THE EFFECTS OF HOST GENETICS AND ENVIRONMENT ON *ZEA MAYS* ENDOPHYTES AND MICROBIAL COMMUNITIES

by

COREY SCHULTZ

(Under the Direction of JASON WALLACE)

ABSTRACT

We need innovation in agriculture to feed a growing world. Understanding and utilizing microbes is an important approach to maintain crop health and increase yield. The research presented here aims to explore how maize genetics and the environment affect the host's interactions with endophytes and the microbiome. In my first project I inoculated diverse maize with potential growth promoting endophytes, and showed that maize cultivar plays an important role in whether growth-promotion occurs or not. In my second project I looked at the role inbreeding had on maize microbiomes in field and greenhouse grown maize. While host genetics were important, differences in maize microbiomes were more strongly associated with environment and tissue. In my final project I looked at bacterial and fungal microbiomes in commercial maize across the United States. I found that tissue, rather than environment, had the largest impact on microbiome structure and networks, and found potential gene pathways important for transition of microbes from one tissue niche to another. Understanding these mechanisms will allow researchers and companies to better utilize endophytes and the microbiome for crop improvement.

INDEX WORDS: MAIZE, ENDOPHYTES, MICROBIOME, GROWTH PROMOTION, MICROBIAL INTERACTIONS

THE EFFECTS OF HOST GENETICS AND ENVIRONMENT ON *ZEA MAYS* ENDOPHYTES AND MICROBIAL COMMUNITIES

by

COREY SCHULTZ

BS, Clemson University, 2018

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2023

THE EFFECTS OF HOST GENETICS AND ENVIRONMENT ON *ZEA MAYS* ENDOPHYTS AND MICROBIAL COMMUNITIES

by

COREY SCHULTZ

Major Professor:	Jason Wallace
Committee:	C.J Tsai
	Y. Anny Chung
	Elizabeth Ottensen

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2023

DEDICATION

I would like to dedicate this dissertation to everyone who helped me through the amazing journey that was graduate school. First, my parents, Bob and Michelle, who supported me and pushed me to push myself. To my sisters, Kate and Amanda, who encouraged me to even when things got tough. To my friends, who truly kept me sane and who made my time at Georgia filled with joy and adventure. The memories and friendships I made here truly made the journey easier. To Jason, for being the best mentor a student could ask for. To Marigold, Cleo, and Lilly, who reminded every morning what I was working towards. And finally to Jordan, who was by my side when I was accepted to UGA, and by my side now that my graduate career has came to a close. I could not have done it without your insight, support, and love.

ACKNOWLEDGEMENTS

I would like to acknowledge all those who helped me throughout my graduate school journey. Firstly, Jason Wallace, whos mentorship and kindness made me the scientist I am today. Jason instilled a passion for investigation, and supported my leadership positions, science communication, and Co-op in industry. My committee members Drs. C.J Tsai, Anny Chung, and Elizabeth Ottesen, whos advice was invaluable. None of this could be possible without the members of the Wallace Lab, who physically assisted me with experiments, helped me formulate analyses, and were a constant support system.

TABLE OF CONTENTS

Page

CHAPTER 1

Introduction to Maize Endophytes and the Microbiome (Literature Review)

The overall goal of my research is to explore and quantify the effect that host genetics and environment has on maize endophytes and its microbial community. In Chapter 2, I focus on the effect that diverse maize germplasm has on single growth promoting endophytes. In Chapter 3, I expand from individual microbes, to analyzing the effect maize genetics, tissue type, and environment have on maize's bacterial community. In Chapter 4, I focus on the effect the environment has on bacteria and fungal communities in commercial maize around the country.

1. The Importance of Maize

1.1 Introduction to maize

Maize (*Zea mays*) shares an ancient polyploid ancestor with most other grass species [1], which then split into a clade that includes sorghum, sugarcane, and many millets [2]. These important food sources grow well, in part, to their C4 photosynthetic process, which means which means it can sustain higher levels of photosynthesis, especially under hot and dry conditions [3]. Maize's wild ancestor, teosinte (*Zea mays* ssp. *parviglumis)* was originally domesticated by indigenous natives from a single domestication event 9,000 years ago in what is today lowland Mexico [2, 4]. There was much population mixing through the years, indicated by modern maize cultivars and landraces sharing introgressions of material from *Zea mays* subsp. *Mexicana* and other *Zea* species [2]. Domestication of maize produced larger ears with more kernels per ear, developing the plant into an important food crop for Native Americans, before proliferating around the world. Maize has consistently been at the forefront of breeding

advandcments. Its success is due, in large part, to heterosis or hybrid vigor, which was identified in 1876 by Charles Darwin in cross-pollinated maize [1]. By the early 1900's, maize breeders were using hybrid maize as the basis for commercial breeding [1]. Modern commercial maize continues to utilize high performing hybrid maize, with inbred lines selected or engineered to be optimized for specific traits [1]. A revolutionary leap in agriculture occurred in the late 20th centrury when genetically engineered maize was introduced in 1997 in the United States [5], with over 92% of US maize being GMO in 2020 [6]. The first maize genome was published in 2009 [7] and 26 high quality genomes of the NAM founders were published in 2021 [8].

1.1.1 Economy of Maize

The economic impact of maize can not be overstated. In 2022 1.2 Billion metric tons of maize were produced around the world [9]. The US was the number one producer of maize and grew 353 million metric tons of it. The USDA valued the total maize export in 2022 at $18.61 Billion [9].While corn is king in the United States, it is a major factor of many other countries. Behind the United States, China, Brazil, Argentina, and the EU lead worldwide maize production [9]. Maize truly is a global crop, it now makes up a major source of food in Africa, Europe, and China. Globally, roughly 19% of calories consumed by humans come from corn [10]. Maize has many uses outside of human consumption in the form of sweetcorn or masa (a form of cornmeal). You can find maize-derived products in almost every aisle of the grocery store. In 2021 the majority of US maize was used for animal feed (38.1%), followed by ethanol used in biofuels (28.8%), exported to other countries (16.3%), Ethanol-Animal Feed Residuals (7.1%), High-Fructose Corn Syrup (2.8%), Glucose and Dextrose Sweeteners (2.5%), starch (1.7%),

Cereal/Other (1.4%), beverage and industrial alcohol (1.1%), seed (0.2%), and other uses (9.7%) [11, 12].

1.1.2 Maize as a Model Organism

Maize has been at the forefront of plant breeding, physiology, and genetics research [4, 13]. Breakthroughs in research often happened first in maize, and then spread to other plants or systems. Maize's reproductive physiology, with male tassels and female silks physically separated on the plant, allows researchers to easily self or cross pollinate plants to tightly regulate experiments. Maize produces several cobs that contain hundreds of large seeds, which make them easy to store, process, and visually assess phenotypes. Tassels create large amount of pollen that can be easily captured and mutagenized. Maize is grown in very different environments and soil types, allowing it to proliferate around the world. The spread of germplasm has led to international cooperation amongst scientist through several NGOs, germplasm populations and repositories, as well as online data bases.

These online resources include powerful tools that can be accessed by scientists around the world. MaizeGDB is the Maize Genetics and Genomics Database [14] http://www.maizegdb.org/ and is critical for understanding the genetic diversity of maize. Gramene is used for comparative genomics of not just maize, but other grasses [15] – www.gramene.org. Maize Cell Genomics Database - [16] is used for sharing information about cellular structure and function. Panzea – is used to access rare alleles in maize and wild relatives – panzea.org. UniformMu is a tool used to share information about transposons in maize [17] . Outside of naturally occurring and induced mutations in maize, there is a massive amount of genetic diversity amongst cultivars and landraces. Maize inbreds and hybrids are grown around the world, and populations and varieties

are available via USDA-Germplasm Resource Information Network (GRIN) and International Maize and Wheat Improvement Center (CIMMYT).

Maize has provided geneticists with a wealth of new discoveries. Genetic recombination and chromosome distance was uncovered in maize [18, 19]. Transposable elements were first described in maize [20]. Pollen is easy to mutagenize with chemicals [21], allowing researchers to discover the role of specific genes and genetic elements. Maize was used to set the early stages of the field of epigenetics [22].

Our current agriculture system relies on high performing hybrid maize lines. This high performance is attributed to hybrid vigor, or heterosis. Heterosis is a phenomena where hybrid offspring will outperform its parents, and can be found many systems outside of maize. Although heterosis is the basis of modern commercial maize breeding, the complete molecular mechanisms behind it are still unknown. Heterosis can cause hybrid offspring to have increased growth rate, yield, biomass, and stress resistance [23, 24]. High quality genomics have shown markers that may impact heterosis in maize [25]. It's been shown that there are changes in gene expression in hybrids, and that these changes may play an important role in heterosis [26]. In hybrid maize, gene expression complementation appears widespread, and maize hybrids express more genes than their parents [27]. Regulatory factors from one parent's genome can interact with target genes from the other parent's genome [28]. Many of these genes are tissue-specific, providing abundant genetic variability for heterosis to act upon [29]. These changes in gene regulation lead to quantifiable changes in protein expression in hybrid maize [30, 31]. Early proteomic research showed that different variants of the same enzyme, or isozymes, between inbred and hybrid maize, could be contributing to increases in yield [32, 33]. Modern analysis has identified differences in the proteome of inbred and hybrid maize through different life

stages and tissues [34, 35]. Specifically, differences in protein expression can help hybrids better perform during germination [31]. A study in the primary roots of popcorn identified 220 proteins that were differentially expressed in hybrid offspring [36]. Changes in the metabolome and proteome in photosynthetic and photorespiratory pathways play a large role in heterosis [37].

1.2. Maize Endophytes and the Microbiome

1.2.1 The bioinoculant market

Recently, a new commercial product has flooded the market promising producers higher yields and better stress tolerance: growth-promoting microbials. The global biostimulants (biological products that stimulate plant growth) market is currently valued at $3.5 billion USD and is expected to grow to $6.2 billion by 2027 [38]. Academia large agricultural conglomerates, and a plethora of startup companies are flocking to the biostimulant and bioinoculate scene. One of the biggest challenges is effectively translating small-scale research to a robust commercial product [39–42].While there are many different active ingredients, and many different application methods (foliar, soil treatment, seed treatment), a growing subset of products contain living microbes that will colonize a plant and provide some level of benefit. These products that colonize the interior tissue of the plant are known as growth promoting endophytes.

While the bioinoculant market surges forward, it's important to note potential dangers microbial bioinoculants pose when used in agriculutural settings [43]. It will take years to understand how long bioinoculants can survive in soil, and how they may proliferate to surrounding environments. While commercial bioinoculants may benefit their target hosts, they could harm other plant species in the ecosystem [44]. Bioinoculant invasions can distrupt important plant-microbe interactions that have crucially coevolved, and are necessary for native

plant survival [45]. Over competitive introduced inoculants can change soil microbiomes that may be important for disease resistence [43]. Monitoring the effects of commercial products before widespread introduction is paramount to ensure our efforts to sustainable feed the world do not unintentionally cause damage to important ecosystems and microbial communities.

1.2.2 Maize Endophytes

Endophytes are microorganisms that live within a plant without being obviously pathogenic [46]. Endophytes have been found in the roots [47], stalks [48], leaves[47]. Endophytes can be inherited vertically through seed, or acquired from the environment, and identified bacterial and fungal maize endophytes are highly diverse [46, 49]. Using domesticated maize and wild ancestors, seed endophytes varied to a similar extant to plant host phylogeny [50]. The authors also found taxa that were considered core seed endophytes, and used a GFP tagged strain to show seed-carried *Burkholderia phytofirmans* successfully colonizes different tissues in the growing plant. Another study determined that in juvenile maize, bacterial and fungal endophytes were largely vertically inherited, not from the environment [51]. Verifying this, another study in hybrid maize showed that offspring shared endophytes from both parents [52]. While some grasses like tall fescue have vertically transmitted symbiotic fungi, which are crucial for plant performance [53–55], thus far no obligate symbionts have been found in maize. When donor seed was planted in an array of different soils, maize endophytes come from both seed and the environment [56]. There are many reports of endophytic bacteria and fungi colonizing maize through the roots, stomata, and wounds. The nitrogen fixing bacteria *Herbaspirillum seropedicae* was found to colonize maize roots surfaces and the interior of the roots. Early on it colonized root axils of secondary roots and intercellular spaces of the root

cortex. It then moved into vascular tissue, and it was reported that the nitrogen fixing *nif* gene was expressed in roots, stems and leaves [57, 58]. *Pseudomonas aureofaciens* was able to invade maize aerial tissues in two ways: (1) through the developing shoot in juvenile maize, (2) vascular transport from the roots [59]. A study explored the effectiveness of five delivery methods of 10 endophytic bacteria in maize. The effectiveness of each method was strain dependent, but individual endophytes were found in the root and stalks using seed inoculation, soil drench, foliar spray, pruned-root dip, and seed inoculation + soil drench [60].

These endophytes can assist plant growth through a number of diverse mechanisms. For example, *Herbaspirillum seropedicae* inoculation increased nitrogen, potassium, and magnesium concentrations in maize plants [61], and can increase the growth rate of maize compared to control plants [62]. *Azospirilum brasilense*, meanwhile, alleviated stress in maize plants under low nitrogen fertilizer (~80% less), increasing kernels per cob, grain weight, and overall productivity to the same levels as plants under normal nitrogen treatment [63]. *Burkholderia* increased phosphate utilization in maize and enhanced growth [64]. *Burkholderia* and *Enterobacter* strains relived salinity stress in maize [65]. In another study *Burkholderia* strains and *Enterobacter* made maize more resilient to drought stress [47]. *Azospirillum* produced the phytohormones abscisic acid and gibberellins that alleviated drought effects in maize [66]. Inoculation of maize and wheat with *Azospirillum brasilense* and *lipoferum* improved yields in both crops [67]. *Trihcoderma* species within the maize stalk protected plants from the pathogen *Fusarium verticilloiodes* through niche competition [68]. Three endophytes from teosinte were used to suppress *Fusarium graminearum*, which causes Gibberella Ear Rot, in greenhouse trials with modern maize. These endophytes also suppressed mycotoxin levels during storage to acceptable levels. These three endophytes produced fusaricidin, an anti-*Fusarium* compound

[69]. Another study found bacterial endophytes from wild and ancient maize relatives exhibited a wide range of anti-fungal properties [70]. A follow-up experiment showed that a *Burkholderia gladioli* strain suppressed the maize fungal pathogen *Sclerotinia homoeocarpa* by swarming and cleaving hyphae [71]. It was shown that inoculation of maize with *Azospirillum basilense* decreased feeding of corn rootworm, who preferred non-inoculated plants [72]. The endophytic fungus *Trochoderma* produced volatiles that decreased plant damage and altered feeding patterns of fall armyworm [73]. Maize interacts with a diverse number of endophyts, who can influence the plan'ts health through many different processes and mechanisms.

1.2.3 The maize microbiome

While sections above described individual microbes introduced to maize through some form of inoculation, these growth promoting endophytes must interact with a diverse community of bacteria, fungi, archaea, algae, viruses, protozoa, and nematodes that naturally exist with and colonize maize. Synthetic communities of maize microbiomes have been created to help study this interactions in controlled environments [74]. In this section I will discuss the maize microbiome mostly in the context of bacteria. These organisms can be beneficial, commensal, or pathogenic, and the same organism can play different roles under different circumstances [75]. For example, bacterial communities in maize landraces can reduce the susceptibility of the host to fusarium ear rot [76]. Bacterial RNA Chaperones, engineered into the plant, have improved grain yield in maize experiencing drought conditions [77]. Bacteria recruited from the soil can adapt to protect maize from chilling stress [78].

Different tissues in maize have distinct and different microbiomes. Bulk soil contains microorganisms existing in the environment, and the rhizosphere consist of organisms that can

survive/are recruited near the surface of the roots by the plants. These soil associated communities outside the plant are more diverse than other microbiomes on/in the plant, and are heavily dependent upon the environment. Bulk soil is often dominated by bacteria from the phylums Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria, and fungi from Ascomycota, Basidomycota, and Glomeromycota [79]. The rhizosphere exhibits some ability to select or enrich for certain potential growth-promoting microbes, especially nitrogen-fixing bacteria [80]. The endosphere of maize is thoroughly reviewed [46], and it's been shown that the endosphere community is less numerous and diverse than bulk soil or rhizosphere [49]. As microbes invade from the exterior, they must adapt to different environments and may face barriers as they travel up the plant [49, 51, 81]. There are distinct differences between the root, stalk, and leaf endosphere [46, 49]. The surface of the above ground portion of maize, or the phyllosphere, is a harsh environment with many stressors [82]. Phyllosphere communities have generally low diversity [83–85], and the maize phyllosphere is dominated by Proteobacteria, Actinobacteria, Bacteroidetes [83].

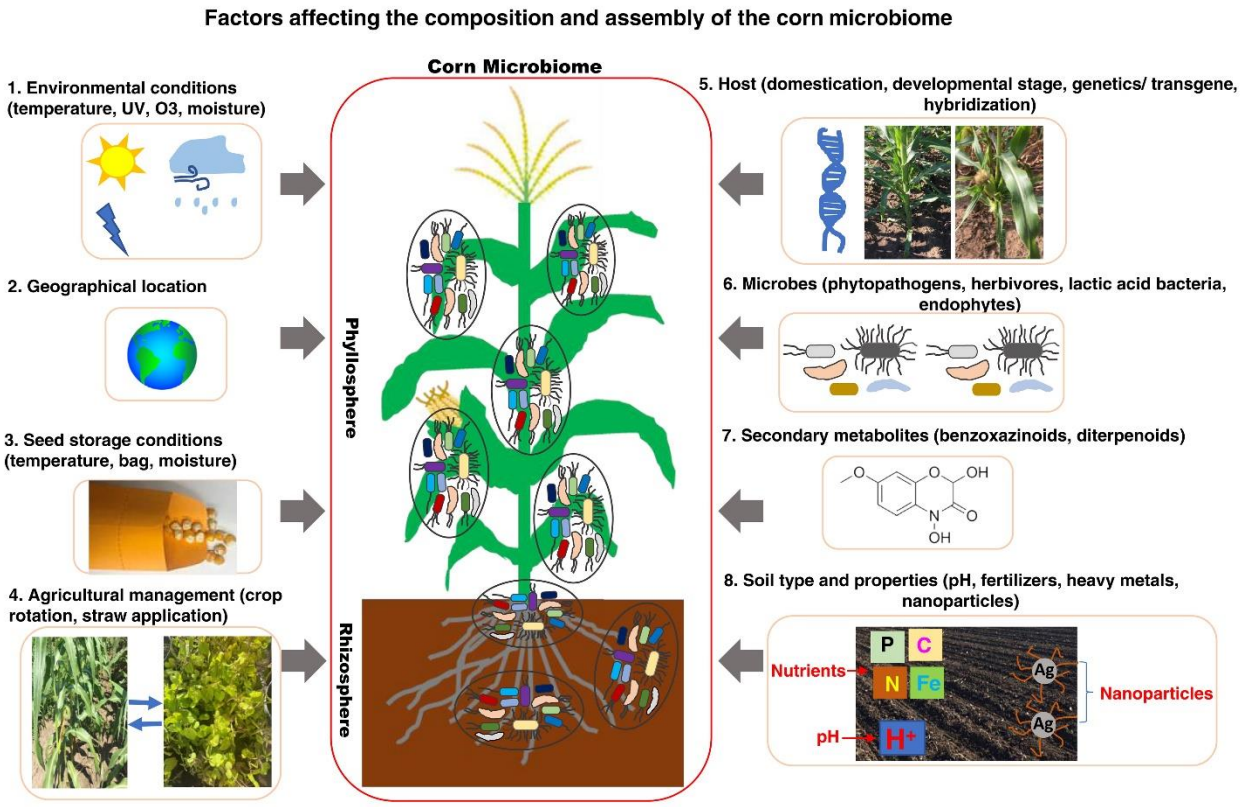


Figure 1. Factors that impact the maize microbiome. Adapted from: https://doi.org/10.1094/PHYTOFR-04-21-0026-RVW

1.2.3.a Host genetics affect microbial interactions

The effects of individual endophytes, and the structure and composition of the microbiome can vary due to maize genetics. Twenty-one *Herbaspirillum* inoculants were tested on two different maize lines, under different field conditions consistent growth promotion was dependent on both nitrogen and genotype [86]. In another study, metabolite expression and growth promotion changed in two different maize genotypes when they were inoculated with two different growth-promoting bacteria, under low and high nitrogen [87]. A study using bacteria that all had nitrogen fixing and P-solubilizing abilities, showed that some of these bacteria could increase maize shoot dry weight, but found a significant maize cultivar-bacteria interaction [88]. A study looking at transgenic maize showed that genetic composition, the

presence of transgenes, and plant stage affected the composition of bacterial and fungal communities of maize leaf endophytes [89].

The genetic diversity of maize allows us to study genetic effects on these interactions in controlled experiments. The maize Nested Association Mapping (NAM) founders consist of 26 inbred lines that represent a massive portion of maize genetic variation, including: 13 tropical lines, 9 temperate lines, 2 sweet corn lines, and 1 popcorn line [90]. This population also contains some European and Chinese germplasm. The 27 maize NAM founders were planted in 5 fields in three states. The microbiomes of these fields clustered by geography, and the authors found that a small subset of rhizosphere taxa seemed to be influenced by host genetics [91]. 300 members of the Goodman Maize Association Panel [92] were grown in the field, and their leaf microbiomes were analyzed from taxa and predicted genetic functionality. A small subset of taxa and metabolic functions were shown to be impacted by host genetics [83]. Eight inbred maize lines were crossed to produce F1 hybrids that were grown in two separate fields, until the bacterial and fungal microbiomes of the leaves and rhizosphere were extracted. They showed a range of microbiome features displayed heterosis within crosses, and that genetics at least partially shape the maize microbiome [93]. A follow-up study showed that in a gnotobiotic or germ-free environment, heterosis of root mass was dependent upon maize seedlings having a microbiome.

Disease resistance seems to play a role in how host genetics shape the microbiome, although the strength of these effects appears to vary. In some cases, inbred lines performed just as well as hybrid offspring in sterile conditions, but they then performed worse when a synthetic community of bacteria were added. This study argues that heterosis may be partially explained by hybrid offspring handling the stress of microbes, even if they aren’t obviously pathogenic,

better than their inbred parents [94]. When maize was infected with southern leaf blight, six loci that correlated with endophyte diversity overlapped with loci controlling resistance. One of these pathways was linked to the gamma-aminobutyric acid pathway, linking high bacterial diversity to disease susceptibility in the field. This suggests that genetically influenced bacteria my suppress other bacteria, and increase resistance to fungal infection in the leaves [95]. A follow-up study with a single maize line (B73) showed that lower bacteria diversity was correlated with increased severity of southern leaf blight [96]. In another study, fungal pathogen resistance QTLs were introgressed into a disease-susceptible maize line. These QTLs did shift the community structure of bacterial and fungal leaf microbiomes, but these effects did not change under different disease pressures, and these effects varied over different fields and years [97].

1.2.3.b Environment affects microbial interactions

The maize microbiome is dependent upon abiotic changes in the environment, as well as changes in the plant host [79]. Differences in soil temperature, soil temperature, and a slew of environmental factors shap what microbes live in a community and are available to maize. There are many examples of changes in the soil and rhizosphere of maize communities due to the environment. The community structure and diversity of rhizosphere bacteria communities is influenced by the stages of plant development and soil type [98]. In tropical soil, soil temperature and plant growth stage changed bacteria diversity in the rhizosphere [99]. While comparing transgenic Bt maize both Bt and soil type affected rhizosphere bacterial communities of maize in the field [100]. Different maize lines, soil types, and Western Corn Rootworm feeding altered both bacterial and fungal rhizosphere communities [101]. In a common garden experiment, levels of organic nutrient availability changed rhizosphere community composition as well as

enzyme activity in maize and summer annuals [102]. Although the interiror of the plant is more sheltered from environmental changes, we see distinct environmental effects on the endosphere. Maize host genetics, field environment, and infection with the pathogen *U. maydis* altered maize fungal endophyte composition [103]. In an experiment exploring the impact of swapping soil on endophytes of diverse and wild maize, a subset of endophytes were unique to single soils [104]. Changes in ultraviolent radiation altered maize leaf surface bacteria communities [105]. Beyond natural environmental factors, agricultural management has a strong effect on the maize microbiome.

There were differences in rhizosphere community and function when comparing organic and inorganic soil fertilizers [106]. A field's cultivation history was shown to have an impact on maize endophytic bacterial diversity [107]. When comparing diversified vs conventional cropping systems, differences were most distinct on the surface of the root during vegetative stage 11, when maize has the highest nitrogen demand. Maize from the diversified cropping system had higher abundances of bacteria implicated in organic matter decomposition on the root surface [108]. In a greenhouse experiment, maize seedlings were planted in soil preceded by either maize, pea, soybean, or sunflower, and then stressed with western corn rootworm or *Fusarium graminearum*.The preceding crop altered the soil microbiome available to the experimental maize. Without stressors, maize seedlings grew better in soil preceded by sunflower or pea. Western corn root damaged maize regardless of soil history, while fungal infection root damage was lower in maize grown in soil that was preceded by sunflower. Stressors had an effect on specific microbial taxa, while soil history had a large effect on rhizpsohere microbiome, with fungi more affected than bacteria [109]. In an experiment exploring the effect of straw application, rhizosphere microbiomes were distinct between the two

soils, previous crop history contributed to community composition, and there were differences in community makeup due to straw application, perhaps in response to oxygen availability [110]. Human activities in other industries often lead to pollution in the soil that provides a unique challenge for agriculture, although there is evidence this stress can be partially alleviated by microbial activity.

Cadmium contamination altered maize and soybean-associated bacteria networks in the soil [111]. In another study, two growth promoting bacteria and salicylic acid helped to remediate chromium toxicity in maize [112]. Another study explored the interaction zinc, cadmium, and lead had on maize in field trials. Rainfall and irrigation affected the bioavailability of these metals in the field. The heavy metals changed the overall diversity of the bacteria in the soil [113]. In another study it was shown that the addition of heavy metals reduced the number of taxa, and this effect was partially reversed through the addition of chelants [114]. Iinorganic (but not organic) phosphate fertilizer reduced the effect that heavy metals had on maize, and improved enzyme activity as well as microbial community structure [115]. Silver nanoparticles are often used for their antimicrobial properties. Nanosilver negatively impacted microbes involved in nitrification and nitrogen cycling, it was shown that phytopathogenic fungi increased (perhaps due to disrupting bacteria that were natural biocontrol agents), and overall stressed maize [116]. Biocontrol agents are an import part of another interaction in the microbiome: microbe – microbe interactions.

1.3. Mechanisms of plant-microbe and microbe-microbe interaction

The diversity of organisms associated with maize is reflected by the diversity of mechanisms in their interactions. Examples of how microbes can act as biocontrol agents have already been discussed [68, 69, 71–73]. Bacteria and fungi often interact with each other in the

soil and throughout the plant [117, 118]. Microbes and plants exude diverse compounds that directly affect their interactions, as well as overall plant health outcome. The next few sections will extend beyond maize, as few papers explicitly use maize to accurately describe these highly complex interactions. An interesting example of these interactions have been identified in maize mucilage, or corn-snot. In a unique system, an indigenous landrace of maize from the Sierra Mixe region of Oaxaca, Mexico grows to enormous heights in low nitrogen soils. This landrace is characterized by extensive aerial roots that secrete carbohydrate-rich mucilage. Within this mucilage, nitrogen fixing bacteria provide the host with 29%-89% of its nitrogen [119]. A unique polysaccharide was found that may be fueling these nitrogen-fixers [120]. The monosaccharide composition of the mucilage is similar to maize root exudes, and could be used as a model to understand how plant exudes shape their associated bacteria [121].

1.3.1 Endophytes functionally differ from exterior counterparts

Organisms living on the interior of the plant face a battery of different environments and stressors, and are exposed to different signaling compounds. *Herbaspirillum seropedicae* is a well-documented nitrogen fixer and growth promoter in maize and other grasses [58, 62, 86, 87, 122]. The genome of this organism has been used to shed light on the mechanisms behind endophytic symbiosis. It is frequently used in growth promoting studies, and understanding the molecular machinery at its desposal is important to understand what may be important for engineering a helpful microbe. *Herbaspirillum seropedicae* strain SmR1's genome contained 4,804 genes. This strain had the capacity to metabolize a wide range of carbon and nitrogen sources. It included type I, II, III, V, and VI secretion systems, and type IV pili (a dynamic structure that allows the organism to react to stimulus and is important for adhesion, motility,

microcolony formation, colonization, and secretion of proteases [123]). This strain can synthesize indole acetic acid (a phytohormone), and contains mechanisms to modulate plant ethylene signaling [124]. A follow-up study compared this organism to the phytopathogen *Herbaspirillum rubrisubalbicans* M1. There were differences in functions related to lipopolysaccharides and adhesion. The pathogenic bacteria had evidence of more recent lateral transfers of genetic material. Although both bacteria had nitrilase (metabolizing nitrogenous compounds) gene sequences, they were not homologous, and may indicate different specificity or efficiency of the strains. Pathogenic *H. rubrisubalbicans* M1 out-competed *H. seropedicae* in attachment assays. The two strains had distinct differences in surface exposed complexes [125]. A group comparing a number of *Herbaspirillum* species found distinct differences in: secretion systems, nitrogen metabolism, respiration, carbohydrate metabolism, plant cell wall degradation, ROS detoxification, and biosynthesis of plant hormones, these differences show how bacteria may adapt to endophytic lifestyles, and develop mechanisms to survive in host specific environments [126].

Three strains of a single species of maize seed endophyte, *P. ananatis*, demonstrated the capacity to interact with maize as a pathogenic, commensal, or beneficial endophyte. These three strains had a genome similarity greater than 99%. Genome assembly and annotation releveled small but significant changes in protein-coding genes and expression. There were differences in genes related to Type IV pilus biogenesis proteins between beneficial and pathogen strains. The beneficial strain lacked some genes related to phage and bacteriophage virulence. Eukaryotic-like protein domains had strain-specific differences. The pathway differences may show how genome changes can change the lifestyle of an endophyte [127]. Moving from examinging individual microbes to the capacity of the community in general, a study in rice roots compared

the metagenomes of bacteria in the rhizosphere and those that colonized the root endosphere and identified some key differences. Bacterial endophytes had increases in motility via flagella, plant-polymer degrading enzymes, iron acquisition and storage, differing secretion systems, detoxification of reactive oxygen species (ROS) and quorum sensing. There were also increases in proteins related to the nitrogen cycle [128].

1.3.2 Endophytes trigger host immune response.

Colonization by beneficial endophytes can induce systemic defense responses that can defend the host from plant pathogens and herbivores through microbe associated molecular patterns (MAMPs) [129, 130]. Beneficial microbes can induce systemic resistance through phytohormones such as jasmonic acid, ethylene, and salicylic acid [131]. Colonization of maize roots by *Trichoderma virens* induced systemic protection against a foliar pathogen. This protection was associated with increases in jasmonic acid production and leaf volatiles. Deleting the SM1 gene from the beneficial fungi caused maize resistance to decrease to levels consistent with control maize. SM1 is secreted by the fungus during colonization, and purified Sm1 elicited a similar protective response in cotton [132]. Rice's response to beneficial and pathogenic fungi overlap by 40%, showcasing distinct differences in gene expression [133]. Recognition of MAMPs triggers responses like the production of reactive oxygen species, nitric oxide, and the expression of defense genes. In one study, two well-studied MAMPs challenged genetically varied maize lines. There was found to be natural variation in the plant's response to MAMP recognition and in turn, disease resistance [130]. Arbuscular mycorrhiza can induce resistance to pathogenic bacteria, fungi, and nematodes. It's been shown that the fungi can suppress salicylic

acid-dependent responses to colonize the plant, and can then stimulate jasmonate-dependent responses in order to bolster the host's systemic immune response [134].

1.3.3 Bacteria and Fungi act as both antagonists and mutualists

Bacteria and fungi interact in the root zone, and these interactions are often antagonistic [118]. The rhizosphere, phylosphere, and endopshere of sugar beet contained bacteria and fungi that had antagonistic mechanisms towards fungi [135]. In another study, increases in fungal density was associated with bacteria enriched for antifungal mechanisms such as siderophores, cyanide and lytic enzymes [136]. Ascomycota and Basidomycota in the root zone can degrade bacterial signaling molecules [137]. Fungi can produce farnesol and fusaric acid which can reduce anti-fungal production in bacteria, as well potential disrupt bacteria communication [138, 139]. On the other hand, intra-kingdom mutualism has been identified in the rhizosphere as well [140, 141]. In a gnotobiotic environment, the legume *Medicago truncatula* was individually inoculated with a nitrogen fixing bacteria, a mycorrhizal fungi, and both. The combination of the two mutualists increase plant growth more than either inoculant on its own. Colonization of the fungi, the bacteria, and both, had unique impacts on gene expression in the microbe as well as the plant. These genes included important functions such as nutrient metabolism, and inoculating the host with both microbes even led to some genes' expression changing direction compared to single inoculates. This interactions is more indirect, and these microbes may still be competing with each other for fixed carbon. The study showed how important multiple mutualists are on host gene expression, as well as gene expression of the inoculants [142].

1.3.4 A diverse range of Metabolites are used in signaling pathways.

Plants and microbes produce secondary metabolites that are critical for cross-kingdom signaling and interactions [143, 144]. These small molecular products are non-essential for growth or reproduction, and can be classified into families such as phenolics, terpenes, steroids, alkaloids, and flavonoids [145]. A review published in 2023 describes 95 terpenoids, 91 phenolics, 31 alkaloids, and 6 other compounds produced by maize [146]. These compounds can regulate root colonization and modulate belowground communities, as reviewed in [146–148]. Bacteria volatile organic compounds can act as biopesticides, growth promoters, and plant defense elicitors [144]. Bacterial volatile organic compounds (VOCs) are often hydrocarbons, ketones, organic acids, esters, alcohols, terpenes, aldehydes, or sulfur/nitrogen containing compounds. Soil bacteria often emit fatty acid derivatives, indole acids, and dimethyl disulfide [149].

Using maize, it was shown that benzoxazinoids (BX) determine root-associated microbiota, conditioning the soil with benzoxazinoids increased plant defense, these effects were genotype-specific, and reached into the next generation of maize planted in that soil as well [150]. Chloroflexi appeared to be enriched in soil with benzoxazinoids, while Glomeromycota tended to be less abundant [150]. Another study used BX knock out lines and wild type parental lines to measure the effect benzoxazinoids had on bacterial and fungal microbiomes in controlled experiments. It was shown that BXs affected the microbiome in the rhizosphere, roots, and shoot endospheres, and that this effect was genotype-specific and plant-age specific [151]. BX levels varied across maize genotypes. Bacteria taxa were both positively and negatively impacted by BX levels, while fungi was mostly positively correlated [151]. Mutating the BX biosynthesis

pathway had major changes on maize root exudes, which in turn altered the rhizosphere community [152]. It was shown that phenolics, flavonoids, and indole-3-acetic acid were crucial in establishing a symbiotic relationship between *Aspergillus nomius* wlg2 and maize roots. Both the bacteria and maize roots secrete these metabolites, and suppressing the metabolites were found to massively reduce the rate of endophyte colonization [153]. 2,3-butanediol produced from *Enterobacter aerogenes* promotes growth in maize, and protects the plant from northern corn leaf blight [154]. VOCs from maize endophytes were found to have antifungal activity on known maize fungal pathogens [155, 156].

1.4. Microbiome Network Analysis

1.4.1 Microbiome Networks

Microbiome co-occurrence network analysis has been widely adopted for exploring correlations of taxa in a number of plants and environments [157]. As demonstrated above, interactions between bacteria, fungi, and their plant hosts are complex and are dependent on a wide range of signaling pathways as well antagonism/mutualism. On top of these biotic interactions, plants are bombarded with a plethora of other factors: changing environments, agriculture management by humans, macro-organisms, and microorganisms such as protists, oomycetes and viruses that may not be captured sequencing data. Beyond predicting potential interaction in the microbiome, network analysis can be helpful in identifying keystone or hub taxa [158]. These taxa are highly associated with other taxa in the microbiome, and their removal would cause a drastic shift in the structure and function of the microbiome [159].

Microbiome data has unique properties that must be considered when applying correlation analyses [160]. This means that microbiome data is “compositional”, and data must be normalized in order to compare the relative abundance of taxa across samples. Normalization of microbiome data through rarefaction or proportions is a hotly debated topic in the field [162],

although microbiome-specific methods are emerging [160]. Microbiome data is also sparse, meaning most of the counts are zeros. The causes challenges for models and correlations, where they must account for a vast number of zeros, and must be accounted for by adding pseudo-counts (adding a specific number, such as one, to every count), agglomerating to higher taxa levels, or using algorithms designed to handle this sparse data. Finally, microbiome data has the capacity to be heterogeneous. Composition of microbial communities can change across environments and in different tissue types as demonstrated above. Unlike transcriptomic data, where most genes are expected to have some level of expression in most samples, often the subset of ASVs or OTUs found across all samples in small [160].

With these caveats in mind, microbiome network analysis has proven to be invaluable for identifying potential microbial interactions, and will continue to improve as environmental data and other kingdoms of life are included [163]. These analyses can give us clues on how the microbiome changes over time, and which taxa may have the most important impact on microbiome, and thus plant health outcomes. Network analysis has been used to examine the role domestication has on rice and maize microbiomes [164, 165]. The microbiomes of seeds from 43 wild and cultivated rice accessions were extracted and analyzed. Rice accessions (genetics) contributed to both bacterial and fungal differences in the seed microbiome. Domestication caused changes in the relative abundance of bacteria and fungi within seeds, and caused hub taxa of co-occurence networks to shift from being dominated by fungi to bacteria, and domesticated rice had simpler networks, or fewer potential interactions [164]. Exploring the role of domestication on microbiome networks was examined in our crop of interest, maize, as well. The rhizosphere microbiome of inbred maize, landraces, and their ancient ancestor were compared in a field experiment. Domestication in maize caused increased bacterial rhizosphere diversity, and

domesticated inbred maize had higher co-occurrence network complexity than landraces or teosinte. Hub taxa were different for the three genetic groups, despite being grown in the same field [165]. Network analyzsis can be used to study the effects of time and environment as well. The bacterial and fungal microbiomes of the exterior and interior of maize roots and leaves, as well as plastic leaves, were examined at three developmental stages in two field experiments. It was shown that plant developmental stage had a stronger influence on community diversity, structure, and networks in leaves compared to the soil. At the seedling stage bacteria dominated networks and crop yield, while fungi became more important in mature maize. Early-stage phylloplane (leaf surface) microbiomes had abundant beneficial bacteria and were enriched for nitrogen cycling genes, while mature phylloplane microbiomes had more saprophytic fungi with increased carbon degradation genes [166]. Another field study examined bacteria and fungi in the soil, rhizosphere, and stalk endosphere at several maturity points. They found nitrogen-fixing bacteria abundance peaked early in maize development and then declined, while populations of maize-associated mycorrhizae fungi peaked later in the season. Positive correlations between nitrogen-fixing bacteria and important fungi were found, suggesting that increased levels of nitrogen may play a role in important fungal proliferation [167]. These interactions may play a role in hose maize uptakes nutrients throughout the season, as different nutrient requirements are needed at different growth stages.

1.4.2 Core Microbiomes

While the stringency of the definition tends to change from study to study, “core” microbiomes are microbial features that are consistently shared. Their consistency hints that these microbes may have some importance for host fitness [168]. Understanding the core

microbiome could be used to understand important functions of a crop's microbial community, which would impact host performance [169]. The core microbiome is often examined in the concept of consistent microorganisms across space [170, 171], and over time [166, 167, 172]. A core microbiome has been identified in a number of species, including Arabidopsis and rice. In 2012, more than 600 *Arabidopsis thaliana* plants from 8 diverse inbred accessions were grown in two diverse soils, and their rhizosphere in endophytes were sequenced. While there were genotype and soil type effects on the microbiome, a core endophyte community was identified [173]. A study using 6 rice culivars looked at the rhizosphere, rhizoplane, and the root endophyte community in three soils in the greenhouse and field. 32 OTUs were found in the root endosphere in all three fields, and 11 of those 32 were also enriched in the greenhouse [171].

In a longitudinal field study, 27 maize inbred lines were planted in three fields, with partial replication 5 years later. 251 OTUs were shared across 95% of samples, and seven OTUs were present in all samples. All seven OTUs were Proteobacteria, and were found in five fields, across three states, and across a 5 year time frame [170]. A greenhouse study using three soils and four maize cultivars found 19 bacteria that were considered core root microbes, despite soil characteristics playing a large role in root microbiome community assembly [174]. A study previously mentioned examined the heritability of the maize leaf microbiome. They defined core OTUs as being present in 80% of leaf samples. These core OTUs were found to make up a large proportion of microbial abundance. Of these core OTUs, only one showed significant heritability. The authors of the study showed that predicted functional annotations were much more heritable than taxa, and suggest that microbiome analyses should move towards function rather than taxonomy [49, 83, 158, 174]. It's been shown that not all taxa are found everywhere [175], but there are large conserved bacterial functions that cross taxonomic barriers, outside of

basic growth funcitons [174]. As sequencing and computational methods get more advanced, microbiome analysis will evolve to better hone in on the function of microorganisms, to better predict the complex interactions, and allow researchers to parse apart the conetext that alters these intereactions, which influence maize's success as an agricultural powerhouse, and model organism.

1.5. Tools for Analyzing Crop-Microbe Interactions

Much has changed in crop breeding and the study of crop-microbe interactions since the late 19th century, when Rhizobium was discovered in 1889 [177], and heterosis in maize was noted by Darwin in his Theory of Evolution [1]. There are many tools for analyzing changes in he microbiome [178], and as previously mentioned, the compositional nature of most microbiome data requires careful consideration to avoid erroneous results. The tools used in the following chapters allow us to interpret phenotypic variation due to host genetics and the environment. We will briefly describe standard statistical and computational pipelines used.

Linear Regression and ANOVA – A regression is a statistical method for establishing the relationship between variables. A model is created with the dependent variable of interest, and one or more independent variables. An ANOVA (analysis of variance) uses a linear model to summarize each variable's impact on the variance of the dependent variable [179, 180]. PERMANOVA is a permutational multivariate anova. It tests for differences in multiple variables over many iterations to avoid spurious results, and can handle data that a traditional ANOVA cannot. Throughout this dissertation, I use variations of simple and mixed linear regressions to analyze how genotype and environment affect phenotypes of interest, such as plant growth and changes in microbial communities.

Microbiome Toolboxes – To make microbial ecology analysis more accessible, a number of groups have developed toolboxes with standard analysis baked in, and the ability to plug in new tools and analyses. These toolkits provide easy documentation, standard outputs, and often helpful tutorials. I performed much of our work around two workflows: QIIME2 [181] during data processing and quality control, and Phyloseq [182] during ecological analysis and visualization. QIIME2 is used on the Linux command line through bash scripting, while Phyloseq is an R package.

Sequence Pre-processing – The microbiome data in this dissertation consist of amplicon sequencing. These are short reads or amplicons, of taxa specific portions of RNA, 16S for bacteria, and ITS for fungi. The amplicons are used to classify taxonomy to microbes present in the sample. The first step when working with sequencing data is to trim and clean it. I used Cutadapt [183] to find and remove 5' and 3' adapter sequences, primers, and any other unwanted features in sequencing data. After removing unwanted sequencing data, I use FastQC [184] to look for samples with low quality reads, and to make decisions about downstream quality cutoffs. This tool allows us to analyze all samples at once. Many aspects of the tool are less important for microbiome amplicon sequencing compared to other high throughput sequencing data; for example in microbiome data we expect duplicate reads and non-normal GC content. We have used both DADA2 [185] and Deblur [186] to trim reads to consistent lengths, and filter reads that don't match quality control thresholds.

Taxonomic classifiers – Once reads are cleaned and trimmed, classifiers must be used to assign taxonomy to a read, so you can build the picture of what a community looks like. In prokaryotes, the 16S ribosomal RNA is used in amplicon sequencing. This gene has slow rates of evolution, allowing it to be used to create phylogenetic trees. While there are several databases that can be

used with taxonomic classifiers, we use two in this dissertation. SILVA [187] is a ribosomal RNA database established by the Max Planck Institute, the Technical University Munich, and Ribocon. In eukaryotes, the nuclear ribosomal internal transcribed spacer (ITS) region is used for amplicon sequencing. The UNITE [188] database is a public collaboration to store an identify eukaryotic ITS amplicons.

Normalization – As samples often have differences in read depths (the amount of reads sequenced in a sample), microbiome data is often normalized through rarefaction, using proportions, or transformations. Rarefaction randomly selects read to a specific read depth, which intrinsically removes some level of data from the analysis. Relative abundance is frequently used to describe turning reads into their proportion of total reads, and then multiplying to an arbitrary sample depth. There are a number of transforming techniques available; I use center log ratio [189] and variance stabilizing transformation [190] at separate points. Normalization methodology is a widely debated topic [162, 191], but throughout this dissertation, I use relative abundance for community level comparisons and log-transformations for differential abundance testing.

Community Diversity - Diversity measures are used to evaluate differences in the microbiome as a whole. Alpha diversity metrics quantify the diversity within a sample so groups of samples can be compared. Alpha diversity metrics like Shannon Entropy and Inverse Simpson Index evaluate both species richness and evenness. Shannon Index is more influenced by rare taxa, while Inverse Simpson is more influenced by highly abundant taxa. Beta diversity compares between sample diversity, often through dissimilarity matrices. These measures can be tree-dependent, or dependent upon a phylogenetic tree of taxa (Unifrac) or independent (Bray-Curtis,

Jaccard). These dissimilarity matrices can be used to measure the extent other variables have on the microbial community [178].

Microbial Networks – Correlation analysis allows us to visualize community structure and their potential interactions, and can drive future hypotheses. Networks are informed through pairwise relationship. Compositional specific correlation algorithms have been recently developed, and I used CCLasso in this thesis [192].

Differential Abundance Testing – Like other -omics data, we are frequently interested in features that are enriched or suppressed in different groups. Differential abundance testing provides the framework for finding statistically significant changes in taxa and microbial function. DESeq2 [190] has been used in gene expression analysis and has been co-opted for microbiome analysis. This tool outputs groups of microbes or predicted genes that are found significantly more in one group than another.

Predicted Functional Genetics – Although changes in community taxonomic make up are important, leveraging functional differences in microbiomes allow for deeper analysis and more robust findings. Amplicon sequencing is cheaper than metaranscriptomics, metaproteomics, and metabolomics, but we can drive functional hypothesis through predicting taxa gene functions. PICRUST2 [193] uses taxa information from amplicon sequencing and predicts gene pathways through phylogeny. This abundance of Kegg Orthology terms [194] can give us rough approximations of what communities may be doing at the molecular level, but these results are very dependent upon data quality and reference genome annotations.

1.6. Outline of Thesis

The goal of this dissertation is to expand upon and quantify the effect that host genetics and environment has on maize endophytes and its microbial community. I ask how maize

genetics and the environment alter the host's interaction with bacteria and fungi, and how these change the microbial community structure and potential function. In Chapter 2 I foucus on maize genetics and individual microbes. In Chapter 3 I look at how maize genetics and the environment impact its bacterial community. In Chapter 4 I focus on the environmental impacts on both bacteria and fungal communities in commercial maize across the country. Throughout the dissertations we see tissue specific changes, showing how different these communites are within the same plant. Our findings highlight the need for controlled experiments to dive deeper into these interactions, as this field can assist feeding the world.

It's been repeatedly shown that crop cultivars interact differently with the same growth-promoting endophyte. In Chapter 2, we inoculated diverse maize inbreds with growth promoting endophytes in order to quantify this interaction. We found *Herbaspirillum seropedicae* and *Serendpita bescii* to differentially promote growth in a number of maize genotypes. Endophytes did not consistently increase maize growth, and we found growth promotion was correlated with endophyte abundance in our trials with *S. bescii*. This work demonstrates the challenges of bringing growth promoting inoculants to market.

In Chapter 3 we then explored how maize genotypes shape its microbiome. We used diverse inbred, hybrid, and open-pollinated lines in two field experiments, as well as a single greenhouse experiment. We found differences in microbiome composition, structure, and predicted functional genomics when comparing these three groups of maize. We found specific taxa and predicted gene functions that differed between inbred and hybrid maize. These findings may shed some light on how the microbiome effects heterosis and hybrid maize performance. These differences due to genetics were much smaller than changes attributed to tissue and environment, which had the largest influence on the maize microbiome.

With this in mind, in Chapter 4 we used publically available bacterial and fungal amplicon sequencing to examine the role environment has on shaping commercial US maize at 30 locations in the United States as part of the Genomes to Fields collaboration. We found Kingdo- specific effects of location and tissue compartments on microbiome communities. Across all sites, we found patterns of antagonism between Bacteria and Fungi. Environmental and soil characteristics were found that impacted specific microbial abundance. Finally, we identified differences in predicted gene functions that may allow exterior living organisms to transition into the Endosphere.

References

1. Bennetzen JL, Hake SC (2009) Handbook of Maize: Genetics and Genomics. Springer Science & Business Media
2. Schnable JC (2015) Genome Evolution in Maize: From Genomes Back to Genes. Annual Review of Plant Biology 66:329–343
3. Brown NJ, Parsley K, Hibberd JM (2005) The future of C4 research – maize, Flaveria or Cleome? Trends in Plant Science 10:215–221
4. Hake S, Ross-Ibarra J (2015) Genetic, evolutionary and plant breeding insights from the domestication of maize. eLife 4:e05861
5. Gewin V (2003) Genetically Modified Corn— Environmental Benefits and Risks. PLoS Biol 1:e8
6. Nutrition C for FS and A (2023) GMO Crops, Animal Food, and Beyond. FDA
7. Schnable PS, Ware D, Fulton RS, et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115
8. Hufford MB, Seetharam AS, Woodhouse MR, et al (2021) De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. Science 373:655–662
9. Corn Explorer. https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0440000. Accessed 11 May 2023
10. (2019) What Are the World's Most Important Staple Foods? In: WorldAtlas. https://www.worldatlas.com/articles/most-important-staple-foods-in-the-world.html. Accessed 11 May 2023

11. World of Corn 2021. https://ncga.com/world-of-corn-iframe#corn-usage-by-segment. Accessed 11 May 2023

12. (2021) From feed to fuel: This is how corn is used around the world. In: World Economic Forum. https://www.weforum.org/agenda/2021/06/corn-industries-sustainability-food-prices/. Accessed 11 May 2023

13. Nannas NJ, Dawe RK (2015) Genetic and Genomic Toolbox of Zea mays. Genetics 199:655–669

14. Lawrence CJ, Harper LC, Schaeffer ML, Sen TZ, Seigfried TE, Campbell DA (2008) MaizeGDB: The Maize Model Organism Database for Basic, Translational, and Applied Research. International Journal of Plant Genomics 2008:e496957

15. Tello-Ruiz MK, Jaiswal P, Ware D (2022) Gramene: A Resource for Comparative Analysis of Plants Genomes and Pathways. Methods Mol Biol 2443:101–131

16. Krishnakumar V, Choi Y, Beck E, Wu Q, Luo A, Sylvester A, Jackson D, Chan AP (2015) A maize database resource that captures tissue-specific and subcellular-localized gene expression, via fluorescent tags and confocal imaging (Maize Cell Genomics Database). Plant Cell Physiol 56:e12

17. Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde R-A, Coalter R, Barkan A (2010) Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. Plant J 63:167–177

18. Mcclintock B, Ware D, Fulton RS, et al (1929) CHROMOSOME MORPHOLOGY IN ZEA MAYS. Science 69:629–629

19. Creighton HB, McClintock B (1931) A Correlation of Cytological and Genetical Crossing-Over in Zea Mays. Proceedings of the National Academy of Sciences 17:492–497

20. McClintock B (1950) The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences 36:344–355

21. Candela H, Hake S (2008) The art and design of genetic screens: maize. Nat Rev Genet 9:192–203

22. Lisch D (2012) Regulation of transposable elements in maize. Current Opinion in Plant Biology 15:511–516

23. Duvick DN (1984) Genetic Contributions to Yield Gains of U.S. Hybrid Maize, 1930 to 1980. In: Genetic Contributions to Yield Gains of Five Major Crop Plants. John Wiley & Sons, Ltd, pp 15–47

24. Duvick DN (1999) Heterosis: Feeding People and Protecting Natural Resources. In: Genetics and Exploitation of Heterosis in Crops. John Wiley & Sons, Ltd, pp 19–29

25. Lai J, Li R, Xu X, et al (2010) Genome-wide patterns of genetic variation among elite maize inbred lines. Nat Genet 42:1027–1030

26. Xiao Y, Jiang S, Cheng Q, et al (2021) The genetic mechanism of heterosis utilization in maize improvement. Genome Biol 22:148

27. Baldauf JA, Marcon C, Lithio A, Vedder L, Altrogge L, Piepho H-P, Schoof H, Nettleton D, Hochholdinger F (2018) Single-Parent Expression Is a General Mechanism Driving Extensive Complementation of Non-syntenic Genes in Maize Hybrids. Current Biology 28:431-437.e4

28. Paschold A, Jia Y, Marcon C, et al (2012) Complementation contributes to transcriptome complexity in maize (Zea mays L.) hybrids relative to their inbred parents. Genome Res 22:2445–2454

29. Zhou P, Hirsch CN, Briggs SP, Springer NM (2019) Dynamic Patterns of Gene Expression Additivity and Regulatory Variation throughout Maize Development. Molecular Plant 12:410–425

30. Guo B, Chen Y, Li C, et al (2014) Maize (Zea mays L.) seedling leaf nuclear proteome and differentially expressed proteins between a hybrid and its parental lines. PROTEOMICS 14:1071–1087

31. Guo B, Chen Y, Zhang G, et al (2013) Comparative Proteomic Analysis of Embryos between a Maize Hybrid and Its Parental Lines during Early Stages of Seed Germination. PLOS ONE 8:e65867

32. Hunter RB, Kannenberg LW (1971) ISOZYME CHARACTERIZATION OF CORN (Zea mays) INBREDS AND ITS RELATIONSHIP TO SINGLE CROSS HYBRID PERFORMANCE. Can J Genet Cytol 13:649–655

33. Heidrich-Sobrinho E, Cordeiro AR (1975) Codominant isoenzymic alleles as markers of genetic diversity correlated with heterosis in maize (Zea mays). Theoret Appl Genetics 46:197–199

34. Xing J, Sun Q, Ni Z (2016) Proteomic patterns associated with heterosis. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1864:908–915

35. Wu X, Liu Y, Zhang Y, Gu R (2021) Advances in Research on the Mechanism of Heterosis in Plants. Frontiers in Plant Science 12:

36. Rockenbach MF, Corrêa CCG, Heringer AS, Freitas ILJ, Santa-Catarina C, Amaral-Júnior AT do, Silveira V (2018) Differentially abundant proteins associated with heterosis in the primary roots of popcorn. PLOS ONE 13:e0197114

37. Li Z, Zhu A, Song Q, Chen HY, Harmon FG, Chen ZJ (2020) Temporal Regulation of the Metabolome and Proteome in Photosynthetic and Photorespiratory Pathways Contributes to Maize Heterosis. The Plant Cell 32:3706–3722

38. Biostimulants Market, Global Industry Size Forecast [Latest]. In: MarketsandMarkets. https://www.marketsandmarkets.com/Market-Reports/biostimulant-market-1081.html. Accessed 11 May 2023

39. Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim S-H (2018) Do Endophytes Promote Growth of Host Plants Under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes. Microb Ecol 75:407–418

40. Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV (2016) From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms. Frontiers in Plant Science 7:

41. Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson AC (2017) Perspectives and challenges of microbial application for crop improvement. Frontiers in Plant Science 8:49

42. Ganeshan S, Kim SH, Vujanovic V (2021) Scaling-up production of plant endophytes in bioreactors: concepts, challenges and perspectives. Bioresources and Bioprocessing 2021 8:1 8:1–16

43. Jack CN, Petipas RH, Cheeke TE, Rowland JL, Friesen ML (2021) Microbial Inoculants: Silver Bullet or Microbial Jurassic Park? Trends in Microbiology 29:299–308

44. Aprahamian AM, Lulow ME, Major MR, Balazs KR, Treseder KK, Maltz MR (2016) Arbuscular mycorrhizal inoculation in coastal sage scrub restoration. Botany 94:493–499

45. Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF (2020) Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148:107874

46. Wallace JG, May G (2018) Endophytes: The Other Maize Genome. In: Bennetzen J, Flint-Garcia S, Hirsch C, Tuberosa R (eds) The Maize Genome. Springer International Publishing, Cham, pp 213–246

47. Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. Environmental and Experimental Botany 97:30–39

48. Roesch LFW, Camargo FAO, Bento FM, Triplett EW (2008) Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. Plant Soil 302:91–104

49. Schultz CR, Johnson M, Wallace JG (2023) Effects of Inbreeding on Microbial Community Diversity of Zea mays. Microorganisms 11:879

50. Johnston-Monje D, Raizada MN (2011) Conservation and Diversity of Seed Associated Endophytes in Zea across Boundaries of Evolution, Ethnography and Ecology. PLoS ONE 6:e20396

51. Johnston-Monje D, Gutiérrez JP, Lopez-Lavalle LAB (2021) Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes. Front Microbiol 12:737616

52. Liu Y, Zuo S, Xu L, Zou Y, Song W (2012) Study on diversity of endophytic bacterial communities in seeds of hybrid maize and their parental lines. Arch Microbiol 194:1001–1012

53. Schardl CL (2001) Epichloë festucae and Related Mutualistic Symbionts of Grasses. Fungal Genetics and Biology 33:69–82

54. Guerre P, Guerre, Philippe (2015) Ergot Alkaloids Produced by Endophytic Fungi of the Genus Epichloë. Toxins 7:773–790

55. Cagnano G, Lenk I, Roulund N, Jensen CS, Cox MP, Asp T (2020) Mycelial biomass and concentration of loline alkaloids driven by complex population structure in Epichloë uncinata and meadow fescue (Schedonorus pratensis). Mycologia 112:474–490

56. Johnston-Monje D, Lundberg DS, Lazarovits G, Reis VM, Raizada MN (2016) Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant and Soil 405:337–355

57. Monteiro RA, Schmidt MA, Baura VA de, Balsanelli E, Wassem R, Yates MG, Randi MAF, Pedrosa FO, Souza EM de (2008) Early colonization pattern of maize (Zea mays L. Poales, Poaceae) roots by Herbaspirillum seropidicae, Burkholderia WP9, and Serendipita bescii seropedicae (Burkholderiales, Oxalobacteraceae). Genetics and Molecular Biology 31:932–937

58. Roncato-Maccari LDB, Ramos HJO, Pedrosa FO, Alquini Y, Chubatsu LS, Yates MG, Rigo LU, Steffens MBR, Souza EM (2003) Endophytic Herbaspirillum seropedicae expresses nif genes in gramineous plants. FEMS Microbiology Ecology 45:39–47

59. Lamb TG, Tonkyn DW, Kluepfel DA (1996) Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. Can J Microbiol 42:1112–1120

60. Bressan W, Borges MT (2004) Delivery methods for introducing endophytic bacteria into maize. BioControl 49:315–322

61. Baldotto MA, Baldotto LEB, Santana RB, Marciano CR (2012) Initial performance of maize in response to NPK fertilization combined with Herbaspirillum seropedicae. Rev Ceres 59:841–849

62. Canellas LP, Balmori DM, Médici LO, Aguiar NO, Campostrini E, Rosa RCC, Façanha AR, Olivares FL (2013) A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (Zea mays L.). Plant and Soil 366:119–132

63. Matsumura EE, Secco VA, Moreira RS, Santos OJAP dos, Hungria M, Oliveira ALM de (2015) Composition and activity of endophytic bacterial communities in field-grown

maize plants inoculated with Azospirillum brasilense. Annals of Microbiology 65:2187–2200

64. Young L-S, Hameed A, Peng S-Y, Shan Y-H, Wu S-P (2013) Endophytic establishment of the soil isolate Burkholderia sp. CC-Al74 enhances growth and P-utilization rate in maize (Zea mays L.). Applied Soil Ecology 66:40–47

65. Akhtar SS, Andersen MN, Naveed M, Zahir ZA, Liu F (2015) Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. Functional Plant Biol 42:770–781

66. Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic Azospirillum in the alleviation of drought effects in maize. Botany 87:455–462

67. Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. Plant Soil 331:413–425

68. Sobowale AA, Cardwell KF, Odebode AC, Bandyopadhyay R, Jonathan SG (2007) Persistence of Trichoderma species within maize stem against Fusarium verticillioides. Archives of Phytopathology and Plant Protection 40:215–231

69. Mousa WK, Shearer CR, Limay-Rios V, Zhou T, Raizada MN (2015) Bacterial endophytes from wild maize suppress Fusarium graminearum in modern maize and inhibit mycotoxin accumulation. Frontiers in Plant Science 6:

70. Shehata HR, Lyons EM, Jordan KS, Raizada MN (2016) Bacterial endophytes from wild and ancient maize are able to suppress the fungal pathogen Sclerotinia homoeocarpa. Journal of Applied Microbiology 120:756–769

71. Shehata HR, Raizada MN (2017) A Burkholderia endophyte of the ancient maize landrace Chapalote utilizes c-di-GMP-dependent and independent signaling to suppress diverse plant fungal pathogen targets. FEMS Microbiology Letters 364:fnx138

72. Santos F, Peñaflor MFGV, Paré PW, Sanches PA, Kamiya AC, Tonelli M, Nardi C, Bento JMS (2014) A Novel Interaction between Plant-Beneficial Rhizobacteria and Roots: Colonization Induces Corn Resistance against the Root Herbivore Diabrotica speciosa. PLOS ONE 9:e113280

73. Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E, Larsen J (2018) The root endophytic fungus Trichoderma atroviride induces foliar herbivory resistance in maize plants. Applied Soil Ecology 124:45–53

74. Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proceedings of the National Academy of Sciences of the United States of America 114:E2450–E2459

75. 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

76. Passera A, Follador A, Morandi S, et al (2021) Bacterial Communities in the Embryo of Maize Landraces: Relation with Susceptibility to Fusarium Ear Rot. Microorganisms 9:2388

77. Castiglioni P, Warner D, Bensen RJ, et al (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiol 147:446–455

78. Beirinckx S, Viaene T, Haegeman A, et al (2020) Tapping into the maize root microbiome to identify bacteria that promote growth under chilling conditions. Microbiome 8:54

79. Singh R, Goodwin SB (2022) Exploring the Corn Microbiome: A Detailed Review on Current Knowledge, Techniques, and Future Directions. PhytoFrontiers™ 2:158–175

80. Yang Y, Wang N, Guo X, Zhang Y, Ye B (2017) Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLOS ONE 12:e0178425

81. Johnston-Monje D, Gutiérrez JP, Becerra Lopez-Lavalle LA (2022) Stochastic Inoculum, Biotic Filtering and Species-Specific Seed Transmission Shape the Rare Microbiome of Plants. Life (Basel) 12:1372

82. Microbial life in the phyllosphere | Nature Reviews Microbiology. https://www.nature.com/articles/nrmicro2910. Accessed 17 May 2023

83. Wallace JG, Kremling KA, Kovar LL, Buckler ES (2018) Quantitative genetics of the maize leaf microbiome. Phytobiomes Journal 2:208–224

84. Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. PLOS ONE 8:e56329

85. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C, Vorholt JA (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proceedings of the National Academy of Sciences 106:16428–16433

86. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

87. Brusamarello-Santos LC, Gilard F, Brulé L, Quilleré I, Gourion B, Ratet P, Souza EM de, Lea PJ, Hirel B (2017) Metabolic profiling of two maize (Zea mays L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria Herbaspirillum seropedicae and Azospirillum brasilense. PLOS ONE 12:e0174576

88. Montañez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro. Applied Soil Ecology 58:21–28

89. da Silva KJ, de Armas RD, Soares CRFS, Ogliari JB (2016) Communities of endophytic microorganisms in different developmental stages from a local variety as well as transgenic and conventional isogenic hybrids of maize. World J Microbiol Biotechnol 32:189

90. McMullen MD, Kresovich S, Villeda HS, et al (2009) Genetic Properties of the Maize Nested Association Mapping Population. Science 325:737–740

91. 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 110:6548–6553

92. Flint-Garcia SA, Thuillet A-C, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM, Buckler ES (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. The Plant Journal 44:1054–1064

93. Wagner MR, Roberts JH, Balint-Kurti P, Holland JB (2020) Heterosis of leaf and rhizosphere microbiomes in field-grown maize. New Phytologist 228:1055–1069

94. Wagner MR, Tang C, Salvato F, et al (2021) Microbe-dependent heterosis in maize. Proc Natl Acad Sci USA 118:e2021965118

95. Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE (2010) Maize Leaf Epiphytic Bacteria Diversity Patterns Are Genetically Correlated with Resistance to Fungal Pathogen Infection. MPMI 23:473–484

96. Manching HC, Balint-Kurti PJ, Stapleton AE (2014) Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by nitrogen fertilization. Frontiers in Plant Science 5:

97. Wagner MR, Busby PE, Balint-Kurti P (2020) Analysis of leaf microbiome composition of near-isogenic maize lines differing in broad-spectrum disease resistance. New Phytologist 225:2152–2165

98. Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Applied Soil Ecology 8:11–18

99. Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil 232:167–180

100. Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14:2539–2551

101. Dematheis F, Zimmerling U, Flocco C, Kurtz B, Vidal S, Kropf S, Smalla K (2012) Multitrophic Interaction in the Rhizosphere of Maize: Root Feeding of Western Corn Rootworm Larvae Alters the Microbial Community Composition. PLOS ONE 7:e37288

102. Emmett BD, Buckley DH, Drinkwater LE (2020) Plant growth rate and nitrogen uptake shape rhizosphere bacterial community composition and activity in an agricultural field. New Phytologist 225:960–973

103. Pan JJ, Baumgarten AM, May G (2008) Effects of host plant environment and Ustilago maydis infection on the fungal endophyte community of maize (Zea mays). New Phytologist 178:147–156

104. Johnston-Monje D, Mousa WK, Lazarovits G, Raizada MN (2014) Impact of swapping soils on the endophytic bacterial communities of pre-domesticated, ancient and modern maize. BMC Plant Biol 14:233

105. Kadivar H, Stapleton AE (2003) Ultraviolet Radiation Alters Maize Phyllosphere Bacterial Diversity. Microb Ecol 45:353–361

106. Wolters B, Jacquiod S, Sørensen SJ, Widyasari-Mehta A, Bech TB, Kreuzig R, Smalla K (2018) Bulk soil and maize rhizosphere resistance genes, mobile genetic elements and microbial communities are differently impacted by organic and inorganic fertilization. FEMS Microbiology Ecology 94:fiy027

107. Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize Endophytic Bacterial Diversity as Affected by Soil Cultivation History. Frontiers in Microbiology 9:

108. Wattenburger CJ, Halverson LJ, Hofmockel KS (2019) Agricultural Management Affects Root-Associated Microbiome Recruitment Over Maize Development. Phytobiomes Journal 3:260–272

109. Benitez M-S, Osborne SL, Lehman RM (2017) Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. Sci Rep 7:15709

110. Maarastawi SA, Frindte K, Linnartz M, Knief C (2018) Crop Rotation and Straw Application Impact Microbial Communities in Italian and Philippine Soils and the Rhizosphere of Zea mays. Frontiers in Microbiology 9:

111. Chen Z, Zheng Y, Ding C, Ren X, Yuan J, Sun F, Li Y (2017) Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. Ecotoxicology and Environmental Safety 145:111–118

112. Islam F, Yasmeen T, Arif MS, Riaz M, Shahzad SM, Imran Q, Ali I (2016) Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. Plant Physiology and Biochemistry 108:456–467

113. Panitlertumpai N, Nakbanpote W, Sangdee A, Boonapatcharoen N, Prasad MNV (2018) Potentially toxic elements to maize in agricultural soils—microbial approach of rhizospheric and bulk soils and phytoaccumulation. Environ Sci Pollut Res 25:23954–23972

114. Vigliotta G, Matrella S, Cicatelli A, Guarino F, Castiglione S (2016) Effects of heavy metals and chelants on phytoremediation capacity and on rhizobacterial communities of maize. Journal of Environmental Management 179:93–102

115. Wu W, Wu J, Liu X, Chen X, Wu Y, Yu S (2017) Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure. Ecotoxicology and Environmental Safety 143:322–329

116. Sillen WMA, Thijs S, Abbamondi GR, De La Torre Roche R, Weyens N, White JC, Vangronsveld J (2020) Nanoparticle treatment of maize analyzed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis. Microbiome 8:127

117. de Menezes AB, Richardson AE, Thrall PH (2017) Linking fungal–bacterial co-occurrences to soil ecosystem function. Current Opinion in Microbiology 37:135–141

118. van Overbeek LS, Saikkonen K (2016) Impact of Bacterial–Fungal Interactions on the Colonization of the Endosphere. Trends in Plant Science 21:230–242

119. Deynze AV, Zamora P, Delaux P-M, et al (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLOS Biology 16:e2006352

120. Amicucci MJ, Galermo AG, Guerrero A, et al (2019) A Strategy for Structural Elucidation of Polysaccharides: Elucidation of a Maize Mucilage that Harbors Diazotrophic Bacteria. Analytical Chemistry acs.analchem.9b00789

121. Bennett AB, Pankievicz VCS, Ané J-M (2020) A Model for Nitrogen Fixation in Cereal Crops. Trends in Plant Science 25:226–235

122. Schultz C, Brantley K, Wallace J (2021) The Role of Genetic Variation in Maize Response to Beneficial Endophytes. 2021.11.03.467096

123. Craig L, Forest KT, Maier B (2019) Type IV pili: dynamics, biophysics and functional consequences. Nat Rev Microbiol 17:429–440

124. Pedrosa FO, Monteiro RA, Wassem R, et al (2011) Genome of Herbaspirillum seropedicae Strain SmR1, a Specialized Diazotrophic Endophyte of Tropical Grasses. PLOS Genetics 7:e1002064

125. Monteiro RA, Balsanelli E, Tuleski T, et al (2012) Genomic comparison of the endophyte Herbaspirillum seropedicae SmR1 and the phytopathogen Herbaspirillum rubrisubalbicans M1 by suppressive subtractive hybridization and partial genome sequencing. FEMS Microbiology Ecology 80:441–451

126. Straub D, Rothballer M, Hartmann A, Ludewig U (2013) The genome of the endophytic bacterium H. frisingense GSF30T identifies diverse strategies in the Herbaspirillum genus to interact with plants. Frontiers in Microbiology 4:

127. Sheibani-Tezerji R, Naveed M, Jehl M-A, Sessitsch A, Rattei T, Mitter B (2015) The genomes of closely related Pantoea ananatis maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. Frontiers in Microbiology 6:

128. Sessitsch A, Hardoim P, Döring J, et al (2012) Functional Characteristics of an Endophyte Community Colonizing Rice Roots as Revealed by Metagenomic Analysis. MPMI 25:28–36

129. Van Wees SCM, Van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448

130. Zhang X, Valdés-López O, Arellano C, Stacey G, Balint-Kurti P (2017) Genetic dissection of the maize (Zea mays L.) MAMP response. Theoretical and Applied Genetics 130:1155–1168

131. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced Systemic Resistance by Beneficial Microbes. Annual Review of Phytopathology 52:347–375

132. Djonović S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM (2007) A Proteinaceous Elicitor Sm1 from the Beneficial Fungus Trichoderma virens Is Required for Induced Systemic Resistance in Maize. Plant Physiology 145:875–889

133. Güimil S, Chang H-S, Zhu T, et al (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proceedings of the National Academy of Sciences 102:8066–8070

134. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth - Artursson - 2006 - Environmental Microbiology - Wiley Online Library. https://ami-journals.onlinelibrary.wiley.com/doi/full/10.1111/j.1462-2920.2005.00942.x. Accessed 16 May 2023

135. Zachow C, Tilcher R, Berg G (2008) Sugar Beet-Associated Bacterial and Fungal Communities Show a High Indigenous Antagonistic Potential Against Plant Pathogens. Microb Ecol 55:119–129

136. de Boer W, de Ridder-Duine AS, Klein Gunnewiek PJA, Smant W, Van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. Soil Biology and Biochemistry 40:1542–1544

137. Uroz S, Heinonsalo J (2008) Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. FEMS Microbiology Ecology 65:271–278

138. Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial–fungal interactions. Nat Rev Microbiol 8:340–349

139. Notz R, Maurhofer M, Dubach H, Haas D, Défago G (2002) Fusaric Acid-Producing Strains of Fusarium oxysporum Alter 2,4-Diacetylphloroglucinol Biosynthetic Gene Expression in Pseudomonas fluorescens CHA0 In Vitro and in the Rhizosphere of Wheat. Applied and Environmental Microbiology 68:2229–2235

140. Jäderlund L, Arthurson V, Granhall U, Jansson JK (2008) Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations. FEMS Microbiology Letters 287:174–180

141. Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA (2014) Newly identified helper bacteria stimulate ectomycorrhizal formation in Populus. Frontiers in Plant Science 5:

142. Afkhami ME, Stinchcombe JR (2016) Multiple mutualist effects on genomewide expression in the tripartite association between Medicago truncatula, nitrogen-fixing bacteria and mycorrhizal fungi. Molecular Ecology 25:4946–4962

143. Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y (2021) Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Frontiers in Plant Science 12:

144. Rani A, Rana A, Dhaka RK, Singh AP, Chahar M, Singh S, Nain L, Singh KP, Minz D (2023) Bacterial volatile organic compounds as biopesticides, growth promoters and plant-defense elicitors: Current understanding and future scope. Biotechnology Advances 63:108078

145. Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q (2018) Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23:762

146. Zhou H, Hua J, Li H, Song X, Luo S Structurally diverse specialized metabolites of maize and their extensive biological functions. Journal of Cellular Physiology. https://doi.org/10.1002/jcp.30955

147. Yu J, Tu X, Huang AC (2022) Functions and biosynthesis of plant signaling metabolites mediating plant–microbe interactions. Nat Prod Rep 39:1393–1422

148. Adedeji AA, Babalola OO (2020) Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. Planta 252:61

149. Tyc Olaf, Song C, Dickschat JS, Vos M, Garbeva P (2017) The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. Trends in Microbiology 25:280–292

150. Hu L, Robert CAM, Cadot S, et al (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun 9:2738

151. Kudjordjie EN, Sapkota R, Steffensen SK, Fomsgaard IS, Nicolaisen M (2019) Maize synthesized benzoxazinoids affect the host associated microbiome. Microbiome 7:59

152. Cotton TEA, Pétriacq P, Cameron DD, Meselmani MA, Schwarzenbacher R, Rolfe SA, Ton J (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J 13:1647–1658

153. Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Tawab A, Khan N (2020) Yucasin and cinnamic acid inhibit IAA and flavonoids biosynthesis minimizing interaction between maize and endophyte Aspergillus nomius. Symbiosis 81:149–160

154. D'alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J, Turlings TCJ (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant, Cell & Environment 37:813–826

155. Tenorio-Salgado S, Tinoco R, Vazquez-Duhalt R, Caballero-Mellado J, Perez-Rueda E (2013) Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4:236–243

156. Xie S, Liu J, Gu S, Chen X, Jiang H, Ding T (2020) Antifungal activity of volatile compounds produced by endophytic Bacillus subtilis DZSY21 against Curvularia lunata. Annals of Microbiology 70:2

157. Lee KK, Kim H, Lee Y-H (2022) Cross-kingdom co-occurrence networks in the plant microbiome: Importance and ecological interpretations. Frontiers in Microbiology 13:

158. Banerjee S, Schlaeppi K, van der Heijden MGA (2018) Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol 16:567–576

159. Heijden MGA van der, Hartmann M (2016) Networking in the Plant Microbiome. PLOS Biology 14:e1002378

160. Jiang D, Armour CR, Hu C, Mei M, Tian C, Sharpton TJ, Jiang Y (2019) Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities. Frontiers in Genetics 10:

161. Hong J, Karaoz U, de Valpine P, Fithian W (2022) To rarefy or not to rarefy: robustness and efficiency trade-offs of rarefying microbiome data. Bioinformatics btac127

162. Hünninghaus M, Dibbern D, Kramer S, Koller R, Pausch J, Schloter-Hai B, Urich T, Kandeler E, Bonkowski M, Lueders T (2019) Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize. Soil Biology and Biochemistry 134:122–130

163. Kim H, Lee KK, Jeon J, Harris WA, Lee Y-H (2020) Domestication of Oryza species eco-evolutionarily shapes bacterial and fungal communities in rice seed. Microbiome 8:20

164. Huang J, Li Y, Ma Y, Li Y, Jin J, Lian T (2022) The rhizospheric microbiome becomes more diverse with maize domestication and genetic improvement. Journal of Integrative Agriculture 21:1188–1202

165. Xiong C, Singh BK, He J-Z, et al (2021) Plant developmental stage drives the differentiation in ecological role of the maize microbiome. Microbiome 2021 9:1 9:1–15

166. Gardner CM, Gerhard WA, Redfern LK, Gunsch CK (2022) Evaluation of developing maize microbiomes and associations among nitrogen cyclers and key fungal taxa. Microbiology 168:001155

167. Toju H, Peay KG, Yamamichi M, et al (2018) Core microbiomes for sustainable agroecosystems. Nature Plants 4:247–257

168. Shade A, Stopnisek N (2019) Abundance-occupancy distributions to prioritize plant core microbiome membership. Current Opinion in Microbiology 49:50–58

169. Walters WA, Jin Z, Youngblut N, et al (2018) Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences of the United States of America 115:7368–7373

170. 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 112:E911–E920

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

172. Lundberg DS, Lebeis SL, Paredes SH, et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90

173. Chen L, Xin X, Zhang J, Redmile-gordon M, Nie G, Wang Q (2019) Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize. Pedosphere 29:360–373

174. Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y (2017) Let the Core Microbiota Be Functional. Trends in Plant Science 22:583–595

175. Ranjard L, Dequiedt S, Chemidlin Prévost-Bouré N, et al (2013) Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. Nat Commun 4:1434

176. Young JM, Kuykendall LD, Martínez-Romero E, Kerr A, Sawada H (2001) A revision of Rhizobium Frank 1889, with an emended description of the genus, and the inclusion of all species of Agrobacterium Conn 1942 and Allorhizobium undicola de Lajudie et al. 1998 as new combinations: Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola and R. vitis. International Journal of Systematic and Evolutionary Microbiology 51:89–103

177. Galloway-Peña J, Hanson B (2020) Tools for Analysis of the Microbiome. Dig Dis Sci 65:674–685

178. Su X, Yan X, Tsai C-L (2012) Linear regression. WIREs Computational Statistics 4:275–294

179. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46

180. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857

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

182. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

183. Andrews, S (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data [Online].

184. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

185. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns | mSystems. https://journals.asm.org/doi/10.1128/mSystems.00191-16. Accessed 5 May 2022

186. 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–D596

187. Nilsson RH, Larsson K-H, Taylor AFS, et al (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264

188. Badri M, Kurtz ZD, Bonneau R, Müller CL (2020) Shrinkage improves estimation of microbial associations under different normalization methods. NAR Genomics and Bioinformatics 2:lqaa100

189. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:1–21

190. McKnight DT, Huerlimann R, Bower DS, Schwarzkopf L, Alford RA, Zenger KR (2019) Methods for normalizing microbiome data: An ecological perspective. Methods in Ecology and Evolution 10:389–400

191. Fang H, Huang C, Zhao H, Deng M (2015) CCLasso: correlation inference for compositional data through Lasso. Bioinformatics 31:3172–3180

192. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688

193. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457-462

194. 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 Advanced Research. https://doi.org/10.1016/J.JARE.2019.03.004

195. Innerebner G, Knief C, Vorholt JA (2011) Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System ▿. Appl Environ Microbiol 77:3202–3210

196. Rojas X, Guo J, Leff JW, McNear DH, Fierer N, McCulley RL (2016) Infection with a Shoot-Specific Fungal Endophyte (Epichloë) Alters Tall Fescue Soil Microbial Communities. Microbial Ecology 72:197–206

197. Akhtar SAS, Andersen MBN, Naveed MD, Zahir ZDA, Liu FA Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. https://doi.org/10.1071/FP15054

198. Jochum MD, McWilliams KL, Pierson EA, Jo YK (2019) Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. PLoS ONE 14:e0225933

199. Naylor D, Degraaf S, Purdom E, Coleman-Derr D (2017) Drought and host selection influence bacterial community dynamics in the grass root microbiome. ISME Journal 11:2691–2704

200. Baldotto LEB, Olivares FL, Bressan-Smith R (2011) Structural interaction between GFP-labeled diazotrophic endophytic bacterium Herbaspirillum seropedicae RAM10 and pineapple plantlets “Vitória.” Brazilian Journal of Microbiology 42:114–125

201. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

202. Caradonia F, Ronga D, Catellani M, Azevedo CVG, Terrazas RA, Robertson-Albertyn S, Francia E, Bulgarelli D (2019) Nitrogen Fertilisers Shape The Composition And Predicted Functions Of The Microbiota Of Field-Grown Tomato Plants. bioRxiv 672162

203. Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Letters in Applied Microbiology 48:542–547

204. Rivas-Franco F, Hampton JG, Narciso J, Rostás M, Wessman P, Saville DJ, Jackson TA, Glare TR (2020) Effects of a maize root pest and fungal pathogen on entomopathogenic fungal rhizosphere colonization, endophytism and induction of plant hormones. Biological Control 150:104347

205. Patel M, Singh S, Vasanthakumari M, Naik S, Manjunatha B, Jadhav S, Ravikanth G, K N G, Shaanker R (2013) Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective. In: Advances in Endophytic Research. pp 177–190

206. Oukala N, Aissat K, Pastor V (2021) Bacterial Endophytes: The Hidden Actor in Plant Immune Responses against Biotic Stress. Plants (Basel) 10:1012

207. Ma Y (2017) Beneficial Bacteria for Disease Suppression and Plant Growth Promotion. In: Plant-Microbe Interactions in Agro-Ecological Perspectives. pp 513–529

208. Zhang W, Mason GA (2022) Modulating the rhizosphere microbiome by altering the cocktail of root secretions. Plant Physiology 188:12–13

209. Sun H, Jiang S, Jiang C, Wu C, Gao M, Wang Q (2021) A review of root exudates and rhizosphere microbiome for crop production. Environmental Science and Pollution Research 2021 1–14

210. Wu L, Kobayashi Y, Wasaki J, Koyama H (2018) Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Science and Plant Nutrition 64:697–704

211. Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480

212. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

213. Bergelson J, Brachi B, Roux F, Vailleau F (2021) Assessing the potential to harness the microbiome through plant genetics. Current Opinion in Biotechnology 70:167–173

214. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A (2020) Plant microbiome–an account of the factors that shape community composition and diversity. Current Plant Biology 23:100161

215. French E, Kaplan I, Iyer-Pascuzzi A, Nakatsu CH, Enders L (2021) Emerging strategies for precision microbiome management in diverse agroecosystems. Nature Plants 7:256–267

216. Roman-reyna V, Pinili D, Borjaa FN, et al (2019) The Rice Leaf Microbiome Has a Conserved Community Structure Controlled by Complex Host-Microbe Interactions. https://doi.org/10.2139/ssrn.3382544

217. Wipf HML, Coleman-Derr D (2021) Evaluating domestication and ploidy effects on the assembly of the wheat bacterial microbiome. PLOS ONE 16:e0248030

218. Gholizadeh S, Mohammadi SA, Salekdeh GH (2022) Changes in root microbiome during wheat evolution. BMC Microbiology 22:64

219. Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, Tschaplinski TJ, Schadt CW (2019) Rhizosphere microbiomes diverge among Populus trichocarpa plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome 7:76

220. Cordovez V, Rotoni C, Dini-Andreote F, Oyserman B, Carrión VJ, Raaijmakers JM (2021) Successive plant growth amplifies genotype-specific assembly of the tomato rhizosphere microbiome. Science of The Total Environment 772:144825

221. Bennetzen JL, Hake S (eds) (2009) Handbook of Maize. https://doi.org/10.1007/978-0-387-77863-1

222. corn : USDA ARS. https://www.ars.usda.gov/oc/timeline/corn/. Accessed 8 Dec 2021

223. Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/commodities/corn. Accessed 28 May 2022

224. USDA - National Agricultural Statistics Service - Publications. https://www.nass.usda.gov/Publications/. Accessed 28 May 2022

225. Dewey,Lee, Nolan, Reagan (2018) A Guide to Corn Production in Georgia 2018. University of Georgia Cooperative Extension

226. Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

227. Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis.

228. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR,, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package.

229. UpSetR: an R package for the visualization of intersecting sets and their properties | Bioinformatics | Oxford Academic. https://academic.oup.com/bioinformatics/article/33/18/2938/3884387. Accessed 5 May 2022

230. Pauvert C (2021) psadd: Additions to phyloseq package for microbiome analysis.

231. Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12:1–10

232. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9:980–989

233. Mueller UG, Juenger TE, Kardish MR, Carlson AL, Burns K, Smith C, Marais DLD Artificial Selection on Microbiomes to Confer Salt-Tolerance to Plants Artificial Microbiome-Selection to Engineer Microbiomes That Confer Salt-Tolerance to Plants 1 2. https://doi.org/10.1101/081521

234. Chiu C-H, Jost L, Chao A (2014) Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers. Ecological Monographs 84:21–44

235. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. ISME J 5:169–172

236. Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M (2016) High taxonomic variability despite stable functional structure across microbial communities. Nat Ecol Evol 1:1–12

237. McCaw ME, Wallace JG, Albert PS, Buckler ES, Birchler JA (2016) Fast-Flowering Mini-Maize: Seed to Seed in 60 Days. Genetics 204:35–42

238. Mehboob I, Zahir Z, Arshad M, Tanveer A, Khalid M (2012) Comparative effectiveness of different rhizobium sp. for improving growth and yield of maize (Zea mays L.). Soil and Environment 31:37–46

239. Estrada GA, Baldani VLD, Oliveira DM de, Urquiaga S, Baldani JI (2013) Selection of phosphate-solubilizing diazotrophic Herbaspirillum and Burkholderia strains and their effect on rice crop yield and nutrient uptake. Plant and Soil 369:115–129

240. Doni F, Suhaimi NSM, Irawan B, Mohamed Z, Mispan MS (2021) Associations of Pantoea with Rice Plants: As Friends or Foes? Agriculture 11:1278

241. Quecine MC, Araújo WL, Rossetto PB, Ferreira A, Tsui S, Lacava PT, Mondin M, Azevedo JL, Pizzirani-Kleiner AA (2012) Sugarcane Growth Promotion by the Endophytic Bacterium Pantoea agglomerans 33.1. Applied and Environmental Microbiology 78:7511–7518

242. Soluch R, Hülter NF, Romero Picazo D, Özkurt E, Stukenbrock EH, Dagan T (2021) Colonization dynamics of Pantoea agglomerans in the wheat root habitat. Environmental Microbiology 23:2260–2273

243. Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (Zea mays) by bacteria: Implications for energy plant biomass production and phytoremediation. Journal of Environmental Management 103:58–64

244. Piacentino D, Grant-Beurmann S, Vizioli C, et al (2021) Gut microbiome and metabolome in a non-human primate model of chronic excessive alcohol drinking. Transl Psychiatry 11:609

245. Coles VJ, Stukel MR, Brooks MT, et al (2017) Ocean biogeochemistry modeled with emergent trait-based genomics. Science 358:1149–1154

246. Levy A, Salas Gonzalez I, Mittelviefhaus M, et al (2017) Genomic features of bacterial adaptation to plants. Nat Genet 50:138–150

247. Bengelsdorf FR, Beck MH, Erz C, Hoffmeister S, Karl MM, Riegler P, Wirth S, Poehlein A, Weuster-Botz D, Dürre P (2018) Chapter Four - Bacterial Anaerobic Synthesis Gas (Syngas) and CO2+H2 Fermentation. In: Sariaslani S, Gadd GM (eds) Advances in Applied Microbiology. Academic Press, pp 143–221

248. Brilli F, Loreto F, Baccelli I (2019) Exploiting Plant Volatile Organic Compounds (VOCs) in Agriculture to Improve Sustainable Defense Strategies and Productivity of Crops. Frontiers in Plant Science 10:

249. Scala A, Allmann S, Mirabella R, Haring MA, Schuurink RC (2013) Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. International Journal of Molecular Sciences 14:17781–17811

250. Stolterfoht H, Rinnofner C, Winkler M, Pichler H (2019) Recombinant Lipoxygenases and Hydroperoxide Lyases for the Synthesis of Green Leaf Volatiles. J Agric Food Chem 67:13367–13392

251. Mosquito S, Bertani I, Licastro D, Compant S, Myers MP, Hinarejos E, Levy A, Venturi V (2020) In Planta Colonization and Role of T6SS in Two Rice Kosakonia Endophytes. MPMI 33:349–363

252. Kamat SS, Raushel FM (2013) The enzymatic conversion of phosphonates to phosphate by bacteria. Current Opinion in Chemical Biology 17:589–596

253. Diversity and abundance of phosphonate biosynthetic genes in nature | PNAS. https://www.pnas.org/doi/10.1073/pnas.1315107110. Accessed 31 May 2022

254. Navarro JA, Durán RV, De la Rosa MA, Hervás M (2005) Respiratory cytochrome c oxidase can be efficiently reduced by the photosynthetic redox proteins cytochrome c6 and plastocyanin in cyanobacteria. FEBS Letters 579:3565–3568

255. Deisenhofer J, Michel H (1993) 17 - Three-Dimensional Structure of the Reaction Center of Rhodopseudomonas viridis. In: Deisenhofer J, Norris JR (eds) Photosynthetic Reaction Center. Academic Press, San Diego, pp 541–558

256. Cardona T (2015) A fresh look at the evolution and diversification of photochemical reaction centers. Photosynth Res 126:111–134

257. Fleischman D (2012) Chapter 51 - Photosynthesis. In: Sperelakis N (ed) Cell Physiology Source Book (Fourth Edition). Academic Press, San Diego, pp 909–924

258. Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF (2000) Variation among Maize Inbred Lines and Detection of Quantitative Trait Loci for Growth at Low Phosphorus and Responsiveness to Arbuscular Mycorrhizal Fungi. Crop Science 40:358–364

259. Whitaker BK, Vaughan MM, McCormick SP (2022) Biocontrol Impacts on Wheat Physiology and Fusarium Head Blight Outcomes Are Bacterial Endophyte Strain and Cultivar Specific. Phytobiomes Journal PBIOMES-08-22-0056-R

260. Abdul Rahman NSN, Abdul Hamid NW, Nadarajah K (2021) Effects of Abiotic Stress on Soil Microbiome. International Journal of Molecular Sciences 22:9036

261. Trivedi P, Batista BD, Bazany KE, Singh BK (2022) Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytologist 234:1951–1959

262. Tiziani R, Miras-Moreno B, Malacrinò A, Vescio R, Lucini L, Mimmo T, Cesco S, Sorgonà A (2022) Drought, heat, and their combination impact the root exudation patterns and rhizosphere microbiome in maize roots. Environmental and Experimental Botany 203:105071

263. Swift JF, Kolp MR, Carmichael A, Ford NE, Hansen PM, Sikes BA, Kleiner M, Wagner MR (2023) Legacy effects of precipitation and land use impact maize growth and microbiome assembly under drought stress. 2023.04.11.536405

264. Methe BA, Hiltbrand D, Roach J, Xu W, Gordon SG, Goodner BW, Stapleton AE (2020) Functional gene categories differentiate maize leaf drought-related microbial epiphytic communities. PLOS ONE 15:e0237493

265. Vescio R, Malacrinò A, Bennett AE, Sorgonà A (2021) Single and combined abiotic stressors affect maize rhizosphere bacterial microbiota. Rhizosphere 17:100318

266. Mukhtar S, Mirza BS, Mehnaz S, Mirza MS, Mclean J, Malik KA (2018) Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. World J Microbiol Biotechnol 34:136

267. Guan Y, Jiang N, Wu Y, Yang Z, Bello A, Yang W (2021) Disentangling the role of salinity-sodicity in shaping soil microbiome along a natural saline-sodic gradient. Science of The Total Environment 765:142738

268. Yuan Y, Brunel C, van Kleunen M, Li J, Jin Z (2019) Salinity-induced changes in the rhizosphere microbiome improve salt tolerance of Hibiscus hamabo. Plant Soil 443:525–537

269. Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Applied Soil Ecology 107:324–333

270. Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host & Microbe 22:142–155

271. Babalola OO, Fadiji AE, Enagbonma BJ, Alori ET, Ayilara MS, Ayangbenro AS (2020) The Nexus Between Plant and Plant Microbiome: Revelation of the Networking Strategies. Frontiers in Microbiology 11:

272. Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Gutjahr C, Falter-Braun P (2019) Systems Biology of Plant-Microbiome Interactions. Molecular Plant 12:804–821

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

274. 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

275. Zheng W, Zhao Z, Gong Q, Zhai B, Li Z (2018) Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil. Soil Biology and Biochemistry 125:54–63

276. Ritter CD, Forster D, Azevedo JAR, Antonelli A, Nilsson RH, Trujillo ME, Dunthorn M (2021) Assessing Biotic and Abiotic Interactions of Microorganisms in Amazonia through Co-Occurrence Networks and DNA Metabarcoding. Microb Ecol 82:746–760

277. Chen M, He S, Li J, Hu W, Ma Y, Wu L, Gang G (2019) Co-occurrence patterns between bacterial and fungal communities in response to a vegetation gradient in a freshwater wetland. Can J Microbiol 65:722–737

278. (2023) Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/data/commodities/corn. Accessed 27 Apr 2023

279. Ling N, Zhu C, Xue C, Chen H, Duan Y, Peng C, Guo S, Shen Q (2016) Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biology and Biochemistry 99:137–149

280. Wu X, Hu H, Li S, Zhao J, Li J, Zhang G, Li G, Xiu W (2022) Chemical fertilizer reduction with organic material amendments alters co-occurrence network patterns of bacterium-fungus-nematode communities under the wheat–maize rotation regime. Plant Soil 473:605–623

281. Bazany KE, Wang J-T, Delgado-Baquerizo M, Singh BK, Trivedi P (2022) Water deficit affects inter-kingdom microbial connections in plant rhizosphere. Environmental Microbiology 24:3722–3734

282. Cobo-Díaz JF, Baroncelli R, Le Floch G, Picot A (2019) Combined Metabarcoding and Co-occurrence Network Analysis to Profile the Bacterial, Fungal and Fusarium Communities and Their Interactions in Maize Stalks. Frontiers in Microbiology 10:

283. Rogers AR, Holland JB (2022) Environment-specific genomic prediction ability in maize using environmental covariates depends on environmental similarity to training data. G3 Genes|Genomes|Genetics 12:jkab440

284. Kick DR, Wallace JG, Schnable JC, et al (2022) Yield Prediction Through Integration of Genetic, Environment, and Management Data Through Deep Learning. 2022.07.29.502051

285. Westhues CC, Simianer H, Beissinger TM (2022) learnMET: an R package to apply machine learning methods for genomic prediction using multi-environment trial data. G3 Genes|Genomes|Genetics 12:jkac226

286. Can High-Resolution Satellite Multispectral Imagery Be Used to Phenotype Canopy Traits and Yield Potential in Field Conditions? https://doi.org/10.13031/trans.14197. Accessed 26 May 2023

287. ImageBreed: Open-access plant breeding web–database for image-based phenotyping - Morales - 2020 - The Plant Phenome Journal - Wiley Online Library. https://acsess.onlinelibrary.wiley.com/doi/full/10.1002/ppj2.20004. Accessed 26 May 2023

288. Wiesner-Hanks T, Wu H, Stewart E, DeChant C, Kaczmar N, Lipson H, Gore MA, Nelson RJ (2019) Millimeter-Level Plant Disease Detection From Aerial Photographs via Deep Learning and Crowdsourced Data. Frontiers in Plant Science 10:

289. Bai G, Ge Y, Scoby D, Leavitt B, Stoerger V, Kirchgessner N, Irmak S, Graef G, Schnable J, Awada T (2019) NU-Spidercam: A large-scale, cable-driven, integrated sensing and robotic system for advanced phenotyping, remote sensing, and agronomic research. Computers and Electronics in Agriculture 160:71–81

290. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522

291. Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18:1403–1414

292. Walters W, Hyde ER, Berg-Lyons D, et al (2016) Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems. https://doi.org/10.1128/MSYSTEMS.00009-15

293. Xie J, Fu Y, Jiang D, Li G, Huang J, Li B, Hsiang T, Peng Y (2008) Intergeneric transfer of ribosomal genes between two fungi. BMC Evolutionary Biology 8:87

294. Free Weather API | Visual Crossing. https://www.visualcrossing.com/weather-api. Accessed 23 May 2023

295. Peschel S, Müller CL, von Mutius E, Boulesteix A-L, Depner M (2021) NetCoMi: network construction and comparison for microbiome data in R. Briefings in Bioinformatics 22:bbaa290

296. Jun W, Barahona M, Yue-Jin T, Hong-Zhong D (2010) Natural Connectivity of Complex Networks. Chinese Phys Lett 27:078902

297. J Fox, S Weisberg (2019) An R Companion to Applied Regression, 3rd Edition. Sage

298. Smith SD (2019) phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. Journal of Open Source Software 4:1442

299. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 57:289–300

300. Bairoch A (2000) The ENZYME database in 2000. Nucleic Acids Res 28:304–305

301. Simon HM, Jahn CE, Bergerud LT, Sliwinski MK, Weimer PJ, Willis DK, Goodman RM (2005) Cultivation of Mesophilic Soil Crenarchaeotes in Enrichment Cultures from Plant Roots. Appl Environ Microbiol 71:4751–4760

302. Madegwa YM, Uchida Y (2021) Land use and season drive changes in soil microbial communities and related functions in agricultural soils. Environmental DNA 3:1214–1228

303. Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

304. Bahram M, Hildebrand F, Forslund SK, et al (2018) Structure and function of the global topsoil microbiome. Nature 560:233–237

305. Bérdy J (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot 65:385–395

306. Li X, Garbeva P, Liu X, klein Gunnewiek PJA, Clocchiatti A, Hundscheid MPJ, Wang X, de Boer W (2020) Volatile-mediated antagonism of soil bacterial communities against fungi. Environmental Microbiology 22:1025–1035

307. Koopman N, Remijas L, Seppen J, Setlow P, Brul S (2022) Mechanisms and Applications of Bacterial Sporulation and Germination in the Intestine. Int J Mol Sci 23:3405

308. Chamkhi I, El Omari N, Benali T, Bouyahya A (2020) Quorum Sensing and Plant-Bacteria Interaction: Role of Quorum Sensing in the Rhizobacterial Community Colonization in the Rhizosphere. In: Quorum Sensing: Microbial Rules of Life. American Chemical Society, pp 139–153

309. Venturi V, Keel C (2016) Signaling in the Rhizosphere. Trends in Plant Science 21:187–198

310. Picazo-Aragonés J, Terrab A, Balao F (2020) Plant Volatile Organic Compounds Evolution: Transcriptional Regulation, Epigenetics and Polyploidy. Int J Mol Sci 21:8956

311. Raza W, Wei Z, Jousset A, Shen Q, Friman V-P (2021) Extended Plant Metarhizobiome: Understanding Volatile Organic Compound Signaling in Plant-Microbe Metapopulation Networks. mSystems 6:e00849-21

312. Rho H, Doty SL, Kim S-H (2018) Estimating microbial respiratory CO2 from endophytic bacteria in rice. Plant Signaling & Behavior 13:e1500067

313. Suryanarayanan TS, Ayesha MS, Shaanker RU (2022) Leaf photosynthesis: do endophytes have a say? Trends in Plant Science 27:968–970

314. Khare E, Mishra J, Arora NK (2018) Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. Frontiers in Microbiology 9:

CHAPTER 2

THE ROLE OF GENETIC VARIATION IN *ZEA MAYS* RESPONSE TO BENEFICIAL ENDOPHYTES[1]

---

[1] Schultz, C.R., Brantley, K.M. & Wallace, J.G. The role of genetic variation in Zea mays response to beneficial endophytes. Plant Growth Regul 98, 167–177 (2022). https://doi.org/10.1007/s10725-022-00842-9

Abstract:

Growth-promoting endophytes have great potential to boost crop production and sustainability. There is, however, a lack of research on how differences in the plant host affect an endophyte's ability to promote growth. We set out to quantify how different maize genotypes respond to specific growth-promoting endophytes. We inoculated genetically diverse maize lines with three different known beneficial endophytes: *Herbaspirillum seropedicae* (a gram-negative bacteria), *Burkholderia* WP9 (a gram-negative bacteria), and *Serendipita vermifera* Subsp. *bescii* (a Basidiomycota fungi). Maize seedlings were grown for 3 weeks under controlled watering and limited nutrient conditions in the greenhouse and assessed for various growth-promotion phenotypes. We found *Herbaspirillum seropedicae* to increase chlorophyll content (p=0.02), plant height (p=0.012), root length (p=0.057), and root volume (p=0.044) significantly in different maize genotypes, while *Burkholderia* WP9 did not promote growth in maize genotypes under these conditions. *Serendipita bescii* significantly increased plant height (p=0.0041), root (p=0.0004) and shoot biomass (p=0.0046) for different maize genotypes, and shoot mass growth promotion correlated (r =0 .58 p = 1.97e-09) with measured fungal abundance. Although plant genetic variation by itself had a strong effect on phenotype, its interaction with the different endophytes was weak, and the endophytes rarely produced consistent effects across different genotypes. This genome-by-genome interaction indicates that the relationship between a plant host and beneficial endophytes is complex, and it may partly explain why many microbe-based growth stimulants fail to translate from laboratory settings to the field. Detangling these interactions will provide a ripe area for future studies to understand how to best harness beneficial endophytes for agriculture.

Keywords: Growth promoting, Crop-microbe interaction, Quantifying variance.

Introduction

Food security is critical to modern global society. However, problems such as soil degradation, climate change, and a growing population will challenge our global food supply in the 21st century [Affairs 2015, Ray et al. 2013]. Improving crop yield by even small percentages results in massive increases in product and reduces the environmental burden of production [Reddy et al. 2020]. Biostimulants are a class of agricultural inputs based on either living organisms or products derived from them, and the use of biostimulants is touted as a sustainable way to improve yield, reduce soil degradation, and provide other ecological benefits [Majeed et al. 2018].

A common class of biostimulants involves the use of endophytes, microbes that live inside plants' tissue [Moran and Sloan 2015, Wallace and May 2018]. Previous studies have identified many ways that endophytes impact their hosts, such as by supplying nutrients (including nitrogen) [Baldotto et al. 2012, Boddey et al. 1991, Matsumura et al. 2015, Young et al. 2013], increasing stress resistance [Akhtar et al. 2015, Arachevaleta et al. 1989, Cohen et al. 2009, Naveed et al. 2014] and increasing crop growth and yield [Akhtar et al. 2015, Canellas et al. 2012, Hungria et al. 2010, Young et al. 2013]. Endophytes can stimulate plant growth in a variety of ways, such as by out-competing pathogens [Sobowale et al. 2007], producing antimicrobial compounds [Mousa et al. 2015, Shehata et al. 2016,Shehata et al. 2017], synthesizing/increasing phytohormones and secondary metabolites [Cohen et al. 2009, Kumara et al. 2013, Rivas-Franco et al. 2020], and mitigating stress [[Akhtar et al. 2015, Arachevaleta et al. 1989, Cohen et al. 2009, Naveed et al. 2014].

The biostimulant market is projected to be worth 11 billion USD by 2027 [Biostimulants Market 2021]. Many such treatments are already commercially available in the form of foliar

sprays, soil treatments, and seed treatments. Both startup companies and large corporations like Bayer and Syngenta are heavily investing in biostimulants [Biostimulants Market 2021], and trends in the scientific literature indicate growing interest in endophyte growth promotion in the public sector as well [Rho et al. 2017]. Despite this, it is incredibly challenging to bring a biostimulant from the lab to market [Parnell et al. 2016, Timmusk et al. 2017]. Many microbes that appear promising in the lab do not produce reliable effects in the field. Although many factors are probably responsible for this issue, one that has received less attention is how different plant genotypes respond to beneficial endophytes.

Several groups have shown that the effects of beneficial microbes vary across genotypes [Alves et al. 2014, Arujo et al. 2013, Brusamarello-Santos et al. 2017], but there has been relatively little quantification of this effect and almost no exploration of the underlying mechanisms. For example, an increase in maize yield due to bacteria in the genus *Herbaspirillum* depended on both the endophyte strain and the maize variety, with *Herbaspirillum seropedicae (*ZAE 94*)* increasing biomass in less than a third of commercial maize genotypes tested [Alves et al. 2014].

These results provide a firm estimate of the degree to which different maize genotypes respond to beneficial endophytes, and how genetic variation among these lines modulates that response. Although not investigated in detail, previous research has shown that the same endophyte impacts different maize inbreds to different degrees, and these differences are likely due to differences in the host's genetics [Alves et al. 2014, Arujo et al. 2013, Brusamarello-Santos et al. 2017, Montanez et al. 2012, Naveed et al. 2014, Riggs et al. 2001, Walters et al. 2018]. Few studies have tried to quantify the effect host genotype has on growth-promoting

interactions. Understanding and effectively utilizing these interactions would be a step towards increasing yield in a sustainable fashion.

In this study we aimed to quantify the effect maize genotype has on an endophyte's ability to promote growth. In a series of three experiments, we inoculated diverse maize lines with one of three different growth-promoting endophytes (*Herbaspirillum seropedicae*, *Burkholderia WP9*, and *Serendipita bescii*) [Monteiro et al. 2012, Young et al. 2013, Ray et al. 2015] and quantified the resulting changes in phenotype. For each microbe, we determined its effect on seedling phenotypes of diverse maize varieties and quantified the effect of maize genetics, microbes, and their interaction. Maize is one of the most important crops in global agriculture. Over a billion tonnes of maize were produced worldwide in 2019 [FAOSTAT 2021].

Materials and Methods

Experimental Design

Maize genotypes were selected from among the Goodman-Buckler diversity panel [Flint-Garcia et al. 2005], with most also being founders of the maize Nested Association Mapping population [Mcmullen et al. 2009] (Table 1). For space reasons, each experiment was subdivided into a series of "grows" including only a subset of plant genotypes. Within each grow, plants were arranged in a randomized complete block design with 5 replicates; in a few cases seedlings died after germination leaving that genotype with four replicates.

Table 1: Maize germplasm

Maize Genotype	GRIN Accession	Experiment(s) Used
A635	PI 693329	1, 2, 3
B73	PI 5504073	1, 3
B97	PI 564682	1, 3
CML 52	PI 595561	1, 3

CML 103	Ames 27081	1, 3
CML 228	Ames 27081	1, 2
CML 333	Ames 27101	3
HP301	PI 587131	3
KI3	Ames 27123	1, 3
KI11	Ames 27124	2, 3
MO17	PI 558532	1
MS71	PI 587137	1, 2, 3
NC350	Ames 27171	1, 2, 3
P39	Ames 28186	2, 3
TX303	Ames 19327	1, 3

Seed Sterilization and Plant Growth

Seeds were surface sterilized for five minutes using 50 mL sterile H_2O, 50 mL of bleach (Clorox), and three drops of Tween 20 (VWR). Seeds were rinsed five times with 100 mL of sterile water, then immersed in a 60° C water bath for 15 minutes to kill existing endophytes [Bacon C.W. 1994]. Seeds (in water) were then allowed to cool and imbibe for 1 hour before placing 10 seeds equidistant from each other in an autoclaved magenta box with 15 ml nutrient agar (1x Hoagland solution [bioWorld 30630038-5] + 15g/L of agar [Caisson Labs])). The box was then parafilmed shut, and the seeds were allowed to germinate for seven days. After 7 days, seedlings were moved to the greenhouse and planted 4 cm deep in 2.37 L pots filled with autoclaved Professional Growing Mix Fafard 3B/Metro-Mix 830 (Sungro Horticulture) and inoculated as described below. Pots were watered three times a week, and plants were grown for an additional 21 days before they were harvested.

Bacterial growth and inoculation

Experiment 1: *H. seropediceae* (ATTC 35892) was grown from a single colony in nutrient broth at 24 °C for 48 hours to an OD of ~0.8. Germinated seeds were placed into the autoclaved soil

and inoculated with 2mL of culture or sterile nutrient broth (control) before being covered by soil. No additional water was applied for 2 days to allow for colonization.

Experiment 2: *Burkholderia* WP9 (Sharon Doty, University of Washington) was grown from a single colony in nutrient broth at 24 °C for 48 hours to an OD of ~0.8. Autoclaved soil was inoculated with 200mL of culture/kg of soil, or with sterile nutrient broth for controls, before being placed into individual pots and germinated seedlings planted as above.

Experiment 3: *Serendipita bescii* (Kelly Craven, Noble Research Institute) was pre-inoculated onto clay bentonite particles [Ray et al. 2015, Rat et al. 2018] by collaborators at the Noble Research Institute. Based on their recommendation, soil was placed into pots a week before sowing and thoroughly washed with water 5 times to leech minerals and nutrients from the media. 100g of clay particles (inoculated or control) were placed in a depression in the soil, with the germinated seed then placed on top and covered.

After 21 days, the above- and below-ground portions of each plant were separated with a sterile razor blade and frozen at -80° C for further phenotyping.

PCR confirmation of colonization

For each experiment, endophyte colonization was confirmed by PCR. About 0.5g of washed root was collected using a sterile razor blade ~8cm from the base of the root. Samples were placed into a 2mL microtube with a sterile metal ball (Daisy BBs) and placed into a GenoGrinder 2010 at 1400 RPMs for 5 minutes. DNA was extracted with a Quick-DNA Fungal/Bacterial kit (Zymo) and DNA quality and concentration checked via Infinite M200 Pro (TECAN). PCR was run on each sample using the specific endophyte primers (Additional File 1) and run on a 1% agarose gel to confirm colonization. Non-inoculated plants served as controls

for greenhouse contamination. PCR program was as follows: 30s at 95 °C, followed by 30 cycles of 15s at 95 °C, 60s at 59 °C, 30s at 68 °C, ending with 5m at 68 °C and hold at 4 °C.

Phenotyping Methods

Phenotyping methods changed as the experiments progressed in an attempt to better capture the impact of the endophyte. Phenotypes were consistent within an experiment.

Plant Height: was measured from the soil line to the tip of the longest/tallest leaf when held upright, and was recorded every week. This method is thus is a combination of plant height and leaf length, and was used to control for different leaf angles.

Chlorophyll: Quantum Yield was measured using a Flouropen FP 100 (Photon Systems Instruments). Measurements were taken from halfway up the most mature leaf on the plant. Three measurements were taken at the same location and averaged. Chlorophyll content was only tested in experiment 1, as the instrument had a large measuring variance.

Leaf Area: The newest mature leaf was gently removed at the collar. Leaves were laid flat and pinned to a white surface next to a 1"-square size marker and a paper with sample identifying information (name & date). Images of each leaf were quantified with EasyLeafArea [Easlon et al 2014], with the following batch parameters: Leaf minimum Green RGB value 15, Leaf Green Ratio (G/R) 1.06, Leaf Green Ratio (G/B) 1.08, Scale Minimum Red RGB value 50, Scale Red Ratio 1.96, Scale area (cm^2) = 6.5 cm^2.

Shoot biomass: The entire aboveground portion of the plant, including the leaf removed to measure leaf area, was dried in a forced-air oven at 37.7 °C for 48 hours before being weighed on a precision balance (VWR 164AC).

Root Phenotypes: Frozen roots were removed from the freezer and washed with warm sterile water while gently rubbing to remove as much soil as possible without damaging the root

system. Root length was measured from the base to the end of the longest root. Root volume was measured by placing the washed & dried roots into a 20 mL graduated cylinder half-filled with water and recording the displacement volume as the roots were submerged. Dry root biomass was measured on a precision balance (VWR 164AC) after air-drying samples for 48 h at 37.7 °C in a forced-air drier.

qPCR for fungal biomass: For Experiment 3 only, relative fungal biomass was estimated using the $2^{\Delta\Delta Ct}$ method [Livak and Schmittgen 2001] to compare the amount of fungal ITS3 to maize CDK (cycline-dependent kinase housekeeping gene) DNA in each sample. Extracted maize root DNA (the same used to confirm colonization, above) was diluted to 12 ng/uL using an Infinite M200 Pro (TECAN). qPCR was performed using primers specific for the *Serendipita* ITS3 gene [Ray et al. 2015] or the maize CDK [Lin et al. 2014] (Table S1). Reactions were performed using SYBR Green I Master Mix (Roche) and the manufacturer's recommended protocol (pre-incubation at 95 °C for 5m, 45 cycles of amplification for 10s at 95 °C, 49.6°C (ITS) or 59.3 °C (CDK) for 18s, and 30s at 72 °C). A single melting curve was performed, with 8 acquisitions/°C. Reactions were run on a Roche LightCycler 480, with two technical replicates for each sample. $2^{\Delta\Delta Ct}$ values were generated from threshold crossing (Ct) values, and then log transformed to visualize *S. bescii* colonization for each maize genotype in R.

Statistics

All statistics were run in R [R Core Team]. Due to space constraints, groups of genotypes ("grows") had to be planted separately throughout each experiment; since these grows were completely confounded with the plant genotype, they were not included in subsequent analyses. ANOVA was performed by fitting a linear model of Phenotype ~ Genotype + Condition + Genotype: Condition, where "Condition" represents either inoculated or control. The resulting

ANOVA table was used to calculate the fraction of total variation contributed by each component and its statistical significance. The Genome x Genome (GxG) interaction represents the fraction of variation explained after accounting for the main effects of maize genotype and the general effect of inoculation. GxG is calculated as $V_{GC} / (V_{GC}+V_e)$, where V_{GC} is the variation due to genotype-by-condition interaction, and V_e is the residual (error) variance. Significant growth differences were determined using a Welch's two-sided t-test between inoculated and control plants of the same genotype. Levene's test was used to check for homogeny of variance for each phenotype.

Results

We tested three separate endophytes for growth promoting abilities in maize inbred lines in three separate greenhouse experiments. Due to space constraints, experiments had to be subdivided into grows, which contained all of the replicates for a genotype. The experimental design was kept identical for grows within the same experiment, with planting date being the only difference. All tested genotypes are listed in Table 1. In these experiments, "genome-by-genome interaction" (GxG) refers to how much variation was due to the specific combination of maize variety and endophyte inoculation. Functionally, GxG represents the differences in how maize varieties respond to an endophyte, so that the larger GxG is the stronger the differences are among maize varieties.

Figure 1. ANOVA analysis

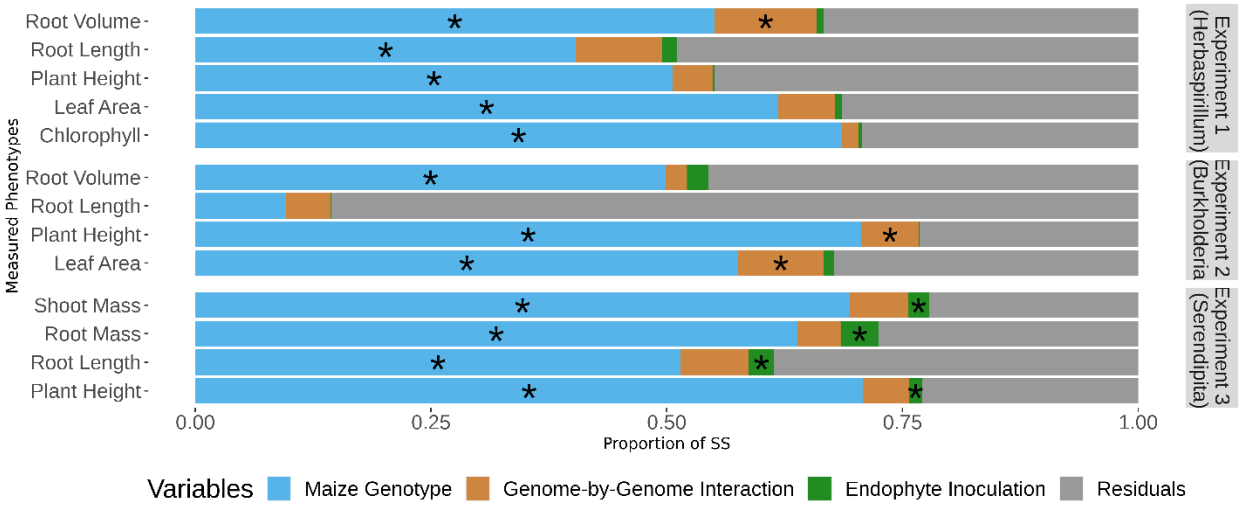


Fig 2 ANOVA analysis. Standard ANOVA was used to break phenotypic variation into the effects of maize genotype, endophyte inoculation, their interaction, and residual error. Asterisks denote significant effects ($p<0.05$). Plant genotype is almost always significant, while only *Serendipita* (Experiment 3) shows a consistent effect of the endophyte. Levene's test was used to check for equality of variances (one of the assumptions of ANOVA), Shoot Mass in Experiment 3 failed the test, so its results should be taken with caution.

Experiment 1

Experiment 1 used the gram-negative bacterium *Herbaspirillum seropediceae* Z67, with liquid culture applied directly to germinated seeds. Phenotypic variation was largely due to the plant genotype (Figure 1), as was expected given the high genetic variation in maize. Inoculation with *H. seropediceae* was not a significant source of variation for any phenotype, and GxG interaction was only statistically significant for root volume ($p=.05$; Figure 1). Root volume had the highest GxG for the entire study (0.243, after accounting for the main effects of genotype and endophyte; Table 2). When looking at individual genotypes instead of the experiment as a whole, *H. seropediceae* increased chlorophyll content, plant height, and root volume (Table 3), though only for 1 maize genotype in each case. *Herbaspirillum* increased growth, though generally only for a couple of maize genotypes. There was also an interesting trend where *H. seropediceae*

increased root length in A635 but decreased it in CML52, though it did not reach statistical significance.

Table 2. Genome by Genome Interaction Calculations

Endophyte	Trait	GxG Interaction (0-1)
	Experiment 1	
Herbaspirillum	Chlorophyll	.057
Herbaspirillum	Leaf Area	0.155
Herbaspirillum	Plant Height	0.091
Herbaspirillum	Root Length	0.157
Herbaspirillum	Root Volume	0.243
	Experiment 2	
Burkholderia	Leaf Area	0.232
Burkholderia	Plant Height	0.218
Burkholderia	Root Length	0.071
Burkholderia	Root Volume	0.027
	Experiment 3	
Serendipita	Plant Height	0.178
Serendipita	Root Length	0.157
Serendipita	Root Mass	0.144
Serendipita	Shoot Mass	0.222

Table 2. Genome x Genome interaction calculations. GxG were calculated as the fraction of phenotypic variance due to Genotype:Inoculation interaction after accounting for the main effects of maize genotype and inoculation.

Experiment 2

Experiment 2 used the gram-negative bacteria *Burkholderia* sp. WP9, with liquid culture applied directly to bulk soil immediately before adding seeds. Again, plant genotype was statistically significant for most measured phenotypes while endophyte inoculation by itself was not significant for any (Figure 1). Plant-endophyte interaction was statistically significant for both plant height and leaf area, with GxG interaction scores of ~0.2 (Table 1). Although *Burkholderia WP9* reportedly boosts maize growth (Sharon Doty, personal communication), we saw no statistically significant growth promotion for any individual phenotypes in any lines that

were tested (Table 3). The fact that GxG interaction overall is significant while no individual genotypes are is probably due to the greater statistical power when looking at the experiment as a whole.

Table 3. Significant Growth Differences

Trait	P < 0.1	P < 0.05
Experiment 1 – *H. Seropedicaea*		
Chlorophyll	CML103(+)	B73(+)
Leaf Area	-	-
Plant Height	-	Mo17(+)
Root Length	CML52(-), A635(+)	-
Root Volume	TX303(+)	CML228(+)
Experiment 2 – *Burkholderia* WP9		
Leaf Area	CML228(-)	-
Plant Height	-	-
Root Length	-	-
Root Volume	-	-
Experiment 3 – *S. bescii*		
Plant Height	-	TX303(+), CML52(+)
Root Length	B73(-), CML103(-), A635(+)	-
Root Mass	KI11(+)	P39(+), CML52(+)
Shoot Mass	-	P39(+), NC350(+), TX303(+)

Table 3. Genotypes that have significant growth promotion for a measured phenotype. Significance was determined by a two-sided t-test of control versus inoculated plants (see Methods). The direction of growth (increase or decrease) is indicated by a "+" or "–" sign after each genotype.

Experiment 3

Experiment 3 used the Basidiomycota fungi *Serendipita bescii*, which was pre-inoculated on sterile clay particles that were placed directly under the germinated seed in the soil. *S. bescii* showed the strongest growth-promoting effects of the three endophytes, with a significant main effect of inoculation for all four measured phenotypes (Figure 1). *S. bescii* increased growth in a

number of genotypes (Table 3; Figure 2), though GxG interaction was not significant in the experiment overall (Figure 1), possibly due to the high variance within some of the lines. A particularly interesting contrast in this experiment involves the maize lines CML52, NC350, P39, and TX303 (Table 3). Inoculation with *S. bescii* increased only the above-ground biomass of NC350 and TX303, only the belowground biomass of CML52, and both traits for P39 (Figure 2).

Figure 2. *Serendipita* growth promotion

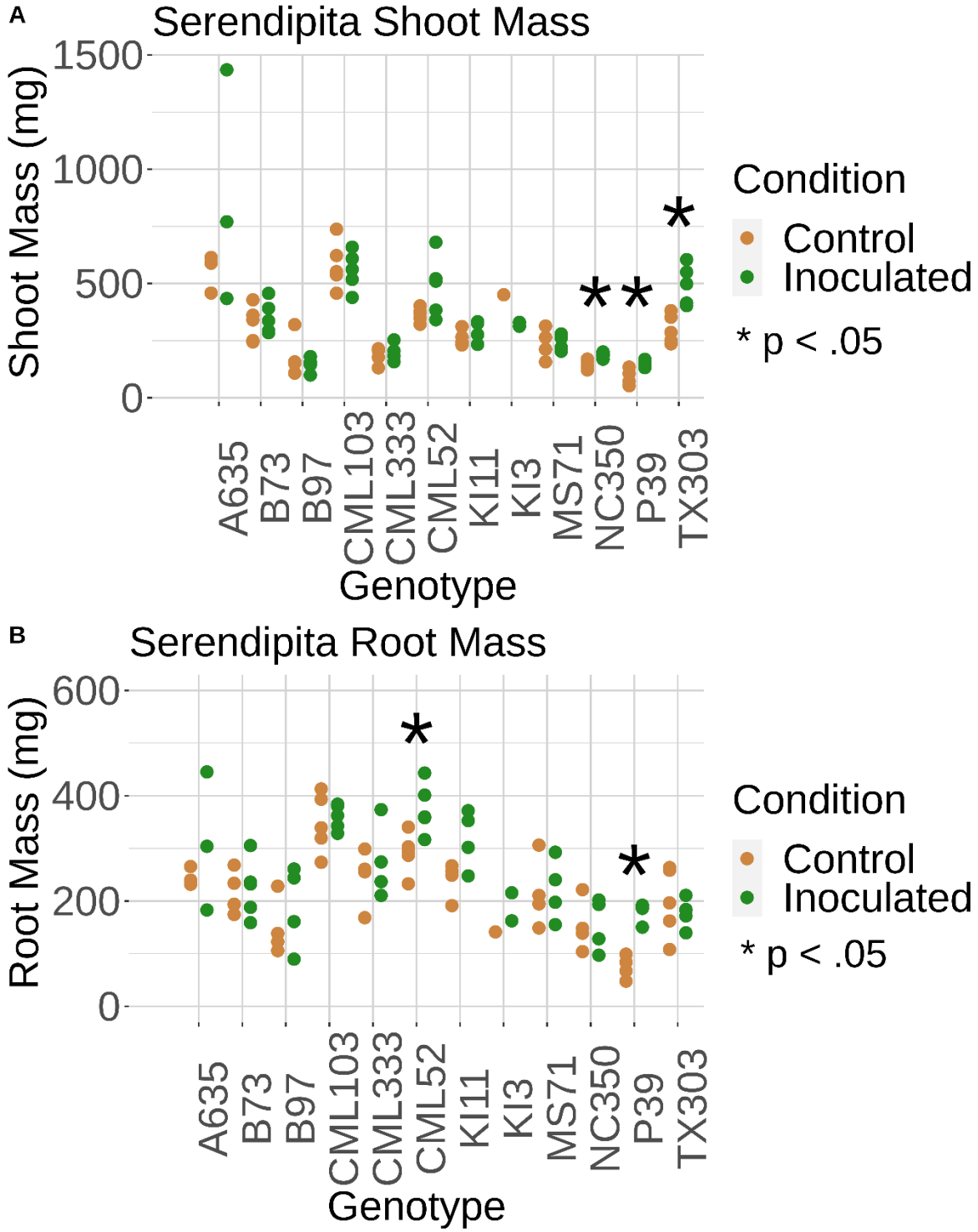


Fig 2 Effect of inoculation of *S. bescii* on different maize gentoypes for (A) shoot and (B) root biomass. Asterisks denote significant growth promotion. Several genotypes show growth promotion in only one of the two compartments; only P39 shows promotion in both.

In all three experiments root endophyte colonization was confirmed by PCR. This was done to ensure inoculates were in fact colonizing maize seedlings, while also confirming that greenhouse care was not cross contaminating plants. All inoculated seedlings contained an appropriate band for its endophyte primers, and all control plants remained negative (data not shown).

We quantified the colonization of *Serendipita* using qPCR of the *Serendipita* fungal ITS3 [Ray et al. 2015] normalized against the maize housekeeping gene CDK [Lin et al. 2014] (Figure 3). Higher levels of *Serendipita* colonization coincided with increased growth in the greenhouse. Maize lines P39, NC350, TX303, and CML52 had high *Serendipita* in their respective grows, and showed growth promotion for at least one phenotype with a $p < 0.05$ (Table 3); two other maize genotypes (A635 and KI11) also had high *S. bescii* abundances but less significant growth promotion ($p < 0.1$; Table 3). Of the remaining lines, MS71 and CML333 showed no growth promotion, while CML103 and B73 had a nonsignificant trend ($p \sim 0.07$) toward growth inhibition.

Figure 3. Differences in *Serendipita* Abundance by Genotype

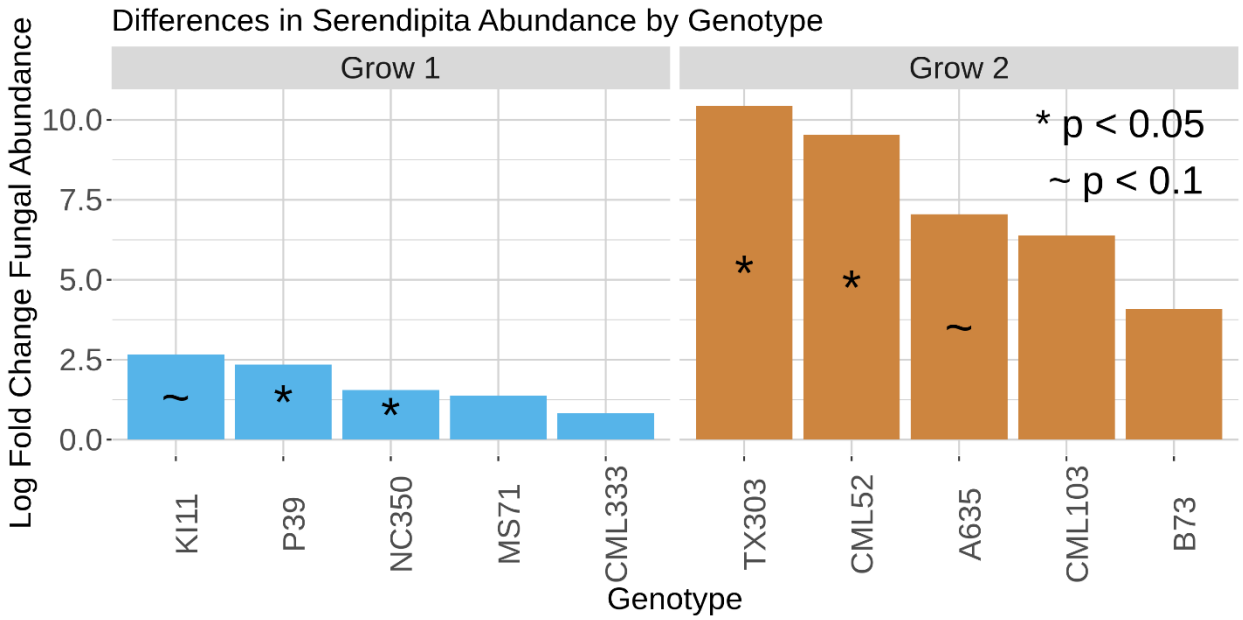


Fig 3 Log fold-change differences in *S. bescii* colonization between control and inoculated plants. The *S. bescii* ITS3 gene was quantified via qPCR and normalized to the maize CDK housekeeping gene. Since "grows" were planted on separate dates for space reasons, they are kept separate in this analysis. Within each grow, higher levels of colonization coincided with significant growth promotion (marked with ~ or *). The least-colonized plants in each grow showed either no promotion or trends toward growth inhibition (Table 2).

Discussion

Our results in this study highlight how three potentially growth-promoting endophytes affect genetically diverse maize.

Herbaspirillum seropedicae is a well-studied gram-negative, growth-promoting endophyte that is commonly used to study nitrogen fixation in symbioses with grasses [Alves et al. 2014, Canellas et al. 2012, Dall'Asta et al. 2018]. It has a broad host range and can colonize sugarcane, rice, wheat, and maize, where it can act as a biofertilizer [Boddey et al. 1991, Majeed et al. 2018, Rosenblueth et al. 2018]. In addition to fixing nitrogen, *H. seropedicae* can solubilize minerals and produce phytohormones [Monteiro et al. 2012]. When inoculated with *Herbaspirillum seropedicae* (Experiment 1), we found several maize genotypes showed increased growth in one of several phenotypes (Chlorophyll content, plant height, root length,

and root volume, Table 3). *Herbaspirillum* did not increase growth in the experiment as a whole, but on the individual genotype level. This aligns with previous studies that have shown that maize yield, metabolite content, and shoot dry weight depend upon the genotype of both the *Herbaspirillum* inoculant, as well as the genotype of the maize host [Alves et al. 2014, Arujo et al. 2013, Brusamarello-Santos et al. 2017]. Genotype was the main driver of phenotypic variance throughout all three experiments (Figure 1). This was expected, as most of the differences in growth are due to the high diversity of the maize lines used, including members of the NAM founder population [Mcmullen et al. 2009]. A lack of significance in the "inoculation" term (Figure 1) indicates that the introduction of *Herbaspirillum* to the system does not cause significant and consistent changes in growth (increase or decrease) across all genotypes. Root volume was the only phenotype to have a significant interaction between genotype and inoculation (Figure 1). This may mean that *Herbaspirillum'*s ability to alter root characteristics may be controlled by its interaction with specific maize lines. Although the trend was not statistically significant, we observed an increase in root length for A635 and a decrease in root length for maize line CML52 when inoculated with maize. These findings suggest that further experiments looking at how *Herbaspirillum* impacts root mass, volume, and length on a larger set of diverse maize germplasm could yield more information on how endophytes impact belowground architecture.

Burkholderia is bacterial genus containing well-studied growth-promoting endophytes. Different species have been shown to enhance growth, yield, and disease resistance [Young et al. 2013] and aid in the uptake of phosphate and nitrogen [Estrada et al. 2012, Young et al. 2013]. *Burkholderia* WP9 was isolated from black cottonwood and has nitrogen fixing abilities [Doty et al. 2009]. Experiment 2, examining *Burkholderia* WP9, indicated significant GxG interaction for

two phenotypes (plant height and leaf area), though an examination of individual varieties did not show statistically significant effects, probably due to the smaller sample size. As with *Herbaspirillum*, the main driver of phenotypic variance in the experiment was maize genotype, and endophyte inoculation alone was not significant across this experiment (Figure 1). Experiment 2 did show that some maize genotypes were slightly hindered by the endophyte, while some received slight growth promotion, highlighting the importance of genotype interactions. Although we confirmed colonization of the roots with this endophyte, we observed no growth promotion at $p<.005$. In Experiment 2 *Burkholderia* was inoculated into the bulk soil that the seedlings were sown into. By not directly inoculating the seeds, *Burkholderia* concentrations may have been too low to significantly impact maize growth. On the other hand, as the maize was harvested after 4 weeks, *Burkholderia* inoculated maize may have shown significant growth promotion if the experiment was extended. Finally, differences in growing environments between our experiment and previous ones may have changed how *Burkholderia* WP9 affected with the maize. Since other studies have shown that this isolate promotes growth in both rice [Khan et al. 2016] and maize (Sharon Doty, personal communication), it may be worth additional investigation.

Serendipita bescii is a Basidiomycota fungus, and fungi in this genus are known to associate with many different plant species as an endomycorrhizae [Ray et al. 2018]. Originally recognized as orchid mutualists, *Serendipita* fungi also promote growth in a number of different plants, including switchgrass [Ray et al. 2015]. It is hypothesized that when serendipitoid fungi colonize root systems, they break down organic manner in the soil and make these nutrients available to the plant [Craven and Ray 2019].

Of the genotypes inoculated with *Serendipita bescii* (Experiment 3), one (P39) showed growth increases both above and below ground (Figures 2 & 3), while three other genotypes (NC350, CML52 and TX303) experienced increases in only one of the two categories. These distinct differences show that not all germplasm may be able to receive growth promotion from *Serendipita.* Similar to *Burkholderia*, inoculation with *Serendipita* decreased the root length of two maize genotypes (B73 and CML103), further emphasizing the potential for unique reactions to endophyte treatment among different genotypes.

As with the previous endophytes, the variance in phenotype in Experiment 3 mainly came from genotype, and there was no statistically significant impact between *Serendipita* and maize genotype. *Serendipita* was the only endophyte to show a main effect of inoculation across all phenotypes (Figure 1), meaning it was the only one of the three endophytes to have a relatively consistent effect on hosts regardless of genotype. When *Serendipita* was introduced to maize, it consistently changed growth when we compared inoculated and controlled plants, through either promoting or hindering growth.

Not only did *Serendipita* have the most consistent effect on hosts, we also showed that the size of this effect may be related to the plants colonization level (Figure 3). Maize genotypes with higher abundance of *Serendipita* showed significant ($p<0.05$) or close to significant ($p<0.1$) growth promotion in the greenhouse. On the other hand, two maize lines (CML103 and B73) had comparatively lower amounts of *Serendipita,* and these lines experienced growth inhibition ($p\sim0.07$). A correlation analysis showed that shoot mass growth promotion correlated ($r = .58$ $p = 1.97e\text{-}09$) with measured fungal abundance. This indicates that stronger growth-promotion phenotypes may be a direct consequence of higher endophyte loads.

These differences in phenotype response could be due to differences in endophyte colonization, as has been shown for other grasses and poplar [Faville et al. 2015, Khan et al. 2016]. Mechanically, Epichloë fungal endophyte biomass has been shown to correlate with the amount of protective alkaloid compounds in their grass hosts [Cagnano et al. 2020, Faville et al. 2015]. Further testing is needed to confirm if endophyte biomass amounts have a direct influence on growth promotion, and to uncover the mechanisms and genetic diversity behind these interactions in maize.

These data demonstrate that maize genotype does have a significant impact on endophyte-mediated growth promotion. In addition, all three endophytes showed indications of decreased growth in at least one maize line, but none of these lines were the same. On the other hand, of the nine maize genotypes tested with both *H. seropedicae* and *S. bescii*, only one (TX303) showed statistically significant growth promotion for both endophytes. Interestingly, this promotion was belowground (Root Volume) for *H. seropedicae* and above ground (Plant Height) for *S. bescii.* This may indicate that these endophytes are activating different pathways or altering the expression of different genes.

This data does not provide insight into the molecular mechanisms of genome-by-genome interaction, but interest in this field is quickly growing. Prior work indicates that these interactions could be impacted by a number of metabolites and pathways. For example, diverse maize genotypes respond differently to microbe-associated molecular patterns (MAMPs), and as a consequence show significant differences in reactive oxygen species, nitric oxide production, and defense gene expression [Wang et al. 2021, Zhang et al. 2017]. It has been suggested that *Herbaspirillum* may regulate the ROS pathway differently in diverse maize roots [Brusamarello-Santos et al. 2017]. Many endophytes produce phytohormones [Cohen et al. 2009, Wallace and

May 2018] and volatile organic compounds [Kumara et al. 2013, Monteiro et al. 2012], which directly impact growth promotion and stress resistance. Future studies may be able to shed light on how host genetic diversity impacts these molecular interactions.

A chief goal of this research was to quantify "Genome-by-Genome interaction" in this system, meaning changes in phenotype that depend on both the maize genotype and the specific endophyte. The GxG interactions we identified explain relatively little phenotypic variance, even after factoring out the effect of maize genotype (Table 1). The strongest GxG effects explain ~20-25% of residual variance after accounting for genotype, although most are half this or less. Looked at another way, GxG interaction generally explained only 10% of the variance that maize genotype did, meaning that the effect was 10 times weaker than the effect of plant genetics. This small effect size may make it challenging to attempt to dissect the underlying genetic components that affect plant-microbe interactions, a conclusion shared by a recent review of genome wide association studies (GWAS) [Bergelson et al. 2021]. One of the few studies investigating this interaction traced the variation in *Arabidopsis*' response to growth-promoting rhizobacteria to several candidate genes, including genes involved in plant-growth processes like transporters and metabolism [Wintermans et al. 2016].

One should keep in mind, however, that the maize varieties in this study were specifically chosen for their high diversity. Diversity in elite breeding programs is generally much lower, so endophyte inoculation may have relatively larger or more consistent effects within elite material. Understanding the nature and extent of this variation could provide a way to improve the development and use of biostimulants in agriculture.

Conclusion

Our findings highlight how growth promoting endophytes interacted differently with diverse maize germplasm. *Herbaspirillum seropedicae* and *Serendipita bescii* differentially promoted growth for several maize genotypes, and even hindered growth in some instances. Interaction between maize genetic variation and endophytes were weak throughout the three experiments, indicating that this genome-by-genome interaction is complex. This interaction may involve regulating endophyte colonization levels, as colonization levels of *Serendipita* correlated with some growth promoted phenotypes. These findings provide insight into the range of responses between plants and microbes, and are especially important for groups developing new bioinoculates and biofertilizers. The high variability of growth promotion across genotypes may partly explain why many beneficial microbes reported in the literature fail to translate to field production. (This is apart from logistical factors such as scalability, shelf life, ease of use, and compatibility with existing formulation [Parnell et al. 2016, Timmusk et al. 2017], all of which also play a role). Our interactions with maize producers in the US indicates that they are both interested in biologicals and concerned about their efficacy. These results imply that new microbial-based products for agriculture should be screened against diverse genotypes early in the process so as to weed out microbes with variable effects. Further studies will show how these interactions occur and how we can best harness them to improve global agriculture.

References

Affairs, U.N.D.o.E.a.S., *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables.* Working Paper No ESA/WP.241, 2015.

Akhtar, Saqib Saleem, et al. "Interactive Effect of Biochar and Plant Growth-Promoting Bacterial Endophytes on Ameliorating Salinity Stress in Maize." *Functional Plant Biology*, vol. 42, no. 8, 2015, p. 770., doi:10.1071/fp15054.

Alves, Gabriela Cavalcanti, et al. "Differential Plant Growth Promotion and Nitrogen Fixation in Two Genotypes of Maize by Several Herbaspirillum Inoculants." *Plant and Soil*, vol. 387, no. 1-2, 2014, pp. 307–321., doi:10.1007/s11104-014-2295-2.

Arachevaleta, M., et al., *Effect of the Tall Fescue Endophyte on Plant-Response to Environmental-Stress.* Agronomy Journal, 1989. **81**(1): p. 83-90

Araujo, Fabio Fernando De, et al. "Híbridos e Variedades De Milho Submetidos à Inoculação De Sementes Com Herbaspirillum Seropedicae*." Semina: Ciências Agrárias*, vol. 34, no. 3, 2013, doi:10.5433/1679-0359.2013v34n3p1043.

BACON, C. W. (1994). A corn seedling assay for resistance tofusarium moniliforme. *Plant Disease, 78*(3), 302. doi:10.1094/pd-78-0302

Baldotto MA, Borges Baldotto LE, Santana RB, Marciano CR (2012) Initial performance of maize in response to NPK fertilization combined with Herbaspirillum seropedicae. Revista Ceres 59

Bergelson, J., Brachi, B., Roux, F., & Vailleau, F. (2021). Assessing the potential to harness the microbiome through plant genetics. *Current Opinion in Biotechnology*, *70*, 167–173. https://doi.org/10.1016/j.copbio.2021.05.007

Biostimulants Market. Market Research Firm. (n.d.). https://www.marketsandmarkets.com/Market-Reports/biostimulant-market-1081.html.

Boddey, R.M., et al., *Biological Nitrogen-Fixation Associated with Sugar-Cane.* Plant and Soil, 1991. **137**(1): p. 111-117.

Brusamarello-Santos, Liziane Cristina, et al. "Metabolic Profiling of Two Maize (Zea Mays L.) Inbred Lines Inoculated with the Nitrogen Fixing Plant-Interacting Bacteria Herbaspirillum Seropedicae and Azospirillum Brasilense." *Plos One*, vol. 12, no. 3, 2017, doi:10.1371/journal.pone.0174576

Cagnano, G., Lenk, I., Roulund, N., Jensen, C. S., Cox, M. P., & Asp, T. (2020). Mycelial biomass and concentration of loline alkaloids driven by complex population structure in Epichloë uncinata and meadow fescue (Schedonorus pratensis). *Mycologia*, *112*(3), 474–490. https://doi.org/10.1080/00275514.2020.1746607

Canellas, Luciano Pasqualoto, et al. "A Combination of Humic Substances and Herbaspirillum Seropedicae Inoculation Enhances the Growth of Maize (Zea Mays L.)." *Plant and Soil*, vol. 366, no. 1-2, 2012, pp. 119–132., doi:10.1007/s11104-012-1382-5.

Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic Azospirillum in the alleviation of drought effects in maize. Botany 87:455–462

Craven, Kelly D., and Prasun Ray. "More than Serendipity: The Potential to Manage Soil Carbon and Emissions While Promoting Low-Input Agriculture with Serendipitoid Mycorrhizae." *Phytobiomes Journal*, vol. 3, no. 3, 2019, pp. 161–164., doi:10.1094/pbiomes-12-18-0058-p

Dall'Asta, Pâmela, et al. "Herbaspirillum Seropedicae Promotes Maize Growth but Fails to Control the Maize Leaf Anthracnose." *Physiology and Molecular Biology of Plants*, vol. 25, no. 1, 2018, pp. 167–176., doi:10.1007/s12298-018-0616-2.

Doty, S. L., Oakley, B., Xin, G., Kang, J. W., Singleton, G., Khan, Z., . . . Staley, J. T. (2009). Diazotrophic endophytes of native black Cottonwood and willow. *Symbiosis, 47*(1), 23-33. doi:10.1007/bf03179967

Easlon, Hsien Ming, and Arnold J. Bloom. "Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area." *Applications in Plant Sciences*, vol. 2, no. 7, 2014, p. 1400033., doi:10.3732/apps.1400033.

Estrada, German Andres, et al. "Selection of Phosphate-Solubilizing Diazotrophic Herbaspirillum and Burkholderia Strains and Their Effect on Rice Crop Yield and Nutrient Uptake." *Plant and Soil*, vol. 369, no. 1-2, 2012, pp. 115–129., doi:10.1007/s11104-012-1550-7.

FAOSTAT. (n.d.). Retrieved March 11, 2021, from http://www.fao.org/faostat/en/#data/QC/visualize

Faville, M. J., Briggs, L., Cao, M., Koulman, A., Jahufer, M. Z., Koolaard, J., & Hume, D. E. (2015). A QTL analysis of host Plant effects on fungal ENDOPHYTE biomass And alkaloid expression in perennial ryegrass. *Molecular Breeding, 35*(8). doi:10.1007/s11032-015-0350-1

Flint-Garcia, S. A., Thuillet, A.-C., Yu, J., Pressoir, G., Romero, S. M., Mitchell, S. E., Doebley, J., Kresovich, S., Goodman, M. M., & Buckler, E. S. (2005). Maize Association population: A High-resolution platform for quantitative trait locus dissection. *The Plant Journal*, *44*(6), 1054–1064. https://doi.org/10.1111/j.1365-313x.2005.02591.x

Hungria, M., et al., *Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil.* Plant and Soil, 2010. **331**(1-2): p. 413-425.

Khan, Z., Rho, H., Firrincieli, A., Hung, S. H., Luna, V., Masciarelli, O., Doty, S. L. (2016). Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Current Plant Biology, 6*, 38-47. doi:10.1016/j.cpb.2016.08.001

Kumara, P. Mohana, et al. "Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective." *Advances in Endophytic Research*, 2013, pp. 177–190., doi:10.1007/978-81-322-1575-2_9.

Lin, F., Jiang, L., Liu, Y., Lv, Y., Dai, H., & Zhao, H. (2014). Genome-wide identification of housekeeping genes in maize. Plant Molecular Biology, 86(4-5), 543-554. doi:10.1007/s11103-014-0246-1

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative pcr and the 2−δδct method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Majeed, A., Muhammad, Z., & Ahmad, H. (2018). Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. *Plant Cell Reports*, *37*(12), 1599–1609. https://doi.org/10.1007/s00299-018-2341-2

Matsumura EE, Secco VA, Moreira RS et al (2015) Composition and activity of endophytic bacterial communities in field-grown maize plants inoculated with Azospirillum brasilense. Ann Microbiol 65:2187–2200

Mcmullen, M. D., et al. "Genetic Properties of the Maize Nested Association Mapping Population." *Science*, vol. 325, no. 5941, 2009, pp. 737–740., doi:10.1126/science.1174320.

Montanez, A., et al., *Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro.* Applied Soil Ecology, 2012. **58**: p. 21-28

Monteiro, Rose Adele, et al. "Herbaspirillum-Plant Interactions: Microscopical, Histological and Molecular Aspects." *Plant and Soil*, vol. 356, no. 1-2, 2012, pp. 175–196., doi:10.1007/s11104-012-1125-7.

Moran N, Sloan D. (2015) "The Hologenome Concept: Helpful or Hollow?" *PLOS Biology*, https://doi.org/10.1371/journal.pbio.1002311

Mousa WK, Shearer CR, Limay-Rios V et al (2015) Bacterial endophytes from wild maize suppress Fusarium graminearum in modern maize and inhibit mycotoxin accumulation. Front Plant Sci 6:805

Naveed, Muhammad, et al. "Increased Drought Stress Resilience of Maize through Endophytic Colonization by Burkholderia Phytofirmans PsJN and Enterobacter Sp. FD17." *Environmental and Experimental Botany*, vol. 97, 2014, pp. 30–39.,

Parnell, J. J., Berka, R., Young, H. A., Sturino, J. M., Kang, Y., Barnhart, D. M., & DiLeo, M. V. (2016). From the lab to The Farm: An industrial perspective of plant Beneficial Microorganisms. *Frontiers in Plant Science, 7*. doi:10.3389/fpls.2016.01110

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ray, D.K., et al., *Yield Trends Are Insufficient to Double Global Crop Production by 2050.* Plos One, 2013. **8**(6).

Ray, Prasun, et al. “A Novel Delivery System for the Root Symbiotic Fungus, Sebacina Vermifera, and Consequent Biomass Enhancement of Low Lignin COMT Switchgrass Lines.” *BioEnergy Research*, vol. 8, no. 3, Dec. 2015, pp. 922–933., doi:10.1007/s12155-015-9636-8.

Ray, Prasun, et al. “Genome Sequence of the Plant Growth Promoting Fungus Serendipita Vermifera Subsp. Bescii: The First Native Strain from North America.” *Phytobiomes Journal*, vol. 2, no. 2, 2018, pp. 62–63., doi:10.1094/pbiomes-04-17-0017-a.

Reddy, G. C. *et al.* Biofertilizers Toward Sustainable Agricultural Development. in *Plant Microbe Symbiosis* (eds. Varma, A., Tripathi, S. & Prasad, R.) 115–128 (Springer International Publishing, 2020). doi:10.1007/978-3-030-36248-5_7.

Rho, H., Hsieh, M., Kandel, S. L., Cantillo, J., Doty, S. L., & Kim, S. (2017). Do endophytes promote growth of host plants under Stress? A meta-analysis on PLANT STRESS mitigation BY ENDOPHYTES. *Microbial Ecology, 75*(2), 407-418. doi:10.1007/s00248-017-1054-3

Riggs, Patrick J., et al. “Enhanced Maize Productivity by Inoculation with Diazotrophic Bacteria.” *Functional Plant Biology*, vol. 28, no. 9, 2001, p. 829., doi:10.1071/pp01045.

Rivas-Franco, Federico, et al. “Effects of a Maize Root Pest and Fungal Pathogen on Entomopathogenic Fungal Rhizosphere Colonization, Endophytism and Induction of Plant Hormones.” *Biological Control*, vol. 150, 2020, p. 104347., doi:10.1016/j.biocontrol.2020.104347.

Rosenblueth, M., Ormeño-Orrillo, E., López-López, A., Rogel, M. A., Reyes-Hernández, B. J., Martínez-Romero, J. C., Reddy, P. M., & Martínez-Romero, E. (2018). Nitrogen Fixation in Cereals. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.01794

Shehata HR, Lyons EM, Jordan KS, Raizada MN (2016) Bacterial endophytes from wild and ancient maize are able to suppress the fungal pathogen Sclerotinia homoeocarpa. J Appl Microbiol 120:756–769

Shehata HR, Raizada MN (2017) A Burkholderia endophyte of the ancient maize landrace Chapalote utilizes c-di-GMP-dependent and independent signaling to suppress diverse plant fungal pathogen targets. FEMS Microbiol Lett 364.

Sobowale AA, Cardwell KF, Odebode AC et al (2007) Persistence of Trichoderma species within maize stem against Fusarium verticillioides. Arch Phytopathol Plant Prot 40:215– 231

Timmusk, S., Behers, L., Muthoni, J., Muraya, A., & Aronsson, A. (2017). Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Science, 8*. doi:10.3389/fpls.2017.00049

Wallace, Jason G., and Georgiana May. "*Endophytes: The Other Maize Genome*." The Maize Genome, 2018, pp. 213–246.,

Walters, William A., et al. "Large-Scale Replicated Field Study of Maize Rhizosphere Identifies Heritable Microbes." *Proceedings of the National Academy of Sciences*, vol. 115, no. 28, 2018, pp. 7368–7373., doi:10.1073/pnas.1800918115.

Wang, Y., Holland, J., & Balint-Kurti, P. (2021). Development and use of a seedling growth retardation assay to quantify and map loci underlying variation in the maize basal defense response. *PhytoFrontiers™*. doi:10.1094/phytofr-12-20-0038-r

Wintermans, P. C., Bakker, P. A., & Pieterse, C. M. (2016). Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. *Plant Molecular Biology*, *90*(6), 623–634. https://doi.org/10.1007/s11103-016-0442-2

Young, Li-Sen, et al. “Endophytic Establishment of the Soil Isolate Burkholderia Sp. CC-Al74 Enhances Growth and P-Utilization Rate in Maize (Zea Mays L.).” *Applied Soil Ecology*, vol. 66, 2013, pp. 40–47., doi:10.1016/j.apsoil.2013.02.001.

Zhang, X., Valdés-López, O., Arellano, C., Stacey, G., & Balint-Kurti, P. (2017). Genetic dissection of the MAIZE (zea Mays L.) mamp response. *Theoretical and Applied Genetics, 130*(6), 1155-1168. doi:10.1007/s00122-017-2876-6

CHAPTER 3

EFFECTS OF INBREEDING ON MICROBIAL COMMUNITY DIVERSITY OF ZEA MAYS[1]

---

[1] Schultz CR, Johnson M, Wallace JG (2023) Effects of Inbreeding on Microbial Community Diversity of Zea mays. Microorganisms 11:879

doi: 10.3390/microorganisms11040879

Abstract:

Heterosis, also known as hybrid vigor, is the basis of modern maize production. The effect of heterosis on maize phenotypes has been studied for decades, but its effect on the maize-associated microbiome is much less characterized. To determine the effect of heterosis on the maize microbiome, we sequenced and compared the bacterial communities of inbred, open pollinated, and hybrid maize. Samples covered three tissue types (Stalk, Root, and Rhizosphere) in two field experiments and one greenhouse experiment. Bacterial diversity was more affected by location and tissue type than genetic background, for both within-sample (alpha) and between-sample (beta) diversity. PERMANOVA analysis similarly showed that tissue type and location had significant effects on the overall community structure, whereas the intra-species genetic background and individual plant genotypes did not. Differential abundance analysis identified only 25 bacterial ASVs that significantly differed between inbred and hybrid maize. Predicted metagenome content was inferred with Picrust2, and it also showed a significantly larger effect of tissue and location than genetic background. Overall, these results indicate that the bacterial communities of inbred and hybrid maize are often more similar than they are different, and that non-genetic effects are generally the largest influences on the maize microbiome.

Keywords: Maize; microbiome; inbreeding; heterosis; genetic variation

1. Introduction

All plants coexist with communities of fungi and bacteria in, on, and around them [46, 195]. These microbes can colonize aboveground surfaces (the phyllosphere), soil near the roots (the rhizosphere), and the interior of plant tissues (the endosphere) [46, 91], and they can significantly contribute to the overall health of the plant [46]. A plant's microbiota—the collection of all microbes associated with it—can benefit the plant by protecting it from pathogens and herbivores [4–6,102], protecting against abiotic stress [47, 77, 198–200], and promoting growth through nutrient acquisition (through nitrogen [N] fixation, phosphate [P] solubilization, siderophore production, etc) [63, 64, 195, 201–203] , and phytohormone production [204–206]. Beneficial endophytes can activate plant immune responses resulting in a level of protection from pathogens [129, 207, 208]. In turn, the host plants affect microbes by changing soil chemistry and secreting signaling compounds [209–212], exuding energy-rich carbon compounds into the rhizosphere [210], and otherwise providing niches for microbes [213].

An active research area in plant-microbe interactions is determining the extent to which plant genetic variation alters the microbial community [195, 214–216]. One motivation for this research is the idea of breeding crops for improved microbial associations [214]. On the inter-species level, it has been shown that host species shapes the bacterial microbiome throughout several niches [100]. Several studies have shown that intra-species host genetics significantly affects microbiome community structure in maize [3, 31, 32, 101,102], rice [164, 217], wheat [218, 219], and other crops [173, 220, 221].

Maize has been a model crop for plant genetics for over 100 years [222], due in a large part to its extensive genetic variation and high economic value (170.7 bushels/acre and $9.2

billion in US exports alone in 2020) [223–225]. Although most commercial maize consists of F1 hybrids [223], most maize microbiome research has been on inbred lines [83, 91, 97, 170], with only a few studies examining the difference between inbred and hybrid maize [93, 94].

F1 hybrids show increased vigor and yield relative to their parents [93], an effect called hybrid vigor or heterosis. Heterosis, which is particularly strong in maize, can manifest as increased growth rate, biomass, stress resistance, and yield [23, 24].

Recently it was shown that field-grown maize displays heterosis in bacterial rhizosphere communities, as well as fungal communities in the rhizosphere and phyllosphere [93]. In addition, heterosis for germination and root biomass was shown, at least in some instances, to depend upon the local microbial community [94]. In this case, heterosis resulted from inbreds performing as well as hybrids in sterile conditions but worse in the presence of microbes. These results indicate that some part of heterosis may be due to hybrids' superior ability to deal with harmful microbes in the environment.

These previous studies focused on the exterior communities of the plant (the rhizosphere and phyllosphere). In this study we sought to characterize how inbreeding and heterosis affects both the interior and exterior bacterial communities of maize by looking at the bacteria of the rhizosphere, root endosphere, and stalk endosphere communities in three differently inbred maize groups (inbreds, F1 hybrids, and open-pollinated varieties). Our primary goals were to (1) characterize the bacterial communities in each compartment for each group, (2) determine aspects of the community that were consistent across them, (3) determine differences in the communities that could be linked to heterosis, and (4) test the hypothesis that hybrid maize may be selecting superior microbial communities.

2. Materials and Methods

2.1. Maize Cultivars

Table 2. Maize genotypes used in the three experiments.

Maize Genotype	GRIN Accession	Genetic Group	Experiment
CML247	PI 692141	Inbred	GH
Mo17xPh207		Hybrid	GH
Mo17	PI 558532	Inbred	GH, Year 2
Reid Yellow Dent	PI 222613	Open Pollinated	GH, Year 1
Ph207	PI 601005	Inbred	GH, Year 1, Year 2
Ph207xB73		Hybrid	GH, Year 1
B73	PI 550473	Inbred	GH, Year 1, Year 2
Oh43	PI 690332	Inbred	GH
B73xCML247		Hybrid	GH
B73xOh43		Hybrid	GH

Mo17xB73		Hybrid	GH
B73xMo17	Ames 19097	Hybrid	GH, Year 2
Hopi_blue	NSL 165817	Open Pollinated	GH
B73xPh207		Hybrid	GH, Year 1
DKC70-27		Hybrid	Year 2
903VIP		Hybrid	Year 2
CML322	PI 690321	Inbred	Year 2
HP301	PI 587131	Inbred	Year 2
Bloody Butcher	Ames 32345	Open Pollinated	Year 1

2.2. Field and Greenhouse Design

Fields were planted in the summer of 2018 (**Year 1**) and 2019 (**Year 2**) at different locations within Iron Horse Research Farm in Watkinsville, Georgia. Plants were grown via standard agronomic practices for the state of Georgia [226]. Our first trial in 2018 consisted of

rhizosphere and stalk samples, there were no root samples for this year. We planted 6 genotypes and sampled 2 plants from each genotype with a single rhizosphere and stalk sample from the same plant [6 Genotypes x 2 Tissues x 2 Biological Reps = 24 Samples]. Our second trial in 2019 consisted of rhizosphere, root, and stalk samples, a single each from the same plant. We used 8 genotypes and 4 reps per genotype [8 Genotypes x 3 Tissues x 4 Biological Reps = 96 Samples]. A randomized complete block design was used in the field and greenhouse.

A single greenhouse experiment was carried out in 2019, looking at rhizosphere, root, and stalk samples within the same plant. For each pot, four seeds were planted in a 5 gallon pot with 90% Professional Growing Mix Fafard 3B/Metro-Mix 830 (Sungro Horticulture) and 10% Vermiculite. Upon emergence pots were thinned down to one plant per pot. Three pots per genotype were grown, and pots were arranged in a randomized block design, with each table in the greenhouse consisting of a block containing all 14 genotypes. [14 Genotypes x 3 Tissues x 3 Biological Reps = 126 Samples].

In total we had 248 samples across the three experiments. Not every genotype was used in all 3 experiments. Table 1 above shows which genotypes were used in each experiment. Supplementary table S5 shows the metadata for each sample, including tissue, experiment, block, number of ASVs, and number of reads.

2.3. Sample Collection and Processing

Plants were harvested in a single day to avoid batch effects. A 10cm section of stalk was cut from the plant 20cm off the ground using sterilized razor blades and gloves. Plants were dug up around the roots, and roots were removed from the center of the root ball and placed into a clean falcon tube for root and rhizosphere samples.

The outer portion of stalks were removed with a sterile razor blade, and the inner tissue (protected from contamination and external microbes) were cut into 1-3mm pieces and loaded into a 2mL conical tube for GenoGrinding (SPEX SamplePrep). Root samples were vortexed on the max setting for 15s in deionized water to separate the rhizosphere from the root. This wash was then centrifuged at 4500g for 10 minutes to prepare the wash for DNA extraction. Roots were then thoroughly cleaned with deionized water to remove any residual rhizosphere. 2-3cm of roots were chopped up with a sterile razor blade and loaded into a 2mL conical tube for GenoGrinding (SPEX SamplePrep).

2.4. DNA Extraction and Sequencing

DNA was extracted with a Quick-DNA Fecal/Soil Microbe 96 Kit (Zymo), following the manufacturer's instructions. 16s rDNA gene amplification was performed using the Earth Microbiome Project 515F(Parada) and 806R(Apprill) primers (Thompson, et.al. 2017) with linkers. Sequences are GTGYCAGCMGCCGCGGTAAGT (515F) and GGACTACNVGGGTWTCTAATCC (806R). Peptide nucleic acids (pPNA and mPNA, to block plastid and mitochondrial amplification, respectively; PNA Bio) were mixed and diluted to 2.5uM each for inclusion in the reaction. The first PCR reaction consisted of 5 μL DNA template, 2 μL of each primer (0.5 μM), 12.5 μL of Hot Start Taq 2X Master Mix (New England Biolabs), 2.5 μL PNA mixture (2.5 μM each), and 1uL of sterile water. The amplification reaction was 95ºC for 45 seconds; twenty cycles of 95ºC for 15 seconds, 78ºC for 10 seconds, 60ºC for 45 seconds, 72ºC for 45 seconds; and finally hold at 4ºC. PCR products were purified with AMPure (Beckman Coulter Life sciences).

Five μL of the first PCR product for each sample was used in the second PCR amplification. The reaction mix consisted of 5 μL first PCR product, 5 μL Nextera i5 and i7

Barcode Primers, 12.5 μL 2x Taq DNA polymerase master mix, and 2.5 μL PNA mix. The second amplification reaction was 95ºC for 45 seconds; 25 cycles of 95ºC for 15 seconds, 78ºC for 10 seconds, 60ºC for 45 seconds, 72ºC for 45 seconds; and finally, 68ºC for 5 minutes followed by hold at 4ºC. The second PCR products were purified using AMPure beads and the cleaned products were eluted in 27uL of sterile water and stored at -20°C until sequencing. Three blanks were used in DNA extraction and library prep. Libraries were sequenced at the Georgia Genomics and Bioinformatics Core on an Illumina MiSeq instrument using one paired-end 250 flowcell. The raw data are available at the NCBI Sequence Read Archive under accession PRJNA924784.

2.5. Bioinformatics

Sequence processing and quality filtering was completed within the QIIME2 version 2019.1 toolbox [181]. Cutadapt [183] was used to trim primers from raw sequences and filter reads that did not reach a Phred score of 26. FastQC was used to visualize read quality [184]. Paired reads were joined with vsearch in QIIME2 [227]. deblur [186] was used to truncate reads to 200 bp. The SILVA 132-99-nb classifier [187] was used to assign taxonomy to ASVs. The original dataset contained 15126 ASVs in 248 samples (excluding blanks). Taxa with no Phylum identity were discarded, as well as ASVs found in blanks, and ambiguous calls. We also removed taxa related to the host, chloroplasts, and mitochondria, as well as reads that were only present once or twice in a sample. Samples with less than 500 reads were removed. ASVs were not agglomerated into OTUs. This left us with 10922 ASVs. For some analysis (like Core ASVs) we only looked at the 938 taxa that were found in all three experiments.

First we compared alpha diversity based on genetic background, tissue, and location. We used Observed ASVs, Shannon, and Simpson index from the phyloseq package [182]. Pairwise

Wilcox tests and Dunn's post-hoc test were used to test for significance with the FSA package [228]. UniFrac distance matrices were generated in QIIME2 for beta diversity and plotted to visually represent sample diversity. To test what variables had the most significant impact on beta diversity, we generated Bray-Curtis distances in phyloseq**,** and then a PERMANOVA was performed using vegan [229]. Alpha and Beta diversity analyses were used on data that was rarefied to 500 reads per sample with our seed set to 18. Differential abundance analysis was used to identify ASVs that occurred in all three experiments that differed between inbred and hybrid maize. DESeq2 [190] was used to fit negative binomial models with an alpha value of 0.001. Core ASVs were defined as present in 50% of samples. UpSet plots were created using the UpSetR [230] package. PiCRUST2 [193] was used to predict functional gene pathways from ASVs using the Kegg Orthology database [194]. Raw KO terms were agglomerated to higher functional pathways, and DESeq2 was used to identify pathways that differed between inbred and hybrid maize compartments, with an alpha value of 0.001. psadd was used to create interactive krona plots of microbiome taxonomy [231, 232]. All bioinformatics scripts and pipelines are available at: https://github.com/wallacelab/paper-schultz-microbiome-2023.

2.6. MiniMaize Inoculation Experiment

Maize lines B73, Mo17, and their F1 hybrid seeds were sterilized via our previously established method [122]. Seeds were surface sterilized with sterile water, bleach, and tween 20 and then placed in a hot water bath. They were then allowed to germinate on Hoagland's agar for seven days to check for contamination. These seeds were then planted in the greenhouse as described above and grown until flowering. Once the silks emerged, stalk sections were sampled from 6 – 12 inches above the soil line. A razor was used to cut a 10 cm x 10 cm square in the

side of mature maize root ball, and roots were removed from the plant to include roots all the way to the center of the pot.

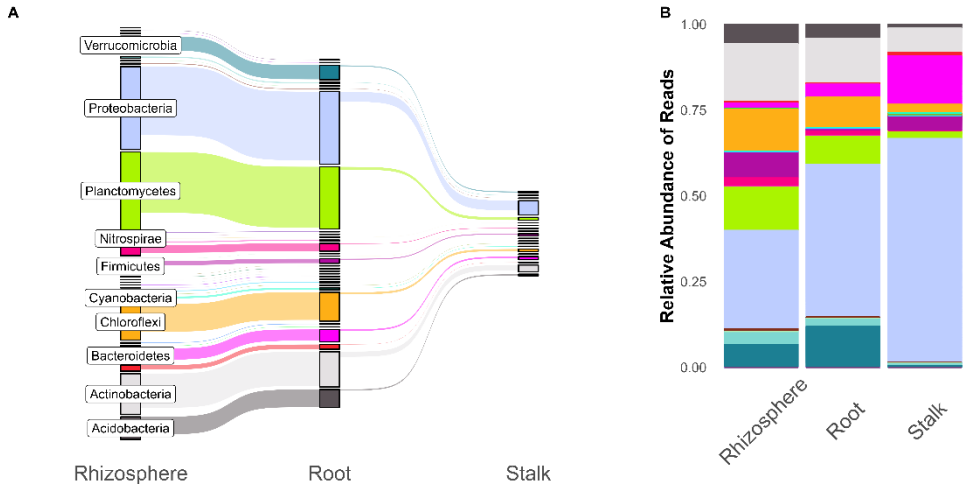
Microbiome extraction was modified from [233, 234]. Stalk samples were cored using a sterilized drill tip. Stalk pulp was placed into a 50mL falcon tube, and filled with 40mLs of MilliQ H_2O, then shaken 50 times and vortexed on max speed for 10 seconds. 30mLs of liquid was decanted into another falcon tube, using the tube cap to exclude large debris. The microbe suspension was centrifuged for 2 minutes at 3,500 RPM to pellet plant debris. 20 mLs of this was filtered through a 2 micrometer Whatman filter. Root samples were placed into a falcon tube without drill tip pulverization, and the same method was used to extract rhizosphere microbiomes.

Sterilized MiniMaize seeds were planted in 2.7L sterilized pots, with autoclave media mixture (same as above). Two sterilized MiniMaize seeds were planted in each pot. Pots were inoculated with 10 mLs of either B73, Mo17, or F1 combined stalk and rhizosphere microbiomes, with 8 pots per treatment. Autoclaved tin foil was placed over the pots for 4 days to ensure no outside microbes were introduced, then plants were thinned to one plant per pot. Plants were allowed to grow for 5 weeks. Shoots were cut at the soil line and placed in brown paper bags. Roots were gently washed of soil and bagged. Above and below ground samples were dried out and weighed.

3. Results

We grew hybrid, inbred, and open pollinated maize lines in two field experiments (2018 and 2019) and one greenhouse experiment. Bacterial microbiomes were extracted from stalks, roots and rhizospheres of the plants, and quantified with QIIME2 and deblur. High-quality reads were retained and classified with SILVA taxonomy classifier. Low-abundance amplicon

sequence variants (ASVs), as well as ASVs associated with mitochondria, chloroplasts, and our blanks, were filtered out. Our final dataset consisted of 241 samples and 10922 ASVs, 938 (9%) of which were present across all three experiments.



(a) (b)

Figure 1. Shared ASVs across plant tissues, compared to relative abundance of reads in plant tissues, colored by phylum.

Throughout the three experiments we found that stalk tissue had lower read depth and fewer associated ASVs compared to the rhizosphere and roots. The rhizosphere had the largest number of ASVs, with a majority of these also found in the roots (Figure 1A). Only a fraction of the microbial community found in the rhizosphere and roots can be found in the stalks, though these shared ASVs accounted for the majority (99%) of stalk reads. While the number of ASVs in a phylum appear to accurately represent the relative abundance of reads in the rhizosphere and root, we see differences in the stalk. The relative abundance of ASVs in the rhizosphere and root relative was roughly in line with the number of unique ASVs. Stalk samples, however, were dominated by Proteobacteria reads (38.9% of stalk ASVs but 66.7% of total read depth). Krona

plots (nested pie chart distributions) of overall community structures, with comparisons for tissue and genetic background, can be found in the Supplemental Materials Dataset S1.

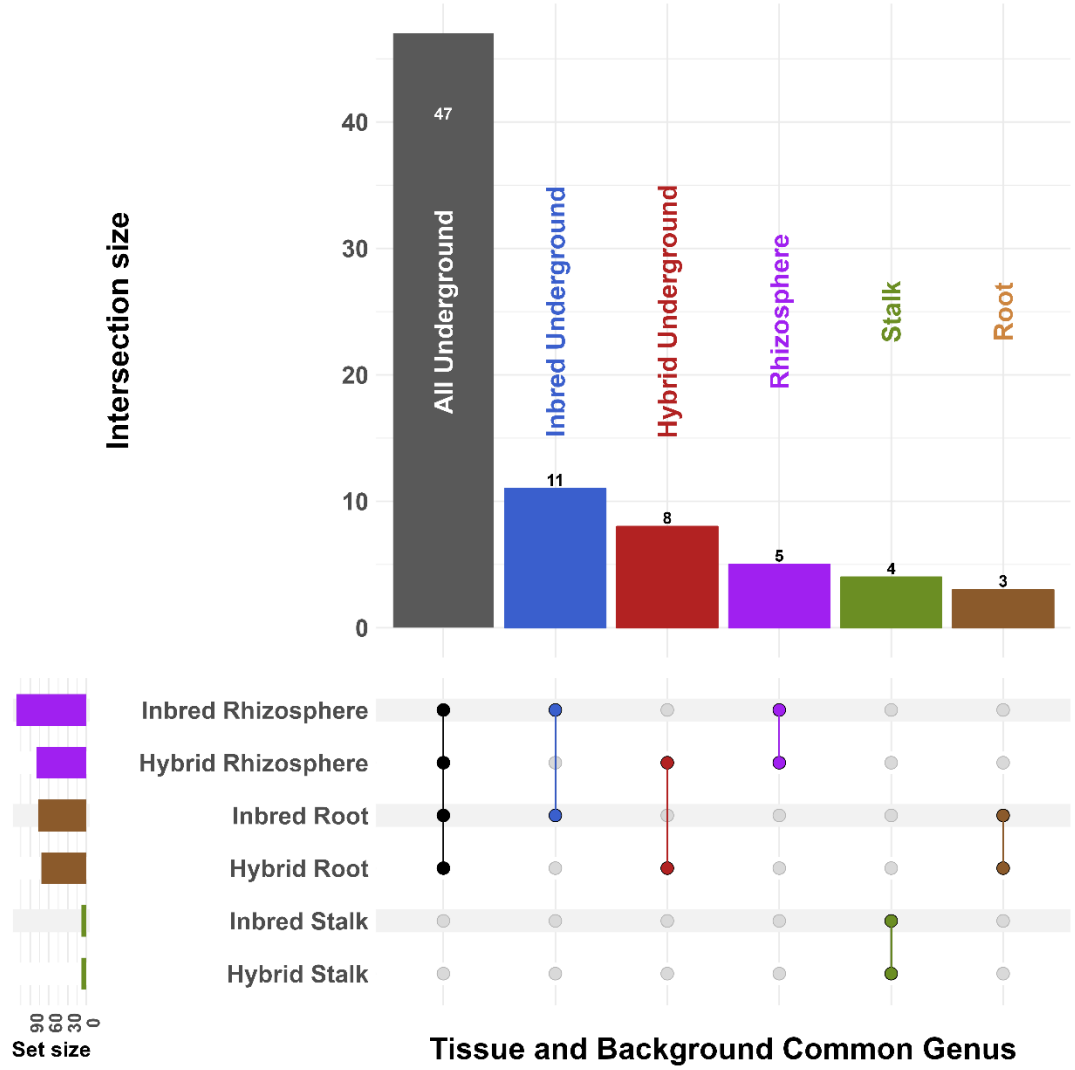


Figure 2. UpSet plot [230] showing intersections of common genera (those found in >50% of samples) based on genetic background and tissue. Intersections with zero counts are not shown. Open pollinated lines share genera in some, but not all of these intersections.

To investigate what taxa were shared by groups of samples, we plotted intersections of common ASVs collapsed at the genus level (Figure 2). We defined common taxa as genera that were found in at least 50% of samples in a group; a table for all taxa and groups can be found in

the Supplemental Materials. Forty-seven genera were shared by at least 50% of samples in inbred and hybrid roots and rhizospheres. 11 genera were shared by inbred roots and rhizospheres but not hybrids, and 8 were shared by hybrids. As a group, the rhizosphere samples contained 5 genera that were not found in the roots, while the roots had 3 genera not found in the rhizosphere. Inbred and hybrid stalk samples shared 4 common microbes that was not found in underground compartments. No genera were shared across all samples and genetic backgrounds. All intersections can be found is Table S1.

Alpha diversity was measured with three common metrics--Observed ASVs, Shannon Entropy, and Hill's q1 [exponential of Shannon Entropy [235] on rarefied data (Figure S1)—and compared using Kruskal-Wallis and Dunn's test. For all tissue types, we find that field samples have higher alpha diversity than their greenhouse counterparts ($p < .001$). Similarly, the root and rhizosphere samples have higher alpha diversity than stalk samples ($p < .001$). Post-hoc tests show that there are no significant differences in alpha diversity comparing inbred, hybrid, or open-pollinated samples across experiments (Table S2).

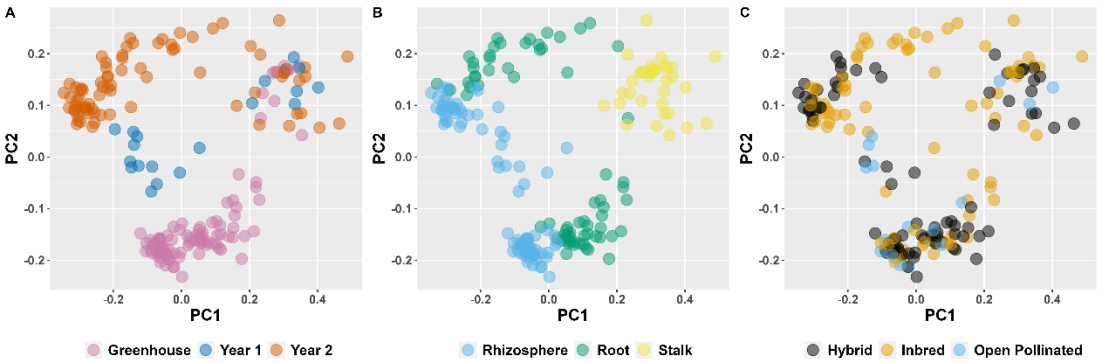


Figure 3. Weighted UniFrac diversity principle coordinates, colorized by experiment (A), tissue type (B), and genetic background (C).

Beta diversity was calculated using the Weighted UniFrac metric [236] (Fig. 3). Samples were most strongly separated based on tissue type, with rhizosphere, root, and stalks strongly

separating from each other. Whether the experiment made a difference depended on the tissue: rhizosphere and root samples were strongly differentiated based on experiment, and stalk samples not at all. Genetic background did not significantly differentiate samples in any compartment. PERMANOVA analysis of Weighted UniFrac distances indicated that experiment and tissue type had the most impact on beta diversity ($p = .001$ and $p = .001$ by Type II ANOVA) (Table S3). Genetic background (inbred/hybrid/open-pollinated) and individual genotype had no significant effect on beta diversity ($p > 0.05$; data not shown).

Table 2. Table of Differentially abundant taxa. DESeq2 was used to compare genetic background, location, and tissue type. Tissue type and location had a much large impact on the number of differentially abundant ASVs than genetic background.

Tissue	Comparison	Number of ASVs
Compare genetic background		
All	Inbred vs Hybrid	61
All	Inbred vs Open Pollinated	76
All	Hybrid vs Open Pollinated	20
Rhizos	Inbred vs Hybrid	2
Rhizos	Inbred vs Open Pollinated	8
Rhizos	Hybrid vs Open Pollinated	5
Roots	Inbred vs Hybrid	11
Roots	Inbred vs Open Pollinated	6
Roots	Hybrid vs Open Pollinated	2
Stalks	Inbred vs Hybrid	14

Stalks	Inbred vs Open Pollinated	7
Stalks	Hybrid vs Open Pollinated	6
Compare locations		
All	Field vs Greenhouse	504
Rhizos	Field vs Greenhouse	192
Roots	Field vs Greenhouse	182
Stalks	Field vs Greenhouse	33
Compare tissues		
-	Stalks vs Rhizos	512
-	Stalks vs Roots	371
-	Roots vs Rhizos	274

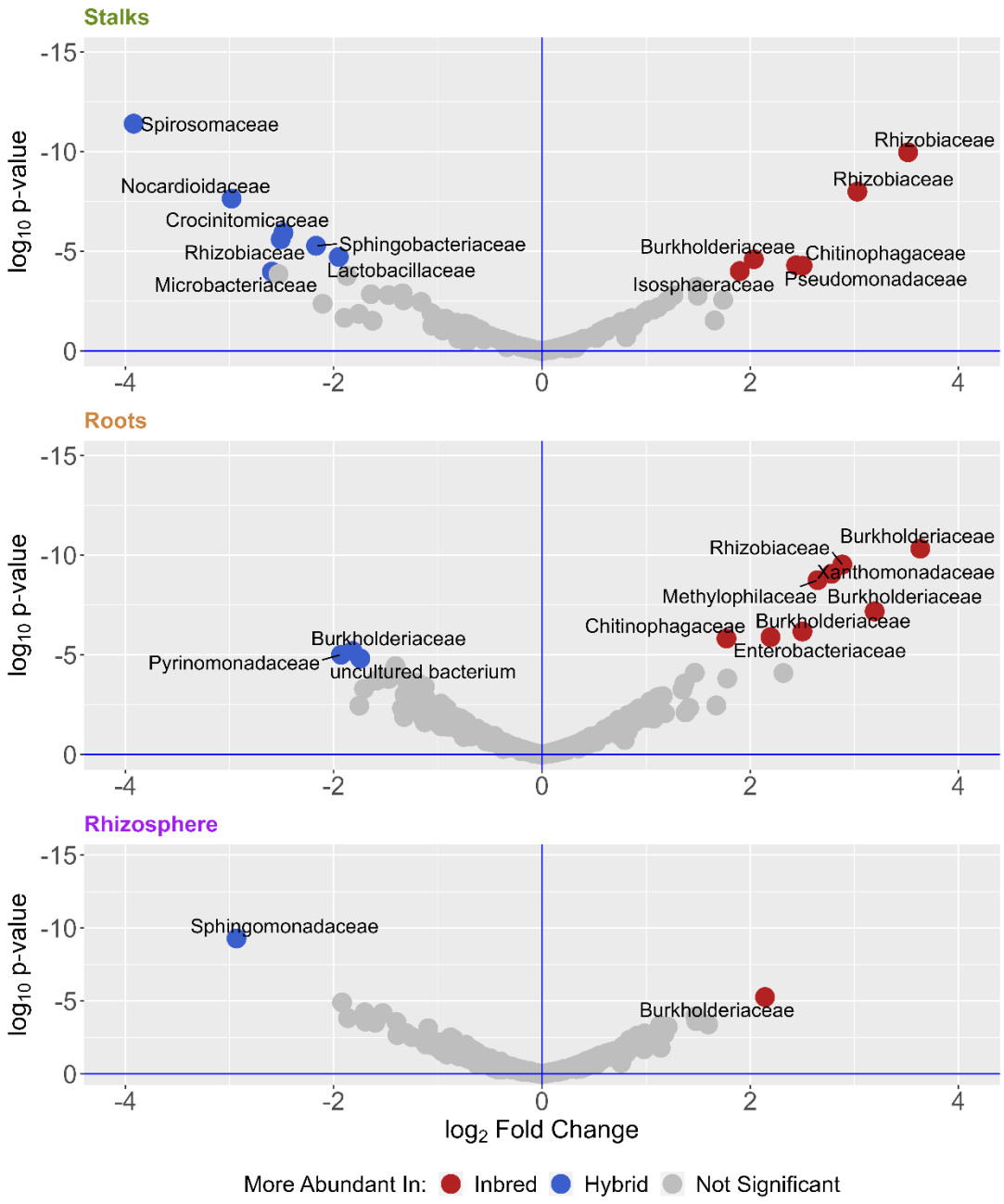


Figure 4. Volcano plots of differentially abundant ASVs. ASVs were more abundant in Inbred (blue) or hybrid (red) determined by DESeq2 with an alpha of .001. Dots represent individual ASVs, which are labeled according to their taxonomic Family. The full list of differentially abundant ASVs is in Supplemental Table 4.

To identify the microbes that are most different between samples, we analyzed differentially abundant microbes using DESeq2 (Table 2 and Fig 4), using the 938 ASVs found in all three experiments. Table 2 shows differential comparisons across tissue type, genetic background, and location. We see that location and tissue type have far more differentially abundant microbes than comparisons of genetic background. Most of these were found in the root and stalks, and many of these were members of the *Burkholderiaceae* and *Rhizobiaceae* families.

Table 3. Differentially abundant PICRUST predicted genomic functions. Agglomerated pathways were grouped based on KEGG pathways before differential abundance was performed (alpha of .001).

Tissue	Comparison	Agglomerated Pathways	Raw Annotations
Compare genetic background			
All	Inbred vs Hybrid	1	261
All	Inbred vs OP	0	846
All	Hybrid vs OP	0	192
Stalks	Inbred vs Hybrid	2	26
Stalks	Inbred vs OP	1	334
Stalks	Hybrid vs OP	0	139
Rhizosphere	Inbred vs Hybrid	0	28
Rhizosphere	Inbred vs OP	0	29
Rhizosphere	Hybrid vs OP	0	67
Root	Inbred vs Hybrid	34	638
Root	Inbred vs OP	0	170

Root	Hybrid vs OP	0	21
Compare locations			
All	Greenhouse vs Field	48	2994
Stalks	Greenhouse vs Field	13	724
Root	Greenhouse vs Field	108	4489
Rhizosphere	Greenhouse vs Field	176	4966
Compare tissues			
-	Stalks vs Roots	139	5261
-	Stalks vs Rhizosphere	165	5901
-	Rhizosphere vs Root	71	3587

Previous work has shown that metabolic functions are a better characterization of microbial communities than 16s-based taxonomy [83, 174, 236].We used PICRUST2 [193] to predict community functional capacity from the 16s sequencing, and DESeq2 to compare differential abundance for comparisons across genetic background, tissue, and location (Table 3). Functional differences were much larger between tissue types and locations than genetic background. When comparing inbred and hybrid tissues, while each compartment had some individually different metabolic functions, only the roots maintained multiple significant differences when individual functional annotations were grouped into metabolic pathways (Table 3). Inbred roots had an increase in predicted gene groups related to metabolism and molecular degradation, while hybrid maize roots had an increase in groups related to carbon transport, electron transfer carriers, and photosynthesis (Figure S2).

Since hybrid maize is generally more fit than inbred maize, we hypothesized that hybrid maize would cultivate a more optimal microbiome. To test this, we grew the inbred lines B73 and Mo17, and their F1 hybrid to maturity from surface-sterilized seeds germinated in vitro. These plants were grown to flowering in the greenhouse, and were allowed to recruit microbiomes from non-sterile potting mix. Fast-Flowering Mini-Maize [238] was surface sterilized and germinated *in vitro*, then inoculated with filtered bacterial microbiomes harvested from stalks and rhizospheres of mature B73, Mo17, their F1 Hybrid, or an autoclaved control. After 28 days, roots and shoots were harvested, dried, and weighed. No significant differences occurred below-ground for the separate inoculation groups. Inoculation with microbiomes from B73 ($p = 0 .048$) and the F1 hybrid ($p = 0.037$) resulted in a small but significant decrease in shoot biomass relative to control (Figure S3). No other differences were significant.

4. Discussion

Our results show that inbreeding has a small but significant effect on maize microbial communities. These effects however, are much smaller than the effect that environment and tissue compartment have on microbiome makeup and predicted function. One limitation of this study is that due to logistical constraints, not all genotypes were present in all three experiments. These results have several implications for maize-microbe interactions.

First, we showed that root communities were very similar to the rhizosphere soil, while only a fraction of these bacteria can be found in stalk tissue (Figure 1). This may be due to a combination of strong filters as bacteria travel up the plant [51, 81], or a larger effect of seed-transmitted microbes in the stalks compared to the roots [51]. Its been shown that endophytes can travel and persist in different tissues in maize [101]. Stalk samples had fewer ASVs overall and lower reads, and but had a similar sized common microbiome when compared to root and

rhizosphere (Figure 2). Although shared taxa were significantly different based on tissues, there were no such differences when comparing inbred and hybrid maize.

Similarly, there were no significant differences in alpha diversity measures based on inbreeding, but location and tissue had large affects (Figure S1). This is similar to the results of Wagner et al. [93], who also did not find significant differences in alpha diversity between inbred and hybrid maize. We found alpha diversity to be higher belowground than above ground, and we found field microbial communities to have higher alpha diversity than greenhouse communities. A similar pattern held for beta diversity, where tissue and experiment had larger impacts than inbreeding, as shown by both PCoA plots and PERMANOVA (Figure 3). These results add to the emerging collection of data that (1) maize tissues have different and distinct microbiomes [83, 91, 170], and (2) environment has a larger impact on plant microbiome assembly than intra-species genetics [80, 83, 91].

When directly comparing inbred versus hybrid communities, we identified 11 differentially abundant ASVs in the roots, 14 the stalks, and 2 in the rhizosphere (Figure 4). This represent a very small number of the ASVs in this dataset, and is much smaller than the hundreds of differential ASVs indicated by other characteristics (Table 2). Most of these differences were found in the roots and stalks, this may indicate the effect of inbreeding may be most pronounced there, compared to the rhizosphere. Perhaps not surprisingly, many of these ASVs belong to groups known to include plant-associated and plant-beneficial bacteria, such as *Rhizobiaceae* and *Burkholderiacea.* Endophytes from both of these families have been shown to have growth promotion potential in maize [64, 78, 239, 240]. We also identified a species of the genus *Pantoea*, which is known to associate with plants [127, 241–243], as well as a species of the

genus *Sphingomonas*, which has been shown to promote growth and can play a role in phytoremediation [244].

Although predicting metabolic capacity from 16s data is less precise than actual metagenomics data, prior work has shown that metabolomics data supports PICRUST2 predictions [245]. It has been found that metabolic functions of the microbiome community may be more important than the taxonomic identity of the individual microbes [174, 236, 245], and Wallace et al [83], found predicted metabolic pathways to be more heritable (meaning affected by host genetics) than individual microbes in the maize leaf microbiome. In our data, we found multiple predicted differences in gene functions in maize roots--but not the stalk or rhizosphere--when comparing inbred and hybrid maize. Similar to taxonomic differences (Table 2), the predicted metabolic differences were much smaller when comparing intra-species inbreeding than comparing locations and tissues (Table 3). Inbred roots had an increase in predicted gene groups related to acetyl-coA activity, molecular degradation of organic compounds, and increases in plant-microbe signaling; while hybrid maize roots had an increase in groups related to ribosome synthesis, energy, and photosynthetic functions (Figure S2). Taxa contributions to these gene groups (Dataset S3) were taxonomically diverse, and most were not found to be differentially abundant in inbred or hybrid roots, highlighting the importance of comparing community function. It has been shown that plant associated bacteria genomes encode more carbohydrate metabolism functions, as well diverse functions related to organic compound metabolism, and plant protein mimicry [247]. Many of these functions were found in bacteria cultured from diverse maize seeds [101].

Inbred plants showed increases in acetyl-coA-transferases and dehydrogenases, implying there may be a difference in anaerobic metabolism, even though there were no known acetogens

[248] in our differentially abundant ASVs. There were also increases in enzymes related to organic compounds that may be produced by the plant or bacteria. Plants produces exudes and can respond to stress through volatile organic compounds (VOCs), while plant microbiomes can also produce a wide array of VOCs that can impact plant health. These VOCs are incredibly varied and include alcohols, aldehydes, acids, esters, fatty acids, and hydrocarbons (reviewed in [143, 249]). We found predicted functions related to esters [143], hydrogen peroxide (plant VOC) ligases [250, 251], and phosphonates, which can protect bacteria and make phosphate available to the plant [252–254]. These increases may indicate differential communication between the plant and the microbiome, or the microbiome's reaction to plant stress response. If heterosis is partly microbe-dependent [94], a thorough investigation of these signaling pathways may reveal mechanisms influencing heterosis in maize.

Hybrid plants had an increase in pathways related to ribosome biogenesis (including synthases and GTPase A), energy production, and photosynthesis/carbon metabolism. Several pathways related to energy production and storage were found increased in hybrid maize. These included oxidative phosphorylation, heme uptake proteins, and electron transport. Outside of these energy related pathways, a number of energy and protein pathways related to phototrophic capabilities were differentially abundant in hybrid maize root bacterial communities. These include: plastocyanin [255], cytochrome c subunit [255], and photo reaction center m [256, 257]; all related to photosynthetic energy production in photo-reactive bacteria [257]. Cyanobacteria ASVs, found within these maize microbiomes (Figure 1), had little effect on the differences in functional gene prediction. Differences in photosynthetic protein and photosynthesis gene groups were due to a wide range of phylums, including Proteobacteria, Chloroflexi, Fimicutes, and Acidobacteria (Dataset S3), all shown to have phototrophic capabilities [258]. These taxa were

not identified as differentially abundant (Figure 4). This analysis indicates that hybrid plants may be selecting for microbial communities that have increased energy production or phototrophic potential. Actual metagenomic data, instead of 16s-based predictions would allow us the parse apart real metabolic differences between the two communities, whereas this analysis focuses on predicted potential of the communities.

When we inoculated MiniMaize with the filtered bacteria microbiome from B73, Mo17, and their F1 hybrid, we saw minor differences in biomass above ground but not below (Figure S3). Specifically, we saw B73 and F1 inoculates decreased plant biomass compared to control inoculated plants, while Mo17 had no significant effect. We hypothesized that hybrid maize may harbor more beneficial bacteria, but our results do not support this conclusion. In a previous study Kaeppler et al. [259] tested the mycorrhizal responsiveness of 28 inbred lines maize lines. It was found that B73 and Mo17 had the largest differences in response to mycorrhizae. Our study used bacterial filtrates, indicating these two lines may consistently respond differently to microbial inoculation.

Our results are somewhat similar with those of Wagner et al., who showed significant differences in inbred and hybrid maize microbial-communities in the field [93], and that at least some effects of heterosis may be due to hybrids' superior ability to deal with harmful microbes [97].

Our primary goals were to (1) characterize the bacterial communities in each compartment for each group, (2) determine aspects of the community that were consistent across groups, (3) determine differences in the communities that could be linked to heterosis, and (4) test the hypothesis that hybrid maize may be selecting superior microbial communities.

We found that (1) bacterial communities in the roots and rhizospheres were very similar to each other, and stalk communities only have a small portion of these bacteria, (2) common bacteria were found across compartments, regardless of inbreeding, (3) intra-species heterosis played a much smaller role on microbial community diversity, composition, and function compared to tissue compartment or location, (4) in a small greenhouse experiment we found hybrid microbiomes failed to benefit inoculated MiniMaize.

5. Conclusions

Modern maize breeding is built on heterosis, and with many new biologicals coming to market, it is paramount to understand how these microbes interact with intra-species plant genetics. The literature has shown that host species plays a large role in shaping niche microbiome, our data show that inbreeding has small and significant effects on taxa and function in maize microbial communities, however, these effects pale in comparison to the effect environment and tissue type have on community composition. With environment playing such a large role in shaping bacterial communities, investigating the extent that maize can recruit specific taxa/function would shed light on how much potential there is to use the plant itself to alter its microbiome. Further work is needed to examine how maize genetic diversity and the environment shape community function. Understanding how the microbiome interacts with maize genetic diversity will allow breeders and scientists to make better use of microbial communities for more sustainable crop production.

References

1. Bennetzen JL, Hake SC (2009) Handbook of Maize: Genetics and Genomics. Springer Science & Business Media

2. Schnable JC (2015) Genome Evolution in Maize: From Genomes Back to Genes. Annual Review of Plant Biology 66:329–343

3. Brown NJ, Parsley K, Hibberd JM (2005) The future of C4 research – maize, Flaveria or Cleome? Trends in Plant Science 10:215–221

4. Hake S, Ross-Ibarra J (2015) Genetic, evolutionary and plant breeding insights from the domestication of maize. eLife 4:e05861

5. Gewin V (2003) Genetically Modified Corn— Environmental Benefits and Risks. PLoS Biol 1:e8

6. Nutrition C for FS and A (2023) GMO Crops, Animal Food, and Beyond. FDA

7. Schnable PS, Ware D, Fulton RS, et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

8. Hufford MB, Seetharam AS, Woodhouse MR, et al (2021) De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. Science 373:655–662

9. Corn Explorer. https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0440000. Accessed 11 May 2023

10. (2019) What Are the World's Most Important Staple Foods? In: WorldAtlas. https://www.worldatlas.com/articles/most-important-staple-foods-in-the-world.html. Accessed 11 May 2023

11. World of Corn 2021. https://ncga.com/world-of-corn-iframe#corn-usage-by-segment. Accessed 11 May 2023

12. (2021) From feed to fuel: This is how corn is used around the world. In: World Economic Forum. https://www.weforum.org/agenda/2021/06/corn-industries-sustainability-food-prices/. Accessed 11 May 2023

13. Nannas NJ, Dawe RK (2015) Genetic and Genomic Toolbox of Zea mays. Genetics 199:655–669

14. Lawrence CJ, Harper LC, Schaeffer ML, Sen TZ, Seigfried TE, Campbell DA (2008) MaizeGDB: The Maize Model Organism Database for Basic, Translational, and Applied Research. International Journal of Plant Genomics 2008:e496957

15. Tello-Ruiz MK, Jaiswal P, Ware D (2022) Gramene: A Resource for Comparative Analysis of Plants Genomes and Pathways. Methods Mol Biol 2443:101–131

16. Krishnakumar V, Choi Y, Beck E, Wu Q, Luo A, Sylvester A, Jackson D, Chan AP (2015) A maize database resource that captures tissue-specific and subcellular-localized gene expression, via fluorescent tags and confocal imaging (Maize Cell Genomics Database). Plant Cell Physiol 56:e12

17. Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde R-A, Coalter R, Barkan A (2010) Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. Plant J 63:167–177

18. Mcclintock B, Ware D, Fulton RS, et al (1929) CHROMOSOME MORPHOLOGY IN ZEA MAYS. Science 69:629–629

19. Creighton HB, McClintock B (1931) A Correlation of Cytological and Genetical Crossing-Over in Zea Mays. Proceedings of the National Academy of Sciences 17:492–497

20. McClintock B (1950) The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences 36:344–355

21. Candela H, Hake S (2008) The art and design of genetic screens: maize. Nat Rev Genet 9:192–203

22. Lisch D (2012) Regulation of transposable elements in maize. Current Opinion in Plant Biology 15:511–516

23. Duvick DN (1984) Genetic Contributions to Yield Gains of U.S. Hybrid Maize, 1930 to 1980. In: Genetic Contributions to Yield Gains of Five Major Crop Plants. John Wiley & Sons, Ltd, pp 15–47

24. Duvick DN (1999) Heterosis: Feeding People and Protecting Natural Resources. In: Genetics and Exploitation of Heterosis in Crops. John Wiley & Sons, Ltd, pp 19–29

25. Lai J, Li R, Xu X, et al (2010) Genome-wide patterns of genetic variation among elite maize inbred lines. Nat Genet 42:1027–1030

26. Xiao Y, Jiang S, Cheng Q, et al (2021) The genetic mechanism of heterosis utilization in maize improvement. Genome Biol 22:148

27. Baldauf JA, Marcon C, Lithio A, Vedder L, Altrogge L, Piepho H-P, Schoof H, Nettleton D, Hochholdinger F (2018) Single-Parent Expression Is a General Mechanism Driving Extensive Complementation of Non-syntenic Genes in Maize Hybrids. Current Biology 28:431-437.e4

28. Paschold A, Jia Y, Marcon C, et al (2012) Complementation contributes to transcriptome complexity in maize (Zea mays L.) hybrids relative to their inbred parents. Genome Res 22:2445–2454

29. Zhou P, Hirsch CN, Briggs SP, Springer NM (2019) Dynamic Patterns of Gene Expression Additivity and Regulatory Variation throughout Maize Development. Molecular Plant 12:410–425

30. Guo B, Chen Y, Li C, et al (2014) Maize (Zea mays L.) seedling leaf nuclear proteome and differentially expressed proteins between a hybrid and its parental lines. PROTEOMICS 14:1071–1087

31. Guo B, Chen Y, Zhang G, et al (2013) Comparative Proteomic Analysis of Embryos between a Maize Hybrid and Its Parental Lines during Early Stages of Seed Germination. PLOS ONE 8:e65867

32. Hunter RB, Kannenberg LW (1971) ISOZYME CHARACTERIZATION OF CORN (Zea mays) INBREDS AND ITS RELATIONSHIP TO SINGLE CROSS HYBRID PERFORMANCE. Can J Genet Cytol 13:649–655

33. Heidrich-Sobrinho E, Cordeiro AR (1975) Codominant isoenzymic alleles as markers of genetic diversity correlated with heterosis in maize (Zea mays). Theoret Appl Genetics 46:197–199

34. Xing J, Sun Q, Ni Z (2016) Proteomic patterns associated with heterosis. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1864:908–915

35. Wu X, Liu Y, Zhang Y, Gu R (2021) Advances in Research on the Mechanism of Heterosis in Plants. Frontiers in Plant Science 12:

36. Rockenbach MF, Corrêa CCG, Heringer AS, Freitas ILJ, Santa-Catarina C, Amaral-Júnior AT do, Silveira V (2018) Differentially abundant proteins associated with heterosis in the primary roots of popcorn. PLOS ONE 13:e0197114

37. Li Z, Zhu A, Song Q, Chen HY, Harmon FG, Chen ZJ (2020) Temporal Regulation of the Metabolome and Proteome in Photosynthetic and Photorespiratory Pathways Contributes to Maize Heterosis. The Plant Cell 32:3706–3722

38. Biostimulants Market, Global Industry Size Forecast [Latest]. In: MarketsandMarkets. https://www.marketsandmarkets.com/Market-Reports/biostimulant-market-1081.html. Accessed 11 May 2023

39. Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim S-H (2018) Do Endophytes Promote Growth of Host Plants Under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes. Microb Ecol 75:407–418

40. Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV (2016) From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms. Frontiers in Plant Science 7:

41. Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson AC (2017) Perspectives and challenges of microbial application for crop improvement. Frontiers in Plant Science 8:49

42. Ganeshan S, Kim SH, Vujanovic V (2021) Scaling-up production of plant endophytes in bioreactors: concepts, challenges and perspectives. Bioresources and Bioprocessing 2021 8:1 8:1–16

43. Jack CN, Petipas RH, Cheeke TE, Rowland JL, Friesen ML (2021) Microbial Inoculants: Silver Bullet or Microbial Jurassic Park? Trends in Microbiology 29:299–308

44. Aprahamian AM, Lulow ME, Major MR, Balazs KR, Treseder KK, Maltz MR (2016) Arbuscular mycorrhizal inoculation in coastal sage scrub restoration. Botany 94:493–499

45. Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF (2020) Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148:107874

46. Wallace JG, May G (2018) Endophytes: The Other Maize Genome. In: Bennetzen J, Flint-Garcia S, Hirsch C, Tuberosa R (eds) The Maize Genome. Springer International Publishing, Cham, pp 213–246

47. Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. Environmental and Experimental Botany 97:30–39

48. Roesch LFW, Camargo FAO, Bento FM, Triplett EW (2008) Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. Plant Soil 302:91–104

49. Schultz CR, Johnson M, Wallace JG (2023) Effects of Inbreeding on Microbial Community Diversity of Zea mays. Microorganisms 11:879

50. Johnston-Monje D, Raizada MN (2011) Conservation and Diversity of Seed Associated Endophytes in Zea across Boundaries of Evolution, Ethnography and Ecology. PLoS ONE 6:e20396

51. Johnston-Monje D, Gutiérrez JP, Lopez-Lavalle LAB (2021) Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes. Front Microbiol 12:737616

52. Liu Y, Zuo S, Xu L, Zou Y, Song W (2012) Study on diversity of endophytic bacterial communities in seeds of hybrid maize and their parental lines. Arch Microbiol 194:1001–1012

53. Schardl CL (2001) Epichloë festucae and Related Mutualistic Symbionts of Grasses. Fungal Genetics and Biology 33:69–82

54. Guerre P, Guerre, Philippe (2015) Ergot Alkaloids Produced by Endophytic Fungi of the Genus Epichloë. Toxins 7:773–790

55. Cagnano G, Lenk I, Roulund N, Jensen CS, Cox MP, Asp T (2020) Mycelial biomass and concentration of loline alkaloids driven by complex population structure in Epichloë uncinata and meadow fescue (Schedonorus pratensis). Mycologia 112:474–490

56. Johnston-Monje D, Lundberg DS, Lazarovits G, Reis VM, Raizada MN (2016) Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant and Soil 405:337–355

57. Monteiro RA, Schmidt MA, Baura VA de, Balsanelli E, Wassem R, Yates MG, Randi MAF, Pedrosa FO, Souza EM de (2008) Early colonization pattern of maize (Zea mays L. Poales, Poaceae) roots by Herbaspirillum seropidicae, Burkholderia WP9, and Serendipita bescii seropedicae (Burkholderiales, Oxalobacteraceae). Genetics and Molecular Biology 31:932–937

58. Roncato-Maccari LDB, Ramos HJO, Pedrosa FO, Alquini Y, Chubatsu LS, Yates MG, Rigo LU, Steffens MBR, Souza EM (2003) Endophytic Herbaspirillum seropedicae expresses nif genes in gramineous plants. FEMS Microbiology Ecology 45:39–47

59. Lamb TG, Tonkyn DW, Kluepfel DA (1996) Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. Can J Microbiol 42:1112–1120

60. Bressan W, Borges MT (2004) Delivery methods for introducing endophytic bacteria into maize. BioControl 49:315–322

61. Baldotto MA, Baldotto LEB, Santana RB, Marciano CR (2012) Initial performance of maize in response to NPK fertilization combined with Herbaspirillum seropedicae. Rev Ceres 59:841–849

62. Canellas LP, Balmori DM, Médici LO, Aguiar NO, Campostrini E, Rosa RCC, Façanha AR, Olivares FL (2013) A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (Zea mays L.). Plant and Soil 366:119–132

63. Matsumura EE, Secco VA, Moreira RS, Santos OJAP dos, Hungria M, Oliveira ALM de (2015) Composition and activity of endophytic bacterial communities in field-grown maize plants inoculated with Azospirillum brasilense. Annals of Microbiology 65:2187–2200

64. Young L-S, Hameed A, Peng S-Y, Shan Y-H, Wu S-P (2013) Endophytic establishment of the soil isolate Burkholderia sp. CC-Al74 enhances growth and P-utilization rate in maize (Zea mays L.). Applied Soil Ecology 66:40–47

65. Akhtar SS, Andersen MN, Naveed M, Zahir ZA, Liu F (2015) Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. Functional Plant Biol 42:770–781

66. Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic Azospirillum in the alleviation of drought effects in maize. Botany 87:455–462

67. Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. Plant Soil 331:413–425

68. Sobowale AA, Cardwell KF, Odebode AC, Bandyopadhyay R, Jonathan SG (2007) Persistence of Trichoderma species within maize stem against Fusarium verticillioides. Archives of Phytopathology and Plant Protection 40:215–231

69. Mousa WK, Shearer CR, Limay-Rios V, Zhou T, Raizada MN (2015) Bacterial endophytes from wild maize suppress Fusarium graminearum in modern maize and inhibit mycotoxin accumulation. Frontiers in Plant Science 6:

70. Shehata HR, Lyons EM, Jordan KS, Raizada MN (2016) Bacterial endophytes from wild and ancient maize are able to suppress the fungal pathogen Sclerotinia homoeocarpa. Journal of Applied Microbiology 120:756–769

71. Shehata HR, Raizada MN (2017) A Burkholderia endophyte of the ancient maize landrace Chapalote utilizes c-di-GMP-dependent and independent signaling to suppress diverse plant fungal pathogen targets. FEMS Microbiology Letters 364:fnx138

72. Santos F, Peñaflor MFGV, Paré PW, Sanches PA, Kamiya AC, Tonelli M, Nardi C, Bento JMS (2014) A Novel Interaction between Plant-Beneficial Rhizobacteria and Roots: Colonization Induces Corn Resistance against the Root Herbivore Diabrotica speciosa. PLOS ONE 9:e113280

73. Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E, Larsen J (2018) The root endophytic fungus Trichoderma atroviride induces foliar herbivory resistance in maize plants. Applied Soil Ecology 124:45–53

74. Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proceedings of the National Academy of Sciences of the United States of America 114:E2450–E2459

75. 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

76. Passera A, Follador A, Morandi S, et al (2021) Bacterial Communities in the Embryo of Maize Landraces: Relation with Susceptibility to Fusarium Ear Rot. Microorganisms 9:2388

77. Castiglioni P, Warner D, Bensen RJ, et al (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiol 147:446–455

78. Beirinckx S, Viaene T, Haegeman A, et al (2020) Tapping into the maize root microbiome to identify bacteria that promote growth under chilling conditions. Microbiome 8:54

79. Singh R, Goodwin SB (2022) Exploring the Corn Microbiome: A Detailed Review on Current Knowledge, Techniques, and Future Directions. PhytoFrontiers^{TM} 2:158–175

80. Yang Y, Wang N, Guo X, Zhang Y, Ye B (2017) Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLOS ONE 12:e0178425

81. Johnston-Monje D, Gutiérrez JP, Becerra Lopez-Lavalle LA (2022) Stochastic Inoculum, Biotic Filtering and Species-Specific Seed Transmission Shape the Rare Microbiome of Plants. Life (Basel) 12:1372

82. Microbial life in the phyllosphere | Nature Reviews Microbiology. https://www.nature.com/articles/nrmicro2910. Accessed 17 May 2023

83. Wallace JG, Kremling KA, Kovar LL, Buckler ES (2018) Quantitative genetics of the maize leaf microbiome. Phytobiomes Journal 2:208–224

84. Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. PLOS ONE 8:e56329

85. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C, Vorholt JA (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proceedings of the National Academy of Sciences 106:16428–16433

86. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

87. Brusamarello-Santos LC, Gilard F, Brulé L, Quilleré I, Gourion B, Ratet P, Souza EM de, Lea PJ, Hirel B (2017) Metabolic profiling of two maize (Zea mays L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria Herbaspirillum seropedicae and Azospirillum brasilense. PLOS ONE 12:e0174576

88. Montañez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro. Applied Soil Ecology 58:21–28

89. da Silva KJ, de Armas RD, Soares CRFS, Ogliari JB (2016) Communities of endophytic microorganisms in different developmental stages from a local variety as well as

transgenic and conventional isogenic hybrids of maize. World J Microbiol Biotechnol 32:189

90. McMullen MD, Kresovich S, Villeda HS, et al (2009) Genetic Properties of the Maize Nested Association Mapping Population. Science 325:737–740

91. 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 110:6548–6553

92. Flint-Garcia SA, Thuillet A-C, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM, Buckler ES (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. The Plant Journal 44:1054–1064

93. Wagner MR, Roberts JH, Balint-Kurti P, Holland JB (2020) Heterosis of leaf and rhizosphere microbiomes in field-grown maize. New Phytologist 228:1055–1069

94. Wagner MR, Tang C, Salvato F, et al (2021) Microbe-dependent heterosis in maize. Proc Natl Acad Sci USA 118:e2021965118

95. Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE (2010) Maize Leaf Epiphytic Bacteria Diversity Patterns Are Genetically Correlated with Resistance to Fungal Pathogen Infection. MPMI 23:473–484

96. Manching HC, Balint-Kurti PJ, Stapleton AE (2014) Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by nitrogen fertilization. Frontiers in Plant Science 5:

97. Wagner MR, Busby PE, Balint-Kurti P (2020) Analysis of leaf microbiome composition of near-isogenic maize lines differing in broad-spectrum disease resistance. New Phytologist 225:2152–2165

98. Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Applied Soil Ecology 8:11–18

99. Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil 232:167–180

100. Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14:2539–2551

101. Dematheis F, Zimmerling U, Flocco C, Kurtz B, Vidal S, Kropf S, Smalla K (2012) Multitrophic Interaction in the Rhizosphere of Maize: Root Feeding of Western Corn Rootworm Larvae Alters the Microbial Community Composition. PLOS ONE 7:e37288

102. Emmett BD, Buckley DH, Drinkwater LE (2020) Plant growth rate and nitrogen uptake shape rhizosphere bacterial community composition and activity in an agricultural field. New Phytologist 225:960–973

103. Pan JJ, Baumgarten AM, May G (2008) Effects of host plant environment and Ustilago maydis infection on the fungal endophyte community of maize (Zea mays). New Phytologist 178:147–156

104. Johnston-Monje D, Mousa WK, Lazarovits G, Raizada MN (2014) Impact of swapping soils on the endophytic bacterial communities of pre-domesticated, ancient and modern maize. BMC Plant Biol 14:233

105. Kadivar H, Stapleton AE (2003) Ultraviolet Radiation Alters Maize Phyllosphere Bacterial Diversity. Microb Ecol 45:353–361

106. Wolters B, Jacquiod S, Sørensen SJ, Widyasari-Mehta A, Bech TB, Kreuzig R, Smalla K (2018) Bulk soil and maize rhizosphere resistance genes, mobile genetic elements and microbial communities are differently impacted by organic and inorganic fertilization. FEMS Microbiology Ecology 94:fiy027

107. Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize Endophytic Bacterial Diversity as Affected by Soil Cultivation History. Frontiers in Microbiology 9:

108. Wattenburger CJ, Halverson LJ, Hofmockel KS (2019) Agricultural Management Affects Root-Associated Microbiome Recruitment Over Maize Development. Phytobiomes Journal 3:260–272

109. Benitez M-S, Osborne SL, Lehman RM (2017) Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. Sci Rep 7:15709

110. Maarastawi SA, Frindte K, Linnartz M, Knief C (2018) Crop Rotation and Straw Application Impact Microbial Communities in Italian and Philippine Soils and the Rhizosphere of Zea mays. Frontiers in Microbiology 9:

111. Chen Z, Zheng Y, Ding C, Ren X, Yuan J, Sun F, Li Y (2017) Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. Ecotoxicology and Environmental Safety 145:111–118

112. Islam F, Yasmeen T, Arif MS, Riaz M, Shahzad SM, Imran Q, Ali I (2016) Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. Plant Physiology and Biochemistry 108:456–467

113. Panitlertumpai N, Nakbanpote W, Sangdee A, Boonapatcharoen N, Prasad MNV (2018) Potentially toxic elements to maize in agricultural soils—microbial approach of rhizospheric and bulk soils and phytoaccumulation. Environ Sci Pollut Res 25:23954–23972

114. Vigliotta G, Matrella S, Cicatelli A, Guarino F, Castiglione S (2016) Effects of heavy metals and chelants on phytoremediation capacity and on rhizobacterial communities of maize. Journal of Environmental Management 179:93–102

115. Wu W, Wu J, Liu X, Chen X, Wu Y, Yu S (2017) Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure. Ecotoxicology and Environmental Safety 143:322–329

116. Sillen WMA, Thijs S, Abbamondi GR, De La Torre Roche R, Weyens N, White JC, Vangronsveld J (2020) Nanoparticle treatment of maize analyzed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis. Microbiome 8:127

117. de Menezes AB, Richardson AE, Thrall PH (2017) Linking fungal–bacterial co-occurrences to soil ecosystem function. Current Opinion in Microbiology 37:135–141

118. van Overbeek LS, Saikkonen K (2016) Impact of Bacterial–Fungal Interactions on the Colonization of the Endosphere. Trends in Plant Science 21:230–242

119. Deynze AV, Zamora P, Delaux P-M, et al (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLOS Biology 16:e2006352

120. Amicucci MJ, Galermo AG, Guerrero A, et al (2019) A Strategy for Structural Elucidation of Polysaccharides: Elucidation of a Maize Mucilage that Harbors Diazotrophic Bacteria. Analytical Chemistry acs.analchem.9b00789

121. Bennett AB, Pankievicz VCS, Ané J-M (2020) A Model for Nitrogen Fixation in Cereal Crops. Trends in Plant Science 25:226–235

122. Schultz C, Brantley K, Wallace J (2021) The Role of Genetic Variation in Maize Response to Beneficial Endophytes. 2021.11.03.467096

123. Craig L, Forest KT, Maier B (2019) Type IV pili: dynamics, biophysics and functional consequences. Nat Rev Microbiol 17:429–440

124. Pedrosa FO, Monteiro RA, Wassem R, et al (2011) Genome of Herbaspirillum seropedicae Strain SmR1, a Specialized Diazotrophic Endophyte of Tropical Grasses. PLOS Genetics 7:e1002064

125. Monteiro RA, Balsanelli E, Tuleski T, et al (2012) Genomic comparison of the endophyte Herbaspirillum seropedicae SmR1 and the phytopathogen Herbaspirillum rubrisubalbicans M1 by suppressive subtractive hybridization and partial genome sequencing. FEMS Microbiology Ecology 80:441–451

126. Straub D, Rothballer M, Hartmann A, Ludewig U (2013) The genome of the endophytic bacterium H. frisingense GSF30T identifies diverse strategies in the Herbaspirillum genus to interact with plants. Frontiers in Microbiology 4:

127. Sheibani-Tezerji R, Naveed M, Jehl M-A, Sessitsch A, Rattei T, Mitter B (2015) The genomes of closely related Pantoea ananatis maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. Frontiers in Microbiology 6:

128. Sessitsch A, Hardoim P, Döring J, et al (2012) Functional Characteristics of an Endophyte Community Colonizing Rice Roots as Revealed by Metagenomic Analysis. MPMI 25:28–36

129. Van Wees SCM, Van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448

130. Zhang X, Valdés-López O, Arellano C, Stacey G, Balint-Kurti P (2017) Genetic dissection of the maize (Zea mays L.) MAMP response. Theoretical and Applied Genetics 130:1155–1168

131. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced Systemic Resistance by Beneficial Microbes. Annual Review of Phytopathology 52:347–375

132. Djonović S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM (2007) A Proteinaceous Elicitor Sm1 from the Beneficial Fungus Trichoderma virens Is Required for Induced Systemic Resistance in Maize. Plant Physiology 145:875–889

133. Güimil S, Chang H-S, Zhu T, et al (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proceedings of the National Academy of Sciences 102:8066–8070

134. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth - Artursson - 2006 - Environmental Microbiology - Wiley Online Library. https://ami-journals.onlinelibrary.wiley.com/doi/full/10.1111/j.1462-2920.2005.00942.x. Accessed 16 May 2023

135. Zachow C, Tilcher R, Berg G (2008) Sugar Beet-Associated Bacterial and Fungal Communities Show a High Indigenous Antagonistic Potential Against Plant Pathogens. Microb Ecol 55:119–129

136. de Boer W, de Ridder-Duine AS, Klein Gunnewiek PJA, Smant W, Van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. Soil Biology and Biochemistry 40:1542–1544

137. Uroz S, Heinonsalo J (2008) Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. FEMS Microbiology Ecology 65:271–278

138. Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial–fungal interactions. Nat Rev Microbiol 8:340–349

139. Notz R, Maurhofer M, Dubach H, Haas D, Défago G (2002) Fusaric Acid-Producing Strains of Fusarium oxysporum Alter 2,4-Diacetylphloroglucinol Biosynthetic Gene Expression in Pseudomonas fluorescens CHA0 In Vitro and in the Rhizosphere of Wheat. Applied and Environmental Microbiology 68:2229–2235

140. Jäderlund L, Arthurson V, Granhall U, Jansson JK (2008) Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations. FEMS Microbiology Letters 287:174–180

141. Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA (2014) Newly identified helper bacteria stimulate ectomycorrhizal formation in Populus. Frontiers in Plant Science 5:

142. Afkhami ME, Stinchcombe JR (2016) Multiple mutualist effects on genomewide expression in the tripartite association between Medicago truncatula, nitrogen-fixing bacteria and mycorrhizal fungi. Molecular Ecology 25:4946–4962

143. Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y (2021) Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Frontiers in Plant Science 12:

144. Rani A, Rana A, Dhaka RK, Singh AP, Chahar M, Singh S, Nain L, Singh KP, Minz D (2023) Bacterial volatile organic compounds as biopesticides, growth promoters and plant-defense elicitors: Current understanding and future scope. Biotechnology Advances 63:108078

145. Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q (2018) Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23:762

146. Zhou H, Hua J, Li H, Song X, Luo S Structurally diverse specialized metabolites of maize and their extensive biological functions. Journal of Cellular Physiology. https://doi.org/10.1002/jcp.30955

147. Yu J, Tu X, Huang AC (2022) Functions and biosynthesis of plant signaling metabolites mediating plant–microbe interactions. Nat Prod Rep 39:1393–1422

148. Adedeji AA, Babalola OO (2020) Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. Planta 252:61

149. Tyc Olaf, Song C, Dickschat JS, Vos M, Garbeva P (2017) The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. Trends in Microbiology 25:280–292

150. Hu L, Robert CAM, Cadot S, et al (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun 9:2738

151. Kudjordjie EN, Sapkota R, Steffensen SK, Fomsgaard IS, Nicolaisen M (2019) Maize synthesized benzoxazinoids affect the host associated microbiome. Microbiome 7:59

152. Cotton TEA, Pétriacq P, Cameron DD, Meselmani MA, Schwarzenbacher R, Rolfe SA, Ton J (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J 13:1647–1658

153. Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Tawab A, Khan N (2020) Yucasin and cinnamic acid inhibit IAA and flavonoids biosynthesis minimizing interaction between maize and endophyte Aspergillus nomius. Symbiosis 81:149–160

154. D'alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J, Turlings TCJ (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant, Cell & Environment 37:813–826

155. Tenorio-Salgado S, Tinoco R, Vazquez-Duhalt R, Caballero-Mellado J, Perez-Rueda E (2013) Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4:236–243

156. Xie S, Liu J, Gu S, Chen X, Jiang H, Ding T (2020) Antifungal activity of volatile compounds produced by endophytic Bacillus subtilis DZSY21 against Curvularia lunata. Annals of Microbiology 70:2

157. Lee KK, Kim H, Lee Y-H (2022) Cross-kingdom co-occurrence networks in the plant microbiome: Importance and ecological interpretations. Frontiers in Microbiology 13:

158. Banerjee S, Schlaeppi K, van der Heijden MGA (2018) Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol 16:567–576

159. Heijden MGA van der, Hartmann M (2016) Networking in the Plant Microbiome. PLOS Biology 14:e1002378

160. Jiang D, Armour CR, Hu C, Mei M, Tian C, Sharpton TJ, Jiang Y (2019) Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities. Frontiers in Genetics 10:

161. Hong J, Karaoz U, de Valpine P, Fithian W (2022) To rarefy or not to rarefy: robustness and efficiency trade-offs of rarefying microbiome data. Bioinformatics btac127

162. Hünninghaus M, Dibbern D, Kramer S, Koller R, Pausch J, Schloter-Hai B, Urich T, Kandeler E, Bonkowski M, Lueders T (2019) Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize. Soil Biology and Biochemistry 134:122–130

163. Kim H, Lee KK, Jeon J, Harris WA, Lee Y-H (2020) Domestication of Oryza species eco-evolutionarily shapes bacterial and fungal communities in rice seed. Microbiome 8:20

164. Huang J, Li Y, Ma Y, Li Y, Jin J, Lian T (2022) The rhizospheric microbiome becomes more diverse with maize domestication and genetic improvement. Journal of Integrative Agriculture 21:1188–1202

165. Xiong C, Singh BK, He J-Z, et al (2021) Plant developmental stage drives the differentiation in ecological role of the maize microbiome. Microbiome 2021 9:1 9:1–15

166. Gardner CM, Gerhard WA, Redfern LK, Gunsch CK (2022) Evaluation of developing maize microbiomes and associations among nitrogen cyclers and key fungal taxa. Microbiology 168:001155

167. Toju H, Peay KG, Yamamichi M, et al (2018) Core microbiomes for sustainable agroecosystems. Nature Plants 4:247–257

168. Shade A, Stopnisek N (2019) Abundance-occupancy distributions to prioritize plant core microbiome membership. Current Opinion in Microbiology 49:50–58

169. Walters WA, Jin Z, Youngblut N, et al (2018) Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences of the United States of America 115:7368–7373

170. 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 112:E911–E920

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

172. Lundberg DS, Lebeis SL, Paredes SH, et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90

173. Chen L, Xin X, Zhang J, Redmile-gordon M, Nie G, Wang Q (2019) Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize. Pedosphere 29:360–373

174. Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y (2017) Let the Core Microbiota Be Functional. Trends in Plant Science 22:583–595

175. Ranjard L, Dequiedt S, Chemidlin Prévost-Bouré N, et al (2013) Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. Nat Commun 4:1434

176. Young JM, Kuykendall LD, Martínez-Romero E, Kerr A, Sawada H (2001) A revision of Rhizobium Frank 1889, with an emended description of the genus, and the inclusion of all species of Agrobacterium Conn 1942 and Allorhizobium undicola de Lajudie et al. 1998 as new combinations: Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola and R. vitis. International Journal of Systematic and Evolutionary Microbiology 51:89–103

177. Galloway-Peña J, Hanson B (2020) Tools for Analysis of the Microbiome. Dig Dis Sci 65:674–685

178. Su X, Yan X, Tsai C-L (2012) Linear regression. WIREs Computational Statistics 4:275–294

179. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46

180. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857

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

182. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

183. Andrews, S (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data [Online].

184. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

185. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns | mSystems. https://journals.asm.org/doi/10.1128/mSystems.00191-16. Accessed 5 May 2022

186. 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–D596

187. Nilsson RH, Larsson K-H, Taylor AFS, et al (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264

188. Badri M, Kurtz ZD, Bonneau R, Müller CL (2020) Shrinkage improves estimation of microbial associations under different normalization methods. NAR Genomics and Bioinformatics 2:lqaa100

189. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:1–21

190. McKnight DT, Huerlimann R, Bower DS, Schwarzkopf L, Alford RA, Zenger KR (2019) Methods for normalizing microbiome data: An ecological perspective. Methods in Ecology and Evolution 10:389–400

191. Fang H, Huang C, Zhao H, Deng M (2015) CCLasso: correlation inference for compositional data through Lasso. Bioinformatics 31:3172–3180

192. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688

193. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457-462

194. 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 Advanced Research. https://doi.org/10.1016/J.JARE.2019.03.004

195. Innerebner G, Knief C, Vorholt JA (2011) Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System ▿. Appl Environ Microbiol 77:3202–3210

196. Rojas X, Guo J, Leff JW, McNear DH, Fierer N, McCulley RL (2016) Infection with a Shoot-Specific Fungal Endophyte (Epichloë) Alters Tall Fescue Soil Microbial Communities. Microbial Ecology 72:197–206

197. Akhtar SAS, Andersen MBN, Naveed MD, Zahir ZDA, Liu FA Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. https://doi.org/10.1071/FP15054

198. Jochum MD, McWilliams KL, Pierson EA, Jo YK (2019) Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. PLoS ONE 14:e0225933

199. Naylor D, Degraaf S, Purdom E, Coleman-Derr D (2017) Drought and host selection influence bacterial community dynamics in the grass root microbiome. ISME Journal 11:2691–2704

200. Baldotto LEB, Olivares FL, Bressan-Smith R (2011) Structural interaction between GFP-labeled diazotrophic endophytic bacterium Herbaspirillum seropedicae RAM10 and pineapple plantlets “Vitória.” Brazilian Journal of Microbiology 42:114–125

201. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

202. Caradonia F, Ronga D, Catellani M, Azevedo CVG, Terrazas RA, Robertson-Albertyn S, Francia E, Bulgarelli D (2019) Nitrogen Fertilisers Shape The Composition And Predicted Functions Of The Microbiota Of Field-Grown Tomato Plants. bioRxiv 672162

203. Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Letters in Applied Microbiology 48:542–547

204. Rivas-Franco F, Hampton JG, Narciso J, Rostás M, Wessman P, Saville DJ, Jackson TA, Glare TR (2020) Effects of a maize root pest and fungal pathogen on entomopathogenic fungal rhizosphere colonization, endophytism and induction of plant hormones. Biological Control 150:104347

205. Patel M, Singh S, Vasanthakumari M, Naik S, Manjunatha B, Jadhav S, Ravikanth G, K N G, Shaanker R (2013) Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective. In: Advances in Endophytic Research. pp 177–190

206. Oukala N, Aissat K, Pastor V (2021) Bacterial Endophytes: The Hidden Actor in Plant Immune Responses against Biotic Stress. Plants (Basel) 10:1012

207. Ma Y (2017) Beneficial Bacteria for Disease Suppression and Plant Growth Promotion. In: Plant-Microbe Interactions in Agro-Ecological Perspectives. pp 513–529

208. Zhang W, Mason GA (2022) Modulating the rhizosphere microbiome by altering the cocktail of root secretions. Plant Physiology 188:12–13

209. Sun H, Jiang S, Jiang C, Wu C, Gao M, Wang Q (2021) A review of root exudates and rhizosphere microbiome for crop production. Environmental Science and Pollution Research 2021 1–14

210. Wu L, Kobayashi Y, Wasaki J, Koyama H (2018) Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Science and Plant Nutrition 64:697–704

211. Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480

212. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

213. Bergelson J, Brachi B, Roux F, Vailleau F (2021) Assessing the potential to harness the microbiome through plant genetics. Current Opinion in Biotechnology 70:167–173

214. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A (2020) Plant microbiome–an account of the factors that shape community composition and diversity. Current Plant Biology 23:100161

215. French E, Kaplan I, Iyer-Pascuzzi A, Nakatsu CH, Enders L (2021) Emerging strategies for precision microbiome management in diverse agroecosystems. Nature Plants 7:256–267

216. Roman-reyna V, Pinili D, Borjaa FN, et al (2019) The Rice Leaf Microbiome Has a Conserved Community Structure Controlled by Complex Host-Microbe Interactions. https://doi.org/10.2139/ssrn.3382544

217. Wipf HML, Coleman-Derr D (2021) Evaluating domestication and ploidy effects on the assembly of the wheat bacterial microbiome. PLOS ONE 16:e0248030

218. Gholizadeh S, Mohammadi SA, Salekdeh GH (2022) Changes in root microbiome during wheat evolution. BMC Microbiology 22:64

219. Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, Tschaplinski TJ, Schadt CW (2019) Rhizosphere microbiomes diverge among Populus trichocarpa plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome 7:76

220. Cordovez V, Rotoni C, Dini-Andreote F, Oyserman B, Carrión VJ, Raaijmakers JM (2021) Successive plant growth amplifies genotype-specific assembly of the tomato rhizosphere microbiome. Science of The Total Environment 772:144825

221. Bennetzen JL, Hake S (eds) (2009) Handbook of Maize. https://doi.org/10.1007/978-0-387-77863-1

222. corn : USDA ARS. https://www.ars.usda.gov/oc/timeline/corn/. Accessed 8 Dec 2021

223. Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/commodities/corn. Accessed 28 May 2022

224. USDA - National Agricultural Statistics Service - Publications. https://www.nass.usda.gov/Publications/. Accessed 28 May 2022

225. Dewey,Lee, Nolan, Reagan (2018) A Guide to Corn Production in Georgia 2018. University of Georgia Cooperative Extension

226. Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

227. Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis.

228. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR,, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package.

229. UpSetR: an R package for the visualization of intersecting sets and their properties | Bioinformatics | Oxford Academic. https://academic.oup.com/bioinformatics/article/33/18/2938/3884387. Accessed 5 May 2022

230. Pauvert C (2021) psadd: Additions to phyloseq package for microbiome analysis.

231. Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12:1–10

232. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9:980–989

233. Mueller UG, Juenger TE, Kardish MR, Carlson AL, Burns K, Smith C, Marais DLD Artificial Selection on Microbiomes to Confer Salt-Tolerance to Plants Artificial Microbiome-Selection to Engineer Microbiomes That Confer Salt-Tolerance to Plants 1 2. https://doi.org/10.1101/081521

234. Chiu C-H, Jost L, Chao A (2014) Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers. Ecological Monographs 84:21–44

235. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. ISME J 5:169–172

236. Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M (2016) High taxonomic variability despite stable functional structure across microbial communities. Nat Ecol Evol 1:1–12

237. McCaw ME, Wallace JG, Albert PS, Buckler ES, Birchler JA (2016) Fast-Flowering Mini-Maize: Seed to Seed in 60 Days. Genetics 204:35–42

238. Mehboob I, Zahir Z, Arshad M, Tanveer A, Khalid M (2012) Comparative effectiveness of different rhizobium sp. for improving growth and yield of maize (Zea mays L.). Soil and Environment 31:37–46

239. Estrada GA, Baldani VLD, Oliveira DM de, Urquiaga S, Baldani JI (2013) Selection of phosphate-solubilizing diazotrophic Herbaspirillum and Burkholderia strains and their effect on rice crop yield and nutrient uptake. Plant and Soil 369:115–129

240. Doni F, Suhaimi NSM, Irawan B, Mohamed Z, Mispan MS (2021) Associations of Pantoea with Rice Plants: As Friends or Foes? Agriculture 11:1278

241. Quecine MC, Araújo WL, Rossetto PB, Ferreira A, Tsui S, Lacava PT, Mondin M, Azevedo JL, Pizzirani-Kleiner AA (2012) Sugarcane Growth Promotion by the Endophytic Bacterium Pantoea agglomerans 33.1. Applied and Environmental Microbiology 78:7511–7518

242. Soluch R, Hülter NF, Romero Picazo D, Özkurt E, Stukenbrock EH, Dagan T (2021) Colonization dynamics of Pantoea agglomerans in the wheat root habitat. Environmental Microbiology 23:2260–2273

243. Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (Zea mays) by bacteria: Implications for energy plant biomass production and phytoremediation. Journal of Environmental Management 103:58–64

244. Piacentino D, Grant-Beurmann S, Vizioli C, et al (2021) Gut microbiome and metabolome in a non-human primate model of chronic excessive alcohol drinking. Transl Psychiatry 11:609

245. Coles VJ, Stukel MR, Brooks MT, et al (2017) Ocean biogeochemistry modeled with emergent trait-based genomics. Science 358:1149–1154

246. Levy A, Salas Gonzalez I, Mittelviefhaus M, et al (2017) Genomic features of bacterial adaptation to plants. Nat Genet 50:138–150

247. Bengelsdorf FR, Beck MH, Erz C, Hoffmeister S, Karl MM, Riegler P, Wirth S, Poehlein A, Weuster-Botz D, Dürre P (2018) Chapter Four - Bacterial Anaerobic Synthesis Gas (Syngas) and CO2+H2 Fermentation. In: Sariaslani S, Gadd GM (eds) Advances in Applied Microbiology. Academic Press, pp 143–221

248. Brilli F, Loreto F, Baccelli I (2019) Exploiting Plant Volatile Organic Compounds (VOCs) in Agriculture to Improve Sustainable Defense Strategies and Productivity of Crops. Frontiers in Plant Science 10:

249. Scala A, Allmann S, Mirabella R, Haring MA, Schuurink RC (2013) Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. International Journal of Molecular Sciences 14:17781–17811

250. Stolterfoht H, Rinnofner C, Winkler M, Pichler H (2019) Recombinant Lipoxygenases and Hydroperoxide Lyases for the Synthesis of Green Leaf Volatiles. J Agric Food Chem 67:13367–13392

251. Mosquito S, Bertani I, Licastro D, Compant S, Myers MP, Hinarejos E, Levy A, Venturi V (2020) In Planta Colonization and Role of T6SS in Two Rice Kosakonia Endophytes. MPMI 33:349–363

252. Kamat SS, Raushel FM (2013) The enzymatic conversion of phosphonates to phosphate by bacteria. Current Opinion in Chemical Biology 17:589–596

253. Diversity and abundance of phosphonate biosynthetic genes in nature | PNAS. https://www.pnas.org/doi/10.1073/pnas.1315107110. Accessed 31 May 2022

254. Navarro JA, Durán RV, De la Rosa MA, Hervás M (2005) Respiratory cytochrome c oxidase can be efficiently reduced by the photosynthetic redox proteins cytochrome c6 and plastocyanin in cyanobacteria. FEBS Letters 579:3565–3568

255. Deisenhofer J, Michel H (1993) 17 - Three-Dimensional Structure of the Reaction Center of Rhodopseudomonas viridis. In: Deisenhofer J, Norris JR (eds) Photosynthetic Reaction Center. Academic Press, San Diego, pp 541–558

256. Cardona T (2015) A fresh look at the evolution and diversification of photochemical reaction centers. Photosynth Res 126:111–134

257. Fleischman D (2012) Chapter 51 - Photosynthesis. In: Sperelakis N (ed) Cell Physiology Source Book (Fourth Edition). Academic Press, San Diego, pp 909–924

258. Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF (2000) Variation among Maize Inbred Lines and Detection of Quantitative Trait Loci for Growth at Low Phosphorus and Responsiveness to Arbuscular Mycorrhizal Fungi. Crop Science 40:358–364

259. Whitaker BK, Vaughan MM, McCormick SP (2022) Biocontrol Impacts on Wheat Physiology and Fusarium Head Blight Outcomes Are Bacterial Endophyte Strain and Cultivar Specific. Phytobiomes Journal PBIOMES-08-22-0056-R

260. Abdul Rahman NSN, Abdul Hamid NW, Nadarajah K (2021) Effects of Abiotic Stress on Soil Microbiome. International Journal of Molecular Sciences 22:9036

261. Trivedi P, Batista BD, Bazany KE, Singh BK (2022) Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytologist 234:1951–1959

262. Tiziani R, Miras-Moreno B, Malacrinò A, Vescio R, Lucini L, Mimmo T, Cesco S, Sorgonà A (2022) Drought, heat, and their combination impact the root exudation patterns and rhizosphere microbiome in maize roots. Environmental and Experimental Botany 203:105071

263. Swift JF, Kolp MR, Carmichael A, Ford NE, Hansen PM, Sikes BA, Kleiner M, Wagner MR (2023) Legacy effects of precipitation and land use impact maize growth and microbiome assembly under drought stress. 2023.04.11.536405

264. Methe BA, Hiltbrand D, Roach J, Xu W, Gordon SG, Goodner BW, Stapleton AE (2020) Functional gene categories differentiate maize leaf drought-related microbial epiphytic communities. PLOS ONE 15:e0237493

265. Vescio R, Malacrinò A, Bennett AE, Sorgonà A (2021) Single and combined abiotic stressors affect maize rhizosphere bacterial microbiota. Rhizosphere 17:100318

266. Mukhtar S, Mirza BS, Mehnaz S, Mirza MS, Mclean J, Malik KA (2018) Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. World J Microbiol Biotechnol 34:136

267. Guan Y, Jiang N, Wu Y, Yang Z, Bello A, Yang W (2021) Disentangling the role of salinity-sodicity in shaping soil microbiome along a natural saline-sodic gradient. Science of The Total Environment 765:142738

268. Yuan Y, Brunel C, van Kleunen M, Li J, Jin Z (2019) Salinity-induced changes in the rhizosphere microbiome improve salt tolerance of Hibiscus hamabo. Plant Soil 443:525–537

269. Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Applied Soil Ecology 107:324–333

270. Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host & Microbe 22:142–155

271. Babalola OO, Fadiji AE, Enagbonma BJ, Alori ET, Ayilara MS, Ayangbenro AS (2020) The Nexus Between Plant and Plant Microbiome: Revelation of the Networking Strategies. Frontiers in Microbiology 11:

272. Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Gutjahr C, Falter-Braun P (2019) Systems Biology of Plant-Microbiome Interactions. Molecular Plant 12:804–821

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

274. 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

275. Zheng W, Zhao Z, Gong Q, Zhai B, Li Z (2018) Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil. Soil Biology and Biochemistry 125:54–63

276. Ritter CD, Forster D, Azevedo JAR, Antonelli A, Nilsson RH, Trujillo ME, Dunthorn M (2021) Assessing Biotic and Abiotic Interactions of Microorganisms in Amazonia through Co-Occurrence Networks and DNA Metabarcoding. Microb Ecol 82:746–760

277. Chen M, He S, Li J, Hu W, Ma Y, Wu L, Gang G (2019) Co-occurrence patterns between bacterial and fungal communities in response to a vegetation gradient in a freshwater wetland. Can J Microbiol 65:722–737

278. (2023) Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/data/commodities/corn. Accessed 27 Apr 2023

279. Ling N, Zhu C, Xue C, Chen H, Duan Y, Peng C, Guo S, Shen Q (2016) Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biology and Biochemistry 99:137–149

280. Wu X, Hu H, Li S, Zhao J, Li J, Zhang G, Li G, Xiu W (2022) Chemical fertilizer reduction with organic material amendments alters co-occurrence network patterns of bacterium-fungus-nematode communities under the wheat–maize rotation regime. Plant Soil 473:605–623

281. Bazany KE, Wang J-T, Delgado-Baquerizo M, Singh BK, Trivedi P (2022) Water deficit affects inter-kingdom microbial connections in plant rhizosphere. Environmental Microbiology 24:3722–3734

282. Cobo-Díaz JF, Baroncelli R, Le Floch G, Picot A (2019) Combined Metabarcoding and Co-occurrence Network Analysis to Profile the Bacterial, Fungal and Fusarium Communities and Their Interactions in Maize Stalks. Frontiers in Microbiology 10:

283. Rogers AR, Holland JB (2022) Environment-specific genomic prediction ability in maize using environmental covariates depends on environmental similarity to training data. G3 Genes|Genomes|Genetics 12:jkab440

284. Kick DR, Wallace JG, Schnable JC, et al (2022) Yield Prediction Through Integration of Genetic, Environment, and Management Data Through Deep Learning. 2022.07.29.502051

285. Westhues CC, Simianer H, Beissinger TM (2022) learnMET: an R package to apply machine learning methods for genomic prediction using multi-environment trial data. G3 Genes|Genomes|Genetics 12:jkac226

286. Can High-Resolution Satellite Multispectral Imagery Be Used to Phenotype Canopy Traits and Yield Potential in Field Conditions? https://doi.org/10.13031/trans.14197. Accessed 26 May 2023

287. ImageBreed: Open-access plant breeding web–database for image-based phenotyping - Morales - 2020 - The Plant Phenome Journal - Wiley Online Library. https://acsess.onlinelibrary.wiley.com/doi/full/10.1002/ppj2.20004. Accessed 26 May 2023

288. Wiesner-Hanks T, Wu H, Stewart E, DeChant C, Kaczmar N, Lipson H, Gore MA, Nelson RJ (2019) Millimeter-Level Plant Disease Detection From Aerial Photographs via Deep Learning and Crowdsourced Data. Frontiers in Plant Science 10:

289. Bai G, Ge Y, Scoby D, Leavitt B, Stoerger V, Kirchgessner N, Irmak S, Graef G, Schnable J, Awada T (2019) NU-Spidercam: A large-scale, cable-driven, integrated sensing and robotic system for advanced phenotyping, remote sensing, and agronomic research. Computers and Electronics in Agriculture 160:71–81

290. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522

291. Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18:1403–1414

292. Walters W, Hyde ER, Berg-Lyons D, et al (2016) Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for

Microbial Community Surveys. mSystems. https://doi.org/10.1128/MSYSTEMS.00009-15

293. Xie J, Fu Y, Jiang D, Li G, Huang J, Li B, Hsiang T, Peng Y (2008) Intergeneric transfer of ribosomal genes between two fungi. BMC Evolutionary Biology 8:87

294. Free Weather API | Visual Crossing. https://www.visualcrossing.com/weather-api. Accessed 23 May 2023

295. Peschel S, Müller CL, von Mutius E, Boulesteix A-L, Depner M (2021) NetCoMi: network construction and comparison for microbiome data in R. Briefings in Bioinformatics 22:bbaa290

296. Jun W, Barahona M, Yue-Jin T, Hong-Zhong D (2010) Natural Connectivity of Complex Networks. Chinese Phys Lett 27:078902

297. J Fox, S Weisberg (2019) An R Companion to Applied Regression, 3rd Edition. Sage

298. Smith SD (2019) phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. Journal of Open Source Software 4:1442

299. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 57:289–300

300. Bairoch A (2000) The ENZYME database in 2000. Nucleic Acids Res 28:304–305

301. Simon HM, Jahn CE, Bergerud LT, Sliwinski MK, Weimer PJ, Willis DK, Goodman RM (2005) Cultivation of Mesophilic Soil Crenarchaeotes in Enrichment Cultures from Plant Roots. Appl Environ Microbiol 71:4751–4760

302. Madegwa YM, Uchida Y (2021) Land use and season drive changes in soil microbial communities and related functions in agricultural soils. Environmental DNA 3:1214–1228

303. Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

304. Bahram M, Hildebrand F, Forslund SK, et al (2018) Structure and function of the global topsoil microbiome. Nature 560:233–237

305. Bérdy J (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot 65:385–395

306. Li X, Garbeva P, Liu X, klein Gunnewiek PJA, Clocchiatti A, Hundscheid MPJ, Wang X, de Boer W (2020) Volatile-mediated antagonism of soil bacterial communities against fungi. Environmental Microbiology 22:1025–1035

307. Koopman N, Remijas L, Seppen J, Setlow P, Brul S (2022) Mechanisms and Applications of Bacterial Sporulation and Germination in the Intestine. Int J Mol Sci 23:3405

308. Chamkhi I, El Omari N, Benali T, Bouyahya A (2020) Quorum Sensing and Plant-Bacteria Interaction: Role of Quorum Sensing in the Rhizobacterial Community Colonization in the Rhizosphere. In: Quorum Sensing: Microbial Rules of Life. American Chemical Society, pp 139–153

309. Venturi V, Keel C (2016) Signaling in the Rhizosphere. Trends in Plant Science 21:187–198

310. Picazo-Aragonés J, Terrab A, Balao F (2020) Plant Volatile Organic Compounds Evolution: Transcriptional Regulation, Epigenetics and Polyploidy. Int J Mol Sci 21:8956

311. Raza W, Wei Z, Jousset A, Shen Q, Friman V-P (2021) Extended Plant Metarhizobiome: Understanding Volatile Organic Compound Signaling in Plant-Microbe Metapopulation Networks. mSystems 6:e00849-21

312. Rho H, Doty SL, Kim S-H (2018) Estimating microbial respiratory CO2 from endophytic bacteria in rice. Plant Signaling & Behavior 13:e1500067

313. Suryanarayanan TS, Ayesha MS, Shaanker RU (2022) Leaf photosynthesis: do endophytes have a say? Trends in Plant Science 27:968–970

314. Khare E, Mishra J, Arora NK (2018) Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. Frontiers in Microbiology 9:

CHAPTER 4

The Landscape of Maize-Associated Bacteria and Fungi Across the United States

Abstract

The maize microbiome consists of microbes that are associated with plants, and can be shaped by the host plant, the environment, and microbial partners, some of which can impact plant performance. We used a public dataset to analyze bacteria and fungi in the soil, rhizosphere, roots, and leaves of commercial maize at 30 locations across the US. We found that both tissue type and location had significant effects on community structure and makeup, although the patterns differed in bacteria and fungi based on tissue type. We also found many differences in predicted microbial gene pathways between tissues, with location also shaping predicted functional gene profiles. We found a pattern of potential interaction between fungi and bacteria, and potential intra-kingdom mutualism, in microbiome networks. The robustness of these networks was dependent upon tissue, with endophytes in leaves and roots showing significantly higher natural connectivity. Within a tissue, this connectivity was relatively stable across locations. We identified environment and soil characteristics that may impact tissue specific microbial abundance. Sulfate level in the soil was positively correlated with Proteobacteria abundance, but negatively correlated with Firmicutes abundance in the roots and leafs. Ascomycota appears to be affected by different environmental variables in each tissue. We also identified gene functions and enzymes which may be necessary to allow microbes to transition across compartments and become endophytes.

Introduction

Communities of fungi and bacteria live in, on, and around all plants [46, 195]. These microbes can colonize surfaces of tissue above ground (the phylosphere), soil near the root surface (the rhizosphere), and inside plant tissue (the endosphere) [46, 91], and they can have a large impact on plant health [46]. A plant's microbiome—the community of microbes affiliated with it—can benefit the plant by protecting against abiotic stress [47, 77, 198–200], defending against pathogens and herbivores [55, 76, 196, 197], producing phytohormones [204–206], and promoting growth through nutrient acquisition (N fixation, P solubilization, siderophore production, etc.) [63, 64, 195, 201–203]. Microorganisms can jumpstart plant immune systems resulting in higher levels of protections from pathogens [129, 207, 208]. On the other side, host plants can affect microbes by alterer soil chemistry and producing signaling compounds [209–212]. Plants can also secrete energy-rich carbon compounds ("root exudates") into the environment [210] and otherwise provide habitat for a wide range of organisms [213].

Plant genetics impact interactions with both individual beneficial microorganisms [86, 87, 122, 260], as well as the overall microbiome community [49, 50, 76, 83, 91, 93]. The environment provides most of the microbes for community recruitment (outside of seed endophytes [50]), and it has a direct impact on soil [261], plants [262], and maize [79] microbial community structure. Changes in the environment and abiotic stress such as drought [200, 263–265], heat [263, 266]], soil salinity [267–269], and fertilizer [102, 106, 270] have all been shown to cause changes in a plant's microbiome.

Network analysis in plant microbial communities has been widely adopted to uncover underlying correlations amongst bacteria and fungi, [157, 159, 271–273] and many of these correlations have been found to support antagonistic between the two kingdoms [135, 136, 139].

This analysis can identify cross-kingdom interactions, as well as identify hub taxa, taxa that are highly connected and have the potential to influence microbiome network stability (which describes the proportion of correlations amongst taxa) [164, 274, 275]. There have been few studies examining the impact of biotic and abiotic environmental factors have on cross kingdom network interactions [276–278].

Maize's high economic value ($9.2 billion in US exports alone in 2020) [223, 279] and extensive genetic variation have made it a model organism for the last century [222]. Although many studies have looked at the maize microbiome [49, 50, 83, 91, 93, 97, 170], the majority of these studies use inbred research lines, while few looked at normal production conditions or used commercial varieties [280–283]. As microbial interactions can be effected by host genetics, current understanding and assumption about the maize microbiome using dedicated inbred lines may not translate to high performing commercial varieties.

The Genome to Fields Initiative is a large public collaboration to expand our knowledge about Genome by Environment interactions. Innovations in phenotyping have been developed to explore how weather, soil, and management practices effect crop performance. This collaboration between crop scientists, computation scientists, and engineers spans universities, government agencies, and industry. A number of publications have emerged from this collaboration, especially in the fields of GxE and Genomic Selection [284–286], Phenotyping [287–289], and robotics [290]. Microbiome data has been collected for inbred and their hybrid maize over several years [Li et al. *in preparation*], but these cultivars are research lines which may not reflect high performance commercial maize used by producers. In 2017 Indigo Ag sampled the microbiome of commercial maize border corn in 30 locations across the US, allowing us to fill a crucial gap in current research: 'differences in the microbiome of high-

performing maize across environments. This data set allows us to investigate bacterial and fungal community structure, interaction, and function in the soil, rhizosphere, roots, and leaves. In this study we used an unanalyzed public dataset of maize-associated fungi and bacteria at 30 locations across the United States to (1) characterize bacterial and fungal communities, (2) identify hub taxa and inter-kingdom interactions (3) identify the impact environment has on microbiome networks, and (4) identify the core functions of the commercial maize microbiome.

Methods

2.1 Data Acquisition

To briefly describe how the dataset was generated: Plants in the V3-V5 stage as well as a trowel of soil were dug up, bagged, placed into a cooler and mailed overnight for processing. DNA was extracted from samples, and bacterial and fungal amplicons were sequenced. For bacteria, the 16S sequencing used modified 515F-806R primer pairs [291–293]. For fungi, the ITS region was amplified with the P-ITS1 and P-ITS4 primers [294]

The Genomes to Fields and Indigo Ag Microbiome Data 2017 is publicly available and was downloaded from Cyverse data commons (DOI: 10.25739/htck-sw56). Weather, soil, and field meta data was also downloaded from Cyverse data commons (DOI:10.25739/frmv-wj25). Due to lack of meta data for a large percentage of samples, supplemental weather data was queried from Visual Crossing Weather API [295]. A number of daily weather variables, including solar, temperature, and precipitation metrics, were queried from March 1st 2017 – August 1st 2017 and then the average was used for each location.

2.2 Bioinformatics

<u>Amplicon Sequence Variant Calling</u>

Amplicon sequence quality filtering and processing was performed with version 2022.11 of the QIIME2 toolbox [181]. Primers were trimmed from raw sequences, and reads below a Phred score of 20 were dropped using Cutadapt [183]. FastQC [184] was used to visually confirm read quality. Paired reads were then joined via vsearch in QIIME2 [227]. Dada2 [185] was used to trim fungal reads to 250 bp, and bacteria reads to 200 bp, and cluster ASVs. Unite version ver9_99_16.10.2022 [188] and SILVA-138-99 [187] dataframes were used with QIIME2's classifier to assign taxonomy to fungal and bacterial ASVs, respectively. The initial (raw) dataset contains 213,990 bacteria ASVs in 604 samples, and 95,058 fungal ASVs in 583 samples. We removed ASV's from a sample that (1) were unidentified at the phylum level, (2) were identified as mitochondria or chloroplasts, or (3) had two or fewer total reads in that sample. Samples with fewer than 500 remaining reads were dropped from the analysis. This resulted in a final data set of 6,578 ASVs from both kingdoms across 1,073 samples (with bacteria and fungi separated). For analyses looking at environmental covariates, we used a subset of 734 samples with the necessary metadata. When we combined bacteria and fungal samples from the same extraction (single tissue in a single plant), we had 496 samples that had both bacteria and fungal reads associated with it.

Diversity Metrics

Alpha and beta diversity were compared separately for bacteria and fungi, ASV count data was transformed into proportional data or relative abundance (total sum scaling), where each sample had a read depth of 10,000 reads. We compared alpha diversity based on tissue, location, and plant stage at harvest, using the metrics of observed ASVs, Shannon, and Simpson indices, from the phyloseq package [182]. A marginal PERMANOVA from the vegan package [229] was used for each alpha diversity metric generated in phyloseq to test the relationship

between metadata variables and community diversity. The marginal PERMANOVA was used to ignore term order in the model, and analyze the model term with all other terms. Bray-Curtis (tree-independent) distance matrices were generated in phyloseq for beta diversity of fungi. These measures were plotted, and the effect metadata variables had on community diversity was tested with PERMANOVA again in vegan. We then subset our data based on tissue type, and combined samples within a single field. A mantel test in vegan was used to compare Bray-Curtis distance with geographic distance between fields.

Network Analysis

Co-occurrence networks were built using the NetCoMi package [296] to explore correlations of bacteria and fungi. Our dataset was subset into four tissue types, and then agglomerated to the phylum level in phyloseq. This left us with 48 phyla (28 bacteria, 1 Archaea and 19 fungal). We then created our correlation network using the following parameters: we used CCLasso [192] a correlation algorithm specifically designed for compositional data, added pseudo counts of 1 to account for taxa with 0 reads, had a rho (correlation) threshold of 0.8, and normalized the data with total sum scaling [189], also known as proportions or relative abundance. These strict parameters were used to ensure we were finding the strongest and most significant correlations amongst phyla.

We used Natural Connectivity [297] to identify the effects of tissue, location, and environmental variables had on network connectivity. Natural Connectivity is a measure of network robustness, and quantifies the proportion of hubs connected to one another. This was done to ensure we captured a more holistic view of microbiome interactions. We subset our ASV data based on samples from the same tissue in a single location(~5 samples). Networks were created in NetCoMi with the following parameters: we used spearman correlation, with Centered

log-ratio normalization method [189] as spearman is not compositionally aware. Our rho (correlation) threshold was decreased to 0.5 to capture a larger proportion of potential interactions. We tested whether environmental covariates impacted network connectivity by performing Type II ANOVA using the car package in R [298], environmental variables were centered and scaled prior to testing.

Taxa Correlations with Environment

Correlation of microbial phyla to environmental variables was carried out with the phylosmith package [299]. We transformed our ASV data to relative abundance, agglomerated to the phylum level, added pseudocounts of 1, and then subset by tissue. A Spearman correlation test was run on bacteria and fungi separately. To account for multiple testing, p values were adjusted using the Benjamini & Hochberg (False Discovery Rate) method [300].

Predicted Functional Genetics

PICRUST2's [193] standard pipeline was used to investigate predicted functional gene pathways for bacterial and fungal communities. For 16s reads, raw KO terms were agglomerated to KEGG gene families terms [194], while ITS reads used raw Enzyme Classification [301] numbers, as support for ITS functionality is more limited. Core functions were filtered to include pathways that were abundant in 90% of a single tissue across all locations. These pathways were transformed with a variance stabilizing transformation using DEseq2 [190] in order to visualize functional gene profiles. DEseq2 was then used to identify differentially abundant functional pathways between tissues, with an alpha value of .001.

Results

Diversity Analysis

We used public 16S and ITS sequences from the 2017 Genomes 2 Field microbiome dataset. After quality control and filtering, our final dataset consisted of 6,578 ASVs across 1,073 samples, consisting of four tissues in 30 locations across the US. Our tissue types could also be classified as endophytes (root and leaf) or exterior (soil and rhizosphere). Throughout the experiment, we found that leaf and root tissue had fewer associated ASVs compared to root wash and soil samples. At the phylum level, soil and rhizosphere have more similar taxonomic diversity and relative abundance profiles, while root and leaf samples are more similar to each other (Figure 1). In all four tissues bacterial microbiomes are dominated by Proteobacteria reads, while fungal microbiomes are dominated by Ascomycota reads.

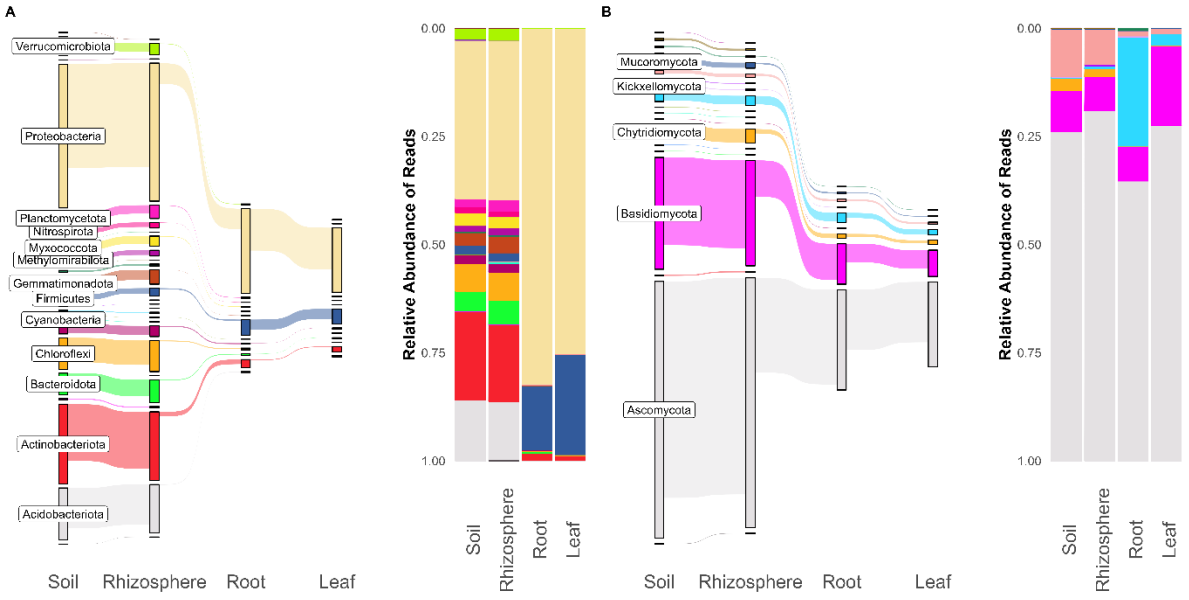


Figure 3. Shared ASVs across plant tissues compared to the relative abundance of reads in plant tissues in (A) bacteria and (B) fungi. Segments are colored by phylum.

ASV data was transformed into proportions (relative abundance) at a read depth of 10,000 reads per sample. Alpha diversity was measured with three common metrics, Observed ASVs, Shannon entropy, and Simpson entropy (Supp Figure 1). Comparisons were made using a marginal PERMANOVA analysis (Supp Table 1). Across all samples we found that bacteria

and fungi diversity was chiefly affected by both tissue type and location (Field). When looking just within individual tissue types, bacteria and fungi show opposite effects. Both show a significant association with sample location and not with plant growth stage, but for Bacteria this is only in the soil and rhizosphere, while for fungi it is only in roots and leaves.

We calculated Beta diversity using the weighted UniFrac and Bray Curtis metrics for Bacteria, and Bray Curtis for Fungi (Figure 2). Samples segregated strongly due to tissue type, with less notable segregation based on location (Supp Figure 2). Type II PERMANOVA of weighted UniFrac distances (bacteria) and Bray Curtis distances (fungi) showed that tissue and location had a significant impact on both bacterial and fungal Beta Diversity ($p < .01$) (Supp Table 2). We compared Bray-Curtis distances and geographic distances between locations using a mantel test, and found that Soil and Rhizosphere Bray-Curtis distances correlated with geographic distance between fields ($p < 05$) (Supp Table x).

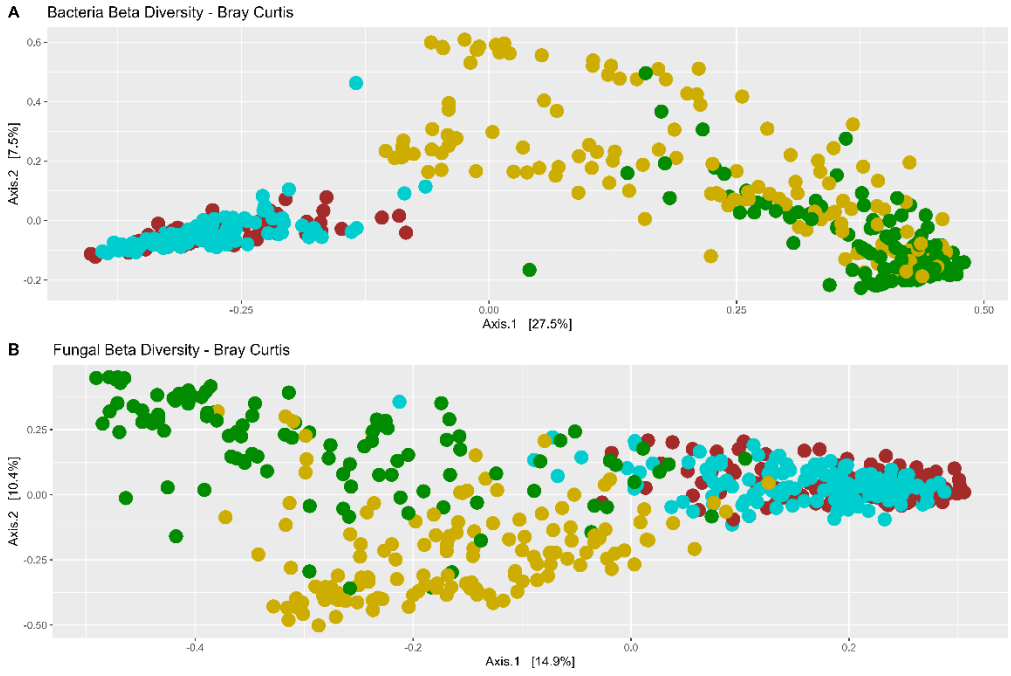


Figure 4. Weighted UniFrac (bacteria, right) and Bray-Curtis (bacteria – top, fungi - bottom) diversity principle coordinates colorized by tissue type, and location within tissue type.

We looked to see if differences in environmental and soil measures had an impact on microbial abundance. We used a subset of 2/3 of our samples, as 10 locations were missing soil data. Figure 3 shows the impact environmental covariates have on a number of bacteria and fungi taxa at the phylum level, as this taxa level was the most impacted by environmental factors. Supp Table 3 contains all significant correlations at the genus level. Overall environmental variables appeared to have impacted taxa in the soil and rhizosphere more than the roots and leaves, and environmental variables affected more fungi taxa than bacteria.

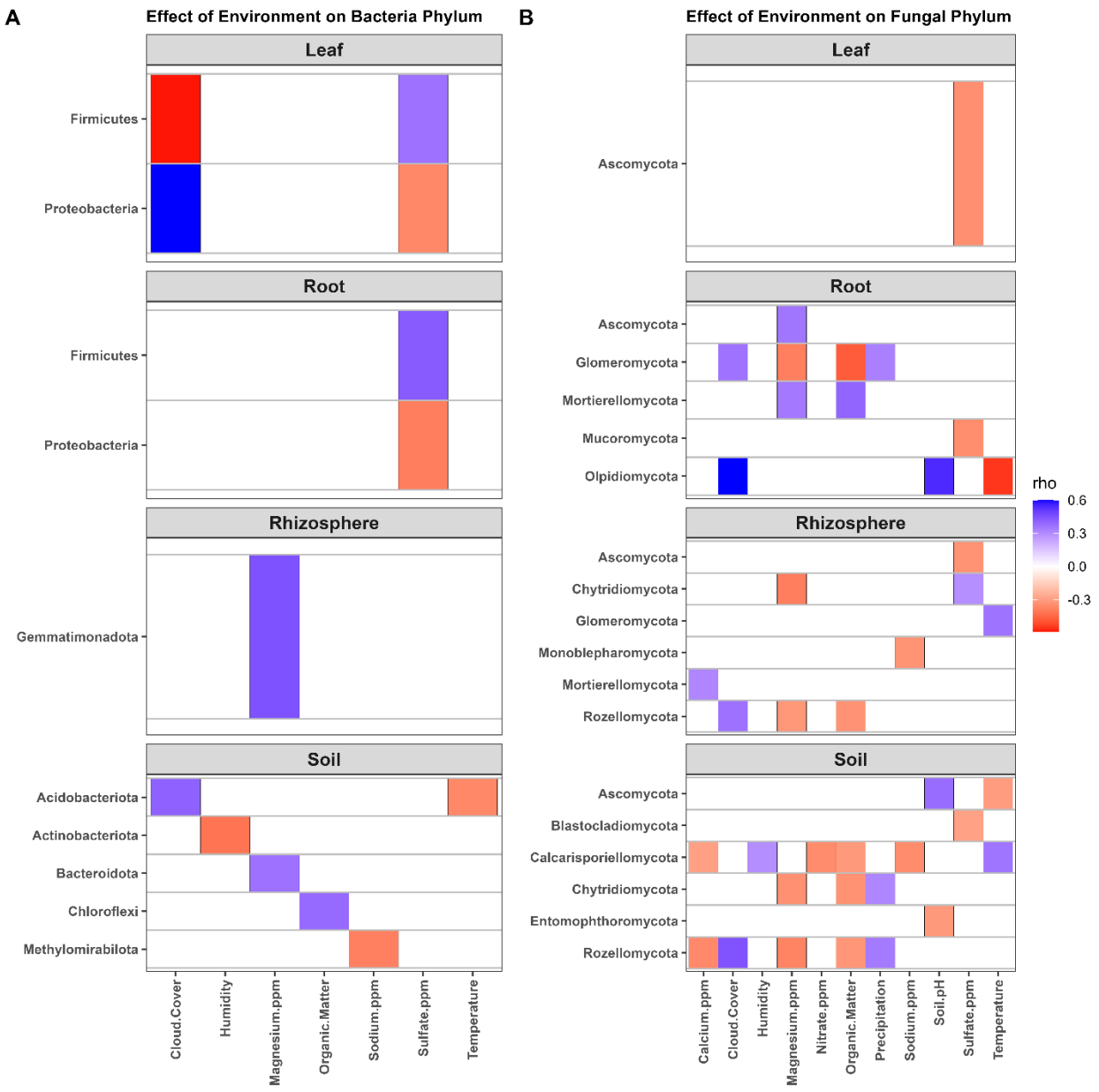


Figure 3. Correlation of bacterial (A) and fungal (B) phyla with environmental parameters. Phylums (Y axis) are significantly correlated with weather and soil covariates (X axis) when there is a colored block present. The color of the block indicates the rho (correlation), showing if the correlation is positive or negative.

Network Analysis

We then looked at the natural connectivity of networks at the ASV level (Figure 4). Natural Connectivity is a measure of network robustness, and to capture a more granular view we created networks of a single tissue type in a single location using a less strict rho cutoff of 0.3. A Type II ANOVA showed that network natural connectivity was dependent upon tissue type, but not location or the number of taxa in the network (Supp Table 3). We found that leaves and roots had higher natural connectivity than the rhizosphere and soil. While Figure 4 shows natural connectivity at the ASV level, this pattern is consistent across taxa level. Supp Figure 4 shows this analysis at the Phylum level. When we used ANOVAs to see if environmental covariates had an impact on tissue specific network connectivity, the only significant relationships were organic matter and calcium concentration on soil samples (Supp Table 4).

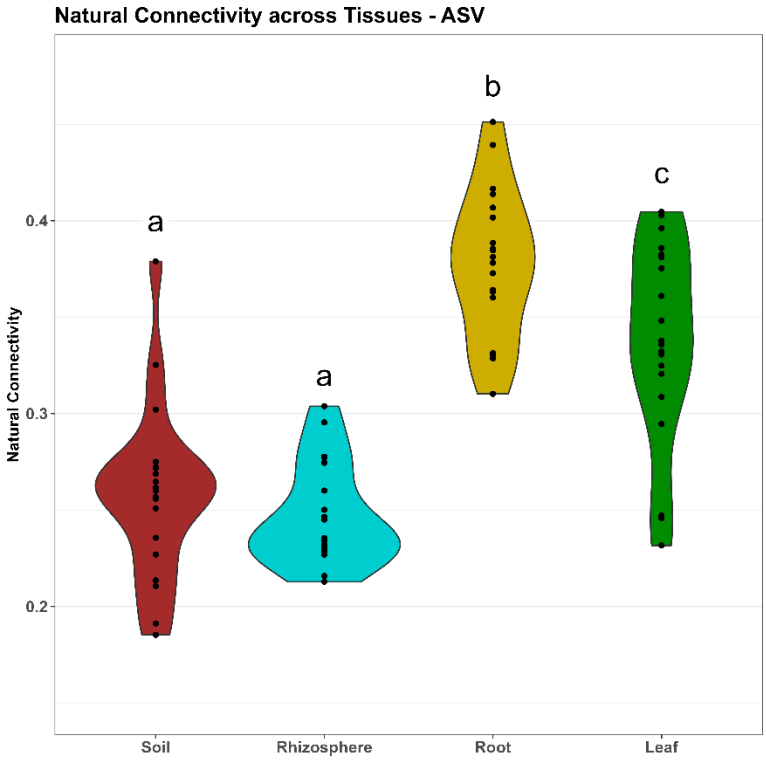


Figure 4. Natural connectivity of samples in a single location, subset by tissue type.

We next constructed networks of our four sample types at the phylum level to investigate potential antagonistic and mutualistic relationships between different bacteria and fungi taxa (Figure 5). Taxonomy was agglomerated at the phylum level, as networks at lower levels are too large and complicated to easily draw conclusions about specific taxa. In order to identify only the strongest relationships, we used a conservative approach with a rho (correlation coefficient) cutoff of 0.8. Across all four sample types, we see bacteria and fungi positively correlated with central hub taxa, which are fungi in the rhizosphere, roots, and leaves. Actinobacteria, Basidomycota, and Mucoromycota were negativily correlated with these central taxa. The archea phylum Crenarchaeota is positivily correlated with the bacteria Verrucomicrobiota in the roots and leaves. There are more significant correlations in endophyte networks than exterior microbiome networks.

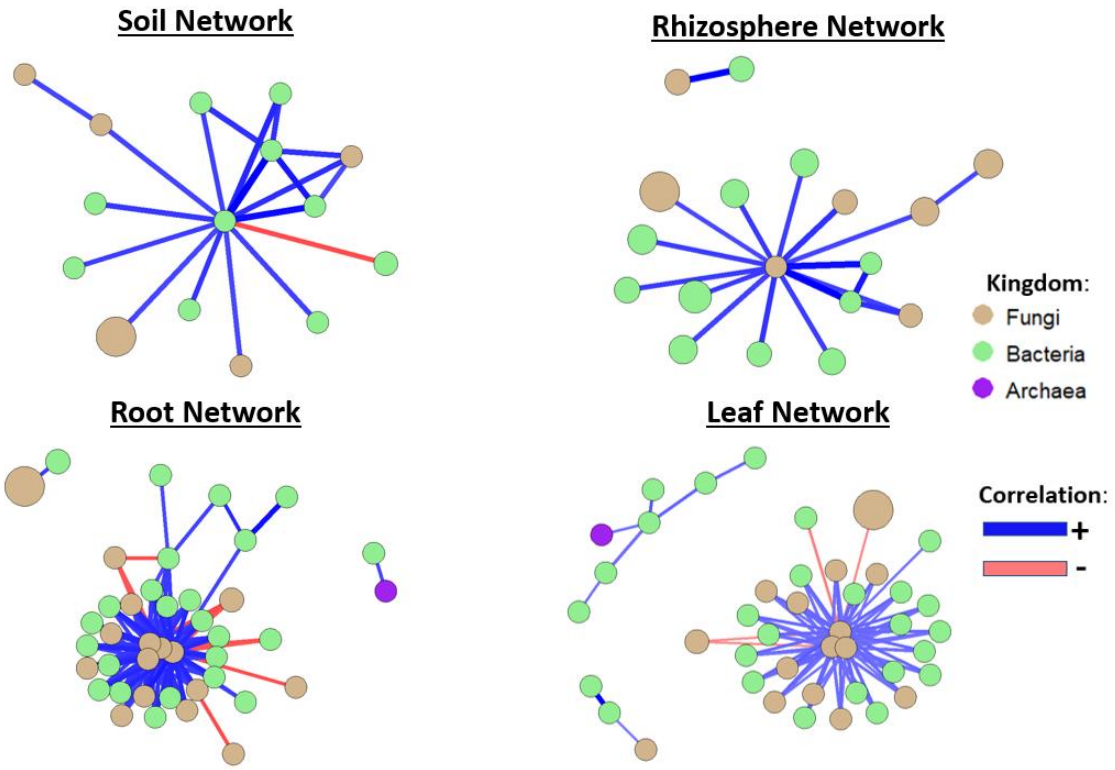


Figure 5. Network of strongly correlated phylums in the soil (A), rhizosphere (B), roots (c) and leaves (d). Edge thickness indicates correlation strength. Hub nodes are labelled with their phyla; a fully labeled version of this figure is in Supp Fig 2.

Imputed Metagenomics

We used PICRUST2 [193] to predict community core functional gene pathways from 16s and ITS sequences. For the heatmap, these gene pathways were normalized with a variance-stabilizing transformation, and were then clustered by samples (Figure 6) to visualize differences in functional gene profiles. For both bacteria and fungi functional profiles, we saw samples cluster into two groups: exterior organisms and endophytes. These two groups were more similar to each other, and show that there are distinct differences between core microbiome functions based on niche. As shown above, microbial communities of the exterior and interior are more similar to themselves, so the clustering of imputed metagenomics may be a reflection this. These transformed values were turned into a Bray Curtis distance matrix, and a PERMANOVA showed that both tissue and location had significant effects on functional gene composition ($p < .001$). We then used DESeq2 [190] to identify differentially abundant pathways between individual tissues, as well as a single tissue compared to the other three (Table 1). We found large differences in all comparisons, with one exception: Across all locations soil and rhizosphere core gene functions are almost identical.

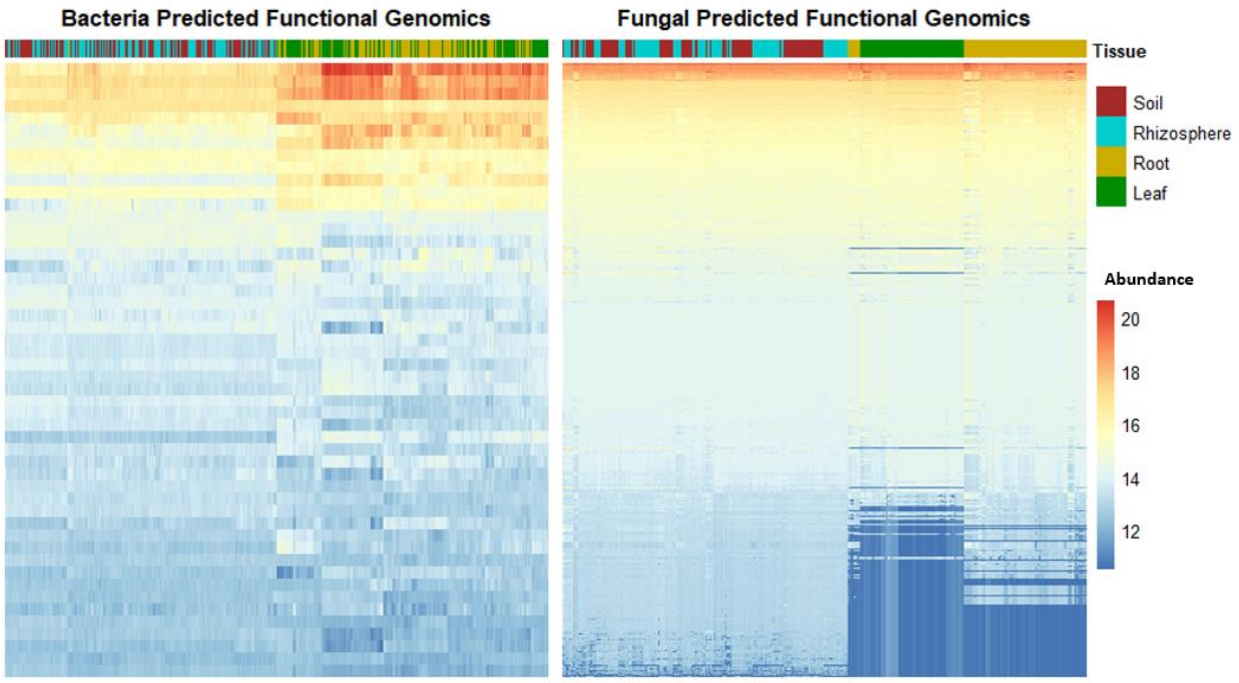


Figure 6. Heat maps of functional profiles for each sample. Samples are clustered based on similarity, and predicted genes are colored by high (red) or low (blue) abundance.

Table 3. Number of differentially abundant core predicted gene functions by tissue.

Tissue 1	Tissue 2	Bacteria KEGG Terms	Fungi EC terms
Soil	All	137	279
Rhizosphere	All	133	276
Root	All	132	310

Leaf	All	148	338
Soil	Rhizosphere	2	3
Soil	Root	153	337
Soil	Leaf	160	357
Rhizosphere	Root	152	337
Rhizosphere	Leaf	155	353
Root	Leaf	133	271
Inside Host	Outside Host	154	329

We wanted to explore the functional differences of microbes at three distinct transitions: (1) the difference between microbes in bulk soil, and those found in the rhizosphere; (2) the transition of microbes from exterior to endophytes; (3) changes in gene functions in endophytes found in the roots, and those found in the leaves. Supp Data 1 contains all of the differentially abundant comparisons.

Bacteria in the soil had an increase in sporulation gene pathways compared to their counterparts in the rhizosphere. Rhizosphere associated bacteria had increases in hormone biosynthesis. Soil associated fungi had increases in: Nucleoside-triphosphate—adenylate kinase and Magnesium-importing ATPase. Rhizosphere associated fungi had an increase in Farnesol dehydrogenase.

Exterior bacteria had distinct differences from bacteria endophytes in the roots and leaves. Bacteria outside the plant had the largest differences in gene pathways related to:

biosynthesis of carotenoids, phytohormones, flavonoids, and other potential volatile organic compounds (VOCs); degradation of atrazine; and pathways related to proteasomes and lysosome. Bacteria endophytes had increases in pathways related to: biosynthesis of peptides, bacterial toxins, metabolism and digestion of: carbohydrates amino acids, lipids; mineral absorption, electron transfer carriers, and increases in flagellar assembly. Fungi's strongest differences were in specific dehydrogenases inside and outside of the plant.

Root bacteria endophytes were enriched for specialized metabolism of VOC pathways including benzoids, phenylpropanoids, and flavonoids. They also have an increased production of streptomycin. Leaf bacteria endophytes had increases energy metabolism such as: Ribosome pathways, electron transfer carriers; amino acid, carbohydrate, and ether lipid metabolism; and the biosynthesis of steroids and carotenoids. Root fungi had large increases in pathways related to membrane transportation: Xenobiotic-transporting ATPase, glyoxylate reductase, molybdate-transporting ATPase, and ethanolamine kinase. Leaf fungi had increases in: farnesol dehydrogenase, sulfatases, and pathways related to carbohydrate and sugar metabolism

Discussion

Our results show that tissue type and the environment have significant and strong effects on both bacterial and fungal communities in maize. While previous work has shown that host genotype affects microbiome structure and function [49, 50, 83, 93], we were unable to take the genetics of our maize into account in this paper. The genetics of this commercial maize are proprietary information, and in several cases, we were unable to positively identify which variety was used.

We show that the exterior microbial community is more similar to itself than the endophyte community (Figure 1), and we see a steep drop off of the number of taxa found in the Endosphere of the roots and leaves. We may be able to attribute this to barriers microbes face when colonizing the interior of the plant, as its been shown that endophytes have genetic adaptions compared to exterior brethren [124–126, 128], and several studies have provided evidence of this steep drop off in taxa between the exterior of the plant and the interior [49, 51, 84, 93].

We found that plant tissue and field location both had a statistically significant impact on alpha diversity, with larger separation due to tissue rather than location (Supp Figure 1). When looking within tissue types, we found distinct differences in bacterial and fungal microbiomes. Bacteria communities outside the plant were dependent upon location, as were fungi communities inside the plant. Tissue and location also had significant effect on beta diversity across all samples. Bray-Curtis distance matrices for both bacteria and fungi in the soil and rhizosphere were correlated with actual distance between sites, indicating that microbial communities inside the plant are not dependent soley on biogeography. Throughout the literature we find that tissue type and the environment have a strong impact on microbiome assembly [49, 80, 83, 91, 93, 170], however, this is the first large scale study with the power to show that tissue compartments can be more similar across the country, than they are to other compartments in the same field.

Fungi appear to be more sensitive to environmental changes, as there were far more interactions between fungi and the environment, specifically in the soil, rhizosphere, and roots. We also see the same group of fungal taxa correlated with different environmental covariates in different tissue compartments. For instance, Rozellomycota, which lack chitinous cell walls, are consistently correlated with cloud cover, magnesium, and organic matter in both the soil and

rhizosphere. Yet, it appears that the transition from soil to rhizosphere may alleviate some of the effects that calcium and precipitation have on this taxa. Glomeromycota is only correlated with temperature in the soil samples, yet as a root endophyte it is correlated with cloud cover, magnesium, organic matter, and precipitation. Ascomycota is negatively correlated with sulfate in the soil, rhizosphere and leaves, but positively correlated with magnesium in the root. These correlations tell a story of tissue-specific interactions with the environment, where different niches can perhaps alleviate or enhance a microbe's sensitivity to weather and soil variables. In this analysis we consistently see cloud cover correlate with taxa abundance. We assume cloud cover is not directly affecting microbes, but is instead a proxy for some other variable(s). Although it correlates with other covariates like solar radiation, solar energy, and UV index, these variables do not correlate with the same taxa, indicating that there is some other variable or combination of variables that cloud cover is a proxy for. We hypothesize that cloud cover may be a proxy for multiple locations or regions of the country. For example, there is some clustering of individual phylum at the location level (New York fields have similar Firmicutes abundance), and cloud cover correlates well with Fimicutes abundance in these fields.

We then investigated natural connectivity of networks in a single tissue, at a single location, at the ASV level (Figure 4). As a single measure describing the amount of correlation between microbes, we view natural connectivity as a measure of potential interactions. A high natural connectivity may indicate that there could me more real biological interactions. At this granular level, we found that the endosphere has a significantly higher natural connectivity than the soil and rhizosphere. Although the soil and rhizosphere microbiome is larger and more diverse than the endosphere, it appears that endosphere ASVs are more connected with each other. This trend also holds when taxa are agglomerated to the phylum level (Supp Figure 4).

This indicates that within a single location, more microorganisms may be interacting with each other inside the plant, perhaps weakly.

We found consistent patterns of inter-kingdom antagonism and intra-kingdom mutualism between bacteria and fungi. Figure 5 demonstrates large patterns of correlations across all 30 locations at the phylum level. Although networks in the soil and rhizosphere were much larger and more complex than in the endosphere, exterior networks were not larger than endopshere networks. Interestingly, in the plant-interior samples the Archaea phylum Crenarchaeota is strongly associated with the bacteria Verrucomicrobiota. Although not often discussed, Archaea have been found to partake in the nitrogen cycle in agricultural soils [302–304], and their interactions with bacteria and fungi provide an interesting area for further research. Across all tissues we see positive correlations between bacteria and fungi with hub taxa, with very few negative correlations. These correlations may inticate interactions in these communities. It's been shown that bacteria can act as mutualists with mycorrhizal fungi and can increase growth promotion in the host [140, 141], and in a gnotobiotic experiment, it was shown that a nitrogen fixing bacteria and a mycorrhizal fungi acted as mutualists to increase growth promotion and drastically alter gene expression in the host plant and fungus [142]. The literature frequently deomonstrates antagonism between bacteria and fungi [117]. It has been shown that fungi often secrete anti-biotics [305, 306], and can disrupt bacteria communication in forest soils [137]. Soil bacteria can suppress fungi through volatile secretions [307], and one study showed that increases in fungal density was associated with enrichment of bacteria that possessed antifungal mechanisms, such as siderophores, cyanide, and lytic enzymes [136]. These correlations can only hint at potential interactions, interactions that may be indirect (where environmental factors regulate their abundance such as Figure 3), or direct interaction between these organisms.

Further studies of maize tissue microenvironments could elucidate what is causing these differences by examining resource availability, host defense stressors, and quantifying levels of molecular antagonism amongst the community in each compartment. Our inferred metagenomics analysis found community differences that support differences in antagonism throughout maize tissue compartments.

We wanted to explore the functional differences of microbes at three distinct transitions: (1) the difference between microbes in bulk soil, and those found in the rhizosphere; (2) the transition of microbes from outside the plant to within; (3) changes in gene functions in endophytes found in the roots, and those found in the leaves. Supp Data 1 contains all of the differentially abundant comparisons. Our functional gene profiles showed clear and distinct clustering of samples from within or outside plant tissue (Figure 6.) which is consistent with the rest of our findings. When looking for differentially abundant core pathways, we found massive differences in gene profiles for both bacterial and fungal communities (Table 1); the only exception was comparing soil to rhizosphere.

Bacteria in the soil had an increase in sporulation gene pathways compared to their counterparts in the rhizosphere. Sporulation results in metabolically inactive and resistant spores [308], and this process leaves highly resistant dormant spores that can activate when conditions improve. This implies that Bacteria in the rhizosphere become less dormant, perhaps association with maize roots or their exudes can protect bacteria from environmental stressors, just like bacteria can protect maize from abiotic stress [47, 76, 78]. It may also indicate that the soil environment doesn't favor spore-forming groups in general. Rhizosphere associated bacteria also had increases in hormone biosynthesis. These phytohormones can be beneficial to the plant and are important in microbe-host communication [204–206], and its believed that the host can select

for bacteria in the rhizosphere that can promote growth [80]. Rhizosphere associated fungi had an increase in Farnesol dehydrogenase. Farnesol can be used in fungi to fungi communication, as well as an antagonist against bacteria quorum sensing [138]. It's been shown that bacteria form biofilms on plant roots and this interaction is important for plant growth promotion [309], which could lead to fungi antagonism disrupting this quorum sensing as the fungus also associates with the maize root.

Exterior bacteria were enriched for pathways the can be part of soluble molecule metabolism as well as known VOC metabolism (phenol, terpenes, hydrocarbons) compared to endophytes. The rhizosphere is a hotspot for signaling between the plant host and microbes as reviewed in [143, 206, 249, 310–312]. Root exudes account for almost 10% of photosynthetically fixed carbon and 15% of plant nitrogen [310]. These compounds released from the plant can shape the microbiome, recruit beneficial organism, and can inhibit bacteria. For example it was shown that maize's production of benzoxazinoids, can shape bacteria and fungi abundance in the rhizosphere [150–152]. Phenolics, flavonoids, and indole-3-acetic acid were crucial in recruiting *Aspergillus nominus* wlg2 to the rhizosphere [153]. We found enriched pathways for compounds from these groups and more, in exterior bacteria from around the country. Endophytes were more mobile, and could metabolize more energy rich compounds. The interior of the plant is energy-rich compared to field soil. It also had a type IV pili, which is important for bacteria motility [124]. A comparison of *Herbaspirillum* species found grass endophytic species had increases in carbohydrate and nitrogen metabolism, and plant cell wall degradation compared to their free-living counterparts [126]. A study of the rice microbiome also found increases motility via flagella, and plant-polymer degrading enzymes [128].

When we compared root endophytes to leaf endophytes, we found potential similarities between bacteria and fungi. Root bacteria were enriched for VOC metabolism (phenols, terpenes, hydrocarbons), and fungi were enriched for enzymes related to membrane transport of inorganic compouns. These mechanisms may be ways to adapt to, and take advantage of, the large diversity of molecular signaling that occurs between microbes and the host [143, 148, 206]. Both bacteria and fungi in the leaves had increases in pathways related to carbohydrate metabolism and energy pathways. In the leaves these organisms have direct access to photosynthates, which bacteria and fungi use as an energy source [313]. It has been proposed that endophytes play a role in plant photosynthesis through a number of mechanisms, including carbon-dioxide assimilation [313, 314]. In rice, it was estimated that microbial respiration accounted for 57% of carbon-dioxide in the plant [313].

Molecular interactions between bacteria, fungi, and their host, are highly complex and is a blossoming area of research [117, 118, 143, 249, 311, 315]. If we assume our metagenome functions represent an accurate view of the microbes in each compartment, then it implies that there are large shifts in community function during key transitions: (1) Bacteria have more genes related to phytohormone production and fungi have more genes related to farnesol dehydrogenase when moving from soil to the root surface. (2) A larger fraction of bacteria outside the plant are capable of degrading and producing more VOC's than endophytes, and endophytes have the capacity to be more mobile, and have the capacity to take advantage of more available resources. Fungi dehydrogenase diversity show endophytes have genes capable of metabolizing different plant-exudes compared to their soil and rhizosphere associated brethren. (3) More root bacteria can metabolize VOC's than leaf bacteria, and leaf bacteria overall have more genes related to energy pathways. Root fungi have more pathways related to membrane

transport, while leaf fungi have the more genes related to metabolizing carbohydrates from the plant and increases in farnesol related genes again. In every comparison, we see differences in metabolites that may be involved with chemical antagonism, whether it be defense mechanisms from the host, antibiotics, or potential anti-fungals. Previous work has shown that predicted metabolic functional pathways correlate with actual metabolomics data in support of PICRUST2 predictions [245]. However, it must be noted that ITS prediction accuracy is much lower than 16s prediction accuracy [193].

In this study we sought to (1) characterize bacterial and fungal communities, (2) identify inter-kingdom interactions (3) identify the impact environment has on microbiome networks, and (4) identify the core functions of the commercial maize microbiome. We found that (1) communities of bacteria and fungi were more strongly influenced by tissue than environment, and that across the US these communities in the soil, rhizosphere, roots, and leaves are dominated by Proteobacteria and Ascomycota; (2) we found patterns of potential interactions between fungi and bacteria in all four tissues, and found that endosphere networks are more tightly connected; (3) we found that the environment effected individual taxa, community structure, and function, but did not affect the natural connectivity of networks; (4) we found tissue type and location significantly affect predicted gene function profiles, and that the functional pathways of communities vary to deal with very specific environments.

Conclusion

The maize microbiome is well studied, and understanding the interactions between host and microbial community is crucial to make further strides in crop protection and enhancement. Prior research has shown that the host and environment play a large role in microbial community

assembly. In this paper we examined the structure, potential function, and interactions of maize bacteria and fungi communities across 30 locations in the United States. We found tissue niche had a larger effect on the maize microbiome than location. Tissue compartments around the country are structurally and functionally more similar to each other than to other tissues in the same field. Throughout our analysis, we find distinct differences between exterior microbes versus endophytes, including predicted gene functions that may be critical for endophyte colonization. This work has proposed that there are distinct differences in the general functions of tissue-specific microbiomes, but further projects are necessary to validate them. In the future, controlled environment studies, synthetic communities, and meta-transcriptomics could help elucidate exactly how these interactions shape plant host growth and health.

References

1. Bennetzen JL, Hake SC (2009) Handbook of Maize: Genetics and Genomics. Springer Science & Business Media

2. Schnable JC (2015) Genome Evolution in Maize: From Genomes Back to Genes. Annual Review of Plant Biology 66:329–343

3. Brown NJ, Parsley K, Hibberd JM (2005) The future of C4 research – maize, Flaveria or Cleome? Trends in Plant Science 10:215–221

4. Hake S, Ross-Ibarra J (2015) Genetic, evolutionary and plant breeding insights from the domestication of maize. eLife 4:e05861

5. Gewin V (2003) Genetically Modified Corn— Environmental Benefits and Risks. PLoS Biol 1:e8

6. Nutrition C for FS and A (2023) GMO Crops, Animal Food, and Beyond. FDA

7. Schnable PS, Ware D, Fulton RS, et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

8. Hufford MB, Seetharam AS, Woodhouse MR, et al (2021) De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. Science 373:655–662

9. Corn Explorer. https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0440000. Accessed 11 May 2023

10. (2019) What Are the World's Most Important Staple Foods? In: WorldAtlas. https://www.worldatlas.com/articles/most-important-staple-foods-in-the-world.html. Accessed 11 May 2023

11. World of Corn 2021. https://ncga.com/world-of-corn-iframe#corn-usage-by-segment. Accessed 11 May 2023

12. (2021) From feed to fuel: This is how corn is used around the world. In: World Economic Forum. https://www.weforum.org/agenda/2021/06/corn-industries-sustainability-food-prices/. Accessed 11 May 2023

13. Nannas NJ, Dawe RK (2015) Genetic and Genomic Toolbox of Zea mays. Genetics 199:655–669

14. Lawrence CJ, Harper LC, Schaeffer ML, Sen TZ, Seigfried TE, Campbell DA (2008) MaizeGDB: The Maize Model Organism Database for Basic, Translational, and Applied Research. International Journal of Plant Genomics 2008:e496957

15. Tello-Ruiz MK, Jaiswal P, Ware D (2022) Gramene: A Resource for Comparative Analysis of Plants Genomes and Pathways. Methods Mol Biol 2443:101–131

16. Krishnakumar V, Choi Y, Beck E, Wu Q, Luo A, Sylvester A, Jackson D, Chan AP (2015) A maize database resource that captures tissue-specific and subcellular-localized gene expression, via fluorescent tags and confocal imaging (Maize Cell Genomics Database). Plant Cell Physiol 56:e12

17. Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde R-A, Coalter R, Barkan A (2010) Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. Plant J 63:167–177

18. Mcclintock B, Ware D, Fulton RS, et al (1929) CHROMOSOME MORPHOLOGY IN ZEA MAYS. Science 69:629–629

19. Creighton HB, McClintock B (1931) A Correlation of Cytological and Genetical Crossing-Over in Zea Mays. Proceedings of the National Academy of Sciences 17:492–497

20. McClintock B (1950) The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences 36:344–355

21. Candela H, Hake S (2008) The art and design of genetic screens: maize. Nat Rev Genet 9:192–203

22. Lisch D (2012) Regulation of transposable elements in maize. Current Opinion in Plant Biology 15:511–516

23. Duvick DN (1984) Genetic Contributions to Yield Gains of U.S. Hybrid Maize, 1930 to 1980. In: Genetic Contributions to Yield Gains of Five Major Crop Plants. John Wiley & Sons, Ltd, pp 15–47

24. Duvick DN (1999) Heterosis: Feeding People and Protecting Natural Resources. In: Genetics and Exploitation of Heterosis in Crops. John Wiley & Sons, Ltd, pp 19–29

25. Lai J, Li R, Xu X, et al (2010) Genome-wide patterns of genetic variation among elite maize inbred lines. Nat Genet 42:1027–1030

26. Xiao Y, Jiang S, Cheng Q, et al (2021) The genetic mechanism of heterosis utilization in maize improvement. Genome Biol 22:148

27. Baldauf JA, Marcon C, Lithio A, Vedder L, Altrogge L, Piepho H-P, Schoof H, Nettleton D, Hochholdinger F (2018) Single-Parent Expression Is a General Mechanism Driving Extensive Complementation of Non-syntenic Genes in Maize Hybrids. Current Biology 28:431-437.e4

28. Paschold A, Jia Y, Marcon C, et al (2012) Complementation contributes to transcriptome complexity in maize (Zea mays L.) hybrids relative to their inbred parents. Genome Res 22:2445–2454

29. Zhou P, Hirsch CN, Briggs SP, Springer NM (2019) Dynamic Patterns of Gene Expression Additivity and Regulatory Variation throughout Maize Development. Molecular Plant 12:410–425

30. Guo B, Chen Y, Li C, et al (2014) Maize (Zea mays L.) seedling leaf nuclear proteome and differentially expressed proteins between a hybrid and its parental lines. PROTEOMICS 14:1071–1087

31. Guo B, Chen Y, Zhang G, et al (2013) Comparative Proteomic Analysis of Embryos between a Maize Hybrid and Its Parental Lines during Early Stages of Seed Germination. PLOS ONE 8:e65867

32. Hunter RB, Kannenberg LW (1971) ISOZYME CHARACTERIZATION OF CORN (Zea mays) INBREDS AND ITS RELATIONSHIP TO SINGLE CROSS HYBRID PERFORMANCE. Can J Genet Cytol 13:649–655

33. Heidrich-Sobrinho E, Cordeiro AR (1975) Codominant isoenzymic alleles as markers of genetic diversity correlated with heterosis in maize (Zea mays). Theoret Appl Genetics 46:197–199

34. Xing J, Sun Q, Ni Z (2016) Proteomic patterns associated with heterosis. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1864:908–915

35. Wu X, Liu Y, Zhang Y, Gu R (2021) Advances in Research on the Mechanism of Heterosis in Plants. Frontiers in Plant Science 12:

36. Rockenbach MF, Corrêa CCG, Heringer AS, Freitas ILJ, Santa-Catarina C, Amaral-Júnior AT do, Silveira V (2018) Differentially abundant proteins associated with heterosis in the primary roots of popcorn. PLOS ONE 13:e0197114

37. Li Z, Zhu A, Song Q, Chen HY, Harmon FG, Chen ZJ (2020) Temporal Regulation of the Metabolome and Proteome in Photosynthetic and Photorespiratory Pathways Contributes to Maize Heterosis. The Plant Cell 32:3706–3722

38. Biostimulants Market, Global Industry Size Forecast [Latest]. In: MarketsandMarkets. https://www.marketsandmarkets.com/Market-Reports/biostimulant-market-1081.html. Accessed 11 May 2023

39. Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim S-H (2018) Do Endophytes Promote Growth of Host Plants Under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes. Microb Ecol 75:407–418

40. Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV (2016) From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms. Frontiers in Plant Science 7:

41. Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson AC (2017) Perspectives and challenges of microbial application for crop improvement. Frontiers in Plant Science 8:49

42. Ganeshan S, Kim SH, Vujanovic V (2021) Scaling-up production of plant endophytes in bioreactors: concepts, challenges and perspectives. Bioresources and Bioprocessing 2021 8:1 8:1–16

43. Jack CN, Petipas RH, Cheeke TE, Rowland JL, Friesen ML (2021) Microbial Inoculants: Silver Bullet or Microbial Jurassic Park? Trends in Microbiology 29:299–308

44. Aprahamian AM, Lulow ME, Major MR, Balazs KR, Treseder KK, Maltz MR (2016) Arbuscular mycorrhizal inoculation in coastal sage scrub restoration. Botany 94:493–499

45. Mawarda PC, Le Roux X, Dirk van Elsas J, Salles JF (2020) Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148:107874

46. Wallace JG, May G (2018) Endophytes: The Other Maize Genome. In: Bennetzen J, Flint-Garcia S, Hirsch C, Tuberosa R (eds) The Maize Genome. Springer International Publishing, Cham, pp 213–246

47. Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. Environmental and Experimental Botany 97:30–39

48. Roesch LFW, Camargo FAO, Bento FM, Triplett EW (2008) Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. Plant Soil 302:91–104

49. Schultz CR, Johnson M, Wallace JG (2023) Effects of Inbreeding on Microbial Community Diversity of Zea mays. Microorganisms 11:879

50. Johnston-Monje D, Raizada MN (2011) Conservation and Diversity of Seed Associated Endophytes in Zea across Boundaries of Evolution, Ethnography and Ecology. PLoS ONE 6:e20396

51. Johnston-Monje D, Gutiérrez JP, Lopez-Lavalle LAB (2021) Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes. Front Microbiol 12:737616

52. Liu Y, Zuo S, Xu L, Zou Y, Song W (2012) Study on diversity of endophytic bacterial communities in seeds of hybrid maize and their parental lines. Arch Microbiol 194:1001–1012

53. Schardl CL (2001) Epichloë festucae and Related Mutualistic Symbionts of Grasses. Fungal Genetics and Biology 33:69–82

54. Guerre P, Guerre, Philippe (2015) Ergot Alkaloids Produced by Endophytic Fungi of the Genus Epichloë. Toxins 7:773–790

55. Cagnano G, Lenk I, Roulund N, Jensen CS, Cox MP, Asp T (2020) Mycelial biomass and concentration of loline alkaloids driven by complex population structure in Epichloë uncinata and meadow fescue (Schedonorus pratensis). Mycologia 112:474–490

56. Johnston-Monje D, Lundberg DS, Lazarovits G, Reis VM, Raizada MN (2016) Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant and Soil 405:337–355

57. Monteiro RA, Schmidt MA, Baura VA de, Balsanelli E, Wassem R, Yates MG, Randi MAF, Pedrosa FO, Souza EM de (2008) Early colonization pattern of maize (Zea mays L. Poales, Poaceae) roots by Herbaspirillum seropidicae, Burkholderia WP9, and Serendipita bescii seropedicae (Burkholderiales, Oxalobacteraceae). Genetics and Molecular Biology 31:932–937

58. Roncato-Maccari LDB, Ramos HJO, Pedrosa FO, Alquini Y, Chubatsu LS, Yates MG, Rigo LU, Steffens MBR, Souza EM (2003) Endophytic Herbaspirillum seropedicae expresses nif genes in gramineous plants. FEMS Microbiology Ecology 45:39–47

59. Lamb TG, Tonkyn DW, Kluepfel DA (1996) Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. Can J Microbiol 42:1112–1120

60. Bressan W, Borges MT (2004) Delivery methods for introducing endophytic bacteria into maize. BioControl 49:315–322

61. Baldotto MA, Baldotto LEB, Santana RB, Marciano CR (2012) Initial performance of maize in response to NPK fertilization combined with Herbaspirillum seropedicae. Rev Ceres 59:841–849

62. Canellas LP, Balmori DM, Médici LO, Aguiar NO, Campostrini E, Rosa RCC, Façanha AR, Olivares FL (2013) A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (Zea mays L.). Plant and Soil 366:119–132

63. Matsumura EE, Secco VA, Moreira RS, Santos OJAP dos, Hungria M, Oliveira ALM de (2015) Composition and activity of endophytic bacterial communities in field-grown maize plants inoculated with Azospirillum brasilense. Annals of Microbiology 65:2187–2200

64. Young L-S, Hameed A, Peng S-Y, Shan Y-H, Wu S-P (2013) Endophytic establishment of the soil isolate Burkholderia sp. CC-Al74 enhances growth and P-utilization rate in maize (Zea mays L.). Applied Soil Ecology 66:40–47

65. Akhtar SS, Andersen MN, Naveed M, Zahir ZA, Liu F (2015) Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. Functional Plant Biol 42:770–781

66. Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic Azospirillum in the alleviation of drought effects in maize. Botany 87:455–462

67. Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. Plant Soil 331:413–425

68. Sobowale AA, Cardwell KF, Odebode AC, Bandyopadhyay R, Jonathan SG (2007) Persistence of Trichoderma species within maize stem against Fusarium verticillioides. Archives of Phytopathology and Plant Protection 40:215–231

69. Mousa WK, Shearer CR, Limay-Rios V, Zhou T, Raizada MN (2015) Bacterial endophytes from wild maize suppress Fusarium graminearum in modern maize and inhibit mycotoxin accumulation. Frontiers in Plant Science 6:

70. Shehata HR, Lyons EM, Jordan KS, Raizada MN (2016) Bacterial endophytes from wild and ancient maize are able to suppress the fungal pathogen Sclerotinia homoeocarpa. Journal of Applied Microbiology 120:756–769

71. Shehata HR, Raizada MN (2017) A Burkholderia endophyte of the ancient maize landrace Chapalote utilizes c-di-GMP-dependent and independent signaling to suppress diverse plant fungal pathogen targets. FEMS Microbiology Letters 364:fnx138

72. Santos F, Peñaflor MFGV, Paré PW, Sanches PA, Kamiya AC, Tonelli M, Nardi C, Bento JMS (2014) A Novel Interaction between Plant-Beneficial Rhizobacteria and Roots: Colonization Induces Corn Resistance against the Root Herbivore Diabrotica speciosa. PLOS ONE 9:e113280

73. Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E, Larsen J (2018) The root endophytic fungus Trichoderma atroviride induces foliar herbivory resistance in maize plants. Applied Soil Ecology 124:45–53

74. Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proceedings of the National Academy of Sciences of the United States of America 114:E2450–E2459

75. 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

76. Passera A, Follador A, Morandi S, et al (2021) Bacterial Communities in the Embryo of Maize Landraces: Relation with Susceptibility to Fusarium Ear Rot. Microorganisms 9:2388

77. Castiglioni P, Warner D, Bensen RJ, et al (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiol 147:446–455

78. Beirinckx S, Viaene T, Haegeman A, et al (2020) Tapping into the maize root microbiome to identify bacteria that promote growth under chilling conditions. Microbiome 8:54

79. Singh R, Goodwin SB (2022) Exploring the Corn Microbiome: A Detailed Review on Current Knowledge, Techniques, and Future Directions. PhytoFrontiers^{TM} 2:158–175

80. Yang Y, Wang N, Guo X, Zhang Y, Ye B (2017) Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLOS ONE 12:e0178425

81. Johnston-Monje D, Gutiérrez JP, Becerra Lopez-Lavalle LA (2022) Stochastic Inoculum, Biotic Filtering and Species-Specific Seed Transmission Shape the Rare Microbiome of Plants. Life (Basel) 12:1372

82. Microbial life in the phyllosphere | Nature Reviews Microbiology. https://www.nature.com/articles/nrmicro2910. Accessed 17 May 2023

83. Wallace JG, Kremling KA, Kovar LL, Buckler ES (2018) Quantitative genetics of the maize leaf microbiome. Phytobiomes Journal 2:208–224

84. Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. PLOS ONE 8:e56329

85. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C, Vorholt JA (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proceedings of the National Academy of Sciences 106:16428–16433

86. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

87. Brusamarello-Santos LC, Gilard F, Brulé L, Quilleré I, Gourion B, Ratet P, Souza EM de, Lea PJ, Hirel B (2017) Metabolic profiling of two maize (Zea mays L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria Herbaspirillum seropedicae and Azospirillum brasilense. PLOS ONE 12:e0174576

88. Montañez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro. Applied Soil Ecology 58:21–28

89. da Silva KJ, de Armas RD, Soares CRFS, Ogliari JB (2016) Communities of endophytic microorganisms in different developmental stages from a local variety as well as

transgenic and conventional isogenic hybrids of maize. World J Microbiol Biotechnol 32:189

90. McMullen MD, Kresovich S, Villeda HS, et al (2009) Genetic Properties of the Maize Nested Association Mapping Population. Science 325:737–740

91. 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 110:6548–6553

92. Flint-Garcia SA, Thuillet A-C, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM, Buckler ES (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. The Plant Journal 44:1054–1064

93. Wagner MR, Roberts JH, Balint-Kurti P, Holland JB (2020) Heterosis of leaf and rhizosphere microbiomes in field-grown maize. New Phytologist 228:1055–1069

94. Wagner MR, Tang C, Salvato F, et al (2021) Microbe-dependent heterosis in maize. Proc Natl Acad Sci USA 118:e2021965118

95. Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE (2010) Maize Leaf Epiphytic Bacteria Diversity Patterns Are Genetically Correlated with Resistance to Fungal Pathogen Infection. MPMI 23:473–484

96. Manching HC, Balint-Kurti PJ, Stapleton AE (2014) Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by nitrogen fertilization. Frontiers in Plant Science 5:

97. Wagner MR, Busby PE, Balint-Kurti P (2020) Analysis of leaf microbiome composition of near-isogenic maize lines differing in broad-spectrum disease resistance. New Phytologist 225:2152–2165

98. Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Applied Soil Ecology 8:11–18

99. Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil 232:167–180

100. Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Molecular Ecology 14:2539–2551

101. Dematheis F, Zimmerling U, Flocco C, Kurtz B, Vidal S, Kropf S, Smalla K (2012) Multitrophic Interaction in the Rhizosphere of Maize: Root Feeding of Western Corn Rootworm Larvae Alters the Microbial Community Composition. PLOS ONE 7:e37288

102. Emmett BD, Buckley DH, Drinkwater LE (2020) Plant growth rate and nitrogen uptake shape rhizosphere bacterial community composition and activity in an agricultural field. New Phytologist 225:960–973

103. Pan JJ, Baumgarten AM, May G (2008) Effects of host plant environment and Ustilago maydis infection on the fungal endophyte community of maize (Zea mays). New Phytologist 178:147–156

104. Johnston-Monje D, Mousa WK, Lazarovits G, Raizada MN (2014) Impact of swapping soils on the endophytic bacterial communities of pre-domesticated, ancient and modern maize. BMC Plant Biol 14:233

105. Kadivar H, Stapleton AE (2003) Ultraviolet Radiation Alters Maize Phyllosphere Bacterial Diversity. Microb Ecol 45:353–361

106. Wolters B, Jacquiod S, Sørensen SJ, Widyasari-Mehta A, Bech TB, Kreuzig R, Smalla K (2018) Bulk soil and maize rhizosphere resistance genes, mobile genetic elements and microbial communities are differently impacted by organic and inorganic fertilization. FEMS Microbiology Ecology 94:fiy027

107. Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize Endophytic Bacterial Diversity as Affected by Soil Cultivation History. Frontiers in Microbiology 9:

108. Wattenburger CJ, Halverson LJ, Hofmockel KS (2019) Agricultural Management Affects Root-Associated Microbiome Recruitment Over Maize Development. Phytobiomes Journal 3:260–272

109. Benitez M-S, Osborne SL, Lehman RM (2017) Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. Sci Rep 7:15709

110. Maarastawi SA, Frindte K, Linnartz M, Knief C (2018) Crop Rotation and Straw Application Impact Microbial Communities in Italian and Philippine Soils and the Rhizosphere of Zea mays. Frontiers in Microbiology 9:

111. Chen Z, Zheng Y, Ding C, Ren X, Yuan J, Sun F, Li Y (2017) Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. Ecotoxicology and Environmental Safety 145:111–118

112. Islam F, Yasmeen T, Arif MS, Riaz M, Shahzad SM, Imran Q, Ali I (2016) Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. Plant Physiology and Biochemistry 108:456–467

113. Panitlertumpai N, Nakbanpote W, Sangdee A, Boonapatcharoen N, Prasad MNV (2018) Potentially toxic elements to maize in agricultural soils—microbial approach of rhizospheric and bulk soils and phytoaccumulation. Environ Sci Pollut Res 25:23954–23972

114. Vigliotta G, Matrella S, Cicatelli A, Guarino F, Castiglione S (2016) Effects of heavy metals and chelants on phytoremediation capacity and on rhizobacterial communities of maize. Journal of Environmental Management 179:93–102

115. Wu W, Wu J, Liu X, Chen X, Wu Y, Yu S (2017) Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure. Ecotoxicology and Environmental Safety 143:322–329

116. Sillen WMA, Thijs S, Abbamondi GR, De La Torre Roche R, Weyens N, White JC, Vangronsveld J (2020) Nanoparticle treatment of maize analyzed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis. Microbiome 8:127

117. de Menezes AB, Richardson AE, Thrall PH (2017) Linking fungal–bacterial co-occurrences to soil ecosystem function. Current Opinion in Microbiology 37:135–141

118. van Overbeek LS, Saikkonen K (2016) Impact of Bacterial–Fungal Interactions on the Colonization of the Endosphere. Trends in Plant Science 21:230–242

119. Deynze AV, Zamora P, Delaux P-M, et al (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLOS Biology 16:e2006352

120. Amicucci MJ, Galermo AG, Guerrero A, et al (2019) A Strategy for Structural Elucidation of Polysaccharides: Elucidation of a Maize Mucilage that Harbors Diazotrophic Bacteria. Analytical Chemistry acs.analchem.9b00789

121. Bennett AB, Pankievicz VCS, Ané J-M (2020) A Model for Nitrogen Fixation in Cereal Crops. Trends in Plant Science 25:226–235

122. Schultz C, Brantley K, Wallace J (2021) The Role of Genetic Variation in Maize Response to Beneficial Endophytes. 2021.11.03.467096

123. Craig L, Forest KT, Maier B (2019) Type IV pili: dynamics, biophysics and functional consequences. Nat Rev Microbiol 17:429–440

124. Pedrosa FO, Monteiro RA, Wassem R, et al (2011) Genome of Herbaspirillum seropedicae Strain SmR1, a Specialized Diazotrophic Endophyte of Tropical Grasses. PLOS Genetics 7:e1002064

125. Monteiro RA, Balsanelli E, Tuleski T, et al (2012) Genomic comparison of the endophyte Herbaspirillum seropedicae SmR1 and the phytopathogen Herbaspirillum rubrisubalbicans M1 by suppressive subtractive hybridization and partial genome sequencing. FEMS Microbiology Ecology 80:441–451

126. Straub D, Rothballer M, Hartmann A, Ludewig U (2013) The genome of the endophytic bacterium H. frisingense GSF30T identifies diverse strategies in the Herbaspirillum genus to interact with plants. Frontiers in Microbiology 4:

127. Sheibani-Tezerji R, Naveed M, Jehl M-A, Sessitsch A, Rattei T, Mitter B (2015) The genomes of closely related Pantoea ananatis maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. Frontiers in Microbiology 6:

128. Sessitsch A, Hardoim P, Döring J, et al (2012) Functional Characteristics of an Endophyte Community Colonizing Rice Roots as Revealed by Metagenomic Analysis. MPMI 25:28–36

129. Van Wees SCM, Van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448

130. Zhang X, Valdés-López O, Arellano C, Stacey G, Balint-Kurti P (2017) Genetic dissection of the maize (Zea mays L.) MAMP response. Theoretical and Applied Genetics 130:1155–1168

131. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced Systemic Resistance by Beneficial Microbes. Annual Review of Phytopathology 52:347–375

132. Djonović S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM (2007) A Proteinaceous Elicitor Sm1 from the Beneficial Fungus Trichoderma virens Is Required for Induced Systemic Resistance in Maize. Plant Physiology 145:875–889

133. Güimil S, Chang H-S, Zhu T, et al (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proceedings of the National Academy of Sciences 102:8066–8070

134. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth - Artursson - 2006 - Environmental Microbiology - Wiley Online Library. https://ami-journals.onlinelibrary.wiley.com/doi/full/10.1111/j.1462-2920.2005.00942.x. Accessed 16 May 2023

135. Zachow C, Tilcher R, Berg G (2008) Sugar Beet-Associated Bacterial and Fungal Communities Show a High Indigenous Antagonistic Potential Against Plant Pathogens. Microb Ecol 55:119–129

136. de Boer W, de Ridder-Duine AS, Klein Gunnewiek PJA, Smant W, Van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. Soil Biology and Biochemistry 40:1542–1544

137. Uroz S, Heinonsalo J (2008) Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. FEMS Microbiology Ecology 65:271–278

138. Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial–fungal interactions. Nat Rev Microbiol 8:340–349

139. Notz R, Maurhofer M, Dubach H, Haas D, Défago G (2002) Fusaric Acid-Producing Strains of Fusarium oxysporum Alter 2,4-Diacetylphloroglucinol Biosynthetic Gene Expression in Pseudomonas fluorescens CHA0 In Vitro and in the Rhizosphere of Wheat. Applied and Environmental Microbiology 68:2229–2235

140. Jäderlund L, Arthurson V, Granhall U, Jansson JK (2008) Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations. FEMS Microbiology Letters 287:174–180

141. Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA (2014) Newly identified helper bacteria stimulate ectomycorrhizal formation in Populus. Frontiers in Plant Science 5:

142. Afkhami ME, Stinchcombe JR (2016) Multiple mutualist effects on genomewide expression in the tripartite association between Medicago truncatula, nitrogen-fixing bacteria and mycorrhizal fungi. Molecular Ecology 25:4946–4962

143. Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y (2021) Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Frontiers in Plant Science 12:

144. Rani A, Rana A, Dhaka RK, Singh AP, Chahar M, Singh S, Nain L, Singh KP, Minz D (2023) Bacterial volatile organic compounds as biopesticides, growth promoters and plant-defense elicitors: Current understanding and future scope. Biotechnology Advances 63:108078

145. Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q (2018) Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23:762

146. Zhou H, Hua J, Li H, Song X, Luo S Structurally diverse specialized metabolites of maize and their extensive biological functions. Journal of Cellular Physiology. https://doi.org/10.1002/jcp.30955

147. Yu J, Tu X, Huang AC (2022) Functions and biosynthesis of plant signaling metabolites mediating plant–microbe interactions. Nat Prod Rep 39:1393–1422

148. Adedeji AA, Babalola OO (2020) Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. Planta 252:61

149. Tyc Olaf, Song C, Dickschat JS, Vos M, Garbeva P (2017) The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. Trends in Microbiology 25:280–292

150. Hu L, Robert CAM, Cadot S, et al (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun 9:2738

151. Kudjordjie EN, Sapkota R, Steffensen SK, Fomsgaard IS, Nicolaisen M (2019) Maize synthesized benzoxazinoids affect the host associated microbiome. Microbiome 7:59

152. Cotton TEA, Pétriacq P, Cameron DD, Meselmani MA, Schwarzenbacher R, Rolfe SA, Ton J (2019) Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J 13:1647–1658

153. Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Tawab A, Khan N (2020) Yucasin and cinnamic acid inhibit IAA and flavonoids biosynthesis minimizing interaction between maize and endophyte Aspergillus nomius. Symbiosis 81:149–160

154. D'alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J, Turlings TCJ (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant, Cell & Environment 37:813–826

155. Tenorio-Salgado S, Tinoco R, Vazquez-Duhalt R, Caballero-Mellado J, Perez-Rueda E (2013) Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4:236–243

156. Xie S, Liu J, Gu S, Chen X, Jiang H, Ding T (2020) Antifungal activity of volatile compounds produced by endophytic Bacillus subtilis DZSY21 against Curvularia lunata. Annals of Microbiology 70:2

157. Lee KK, Kim H, Lee Y-H (2022) Cross-kingdom co-occurrence networks in the plant microbiome: Importance and ecological interpretations. Frontiers in Microbiology 13:

158. Banerjee S, Schlaeppi K, van der Heijden MGA (2018) Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol 16:567–576

159. Heijden MGA van der, Hartmann M (2016) Networking in the Plant Microbiome. PLOS Biology 14:e1002378

160. Jiang D, Armour CR, Hu C, Mei M, Tian C, Sharpton TJ, Jiang Y (2019) Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities. Frontiers in Genetics 10:

161. Hong J, Karaoz U, de Valpine P, Fithian W (2022) To rarefy or not to rarefy: robustness and efficiency trade-offs of rarefying microbiome data. Bioinformatics btac127

162. Hünninghaus M, Dibbern D, Kramer S, Koller R, Pausch J, Schloter-Hai B, Urich T, Kandeler E, Bonkowski M, Lueders T (2019) Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize. Soil Biology and Biochemistry 134:122–130

163. Kim H, Lee KK, Jeon J, Harris WA, Lee Y-H (2020) Domestication of Oryza species eco-evolutionarily shapes bacterial and fungal communities in rice seed. Microbiome 8:20

164. Huang J, Li Y, Ma Y, Li Y, Jin J, Lian T (2022) The rhizospheric microbiome becomes more diverse with maize domestication and genetic improvement. Journal of Integrative Agriculture 21:1188–1202

165. Xiong C, Singh BK, He J-Z, et al (2021) Plant developmental stage drives the differentiation in ecological role of the maize microbiome. Microbiome 2021 9:1 9:1–15

166. Gardner CM, Gerhard WA, Redfern LK, Gunsch CK (2022) Evaluation of developing maize microbiomes and associations among nitrogen cyclers and key fungal taxa. Microbiology 168:001155

167. Toju H, Peay KG, Yamamichi M, et al (2018) Core microbiomes for sustainable agroecosystems. Nature Plants 4:247–257

168. Shade A, Stopnisek N (2019) Abundance-occupancy distributions to prioritize plant core microbiome membership. Current Opinion in Microbiology 49:50–58

169. Walters WA, Jin Z, Youngblut N, et al (2018) Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences of the United States of America 115:7368–7373

170. 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 112:E911–E920

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

172. Lundberg DS, Lebeis SL, Paredes SH, et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90

173. Chen L, Xin X, Zhang J, Redmile-gordon M, Nie G, Wang Q (2019) Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize. Pedosphere 29:360–373

174. Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y (2017) Let the Core Microbiota Be Functional. Trends in Plant Science 22:583–595

175. Ranjard L, Dequiedt S, Chemidlin Prévost-Bouré N, et al (2013) Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. Nat Commun 4:1434

176. Young JM, Kuykendall LD, Martínez-Romero E, Kerr A, Sawada H (2001) A revision of Rhizobium Frank 1889, with an emended description of the genus, and the inclusion of all species of Agrobacterium Conn 1942 and Allorhizobium undicola de Lajudie et al. 1998 as new combinations: Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola and R. vitis. International Journal of Systematic and Evolutionary Microbiology 51:89–103

177. Galloway-Peña J, Hanson B (2020) Tools for Analysis of the Microbiome. Dig Dis Sci 65:674–685

178. Su X, Yan X, Tsai C-L (2012) Linear regression. WIREs Computational Statistics 4:275–294

179. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46

180. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857

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

182. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

183. Andrews, S (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data [Online].

184. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

185. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns | mSystems. https://journals.asm.org/doi/10.1128/mSystems.00191-16. Accessed 5 May 2022

186. 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–D596

187. Nilsson RH, Larsson K-H, Taylor AFS, et al (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264

188. Badri M, Kurtz ZD, Bonneau R, Müller CL (2020) Shrinkage improves estimation of microbial associations under different normalization methods. NAR Genomics and Bioinformatics 2:lqaa100

189. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:1–21

190. McKnight DT, Huerlimann R, Bower DS, Schwarzkopf L, Alford RA, Zenger KR (2019) Methods for normalizing microbiome data: An ecological perspective. Methods in Ecology and Evolution 10:389–400

191. Fang H, Huang C, Zhao H, Deng M (2015) CCLasso: correlation inference for compositional data through Lasso. Bioinformatics 31:3172–3180

192. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38:685–688

193. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457-462

194. 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 Advanced Research. https://doi.org/10.1016/J.JARE.2019.03.004

195. Innerebner G, Knief C, Vorholt JA (2011) Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System ▿. Appl Environ Microbiol 77:3202–3210

196. Rojas X, Guo J, Leff JW, McNear DH, Fierer N, McCulley RL (2016) Infection with a Shoot-Specific Fungal Endophyte (Epichloë) Alters Tall Fescue Soil Microbial Communities. Microbial Ecology 72:197–206

197. Akhtar SAS, Andersen MBN, Naveed MD, Zahir ZDA, Liu FA Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. https://doi.org/10.1071/FP15054

198. Jochum MD, McWilliams KL, Pierson EA, Jo YK (2019) Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. PLoS ONE 14:e0225933

199. Naylor D, Degraaf S, Purdom E, Coleman-Derr D (2017) Drought and host selection influence bacterial community dynamics in the grass root microbiome. ISME Journal 11:2691–2704

200. Baldotto LEB, Olivares FL, Bressan-Smith R (2011) Structural interaction between GFP-labeled diazotrophic endophytic bacterium Herbaspirillum seropedicae RAM10 and pineapple plantlets “Vitória.” Brazilian Journal of Microbiology 42:114–125

201. Alves GC, Videira SS, Urquiaga S, Reis VM (2015) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant and Soil 387:307–321

202. Caradonia F, Ronga D, Catellani M, Azevedo CVG, Terrazas RA, Robertson-Albertyn S, Francia E, Bulgarelli D (2019) Nitrogen Fertilisers Shape The Composition And Predicted Functions Of The Microbiota Of Field-Grown Tomato Plants. bioRxiv 672162

203. Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Letters in Applied Microbiology 48:542–547

204. Rivas-Franco F, Hampton JG, Narciso J, Rostás M, Wessman P, Saville DJ, Jackson TA, Glare TR (2020) Effects of a maize root pest and fungal pathogen on entomopathogenic fungal rhizosphere colonization, endophytism and induction of plant hormones. Biological Control 150:104347

205. Patel M, Singh S, Vasanthakumari M, Naik S, Manjunatha B, Jadhav S, Ravikanth G, K N G, Shaanker R (2013) Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective. In: Advances in Endophytic Research. pp 177–190

206. Oukala N, Aissat K, Pastor V (2021) Bacterial Endophytes: The Hidden Actor in Plant Immune Responses against Biotic Stress. Plants (Basel) 10:1012

207. Ma Y (2017) Beneficial Bacteria for Disease Suppression and Plant Growth Promotion. In: Plant-Microbe Interactions in Agro-Ecological Perspectives. pp 513–529

208. Zhang W, Mason GA (2022) Modulating the rhizosphere microbiome by altering the cocktail of root secretions. Plant Physiology 188:12–13

209. Sun H, Jiang S, Jiang C, Wu C, Gao M, Wang Q (2021) A review of root exudates and rhizosphere microbiome for crop production. Environmental Science and Pollution Research 2021 1–14

210. Wu L, Kobayashi Y, Wasaki J, Koyama H (2018) Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Science and Plant Nutrition 64:697–704

211. Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480

212. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

213. Bergelson J, Brachi B, Roux F, Vailleau F (2021) Assessing the potential to harness the microbiome through plant genetics. Current Opinion in Biotechnology 70:167–173

214. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A (2020) Plant microbiome–an account of the factors that shape community composition and diversity. Current Plant Biology 23:100161

215. French E, Kaplan I, Iyer-Pascuzzi A, Nakatsu CH, Enders L (2021) Emerging strategies for precision microbiome management in diverse agroecosystems. Nature Plants 7:256–267

216. Roman-reyna V, Pinili D, Borjaa FN, et al (2019) The Rice Leaf Microbiome Has a Conserved Community Structure Controlled by Complex Host-Microbe Interactions. https://doi.org/10.2139/ssrn.3382544

217. Wipf HML, Coleman-Derr D (2021) Evaluating domestication and ploidy effects on the assembly of the wheat bacterial microbiome. PLOS ONE 16:e0248030

218. Gholizadeh S, Mohammadi SA, Salekdeh GH (2022) Changes in root microbiome during wheat evolution. BMC Microbiology 22:64

219. Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, Tschaplinski TJ, Schadt CW (2019) Rhizosphere microbiomes diverge among Populus trichocarpa plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome 7:76

220. Cordovez V, Rotoni C, Dini-Andreote F, Oyserman B, Carrión VJ, Raaijmakers JM (2021) Successive plant growth amplifies genotype-specific assembly of the tomato rhizosphere microbiome. Science of The Total Environment 772:144825

221. Bennetzen JL, Hake S (eds) (2009) Handbook of Maize. https://doi.org/10.1007/978-0-387-77863-1

222. corn : USDA ARS. https://www.ars.usda.gov/oc/timeline/corn/. Accessed 8 Dec 2021

223. Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/commodities/corn. Accessed 28 May 2022

224. USDA - National Agricultural Statistics Service - Publications. https://www.nass.usda.gov/Publications/. Accessed 28 May 2022

225. Dewey,Lee, Nolan, Reagan (2018) A Guide to Corn Production in Georgia 2018. University of Georgia Cooperative Extension

226. Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

227. Ogle DH, Doll JC, Wheeler P, Dinno A (2022) FSA: Fisheries Stock Analysis.

228. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR,, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package.

229. UpSetR: an R package for the visualization of intersecting sets and their properties | Bioinformatics | Oxford Academic. https://academic.oup.com/bioinformatics/article/33/18/2938/3884387. Accessed 5 May 2022

230. Pauvert C (2021) psadd: Additions to phyloseq package for microbiome analysis.

231. Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12:1–10

232. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9:980–989

233. Mueller UG, Juenger TE, Kardish MR, Carlson AL, Burns K, Smith C, Marais DLD Artificial Selection on Microbiomes to Confer Salt-Tolerance to Plants Artificial Microbiome-Selection to Engineer Microbiomes That Confer Salt-Tolerance to Plants 1 2. https://doi.org/10.1101/081521

234. Chiu C-H, Jost L, Chao A (2014) Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers. Ecological Monographs 84:21–44

235. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. ISME J 5:169–172

236. Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M (2016) High taxonomic variability despite stable functional structure across microbial communities. Nat Ecol Evol 1:1–12

237. McCaw ME, Wallace JG, Albert PS, Buckler ES, Birchler JA (2016) Fast-Flowering Mini-Maize: Seed to Seed in 60 Days. Genetics 204:35–42

238. Mehboob I, Zahir Z, Arshad M, Tanveer A, Khalid M (2012) Comparative effectiveness of different rhizobium sp. for improving growth and yield of maize (Zea mays L.). Soil and Environment 31:37–46

239. Estrada GA, Baldani VLD, Oliveira DM de, Urquiaga S, Baldani JI (2013) Selection of phosphate-solubilizing diazotrophic Herbaspirillum and Burkholderia strains and their effect on rice crop yield and nutrient uptake. Plant and Soil 369:115–129

240. Doni F, Suhaimi NSM, Irawan B, Mohamed Z, Mispan MS (2021) Associations of Pantoea with Rice Plants: As Friends or Foes? Agriculture 11:1278

241. Quecine MC, Araújo WL, Rossetto PB, Ferreira A, Tsui S, Lacava PT, Mondin M, Azevedo JL, Pizzirani-Kleiner AA (2012) Sugarcane Growth Promotion by the Endophytic Bacterium Pantoea agglomerans 33.1. Applied and Environmental Microbiology 78:7511–7518

242. Soluch R, Hülter NF, Romero Picazo D, Özkurt E, Stukenbrock EH, Dagan T (2021) Colonization dynamics of Pantoea agglomerans in the wheat root habitat. Environmental Microbiology 23:2260–2273

243. Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (Zea mays) by bacteria: Implications for energy plant biomass production and phytoremediation. Journal of Environmental Management 103:58–64

244. Piacentino D, Grant-Beurmann S, Vizioli C, et al (2021) Gut microbiome and metabolome in a non-human primate model of chronic excessive alcohol drinking. Transl Psychiatry 11:609

245. Coles VJ, Stukel MR, Brooks MT, et al (2017) Ocean biogeochemistry modeled with emergent trait-based genomics. Science 358:1149–1154

246. Levy A, Salas Gonzalez I, Mittelviefhaus M, et al (2017) Genomic features of bacterial adaptation to plants. Nat Genet 50:138–150

247. Bengelsdorf FR, Beck MH, Erz C, Hoffmeister S, Karl MM, Riegler P, Wirth S, Poehlein A, Weuster-Botz D, Dürre P (2018) Chapter Four - Bacterial Anaerobic Synthesis Gas (Syngas) and CO2+H2 Fermentation. In: Sariaslani S, Gadd GM (eds) Advances in Applied Microbiology. Academic Press, pp 143–221

248. Brilli F, Loreto F, Baccelli I (2019) Exploiting Plant Volatile Organic Compounds (VOCs) in Agriculture to Improve Sustainable Defense Strategies and Productivity of Crops. Frontiers in Plant Science 10:

249. Scala A, Allmann S, Mirabella R, Haring MA, Schuurink RC (2013) Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. International Journal of Molecular Sciences 14:17781–17811

250. Stolterfoht H, Rinnofner C, Winkler M, Pichler H (2019) Recombinant Lipoxygenases and Hydroperoxide Lyases for the Synthesis of Green Leaf Volatiles. J Agric Food Chem 67:13367–13392

251. Mosquito S, Bertani I, Licastro D, Compant S, Myers MP, Hinarejos E, Levy A, Venturi V (2020) In Planta Colonization and Role of T6SS in Two Rice Kosakonia Endophytes. MPMI 33:349–363

252. Kamat SS, Raushel FM (2013) The enzymatic conversion of phosphonates to phosphate by bacteria. Current Opinion in Chemical Biology 17:589–596

253. Diversity and abundance of phosphonate biosynthetic genes in nature | PNAS. https://www.pnas.org/doi/10.1073/pnas.1315107110. Accessed 31 May 2022

254. Navarro JA, Durán RV, De la Rosa MA, Hervás M (2005) Respiratory cytochrome c oxidase can be efficiently reduced by the photosynthetic redox proteins cytochrome c6 and plastocyanin in cyanobacteria. FEBS Letters 579:3565–3568

255. Deisenhofer J, Michel H (1993) 17 - Three-Dimensional Structure of the Reaction Center of Rhodopseudomonas viridis. In: Deisenhofer J, Norris JR (eds) Photosynthetic Reaction Center. Academic Press, San Diego, pp 541–558

256. Cardona T (2015) A fresh look at the evolution and diversification of photochemical reaction centers. Photosynth Res 126:111–134

257. Fleischman D (2012) Chapter 51 - Photosynthesis. In: Sperelakis N (ed) Cell Physiology Source Book (Fourth Edition). Academic Press, San Diego, pp 909–924

258. Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF (2000) Variation among Maize Inbred Lines and Detection of Quantitative Trait Loci for Growth at Low Phosphorus and Responsiveness to Arbuscular Mycorrhizal Fungi. Crop Science 40:358–364

259. Whitaker BK, Vaughan MM, McCormick SP (2022) Biocontrol Impacts on Wheat Physiology and Fusarium Head Blight Outcomes Are Bacterial Endophyte Strain and Cultivar Specific. Phytobiomes Journal PBIOMES-08-22-0056-R

260. Abdul Rahman NSN, Abdul Hamid NW, Nadarajah K (2021) Effects of Abiotic Stress on Soil Microbiome. International Journal of Molecular Sciences 22:9036

261. Trivedi P, Batista BD, Bazany KE, Singh BK (2022) Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytologist 234:1951–1959

262. Tiziani R, Miras-Moreno B, Malacrinò A, Vescio R, Lucini L, Mimmo T, Cesco S, Sorgonà A (2022) Drought, heat, and their combination impact the root exudation patterns and rhizosphere microbiome in maize roots. Environmental and Experimental Botany 203:105071

263. Swift JF, Kolp MR, Carmichael A, Ford NE, Hansen PM, Sikes BA, Kleiner M, Wagner MR (2023) Legacy effects of precipitation and land use impact maize growth and microbiome assembly under drought stress. 2023.04.11.536405

264. Methe BA, Hiltbrand D, Roach J, Xu W, Gordon SG, Goodner BW, Stapleton AE (2020) Functional gene categories differentiate maize leaf drought-related microbial epiphytic communities. PLOS ONE 15:e0237493

265. Vescio R, Malacrinò A, Bennett AE, Sorgonà A (2021) Single and combined abiotic stressors affect maize rhizosphere bacterial microbiota. Rhizosphere 17:100318

266. Mukhtar S, Mirza BS, Mehnaz S, Mirza MS, Mclean J, Malik KA (2018) Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome. World J Microbiol Biotechnol 34:136

267. Guan Y, Jiang N, Wu Y, Yang Z, Bello A, Yang W (2021) Disentangling the role of salinity-sodicity in shaping soil microbiome along a natural saline-sodic gradient. Science of The Total Environment 765:142738

268. Yuan Y, Brunel C, van Kleunen M, Li J, Jin Z (2019) Salinity-induced changes in the rhizosphere microbiome improve salt tolerance of Hibiscus hamabo. Plant Soil 443:525–537

269. Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Applied Soil Ecology 107:324–333

270. Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host & Microbe 22:142–155

271. Babalola OO, Fadiji AE, Enagbonma BJ, Alori ET, Ayilara MS, Ayangbenro AS (2020) The Nexus Between Plant and Plant Microbiome: Revelation of the Networking Strategies. Frontiers in Microbiology 11:

272. Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Gutjahr C, Falter-Braun P (2019) Systems Biology of Plant-Microbiome Interactions. Molecular Plant 12:804–821

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

274. 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

275. Zheng W, Zhao Z, Gong Q, Zhai B, Li Z (2018) Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil. Soil Biology and Biochemistry 125:54–63

276. Ritter CD, Forster D, Azevedo JAR, Antonelli A, Nilsson RH, Trujillo ME, Dunthorn M (2021) Assessing Biotic and Abiotic Interactions of Microorganisms in Amazonia through Co-Occurrence Networks and DNA Metabarcoding. Microb Ecol 82:746–760

277. Chen M, He S, Li J, Hu W, Ma Y, Wu L, Gang G (2019) Co-occurrence patterns between bacterial and fungal communities in response to a vegetation gradient in a freshwater wetland. Can J Microbiol 65:722–737

278. (2023) Corn. In: USDA Foreign Agricultural Service. https://www.fas.usda.gov/data/commodities/corn. Accessed 27 Apr 2023

279. Ling N, Zhu C, Xue C, Chen H, Duan Y, Peng C, Guo S, Shen Q (2016) Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biology and Biochemistry 99:137–149

280. Wu X, Hu H, Li S, Zhao J, Li J, Zhang G, Li G, Xiu W (2022) Chemical fertilizer reduction with organic material amendments alters co-occurrence network patterns of bacterium-fungus-nematode communities under the wheat–maize rotation regime. Plant Soil 473:605–623

281. Bazany KE, Wang J-T, Delgado-Baquerizo M, Singh BK, Trivedi P (2022) Water deficit affects inter-kingdom microbial connections in plant rhizosphere. Environmental Microbiology 24:3722–3734

282. Cobo-Díaz JF, Baroncelli R, Le Floch G, Picot A (2019) Combined Metabarcoding and Co-occurrence Network Analysis to Profile the Bacterial, Fungal and Fusarium Communities and Their Interactions in Maize Stalks. Frontiers in Microbiology 10:

283. Rogers AR, Holland JB (2022) Environment-specific genomic prediction ability in maize using environmental covariates depends on environmental similarity to training data. G3 Genes|Genomes|Genetics 12:jkab440

284. Kick DR, Wallace JG, Schnable JC, et al (2022) Yield Prediction Through Integration of Genetic, Environment, and Management Data Through Deep Learning. 2022.07.29.502051

285. Westhues CC, Simianer H, Beissinger TM (2022) learnMET: an R package to apply machine learning methods for genomic prediction using multi-environment trial data. G3 Genes|Genomes|Genetics 12:jkac226

286. Can High-Resolution Satellite Multispectral Imagery Be Used to Phenotype Canopy Traits and Yield Potential in Field Conditions? https://doi.org/10.13031/trans.14197. Accessed 26 May 2023

287. ImageBreed: Open-access plant breeding web–database for image-based phenotyping - Morales - 2020 - The Plant Phenome Journal - Wiley Online Library. https://acsess.onlinelibrary.wiley.com/doi/full/10.1002/ppj2.20004. Accessed 26 May 2023

288. Wiesner-Hanks T, Wu H, Stewart E, DeChant C, Kaczmar N, Lipson H, Gore MA, Nelson RJ (2019) Millimeter-Level Plant Disease Detection From Aerial Photographs via Deep Learning and Crowdsourced Data. Frontiers in Plant Science 10:

289. Bai G, Ge Y, Scoby D, Leavitt B, Stoerger V, Kirchgessner N, Irmak S, Graef G, Schnable J, Awada T (2019) NU-Spidercam: A large-scale, cable-driven, integrated sensing and robotic system for advanced phenotyping, remote sensing, and agronomic research. Computers and Electronics in Agriculture 160:71–81

290. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522

291. Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18:1403–1414

292. Walters W, Hyde ER, Berg-Lyons D, et al (2016) Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for

Microbial Community Surveys. mSystems. https://doi.org/10.1128/MSYSTEMS.00009-15

293. Xie J, Fu Y, Jiang D, Li G, Huang J, Li B, Hsiang T, Peng Y (2008) Intergeneric transfer of ribosomal genes between two fungi. BMC Evolutionary Biology 8:87

294. Free Weather API | Visual Crossing. https://www.visualcrossing.com/weather-api. Accessed 23 May 2023

295. Peschel S, Müller CL, von Mutius E, Boulesteix A-L, Depner M (2021) NetCoMi: network construction and comparison for microbiome data in R. Briefings in Bioinformatics 22:bbaa290

296. Jun W, Barahona M, Yue-Jin T, Hong-Zhong D (2010) Natural Connectivity of Complex Networks. Chinese Phys Lett 27:078902

297. J Fox, S Weisberg (2019) An R Companion to Applied Regression, 3rd Edition. Sage

298. Smith SD (2019) phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. Journal of Open Source Software 4:1442

299. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 57:289–300

300. Bairoch A (2000) The ENZYME database in 2000. Nucleic Acids Res 28:304–305

301. Simon HM, Jahn CE, Bergerud LT, Sliwinski MK, Weimer PJ, Willis DK, Goodman RM (2005) Cultivation of Mesophilic Soil Crenarchaeotes in Enrichment Cultures from Plant Roots. Appl Environ Microbiol 71:4751–4760

302. Madegwa YM, Uchida Y (2021) Land use and season drive changes in soil microbial communities and related functions in agricultural soils. Environmental DNA 3:1214–1228

303. Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

304. Bahram M, Hildebrand F, Forslund SK, et al (2018) Structure and function of the global topsoil microbiome. Nature 560:233–237

305. Bérdy J (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot 65:385–395

306. Li X, Garbeva P, Liu X, klein Gunnewiek PJA, Clocchiatti A, Hundscheid MPJ, Wang X, de Boer W (2020) Volatile-mediated antagonism of soil bacterial communities against fungi. Environmental Microbiology 22:1025–1035

307. Koopman N, Remijas L, Seppen J, Setlow P, Brul S (2022) Mechanisms and Applications of Bacterial Sporulation and Germination in the Intestine. Int J Mol Sci 23:3405

308. Chamkhi I, El Omari N, Benali T, Bouyahya A (2020) Quorum Sensing and Plant-Bacteria Interaction: Role of Quorum Sensing in the Rhizobacterial Community Colonization in the Rhizosphere. In: Quorum Sensing: Microbial Rules of Life. American Chemical Society, pp 139–153

309. Venturi V, Keel C (2016) Signaling in the Rhizosphere. Trends in Plant Science 21:187–198

310. Picazo-Aragonés J, Terrab A, Balao F (2020) Plant Volatile Organic Compounds Evolution: Transcriptional Regulation, Epigenetics and Polyploidy. Int J Mol Sci 21:8956

311. Raza W, Wei Z, Jousset A, Shen Q, Friman V-P (2021) Extended Plant Metarhizobiome: Understanding Volatile Organic Compound Signaling in Plant-Microbe Metapopulation Networks. mSystems 6:e00849-21

312. Rho H, Doty SL, Kim S-H (2018) Estimating microbial respiratory CO2 from endophytic bacteria in rice. Plant Signaling & Behavior 13:e1500067

313. Suryanarayanan TS, Ayesha MS, Shaanker RU (2022) Leaf photosynthesis: do endophytes have a say? Trends in Plant Science 27:968–970

314. Khare E, Mishra J, Arora NK (2018) Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. Frontiers in Microbiology 9:

CHAPTER 5

Conclusions

The microbial community can have a significant effect on its host's health and yield in crop plants. With a number of new microbial products on the product, and the potential for bacteria and fungi to benefit farmers, maize endophytes and the microbiome are an expanding area of research. In these projects I aimed to asses how maize genetics and the environment shape interactions between maize and its microbes, as well as interactions among the microbes. I examined a number of tissues in maize below and above ground, and note sharp differences in microbial communities living outside the plant compared to inside.

In our first project I inoculated a subset of the NAM founders with two bacteria and a fungus that had the potential to promote growth. My findings highlight how growth-promoting endophytes interacted differently with diverse maize germplasm. *Herbaspirillum seropedicae* and *Serendipita bescii* differentially promoted growth for several maize genotypes, and even hindered growth in some instances. *Herbaspirillum seropedicae* increased chlorophyll content, plant height, root length, and root volume. *Serendipita bescii* increased plant height, root biomass, and shoot biomass. I showed that there is a complex interaction between maize cultivar and individual endophytes. This interaction may involve regulating endophyte colonization levels, as colonization levels of *Serendipita* correlated with some growth-promotion phenotypes. These findings provide insight into the range of responses between plants and microbes, and are especially important for groups developing new bioinoculates and biofertilizers. Many farmers struggle with consistent performance of microbial products, and the high variability of growth promotion across genotypes showcased in this project may partly explain why many beneficial microbes reported in the literature fail to translate to field production.

In my second project we sought to examine how inbreeding affects maize microbiomes in different tissues using inbred, hybrid, and open-pollinated maize lines grown in the field and the greenhouse. My data show that inbreeding has small and significant effects on taxa and function in maize microbial communities, however, these effects pale in comparison to the effect environment and tissue type have on community composition. Differential abundance testing found 25 bacteria ASVs that differed between inbred and hybrid maize, while there were massive differences when comparing tissue types and environment. Using predicted functional genomics we found pathways that may be involved with heterosis, and showed that functionality of the microbiome may be more important than taxonomic descriptions.

In my third project, I sought two expand upon the findings of project 2 by analyzing how the environment effected microbiomes in several tissues in 30 locations across the United States. In this project we examined the structure, potential function, and interactions of maize bacteria and fungi communities. I found tissue had a larger effect on the maize microbiome than location, indicating that tissue compartments around the country are structurally and functionally more similar to each other than to other tissues in the same field. Throughout my analysis, I find distinct differences between exterior microbes versus endophytes, including predicted gene functions that may be critical for endophyte colonization.

The maize microbiome is a well researched field of study, and this work expands upon factors that effect these complex interactions. Understanding how maize interacts with beneficial microbes, and its greater microbial community, will allow us to leverage the abilities of bacteria and fungi to increase yield and keep maize healthier. To feed a growing population we need innovation in agriculture, and using microbes in a safe and responsible manner, have the potential to supplement our farming system and have a positive impact on growers and

consumers. I have generated several hypothesis throughout these projects that require further research and validation. Follow up studies exploring (1) How we can ensure beneficial microbes will work with genetically diverse maize in different environments and (2) Understand how maize genetics and the environment change microbiome function throughout plant tissues, will allow us to understand more of the mechanisms behind these complex interactions. This work argues for the adoption of microbiome function in conjunction than taxa as the unit of analysis, and several follow up studies using metabolomics and metatranscriptomics have been proposed.