovil CM d. S, Bittman S, Brito LM, Goss MJ, Hunt D, Serra J, Gourley C, Aarons S, Skiba U, Amon B, *et al.* 2020. Chapter 22 - Climate-resilient and smart agricultural management tools to cope with climate change-induced soil quality decline. In: Prasad MNV, Pietrzykowski M, eds. Climate Change and Soil Interactions. Elsevier, 613–662.

Dastogeer KMG, Li H, Sivasithamparam K, Jones MGK, Wylie SJ. **2018**. Fungal endophytes and a virus confer drought tolerance to Nicotiana benthamiana plants through modulating osmolytes, antioxidant enzymes and expression of host drought responsive genes. *Environmental and experimental botany* **149**: 95–108.

De Cáceres M, Legendre P. **2009**. Associations between species and groups of sites: indices

and statistical inference. *Ecology* **90**: 3566–3574.

De Graaff M-A, Classen AT, Castro HF, Schadt CW. **2010**. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *The New phytologist* **188**: 1055–1064.

Delhaize E, Ryan PR. **1995**. Aluminum Toxicity and Tolerance in Plants. *Plant physiology* **107**: 315–321.

Deng S, Caddell D, Yang J, Dahlen L, Washington L, Coleman-Derr D. Genome wide association study reveals plant loci controlling heritability of the rhizosphere microbiome.

Deng B, Du W, Liu C, Sun W, Tian S, Dong H. **2012**. Antioxidant response to drought, cold and nutrient stress in two ploidy levels of tobacco plants: low resource requirement confers polytolerance in polyploids? *Plant Growth Regulation* **66**: 37–47.

Deng S, -L. Wipf HM, Pierroz G, Raab TK, Khanna R, Coleman-Derr D. **2019**. A Plant Growth-Promoting Microbial Soil Amendment Dynamically Alters the Strawberry Root Bacterial Microbiome. *Scientific reports* **9**.

Derpsch R. **2003**. Conservation Tillage, No-Tillage and Related Technologies. In: García-Torres L, Benites J, Martínez-Vilela A, Holgado-Cabrera A, eds. Conservation Agriculture: Environment, Farmers Experiences, Innovations, Socio-economy, Policy. Dordrecht: Springer Netherlands, 181–190.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. **2006**. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology* **72**: 5069–5072.

Dhanyalakshmi KH, Mounashree DC, Vidyashree DN, Earanna N, Nataraja KN. **2019**. Options and opportunities for manipulation of drought traits using endophytes in crops. *Plant Physiology Reports*: 1–8.

Dixon P. **2003**. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* **14**: 927–930.

Docherty KM, Borton HM, Espinosa N, Gebhardt M, Gil-Loaiza J, Gutknecht JLM, Maes PW, Mott BM, Parnell JJ, Purdy G, *et al.* 2015. Key Edaphic Properties Largely Explain Temporal and Geographic Variation in Soil Microbial Communities across Four Biomes. *PloS one* **10**: e0135352.

Dodd JC, Jeffries P. **1986**. Early development of vesicular-arbuscular mycorrhizas in autumn-sown cereals. *Soil biology & biochemistry* **18**: 149–154.

Dong W, Liu E, Yan C, Zhang H, Zhang Y. **2017**. Changes in the composition and diversity of topsoil bacterial, archaeal and fungal communities after 22 years conventional and no-tillage

managements in Northern China. *Archives of Agronomy and Soil Science* **63**: 1369–1381.

Dorr de Quadros P, Zhalnina K, Davis-Richardson A, Fagen JR, Drew J, Bayer C, Camargo FAO, Triplett EW. **2012**. The Effect of Tillage System and Crop Rotation on Soil Microbial Diversity and Composition in a Subtropical Acrisol. *Diversity* **4**: 375–395.

Drummelsmith J. **2020**. The impact of cover crops and crop residue removal on soil microbial community abundance, diversity and soil health in a medium-term cover crop field trial in Southwest Ontario.

Dubcovsky J, Dvorak J. **2007**. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* **316**: 1862–1866.

Dumontet S, Mazzatura A, Casucci C, Perucci P. **2001**. Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. *Biology and fertility of soils* **34**: 411–416.

Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. **2018**. Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. *Cell* **175**: 973–983.e14.

Edgar RC. **2004**. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* **32**: 1792–1797.

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

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. **2015**. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America* **112**: E911–20.

Elad Y, Pertot I. **2014**. Climate Change Impacts on Plant Pathogens and Plant Diseases. *Journal of Crop Improvement* **28**: 99–139.

El-Daim IAA, Bejai S, Meijer J. **2014**. Improved heat stress tolerance of wheat seedlings by bacterial seed treatment. *Plant and soil* **379**: 337–350.

Engelmoer DJP, Behm JE, Toby Kiers E. **2014**. Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance. *Molecular ecology* **23**: 1584–1593.

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, *et al.* 2017. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Frontiers in plant science* **8**: 1147.

Faith DP. **1992**. Conservation evaluation and phylogenetic diversity. *Biological conservation*

61: 1–10.

FAO. **2011**. Save and grow: a policymaker's guide to the sustainable intensification of smallholder crop production.

FAO. **2019**. *The State of Food Security and Nutrition in the World 2019: Safeguarding against economic slowdowns and downturns*. Food & Agriculture Org.

FAOSTAT. **2020**. Food and agriculture data. *Statistics division of food and agriculture organization of the United Nations*.

Feng Y, Motta AC, Reeves DW, Burmester CH, van Santen E, Osborne JA. **2003**. Soil microbial communities under conventional-till and no-till continuous cotton systems. *Soil biology & biochemistry* **35**: 1693–1703.

Feng K, Zhang Z, Cai W, Liu W, Xu M, Yin H, Wang A, He Z, Deng Y. **2017**. Biodiversity and species competition regulate the resilience of microbial biofilm community. *Molecular ecology* **26**: 6170–6182.

Fierer N. **2017**. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature reviews. Microbiology* **15**: 579–590.

Finney DM, Buyer JS, Kaye JP. **2017**. Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*.

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. **2018**. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E1157–E1165.

Forrester NJ, Ashman T-L. **2018**. The direct effects of plant polyploidy on the legume–rhizobia mutualism. *Annals of Botany* **121**: 209–220.

Forrester NJ, Ashman T-L. **2020**. Autopolyploidy alters nodule-level interactions in the legume-rhizobium mutualism. *American journal of botany* **107**: 179–185.

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

Galand PE, Lucas S, Fagervold SK, Peru E, Pruski AM, Vétion G, Dupuy C, Guizien K. **2016**. Disturbance Increases Microbial Community Diversity and Production in Marine Sediments. *Frontiers in microbiology* **7**: 1950.

Gao C, Montoya L, Xu L, Madera M, Hollingsworth J, Purdom E, Hutmacher RB, Dahlberg JA, Coleman-Derr D, Lemaux PG, *et al.* 2019. Strong succession in arbuscular

mycorrhizal fungal communities. *The ISME journal* **13**: 214–226.

Gargallo-Garriga A, Preece C, Sardans J, Oravec M, Urban O, Peñuelas J. **2018**. Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific reports* **8**: 12696.

Getzke F, Thiergart T, Hacquard S. **2019**. Contribution of bacterial-fungal balance to plant and animal health. *Current opinion in microbiology* **49**: 66–72.

Glick BR, Penrose DM, Li J. **1998**. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of theoretical biology* **190**: 63–68.

Gómez P, Buckling A. **2013**. Real-time microbial adaptive diversification in soil. *Ecology letters* **16**: 650–655.

Gosling P, Hodge A, Goodlass G, Bending GD. **2006**. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, ecosystems & environment* **113**: 17–35.

Gregory PJ. **2011**. Matthew P. Reynolds (Ed): Climate change and crop production. *Food Security* **3**: 111–112.

Griesbach RJ, Kamo KK. **1996a**. The effect of induced polyploidy on the flavonols of Petunia 'Mitchell'. *Phytochemistry* **42**: 361–363.

Griesbach RJ, Kamo KK. **1996b**. The effect of induced polyploidy on the flavonols of Petunia 'Mitchell'. *Phytochemistry* **42**: 361–363.

Griffin EA, Harrison JG, Kembel SW, Carrell AA, Joseph Wright S, Carson WP. **2019**. Plant host identity and soil macronutrients explain little variation in sapling endophyte community composition: Is disturbance an alternative explanation? *Journal of Ecology* **107**: 1876–1889.

Griffiths RI, Thomson BC, James P, Bell T, Bailey M, Whiteley AS. **2011**. The bacterial biogeography of British soils. *Environmental microbiology* **13**: 1642–1654.

Guo F, Zhang T. **2014**. Detecting the nonviable and heat-tolerant bacteria in activated sludge by minimizing DNA from dead cells. *Microbial ecology* **67**: 829–836.

Hacquard S. **2016**. Disentangling the factors shaping microbiota composition across the plant holobiont. *The New phytologist* **209**: 454–457.

Haichar F el Z, el Zahar Haichar F, Santaella C, Heulin T, Achouak W. **2014**. Root exudates mediated interactions belowground. *Soil Biology and Biochemistry* **77**: 69–80.

Hallama M, Pekrun C, Lambers H, Kandeler E. **2019**. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant and Soil*

434: 7–45.

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

Hartman K, Tringe SG. **2019**. Interactions between plants and soil shaping the root microbiome under abiotic stress. *Biochemical Journal* **476**: 2705–2724.

Hayakawa M, Yamamura H, Nakagawa Y, Kawa Y, Hayashi Y, Misonou T, Kaneko H, Kikushima N, Takahashi T, Yamasaki S, *et al.* 2010. Taxonomic Diversity of Actinomycetes Isolated from Swine Manure Compost. 日本放線菌学会誌 **advpub**: 1009280039–1009280039.

He P, Friebe BR, Gill BS, Zhou J-M. **2003**. Allopolyploidy alters gene expression in the highly stable hexaploid wheat. *Plant molecular biology* **52**: 401–414.

van der Heijden MGA, Bruin S de, Luckerhoff L, van Logtestijn RSP, Schlaeppi K. **2016**. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *The ISME journal* **10**: 389–399.

Helgason BL, Walley FL, Germida JJ. **2009**. Fungal and Bacterial Abundance in Long-Term No-Till and Intensive-Till Soils of the Northern Great Plains. *Soil Science Society of America journal. Soil Science Society of America* **73**: 120–127.

Hill RL, Horton R, Cruse RM. **1985**. Tillage Effects on Soil Water Retention and Pore Size Distribution of Two Mollisols. *Soil Science Society of America Journal* **49**: 1264–1270.

Hobbs PR, Sayre K, Gupta R. **2008**. The role of conservation agriculture in sustainable agriculture. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **363**: 543–555.

Hofberger JA, Lyons E, Edger PP, Chris Pires J, Eric Schranz M. **2013**. Whole genome and tandem duplicate retention facilitated glucosinolate pathway diversification in the mustard family. *Genome biology and evolution* **5**: 2155–2173.

Holmes AJ, Bowyer J, Holley MP, O'Donoghue M, Montgomery M, Gillings MR. **2000**. Diverse, yet-to-be-cultured members of the Rubrobacter subdivision of the Actinobacteria are widespread in Australian arid soils. *FEMS microbiology ecology* **33**: 111–120.

Hontoria C, García-González I, Quemada M, Roldán A, Alguacil MM. **2019**. The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. *The Science of the total environment* **660**: 913–922.

Hubbard M, Germida J, Vujanovic V. **2012**. Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany* **90**: 137–149.

Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Tennessen K,

Palaniappan K, Szeto E, Pillay M, Chen I-MA, *et al.* 2016. The standard operating procedure of the DOE-JGI Metagenome Annotation Pipeline (MAP v.4). *Standards in genomic sciences* **11**: 17.

Hussain HA, Men S, Hussain S, Chen Y, Ali S, Zhang S, Zhang K, Li Y, Xu Q, Liao C, *et al.* 2019. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Scientific reports* **9**: 3890.

Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. **2010**. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC bioinformatics* **11**: 119.

Iannucci A, Fragasso M, Beleggia R, Nigro F, Papa R. **2017**. Evolution of the Crop Rhizosphere: Impact of Domestication on Root Exudates in Tetraploid Wheat (Triticum turgidum L.). *Frontiers in plant science* **8**: 2124.

Jackson S, Chen ZJ. **2010**. Genomic and expression plasticity of polyploidy. *Current opinion in plant biology* **13**: 153–159.

James C. **2003**. Global review of commercialized transgenic crops. *Current science* **84**: 303–309.

Kabir Z, Koide RT. **2002**. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA. *Plant and soil* **238**: 205–215.

Kabir Z, O'Halloran IP, Fyles JW, Hamel C. **1997a**. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization : Hyphal density and mycorrhizal root colonization. *Plant and soil* **192**: 285–293.

Kabir Z, O'Halloran IP, Hamel C. **1997b**. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance. *Mycorrhiza* **7**: 197–200.

Kabir Z, Rhamoun M, Lazicki P, Horwath W. **2008**. Cover crops and conservation tillage increase mycorrhizal colonization of corn and tomato roots. *Sustainable Agriculture Farming System Project* **9**.

Kang Y, Khan S, Ma X. **2009**. Climate change impacts on crop yield, crop water productivity and food security – A review. *Progress in natural science: communication of state key laboratories of China* **19**: 1665–1674.

Kennedy AC, Smith KL. **1995**. Soil microbial diversity and the sustainability of agricultural soils. *Plant and soil* **170**: 75–86.

Kim Y-G, Udayanga KGS, Totsuka N, Weinberg JB, Núñez G, Shibuya A. **2014**. Gut Dysbiosis Promotes M2 Macrophage Polarization and Allergic Airway Inflammation via

Fungi-Induced PGE2. *Cell host & microbe* **15**: 95–102.

Knegt B, Jansa J, Franken O, Engelmoer DJP, Werner GDA, Bücking H, Toby Kiers E. **2016**. Host plant quality mediates competition between arbuscular mycorrhizal fungi. *Fungal ecology* **20**: 233–240.

Koch A, McBratney A, Adams M, Field D, Hill R, Crawford J, Minasny B, Lal R, Abbott L, O'Donnell A, *et al.* 2013. Soil Security: Solving the Global Soil Crisis. *Global Policy* **4**: 434–441.

Kohler J, Hernández JA, Caravaca F, Roldán A. **2008**. Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. *Functional plant biology: FPB* **35**: 141–151.

Kour D, Rana KL, Yadav N, Yadav AN, Kumar A, Meena VS, Singh B, Chauhan VS, Dhaliwal HS, Saxena AK. **2019**. Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture. In: Kumar A, Meena VS, eds. Plant Growth Promoting Rhizobacteria for Agricultural Sustainability : From Theory to Practices. Singapore: Springer Singapore, 19–65.

Kuramae E, Gamper H, van Veen J, Kowalchuk G. **2011**. Soil and plant factors driving the community of soil-borne microorganisms across chronosequences of secondary succession of chalk grasslands with a neutral pH. *FEMS microbiology ecology* **77**: 285–294.

Kuramae EE, Yergeau E, Wong LC, Pijl AS, van Veen JA, Kowalchuk GA. **2012**. Soil characteristics more strongly influence soil bacterial communities than land-use type. *FEMS microbiology ecology* **79**: 12–24.

Lal R, Regnier E, Eckert DJ, Edwards WM, Hammond R. **1991**. Expectations of cover crops for sustainable agriculture. *cover crops for clean water. Hargrove WL Soil and water conservation society publication, Ankey, USA*: 1–11.

Lamaoui M, Jemo M, Datla R, Bekkaoui F. **2018**. Heat and Drought Stresses in Crops and Approaches for Their Mitigation. *Frontiers in chemistry* **6**: 26.

Lareen A, Burton F, Schäfer P. **2016**. Plant root-microbe communication in shaping root microbiomes. *Plant molecular biology* **90**: 575–587.

Lauber CL, Hamady M, Knight R, Fierer N. **2009**. Soil pH as a predictor of soil bacterial community structure at the continental scale: a pyrosequencing-based assessment. *Applied and environmental microbiology*.

Lavania UC, Srivastava S, Lavania S, Basu S, Misra NK, Mukai Y. **2012a**. Autopolyploidy differentially influences body size in plants, but facilitates enhanced accumulation of secondary metabolites, causing increased cytosine methylation. *The Plant Journal* **71**: 539–549.

Lavania UC, Srivastava S, Lavania S, Basu S, Misra NK, Mukai Y. **2012b**. Autopolyploidy

differentially influences body size in plants, but facilitates enhanced accumulation of secondary metabolites, causing increased cytosine methylation. *The Plant journal: for cell and molecular biology* **71**: 539–549.

Leggett M, Gleddie S, Holloway G. **2001**. Phosphate-Solubilizing Microorganisms and Their Use. In: Plant Nutrient Acquisition. Springer Japan, 299–318.

Letunic I, Bork P. **2007**. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23**: 127–128.

Levin SA. **1983**. Coevolution. In: Population Biology. Springer Berlin Heidelberg, 328–334.

Levin DA. **2002a**. *The Role of Chromosomal Change in Plant Evolution*. Oxford University Press, USA.

Levin DA. **2002b**. *The Role of Chromosomal Change in Plant Evolution*. Oxford University Press.

Levin SA, Segel LA, Adler FR. **1990**. Diffuse coevolution in plant-herbivore communities. *Theoretical Population Biology* **37**: 171–191.

Lewin GR, Carlos C, Chevrette MG, Horn HA, McDonald BR, Stankey RJ, Fox BG, Currie CR. **2016**. Evolution and Ecology of Actinobacteria and Their Bioenergy Applications. *Annual review of microbiology* **70**: 235–254.

Likar M, Stres B, Rusjan D, Potisek M, Regvar M. **2017**. Ecological and conventional viticulture gives rise to distinct fungal and bacterial microbial communities in vineyard soils. *Applied Soil Ecology* **113**: 86–95.

Liu J, Li J, Feng L, Cao H, Cui Z. **2010**. An improved method for extracting bacteria from soil for high molecular weight DNA recovery and BAC library construction. *The Journal of Microbiology* **48**: 728–733.

Lugtenberg B, Kamilova F. **2009**. Plant-growth-promoting rhizobacteria. *Annual review of microbiology* **63**: 541–556.

Lukashin A. **1998**. GeneMark.hmm: new solutions for gene finding. *Nucleic Acids Research* **26**: 1107–1115.

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

Lupwayi NZ, Rice WA, Clayton GW. **1998**. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil biology & biochemistry* **30**: 1733–1741.

Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B, Quero

JL, García-Gómez M, Gallardo A, Ulrich W, *et al.* 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 15684–15689.

Magadum S, Banerjee U, Murugan P, Gangapur D, Ravikesavan R. **2013**. Gene duplication as a major force in evolution. *Journal of genetics* **92**: 155–161.

Magoc T, Salzberg SL. **2011**. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**: 2957–2963.

Mainali KP, Heckathorn SA, Wang D, Weintraub MN, Frantz JM, Hamilton EW 3rd. **2014**. Impact of a short-term heat event on C and N relations in shoots vs. roots of the stress-tolerant C4 grass, Andropogon gerardii. *Journal of plant physiology* **171**: 977–985.

Malinowski DP, Belesky DP. **2000**. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop science* **40**: 923–940.

Mann L, Tolbert V, Cushman J. **2002**. Potential environmental effects of corn (*Zea mays* L.) stover removal with emphasis on soil organic matter and erosion. *Agriculture, ecosystems & environment* **89**: 149–166.

Mapelli F, Marasco R, Rolli E, Barbato M, Cherif H, Guesmi A, Ouzari I, Daffonchio D, Borin S. **2013**. Potential for Plant Growth Promotion of Rhizobacteria Associated with Salicornia Growing in Tunisian Hypersaline Soils. *BioMed research international* **2013**: 1–13.

Marschner H, Marschner P. **1995**. *Mineral Nutrition of Higher Plants*. Gulf Professional Publishing.

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

Matsuoka Y, Takumi S, Nasuda S. **2014**. Genetic mechanisms of allopolyploid speciation through hybrid genome doubling: novel insights from wheat (Triticum and Aegilops) studies. *International review of cell and molecular biology* **309**: 199–258.

Mayak S, Tirosh T, Glick BR. **2004**. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant science: an international journal of experimental plant biology* **166**: 525–530.

McConkey B. **2003**. Crop rotation and tillage impact on carbon sequestration in Canadian prairie soils. *Soil and Tillage Research* **74**: 81–90.

McDevitt-Irwin JM, Garren M, McMinds R, Thurber RV, Baum JK. **2019**. Variable interaction outcomes of local disturbance and El Niño-induced heat stress on coral microbiome alpha and beta diversity. *Coral Reefs* **38**: 331–345.

McMurdie PJ, Holmes S. **2013**. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**: e61217.

Mendes R, Garbeva P, Raaijmakers JM. **2013**. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews* **37**: 634–663.

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, *et al.* 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **332**: 1097–1100.

Micallef SA, Channer S, Shiaris MP, Colón-Carmona A. **2009a**. Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant Signaling & Behavior* **4**: 777–780.

Micallef SA, Shiaris MP, Colón-Carmona A. **2009b**. Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. *Journal of experimental botany* **60**: 1729–1742.

Miguez FE, Bollero GA. **2005**. Review of Corn Yield Response under Winter Cover Cropping Systems Using Meta-Analytic Methods. *Crop science* **45**: 2318–2329.

Minic Z. **2008**. Physiological roles of plant glycoside hydrolases. *Planta* **227**: 723–740.

Mitchell JP, Shrestha A, Horwath WR, Southard RJ, Madden N, Veenstra J, Munk DS. **2015**. Tillage and Cover Cropping Affect Crop Yields and Soil Carbon in the San Joaquin Valley, California. *Agronomy journal* **107**: 588–596.

Moebius-Clune BN, van Es HM, Idowu OJ, Schindelbeck RR, Moebius-Clune DJ, Wolfe DW, Abawi GS, Thies JE, Gugino BK, Lucey R. **2008**. Long-Term Effects of Harvesting Maize Stover and Tillage on Soil Quality. *Soil Science Society of America journal. Soil Science Society of America* **72**: 960–969.

de M. Sá JC, Cerri CC, Dick WA, Lal R, Filho SPV, Piccolo MC, Feigl BE. **2001**. Organic Matter Dynamics and Carbon Sequestration Rates for a Tillage Chronosequence in a Brazilian Oxisol. *Soil Science Society of America journal. Soil Science Society of America* **65**: 1486–1499.

Murugan R, Koch H-J, Joergensen RG. **2014**. Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths. *Biology and fertility of soils* **50**: 487–498.

Nakayasu ES, Nicora CD, Sims AC, Burnum-Johnson KE, Kim Y-M, Kyle JE, Matzke MM, Shukla AK, Chu RK, Schepmoes AA, *et al.* 2016. MPLEx: a Robust and Universal Protocol for Single-Sample Integrative Proteomic, Metabolomic, and Lipidomic Analyses. *mSystems* **1**.

Navarro-Noya YE, Gómez-Acata S, Montoya-Ciriaco N, Rojas-Valdez A, Suárez-Arriaga

MC, Valenzuela-Encinas C, Jiménez-Bueno N, Verhulst N, Govaerts B, Dendooven L. **2013**. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. *Soil biology & biochemistry* **65**: 86–95.

Naylor D, DeGraaf S, Purdom E, Coleman-Derr D. **2017**. Drought and host selection influence bacterial community dynamics in the grass root microbiome. *The ISME journal* **11**: 2691–2704.

Nearing MA, Pruski FF, O'Neal MR. **2004**. Expected climate change impacts on soil erosion rates: A review. *Journal of Soil and Water Conservation* **59**: 43–50.

Neelam AA, Gaur A, Bhalla E, Gupta SR. **2010**. Soil aggregate carbon and diversity of mycorrhiza as affected by tillage practices in a rice-wheat cropping system in northern India. *Int J Ecol Environ Sci* **36**: 233–243.

Neely CL, Beare MH, Hargrove WL, Coleman DC. **1991**. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. *Soil biology & biochemistry* **23**: 947–954.

Nessner Kavamura V, Taketani RG, Lançoni MD, Andreote FD, Mendes R, Soares de Melo I. **2013**. Water regime influences bulk soil and Rhizosphere of Cereus jamacaru bacterial communities in the Brazilian Caatinga biome. *PloS one* **8**: e73606.

Nivelle E, Verzeaux J, Habbib H, Kuzyakov Y, Decocq G, Roger D, Lacoux J, Duclercq J, Spicher F, Nava-Saucedo J-E, *et al.* 2016. Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization. *Applied soil ecology: a section of Agriculture, Ecosystems & Environment* **108**: 147–155.

Niwa Y, Sasaki Y. **2003**. Plant self-defense mechanisms against oxidative injury and protection of the forest by planting trees of triploids and tetraploids. *Ecotoxicology and environmental safety* **55**: 70–81.

Noguchi H, Taniguchi T, Itoh T. **2008**. MetaGeneAnnotator: Detecting Species-Specific Patterns of Ribosomal Binding Site for Precise Gene Prediction in Anonymous Prokaryotic and Phage Genomes. *DNA Research* **15**: 387–396.

Nuccio EE, Starr E, Karaoz U, Brodie EL, Zhou J, Tringe S, Malmstrom RR, Woyke T, Banfield JF, Firestone MK, *et al.* Niche differentiation is spatially and temporally regulated in the rhizosphere.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, *et al.* 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (RK Pachauri and L Meyer, Eds.). Geneva, Switzerland: IPCC.

Panchy N, Lehti-Shiu M, Shiu S-H. **2016**. Evolution of Gene Duplication in Plants. *Plant*

physiology **171**: 2294–2316.

Panel ME. **2014**. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. *Ecosystem Services*.

Peay KG, Kennedy PG, Talbot JM. **2016**. Dimensions of biodiversity in the Earth mycobiome. *Nature reviews. Microbiology* **14**: 434–447.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. **2013**. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 6548–6553.

Pérez-Jaramillo JE, Carrión VJ, de Hollander M, Raaijmakers JM. **2018**. The wild side of plant microbiomes. *Microbiome* **6**: 143.

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. **2016a**. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology* **90**: 635–644.

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. **2016b**. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant molecular biology* **90**: 635–644.

Peters RD, Sturz AV, Carter MR, Sanderson JB. **2003**. Developing disease-suppressive soils through crop rotation and tillage management practices. *Soil and Tillage Research* **72**: 181–192.

Ponsford JCB, Hubbard CJ, Harrison JG, Maignien L, Alex Buerkle C, Weinig C. Whole-genome duplication and host genotype affect rhizosphere microbial communities.

Powell AF, Doyle JJ. **2016**. Enhanced rhizobial symbiotic capacity in an allopolyploid species of Glycine (Leguminosae). *American journal of botany* **103**: 1771–1782.

Price MN, Dehal PS, Arkin AP. **2010**. FastTree 2--approximately maximum-likelihood trees for large alignments. *PloS one* **5**: e9490.

Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, Lind EM, Seabloom EW, Adler PB, Bakker JD, *et al.* 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology letters* **18**: 85–95.

Qin W, Liu C, Jiang W, Xue Y, Wang G, Liu S. **2019**. A coumarin analogue NFA from endophytic Aspergillus fumigatus improves drought resistance in rice as an antioxidant. *BMC microbiology* **19**: 50.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research* **41**: D590–6.

Ramirez-Villanueva DA, Bello-López JM, Navarro-Noya YE, Luna-Guido M, Verhulst N,

Govaerts B, Dendooven L. **2015**. Bacterial community structure in maize residue amended soil with contrasting management practices. *Applied soil ecology: a section of Agriculture, Ecosystems & Environment* **90**: 49–59.

Renny-Byfield S, Wendel JF. **2014**. Doubling down on genomes: polyploidy and crop plants. *American journal of botany* **101**: 1711–1725.

Rho M, Tang H, Ye Y. **2010**. FragGeneScan: predicting genes in short and error-prone reads. *Nucleic Acids Research* **38**: e191–e191.

Riah-Anglet W, Trinsoutrot-Gattin I, Martin-Laurent F, Laroche-Ajzenberg E, Norini M-P, Latour X, Laval K. **2015**. Soil microbial community structure and function relationships: A heat stress experiment. *Applied soil ecology: a section of Agriculture, Ecosystems & Environment* **86**: 121–130.

Rincon-Florez VA, Carvalhais LC, Dang YP, Schenk PM. **2016**. Soil Microbial Community Interactions Under Tillage Systems in Australia. In: Hakeem KR, Akhtar MS, Abdullah SNA, eds. Plant, Soil and Microbes: Volume 1: Implications in Crop Science. Cham: Springer International Publishing, 93–102.

Ritz K, Young IM. **2004**. Interactions between soil structure and fungi. *The mycologist* **18**: 52–59.

Rizhsky L, Liang H, Mittler R. **2002**. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant physiology* **130**: 1143–1151.

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R. **2004**. When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant physiology* **134**: 1683–1696.

Robinson MD, Oshlack A. **2010**. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome biology* **11**: R25.

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS. **2008**. Stress tolerance in plants via habitat-adapted symbiosis. *The ISME journal* **2**: 404–416.

Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, Gandolfi C, Casati E, Previtali F, Gerbino R, *et al.* 2015. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental microbiology* **17**: 316–331.

Rosner K, Bodner G, Hage-Ahmed K, Steinkellner S. **2018**. Long-term Soil Tillage and Cover Cropping Affected Arbuscular Mycorrhizal Fungi, Nutrient Concentrations, and Yield in Sunflower. *Agronomy journal* **110**: 2664–2672.

Roucou A, Violle C, Fort F, Roumet P, Ecarnot M, Vile D. **2018**. Shifts in plant functional

strategies over the course of wheat domestication. *Journal of Applied Ecology* **55**: 25–37.

Rousk J, Bååth E. **2011**. Growth of saprotrophic fungi and bacteria in soil. *FEMS microbiology ecology* **78**: 17–30.

Saleem M, Law AD, Sahib MR, Pervaiz ZH, Zhang Q. **2018**. Impact of root system architecture on rhizosphere and root microbiome. *Rhizosphere* **6**: 47–51.

Saleh B, Allario T, Dambier D, Ollitrault P, Morillon R. **2008**. Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. *Comptes rendus biologies* **331**: 703–710.

Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswarlu B. **2010**. Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant growth regulation* **62**: 21–30.

Santos-Medellín C, Edwards J, Liechty Z, Nguyen B, Sundaresan V. **2017**. Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes. *mBio* **8**.

Santoyo G, Hernández-Pacheco C, Hernández-Salmerón J, Hernández-León R. **2017**. The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. *Spanish journal of agricultural research = Revista de investigacion agraria* **15**: e03R01.

Sasse J, Martinoia E, Northen T. **2018**. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in plant science* **23**: 25–41.

Sathya A, Vijayabharathi R, Gopalakrishnan S. **2017**. Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech* **7**: 102.

Schlemper TR, Leite MFA, Lucheta AR, Shimels M, Bouwmeester HJ, van Veen JA, Kuramae EE. **2017**. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS microbiology ecology* **93**.

Schloss PD, Handelsman J. **2006**. Toward a census of bacteria in soil. *PLoS computational biology* **2**: e92.

Schmidt R, Gravuer K, Bossange AV, Mitchell J, Scow K. **2018**. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PloS one* **13**: e0192953.

Schmidt R, Mitchell J, Scow K. **2019**. Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. *Soil biology & biochemistry* **129**: 99–109.

Schutter M, Sandeno J, Dick R. **2001**. Seasonal, soil type, and alternative management

influences on microbial communities of vegetable cropping systems. *Biology and fertility of soils* **34**: 397–410.

Shah AN, Tanveer M, Shahzad B, Yang G, Fahad S, Ali S, Bukhari MA, Tung SA, Hafeez A, Souliyanonh B. **2017**. Soil compaction effects on soil health and crop productivity: an overview. *Environmental science and pollution research international* **24**: 10056–10067.

Shirley C, Bowman G, Cramer C. **1998**. *Managing cover crops profitably*. Burlington, VT: Sustainable Agriculture Publications.

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, *et al.* 2001. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Applied and environmental microbiology* **67**: 2469–2475.

Simmons T, Caddell DF, Deng S, Coleman-Derr D. **2018**. Exploring the Root Microbiome: Extracting Bacterial Community Data from the Soil, Rhizosphere, and Root Endosphere. *Journal of Visualized Experiments*.

Simmons T, Styer AB, Pierroz G, Gonçalves AP, Pasricha R, Hazra AB, Bubner P, Coleman-Derr D. **2020**. Drought Drives Spatial Variation in the Millet Root Microbiome. *Frontiers in plant science* **11**: 599.

Singh D, Jain P, Gupta A, Nema R. **2013**. Soil diversity: a key for natural management of biological and chemical constitute to maintain soil health & fertility. *International Journal of Bio-Science and Bio-Technology* **5**: 41–50.

Six J, Frey SD, Thiet RK, Batten KM. **2006**. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Science Society of America journal. Soil Science Society of America* **70**: 555–569.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, Depamphilis CW, Wall PK, Soltis PS. **2009**. Polyploidy and angiosperm diversification. *American journal of botany* **96**: 336–348.

Souza RC, Cantão ME, Vasconcelos ATR, Nogueira MA, Hungria M. **2013**. Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. *Applied soil ecology: a section of Agriculture, Ecosystems & Environment* **72**: 49–61.

Stavi I, Bel G, Zaady E. **2016**. Soil functions and ecosystem services in conventional, conservation, and integrated agricultural systems. A review. *Agronomy for Sustainable Development* **36**.

Stevenson A, Hallsworth JE. **2014**. Water and temperature relations of soil Actinobacteria. *Environmental microbiology reports* **6**: 744–755.

Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ. **2018**. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E5213–E5222.

Strock JS, Porter PM, Russelle MP. **2004**. Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. *Journal of environmental quality* **33**: 1010–1016.

Sudová R, Pánková H, Rydlová J, Münzbergová Z, Suda J. **2014**. Intraspecific ploidy variation: A hidden, minor player in plant-soil-mycorrhizal fungi interactions. *American journal of botany* **101**: 26–33.

Sugiyama S. **1998**. Differentiation in competitive ability and cold tolerance between diploid and tetraploid cultivars in Lolium perenne. *Euphytica/ Netherlands journal of plant breeding* **103**: 55–59.

Sugiyama S-I. **2006**. Responses of shoot growth and survival to water stress gradient in diploid and tetraploid populations ofLolium multiflorumandL. perenne. *Grassland Science* **52**: 155–160.

Sukumar P, Legué V, Vayssières A, Martin F, Tuskan GA, Kalluri UC. **2013**. Involvement of auxin pathways in modulating root architecture during beneficial plant-microorganism interactions. *Plant, cell & environment* **36**: 909–919.

Sundermeier AP, Islam KR, Raut Y, Reeder RC, Dick WA. **2011**. Continuous No-Till Impacts on Soil Biophysical Carbon Sequestration. *Soil Science Society of America journal. Soil Science Society of America* **75**: 1779–1788.

Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. **2014**. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PloS one* **9**: e105592.

Tang C-S, Cai W-F, Kohl K, Nishimoto RK. **1994**. Plant Stress and Allelopathy. *ACS Symposium Series*: 142–157.

Tatusov RL, Galperin MY, Natale DA, Koonin EV. **2000**. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic acids research* **28**: 33–36.

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T. **2016**. Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing. *Applied and environmental microbiology* **82**: 7217–7226.

Těšitelová T, Jersáková J, Roy M, Kubátová B, Těšitel J, Urfus T, Trávníček P, Suda J. **2013**. Ploidy-specific symbiotic interactions: divergence of mycorrhizal fungi between cytotypes of the Gymnadenia conopsea group (Orchidaceae). *The New phytologist* **199**: 1022–1033.

Thonar C, Frossard E, Smilauer P, Jansa J. **2014**. Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. *Molecular ecology* **23**: 733–746.

Tremblay J, Singh K, Fern A, Kirton ES, He S, Woyke T, Lee J, Chen F, Dangl JL, Tringe SG. **2015**. Primer and platform effects on 16S rRNA tag sequencing. *Frontiers in microbiology* **6**: 771.

Trivedi P, Schenk PM, Wallenstein MD, Singh BK. **2017**. Tiny Microbes, Big Yields: enhancing food crop production with biological solutions. *Microbial biotechnology* **10**: 999–1003.

Turmel M-S, Speratti A, Baudron F, Verhulst N, Govaerts B. **2015**. Crop residue management and soil health: A systems analysis. *Agricultural Systems* **134**: 6–16.

Uselman SM, Qualls RG, Thomas RB. **2000**. Effects of increased atmospheric CO 2, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (Robinia pseudoacacia L.). *Plant and soil* **222**: 191–202.

Vives-Peris V, Molina L, Segura A, Gómez-Cadenas A, Pérez-Clemente RM. **2018**. Root exudates from citrus plants subjected to abiotic stress conditions have a positive effect on rhizobacteria. *Journal of plant physiology* **228**: 208–217.

van der Voort M, Kempenaar M, van Driel M, Raaijmakers JM, Mendes R. **2016**. Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecology letters* **19**: 375–382.

Vukicevich E, Lowery T, Bowen P, Úrbez-Torres JR, Hart M. **2016**. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agronomy for Sustainable Development* **36**: 48.

Wagner MR, Lundberg DS, Del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. **2016**. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature communications* **7**: 12151.

Wakita Y, Shimomura Y, Kitada Y, Yamamoto H, Ohashi Y, Matsumoto M. **2018**. Taxonomic classification for microbiome analysis, which correlates well with the metabolite milieu of the gut. *BMC microbiology* **18**: 188.

Wallenstein MD, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH. **2006**. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest ecology and management* **222**: 459–468.

Wang Q, Cen Z, Zhao J. **2015**. The survival mechanisms of thermophiles at high temperatures: an angle of omics. *Physiology* **30**: 97–106.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007a**. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental*

Microbiology **73**: 5261–5267.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007b**. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology* **73**: 5261–5267.

Wang Y, Xu J, Shen J, Luo Y, Scheu S, Ke X. **2010**. Tillage, residue burning and crop rotation alter soil fungal community and water-stable aggregation in arable fields. *Soil and Tillage Research* **107**: 71–79.

Waring BG, Averill C, Hawkes CV. **2013**. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. *Ecology letters* **16**: 887–894.

Wei F, Zhao L, Xu X, Feng H, Shi Y, Deakin G, Feng Z, Zhu H. **2019**. Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in Plant Science* **10**.

Welbaum GE, Sturz AV, Dong Z, Nowak J. **2004**. Managing Soil Microorganisms to Improve Productivity of Agro-Ecosystems. *Critical reviews in plant sciences* **23**: 175–193.

Wilhelm WW, Johnson JMF, Hatfield JL, Voorhees WB, Linden DR. **2004**. Crop and soil productivity response to corn residue removal. *Agronomy journal* **96**: 1–17.

Williams A, de Vries FT. **2020**. Plant root exudation under drought: implications for ecosystem functioning. *The New phytologist* **225**: 1899–1905.

Wood CW, Edwards JH. **1992**. Agroecosystem management effects on soil carbon and nitrogen. *Agriculture, ecosystems & environment* **39**: 123–138.

Wortman SE, Francis CA, Lindquist JL. **2012**. Cover Crop Mixtures for the Western Corn Belt: Opportunities for Increased Productivity and Stability. *Agronomy journal* **104**: 699–705.

Wu W, Duncan RW, Ma B-L. **2017**. Quantification of canola root morphological traits under heat and drought stresses with electrical measurements. *Plant and soil* **415**: 229–244.

Xu L, Coleman-Derr D. **2019**. Causes and consequences of a conserved bacterial root microbiome response to drought stress. *Current opinion in microbiology* **49**: 1–6.

Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim Y-M, Zink EM, Engbrecht KM, Wang Y, *et al.* 2018. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E4284–E4293.

Yin C, Mueth N, Hulbert S, Schlatter D, Paulitz TC, Schroeder K, Prescott A, Dhingra A. **2017**. Bacterial Communities on Wheat Grown Under Long-Term Conventional Tillage and

No-Till in the Pacific Northwest of the United States. *Phytobiomes Journal* **1**: 83–90.

Zachow C, Müller H, Tilcher R, Berg G. **2014**. Differences between the rhizosphere microbiome of Beta vulgaris ssp. maritima-ancestor of all beet crops-and modern sugar beets. *Frontiers in microbiology* **5**: 415.

Zamioudis C, Pieterse CMJ. **2012**. Modulation of host immunity by beneficial microbes. *Molecular plant-microbe interactions: MPMI* **25**: 139–150.

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A. **2018**. Plant adaptations to the combination of drought and high temperatures. *Physiologia plantarum* **162**: 2–12.

Zerrouk IZ, Benchabane M, Khelifi L, Yokawa K, Ludwig-Müller J, Baluska F. **2016**. A Pseudomonas strain isolated from date-palm rhizospheres improves root growth and promotes root formation in maize exposed to salt and aluminum stress. *Journal of plant physiology* **191**: 111–119.

Zhang H, Xie X, Kim M-S, Kornyeyev DA, Holaday S, Paré PW. **2008**. Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *The Plant journal: for cell and molecular biology* **56**: 264–273.

Zimin AV, Puiu D, Hall R, Kingan S, Clavijo BJ, Salzberg SL. **2017**. The first near-complete assembly of the hexaploid bread wheat genome, Triticum aestivum. *GigaScience* **6**: 1–7.