Title

The role of phyllosphere microbiome in plant health: Understanding the complex processes that shape the services bacteria provide for their host organism

Author

Mehlferber, Elijah Curtis Pederson

Publication Date

2022

The role of phyllosphere microbiome in plant health: Understanding the complex processes that shape the services bacteria provide for their host organism

By

Elijah Curtis Pederson Mehlferber

A dissertation submitted in partial satisfaction of the
requirements of the degree of
Doctor of Philosophy
in
Integrative Biology
in the
Graduate Division
of the
University of California, Berkeley

Committee in charge:

Professor Britt Koskella, Chair
Professor Mike Boots
Professor Steve Lindow

Summer 2022

Abstract

The role of phyllosphere microbiome in plant health: Understanding the complex processes that shape the services bacteria provide for their host organism

by

Elijah Curtis Pederson Mehlferber

Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor Britt Koskella, Chair

Host associated microbial communities can provide a broad set of functions to the organisms they inhabit. However, while these communities can be critically important to host health and development, the interactions and mechanisms that underly these functions, as well as the context in which they matter, are less well understood. The work presented here seeks to provide a better understanding of these host-microbial interactions by utilizing a plant phyllosphere (the bacteria inhabiting the aboveground portion of the plant) model system, and a model microbial community (Synthetic Community) that is a simplified representation of the bacteria that would interact with the plant in a natural setting.

This research begins by outlining the development of the Synthetic Community that will be used throughout the rest of the work to investigate these host-microbe interactions. This chapter highlights the methods used to build the community, the resources that were developed to aid in the use of the community, and a selection of experiments in which the community is initially used. These initial experiments show the importance of understanding functional potential when predicting interactions among species, as well as the ability of individually protective species to work synergistically to yield better outcomes when their host is challenged by a pathogen.

In the following chapter, the Synthetic Community is used to investigate the importance of the phyllosphere to the development and reproductive success of the host plant, specifically looking at colonization and growth of tomato plants. This begins with the hypothesis that phyllosphere interactions will be disrupted in a greenhouse setting where microbial dispersal is limited, and that adding the synthetic community will yield important benefits to the host plant, allowing for a better understanding of the role of these bacteria in plant development. In a series of experiments taking place over several trials, I confirm that greenhouse-grown plants have a depauperate phyllosphere microbiome and that the addition of the Synthetic Community is responsible for a clear and repeatable increase in fruit production in this setting, but not in the field. I further show that this effect is synergistic with the addition of micronutrient-based soil amendments, and that modulating nutrition through conventional fertilizer can alter the protective capacity of these communities. These results suggest that greenhouse environments have poor phyllosphere microbiome establishment, with negative impacts on the plant. The results also implicate the phyllosphere microbiome as a key component of plant fitness,

emphasizing that these communities have a clear role to play in the ecology and evolution of plant communities.

In the final chapter, I focus on the role that host condition plays in modulating the reliance on the microbiome. To do this, I look at the effect of whole-genome duplication in *Arabidopsis thaliana* on the phyllosphere microbiome, using the Synthetic Community, and determine the interacting impacts of ploidy and microbiome on disease outcome. This chapter shows polyploids fare better against the pathogen than diploids, regardless of microbial inoculation, while diploids harboring an intact microbiome have lower pathogen densities than those without. In addition, diploids have elevated numbers of defense-related genes that are differentially expressed in the presence of their phyllosphere microbiota, while polyploids exhibit some constitutively activated defenses regardless of colonization by the synthetic community. These results imply that whole-genome duplication can enhance immunity resulting in a decreased dependence on the microbiome for protection against pathogens.

To those who helped me along the way, but are no longer with us:
Roger, Dana, Teri, Walter, Kathryn, and Frank.

Table of Contents

Chapter 1: Introduction

Chapter 2: The design and use of a phyllosphere synthetic community

Chapter 3: Early phyllosphere microbial associations impact plant reproductive success

Chapter 4: Polyploidy and microbiome associations mediate similar responses to pathogens in Arabidopsis

Acknowledgements

I'm not sure if it would be possible to adequately express the level of thanks I have for the enumerable people throughout my life who have helped me reach this moment. With that in mind, I will attempt to do my best, and to keep things roughly in chronological order.

I would first like to thank my family, especially my mother and father for instilling a love of learning in me from an early age. With this, they set me upon the path that would lead all the way through graduate school, and I wouldn't have made it all this way without their love and support. I would like to thank all the teachers that I had throughout grade, middle and high school, and specifically mention Kelly York, my 9thgrade biology teacher who encouraged me to consider a PhD in science as a potential career.

There is no way that I would be here without the help of Drs. Trish and Allen Moore, who I worked with throughout my time as an undergraduate at the University of Georgia. Trish gave me a level of scientific independence that most undergraduates never get, and my experience leading projects across her and Allen's labs cemented my interest in continuing research as my career. I would also like to thank my friends throughout college, without them and their support I wouldn't have made it nearly this far.

Throughout graduate school I have had the wonderful opportunity to work with some of the most amazing scientists in the world, first and foremost my advisor Dr. Britt Koskella. From the first time I spoke to Britt I knew that she had two qualities that would make her an amazing mentor: creativity and compassion. Being smart can only get you so far in science, and Britt certainly has that going for her, but she also has a willingness to strike out and try new things that pushes her science, and the science of those working around her, to another level. She has, hands down, absolutely the best experimental designs of anyone I have worked with, and somehow, she always manages to find a way for a project to ask that one extra question. Beyond that though, she truly cares about her students and colleagues, in an almost unprecedented way. Whenever anyone has asked what lab I'm in, the next comment is invariably "Wow, you're so lucky, Britt is amazing". And they're right, because I am incredibly lucky to have found her lab, without it, not only would I not be the scientist that I am today, but I may not have been a scientist at all.

Besides the amazingness that Britt adds to the Koskella lab, the rest of the lab follows suit in terms of their warmth and wickedly cool science. Without Norma and Cathy my first few years of graduate school would have been impossibly hard, and as our lab has grown it has been amazing to see how we've all developed as scientists together. I owe a number of lab members thanks for amazing conversations throughout the years, both about science and life, and I would like to specifically thank Reena, Tristan, Isabella, Alina, Kyle, Wenke, Kama, Kate, Emily, and Julia. For Asa, Claire, Dominique and Milo, while our interactions have been somewhat limited by COVID, I can tell that I'm leaving the lab in good hands, and I can't wait to see how you and future lab members shape its direction.

I also owe a very special thanks to the undergraduates that have worked with me throughout their time at Berkeley, specifically Rachel, Elodie, Nikita, James and Lina. All of you helped me immeasurably throughout the process of completing this PhD, and, while I hope you learned something from me, I know I have learned more from working with all of you.

Beyond the members of my lab, I would like to thank the Integrative Biology department for fostering an environment where we can learn and grow as scientists, and all the friends I have made along the way throughout the many labs. Especially, thank you to all the members of my cohort, and the honorary ones as well. Without you this process would have been lonely and intolerably boring, and I couldn't have been luckier to find this group of people to go through grad school with. Even (or especially) the times we were in lab late into the night were fun with all of you around.

Some other members of the IB (and broader Berkeley) community deserve special recognition, specifically the members of my qualifying exam committee, Devin Coleman-Derr, Noah Whiteman, Wayne Sousa, Mike Boots and Steve Lindow. While this process was certainly hard, I don't think there was a point in graduate school, or maybe even ever, where I learned so much in so little time. I still look back on my development as a scientist as a clear delineation between before and after the exam, and I am eternally grateful for your parts in making this process both as enjoyable and productive as possible.

I would like to further thank Mike and Steve, both members of my dissertation committee, for all the amazing chats that we have had throughout this process. Both of you have been so incredibly generous with your time and your ideas and have been truly influential in me becoming the scientist I am today.

Finally, last but most certainly not least, I am eternally grateful to my wife, Hannah Wargowsky, for all her support throughout this process. Five years ago, she decided to move out to California with me when I accepted my offer at Berkeley, and I truly would not have made it without her by my side. Nothing in life is easy, especially not grad school, and she has been there to support me throughout the toughest times, to help console me after every failure, and to help me celebrate at every triumph. From my first year at Berkeley, to our tiny first apartment together, to the beginning of COVID and driving across the country 6 times, she has been there with me throughout all of this. All this time that I have been growing and developing as a scientist she has also been helping me grow as a person. She is smart, and compassionate, and she cares deeply about what it means to be good. And even more, she cares enough to push everyone around her to be better. I wouldn't be here if not for the help of all the amazing people along the way, but for every person of which that is true, most especially her.

As an addendum, I would like to specifically mention my sister, Linden, and my sisters in law Isabel and Olivia, as well as my parents in law Kim and Kent, you have each helped me so much along this path. Also, my dog Juniper, while I'm not sure she knows what I've been doing these five years, she has been immeasurably supportive throughout the process.

Chapter 1. Introduction

1.1 Host-Microbiome Interactions

Most multi-cellular organisms are closely associated with a diverse consortia of microbial species, collectively termed (inclusive of their genes and environment) the microbiome (Berg *et al.*, 2020). These microbes, which are primarily bacterial or fungal, live in, or on their hosts, and are capable of modulating host outcomes through the various functions that they provide. The importance of these host-associated microbial communities is of broad interest across scientific fields, comprising studies focused on their roles in the health, development, and evolution of their hosts (Stappenbeck & Virgin, 2016; Foster *et al.*, 2017; Dominguez-Bello *et al.*, 2019; Malard *et al.*, 2021). For example, the gut microbial community of *Drosophila melanogaster* has been shown to influence development and reproductive success, as well as life span, with more diverse communities linked to greater fecundity but also shorter life spans (Gould *et al.*, 2018). Meanwhile, in plants, it has been shown that exposure to certain non-pathogenic bacteria can induce long lasting defense responses that protect against future pathogen establishment (Conrath *et al.*, 2002).

In fact, these interactions, and their fitness consequences, are so profound that some scientists argue that the host and its microbiome should be considered a single evolutionary unit, the; hologenome (Zilber-Rosenberg & Rosenberg, 2008; Bordenstein & Theis, 2015). This theory proposes that the symbiotic relationships between hosts and their associated microbial communities influence the fitness of the collective "holobiont", these communities are capable of being transmitted transgenerationally, allowing for the inheritance of microbial associations, and that differences in the composition of microbial communities represent variation on which selection can act. Consequently, this "super-organism" should be considered to have a single "hologenome", which can be shaped by evolutionary forces. While the interactions between some species and their symbionts fulfill the requirements to be linked as a single unit of selection, for example aphids and their obligate intercellular bacteria *Buchnera* (Douglas, 1998; Braendle *et al.*, 2003), it is unclear, and hotly debated, to what extent this idea can be generalized across host associated microbial communities (Leggat *et al.*, 2007; Moran & Sloan, 2015; Theis *et al.*, 2016). Despite this controversy, it is clear that these communities can be critically important to their hosts, and their interactions and functions warrant further study.

When attempting to study the importance of a host-associated microbial community there are several questions to ask. First, what functions can this community perform for its host? Are these microbes important in the development of the host? Can they provide disease protection? How do they influence host fitness? Second, it is important to understand the mechanisms by which these benefits take place. Do certain bacteria produce inhibitory compounds that prevent pathogen colonization? Do the functions arise from emergent properties of the interactions within the system? Is the diversity of the community important? Etc. Finally, there is the question as to when these interactions are important. Like in the hygiene hypothesis, which posits that the immune system requires exposure to pathogens early in its development in order to be optimally functional (Okada *et al.*, 2010), there could be certain times throughout the development of the host where they are receptive to the effects of their microbiome, or certain conditions under which the microbial species are more or less important (Metcalf *et al.*, 2019).

1.2 The Phyllosphere Microbiome

To better understand the importance of host-associated microbial communities, including the functions they can perform, the mechanisms underlying those functions, and the context in which they are important, a model system must first be selected. For the purposes of this dissertation, the research herein focuses on understanding the contribution of the plant associated phyllosphere microbiome (comprising species living on the aerial surfaces of the plant, primarily the leaves), and its functional importance to the host. Plant associated microbial communities are known to provide a broad set of functions for their host organisms, including the aforementioned drought protection, aiding in nutrient acquisition (Adesemoye *et al.*, 2008), and protection from pathogen development (Innerebner *et al.*, 2011). The bacteria that interact with the plant can be broadly characterized as inhabiting three different locations, either internal to the plant, the endosphere, external and in the soil, the rhizosphere, or external and on the aboveground surface (primarily the leaves), the phyllosphere (Dastogeer *et al.*, 2020).

Certainly, the bacteria residing in each of these locations have important implications for host fitness, but the Phyllosphere is both a conveniently tractable system, and relatively less is known about the function and importance of its microbial inhabitants. Compared to the rhizosphere, a greater proportion of bacterial species are culturable (Burch *et al.*, 2016), further, these species can be easily applied throughout the life of the plant and can be sampled with minimal destructive sampling, or, in some cases, capable leaving the surface intact (Remus-Emsermann *et al.*, 2014). Additionally, the Phyllosphere constantly recruits species from surrounding plants (Šantl-Temkiv *et al.*, 2018; Meyer *et al.*, 2022) and a large pool of both atmospheric (Bowers *et al.*, 2011; Ottesen *et al.*, 2016) and soil microbes (Bodenhausen *et al.*, 2013a; Copeland *et al.*, 2015), making it a convenient model to study ecological process such as competition, immigration and invasion (Meyer & Leveau, 2012).

The phyllosphere is made up of a diverse consortia of bacteria, with densities ranging from 10^6 to 10^7 cells per square centimeter on leaves (Lindow & Brandl, 2003). These epiphytes must contend with a hostile environment, encountering high levels of UV stress, temperature changes, and desiccation (Jacobs *et al.*, 2005; Beattie, 2011). Additionally, the phyllosphere is believed to be a very heterogeneous habitat with regard to resource distribution, with some patches able to support high levels of bacterial growth, interspersed across what might be considered a microbial wasteland (Remus-Emsermann *et al.*, 2012). Despite hosting many species, it is unclear the extent to which most of these bacteria are adapted to this environment or are unfortunate accidental colonizers. A recent study showed that passaging phyllosphere associated microbes in a greenhouse environment led to significant shifts in community composition over time, potentially indicating that many of these species are unable to successfully persist on the leaf surface over time (Morella *et al.*, 2020). While the bacteria inhabiting the phyllosphere have been investigated in terms of their ability to prevent disease (Mercier & Lindow, 2001; Innerebner *et al.*, 2011; Berg & Koskella, 2018; Morella *et al.*, 2019; Qin *et al.*, 2019), relatively little is known about the mechanisms or conditions that influence this protection, and even less is known about the other functions that they might perform for their hosts.

The potential functions of the phyllosphere associated microbiome can be grouped into several (sometimes overlapping) sub-categories, enhanced nutrient acquisition, phytohormone modification, buffering against environmental stress, and the biocontrol of pathogens (Glick, 2012), though broadly they function by either allowing the plant to achieve greater development than would otherwise be possible, or mitigating harm that would otherwise come to the host (Finkel *et al.*, 2017; Vannier *et al.*, 2019; Trivedi *et al.*, 2021; Ke *et al.*, 2021). These functions are better understood in the context of the below-ground plant associated communities, and the mechanisms that have been elucidated in those compartments will be explored in greater detail in the following section.

1.3 Mechanisms of Growth Promotion

The ability of certain microbes to enhance plant growth has been exploited for millennia (Finkel *et al.*, 2017), and the mechanisms by which they are able to promote that growth have been a topic of scientific curiosity since at least the late 1970's with the introduction of the term plant growth promoting rhizobacteria (PGPR) to describe bacteria that enhanced growth through biocontrol of pathogens (Kloepper *et al.*, 1980). Since that time, the term has been modified to plant growth promoting bacteria (PGPB) as it has become clear that the mechanisms of growth promotion are not limited to inhabitants of the rhizosphere, and to include other mechanisms of growth enhancement beyond disease suppression (Bashan & Holguin, 1998). As previously mentioned, this growth promotion can occur through several mechanisms that either enhance plant growth or stress tolerance, and there is reason to believe that these mechanisms could be occurring in the phyllosphere as well.

First, plant associated bacteria can directly enhance the growth of their host through aiding in the acquisition of nutrients. This can occur either through the production of those compounds themselves, a common example being the release of ammonia (NH3) through nitrogen fixation (Mus *et al.*, 2016), or by concentrating compounds that are already available but difficult for the plant to access, such as increased iron acquisition as a result of bacterially produced siderophores (Sharma *et al.*, 2003). Nitrogen fixation has been previously described in phyllosphere associated bacteria (RUINEN, 1965), and has been linked to increased yield (Pati & Chandra, 1981). Further, these bacteria can produce phytohormones that stimulate plant growth, or modify the functions of the plant immune system, with auxin and ethylene being two key examples (Remans *et al.*, 2008; Galland *et al.*, 2012). Bacteria inhabiting the phyllosphere are frequently reported to produce auxin (Lee *et al.*, 2004; Ali *et al.*, 2009), which has been reported to have both positive (Etesami *et al.*, 2015) and negative (Lindow *et al.*, 1998) impacts on plant outcomes.

Growth promotion can also happen through mechanisms that buffer abiotic and biotic stress, allowing the plant to grow more efficiently. A common example of abiotic stress reduction in plants is the increase in drought tolerance achieved through the recruitment of Actinobacteria in the rhizosphere (Xu *et al.*, 2018). Some mechanism of increased drought tolerance have also been reported through the application of phyllosphere bacteria (K *et al.*, 2020). Finally, these bacteria can benefit their host through protection against pathogen establishment. This can occur through direct inhibition of pathogen growth through chemical production or competition (Sturz & Christie, 2003; Compant *et al.*, 2010), or through the activation of induced systemic resistance (ISR) or priming, by the plant recognition of common bacterial epitopes such as flagellin (Felix *et al.*, 1999a; Conrath *et al.*, 2002; Mauch-Mani *et al.*, 2017). This is the most commonly studied aspect of phyllosphere associated microbial growth promotion, with several studies showing that certain bacteria, or communities, can effective prevent pathogen colonization and disease (Innerebner *et al.*, 2011; Berg & Koskella, 2018).

Again, while the potential for the functions highlighted throughout this section to take place is broadly understood, key questions remain about the exact benefits that occur, the circumstances under which they are important, and the mechanisms that influence how communities provide them.

1.4 The Synthetic Community Approach

A useful approach to understanding complex bacterial communities, and their interactions with their host organism, is to use a defined collection of species that mimic the natural diversity of the natural communities but at a tractable level of complexity. This is called the Synthetic Community approach, and was pioneered in a mouse gut model system (Faith *et al.*, 2011; McNulty *et al.*, 2011), and eventually used in the phyllosphere (Bodenhausen *et al.*, 2014). This approach allows for a reduced complexity community to be developed and then manipulated to investigate the functions that the phyllosphere will provide, the interactions that underly these functions, and the context in which they are important. The principles of designing SynCom's, and their use will be expanded upon in the second chapter of this dissertation, within which I design a model phyllosphere community.

1.5 Concluding introductory remarks

In the following chapters, I describe three projects that provide, at least in part, a clearer accounting of the complex interactions underlying the functions that phyllosphere bacterial communities can provide for their host organisms. In Chapter 2, I report the process of designing a SynCom to use as a model system in investigating these phyllosphere-microbiome interactions. This community, named PhylloStart, contains 16 species that are broadly representative of the species seen on field grown tomato plants, our model plant system. This chapter also highlights the resources that were developed to aid with the use of the community, including performing whole genome sequencing to understand the phylogenetic and functional traits of the community, measuring colony morphology to allow for identifying and distinguishing these species, and performing a resource utilization assay to understand the patterns in resource use across the members of the community. Finally, two preliminary experiments using these resources are reported, showing that members of the community have different competitive interactions with the model pathogen *Pseudomonas syringae* DC3000, potentially relating to their resource use patterns, and also that different combinations of species can lead to different levels of additive or subtractive disease protection.

In Chapter 3, I use the PhylloStart SynCom to understand the importance of phyllosphere communities in the development and reproductive success of tomato plants in the greenhouse. This environment provides an ideal natural experiment, as I show that these plants are depauperate of their natural communities, allowing for the inoculation of the SynCom and a comparison of the performance of plants that were and were not supplemented with these bacteria throughout the course of their development. Repeated over the course of several growing seasons, these experiments show that the plants with a complex phyllosphere community have significantly more flowers and yield more fruit (without any tradeoff in fruit size) than the plants that are relatively un-colonized. Comparing these plants to tomatoes that are grown in the field, where they can be colonized by a more complex natural community, we see that the effect of phyllosphere supplementation is only important in the greenhouse. Further, we show two examples of the community providing broad biotic and abiotic stress resistance in the greenhouse environment, in cases of over-fertilization with a micronutrient amendment (which otherwise decreases yield), and during powdery mildew disease incidence (a common threat to greenhouse grown plants). Finally, we employ a growth chamber based experiment to show that the community is able to provide protection against pathogen development in cases of plant nutrient stress, but that the protective benefits of this community might be limited in a higher nutrient environment. In total, these experiments show that the phyllosphere community is critical for the reproductive success of the plant, and that it can provide broad protection from biotic and abiotic stress.

Finally, in Chapter 4, the PhylloStart community is used to investigate the role of host condition in their reliance on the community to provide functions, in this case, disease protection. Leveraging an Arabidopsis model system, we first compare the development of the community across whole genome duplicated polyploid lines and their diploid ancestors. Interestingly, we see no conserved difference in the composition of these communities across ploidy level, which might seem to indicate that there would be no effect on disease outcomes. However, this is not the case, and when challenging these plants, as well as plants that were not inoculated with PhylloStart, with the pathogen *P. syringae* DC3000, we see a significant interaction between ploidy, the microbiome and pathogen development. Despite hosting relatively similar communities, the diploid plants require the presence of these bacteria to mount a defense against the pathogen, while the polyploids are broadly protected regardless of having a phyllosphere community or not. Using RNA sequencing we show that diploids respond to the community through a complex modulation of their gene expression patterns, including numerous defense associated genes, while the polyploids respond through relatively fewer changes in expression. Further, focusing on genes that are associated with DC3000 response, and are upregulated in the diploids in response to community exposure, we show that the polyploids constitutively express these at higher levels regardless of having a Phyllosphere microbiome. These results highlight the importance of context in terms of the functions that a community might provide for its host, as under certain conditions the host might rely on these bacteria.

Chapter 2. The design and use of a phyllosphere synthetic community

2.1 Introduction

Most living organisms harbor a diverse collection of microbial species, many of which play important roles in modulating their host's health. Plants, for instance, host bacteria both in their belowground compartments, the rhizosphere and root endosphere, as well as on their above ground surfaces, primarily their leaves. The role of the microbes residing in the rhizosphere and endosphere is a subject of great fascination, and studies have shown them to be important in a variety of circumstances, such as the modulation of flowering time (Wagner *et al.*, 2014), inducing resistance to disease (Hu *et al.*, 2016), and conferring tolerance to drought conditions (Naylor *et al.*, 2017). Recently, the field has begun to take greater interest in the bacteria inhabiting the aboveground portion of the plant, the phyllosphere (Lindow & Brandl, 2003; Knief *et al.*, 2010; Vorholt, 2012; Bodenhausen *et al.*, 2014; Copeland *et al.*, 2015).

The phyllosphere contains a diverse collection of bacterial residents, which can impact host health through the production of phytohormones (such as auxin) that influence their host's growth and development (Lee *et al.*, 2004; Meir *et al.*, 2010), as well as through the direct (due to competition for resources) and indirect (through activation of the plant immune system) protection from pathogens (Morella *et al.*, 2019). This compartment represents a tractable model for understanding inter-microbial and host-microbial dynamics due to its ease of manipulation (Meyer & Leveau, 2012), and the high oxygen environment lends to a relatively high proportion of culturable strains. Beyond its importance to theory, the phyllosphere is also implicated in global resource cycling, for example, through the activity of methane degrading microorganisms (Knief *et al.*, 2010) or nitrate assimilation (Parangan-Smith & Lindow, 2013), and is of critical interest for its potential applications in agriculture (de Souza *et al.*, 2020). As such, this compartment represents an ideal system to study the intersection of microbial dynamics and host health, lending itself to useful modeling and a clear path to applied science.

Despite being well poised as a model environment to study host-microbial dynamics, there are several practical issues to consider when working with the phyllosphere and its microbiota. First, the species associated with this compartment can be highly variable, with individuals arriving from wind and rain (Lindow & Brandl, 2003; Vorholt, 2012), from other nearby plant species (Meyer *et al.*, 2022) but also through larger patterns of aerial bacterial dispersal (Bowers *et al.*, 2011), though it is important to point out that these communities are in many ways distinct from broader airborne communities (Lymperopoulou *et al.*, 2016). Second, while there are strong selective forces shaping the composition of communities inhabiting this compartment, with these bacteria having to contend with desiccation stress, UV stress, as well as the pressures of the plant immune system, it is still unclear how many of these organisms are true phyllosphere residents versus unfortunate bystanders. Indeed, there is significant overlap between the species that colonize leaf surfaces and other non-organic surfaces nearby (Ottesen *et al.*, 2016), though there is the added complication that the arriving species on these non-leaf surfaces may be dispersing from populations that successfully established on nearby leaves. These issues can make it difficult to assess which species might play important roles in their communities or underly benefits to their host's fitness. Third, the sheer diversity of phyllosphere associated bacteria can make it difficult to ascertain the importance of individual species. Across various studies a vast number of unique phyllosphere inhabitants have been identified through 16s rRNA amplicon sequencing, with, for example; 1556 unique OTUs (Operational Taxonomic Unit, individual sequences binned at 97% similarity) identified in (Copeland *et al.*, 2015), 5476 OTUs identified in (Redford *et al.*, 2010), and a staggering 19,818 ASVs (Absolute Sequence Variants, a technique used to reconstruct and group exact sequences through the DADA2 pipeline) identified in (Meyer *et al.*, 2022). Some studies have worked around these problems by using communities sourced directly from the environment (Morella *et al.*, 2020), and while they are quite successful at identifying generalizable trends, it can be difficult to study specific mechanisms as they are inherently un-repeatable, their exact starting community can never be sampled twice.

One popular approach to address these types of issues is to utilize a defined collection of bacterial species that are representative of the natural diversity from the system in question, but at tractable complexity. These Synthetic Community's (commonly called SynComs) were first applied in a mouse gut model to investigate the role that diet plays in influencing gut bacterial community composition, as well as understanding the influence of probiotic strains on community structure and function (Faith *et al.*, 2011; McNulty *et al.*, 2011). The approach has gained traction among a number of different systems as a tool for understanding both ecological and host-microbial interactions (Großkopf & Soyer, 2014; Bodenhausen *et al.*, 2014), and has been used in a number of studies within the plant-microbiome field, for example to keystone species in root communities (Niu *et al.*, 2017), probe the role of diversity in disease protection in the rhizosphere (Hu *et al.*, 2016), and understand priority effects in phyllosphere colonization (Carlström *et al.*, 2019).

When designing a SynCom there are a number of choices that can be made (given an excellent and through review in Vorholt 2017), but these decisions break down essentially to how many, and which, species to include. A greater number of species will allow for a more faithful reproduction of the natural system and will be more likely to include species that play important roles in the community. However, a larger SynCom is inherently harder to work with, increasing combinatorial load and experimental complexity. Meanwhile, the choice of which species to include follows primarily from a consideration of the purpose of the SynCom. For example, a community that is designed to replicate a specific function of interested must at least include the species necessary to recapitulate that function, and so may initially include a very diverse assemblage, while a study focused on the role of strain variation in community dynamics might include relatively few unique species, but with several within strain variants of each.

By generating a model phyllosphere SynCom I was able to gain insight into what functions, broadly, these microbiota are playing in their host fitness and development, and how microbial, and host-microbial interactions can influence those functions. In this chapter I describe both the rationale behind and development of this SynCom, which has allowed me to address several key questions regarding how phyllosphere communities interact with their host plants (as described in chapters 3 and 4), as well as the genomic and phenotypic resources that I developed to aid in the use of this model system.

2.2 Methods

2.2.1 Strain Collection

PhylloStart was designed to mimic the composition of a field grown tomato phyllosphere community, but at reduced complexity to remain tractable. The community was initially designed based on communities sequenced from tomato plants in the student organic garden at UC Davis (Tab 1). The full methods associated with this sequencing is available in (Morella *et al.*, 2020) and are described in brief below. Leaves were collected from nine different sites spread across four fields and stored on ice before being transferred into 4ºC until processing. Sterile phosphate freezing buffer was added to each bag and the leaves were sonicated for 10 minutes and pooled by site before being pelleted for 10 minutes at 4000xG and frozen in glycerol freezing buffer until sequencing. DNA was extracted using the Qiagen PowerSoil DNA-extraction kit, with buffer controls for each set of extractions. Libraries were prepared using primers targeting the 16s rRNA gene V3-V4 region, and were sequenced via 300-paired end reads using Illumina MiSeq V3 at the The California Institute for Quantitative Biosciences (QB3) at the University of California, Berkeley (UC Berkeley). PNAs were added to reduce contamination due to chloroplast and mitochondria (Lundberg *et al.*, 2013). Sequences were processed in *mothur* (Schloss *et al.*, 2009) using the MiSeq SOP (Kozich *et al.*, 2013), with a 97% similarity cut-off for defining OTUs. The Silva reference database (Quast *et al.*, 2013) was used for sequence alignment and taxonomic assignment.

In order to select candidate species for the SynCom, all samples were pooled and species were ranked based on total relative abundance across samples. We found five bacterial families that were present across all pooled samples with a relative abundance greater than 1% these were (in decreasing order), Enterobacteriaceae, Pseudomonadaceae, Oxalobacteraceae, Bacillaceae, and Microbacteriaceae. To collect representative species from each of these families isolates leaves were collected from the UC Davis student organic garden (as previously described), and also from the endpoint of a greenhouse selection experiment (Morella *et al.*, 2020). These isolates were initially plated on KB and LB agar, followed by MacConkey, and 1% Tryptic Soy agar plates to isolate more fastidious species. In total, 93 potential isolates showing differential colony morphology were collected, and identified through 16s sanger sequencing [Include primer], using NCBI's BLASTn to match them to the closest sequenced relative. Of these 93 isolates, 43 were selected for whole genome sequencing, the methods of which are described below. After sequencing, several isolates were identified to be strain level variants, and so 16 species were selected as unique strains/species for inclusion into the working stock of the PhylloStart SynCom. These 16 species are representative at the family level of 97.8% of the bacterial families that were found at a relative abundance greater than 1% in this dataset, including the Enterobacteriaceae, Oxalobacteraceae, Pseudomonadaceae, Bacillaceae, Microbacteriaceae, with the addition of a member of the family Brevibacteriaceae that was identified at a high prevalence on our plated field samples during collection and so included. Within these 16 species, several families, including Enterobacteriaceae and Pseudomonadaceae had several unique species that were included, which is representative of their relative dominance in both our initial phyllosphere sequencing and throughout the literature (Vorholt, 2012; Ottesen *et al.*, 2013). After whole genome sequencing strain identification was performed by sequencing the genomes of each bacteria and matching the sequences for the 16s rRNA using BLAST to publicly available databases on NCBI.

2.2.2 Genome sequencing

The 43 isolates selected for whole genome sequencing were grown from their freezer stocks in 5ml KB for 3 days, including a negative control for freezer contamination and another for KB contamination. The samples were spun down at 4000xG for 10 minutes, after which the supernatant was removed, and lysis buffer was added directly to the tubes. Afterwards, they were transferred into a 2ml tub for DNA extraction using the DNeasy Blood and Tissue kit from QIAGEN, following either the Gram Negative or Gram Positive DNA extraction protocols based on their identification through 16s Sanger sequencing. Libraries were prepared for paired 150-paired-end reads in Illumina's HiSeq4000 platform (Illumina) at The California Institute for Quantitative Biosciences (QB3) at the University of California, Berkeley (UC Berkeley).

2.2.3 Genome assembly and functional predictions

The quality of the resulting paired-end Illumina reads was assessed using Fastqc v0.11.8, and reads were trimmed using Sickle v1.33. The reads were then assembled using Spades v3.13.1 (Bankevich *et al.*, 2012). After assembly, genomes were annotated using Prokka v1.12 (Seemann, 2014), and functional pathways were identified using MinPath (Ye & Doak, 2009). The 16s region of each bacteria was identified using BARNAPP 0.9, and the entire 16s gene was used to identify them through their closest match via BLAST to the NCBI 16s database. After the 16 species were selected for inclusion into the PhylloStart working community. These genomes were further quality controlled and analyzed using tools available in KBase. First the completeness of their genome assembly was assessed using CheckM v1.0.18, with default parameters, which calculates a set of genes with only one copy present across closely related species and identifies how many of those genes are present in the assembly (completeness) and if any genes are present at higher-than-expected abundances (>1, meaning contamination). Detailed assembly statistics for each PhylloStart member are shown in Table 2. Then, functional annotation was performed again using RAStK v1.073, with default parameters. The data illustrated in Figure 1 represents functional annotation using RAStK, functional annotations from Prokka are not included. Phylogenetic tree construction was performed using MUSCLE (Madeira *et al.*, 2022) to align the 16s v4 sequencing, and the tree was then constructed using the EMBL-EBI Simple Phylogeny program using default parameters, with *Thermococcus piezophilus* as the outgroup. The resulting tree was visualized using using iTOL (Interactive Tree of Life) with branch lengths included and default parameters.

2.2.4 Bacterial phenotypic identification

Each member of the PhylloStart community was grown for 3 days in a 50ml tube with KB broth. After which point, they were dilution streaked to generate single colonies under the flame onto one half of a square KB agar plate (leaving the other half of the plate for the next member) and allowed to develop overnight. Photos were taken and used to morphologically describe the species. Photo analysis was performed in R using *magick*.

2.2.5 Resource utilization assay (Biolog Plates)

Each member of the PhylloStart SynCom was grown for 3 days in 4x 2ml tubes KB broth shaking at setting 150 on a VWR orbital shaker at 28ºC. After that time tubes were spun down in a centrifuge at 3500rpm for 10 minutes, and the supernatant was removed. Bacteria were then resuspended in 1ml of MgCL, and combined in a 15ml falcon tube. Bacterial abundance was established by reading optical density at OD=600 in triplicate for each member. Bacteria were adjusted to a density of OD600 = 0.02 and were added into the Biolog Ecolplate at a volume of 150ul per well. Biolog Ecoplates were read at OD590 and OD750 three times the first day, two times the second and third day, and then again at seven days to establish that they had indeed reached carrying capacity by the end of the third day (which was found to be true for each species tested). In between readings each plate was stored statically in the incubator at [temperature], and plates were stacked to prevent fogging which would inhibit optical density readings. The Biolog readings for the model pathogen *P. syringae* DC3000 were performed at a later time, using the methods described above, but incubated in the plate reader statically at 25ºC for 50 hours with OD590 and OD750 readings taken every 8 minutes.

2.2.6 In Planta Competition Assay

To assess microbial growth and competition *in planta*, tomato (cultivar moneymaker) seeds were grown in 15ml clear falcon tubes containing approximately 5ml of 1% water agar. Seeds were sterilized by shaking with 70% ethanol for one minute, followed by 5 minutes of shaking in a solution containing 3% NaOCl w + 0.02% Tween20, then rinsed five times with autoclaved ddH_2O. A selection of seeds were placed on KB agar plates and incubated for 3 days at 28ºC to test for sterility. Seeds were placed into a 21ºC dark chamber until germination, at which point they were transferred into a growth chamber at 28ºC with a 15h-9h light/dark photoperiod. The selected PhylloStart strains were grown from freezer stocks overnight in liquid KB broth at 28ºC, and after transferring them into 10Mm $MgCl_2$, density was adjusted to OD600 = 0.002, and 0.02% SilWet was added. Plants were inoculated 10 days post germination by flooding the tubes with the bacterial suspension, after which they were allowed to sit for five minutes before the solution was poured off and the plants were left to dry for 1.5 hours in a biosafety cabinet. For co-inoculation treatments the two microbial suspensions were added at equal volumes and mixed via vortexing for 15 seconds prior to inoculation. After seedlings had dried they were transferred back into the growth chamber.

Microbial growth was assessed seven days after the initial inoculation. Prior to homogenization, seedlings were removed from their tubes under the flame with sterile tweezers and transferred into a sterile homogenization tube (1.5 ml, MP Biomedicals). This tube was then weighed on a precision scale and this measurement was used to normalize bacterial density to plant mass. Subsequently, 10 mM $MgCl_2$ was added and the plant were homogenized in a FastPrep-24 G5 (MP Biomedicals) at 4m/s for 60s using two sterile ceramic beads. Bacterial density was quantified by plating out serial dilutions and counting CFUs after incubation at 28ºC for 24 and 48 hours. In the co-inoculation experiments the two sets of bacteria were enumerated by differentiating between their colony morphologies, with the aid of a dilution set at 10^5 spread onto round petri dishes with glass beads to allow for better resolution and differentiation of morphology and CFU number.

2.2.7 Disease Assay

The plants used in the disease protection assay were prepared largely as described in the previous section, with the following adjustments. First, seeds were allowed to soak in the 3% NaOCl w + 0.02% Tween20 for 20 minutes instead of five, and they were rinsed in autoclaved ddH_2O three times instead of five. Three days after inoculation with *Pantoea*, or *Pantoea* and another focal species, of interest *P. syringae* DC3000 was added at a density of OD600= 0.0002 in 10Mm $MgCl_2$ with .01% SilWet. Seedlings were randomized and disease progression was tracked for the following seven days, with the plants being scored using a standard disease severity scoring index: 1 = mildly diseased, 2 = moderately diseased, 3 = severely diseased, and 4 = loss of leaf or death (Morella *et al.*, 2019). To prevent un-conscious bias in disease scoring plant treatment was hidden until the completion of the experiment.

2.2.8 Data Analysis

Genomic functional similarity and resource usage similarity were assessed in R using a PERMANOVA from the *adonis2* package with default parameters. Competition outcomes were analyzed using the *t_test* function from the *rstatix* package with default parameters to compare growth of the focal species alone to growth with the competitor, and p-values were adjusted for multiple comparison testing using the Holm-Bonferroni method. Disease assay data was first transformed into Area Under the Disease Progression Curve (AUDPC) (Madden & Hughes, 2017) and then analyzed using *t_test* to compare the disease progression for P. syringae DC3000 alone to each other combination of species, again using the Holm-Bonferroni method for multiple test adjustment.

2.3 Results

2.3.1 Genomic Information

A total of 43 genomes were sequenced and assembled, with 27 of the isolates representing strain level variants of other species. As a result, 16 unique species were selected that represent 97.8% of the bacterial families that were found at a relative abundance greater than 1% in the dataset that we used to guide the design of this community. Genome length varied from 3.16 Mb in SC13 to 6.3 Mb in SC35, with an average length of 4.7 Mb. Meanwhile, GC content ranged from 34.69% in SC35 to 73.28% in SC1, with an average of 59.48% (Tab 2).

Genomic assembly results in a collection of contigs, which are the longest sections of DNA that can be resolved from the assembly of individual reads. Contig number is generally regarded as an indication of assembly quality, with the goal of a complete assembly being the recovery of a (or some number of) circular chromosome/s representing the completely resolved structure of the bacterial genome. However, achieving a circularized genome is only currently possible through a combination of short (for example Illumina HiSeq, used for their accuracy) and long (for example Pacbio, used to understand genome structure) read sequencing technologies, and so many bacterial genomes (such as the current draft Genomes of the PhylloStart SynCom) are only assembled using short reads. These 16 genomic assemblies resulted in a median contig assembly number of 26, with a range of as few as 4 contigs and as many as 263, though that number is considered an outlier and most of the assemblies had a much lower contig number.

Genome quality is also frequently measured in terms of contiguity, which is represented by a score known as N50. N50 is calculated by ordering all contigs from longest to shortest, identifying the longest contigs that have summed base pair counts representing 50% of the genome length, and then taking the length of the shortest contig in that group. Across the PhylloStart genomes the median was 0.513 Mb, with the longest being 1.71 Mb, and the shortest (again an outlier) being 0.096 Mb. In general, a score of over 1 Mb is considered good in a context of long read sequencing, so with 3 genomes above 1 Mb, and 6 in total above .8 Mb, these represent relatively contiguous genomic assemblies for using short read technology.

Genome assembly can also be assessed in terms of completeness, and while this can be a difficult metric to measure objectively, there are several tools available that are capable of estimating completeness by calculating a list of phylogenetically conserved essential genes that appear once per genome, and using that to measure the assembled genome's completeness (is it missing any marker genes) or contamination (does it have any copies >1). These metrics were calculated using the package CheckM, with an average completeness of 99.5% and an average contamination of 0.69%.

Over 400 functional pathways (412) were identified across all genomes using the RasTK annotation pipeline. The mean number of assigned genes was 1261, with a range of between 803 and 1600 genes per genome being assigned to a functional category (Tab 2). As illustrated in Figure 1 most of the annotated genes were associated with Amino Acid and Carbohydrate related functions (synthesis and metabolism), with a high number of genes also being associated with Potassium metabolism, DNA metabolism, Cofactors etc., and in the Pseudomonads and Enterobacteriaceae, Stress response. Phylogenetic similarity is commonly used as a proxy for functional similarity, with the assumption being that more closely related species should share a larger proportion of their functional capacity. It is therefore unsurprising that when looking at the similarity in annotated pathway content across members of the PhylloStart community we see a pattern of more closely related taxa sharing a higher percentage of functional pathways (Figure 2). Analyzing this pattern with an adonis PERMANOVA (permutational analysis of variance), we see that family is a significant factor in structuring dissimilarity ($p = 0.001$).

2.3.3 Phenotypic Characterization

For morphological descriptions and exemplar images of the bacteria in the PhylloStart SynCom, each member was plated onto KB agar and allowed to develop for 3 days at 28ºC. *F. endophyticum* presents punctiform colonies, with a smooth surface, and a semi-transparent yellow pigmentation. *P. aggolomerans* develops large round colonies, with a convex surface and a highly mucoid yellow/clear appearance. *C. herbarum* shows punctiform colonies with a smooth surface and opaque orange pigmentation. *E. tasmaniensis* presents large slightly raised round colonies with a smooth surface opaque yellow pigmentation. *B. frigoritolerans* develops very large circular colonies with a smooth raised appearance and a tan/white pigmentation. *M. aurea* develops small slightly raised round colonies with a smooth surface, semi-transparent yellow pigmentation. *P. asturiensis* shows very large irregular shaped raised colonies with a somewhat mucoid appearance and a dark yellow or orange color, further, it exhibits weak fluorescence on KB. *E. sibiricum* develops medium sized circular colonies with a flat appearance and a slightly translucent white pigmentation. *P. rhizosphaerae* presents circular small colonies with a flat appearance and a semi-transparent yellow pigmentation and exhibits weak fluorescence on KB. *P. moraviensis* shows small slightly raised round colonies with an opaque white color showing strong fluorescence on KB. *R. festucase* develops punctiform colonies with a very slight yellow or white opaque tint. *C. pusillum* also exhibits punctiform colonies that are slightly raised with an opaque yellow pigmentation. *P. rhodesiae* develops small round colonies that are slightly raised with a semi-transparent white tint and exhibits strong fluorescence on KB. *P. allii* presents large yellow colonies with a convex surface that are highly mucoid. *M. oleivorans* shows punctiform white/yellow colonies with a slightly raised appearance. Finally*, B. wiedmannii* develops large round colonies with a crateriform elevation and an extensive filiform margin.

2.3.4 Resource Utilization

Resource utilization patterns were determined using the Biolog Ecoplate system, described in detail within the methods section. An OD590 greater than 0.1 was judged to indicate consumption of that resource. Overall, resource consumption was highest on Carbohydrates, Amino Acids, and Carbocylic Acid based carbon sources, with relatively lower consumption of carbon sourced from Phenols, Polymers and Amines. Resource utilization showed a strong phylogenetic signature (Figure 4 and Figure 5), with the Enterobacteriacia consuming the greatest number of resources with the highest affinity (Figure 6), and the Pseudomonads consuming a similar number of resources, but with a lower preference towards them (Figure 5). Importantly, while the number of resources that the Enterobacteraicia and the Pseudomonadacia consumed were similar, the resource utilization profiles (Figure 5) analyzed using an adonis PERMANOVA showed a significant effect of family identity ($p = 0.001$). The model pathogen, *Pseudomonas syringae* DC3000 showed a similar pattern of resource consumption to the other Pseudomonadacia in the PhylloStart community (Figure 4, Figure 5, Figure 6).

2.3.5 In Planta Competition

To determine *in planta* competitive interactions between the different species in the PhylloStart community, representative members of each group, as well as the model pathogen, *P. syringae* DC3000 were applied to tomato plants both alone and in pairs. All data were log- transformed and analyzed using pairwise t-tests with Holm–Bonferroni multiple test correction, to determine if there were significant differences between growth alone and growth with each challenger. All representative bacteria grew to relatively high abundances (Figure 7), with *P. moraviensis* reaching an average of 3 x 10^8 CFU/g of plant mass, *P. agglomerans* reaching an average of 1.5 x 10^8 CFU/g, *B. wiedmannii* developing to an average of 1.7 x 10^8 CFU/g, and DC3000 reaching 2.4 x 10^8 CFU/g. *P. moraviensis* showed no significant reduction in growth when it was grown in combination each other representative species, while *P. agglomerans* density was only significantly reduced when grown in combination with DC3000 ($p = 0.001$). *B. widmannii*'s development was significantly impeded by every competitor ($p < 0.001$, $p < 0.001$, and $p = 0.003$ for growth with *P. moraviensis*, *P. agglomerans*, and DC3000 respectively). Finally, DC3000 abundance was only significantly reduced by the presence of *P. agglomerans* ($p = 0.02$), though there was as trend of reduced abundance in the presence of *B. wiedmannii* ($p = 0.08$).

2.3.6 Additive Disease Protection

Given that *P. agglomerans* significantly reduced the DC3000 pathogen development on the leaf surface, an experiment was developed to determine if pairing it with another member of the PhylloStart community could lead to enhanced protection against disease development. This experiment compared the disease protective effect on developing tomato seedlings in a sterile environment, inoculated either with DC3000 alone, or previously inoculated with *P. agglomerans,* or *P. agglomerans* paired with *B. wiedmannii, E. tasmaniensis, M. aurea,* or *R. festucae*. Disease outcomes were transformed into a single metric, area under the disease progression curve (AUDPC), and results were analyzed with an ANOVA, which identified treatment as a significant factor ($p = 0.03$). Post-hoc analysis with a TukeyHSD indicated that only the combination of *P. agglomerans* and *B. wiedmannii* provided a significant reduction in disease development throughout the course of the assay ($p = 0.045$), though there was a trend of lower disease curves in *P. agglomerans* and each of the other species (Figure 8). Notably, this was not the case with the addition of *E. tasmaniensis* to *P. agglomerans*, which showed a nearly identical disease progression curve to DC3000 alone. Scoring disease progression is an inherently qualitative process, and as such, future experiments to quantitatively measure changes in *P. syringae* DC3000 abundance under these conditions might be useful to further establish these protective effects.

2.4 Discussion

This chapter described the process of developing a SynCom to investigate the interactions between the plant host and their associated phyllosphere microbiota. After assessing 93 potential strains, followed by the sequencing and assembly of 43 candidate taxa, 16 unique species were selected from the other 27 strain level variants for inclusion in the PhylloStart synthetic bacterial community. Genome assembly was largely successful, with a median contig number of 26, a median N50 of 0.513 Mb, and an average genome completeness of 99.5%. The two species with the highest contig number and the lowest N50, both outliers, were SC6, *Brevibacterium frigoritolerans* (with 80 contigs and an N50 of 0.12) and SC35, *Bacillus wiedmannii* (with 263 contigs and an N50 of 0.10), representing the phyla Actinobacteria and a Firmicutes respectively. Interestingly, CheckM scored both assemblies as being high quality, with a completeness of 98.91% and 99.43%, and with contamination scores of 3.33% and 0%, for *B. frigoritolerans* and *B. wiedmannii* respectively. The primary cause of poor assembly outcomes is insufficient sequencing depth (coverage), with most sequencing experiments aiming for 50x or 100x (Segerman, 2020) however, this is likely not an issue with these particular sequences, as SC6 had 322x coverage, and SC35 had 187x coverage, which should be more than sufficient. The most likely cause of these poor assembly scores is due to the bacteria's relatively low GC content (40% for *B. frigoritolerans* and 35% for *B. wiedmannii*), which has been linked to issues with uneven read coverage during library preparation, and difficulties during genome assembly (Oyola *et al.*, 2012; Browne *et al.*, 2020). Even so, there are other potential causes of high contig number, including a relatively high frequency of repeat sequences (Phillippy *et al.*, 2008), or the presence of insertion sequences (Smits, 2019). Issues arising due to GC content could be addressed by optimizing the library preparation using PCR additives (Browne *et al.*, 2020), though in general, assemblies can be improved through additional long-read (PacBio) sequencing in order to correctly map the location of contig sequences. Combining short and long-read sequencing to obtain complete genome structural resolution is becoming increasingly popular, though cost per genome for long-read sequencing is still relatively high, around $250 per genome at the time of writing this (Derakhshani *et al.*, 2020).

The combination of functional annotation, phenotypic characterization, and resource utilization data lay the groundwork for using this community as a resource to understand interactions between bacterial species, and to understand how these species might provide functions for their host organism, such as pathogen protection. Morphological characterization of each bacterial species facilitated the *in planta* competition experiment, allowing for accurate counts of each member to be distinguished through simple CFU plating, though it is important to point out that competing species could alter plating efficiency, and the utilization of culture independent quantification techniques such as ddPCR might improve reliability. Meanwhile, the functional annotation and carbon utilization patterns allowed for predictions of bacterial interactions. These data both showed that phylogenetic grouping was a significant predictor of functional similarity, which is generally accepted to be a good indication of competitive interactions due to their similar resource use patterns (Violle *et al.*, 2011; Morrissey *et al.*, 2016; Russel *et al.*, 2017).

While phylogenetic similarity may be a good indicator of functional overlap and resource use patterns, it does not necessarily predict competitive interactions a-priori. For example, when testing pairwise strain competition *in planta*, we see that *P. agglomerans* (an Enterobacter) and the pathogen *P. syringae* DC3000 (a Pseudomonad) both significantly reduce the other's growth, despite not being particularly closely related, while *P. moraviensis* (another Pseudomonad), does not significantly alter DC3000 growth or vice versa. For one thing, it is important to point out that the specific carbon sources in Biolog Ecoplates are not necessarily available on the leaf surface, whose primary carbon resources are Glucose, Sucrose, and Fructose (Tukey, 1970; Leveau & Lindow, 2001). This does not diminish the utility of the Biolog resource model though, as it still gives important information on resource utilization and growth rates, allowing us to predict performance with any given resource, as, on average, the species that consumes the greatest number of resources will be most likely to consume some other un-tested resource. Further, the extent to which resource overlap is a useful metric may vary among the species tested, as previous work with Biolog plates found a higher overlap of resource utilization between successful competitors and the pathogen they were able to exclude (Innerebner *et al.*, 2011).

In this environment, resource utilization rate may be more important in predicting competitive outcomes, as we see that the Enterobacteracea are, on average, able to consume more resources within the tested timeframe than any member of the Pseudomonadaceae. A faster basal growth rate is associated with a higher likelihood of invasion success (Kurkjian *et al.*, 2021), which may explain why *P. agglomerans* inhibits DC3000 growth while *P. moraviensis* does not. Another potential explanation for this effect is the known antagonism between some *Pantoea* species and *P. syringae*, with a study showing a significant reduction in live cells when these two species physically interact on bean leaves (Monier & Lindow, 2005). Further, that study noted that there was an asymmetric effect of the interaction between the two species, with more dead *P. agglomerans* cells than *P. syringae*, an effect that we recapitulate in our study as evidenced by a greater reduction in the density of *P. agglomerans* in the presence of DC3000 than is experienced by DC3000 in the presence of *P. agglomerans*. Further evidence of this as a potential mechanism is provided by the lack of reciprocal inhibition between *P. agglomerans* and the other tested Pseudomonad, *P. moraviensis*. One potential mechanism for these interactions could be through the Type IV secretion system, where toxic effectors are directly injected into neighboring rival cells (Sgro *et al.*, 2019), though it is unclear if either of these bacteria possess these capabilities. Further genomic and experimental work would be needed to investigate this possibility.

Considering these results, it is important to note that competition for resources is not the only mechanism that could lead to efficient exclusion of an invading pathogen. For example, the competing species could produce inhibitory compounds (Helfrich *et al.*, 2018) or alter the plant immune system to enhance its defense against pathogen establishment (Vogel *et al.*, 2016; Mauch-Mani *et al.*, 2017). This is illustrated in the case of *B. wiedmannii*, which is a poor performer in the Biolog tests, with a low resource utilization rate and restricted resource breadth. As predicted*, B. wiedmannii* also competes poorly on the plant surface, having its maximum density significantly reduced across all tested pairings. However, despite this poor performance, it is also capable of reducing the abundance of DC3000, and while this reduction does not reach significance, a clear trend is seen. Likewise, its own abundance, through still significantly reduced in the presence of DC3000, is qualitatively less impacted than when compared to its abundance in competition with any other species. Bacillus species have been identified as modulators of the plant immune system, and studies have found that their presence in rhizosphere communities can induce protection against *P. syringae* infections in the leaves (Niu *et al.*, 2011). Further experiments will need to be done to determine the exact mechanism of *B. wiedmanni's* negative impact on DC3000 growth.

Given the clear effect of *P. agglomerans* on the development of DC3000 on these plants, an experiment was designed to determine if this protection would be altered with the inclusion of an additional species. Notably, the impact on disease protection was strain dependent, with the addition of *M. aurea* and *R. festucae* having no qualitative difference from the addition of *P. agglomerans* alone, while the addition of *B. wiedmannii* was the only treatment that provided a significant reduction in disease development from the DC3000 alone control (through it should be noted that each of these other treatments did lead to a qualitative decrease in disease symptoms). The effectiveness of this pairing is unsurprising, given that both species can reduce DC3000 density alone, through it shows that community induced disease protection can show additive effects, highlighting the importance of having multiple pathogen antagonistic strains in a community. Research in the rhizosphere has indicated that certain combinations of bacteria can induce the production of anti-microbial compounds that would not be produced when cultured alone (Mendes *et al.*, 2011; Garbeva *et al.*, 2011), so it is also possible that these effects could be emergent rather than just additive. It is also important to note that there was no qualitative difference in the disease curves produced by DC3000 alone and competing with *P. aggolmerans* supplemented with *E. tasmaniensis*, which both trended higher than the addition of *P. aggolmerans* alone. It is possible that, similar to how certain species parings can lead to enhanced disease protection, others could lead to a decrease in the effectiveness of a strain's disease protective capacity. This highlights the importance of understanding the interactions between strains, and their impact on the host, when designing communities with the purpose of reducing disease incidence in their hosts.

2.5 Perspective

This chapter highlights the resources that I have developed to aid in using the PhylloStart SynCom as a model system for understanding the functions that microbes can provide to their host organism, as well as presenting some experiments investigating these interactions. The following two chapters will highlight two projects utilizing the Phyllostart SynCom to investigate the importance of the phyllosphere community in the reproductive success of their plant hosts, and the role that plant status plays in modulating the reliance on the community for disease protection. It is important the note that these projects, and the SynCom resources developed for them, represent only a small fraction of the potential for this model system. In the following paragraphs I will outline a small selection of further resources that could be developed to aid in the use of this system, and some of the open questions these would allow the system to address in the field of plant phyllosphere microbiome interactions and host microbial interactions more broadly.

One of the primary limitations in performing experiments with a Syn Comm system is determining individual bacterial abundances. The primary method for assaying individual abundance at some endpoint, or changes in abundance throughout time, are through either 16S rRNA Amplicon sequencing or CFU plating. Amplicon sequencing is relatively costly, and with a simplified community and a limit to the number of samples that can be multiplexed, most of the sequencing depth is wasted. On the other hand, CFU plating, while cheap, can become difficult when pairing multiple species with similar morphologies. Considering these limitations, it would be useful to develop an alternative method of assaying individual densities among PhylloStart communities. One option, which represents likely the easiest and cheapest method for assaying individual density is through the development of an antibiotic resistant mutant of each PhylloStart strain. Rifampicin is an attractive option, as it is relatively easy to develop resistance to through de novo selection, resulting from a mutation in the rpoB gene (Goldstein, 2014) and it has been used for some time as marker to recover focal bacteria from environmental studies (Glandorf *et al.*, 1992). With this being said, it has been shown to induce pleiotropic changes in fitness (Sonenshein *et al.*, 1974; Jin & Gross, 1989), so it would be wise to re-assay relevant phenotypic traits in these mutants. Another relatively popular option used to study phyllosphere associated bacteria would be through the introduction of fluorescent tags (Remus-Emsermann & Schlechter, 2018), which, while it can be time consuming and technically difficult to accomplish, would allow for assessment of both focal bacterial densities, and through fluorescent microscopy, the resolution of spatial structuring of a focal species, or, multiple species through the use of several different fluorescent tags. Finally, droplet digital PCR (ddPCR) can be employed to exactly quantify the density of a certain focal species using a strain specific primer (a technique used to assay pathogen density in chapter 3), or multiple strains through probe multiplexing (Morella *et al.*, 2018b). In ddPCR the sample is split into tens of thousands of individual droplets in which a PCR reaction takes place, and positive samples can be identified by a counting machine that measures the presence of probe fluorescence in each droplet. This method requires the identification of sequences in each PhylloStart member that are divergent enough to prevent off target amplification, but in practice this is quite achievable, for example, a section of the *rpoB* gene was found in *P. syringae* DC3000 whose targeted primers did not amplify any of the closely related PhylloStart Pseudomonad strains.

Another important question, both in terms of phyllosphere microbial ecology, and ecology on a broader scale, relates to understanding how competing species can coexist. Studies leveraging SynCom approaches to address these questions can often yield surprising results, with a recent study showing that complex communities can be maintained in single carbon resource environments through cross feeding between species (Goldford *et al.*, 2018). To further utilize the PhylloStart community as a resource for understanding coexistence and competition in microbial communities it is necessary to employ both metabolic modeling and further *in vitro* competition assays. Using the genomic resources that are available from sequencing these strains flux balance analysis (FBA) models, which use a constraint-based modeling strategy to predict phenotypic responses to specific resources (O'Brien *et al.*, 2015; Cuevas *et al.*, 2016), can help formulate a-priori hypotheses about bacterial competition and cooperation on specific resources. In addition to developing resource utilization models, a further set of *in vitro* resource utilization assays would clarify bacterial coexistence patterns. The first goal should be to assay the utilization of Glucose, Sucrose, and Fructose that are prevalent on the leaf surface (Leveau & Lindow, 2001) at various concentrationss across each species in the PhylloStart community. Preliminary data (Supplemental Data) gathered on a subset of the PhylloStart species indicates that they have different minimum uptake concentrations, below which they cannot effectively obtain the resource from their environment, which could allow different species to persist under different local resource conditions on the leaf surface. Further study of the hypothetical and actual resource use patterns of the PhylloStart members will allow for a better understanding of the processes influencing microbial coexistence in the phyllosphere habitat.

Beyond being just a system to study microbial ecology, the phyllosphere is also a key interface through which bacteria and their plant hosts can interact. As shown in the third chapter, these phyllosphere bacteria are critically important for the development and reproductive success of their hosts. To better understand the mechanisms underlying these observations, further experiments assaying plant growth promotion should be employed, combining both phenotypic and transcriptomic approaches. The simplest experiment would utilize growth pouches, a frequently used technique to determine the growth promoting (or inhibiting) effects of different bacterial strains (Penrose & Glick, 2003). In the growth pouch assay a sterilized paper pouch is inoculated with a bacterial strain and seeds are allowed to germinate at the top of the paper, as they grow their roots will spread down the pouch, allowing for measurement of changes in growth phenotype due to bacterial exposure. This method, combined with the functional analysis of the bacteria's genome, should allow for the identification of putative mechanisms by which the species interacts directly with its host, though it is important to note that the plant roots may be more sensitive to bacterial signals than the leaves would be. Further analysis of plant gene expression in response to different bacteria, or combinations of bacteria would then allow for a determination of the mechanisms by which they confer these phenotypic changes (similar to how this method is employed in chapter 4 to determine the changes in reliance on PhylloStart species for protection in polyploids).

These approaches: developing mechanisms to assay bacterial abundance, measuring and predicting comprehensive resource overlap, and quantifying plant growth promotion, are only a small subset of the many future directions in which the PhylloStart community could be developed as a resource. Other potentially interesting tools could include a paired phage library to measure the impact of bacteriophage on phyllosphere growth of various species, a topic of current interest (Morella *et al.*, 2018a), or to model apparent competition among species (Brockhurst *et al.*, 2006). Another useful resource for the community would be a measurement of direct and indirect inhibition between strains, an important component beyond resource overlap that influences coexistence and community composition (Helfrich *et al.*, 2018). Beyond the purely ecological questions that can be tackled with a SynCom model system, this resource also provides the opportunity to understand how these interactions might be shaped over longer time scales through experimental evolution.

In conclusion, it should be clear that the development of a SynCom model system represents an ever-evolving resource that can be utilized to study a variety of plant microbial, ecological, and even evolutionary, interactions. The next chapters of this dissertation will highlight a selection of projects that have leveraged this SynCom resource, and the role that is has played in furthering the understanding of the complex interactions in the phyllosphere microbiome.

2.6 Tables and Figures

Count	*Kingdom*	*Phylum*	*Class*	*Order*	*Family*	*Genus*	*Percent_Abundance*
7716	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	58.40
1294	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	9.80
946	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	7.20
811	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	6.10
688	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	5.20
524	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Exiguobacterium	4.00
324	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Rahnella	2.50
166	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae_unclassified	1.30
118	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Exiguobacterium	0.90
88	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.70
51	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.40
47	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	0.40
26	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.20
23	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	0.20
22	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.20
22	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.20
20	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	0.20
19	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	0.10
17	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	0.10
16	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Curtobacterium	0.10
16	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
16	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobiaceae_unclassified	0.10
13	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
13	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.10
12	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
11	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonadaceae_ge	0.10
10	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	0.10
10	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
9	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae_unclassified	0.10
9	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.10
9	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.10
9	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae_unclassified	0.10
8	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.10
8	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.10
7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.10
7	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.10
7	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Paenarthrobacter	0.10

Table 1. Species abundance from field sequencing used to identify candidate taxa for inclusion in the PhylloStart SynCom.

Number	*Genome*	*Name*	*Origin*	*Contigs*	*GC_Content*	*Genome_Size*	*N50*	*Genes_Identified*	*Pathways_Identified*	*Completeness*	*Contamination*
1	SC1	*Frigoribacterium endophyticum*	Field	12	0.73	3198427	1.64	803	213	98.38	0.51
2	SC2	*Pantoea agglomerans*	Field	32	0.55	4749059	0.32	1426	276	100.00	0.52
3	SC3	*Curtobacterium herbarum*	Field	11	0.71	3593861	0.90	876	218	98.99	1.01
4	SC4	*Erwinia tasmaniensis*	Field	41	0.56	4584383	0.22	1348	268	99.99	0.00
6	SC6	*Brevibacterium frigoritolerans*	Field	80	0.40	5599015	0.12	1583	285	98.91	3.33
9	SC9	*Massilia aurea*	Field	22	0.64	5250572	1.57	1438	262	100.00	0.96
10	SC10	*Pseudomonas asturiensis*	Passaging	34	0.59	6034348	0.49	1540	284	100.00	0.17
13	SC13	*Exiguobacterium sibiricum*	Field	14	0.47	3156318	0.86	966	236	100.00	0.33
14	SC14	*Pseudomonas rhizosphaerae*	Field	14	0.62	4740976	0.54	1332	275	98.38	0.14
16	SC16	*Pseudomonas moraviensis*	Field	43	0.60	5938292	0.33	1535	288	100.00	0.11
21	SC21	*Rathayibacter festucae*	Field	12	0.72	4398498	0.72	985	240	99.41	2.36
23	SC23	*Curtobacterium pusillum*	Field	10	0.72	3804492	0.95	948	231	98.93	1.01
25	SC25	*Pseudomonas rhodesiae*	Passaging	56	0.61	5744567	0.22	1600	288	100.00	0.11
26	SC26	*Pantoea allii*	Passaging	30	0.55	4838685	0.25	1423	274	100.00	0.49
33	SC33	*Microbacterium oleivorans*	Field	4	0.69	3162261	1.71	876	222	99.49	0.00
35	SC35	*Bacillus wiedmannii*	Field	263	0.35	6318919	0.10	1510	270	99.43	0.00

Table 2. Summary table of Genomic information for members of the PhylloStart SynCom, including contig number, GC content, genome size, N50 score, number of unique genes identified using RAStK , number of pathways identified using RAStK, completeness assessed with CheckM, and contamination assessed with CheckM.

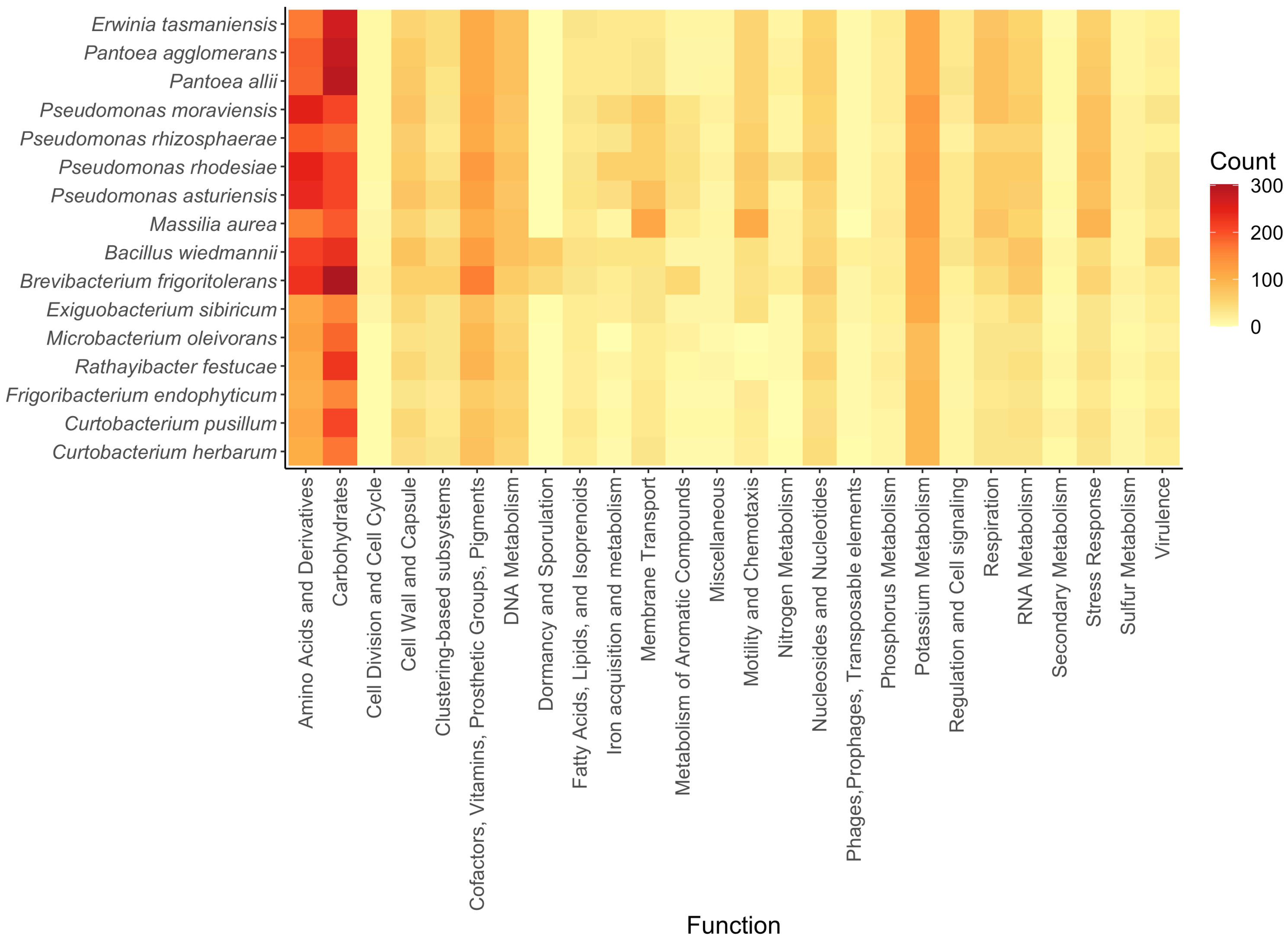


Figure 1. Heatmap of functional pathways assigned to each PhylloStart member, grouped broadly by function. Most assigned functions group into Carbohydrate or Amino Acid utilization, with a relatively high frequency of Potassium metabolism and Cofactors, Vitamins, Prostehtic Groups and Pigments.

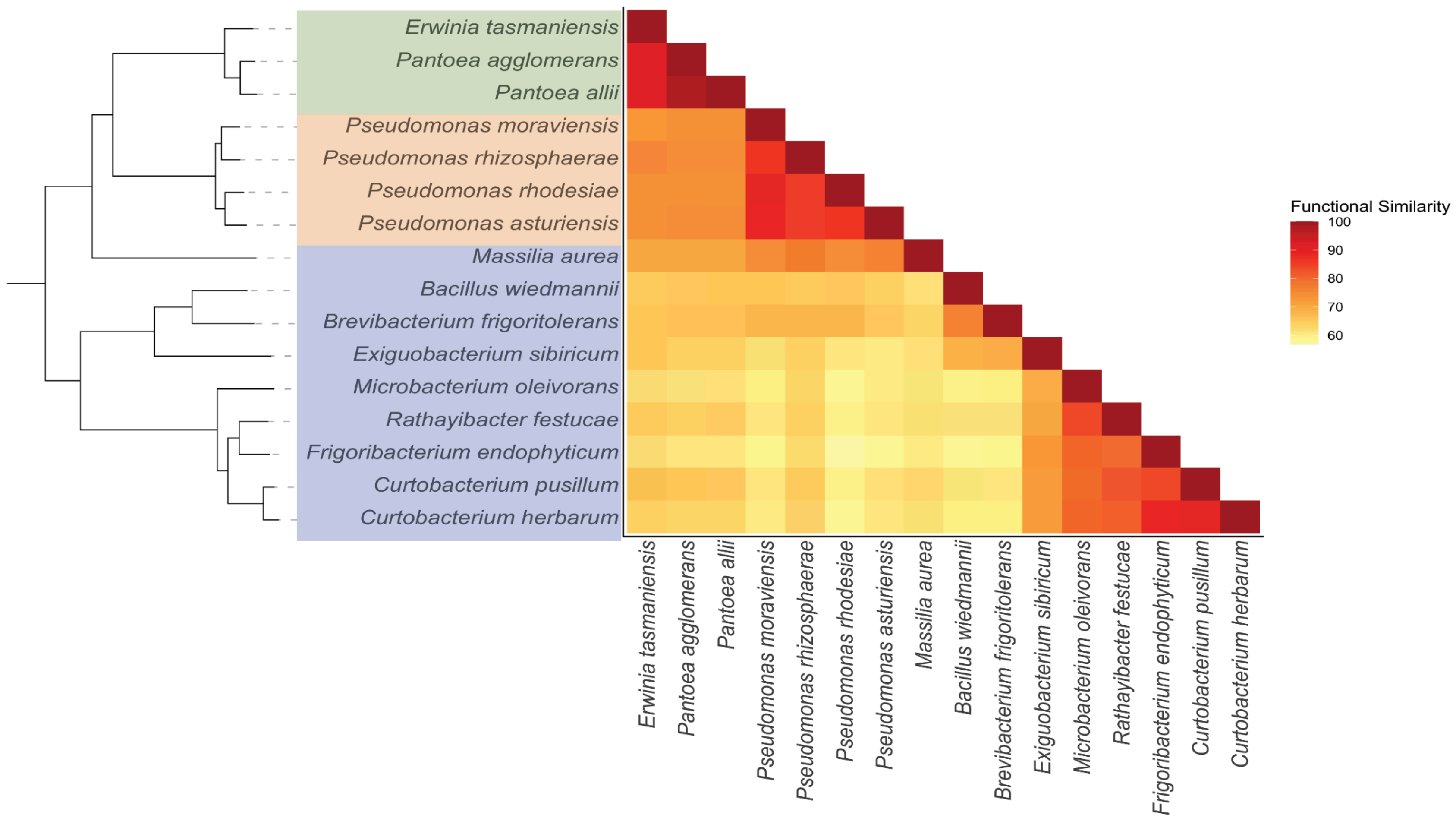
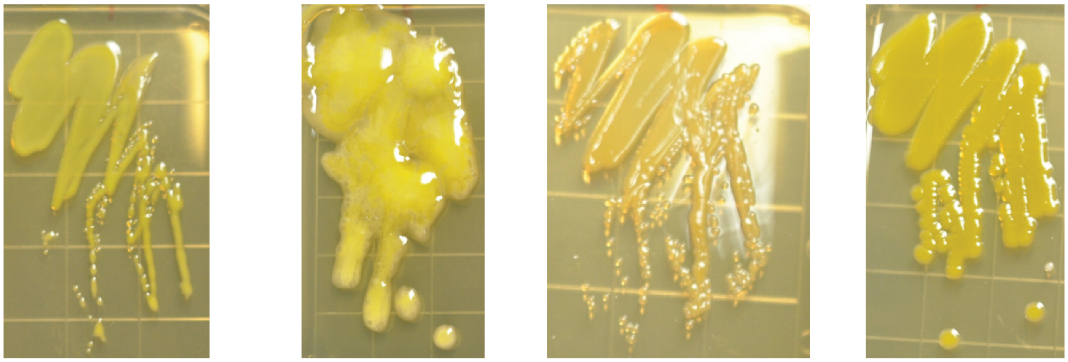
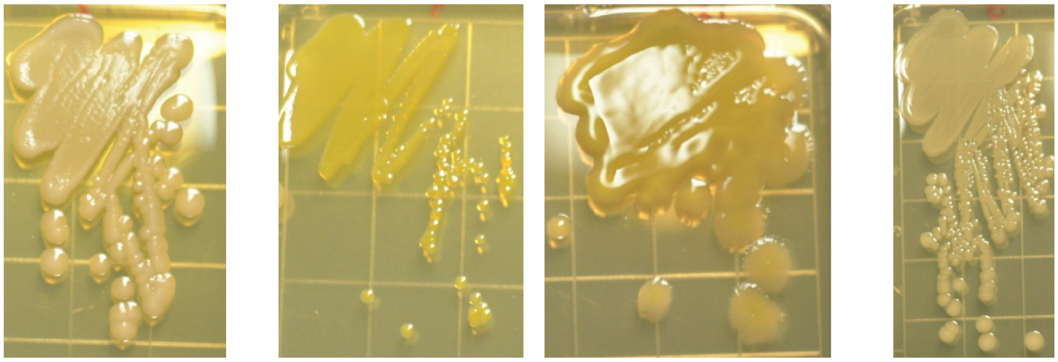


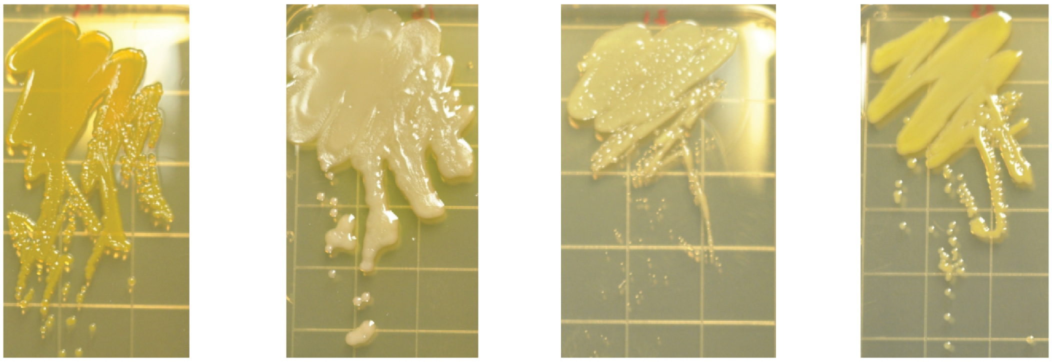
Figure 2. Heatmap representing functional similarity between pairs of PhylloStart species. The major phylogenetic groups are colored, with the Enterobacter in green, Pseudomonad in orange, and the rest of the species in blue. Overall, there is a strong pattern of functional similarity within phylogenetic groups. Analysis with a PERMANOVA indicates that family is a significant factor in functional similarity.



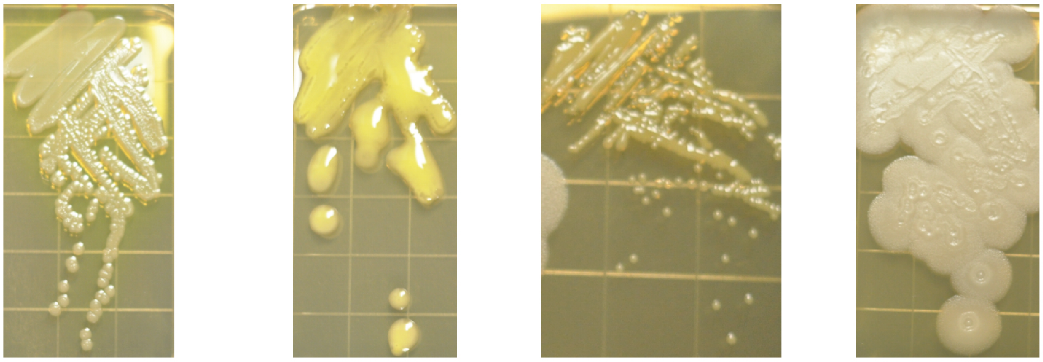
SC1: *F. endophyticum* **SC2:** *P. agglomerans* **SC3:** *C. herbarum* **SC4:** *E. tasmaniensis*



SC6: *B. frigoritolerans* **SC9:** *M. aurea* **SC10:** *P. asturiensis* **SC13:** *E. sibiricum*



SC14: *P. rhizosphaerae* **SC16:** *P. moraviensis* **SC21:** *R. festucae* **SC23:** *C. pusillum*



SC25: *P. rhodesiae* **SC26:** *P. allii* **SC33:** *M. oleivorans* **SC35:** *B. wiedmannii*

Figure 3. Exemplar photos of each member of the PhylloStart SynCom that were used to describe their morphology.

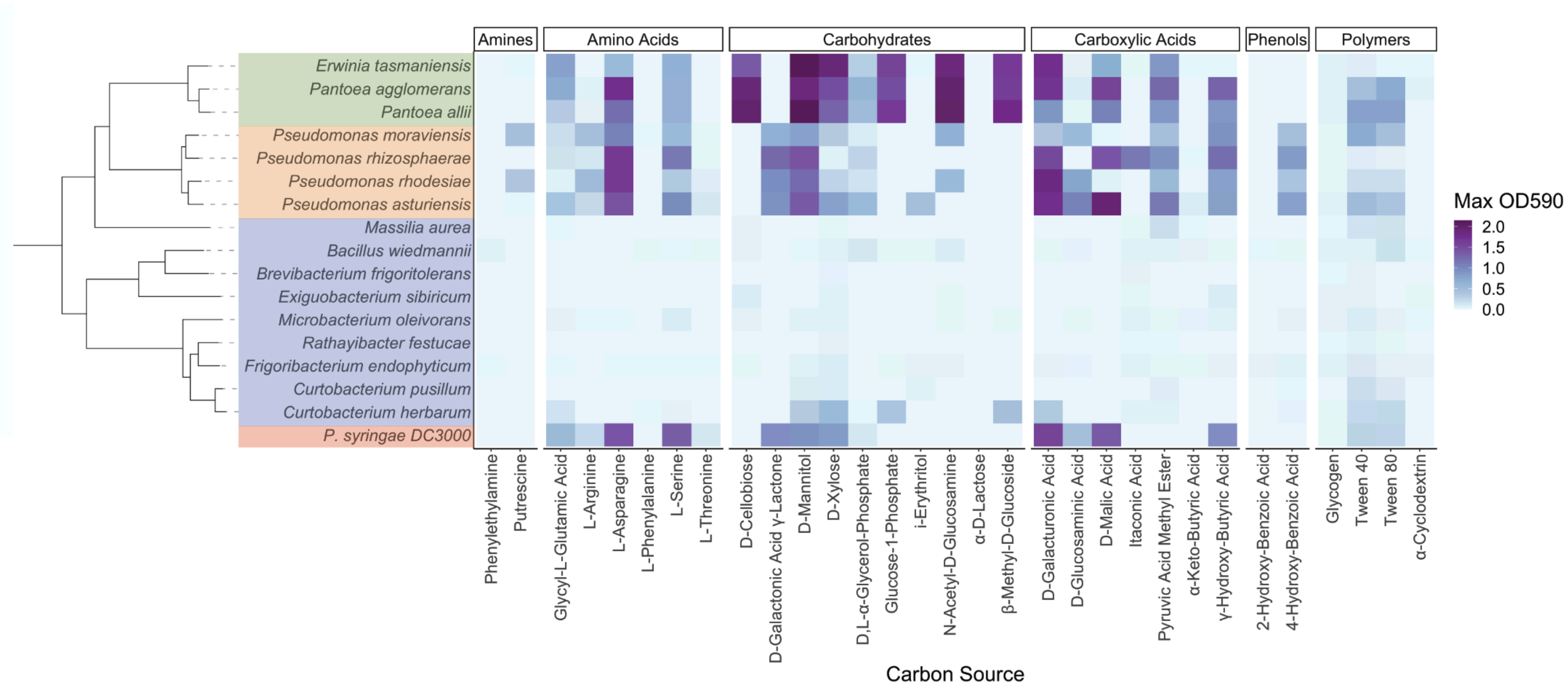


Figure 4. Utilization of resrouces in the Biolog Ecoplates, as measured by color development at OD590 across members of the PhylloStart community, as well as the model pathogen *P. syringae* DC3000. The major phylogenetic groups are colored, with the Enterobacter in green, Pseudomonad in orange, and the rest of the species in blue.

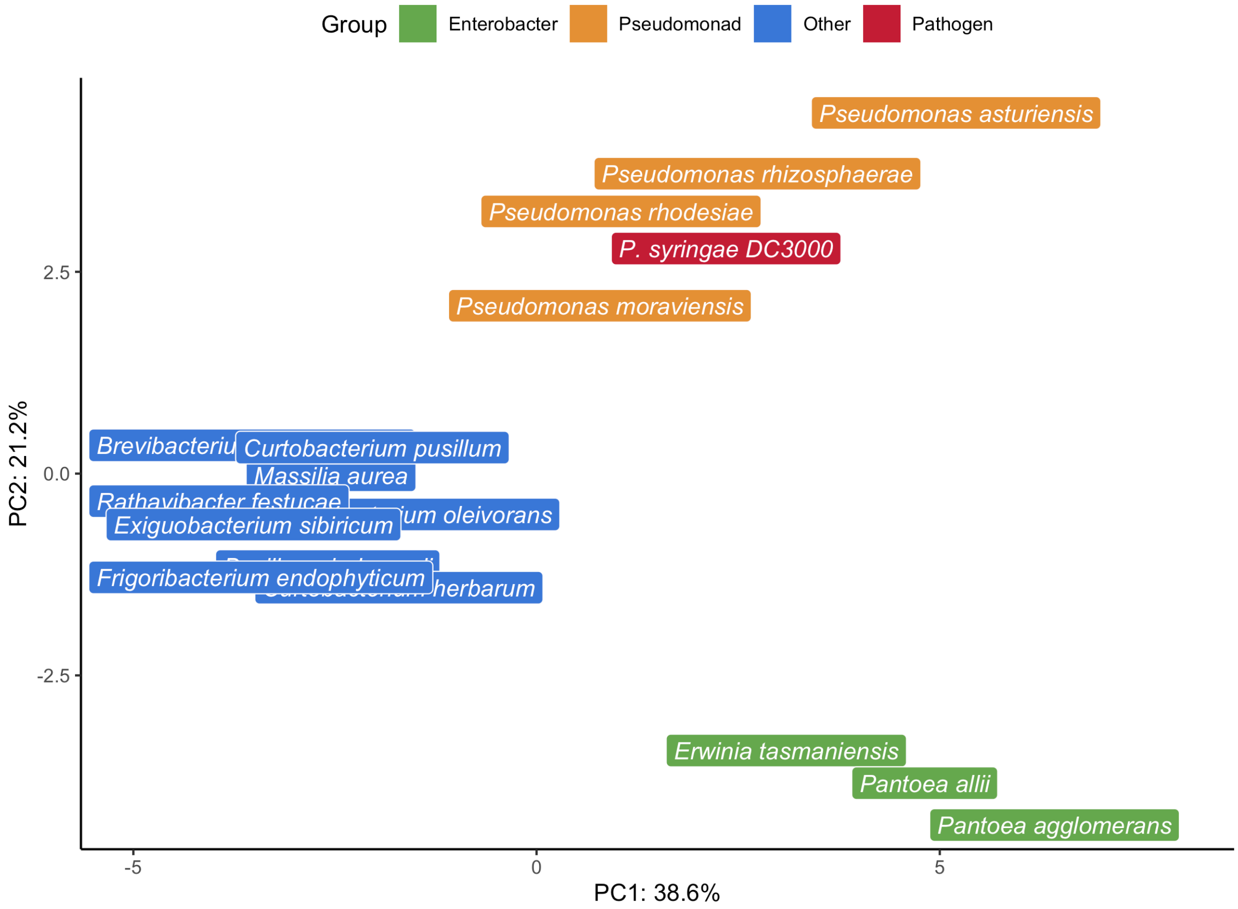


Figure 5. PCA depicting resource utilization patterns across members of the PhylloStart community, as well as the pathogen *P. syringae* DC3000, in Biolog Ecoplates. More closely related species show more similar resource utlization, both in terms of the specific resources that they are capable of consuming and the rate at which they consume them (measured by the amount consumed in the allocated assay time), family is identified as a signficant effect in a PERMANOVA analysis of the resource utilization patterns.

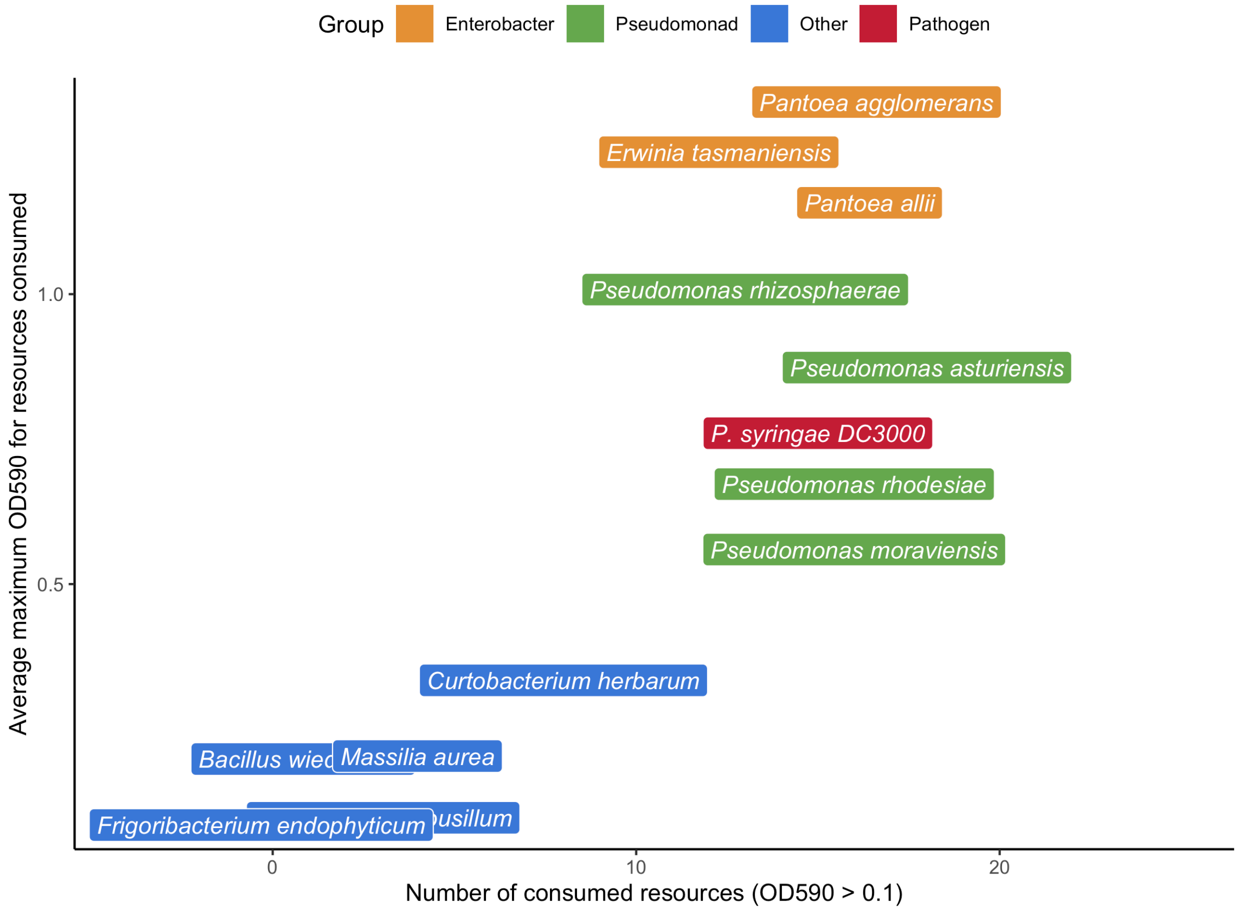


Figure 6. Scatter plot depicting the number of consumed resources (measured as an OD > 0.1 on that resource) and the average maximum OD590 (intenstiy of resource use) for each member of the PhylloStart community and the model pathogen *P. syringae* DC3000. The Enterobacter and Psuedomonad groups consume a similar number of resources, though importantly, the resource overlap differs, with the Enterobacter's showing a higher affinity for the resources that they can consume (higher average OD590). Meanwhile, the "other" group, consisting of the rest of the PhylloStart community, does not consume a large number of resources, and does not, on average, have a particularly strong affinity for those resources.

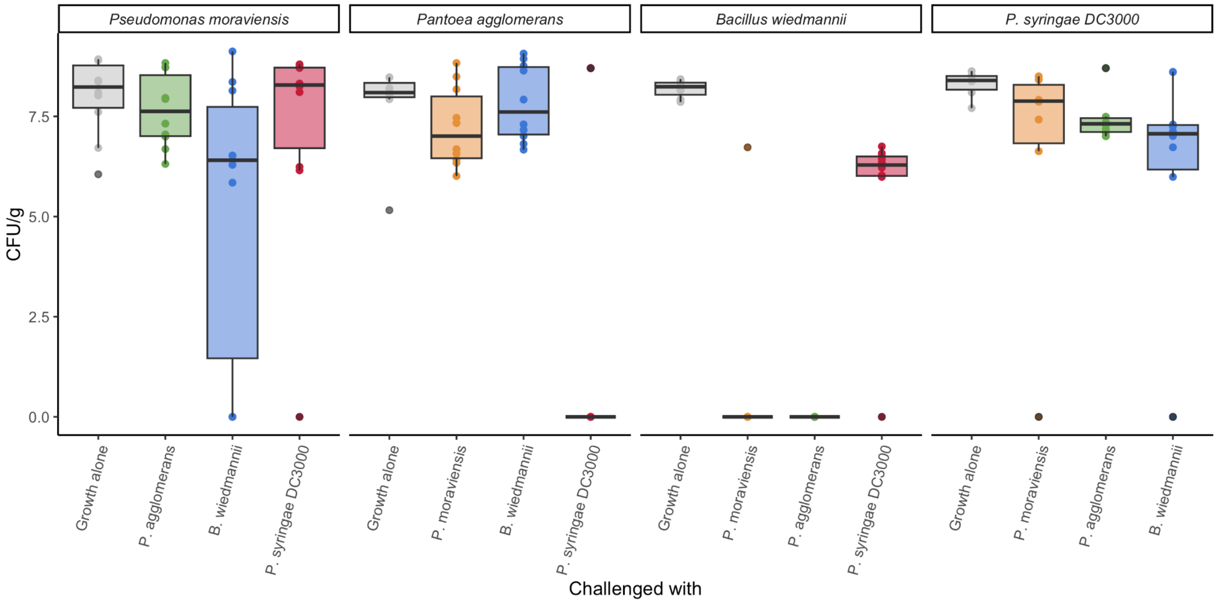


Figure 7. Barplots depicting the reciprocal effects of pairwise co-inoculation onto tomato plants for representative members of the PhylloStart community. Each focal member is identified on the top axis, with the plot representing their density (in CFUs) in response to co-culture with the identified species on the bottom axis, compared to their growth alone in grey. *P. moraviensis* growth is not significantly impacted by any other species, while *P. agglomerans* is significantly reduced by co-inoculation with the pathogen *P. syringae* DC3000. Meanwhile, *B. wiedmanni'*s growth is significantly reduced in the presence of all other tested species, though qualitatively it is the least reduced when paired with DC3000. Finally, DC3000 density is only significantly reduced in the presence of *P. agglomerans*, though there is a trend towards lower abundance in the presence of *B. wiedmanni*.

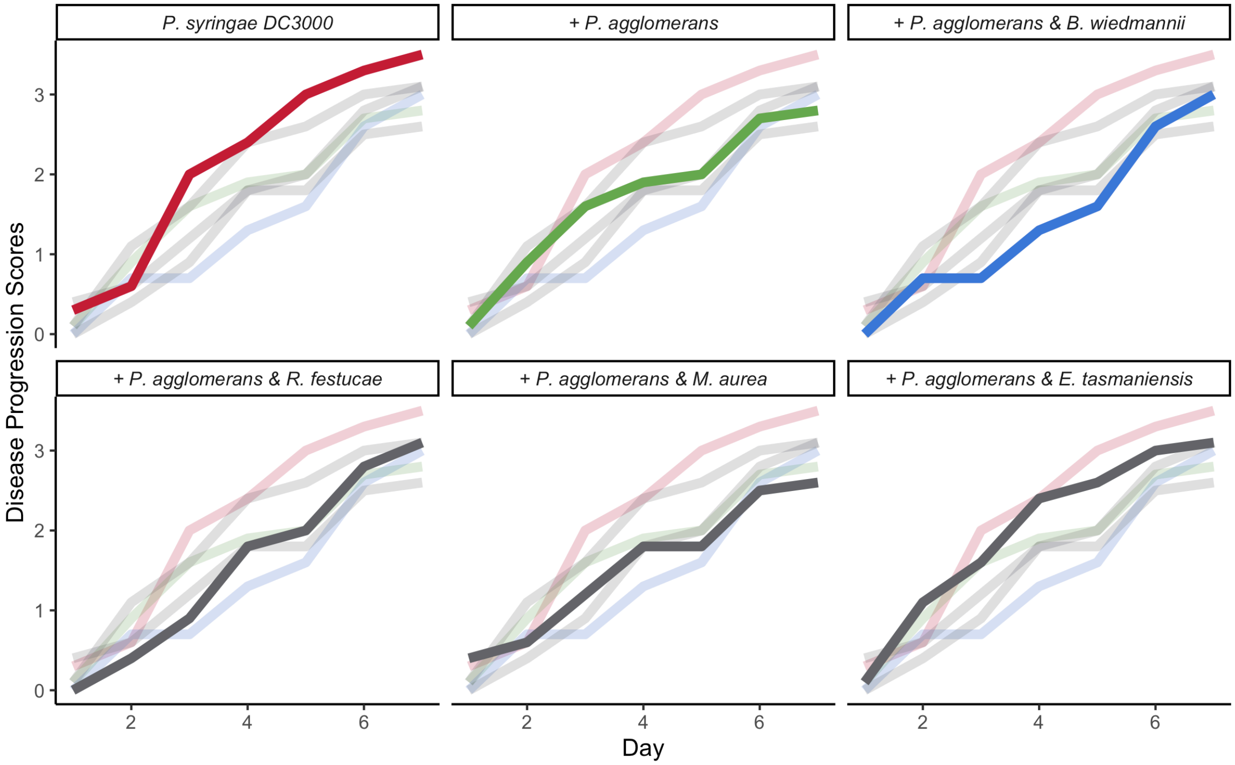


Figure 8. Disease progression curves for seedlings grown with the pathogen *P. syringae* DC3000, either alone or co-inoculated with *P. agglomerans*, or some pairing of *P. agglomerans* and another PhylloStart member. Interestingly, only the combination of *P. agglomerans* and *B. wiedmanni* lead to a significantly lower area under the disease progression curve (AUDPC), though there was as clear trend of lower disease in the *P. agglomerans* alone. No other pairing produced an effect that was significantly different from *P. agglomerant* alone, though co-culture with *E. tasmiensis* did show a trend of less protection.

Chapter 3. Early phyllosphere microbial associations impact plant reproductive success

Parts of this chapter have been adapted from the following with permission:

Mehlferber, Elijah C., Kent F. McCue, Reena Debray, Griffin Kaulbach, Jon E. Ferrel, Rajnish Khanna, and Britt Koskella. "Early phyllosphere microbial associations impact plant reproductive success." *bioRxiv* (2022).

3.1 Introduction

Microbial associations have been shown to be critical in the development and functioning of plant and animal host organisms (Shin *et al.*, 2011; Wagner *et al.*, 2014). For plants, there exists a wealth of data on how the root and soil-associated microbial communities can shape plant growth, competition with neighbors, disease resistance, and nutrient uptake (Berendsen *et al.*, 2012). In contrast to the well-defined role of belowground plant-associated communities, less is known about the importance of bacteria inhabiting the above-ground portion of the plant, the phyllosphere. Thus far, the investigation of phyllosphere microbiomes has generally been limited to their role in protection from disease, such as in cases of pear fire blight (Mercier & Lindow, 2001), tobacco wildfire disease (Qin *et al.*, 2019), or tomato bacterial speck (Innerebner *et al.*, 2011; Morella *et al.*, 2019) and frost injury and fruit russet (Lindow pubs). Although some evidence suggests that these microbial communities can have key functions beyond disease resistance (Stone *et al.*, 2018), for example through nitrogen fixation (Fürnkranz *et al.*, 2008) or the production of growth-regulating signals (Madhaiyan *et al.*, 2006), there remains limited direct evidence for their role in plant growth or yield.

The phyllosphere is inhabited by a relatively diverse consortia of bacteria, with densities ranging from 10^6 to 10^7 cells per square centimeter (Lindow & Brandl, 2003). These epiphytic bacteria are subject to a hostile environment, often encountering high levels of UV radiation, temperature fluctuations, and desiccation (Jacobs *et al.*, 2005; Beattie, 2011). The majority of phyllosphere-inhabiting bacteria are believed to arrive via aerial transmission, including wind and rain (Vorholt, 2012; Ottesen *et al.*, 2016), and much of this transmission is likely to originate from neighboring plants (Šantl-Temkiv *et al.*, 2018; Meyer *et al.*, 2022) . In contrast, plants that are grown in greenhouses are relatively isolated from microbial dispersal through wind, rain, and insects or from neighboring plants. In line with this, greenhouse-grown plants have been shown to develop communities distinct from those developing in outdoor environments (Maignien *et al.*, 2014; Marco lettuce mBio). Since greenhouse plants are typically grown in commercial soil mixes, it is also unlikely that they have the full breadth of bacteria available for recruitment from the soil reservoir (Knief *et al.*, 2010). Given this, greenhouse-grown plants are likely depauperate in their microbial associations, providing a unique opportunity to study the importance of the phyllosphere microbiota in a more complex environment, as opposed to a highly constrained gnotobiotic system.

One promising avenue for investigating the causative effects of plant-microbiota interactions in plant health is through the use of synthetic bacterial communities. Ideally, these synthetic communities represent the phylogenetic diversity of natural phyllosphere communities, but at a tractable level of complexity, allowing for repeatable experimentation. This approach has been used to investigate a wide variety of plant-microbial interactions (Bodenhausen *et al.*, 2014;

Bai *et al.*, 2015; Hu *et al.*, 2016; Castrillo *et al.*, 2017; Berg & Koskella, 2018), but these synthetic communities also hold great potential as microbial 'probiotics' or biostimulants. Such microbial amendments would be especially useful in environments where microbial diversity is otherwise reduced and/or where host-microbiome associations have been disrupted by, for example, pathogen establishment or antimicrobial treatments. We examine this question using a defined set of naturally occurring bacteria to establish a synthetic community (herein referred to as 'PhylloStart') that we developed to mimic the composition of microbial communities associated with field-grown tomato plants.

Our primary objective was to determine the importance of phyllosphere-associated microbiota in agriculturally relevant plant traits, under realistic conditions, and so we focused on early microbiome establishment in a greenhouse setting, where we show that initial microbiome recruitment is highly limited. To investigate potential interactions between microbial associations and nutrient status we included a popular commercially available micronutrient supplement (Azomite) at various concentrations. Over a series of trials we demonstrate both long-term establishment of the synthetic microbiome on plants and a highly repeatable and significant increase in plant growth and yield compared to control plants, with an additive effect from the micronutrient supplement. In the case of field-grown plants that were treated in the same way (i.e., sprayed pre-transplantation with PhylloStart), however, we did not see a significant effect of early life phyllosphere amendment. This differential effect of the phyllosphere on plant fitness across the greenhouse and field is likely the result of reduced microbiome colonization in low diversity/dispersal environment such as the greenhouse. Moreover, higher levels of competition and environmental pressures under field conditions could reduce the efficacy of early life application of probiotic treatments, potentially requiring different application protocols. Overall, our results demonstrate that, typically, phyllosphere microbial communities establish poorly under common greenhouse growth conditions and highlight the underappreciated role of the above-ground microbiome in shaping plant fitness.

3.2 Materials and Methods

3.2.1 Plant generation

Seeds of tomato (*Solanum lycopersicum*) variety 'Moneymaker' were surface sterilized by gently shaking in a solution of sodium hypochlorite and Tween 20 for 20 min, followed by two rinses with filter-sterilized H_2O. Seeds were placed into pots containing Sunshine mix number 1 (Sun Gro) soil and germinated in the greenhouse. When the seedlings were 3 to 4 inches tall, they were transplanted into larger pots, and randomly distributed across the greenhouse, where they were grown for the remainder of their development, which was 20 weeks in the first trial, 19 weeks in the second trial and 24 weeks in the third trial. Plants were grown in the greenhouse under controlled conditions with supplemented lights to maintain long days and fans to control high-temperature fluctuations. Liquid nutrient supplementation consisting of Peters Professional 20/20/20 water-soluble fertilizer was applied (1:64 ppm) once per week, as well as a disease suppression program consisting of Floramite and Decathlon at a rate of 1/4 tsp per gallon of water, mixed/agitated, was applied through a controlled sprayer at the rate of 1 to 2 gal per 100 plants. Plants in the field trial were started in the greenhouse and received the same treatment as the plants in the third trial until a time of 7 weeks, when they were moved outside to harden, and subsequently transplanted into the Oxford Tract field at UC Berkeley at 8 weeks. After transplanting into the field plants were watered upon establishment as needed, and then once a week for approximately 6 hours thereafter, and 20-20-20 water-soluble fertilizer was applied during watering at a rate of .93#N/ac.

3.2.2 Bacterial strains

PhylloStart was designed to mimic the composition of a field grown tomato phyllosphere community, but at reduced complexity to remain tractable. The community was initially designed based on communities sequenced from tomato plants in the student organic garden at UC Davis (Supplemental Table 1), and isolates were collected to be representative at the family level. These isolates were collected directly from the UC Davis Student Farm, and from the endpoint of a greenhouse selection experiment (Morella *et al.*, 2020) by plating initially on KB and LB agar plates, followed by MacConkey, and 1% Tryptic Soy agar plates to isolate more fastidious species. The isolates that were selected for inclusion comprise 97.8% of the bacteria that were found at a relative abundance greater than 1% in this dataset, representing the families Enterobacteriaceae, Oxalobacteraceae, Pseudomonadaceae, Bacillaceae, Microbacteriaceae, with the addition of a member of the family Brevibacteriaceae that was identified at a high prevalence on our plated field samples during collection. In total, 16 unique species were selected to comprise PhylloStart (out of the 93 screened isolates), with several members representing species level variation within the selected families. Information on the identity of the PhylloStart synthetic community is available in Supplemental Table 2. Strain identification was performed by sequencing the genomes of each bacteria and matching the sequences for the 16s rRNA using BLAST to publicly available databases on NCBI.

3.2.3 PhylloStart and Azomite application

For preparation of consortia, all strains were grown individually for three days at 28ºC on a media shaker in liquid culture in KB (King's Broth) nutrient broth (King *et al.*, 1954). Cultures were spun down for 10min at 2500g in a centrifuge and the KB supernatant was replaced with fresh KB. The optical density at 600nm (OD600) of each sample was read and the samples were mixed together at densities equal to 0.2. This suspension was frozen in 50/50 KB/Glycerol at -80ºC until inoculation. On the day of inoculation, the community was thawed and resuspended in sterile 10Mm MgCl buffer with 0.01% SilWet surfactant. For the second greenhouse trial and the field trial we included two concentrations of the PhylloStart, one at OD600=0.02 and another diluted 100-fold at OD600=0.0002. Plants were inoculated by spraying either this suspension, or for controls, the same but without the addition of PhylloStart, onto both sides of all leaves until runoff. Plant inoculation time varied among experiments but was generally performed for three consecutive weeks starting either two- or three-weeks post seedling emergence (See Figure 1 for exact timing across experiments).

The micronutrient supplementation differed between trials, with different preparations and quantities of Azomite® (Azomite Mineral Products, Inc., UT), a soil additive and fertilizer derived from volcanic ash that has previously been shown to increase the growth and yield of tomato plants (Noorani Azad *et al.*, 2016; Mehlferber *et al.*, 2022). There were three trials performed (Figure 1). In the first greenhouse trial we used three Azomite treatments, first, with the plants either amended with 5% wt/wt Azomite Granular grade during sowing and transplanting (n = 10), second, with 1g of Azomite Ultrafine grade applied after transplanting to the soil surface at the base of the plant at 7, 9, and 12 weeks after sowing (n = 10), and finally, with both Azomite Granular and Azomite Ultrafine applied as described (n = 10). Trial 1 included a control treatment with no Azomite or PhylloStart (n = 10), as well as a PhylloStart only treatment and a treatment with both PhylloStart and Azomite Granular and Ultrafine (n = 10). In the second greenhouse trial we applied the same Azomite Granular and Ultrafine treatment as described, but with the modification of Azomite Ultrafine concentration, using 1, 2 and 3 grams (n = 3 for each). This experiment included plants that were inoculated with these concentrations of Azomite as well as PhylloStart (n = 3 for each), PhylloStart alone (n = 3), and a control treatment that did not receive either Azomite or PhylloStart (n = 3). In the third greenhouse trial, and the field trial, we included treatments with Azomite Granular and Ultrafine, using 1 and 2 grams in the greenhouse, and 1 and 3 grams in the field (n = 4 for each). Trial 3 included plants that were amended with these concentrations of Azomite and were inoculated (as described in Figure 1) with either a low, or high dose of PhylloStart (n = 4 for each), a PhylloStart only treatment at both concentrations (n = 6 for each), and a control treatment that did not receive either Azomite or PhylloStart (n = 6). A second field trial was performed at UC Davis to confirm the results from our initial field trial, using the same methods as previously described, with the following treatments; PhylloStart (n = 6), Control (n = 6), PhylloStart with 3 grams of Azomite (n = 6) and Control with 3 grams of Azomite (n = 6).

3.2.4 Plant measurements and harvest

Plant height was measured from the soil surface to the terminal node, recorded weekly during vegetative growth in Days After Sowing as indicated (Supplemental Figure 1). Plant width was determined by measuring the combined lengths of the two longest opposing-side branches at the base of each plant, recorded along with plant height. During reproductive growth, total numbers of flowers and fruit attached per plant were counted as indicated (Supplemental Figure 1 and Figs. 3, 4). Tomatoes were weighed individually, and as total harvested weight per plant as described (Supplementary Figure 2, 3, 4). Tomato number and weight were recorded multiple times per plant from onset of fruit production to plant termination in the greenhouse, but these metrics were measured only once after harvest from each individual plant grown in the field.

3.2.5 Leaf sample collection

Leaves were sampled in the first and third experiments to assay the composition of the phyllosphere microbial community. In each case, 5 leaves were collected into 50ml conical tubes from random locations across each plant. These leaves were weighed and 40ml of sterile 10mM $MgCl_2$ was added. They were sonicated for 10 minutes, followed by five seconds of vortexing to ensure that the bacteria separated from the leaves. The bacteria were pelleted, and the supernatant was removed. These samples were frozen at -80ºC until DNA extraction and sequencing.

3.2.6 Pathogen protection experiment

Moneymaker tomato seeds were prepared as described above, then germinated onto plates of 1% water agar. After 1 week, seedlings were transferred to individual pots containing autoclaved soil consisting of calcined clay medium (Profile Porous Ceramic Greens Grade, Sierra Pacific Turf Supply). In the fertilizer treatment, 960 mg of organic fertilizer (0-11-0 Seabird Guano, Down to Earth) was added to each pot at the transplant stage. Plants were randomized with respect to treatment and maintained in a growth chamber at a 15 h day:9 h night cycle for the duration of the experiment.

When plants were three weeks old, PhylloStart communities were applied to leaves at a concentration of OD600=0.02 with 0.01% SilWet surfactant. One week after spraying, an overnight culture of *Pseudomonas syringae* pathovar tomato PT23 was diluted in 10 mM $MgCl_2$ to a concentration of OD600=0.0002. Three leaves per plant were challenged via blunt-end syringe inoculation. At 24 hours post-infection, 3 hole punches (6-mm diameter) were taken from each inoculated leaf (9 total leaf discs per plant). Leaf discs were homogenized in 1 mL 10 mM $MgCl_2$ in a FastPrep-24 5G sample disruption instrument at 4.0 m/s for 40 seconds. *Pseudomonas syringae* population density on leaves was obtained through colony forming unit (CFU) plating.

3.2.7 DNA extractions, qPCR, 16s rRNA amplification, and sequencing

DNA extraction and sequencing was performed by Microbiome Insights using the following protocols. Bacterial pellets were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following MoBio's instructions on a KingFisher robot. For qPCR, bacterial-specific (300 nM 27F, 5′ -AGAGTTTGATCCTGGCTCAG-3′) forward primers coupled to (300 nM 519R, 5′ -ATTACCGCGGCTGCTGG-3′) reverse primers were used to amplify bacterial 16S rRNA. 20 µl reactions using iQ SYBR Green Supermix (Bio-Rad), with 10µl Supermix, 0.6µl Primer F, 0.6µl Primer R, 6.8µl H2O and 2µl template, were run on Applied Biosystems StepOne Plus instrument in triplicate using the following cycle conditions; 95ºC for 3 min., 95ºC 20 sec., 55ºC for 20 sec., 72ºC for 30 sec., return to step two 45 times. For standards, full-length bacterial 16S rRNA gene was cloned into a pCR4-TOPO vector, with Kanamycin-Ampicillin resistance. The total plasmid fragment size is expected to be 5556 bp. A bacterial standard was prepared via. 10-fold serial dilutions, and the copies of 16S was determined by the following: Copy# = (DNA wt. x 6.02E23)/(Fragment Size x 660 x 1E9). Linear regression was used to determine copy numbers of samples, based on CT of standards. Reaction specificity was assessed using a melt curve from 55ºC to 95ºC, held at 0.5ºC increment for 1s. For 16s rRNA amplification and sequencing, bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5’-GTGCCAGCMGCCGCGGTAA-3', and 806R 5’-GGACTACHVGGGTWTCTAAT-3'), as per the protocol of Kozich et al. 2013 (Kozich *et al.*, 2013). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from template-free controls (negative control) and extraction kit reagents processed the same way as the specimens. A positive control from samples consisting of cloned SUP05 DNA, was also included. The only modification to this standard protocol was the addition of peptide nucleic acid (PNA) PCR clamps according to the method developed in Lundberg et al. (Lundberg *et al.*, 2013). In brief, mPNA, to reduce mitochondria amplification and pPNA to reduce chloroplast amplification, were added into the PCR step during library prep at a concentration of 5uM per PNA. The PCR reaction was then modified with the addition of a PNA annealing step at 78ºC for 10s.

3.2.8 Data analysis

Forward and reverse paired-end reads were filtered and trimmed to 230 and 160 base pairs (bps), respectively using the DADA2 pipeline with default parameters (Callahan *et al.*, 2016). Following denoising and merging reads and removing chimeras, DADA2 was used to infer amplicon sequence variants (ASVs), which are analogous to operational taxonomic units (OTUs), and taxonomy was assigned to these ASVs using the DADA2-trained SILVA database. Using DNA extraction and PCR negative controls from 16s sequencing the *decontam* package was implemented using default settings to identify and remove potential contamination from the samples (Davis *et al.*, 2018). The assigned ASVs, read count data, and sample metadata were combined in a *phyloseq* object (McMurdie & Holmes, 2013) for downstream analyses. The *phyloseq* package was used to calculate field and greenhouse beta diversity, and a permutational analysis (PERMANOVA) was performed on data rarified to 400 reads (in order to account for the extraordinarily low read count in untreated greenhouse samples) using the *adonis* function in the *vegan* package (Oksanen *et al.*, 2022).

All plant growth was analyzed using a linear mixed-effects model in R with the function *lme* from the *nlme* package (Pinheiro *et al.*, 2022). Model fit was assessed using ANOVA with the *anova* function to test if the inclusion of additional factors and interactions significantly improved model fit compared to a null model including only the intercept. In each model plant ID was included as a random effect to account for repeat sampling over time.

3.3 Results

3.3.1 PhylloStart community colonizes the plant phyllosphere

We hypothesized that greenhouse-grown plants would be relatively depauperate in their microbial associations. To test this, we inoculated seedlings with the PhylloStart community by spraying the synthetic microbiome inoculum directly onto the leaves over the course of three, weekly applications. Using an adonis PERMANOVA we saw no differences in the communities from plants treated with or without Azomite, F = 0.92, p = 0.543, and so for the sake of simplicity these sequences are not included in the figure (Figure 2).

We used qPCR to estimate the total number of bacteria on the leaves of PhylloStart treated and control plants, finding that there was a significantly higher abundance of bacteria on the inoculated leaves, $t_{18} = -3.97$, $p = 0.003$, with an average of 1327.7 (±950.93 SD) bacterial sequences in the inoculated group, compared to 132 (±52.14 SD) in the controls. Further, in the treated plants, the vast majority of the bacterial sequences were associated with PhylloStart members with an average of 1217.05 (±946.68) PhylloStart-matching sequences per plant. This indicates both that there is robust representation of the PhylloStart on the plant leaves, and that there is minimal development of leaf associated bacteria from the greenhouse environment.

3.3.2 PhylloStart and micronutrient type interact to increase flowering and fruit production

To determine the role of phyllosphere bacteria, and their interaction with micronutrient supplementation, on greenhouse-grown tomato plants we collected data on a variety of plant characteristics throughout their development. This included plant height, width, flowers, fruit on the plant, and the total weight of fruit harvested. We analyzed the data using a linear mixed effects model, selecting only terms identified by an ANOVA to significantly improve the model's fit. For height and width, only the model including time was selected as significant ($p < 0.0001$ for both), and unsurprisingly there was a significant effect of time on these traits, as both height and width increase as the plant grows, for height, $t_{299} = 106.10$, $p < 0.0001$, and for width, $t_{119} = 39.26$, $p < 0.0001$ (Supplemental Figure 1).

When analyzing flowers and on-plant fruit, we found that the full model (including treatment) was significantly better than the null for both (for flowers, $p = 0.0303$, and for tomatoes counted on plant, $p = 0.007$; Supplemental Figure 1). For flowers, we again saw, as expected, a significant effect of time, $t_{294} = 13.31$, $p = <0.0001$, as well as a significant impact of the granular and ultrafine Azomite + PhylloStart on number of flowers per plant, $t_{54} = -2.32$, $p = 0.024$, with a significant interaction term between this treatment and time, $t_{294} = 1.88$, $p = 0.003$ (Supplemental Figure 1). For fruit counted on the plants, we found a significant effect of time, $t_{292} = 12.34$, $p <0.0001$, and a significant effect of the interaction between both the PhylloStart only treatment and time, $t_{292} = 2.21$, $p = 0.027$, and the granular and ultrafine Azomite + PhylloStart treatment and time, $t_{292} = 2.361$, $p = .019$ (Supplemental Figure 1). Given these results, we chose to focus only on the production of fruit in our two subsequent experiments.

3.3.3 PhylloStart inoculation and micronutrient supplementation increase tomato production

The first experiment fruit was harvested in bulk, preventing the statistical analysis of the resulting harvest data. However, there was a qualitative increase in the total weight of tomatoes harvested from plants inoculated with the PhylloStart bacteria, from 8003.92 grams total yield in control to 9705.54 grams in the PhylloStart treatment and 9302.92 grams in the granular and ultrafine Azomite treatment compared to 10990.6 grams for the granular, ultrafine and PhylloStart treatment (Supplemental Figure 2).

After establishing that the primary impacts of the PhylloStart bacteria were in the numbers of flowers (which transition into fruit) and fruit, we repeated the experiment focusing on the total number of tomatoes produced across bacterial conditions and micronutrient supplement. In this experiment, we found a significant increase in the total number of tomatoes produced by plants that were inoculated with PhylloStart bacteria, $t_{24} = 3.81$, $p = 0.001$, with an average of 8.89 (±2.93 SD) tomatoes produced per treated plant per harvest, compared to an average of 7.63 (±4.72 SD) tomatoes in the control group (Figure 3).

Micronutrient supplementation also significantly increased the number of tomatoes produced per harvest, with the 2-gram treatment increasing the average number of tomatoes from 7.63 (±4.72 SD) to 9.89 (±3.41 SD), $t(24) = 3.045$, $p = 0.006$. We found no significant interaction between PhylloStart application and Azomite concentration. The highest level of micronutrient supplementation, however led to a significant decrease in yield, with an average of 4.11 (±2.32 SD) tomatoes produced, $t_{24} = -2.18$, $p = 0.039$ (Figure 3). As it was clear that the highest concentration of micronutrient supplementation was deleterious to the plant. It is interesting that, while not identified as statistically significant in the model, we observed a trend in which the plants treated with 3 grams of micronutrient supplement and the PhylloStart were less severely affected than those treated with the micronutrient supplement alone (Figure 3), indicating that the presence of Phyllosphere bacteria was partially rescuing the plants from this abiotic stress.

In order to rule out that the plants were producing more but smaller tomatoes, we measured both tomato number and weight. Using a linear mixed-effects model, we saw no significant impact of treatment with PhylloStart on the weights of individual tomatoes produced (Supplemental Figure 3). In treated plants, the average tomato fruit weight was 46.05 (±14.99) grams, as compared to an average weight of 46.12 (±11.98) grams in the control group ($t_{24} = 1.556$, $p = 0.133$). However, we did see a significant impact of the micronutrient supplement, with 1 and 2 grams significantly increasing the individual tomato weight, $t_{24} = 3.83$, $p = 0.001$ and $t_{24} = 6.07$, $p < 0.001$ respectively, with average tomato weights of 50.66 (±12.41 SD) grams and 53.46 (±12.39 SD) grams, compared to the control group at a mean of 46.05 (±11.98 SD) grams. Meanwhile, the higher concentration of micronutrient supplementation, at 3 grams per plant, was associated with a significant reduction in the weight of the tomatoes produced, $t_{24} = -2.48$, p = 0.021, with an average weight of 41.26 (±15.55 SD) grams per tomato. Again, there was no significant interaction between the micronutrient supplementation and PhylloStart bacterial application.

3.3.4 Phyllosphere amendment increases fruit production in a dose-dependent manner, and these effects persist under disease pressure

To determine if the increased fruit production in PhylloStart inoculated plants was dose-dependent, we repeated the experiment in the fall of 2020, including both the standard inoculum density (OD600=0.02, High) and a lower density (OD600=0.0002, Low). The trends we see in this experiment are consistent with the results from our first and second trials. Notably, these plants were impacted by powdery mildew, a common disease in greenhouse tomato production. The plants began to show signs of infection around week 13, and by week 15 powdery mildew was uniformly present across the surface of most leaves on each plant, regardless of treatment. The plants were randomly dispersed throughout the greenhouse, and regardless of location we did not see a noticeable difference in presence of powdery mildew, so there is no reason to believe that the impacts of disease (beyond the broad impact to the plants as a whole) would bias these results.

Interestingly, despite this disease pressure, the number of tomatoes produced was again significantly increased in the plants inoculated with the PhylloStart bacteria, but only in those treated with the higher inoculation density, $t_6 = 2.70$, p = 0.036 (Figure 4; A). These plants produced an average of 16 (±11.68 SD) tomatoes per plant per harvest, as opposed to 12 (±8.98 SD) tomatoes in the control group. Unlike in the previous experiment, we did not see any significant impact of the micronutrient supplementation on the numbers of tomatoes produced. We also did not see any significant impact of either PhylloStart application (as expected) or micronutrient supplementation (in contrast to our previous experiment) on the average weight of the tomatoes produced (Supplemental Figure 3).

3.3.5 Phyllosphere amendment limits subsequent colonization of a bacterial pathogen

Previous work in tomato plants has observed that the native microbiota of the phyllosphere is protective against colonization of the foliar pathogen *Pseudomonas syringae* pv tomato, especially under low resource conditions (Berg & Koskella, 2018). We asked whether the reduced community described in this study would be sufficient to replicate this effect. We applied PhylloStart bacteria to three-week-old plants under either nutrient-limited conditions (grown in autoclaved calcined clay medium) or high nutrient conditions (supplemented with an organic phosphorus fertilizer, Seadbird Guano). One week after PhylloStart bacterial inoculation, leaves were infected with *P. syringae* via blunt-end syringe inoculation. Analysis with ANOVA indicated a significant effect of PhylloStart on pathogen abundance. Post-hoc analysis with a Tukey HSD showed that under nutrient limitation, pathogen load was significantly lower on plants inoculated with PhylloStart bacteria than on plants inoculated with a sterile buffer control, indicating a protective effect, t = 2.67, p = 0.037, however, and as previously observed (Berg & Koskella, 2018), this effect disappeared among plants treated with the phosphorus fertilizer, t = 0.07, p = 0.948 (Figure 5).

3.3.6 Greenhouse plants maintain PhylloStart bacteria over time, while field plants did not under the conditions tested

In order to determine if the effects of PhylloStart bacteria on plant reproductive success would be seen in an environment with greater dispersal of phyllosphere bacteria and/or whether early inoculation of plants changed subsequent microbiome assembly in the field, we included a field component in the third trial experiment. We transferred both PhylloStart-inoculated and control plants at the end of treatment into the field for the remainder of their development. These plants were sampled concurrently with the plants from the same cohort that remained in the greenhouse (three weeks after their last inoculation), and their phyllosphere communities were sequenced. Using an ANOVA we found that there is a significant effect of inoculation density on the relative abundance of the PhylloStart-associated bacteria in the greenhouse ($p = 0.002$), which, interrogated with a Tukey HSD, indicated that there was a significantly higher relative abundance in the Phyllostart high treatment compared to the Phyllostart low treatment ($p = 0.015$) and the control ($p = 0.002$). Meanwhile, there was no significant effect of PhylloStart treatment on the relative abundance of the PhylloStart-associated bacteria in the field ($p = 0.432$), (Figure 4; B).

Furthermore, when looking at a PCOA of community similarity using Bray-Curtis distance metrics (Figure 4; D) we see that the PhylloStart-treated greenhouse plants clearly separate out from the control plants, with the plants treated with high concentrations of PhylloStart distinct from the controls, and the plants treated with low concentrations of PhylloStart falling between the controls and the high inoculation. Indeed, when analyzing dissimilarity using an Adonis PERMANOVA, we see that the PhylloStart High treated plants are significantly different in community composition from the controls ($p=0.004$). In contrast, we see no significant difference in PhylloStart relative abundance on the plants that were transplanted to the field, with each treatment containing relatively few of the PhylloStart-associated bacteria. Further, analysis with the Adonis PERMANOVA reveals that there is no significant difference between any of the bacterial treatments in field plants under the conditions tested.

3.3.7 Early PhylloStart inoculation did not impact field-grown plants

Given that the field-grown plants were able to establish bacteria beyond what was initially inoculated, we sought to determine whether there would be differences in tomato production over the development of the plant. Unlike in the greenhouse experiment, where we found a significant effect of PhylloStart on the total number of tomatoes produced, we did not observe any significant effect of phyllosphere amendment on yield in the field (Figure 4; C). An ANOVA to test the appropriateness of including PhylloStart in a general linear mixed-effects model, found that inclusion of this factor did not significantly improve the model compared to the null with only the intercept. Like in the greenhouse, we saw no significant effect of PhylloStart on tomato weight (Supplemental Figure 3). In order to verify these results, we performed another field trial in a subsequent year, finding broadly the same results of no significant effect of PhylloStart on the number of tomatoes harvested. In this second experiment we again did not see any significant improvement of the PhylloStart term when compared to the null in a glmm (Supplemental Figure 4). It remains to be seen whether these amendments can provide benefits in larger field trials, including under biotic or abiotic pressures where we have observed particularly pronounced effects of PhylloStart in the greenhouse.

3.4 Discussion

This study provides robust evidence that the phyllosphere-associated microbiome enhances the reproductive success of the host plant. Our initial experiment established that greenhouse-grown plants develop a significantly more abundant microbial community when inoculated with phyllosphere native bacterial taxa, and that the effect of early exposure to these bacteria persists throughout the development of the plant. With two additional studies, we verified that these early microbial associations lead to a significant increase in the total number (but not size) of fruit produced by greenhouse-grown tomato plants and that these effects are resilient to both biotic and abiotic stressors. We found these effects to be predominant in the greenhouse setting, as plants that were transplanted into a field environment did not appear to further benefit from the initial inoculation of PhylloStart bacteria. Field performance relies on a combination of additional variables that are commonly controlled in the greenhouse setting. Further field trials under a broad spectrum of conditions and locations are needed to determine whether bacterial amendments to the phyllosphere can potentially confer benefits to commercial field tomato production.

Our qPCR assessment of bacterial abundance indicates minimal development of the phyllosphere community in non-treated greenhouse control plants. This supports previous work that found greenhouse-grown plants develop bacterial communities distinct from those in outdoor environments (Maignien *et al.*, 2014). This lack of appreciable bacteria in the absence of amendment allowed us to examine the importance of phyllosphere bacteria to plant fitness by inoculating plants with a microbial community designed to mimic phyllosphere communities of field-grown plants. We find that the presence of PhylloStart bacteria, inoculated during the early development of the plant, is associated with increased flowering and fruit production, and that these plants produce a significantly higher amount of fruit throughout their lifetimes. As expected, given microbial dispersal outside of the greenhouse, we did not see these effects persist when transplanting seedlings into a field setting. In this case, PhylloStart-associated bacteria were not found at significant abundances on these plants after a month in the field, and their initial community structure did not seem to shape the future composition of the phyllosphere communities. This may indicate that priority effects during early microbiome establishment are minimally important in a setting with strong colonization pressure from other sources.

There are various mechanisms by which the phyllosphere bacterial community might provide these essential benefits to its host. These primarily include: 1) through altering the plant hormone signaling, either directly through the production of phytohormones or indirectly through the elicitation of a plant response; 2) by increasing the nutrients available to the plant either through enhanced nutrient fixation or availability; and 3) through reduction of stress, either environmental or due to pathogen pressure (Esİtken *et al.*, 2005; Paul & Nair, 2008; Adesemoye *et al.*, 2008; Beneduzi *et al.*, 2012; Bhattacharyya & Jha, 2012). While our current study does not seek to explain the mechanism underlying observed biostimulant effects, it likely relies on a combination of these. However, that the effects of Azomite fertilization and PhylloStart inoculation acted primarily in an additive fashion throughout our study suggests that altered nutrient acquisition as a result of phyllosphere amendment is not a particularly dominant force. Moreover, that the impact of PhylloStart application on yield seemed to be resilient against both the impacts from over-fertilization and disease across our experiments could indicate that these communities play a particularly important role in buffering host plants against stress.

One potential explanation for this increased reproductive success is linked to the phytohormone auxin (or IAA), which is a major regulator of plant growth, is commonly produced by bacteria inhabiting the phyllosphere (Brandl & Lindow, 1998) and has been linked to increased biomass accumulation in rice and corn (Mwajita *et al.*, 2013; Abadi *et al.*, 2020). In this context, increased fruit yield could be mediated by the action of auxin, decreasing flower abscission (Sexton & Roberts, 1982; Meir *et al.*, 2010), potentially leaving more flowers available to set. In support of this idea, we did observe a significant increase in total flowers on the plant over time in our first greenhouse trial (the only one in which this was explicitly measured) in response to PhylloStart application with Ultrafine Azomite (Supplemental Figure 1). Further, using BLAST to search the genomes of the PhylloStart bacteria, we found that several members (*Bacillus wiedmannii*, *Erwinia tasmaniensis*, *Pantoea agglomerans*, and *Pantoea allii*) have matches for *idpC* (indole-3-pyruvate/phenylpyruvate decarboxylase)*,* a key protein in auxin production (Brandl *et al.*, 2001). Future work will have to be done to confirm that these bacteria can produce auxin *In Planta,* and if this may explain some of their plant beneficial effects.

It is also possible that the PhylloStart bacteria alter the plant's response to environmental cues, allowing the plant to better optimize its growth strategy and invest more resources in reproduction. Recent work has focused on the phenomenon of microbiome-dependent ontogenic timing (MiDOT), by which the presence of certain bacterial species acts as essential cues in the developmental timing of their host organism (Metcalf *et al.*, 2019). For example, the composition of the *Boechera stricta* (a relative of Arabidopsis) soil-associated bacterial community has been found to significantly alter the timing and duration of flowering. There is further evidence using genetic approaches, such as with the Arabidopsis gene AtBBX32 (Khanna, *et al.*, 2009), when introduced into transgenic commercial crops, is responsible for large-scale shifts in the timing of growth responses to external cues like light, which leads to modulation of the timing of reproductive development, including flowering and yield promotion (Holtan *et al.*, 2011; Preuss *et al.*, 2012). These studies show that the plant is capable of large phenotypic changes triggered by its altered abilities to respond to the incumbent environment, which could be achieved through gene modification or alternative approaches capable of influencing plant physiological responses via the assortment of phytohormones or signals of microbial origin. Further research should continue to assess the role that host-associated microbes play in developmental timing.

Interestingly, we saw that the effects of PhylloStart were particularly pronounced under stressful conditions. In our second trial, where we tested a range of Azomite amounts applied to the soil, it was apparent, in contrast to the benefits at lower concentrations, that the highest concentration of micronutrient supplement produced undesirable results in terms of plant growth and productivity. However, the plants treated with PhylloStart bacteria in addition to this high dose did not see a severe reduction in fruit weight or number of fruit produced, showing performance that was broadly similar to the plants that were not treated with any micronutrient supplement. This effect was apparent again in our third trial, in which powdery mildew severely impacted the plants. In this case, we did not see an impact of the micronutrient fertilizer on the number of fruits produced; however, we still saw a significant impact of association with PhylloStart bacteria on fruit number. Throughout these trials we saw no evidence of a significant direct interaction between the nutrient status of the plant (through micronutrient supplementation) and the effect of the PhylloStart bacteria; instead, these two treatments worked additively to increase the total fruit yield further. Given these observations, we were curious if the PhylloStart community would show the same nutrient dependent pathogen protection as found in our lab's previous work using a conventional fertilizer (Berg & Koskella, 2018). Indeed, we found that the addition of this community limited the growth of the pathogen *P. syringae* in nutrient-limited plants, but that these effects were abolished when organic phosphorus fertilizer was added. These results are in line with the stress gradient hypothesis, which posits that inter-species interactions should become more facilitative under adverse conditions (Bertness & Callaway, 1994; David *et al.*, 2020), and highlight the important role that phyllosphere bacterial associations play in stress response. With that in mind, it is also possible that the protection seen in the nutrient-limited plants was due to competition among the PhylloStart species and the pathogen for these nutrients, and so adding the fertilizer lessened the competitive exclusion. Further, we see these responses persist well after the initial exposure to PhylloStart bacteria in their early development. This suggests that there may be a critical window in which the plant is receptive to exposure to phyllosphere-associated microbiota, much like what is posited in the hygiene hypothesis for human-associated microbes.

3.5 Conclusion

In summary, we find that the presence of phyllosphere-associated bacteria has important benefits to their plant host when they are grown in a microbially depauperate greenhouse environment, primarily through an increase in reproductive success as measured by total fruit production, with further evidence for an increase in stress tolerance. These results are important for understanding the role of microbial communities in host outcomes and are broadly relevant in an agricultural context where, for example, 32% of domestic and 56% of imported tomatoes in the United States are grown in greenhouses that may not provide adequate colonization of phyllosphere bacteria (Baskins *et al.*, 2019). Further, we show that bacterial inoculation provides an additive increase in fruit production when applied with a common supplement containing micronutrients, opening avenues for further optimization of agricultural production by harnessing the biostimulant properties of phyllosphere microbes.

3.6 Figures

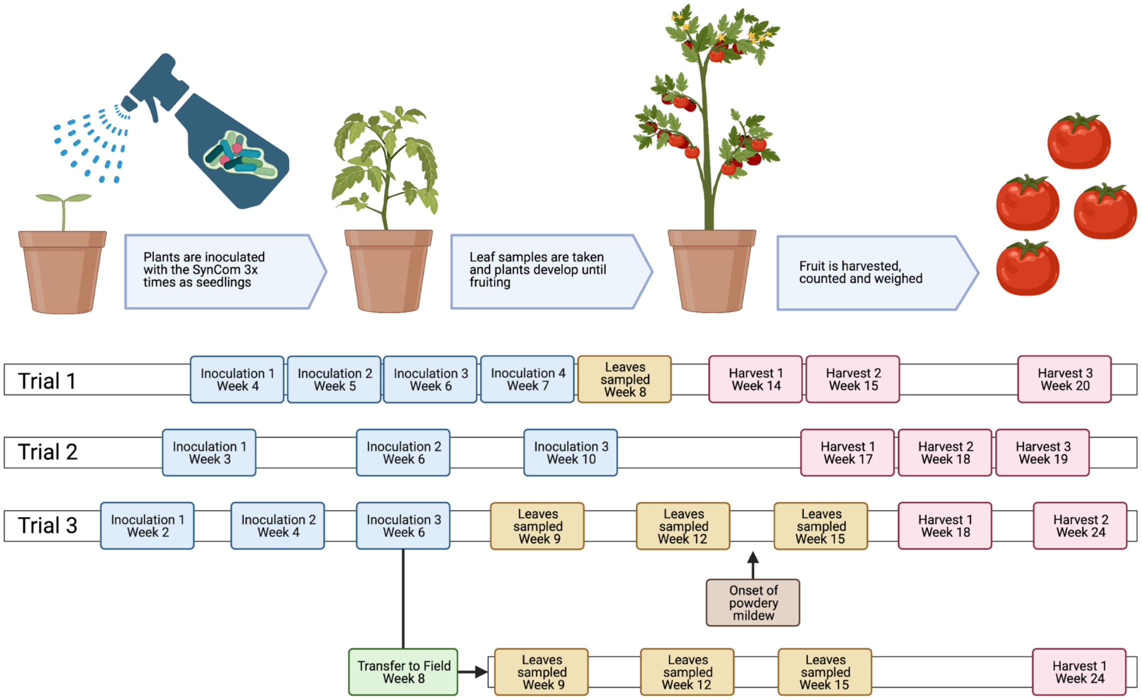


Figure 1. Experimental design for the three greenhouse experiments. Text in Blue indicates the times when the plants were inoculated with the PhylloStart bacteria. Text in Yellow indicates times when leaves were sampled for sequencing. Text in Red indicates timing of tomato harvests. Note the inclusion of the approximate onset of powdery mildew in Trial 3.

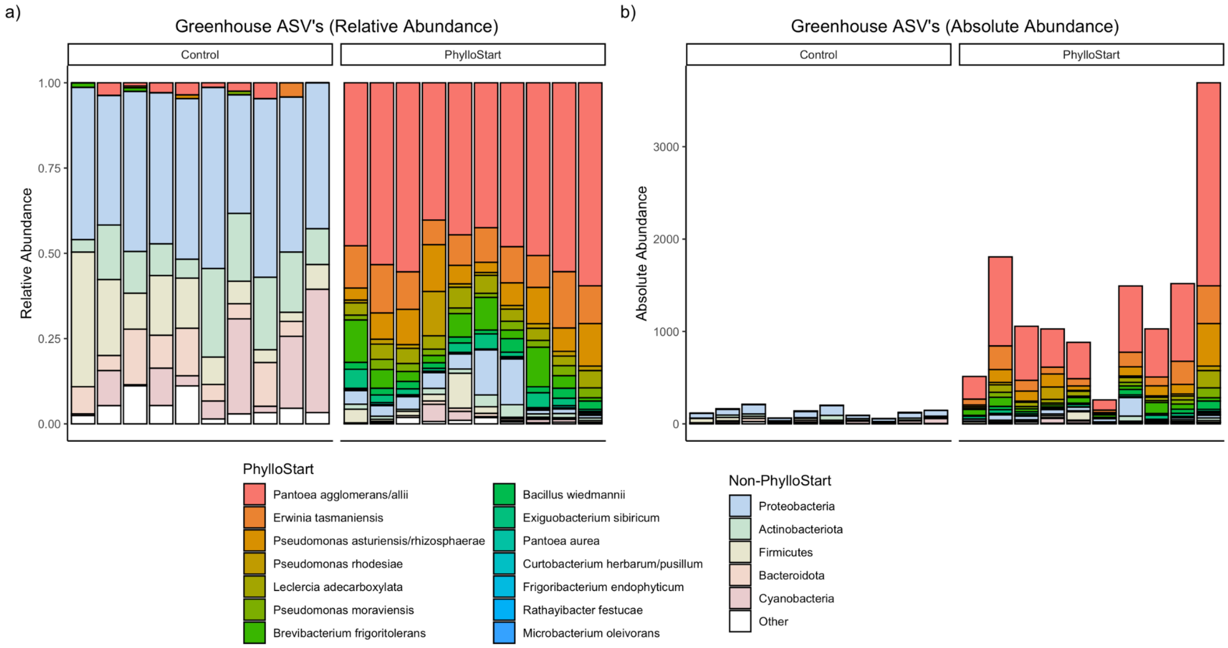


Figure 2. Relative and absolute abundance of ASVs from greenhouse tomato leaves from the first trial. One week after inoculation with the PhylloStart bacteria or a buffer control, only the plants that have been inoculated with PhylloStart have an appreciable number of bacteria residing on their leaves.

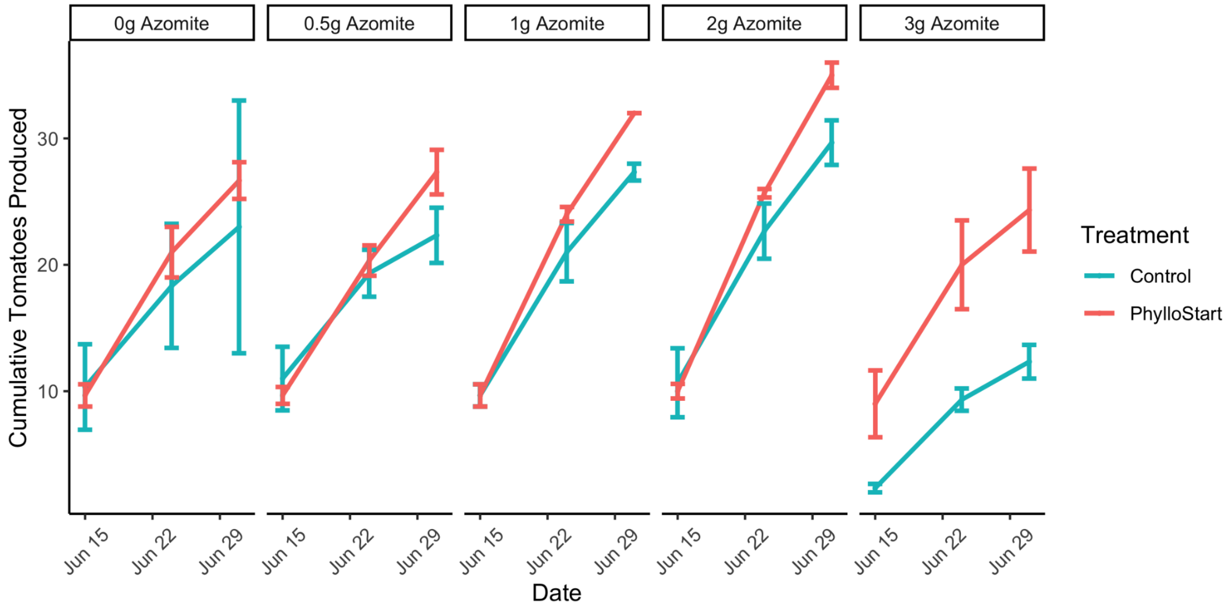


Figure 3. Cumulative number of tomatoes produced across PhylloStart and micronutrient (Azomite) supplemented treatments from the second trial. Both application of the PhylloStart bacteria and Azomite addition lead to a significant increase in the total number of fruit produced. Of note, when adding Azomite in excess (3 grams), total productivity of the control plants was reduced below that of the 0 gram controls. However, when these plants are additionally inoculated with PhylloStart they are rescued to at least the level of the control plants.

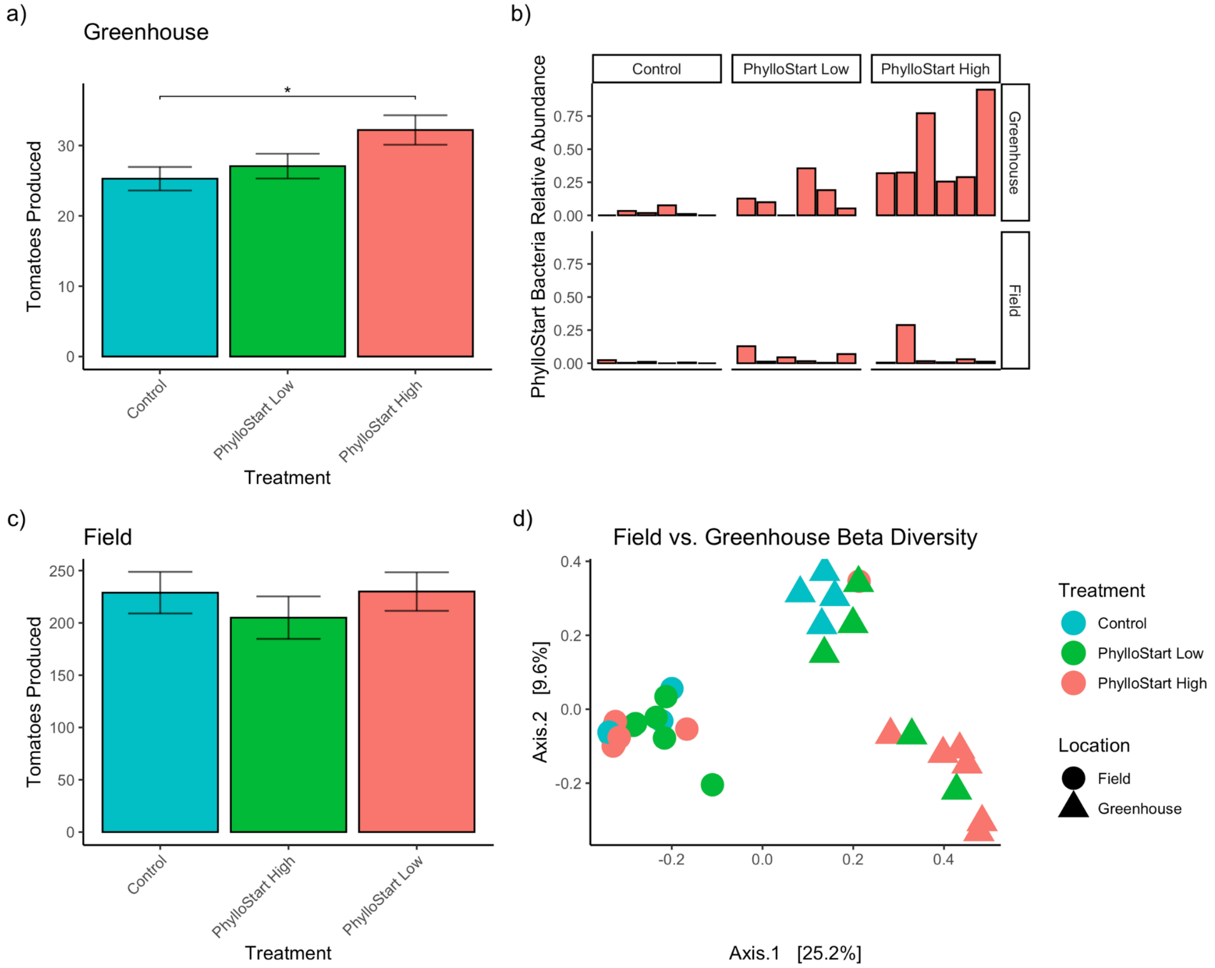


Figure 4. In the third trial greenhouse grown plants show a significant dose dependent effect of PhylloStart, where only the high inoculation density is associated with a significant increase in fruit production. Looking at the relative abundance of PhylloStart bacteria on treated and control plants, one month after inoculation, there are relatively few PhylloStart associated ASVs detected across any of the treatments in the field, while at that time in the greenhouse there are still a marginal number associated with the PhylloStart low density inoculation, and a large portion with the PhylloStart high density inoculation retained on the plants. In contrast to the greenhouse data, there are no significant differences between any of the PhylloStart treatments in the Field grown plants. Finally, looking at Beta Diversity (Bray Dissimilarity), communities group both by treatment and location, with the field communities grouping together, and the greenhouse control and PhylloStart low communities separated from the greenhouse treated PhylloStart high communities.

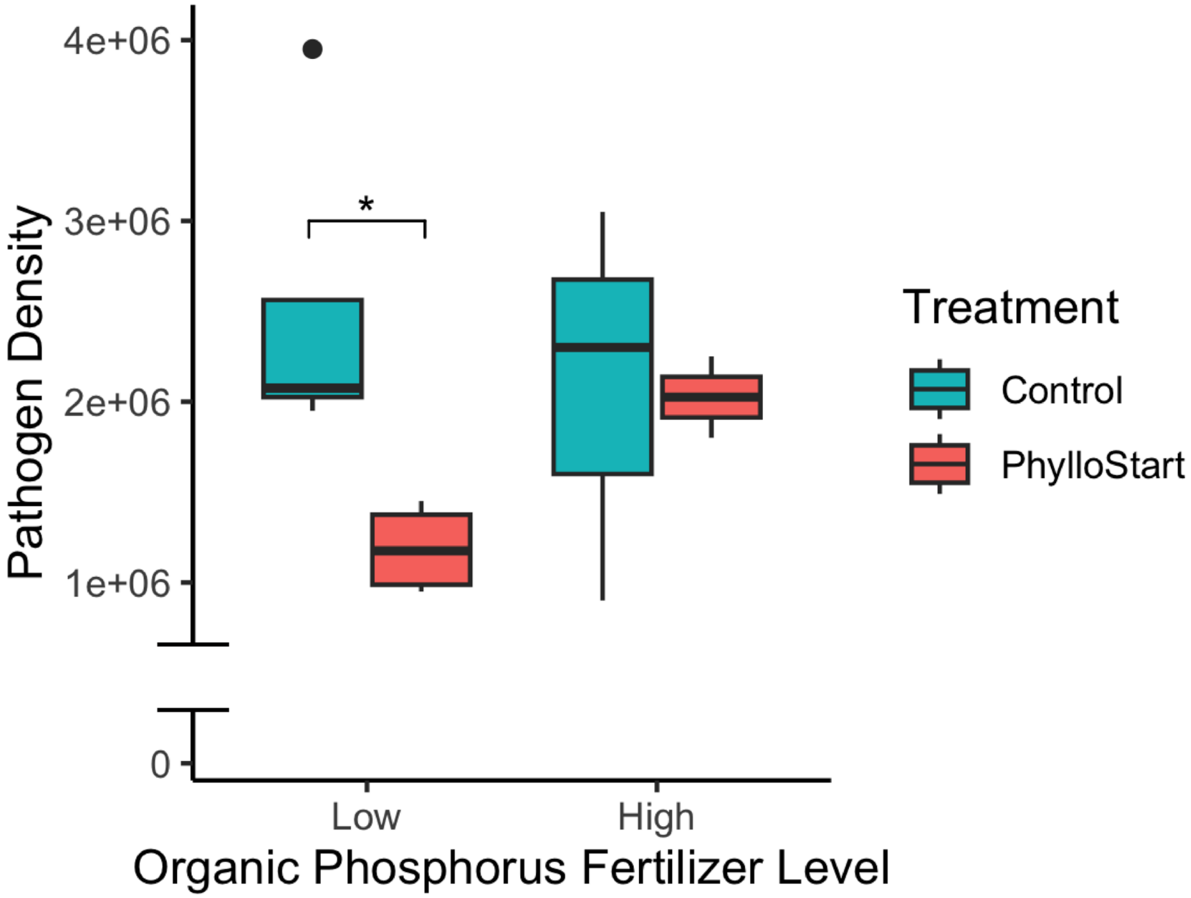
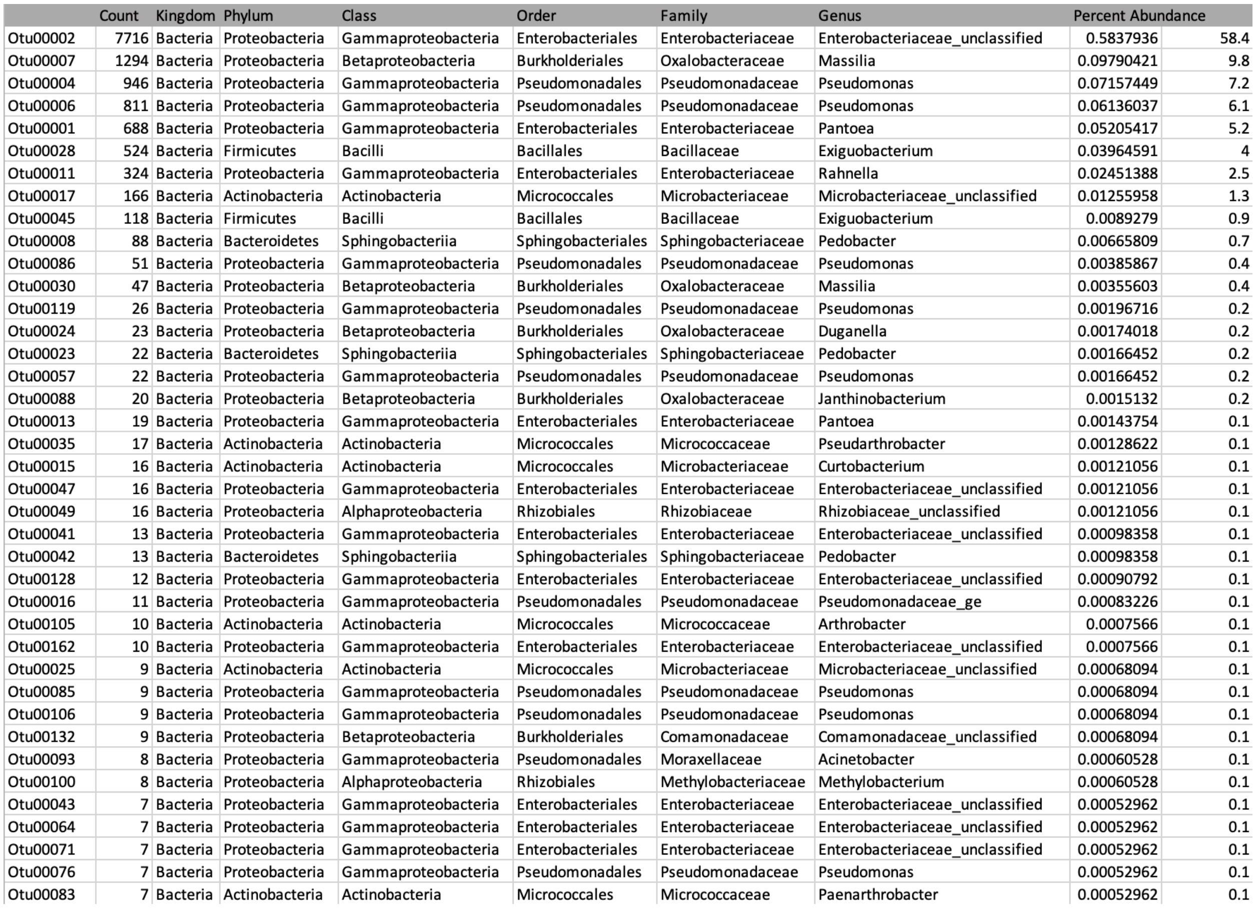


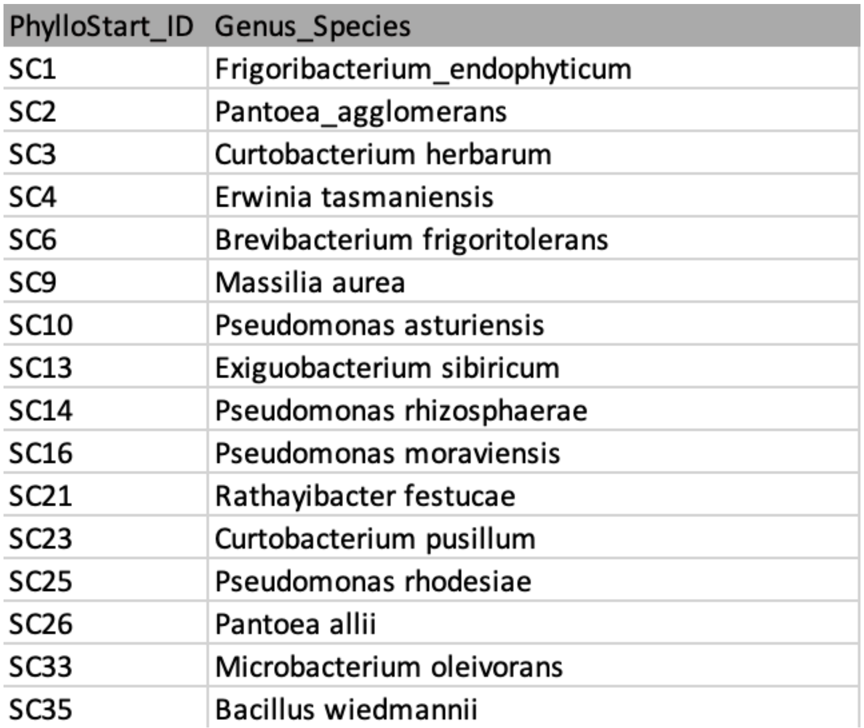
Figure 5. Plants inoculated with PhylloStart bacteria are protected against the establishment of the foliar pathogen P. syringae under low nutrient levels. However, the addition of an organic phosphorus fertilizer leads to a decrease in protection, with both the PhylloStart treated and control plants showing similar levels of pathogen development. Note that, for illustrative purposes, the Y axis is condensed to specific window of density observed on plants and thus does not begin at zero.

3.7 Supplemental Data and Figures



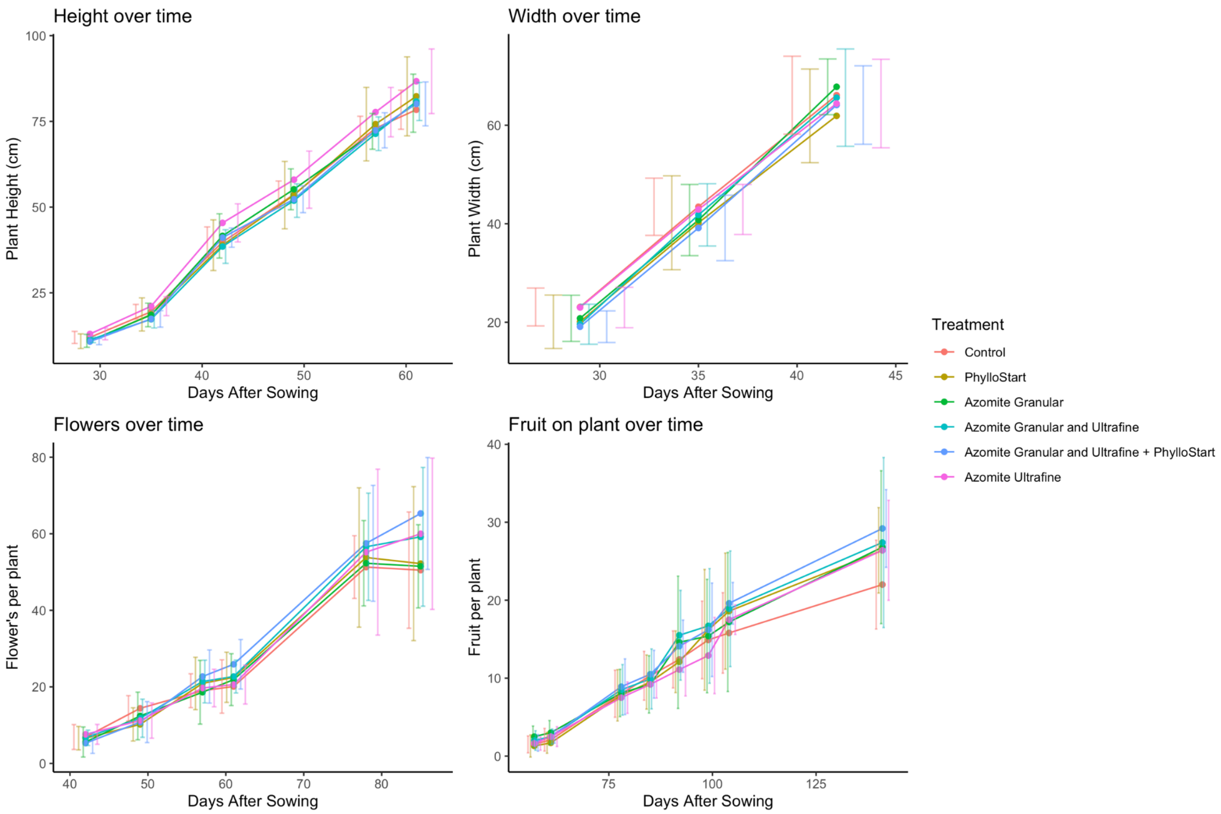
	Count	Kingdom	Phylum	Class	Order	Family	Genus	Percent Abundance	
Otu00002	7716	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.5837936	58.4
Otu00007	1294	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	0.09790421	9.8
Otu00004	946	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.07157449	7.2
Otu00006	811	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.06136037	6.1
Otu00001	688	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	0.05205417	5.2
Otu00028	524	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Exiguobacterium	0.03964591	4
Otu00011	324	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Rahnella	0.02451388	2.5
Otu00017	166	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae_unclassified	0.01255958	1.3
Otu00045	118	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Exiguobacterium	0.0089279	0.9
Otu00008	88	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.00665809	0.7
Otu00086	51	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00385867	0.4
Otu00030	47	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	0.00355603	0.4
Otu00119	26	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00196716	0.2
Otu00024	23	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	0.00174018	0.2
Otu00023	22	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.00166452	0.2
Otu00057	22	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00166452	0.2
Otu00088	20	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	0.0015132	0.2
Otu00013	19	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	0.00143754	0.1
Otu00035	17	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	0.00128622	0.1
Otu00015	16	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Curtobacterium	0.00121056	0.1
Otu00047	16	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00121056	0.1
Otu00049	16	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobiaceae_unclassified	0.00121056	0.1
Otu00041	13	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00098358	0.1
Otu00042	13	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.00098358	0.1
Otu00128	12	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00090792	0.1
Otu00016	11	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonadaceae_ge	0.00083226	0.1
Otu00105	10	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	0.0007566	0.1
Otu00162	10	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.0007566	0.1
Otu00025	9	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae_unclassified	0.00068094	0.1
Otu00085	9	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00068094	0.1
Otu00106	9	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00068094	0.1
Otu00132	9	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae_unclassified	0.00068094	0.1
Otu00093	8	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.00060528	0.1
Otu00100	8	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.00060528	0.1
Otu00043	7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00052962	0.1
Otu00064	7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00052962	0.1
Otu00071	7	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified	0.00052962	0.1
Otu00076	7	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00052962	0.1
Otu00083	7	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Paenarthrobacter	0.00052962	0.1

Supplemental Table 1. Bacterial taxonomic information and abundance from initial sequencing of UC Davis student organic farms.

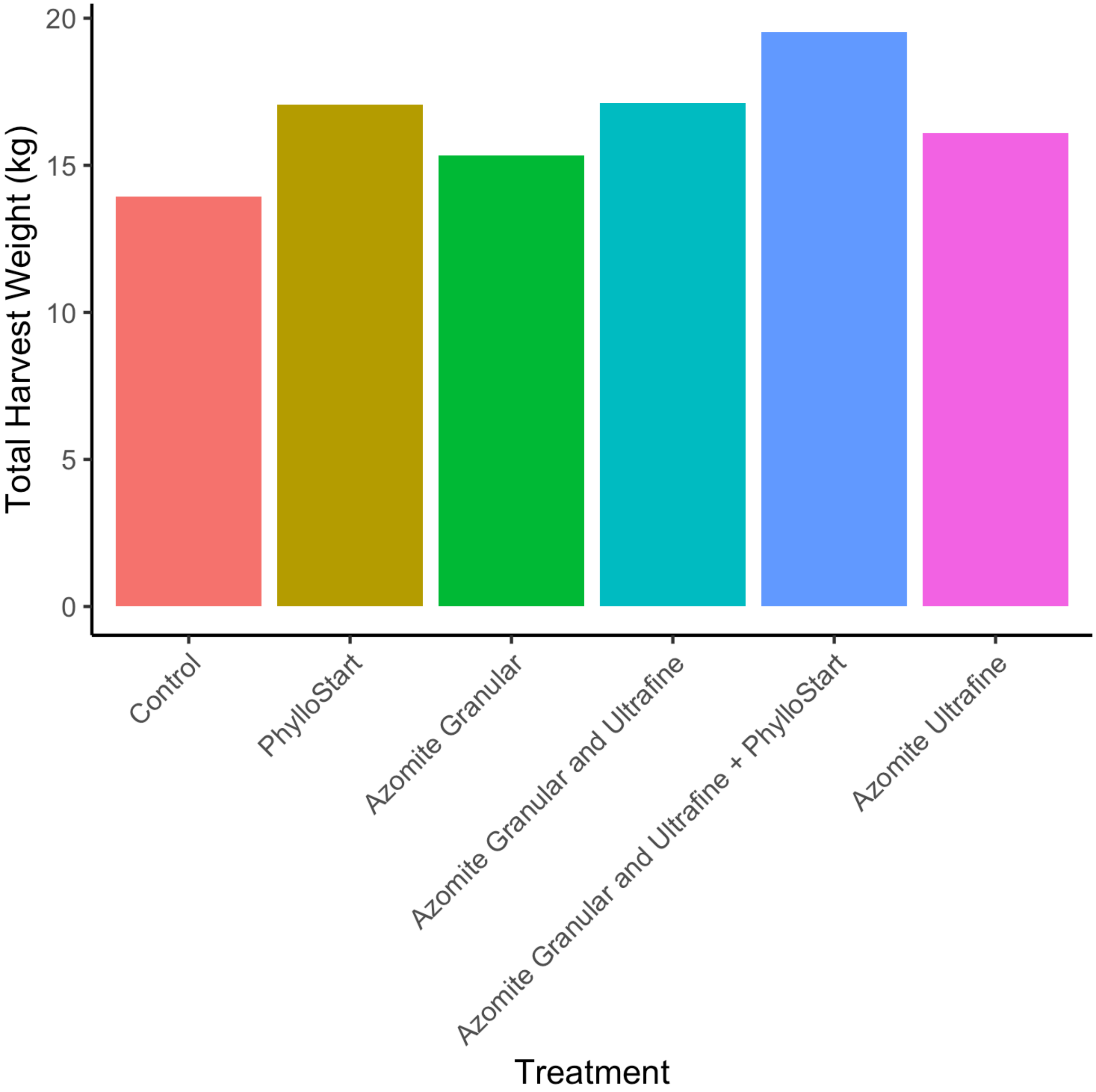


PhylloStart_ID	Genus_Species
SC1	Frigoribacterium_endophyticum
SC2	Pantoea_agglomerans
SC3	Curtobacterium herbarum
SC4	Erwinia tasmaniensis
SC6	Brevibacterium frigoritolerans
SC9	Massilia aurea
SC10	Pseudomonas asturiensis
SC13	Exiguobacterium sibiricum
SC14	Pseudomonas rhizosphaerae
SC16	Pseudomonas moraviensis
SC21	Rathayibacter festucae
SC23	Curtobacterium pusillum
SC25	Pseudomonas rhodesiae
SC26	Pantoea allii
SC33	Microbacterium oleivorans
SC35	Bacillus wiedmannii

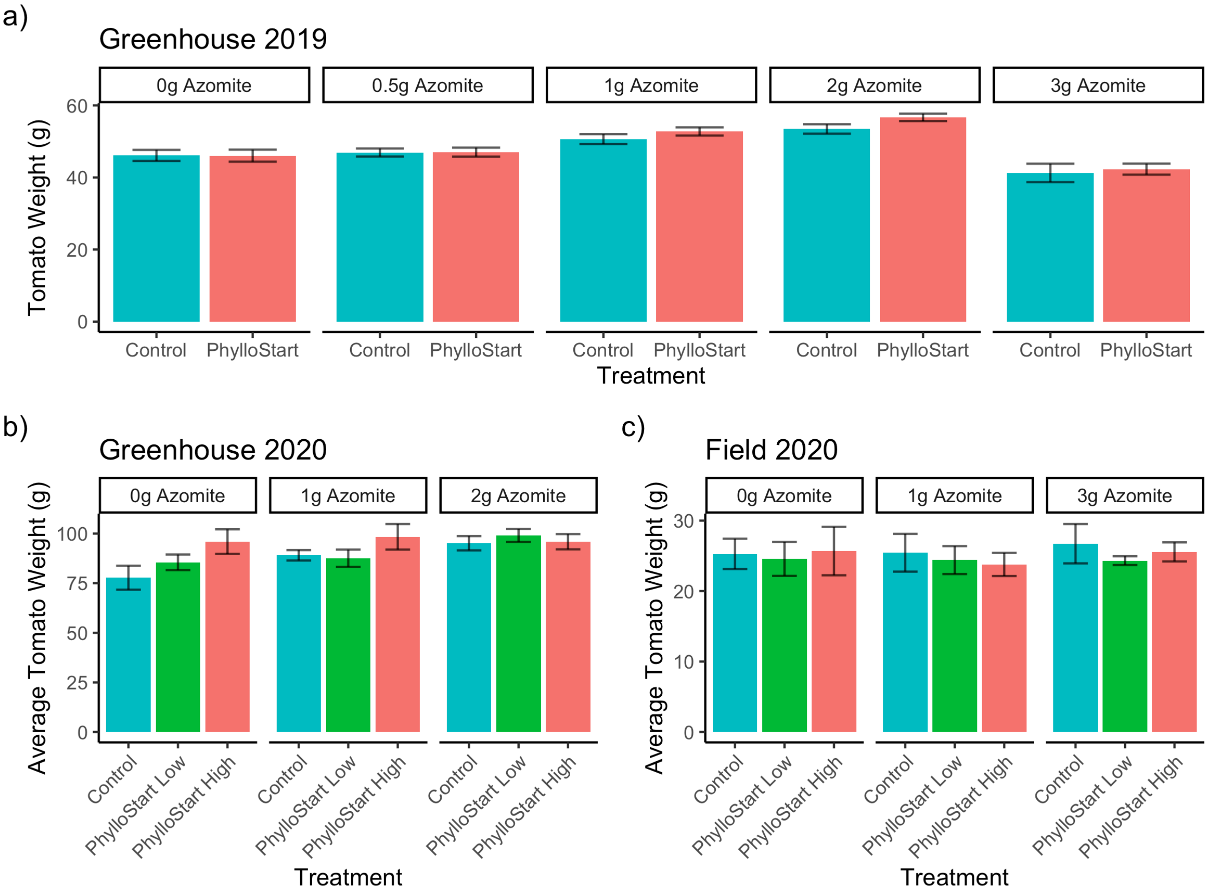
Supplemental Table 2. PhylloStart member identity, as well as 16s sequence and idpC amino acid sequence.



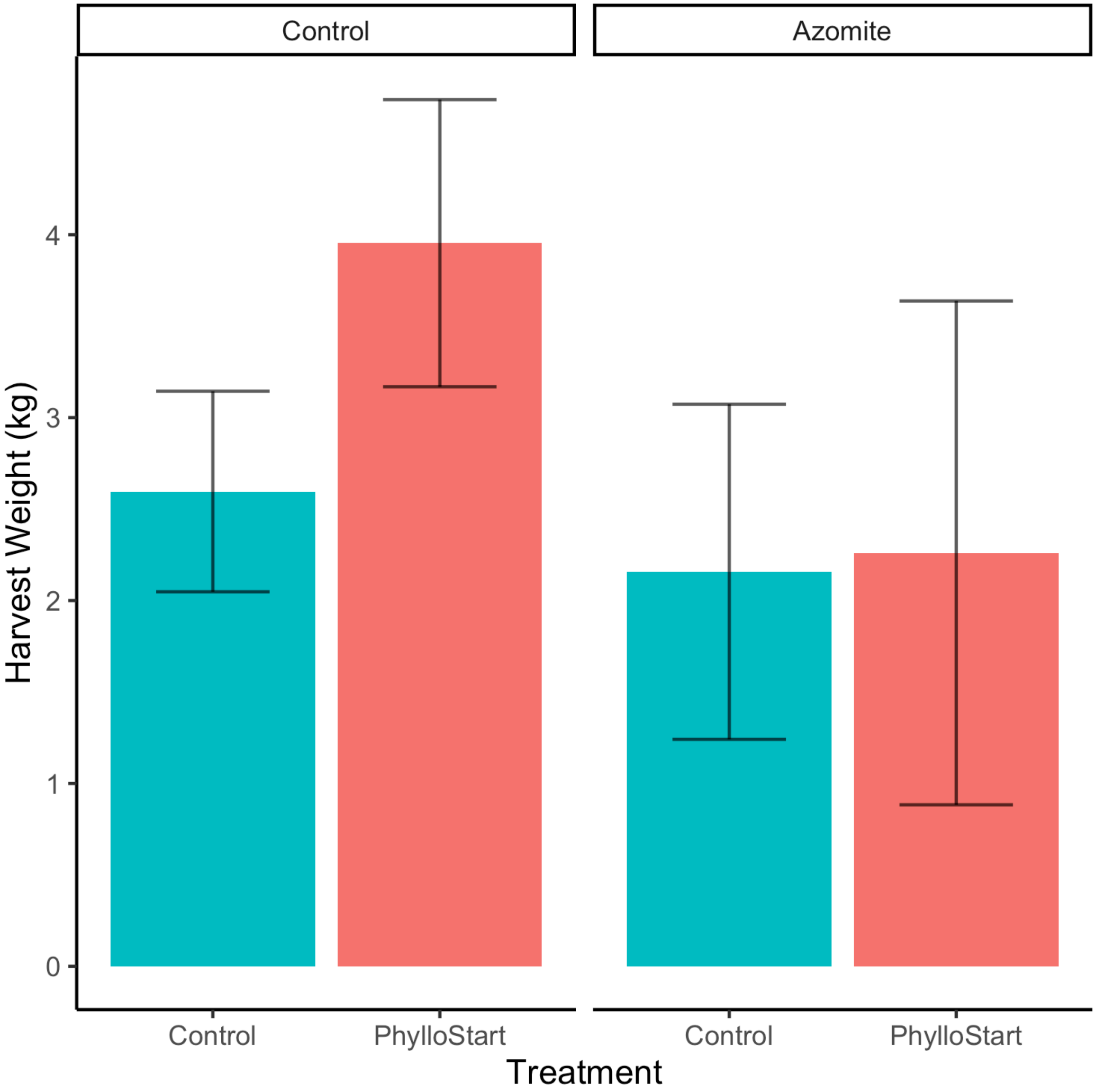
Supplemental Figure 1. Plant metrics measured during the first greenhouse trial. These include plant height, width, flowers, and fruit as measured throughout the growth of the plants. These data were analyzed with a linear mixed effects model selecting only terms that are identified by an ANOVA to lead to a significant improvement in model fit. For height and width only time was identified as a significant factor (p <.0001 and p <.0001 respectively), with height, t(299) = 106.10561, p <.0001, and for width, t(119) = 39.26810, p <.0001, while for both flowers and fruit the full model including PhylloStart and Azomite treatment was found to yield a significantly improved fit (p = .0303 and p = .0074 respectively). Interrogating the full model for flowers there was a significant effect of time, t(294) = 13.314218, p = <.0001, as well as a significant impact of the granular and ultrafine azomite + PhylloStart on flower development, t(54) = -2.317938, p = .0243, with a significant interaction term between this treatment and time, t(294) = 1.881083, p = .0028. Further, for fruit, there was a significant effect of time, t(292) = 12.336256, p <.0001, and significant interactions between both the PhylloStart only treatment and time, t(292) = 2.213625, p = .0276, and the granular and ultrafine azomite + PhylloStart treatment and time, t(292) = 2.361273, p = .0189



Supplemental Figure 2. Final harvest weights for the initial greenhouse trial. Due to sampling methods, in which each treatment was binned, we cannot statistically analyze the total final harvest. However, we do see a trend where Azomite and PhylloStart treatments yield a larger total harvest, with the combination of Azomite Granular and Ultrafine with PhylloStart showing the greatest increase over the control.



Supplemental Figure 3. Tomato weights as measured throughout the greenhouse and field trials. In the second greenhouse trial, several individual tomatoes from each plant were collected and weighed (a). Analyzing the data with a linear mixed effects model indicated that there was no significant impact of treatment with PhylloStart on the weights of individual tomatoes, t(24) = 1.55582, p = .1328, with treated plants having an average tomato weight was 46.05 (±14.99) grams, as compared to an average weight of 46.12 (±11.98) grams in the control group, there was, however, a significant impact of the micronutrient supplement, with 1 and 2 grams significantly increasing the individual tomato weight, t(24) = 3.83212, p = .0008 and t(24) = 6.07488, p < .001 respectively, with average tomato weights of 50.66 (±12.41 SD) grams and 53.46 (±12.39 SD) grams. In the third greenhouse trial (b), average tomato weight was determined based on the number of total fruit and the total harvest weight per plant. Again, there was no significant effect of PhylloStart on tomato weight, and in contrast to the previous trial there was no significant effect of Azomite treatment. Likewise, there was no significant effect of PhylloStart or Azomite on tomato weight in the field trial (c).



Supplemental Figure 4. In order to confirm that there was no effect of PhylloStart treatment on the number of tomatoes in the field, the experiment was repeated in a subsequent year. Again, there was no significant impact of PhylloStart treatment, or in this case micronutrient supplementation on the total harvest weight at the end of the field season.

Chapter 4. Polyploidy and microbiome associations mediate similar responses to pathogens in Arabidopsis

Parts of this chapter have been adapted from the following with permission:

Mehlferber, Elijah C.*, Michael J. Song*, Julianne Naomi Pelaez, Johan Jaenisch, Jeremy E. Coate, Britt Koskella, and Carl J. Rothfels. "Polyploidy and microbiome associations mediate similar responses to pathogens in Arabidopsis." Current Biology (2022).

* These authors contributed equally

4.1 Introduction

Whole-genome duplications (WGDs), or “polyploidizations”, are evolutionary events where the entire genome is doubled. Despite its dramatic nature, WGD is a common mutation in plants (Leitch & Bennett, 1997) and is found throughout the tree of life (Dehal & Boore, 2005). Polyploidy is associated with many novel and potentially adaptive phenotypes including changes to biomass, photosynthesis, water- and nitrogen-use efficiency, and secondary metabolism (Levin, 1983; Huang *et al.*, 2007; Ni *et al.*, 2009; Coate *et al.*, 2012), with polyploids having larger cells and organs and more chloroplasts per cell (Coate *et al.*, 2012). For these reasons, polyploidy is often considered to be a mechanism by which short-term adaptations may arise in response to changes to the environment or stress (Van de Peer *et al.*, 2017). Polyploidy is also implicated in an increased resistance to parasites and pathogens (Oswald & Nuismer, 2007), and there is some experimental evidence that supports this conclusion. For example, in *Actinidia chinensis* (kiwifruit), hexaploids are the most resistant to pathogenic *Pseudomonas syringae,* followed by tetraploids and then diploids (Saei *et al.*, 2018), and inducing polyploidy in *Impatiens walleriana* (cultivated impatiens) confers increased resistance to mildew (Wang *et al.*, 2018).

Another way that plants can achieve increased pathogen resistance is via the associated microbial community (the microbiome), which has also been found to play a critical role in defense against pathogens (Wei *et al.*, 2019; Leopold & Busby, 2020). Both the root and shoot systems of plants host diverse microbial communities, including bacteria, fungi, and other eukaryotes, but these plant systems associate with only a subset of all environmentally available microbes. These associations play important functions in disease or nutrient acquisition (Bulgarelli *et al.*, 2013). Which taxa successfully colonize a given plant can be mediated by the host both directly and indirectly, including through immune responses (Lebeis *et al.*, 2015), coordination of stress and immune system functions (Castrillo *et al.*, 2017), or the production of secondary chemicals(Levin, 1983). Host reliance on the microbiome for disease resistance is now considered as a key determinant of immune system evolution (King & Bonsall, 2017; Metcalf & Koskella, 2019; McLaren & Callahan, 2020), and thus ploidy-induced changes in microbiome-mediated defense could have important consequences for subsequent host evolution. For example, both the employment of the microbiome as well as WGD could potentially allow plants to circumvent the trade-off between growth and defense, but it is not known if WGD would disrupt the recruitment of a beneficial microbiome and perhaps incur negative consequences for growth and/or defense (Thébault *et al.*, 2011; Karasov *et al.*, 2017). As such, a current open, yet critical, question is how whole-genome duplication impacts the interaction between plants and their associated microbiota.

To determine how WGDs alter the interactions between the plant and its above-ground microbiota and the consequences of these altered interactions on pathogen growth, we used seven lines of synthetic autotetraploid accessions of *Arabidopsis thaliana* and their corresponding diploid progenitors (so each of the seven *Arabidopsis* genotypes is included as both diploid and autotetraploid lines), inoculated them with a synthetic community (SynCom) comprising microbial taxa common to the leaf habitat. While the *Arabidopsis* phyllosphere is naturally composed of hundreds of different bacterial species (Bodenhausen *et al.*, 2013), the SynCom is composed of only 16 species to remain tractable. We then determined whether there was a conserved change in bacterial community composition across ploidy level, and if plants of differing ploidy had different transcriptional responses to these bacteria, in particular with respect to priming a plant for defense. To further investigate any effects of these changes on microbiome-mediated pathogen protection, we inoculated these plants, along with untreated controls (plants without the synthetic microbiome), with the model *Arabidopsis* pathogen *Pseudomonas syringae* pv. tomato DC3000 and measured growth during early establishment. By using synthetic autotetraploid accessions of *Arabidopsis* in conjunction with a controlled, synthetic microbial community, we were able to assess the associations between genotype, ploidy level, and the microbiome, determine the extent to which these interactions are mediated through shared transcriptional responses, and quantify the effect of these interactions on pathogen defense.

4.2 Methods

4.2.1 Arabidopsis accessions

14 total lines were used from seven Arabidopsis diploid accessions from natural populations and their colchicine induced autotetraploids: Columbia (Col-0), Warschau (Wa-1), Wassilewskija (Ws-2), Gudow (Gd-1), HR (HR-5), Sorbo (Sorbo), St. Maria d. Feiria (Fei-0). All the autotetraploids produce viable seeds. All experiments were performed on a single cohort of plants that were grown and maintained together.

4.2.2 Plant Growth Conditions

Seeds were surface sterilized by treatment with 70% ethanol v/v for 2 min and then sodium hypochlorite solution (7% available chlorine v/v) containing 0.2% Triton X-100 v/v for 8 min. Samples were then washed seven times with sterile double distilled H2O(Bhardwaj *et al.*, 2011). Seeds were then placed on MS media with .8% agar m/v and cold stratified for two to three days at 4ºC in the dark(Bhardwaj *et al.*, 2011). After germination, seedlings were transferred to a controlled environment in a Conviron growth chamber (Model E15) with a long-day photoperiod (16-h photoperiod) at 22ºC and 55% relative humidity with cool white fluorescent light(Bhardwaj *et al.*, 2011) set at 25% (approximately 200 μmol photons m^{-2} s^{-1}). After seven days the seedlings were transferred to sterile peat and the lighting was changed to short-day conditions (9-h photoperiod) (Innerebner *et al.*, 2011).

4.2.3 Inoculation with synthetic community (SynCom) and infection with pathogen DC3000

The synthetic community is composed of 16 taxa that span the microbial diversity of the tomato phyllosphere. The synthetic community is prepared by growing each bacterial species in a Panasonic incubator (Model MIR-554) at 28.2ºC, shaking at speed setting 140 (VWR Advanced Digital Shaker) for 3 days in Kings Medium B (KB) broth(King *et al.*, 1954), after which the bacteria are centrifuged for 10min at 2500g. The supernatant is removed, and the bacteria are resuspended in 10 mM $MgCl_2$. Bacterial density is measured and adjusted to a density OD600 nm = 0.2. Each species is added in equal volume, and the assembled community is diluted 10 fold in 10 mM $MgCl_2$ to yield a final concentration of OD600 nm = 0.02. Two weeks after germination, each plant was inoculated with either the synthetic community suspended in 10 mM $MgCl_2$ buffer at a density of OD600 nm = 0.02 or just the 10 mM $MgCl_2$ buffer alone. The plants were inoculated by spraying the plant until the leaves were fully saturated, as indicated by runoff. Three weeks after germination (one week post synthetic community inoculation), the plants were spray-inoculated with either the pathogen (*Pseudomonas syringae* pv. tomato DC3000) or a 10 mM $MgCl_2$ buffer. The pathogen was inoculated at a density of OD600 nm = 0.0001 (Innerebner *et al.*, 2011).

4.2.4 Sample Collection

Four sets of samples were collected, the first set one week post inoculation with the SynCom, but immediately prior to inoculation with DC3000 to determine the commensal community composition, the second immediately after inoculation with DC3000, the third 24 hours post inoculation, and the fourth 48 hours post inoculation. All of the plants were approximately at the same stage of development and no plants that showed signs of inflorescence emergence were used in the assay. To sample the aerial portion of the plants, plants were cut just above the roots and transferred the total above-ground biomass into a tube with either 10 mM $MgCl_2$ (for sequencing the SynCom), or into 100mM phosphate buffer (pH 7), for the pathogen inoculated samples. Samples for sequencing were sonicated for 15 minutes in a Branson M5800 sonicating water bath. The resulting leaf wash was then pelleted, the supernatant removed, and frozen at -20ºC until sequencing. Pathogen inoculated samples were bead homogenized using the FastPrep-24 Classic bead beating grinder and lysis system (MP Biomedicals, Inc., CA, USA) and frozen at -20ºC until ddPCR sequencing was performed.

4.2.5 Amplification and Sequencing of Microbial 16S rDNA

Samples were snap frozen on liquid nitrogen and kept at -20ºC and sent to Microbiome Insights for 16S V4 sequencing and qPCR analysis within one month of freezing. Amplification and sequencing were performed according to Microbiome Insights standard protocol: Specimens were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following MoBio's instructions on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5′ -GTGCCAGCMGCCGCGGTAA-3′ , and 806R 5′ -GGACTACHVGGGTWTCTAAT-3′), as per the protocol of Kozich *et al*. (Kozich *et al.*, 2013). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from template-free controls (negative control) and extraction kit reagents processed the same way as the specimens. A positive control from 'S00Z1-' samples consisting of cloned SUP05 DNA, was also included. The only modification to this standard protocol was the addition of PNAs according to the method developed in Lundberg *et al*. (Lundberg *et al.*, 2012), in brief (mPNA, to knock out mitochondria and pPNA to knock out chloroplast) into the PCR step during library prep at a concentration of 5uM per PNA. The PCR reaction was then modified with the addition of a PNA annealing step at 78ºC for 10s.

4.2.6 qPCR assay of Microbial abundance

From the standard methods of Microbiome Insights: Bacterial-specific (300 nM 27F, 5′ - AGAGTTTGATCCTGGCTCAG-3′) forward primers coupled to (300 nM 519R, 5′ - ATTACCGCGGCTGCTGG-3′) reverse primers were used to amplify bacterial 16S rRNA. 20 µl reactions using iQ SYBR Green Supermix (Bio-Rad), with 10µl Supermix, 0.6µl Primer F, 0.6µl Primer R, 6.8µl H2O and 2µl template, were run on Applied Biosystems StepOne Plus instrument in triplicate using the following cycle conditions; 95ºC for 3 min., 95ºC 20 sec., 55ºC for 20 sec., 72ºC for 30 sec., return to step two 45 times. For standards, full-length bacterial 16S rRNA gene was cloned into a pCR4-TOPO vector, with Kanomycin-Ampicillin resistance. The total plasmid fragment size is expected to be 5556 bp. A bacterial standard was prepared via. 10-fold serial dilutions, and the copies of 16S was determined by the following: Copy# = (DNA wt. x 6.02E23)/ (Fragment Size x 660 x 1E9). Linear regression was used to determine copy numbers of samples, based on CT of standards. Reaction specificity was assessed using a melt curve from 55ºC to 95ºC, held at 0.5ºC increment for 1s.

4.2.7 ddPCR assay of pathogen abundance

Absolute bacterial abundance was estimated by performing digital droplet PBR (ddPCR) on homogenized whole plant samples randomized within plate columns using the BIO-RAD QX 200 Droplet Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and custom primers to specifically target and amplify Pseudomonas syringae pv. tomato DC3000. The PCR protocol is as follows: 95ºC for 5 min., 95ºC for 30 sec., 60ºC for 100 sec., return to step two 40 times., 4ºC for 5 min., 90ºC for 5 min., keep at 4ºC overnight.

4.2.8 RNA Sample Collection and Sequencing

For each of three accessions (Columbia (Col-0), Wassilewskija (Ws-2), Sorbo (Sorbo)), six plants of each ploidy level (diploids and induced autotetraploids) were grown in randomized blocks with three plants treated with the synthetic community and three treated with the control buffer, for a total of 36 plants. Single leaves from the largest developmental node of plants at Stage 1.10 (ten rosette leaves >1 mm in length (Boyes *et al.*, 2001)) were collected and directly froze them in liquid nitrogen before subsequent storage at -80C. Tissue was homogenized using a Mini-BeadBeater 8 (BioSpec Products, Bartlesville, OK, USA) following the manufacturer's instructions. RNA was extracted using the Spectrum Plant Total RNA Kit (Merck / MilliporeSigma, MO, USA) according to the manufacturer's recommendations.Three samples per accession were pooled. Samples were sent to Novogene USA Inc. (Sacramento, CA) for library prep (Poly (A) capture, ligation-based addition of adapters and indexes) and sequencing (Illumina NovaSeq 6000, 150bp paired-end reads, 20M reads per sample).

4.2.9 Test for euploidy

Tetraploid samples were tested to assess aneuploidy or euploidy by calculating fold change in relative expression (transcripts per million; TPM) per gene for every pairwise comparison of biological replicates following the methods outlined in Song *et al.*(Song *et al.*, 2020). If there is aneuploidy, the expectation would be to see a large coordinated increase or decrease in TPM for genes on that chromosome, which would be reflected in a shift in fold change of expression relative to the other biological replicates (Supplemental Figure 1). No shift was found and therefore it can be concluded that all tetraploid individuals were euploid.

4.2.10 Microbiome Data Analysis

Forward and reverse paired-end reads were filtered and trimmed to 230 and 160 base pairs (bps), respectively using the DADA2 pipeline with default parameters (Callahan *et al.*, 2016). Following denoising and merging reads and removing chimeras, DADA2 was used to infer amplicon sequence variants (ASVs), which are analogous to operational taxonomic units (OTUs), and taxonomy was assigned to these ASVs using the DADA2-trained SILVA database. Using the negative samples from 16s sequencing the decontam package was implemented using default settings to identify and remove potential contamination from the samples (Davis *et al.*, 2018). The assigned ASVs, read count data, and sample metadata were combined in a phyloseq object (McMurdie & Holmes, 2013) for downstream analyses. Differential microbial changes were calculated using DESeq2 (Love *et al.*, 2014) and the phyloseq package was implemented in R to calculate changes in alpha and beta diversity. For a permutational analysis of variance (PERMANOVA), data was rarified to 90% of the reads of the least abundant sample and the test was performed using the adonis function in the vegan package(Oksanen *et al.*, 2020) in R with 999 permutations to test whether ploidy or genotype had an effect on beta diversity measures.

The test statistics and p-values for the Welch Two-Sample t-test, pairwise ANOVA, and nonparametric multivariate analysis of variance can be found in the results section.

4.2.11 ddPCR assay of pathogen abundance

To assay the pathogen abundance using ddPCR, the default thresholds for identifying positive samples on the Biorad analysis software was used and then the weight of each sample was used to calculate a normalized per gram density of bacteria present on the above-ground plant. The absolute abundance of polyploids and diploid accession pairs across each timepoint was compared to assay how the pathogen interacted with ploidy and microbiome treatment.

The test statistics and p-values for a Linear Mixed Effects Model (nlme) of DC3000 Abundance as a function of the explanatory variables time, ploidy, treatment (SynCom inoculation), and their interactions can be found in Table 1. The test statistics and p-values for a Linear Mixed Effects Model (nlme) for Diploids, with DC3000 Abundance as a function of the explanatory variables Time, Treatment (SynCom inoculation), and their Interactions can be found in Table 2. The test statistics and p-values for a Post hoc Tukey HSD (emmeans) for the Diploid Linear Mixed Effects Model can be found in Table S1.

4.2.12 RNA-seq Data Processing and Analysis

Raw FASTQ files were trimmed and filtered to remove low-quality reads and technical sequences using Trimmomatic (Bolger *et al.*, 2014) with the default settings. Filtered reads were aligned to the Arabidopsis reference sequence (Lamesch *et al.*, 2012) (TAIR10), with HISAT2 (Pertea *et al.*, 2016). HTSeq (Kim *et al.*, 2015) was used to determine read counts per gene for the test for euploidy and DESeq2 was used to analyze differential gene expression(Love *et al.*, 2014) for different experimental comparisons. For DESeq2 analysis gene ontology was assigned using UniProt (UniProt Consortium, 2019). Links to the DESeq2 output data for each of these comparisons can be found in Data S1B. *P. syringae* associated genes were identified using UniProt (UniProt Consortium, 2019) and were analyzed using the rstatix package t_test function (Kassambara, 2021).

4.3 Results

4.3.1 Ploidy level does not impact microbiome composition

To determine if there was a difference in the extent to which diploids versus polyploids recruited their microbiome from the inoculated Synthetic Community, we assayed the communities from 14 SynCom-inoculated plants (one diploid and one tetraploid for each of the seven *Arabidopsis* lines) inoculated with the SynCom by 16S amplicon sequencing one week after their inoculation (immediately prior to pathogen introduction).

The majority (65.8% in diploids and 77.3% in polyploids) of bacteria that we found associated with the plants were from the synthetic community, with *Pantoea*, *Pseudomonas*, and *Exiguobacterium* showing consistently high relative and absolute abundance across samples (Figure 1; A,B). The absolute abundance of bacteria from the synthetic community on the leaves one week after inoculation, as ascertained by qPCR, was not significantly different across ploidy levels (Figure 1; C; standardizing for sample weight: Welch Two-Sample t-test, t= -0.11455, df = 10.076, p = 0.911; Supplemental Figure 3). Further, using DESeq2 we concluded that there were no SynCom-associated bacteria with significantly different abundances across ploidy levels (Data S1A).

Alpha diversity of the established microbiome (measured as species richness, Shannon index, or species evenness) did not differ significantly between diploids and polyploids (pairwise ANOVA, P>0.05; Figure 1; D). Likewise, we found no significant differences in beta diversity (community composition measured with Bray-Curtis dissimilarity) across ploidy level (ADONIS nonparametric multivariate analysis of variance, P>0.05; Figure 1; E).

4.3.2 Polyploids are less susceptible to pathogen establishment

To determine how ploidy level and microbiome inoculation impacted pathogen growth, we used droplet digital PCR (ddPCR) to measure the abundance of DC3000 over time, normalized for the mass of the plant (Morella *et al.*, 2019). Plants were inoculated with the pathogen one week after their treatment with the SynCom (or buffer control). We first sampled 14 plants (both ploidy levels for each line) immediately after inoculation with the pathogen to provide our initial densities (T0). Subsequently, we sampled 28 plants (full factorial sampling across ploidy, Arabidopsis line and SynCom inoculation) at 24 (T1) and 48 (T2) hours after inoculation.

Both time since exposure to the pathogen and the presence of the SynCom significantly impacted pathogen abundance. Analyzing Log10 transformed abundance (linear mixed effects model, p = 0.0018 and p = 0.0031, respectively; lines 2 and 4, Table 1), we found a marginally significant interaction between the two (p = 0.0517; line 6 Table 1). When analyzing the diploid samples alone we found a significant impact of time, treatment (SynCom application), and their interaction on pathogen abundance (p = 0.0001, p = 0.0003, p = 0.01, respectively; lines 2, 3, and 4, Table 2). We performed a Tukey HSD post-hoc test, finding significant differences between the pathogen densities of SynCom-treated samples across timepoints one and two (p = 0.0008; line 1, Table S1), as well as between SynCom-treated samples from timepoint one and control (buffer inoculated) samples from timepoint two (p = 0.0003; line 2, Table S1), and between SynCom-treated and control samples in timepoint one (p = 0.0014; line 3, Table S1). For the polyploids, there was no significant improvement to model fit through the addition of any terms when compared to a null model including only the intercept, indicating that there was no effect of SynCom application on pathogen density in this ploidy level. In addition, there was significantly lower pathogen abundance (Log10 transformed) when comparing the polyploid to diploid plants at the second timepoint for both the control (buffer inoculated) plants (Welch Two-Sample t-test, t = 2.809, df = 4.9939, p = 0.03765), as well as the treated (SynCom

Inoculated) plants (Welch Two-Sample t-test, t = 2.4295, df = 8.211, p = 0.04048; Figure 2), indicating that pathogen reduction in the polyploids was microbiome independent.

4.3.3 Diploid plants exhibit greater response to synthetic community colonization, while polyploids constitutively express certain defense genes

To test whether plants differed in their responses to inoculation with the SynCom, we sampled leaves from three *Arabidopsis* lines (Col-0, Ws-2, and Sorbo) across ploidy level (diploid and polyploid) and both treatments (SynCom vs. buffer inoculation) for RNA sequencing. Leaf samples for transcriptomic analysis were collected at the same time as the samples for 16s amplicon sequencing, prior to pathogen inoculation, but from separate replicate plants (ie. for each accession, ploidy level, and bacterial treatment, two plants were grown; with one being sampled for RNA sequencing and the other for amplicon sequencing).

Analyzing RNA sequences prior to pathogen inoculation, using DESeq2, we found 220 up- or down-regulated genes between SynCom treated and untreated diploid plants, while polyploid plants had only 35 significantly differentially expressed genes (at the 0.1 p-value cut-off; Figure 3; A, B). We chose the 0.1 p-value cutoff to ensure that we captured patterns that might be important but not rise to the standard 0.05 significance level; the results are qualitatively identical when evaluated at a 0.05 p-value cutoff, and those genes that are significant at $p < 0.05$ are indicated in the figure. In general, there is a greater range for differentially expressed genes in the polyploids as compared to the diploids (Figure 4; A,B). Diploid plants showed several clusters of significantly differentially expressed genes when those genes were grouped by function. Many of these groups of genes are associated with defense functions, including, for example, genes associated with the well-characterized phytohormone abscisic acid (ABA; Figure 3; A). Genes associated with hypoxia as well as defense response to bacteria were also significantly up- or down- regulated. Furthermore, several genes associated with ethylene signaling were up-regulated in the SynCom-treated diploids when compared to the control (Figure 3; A), and while it is unclear if ethylene response is essential for P. syringae defense in Arabidopsis (Bent, 1992), it has been implicated in resistance (Guan *et al.*, 2015). When comparing the polyploid treated and control plants we saw a pattern of primarily increased gene expression, with the majority of these being defense related, including ABA, hypoxia, and ethylene signaling related genes (Figure 3; B). When comparing polyploid and diploid plants directly, we see that all significantly differentially expressed genes are upregulated in the polyploids. Several of these genes are associated with stress or defense related functions, including cellular response to hypoxia, general defense response and negative regulation of defense response to bacteria (Figure 3; C).

To focus specifically on the plant responses to the microbial community that might underly the differences in *P. syringae* resistance we observed, we identified genes that are both known to function in *Arabidopsis* response to *P. syringae* infection (by searching the UniProt database (UniProt Consortium, 2019)), and which are expressed at a significantly higher level in the diploids after exposure to the SynCom. When comparing the expression patterns of these genes in the polyploids (Figure 5), we find, for four out of the six genes (AMC4, CYP19-1, STP4 and VDAC1), a pattern of elevated expression regardless of exposure to the SynCom. Further, for the additional two genes (NADK1 and WRKY53), which show significantly reduced expression in the diploids in response to SynCom application, we find that their expression is not significantly reduced in the polyploids.

4.4 Discussion

Overall, we found no significant differences in microbiome establishment--either in composition or diversity--between the diploid or polyploid plants. While it is possible that there are ploidy-dependent effects within certain genotypes, our use of multiple accessions allowed us to rule out a generalized response of the microbiome in this system. The polyploids weighed significantly more across all accessions than did the diploids, consistent with patterns observed in some groups (Pacey *et al.*, 2020), but in contrast to previous work on autotetraploid *Arabidopsis* (Chen, 2010; Ng *et al.*, 2012) and supported a higher total number of commensal bacteria (once we normalized for the weight of the plant, this difference was not significant). Likewise, we did not find any significant differences in the relative abundance of any of the synthetic community members across the two ploidy levels, both of which were primarily colonized by *Pantoea*, *Pseudomonas*, and *Exiguobacterium*. The lack of conserved impact on community composition is in line with work on wheat, where ploidy was found to play a weak and inconsistent role in shaping the below-ground microbiome (Wipf & Coleman-Derr, 2021), but contrasts with previous work on *Arabidopsis* that did find a signature of ploidy in shaping microbial communities (Ponsford *et al.*, 2022). Regardless of the impacts of ploidy on microbiome composition, our study highlights the importance of considering the impact of the microbiome as a function of ploidy, given that, even though we don't see a conserved shift in the community, we do see changes in the way the plants respond to these same bacteria.

To date there has been little study of polyploidy on pathogen response. Although polyploids have been proposed to be more resistant to pathogens (Levin, 1983; Oswald & Nuismer, 2007), empirical studies have generally been inconclusive, i.e. they find evidence for both increased resistance and increased susceptibility (Schoen *et al.*, 1992; Nuismer & Thompson, 2001). Our study leveraged multiple accessions of *Arabidopsis* to discern general patterns between ploidy level and pathogen defense. Overall, we found a trend towards lower pathogen abundance in the polyploid plants regardless of association with a bacterial community, as well as a significant decrease in the abundance of the pathogen in the second timepoint (Figure 2).

Autotetraploids may be more resistant than diploids due to higher expression of defense genes as a consequence of their doubled genome (King *et al.*, 2012). For example, tetraploid *Arabidopsis* accessions acquired increased resistance to copper stress by having increased activation of antioxidative defense (Li *et al.*, 2017). A buttressing of the antioxidant defense system was also found in synthetic tetraploid plants of *Dioscorea zingiberensis* where antioxidant enzymes were over-produced and maintained at high concentration (Zhang *et al.*, 2010). These elevated defense responses generally come with a trade-off--for example, elevated expression of stress-response genes is associated with a fitness cost and slowed growth (Ng *et al.*, 2012). In contrast, our autotetraploids exhibit both greater biomass (Supplemental Figure 3) and higher defensive capacity (Figure 2). Though autotetraploids have double the copy number for all genes, the effect of copy number on gene expression is not necessarily linear. Differences in relative expression may therefore potentially reduce sensitivity to growth-defense trade offs although more work would need to be done to test this hypothesis.

When assessing the effectiveness of the microbiome in protecting the plants of different ploidy levels, we found that the microbiome temporarily arrests pathogen growth on the diploids, while polyploids are protected regardless of exposure to their microbiome (Figure 2). This result is particularly interesting in light of previous work on microbiome-mediated protection by a synthetic microbiome in tomato in which the phyllosphere microbiome was protective against pathogen growth in the absence of fertilizer application, but unimportant when plants had been fertilized prior to microbiome and/or pathogen inoculation (Berg & Koskella, 2018). Plant response to commensal bacterial organisms are complicated, often showing an overlap with the response to pathogens (Vogel *et al.*, 2016). This overlap can be explained in part through the broadly conserved plant responses to common microbial-associated molecular patterns (MAMPs), such as flagellin (Felix *et al.*, 1999), though even these responses can be modulated by a host of commensal interactions, such as repression of conserved epitopes (Colaianni *et al.*, 2021). These responses can be beneficial, through the early activation of broad defense responses (priming) that will then respond more effectively to pathogen exposure (Selosse *et al.*, 2014; Wang *et al.*, 2021). It is possible that this phenomenon plays a role in the increased protection afforded to the diploid plants that have been inoculated with the SynCom, as it may provide a mechanism of priming against potential future pathogens. It is important to note, however, that *Pseudomonas syringae* represents only one plant pathogen, and further study with a variety of other pathogens, representing alternative infective patterns, is needed to determine if there is a generalized effect of ploidy on pathogen defense and microbiome reliance.

As expected, we found that the broad-scale transcriptional profiles of the samples grouped strongly together by accession (Supplemental Figure 2). Nonetheless, when looking across accessions we saw that many of the significant changes in gene expressions associated with microbiome treatment were linked to defense-associated genes (Figure 3; A-B). These genes include those associated with ABA regulation, response to hypoxia, general defense response, and ethylene signaling. ABA is a well-studied plant signaling hormone that is linked to a variety of processes ranging from plant growth, to development and stress response(Yoshida *et al.*, 2019). The function of ABA in defense response is multifaceted, and has been shown to be important in pre-invasion defense, through the closing of stomata in response to microbe-associated molecular patterns (Melotto *et al.*, 2006) (MAMPs), as well as negatively regulating post-invasion defense through the suppression of callose deposition(Clay *et al.*, 2009) and SA dependent resistance (Yasuda *et al.*, 2008; Ton *et al.*, 2009). For all plants that received the synthetic microbiome expression responses were also significantly enriched for GO terms associated with cellular response to hypoxia when compared to the control group. The response to hypoxia requires the ethylene pathway in plants (Fukao & Bailey-Serres, 2004), which is involved in the hormonal control of programmed cell death (Overmyer *et al.*, 2003) and has been shown to influence the composition of the leaf microbial community (Bodenhausen *et al.*, 2013). Responses to pathogens involve increased respiration which creates local hypoxia around the leaf which is otherwise aerobic (Valeri *et al.*, 2021). Similarly, alcohol dehydrogenase, which in plants is involved in NAD+ production, is not only over-expressed in times of low oxygen, but is also induced in response to biotic and abiotic stress and improves responses to pathogens (Shi *et al.*, 2017). All synthetic microbiome-treated plants showed differential expression for several WRKY transcription factors that are linked with defense signaling (Eulgem & Somssich, 2007), as well as CCR4-associated factor 1, which has been shown to play a role in susceptibility to *P. syringae* infection (Liang *et al.*, 2009). Finally, SynCom-treated plants also show a pattern of increased expression in ethylene-activated signaling pathways. Ethylene is another well characterized phytohormone that is responsible for regulation of plant growth, development, and senescence (Iqbal *et al.*, 2017), as well as response to pathogen invasion and modulation of defense response (Ecker & Davis, 1987). These results demonstrate that there are multi-faceted defense responses to inoculation with the SynCom by both diploids and polyploids.

The trade-offs between growth and defense in plants are often metabolic, but can also be due to antagonistic crosstalk between hormones involved in both processes (Karasov *et al.*, 2017). One way to mitigate this tradeoff could be through the association with microbiota that perform similar defenses (Karasov *et al.*, 2017). Consistent with these ideas, the microbiome-treated diploids significantly slowed the progression of DC3000 growth. When comparing the control and inoculated plants, diploids showed nearly six times more differentially expressed genes than did the polyploids, with a mix of up- and down- regulated defense associated genes. In contrast to the diploids, the differentially expressed genes in the polyploid samples were primarily up-regulated, including several defense-related genes (Figure 3: B).

In total, we saw a greater range of genes differentially expressed in the polyploids as compared to the diploids, but with fewer reaching significance (Figure 4; A,B), indicating that their expression was more variable between lines, and that genome duplication events may have had line specific effects on gene expression. Despite this variation, we see that several genes known to function in response to *P. syringae*, which are expressed at higher levels in the SynCom-inoculated diploids compared to the controls, show elevated expression in the polyploids regardless of their exposure to the SynCom (Figure 5). These genes are AMC4, which plays a role in programmed cell death in response to *P. syringae* exposure (Watanabe & Lam, 2011), CYP19-1, involved in the production of reactive oxygen species after infection by *P. syringae* (Pogorelko *et al.*, 2014), STP4, which is induced by pathogen wounding(Truernit *et al.*, 1996), though its link to *P. syringae* response is contested (Bonfig *et al.*, 2006), and VDAC1, which is involved in the maintenance of reactive oxygen species homeostatis and stress response, and has been shown to be upregulated following *P. syringae* infection (Sanyal *et al.*, 2020). This result suggests that the polyploid plants may be protected from *P. syringae* colonization by the constitutive expression of some subset of their defense responses. Further, for the additional two genes, NADK1, which phosphorylates NADH to produce the antioxidant factor NADPH (Berrin *et al.*, 2005) and WRKY53, which promotes *P. syringae* resistance through salicylic acid response pathways (Hu *et al.*, 2012), we see a pattern of decreased expression in the SynCom-treated diploids but not the treated polyploids. It has been shown that commensal species sometimes downregulate defense associated genes in order to successfully colonize (Teixeira *et al.*, 2019), which does not appear to be happening in the polyploids.

This conclusion makes sense in light of our pathogen growth results, as the polyploids are broadly protected regardless of their microbiome, while the diploids require the microbiome to arrest pathogen growth. Consistent with this mechanism, seven out of the 24 *A. thaliana* general non-self response (GNSR) genes (Maier *et al.*, 2021), which have conserved expression changes in the presence of different bacteria, were constitutively expressed in the polyploids, regardless of their exposure to the commensal microbiome. Wang et al. (Wang *et al.*, 2021) shows how polyploidy "potentiates" stress responsive gene expression in response to abiotic stress, which gives some precedence to the finding that polyploids demonstrate some improved defense responses. Our study extends this pattern to include biotic stress. Whether this alteration is adaptive or not would be highly dependent on the environmental context of the plant. For example, in a familiar environment where they could reliably source beneficial bacteria, constitutive activation of certain defense genes could be a waste of otherwise better utilized resources, while in a novel environment this decoupling of defense from microbial associations could be a boon.

Future work could elaborate on the mechanistic underpinnings of these results by inducing auto-polyploidy in known *Arabidopsis* defense mutants and determining their responses to pathogen invasion, for example, using the bak1/bkk1 mutant identified by Vogel *et al*. (Vogel *et al.*, 2016) to be deficient in priming response to commensal species, or by using CRISPR to knock out the *P. syringae* associated genes that we identified in our study.

4.5 Conclusion

This work highlights the important role that plant condition (via polyploidization) plays in the interplay between plants, their associated phyllosphere microbiota, and an invading foliar pathogen. While the presence of the synthetic phyllosphere microbiome was always associated with a pattern of decreased *P. syringae* growth, the effect was only significant in the diploid plants; the polyploids appeared to be broadly protected regardless of the presence of these beneficial bacteria. Our transcriptional results suggest this result is due to a microbiome-independent regulation of defense genes in the polyploids, while the diploids required exposure to the microbiome to induce a sufficient defense response. It is possible that, as a consequence of gene dosage doubling due to WGD, polyploids may have a higher baseline activation of certain defense genes, thus decoupling their defense responses from those induced only by prior microbial associations, though the benefit of this may be context dependent. These results are particularly relevant to understanding the role that domestication, which often involves polyploidization, has played in altering interactions between plants and their associated microbes in agricultural settings. Likewise, the protective effects of the SynCom in diploid plants have important implications for the role of phyllosphere bacterial communities in managing plant disease, both naturally and as an applied supplement. Finally, we note that our results show that a lack of differences in microbiome composition need not imply absence of differences between ploidy levels.

4.6 Tables and Figures

	DC3000 Abundance (Log10)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.15	0.18 – 2.11	**0.033**
time [2]	2.34	1.03 – 3.64	**0.002**
ploidy [4]	0.06	-1.25 – 1.36	0.935
treatment [C]	2.2	0.89 – 3.51	**0.003**
time [2] * ploidy [4]	-1.73	-3.59 – 0.12	0.088
time [2] * treatment [C]	-1.99	-3.83 – -0.14	0.052
ploidy [4] * treatment[C]	-1.4	-3.25 – 0.44	0.164
(time [2] * ploidy [4]) * treatment [C]	0.55	-2.13 – 3.24	0.702
Random Effects			
σ^2	1.46		
$\tau_{00\ \text{eco}}$	0.14		
ICC	0.09		
N_{eco}	7		
Observations	54		
Marginal R^2 / Conditional R^2	0.404 / 0.456		

Table 1. Linear Mixed Effects Model (nlme) of DC3000 Abundance as a function of the explanatory variables time (2 = time point 2, 48 hours post inoculation), ploidy (4 = tetraploid), treatment (SynCom inoculation, C = control), and their interactions.

	DC3000 Abundance (Log10)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.15	0.48 – 1.82	**0.004**
time [2]	2.34	1.39 – 3.29	**<0.001**
treatment [C]	2.2	1.25 – 3.15	**<0.001**
time [2] * treatment [C]	-1.99	-3.33 – -0.64	**0.01**
Random Effects			
σ^2	0.71		
$\tau_{00\ \mathrm{eco}}$	0		
ICC	0		
N_{eco}	7		
Observations	28		
Marginal R^2 / Conditional R^2	0.607 / 0.607		

Table 2. Linear Mixed Effects Model (nlme) for Diploids, with DC3000 Abundance as a function of the explanatory variables Time (2 = time point 2, 48 hours post inoculation), Treatment (SynCom inoculation, C = control), and their interactions.

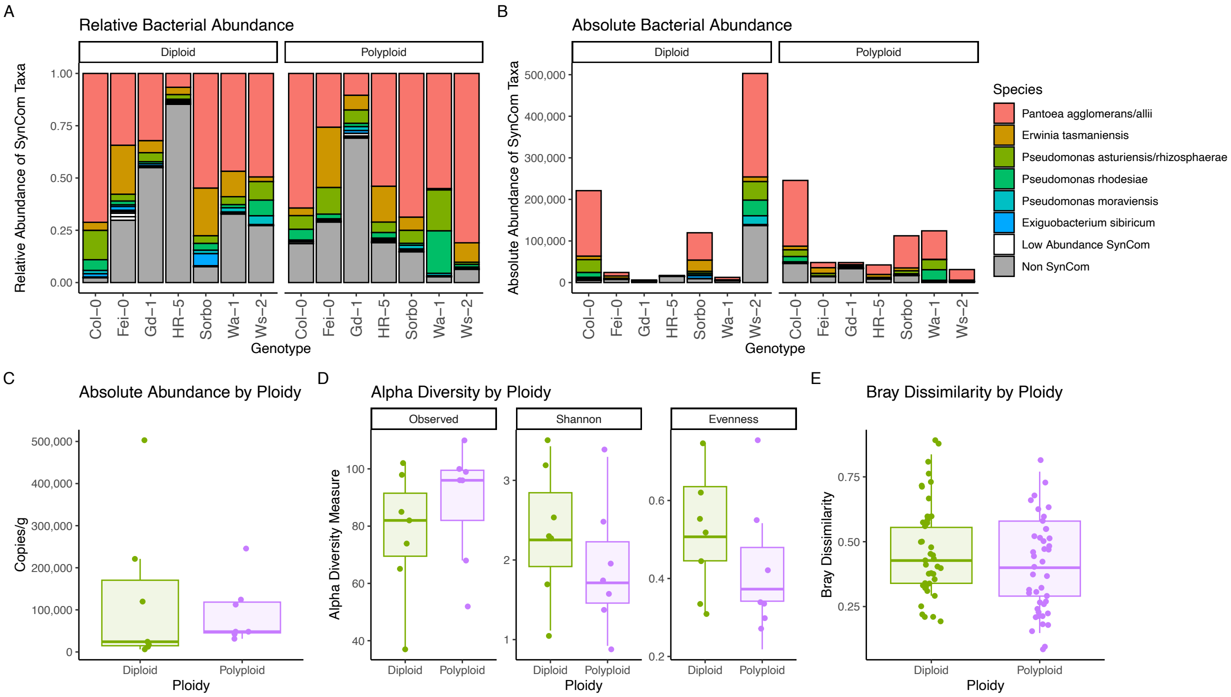


Figure 1. The effect of ploidy on microbiome composition and structure. A) The relative abundance of the SynCom inoculated ASVs across treatments, as well as the residual community. B) The absolute abundance of the microbial communities across treatments. C) There is no significant difference in the absolute abundance of phyllosphere bacteria between the two treatments. D) There is no significant difference in the tested alpha diversity metrics, inducing observed diversity, Shannon diversity, and evenness. However, there is a non-significant trend towards lower Shannon diversity in the polyploid plants, which is driven primarily by their lower evenness. E) Bray-Curtis dissimilarity between plants of each treatment is not significantly different.

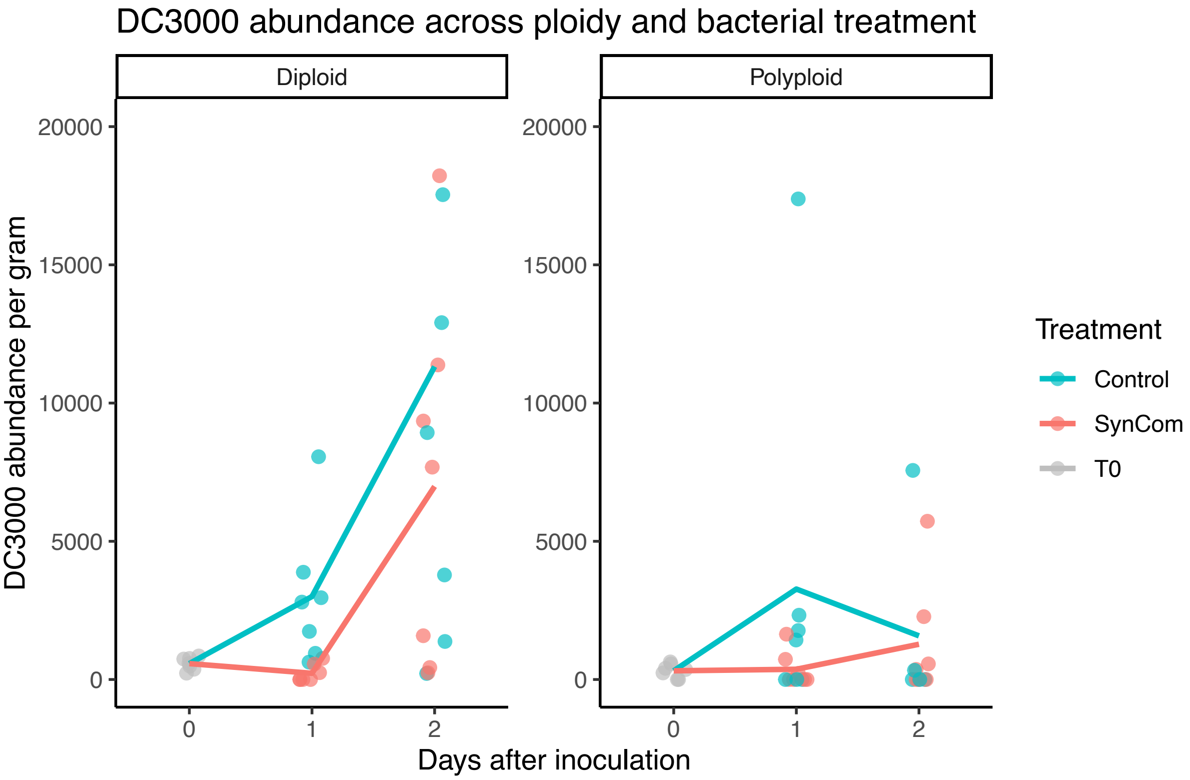


Figure 2. Abundance of the pathogen (normalized as DC3000 count per gram of plant mass) across timepoints and treatments in the diploid and polyploid plants. Samples were taken immediately after inoculation from a subset of plants in each ploidy (n = 14) to establish initial densities (T0), and from each genotype and ploidy across treatments (n = 28) at 24 hours (T1) and 48 hours (T2) after inoculation to determine pathogen growth. Pathogen density was established using ddPCR and was Log10 transformed prior to statistical analysis to yield a more normal distribution. Two days after inoculation, there was significantly less DC3000 detected in polyploids when compared to diploids, across both SynCom Inoculated (p = . 0.04048) and control plants (p = 0.03765).

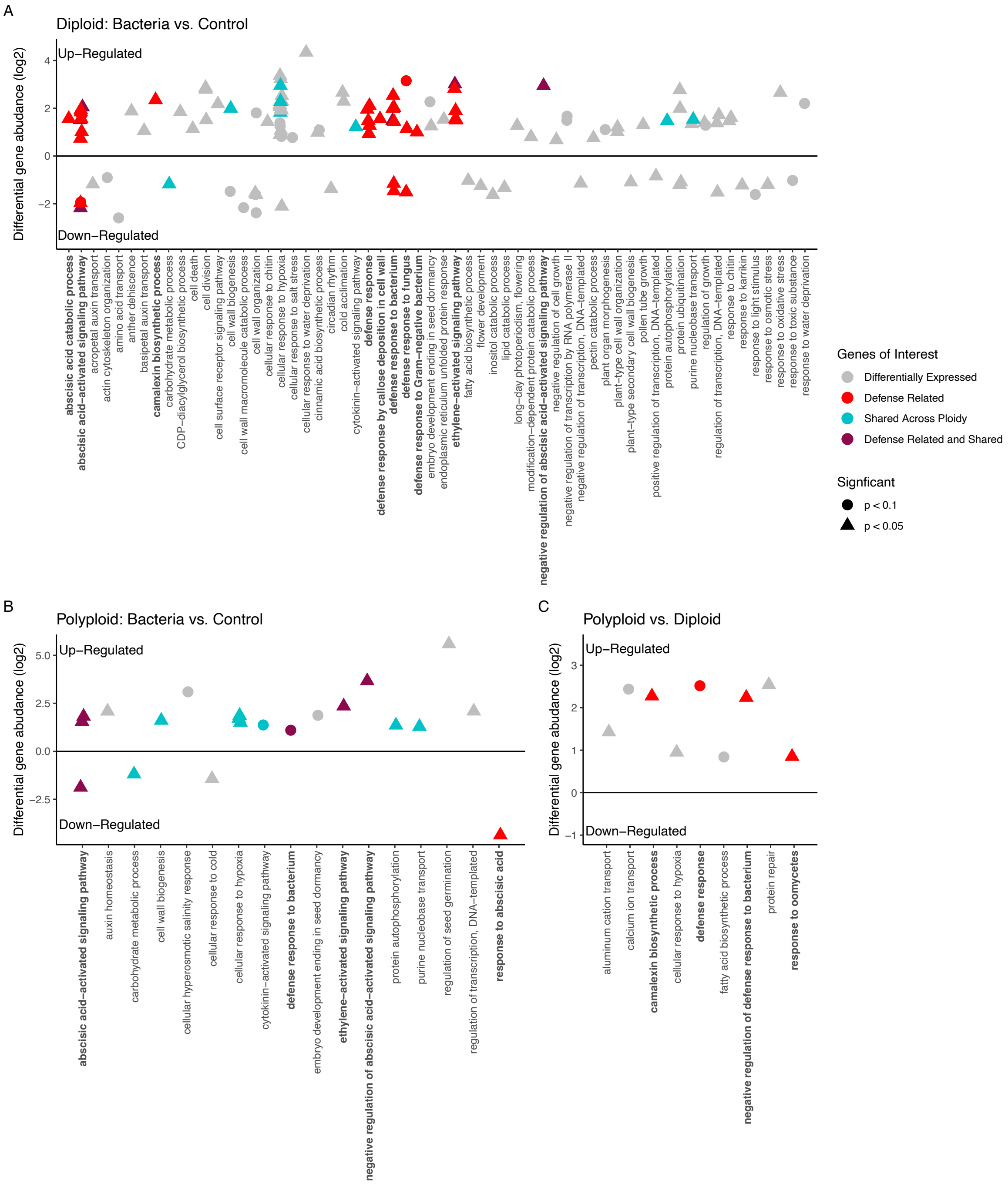


Figure 3. Differential expression of genes highlighted by association with defense response. A) Genes that are significantly differentially expressed ($p < 0.1$) when comparing the microbiome inoculated and control (buffer inoculated) diploid plants. Genes that are associated with defense functions are noted in bold and highlighted in red, while genes that are also differentially expressed in the polyploids are highlighted in blue, and genes that are both defense-associated and shared are highlighted in purple. B) Significantly differentially expressed genes between microbiome inoculated and control polyploids, using the same colors from the previous panel. C) Significantly differentially expressed genes between all polyploid and diploid plants, regardless of treatment, using the same colors as the previous two panels.

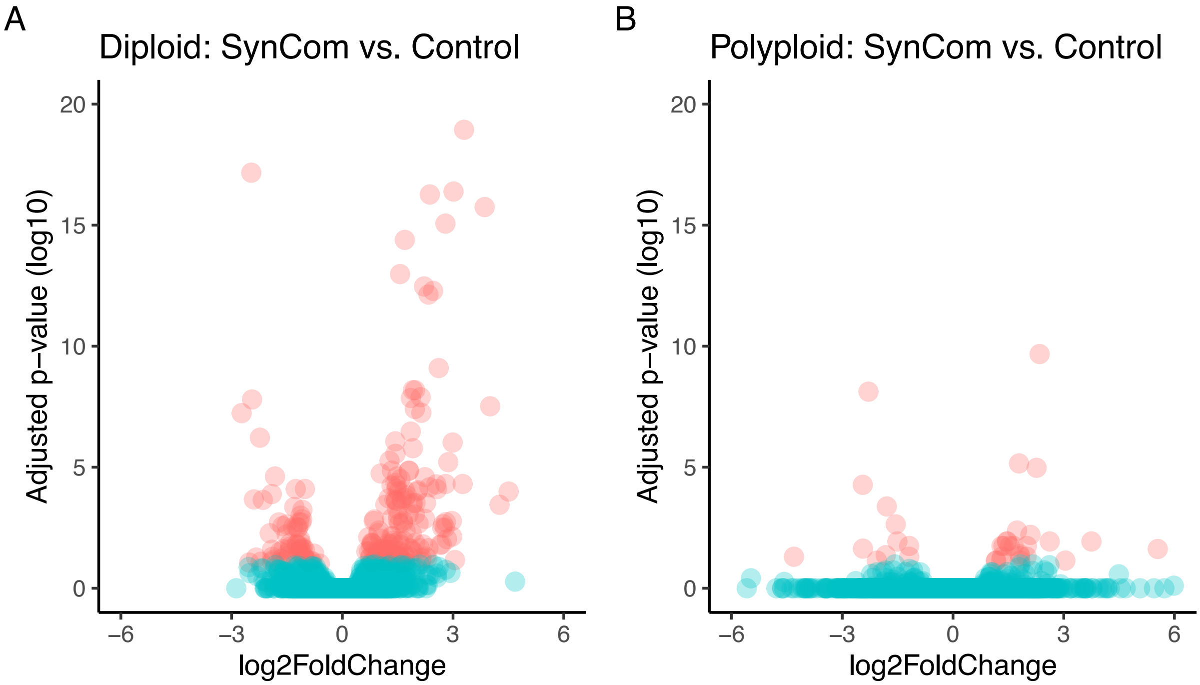


Figure 4. Variability of gene expression between treated (SynCom inoculated) and control (buffer inoculated) plants of both ploidies. A-B) Volcano plots showing proportion of total genes that are significantly differentially expressed (red dots) at the level of adjusted p-value of 0.1 (to account for multiple testing) plotted against their log2-fold changes. Genes that were not statistically significantly differentially expressed are in blue. Comparisons are drawn across SynCom treatment (buffer inoculation vs. SynCom inoculation) for both diploid samples and for polyploid samples.

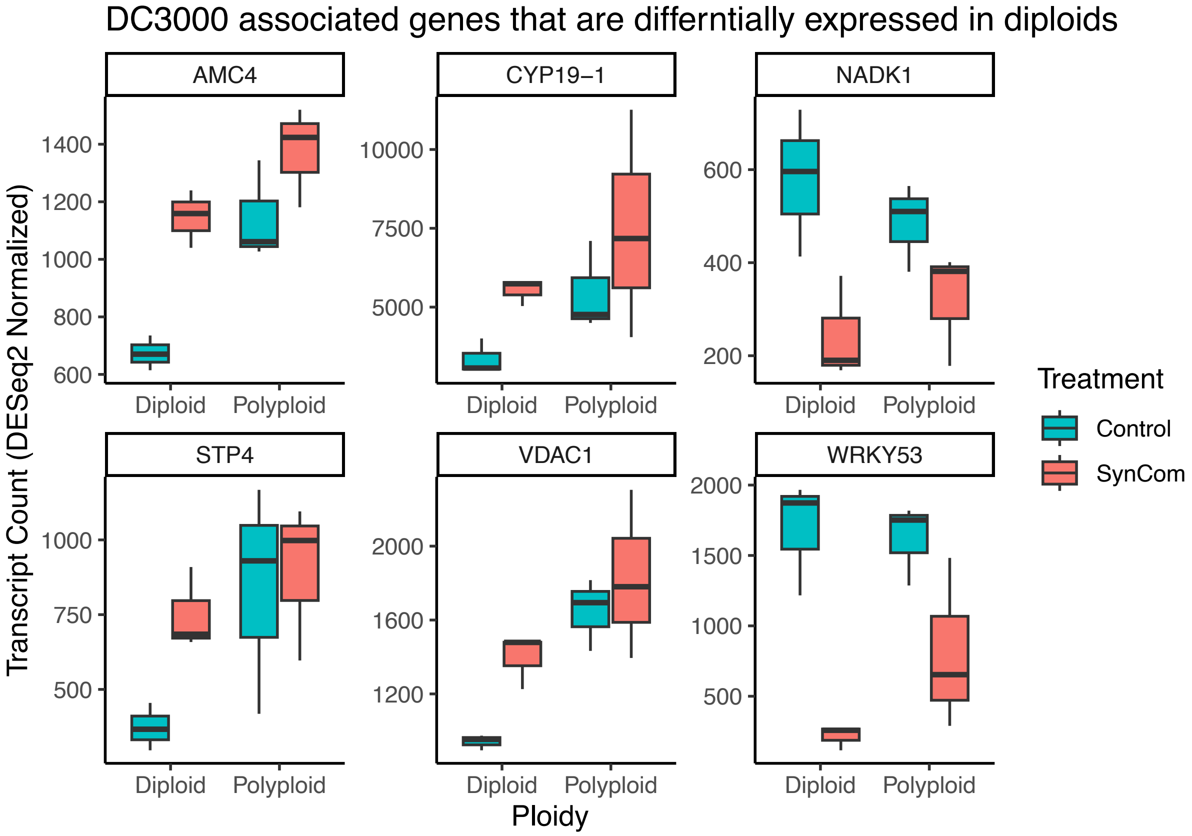
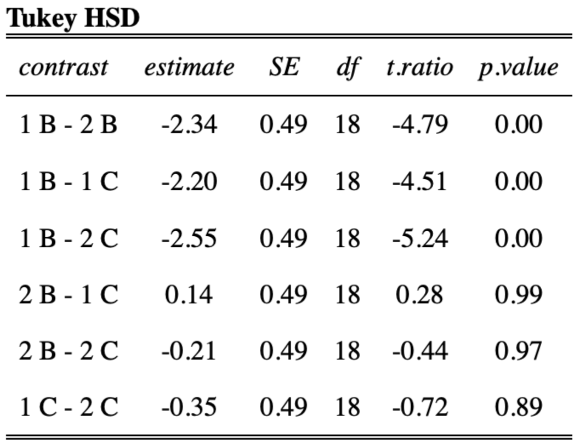


Figure 5. Expression levels of genes that were identified in UniProt as associated with *P. syringae* response, and that are significantly differentially expressed between the treated (SynCom inoculated) and control (buffer inoculated) diploid plants. For four out of the six genes (AMC4, CYP19-1, STP4 and VDAC1) we see a pattern of increased expression in the polyploids regardless of treatment with the SynCom, and for the other two genes (NADK1 and WRKY53) we see that their expression, which is significantly reduced in the diploids, is not significantly altered in the polyploids.

4.7 Supplemental Tables and Figures



Tukey HSD

contrast	*estimate*	*SE*	*df*	*t.ratio*	*p.value*
1 B - 2 B	-2.34	0.49	18	-4.79	0.00
1 B - 1 C	-2.20	0.49	18	-4.51	0.00
1 B - 2 C	-2.55	0.49	18	-5.24	0.00
2 B - 1 C	0.14	0.49	18	0.28	0.99
2 B - 2 C	-0.21	0.49	18	-0.44	0.97
1 C - 2 C	-0.35	0.49	18	-0.72	0.89

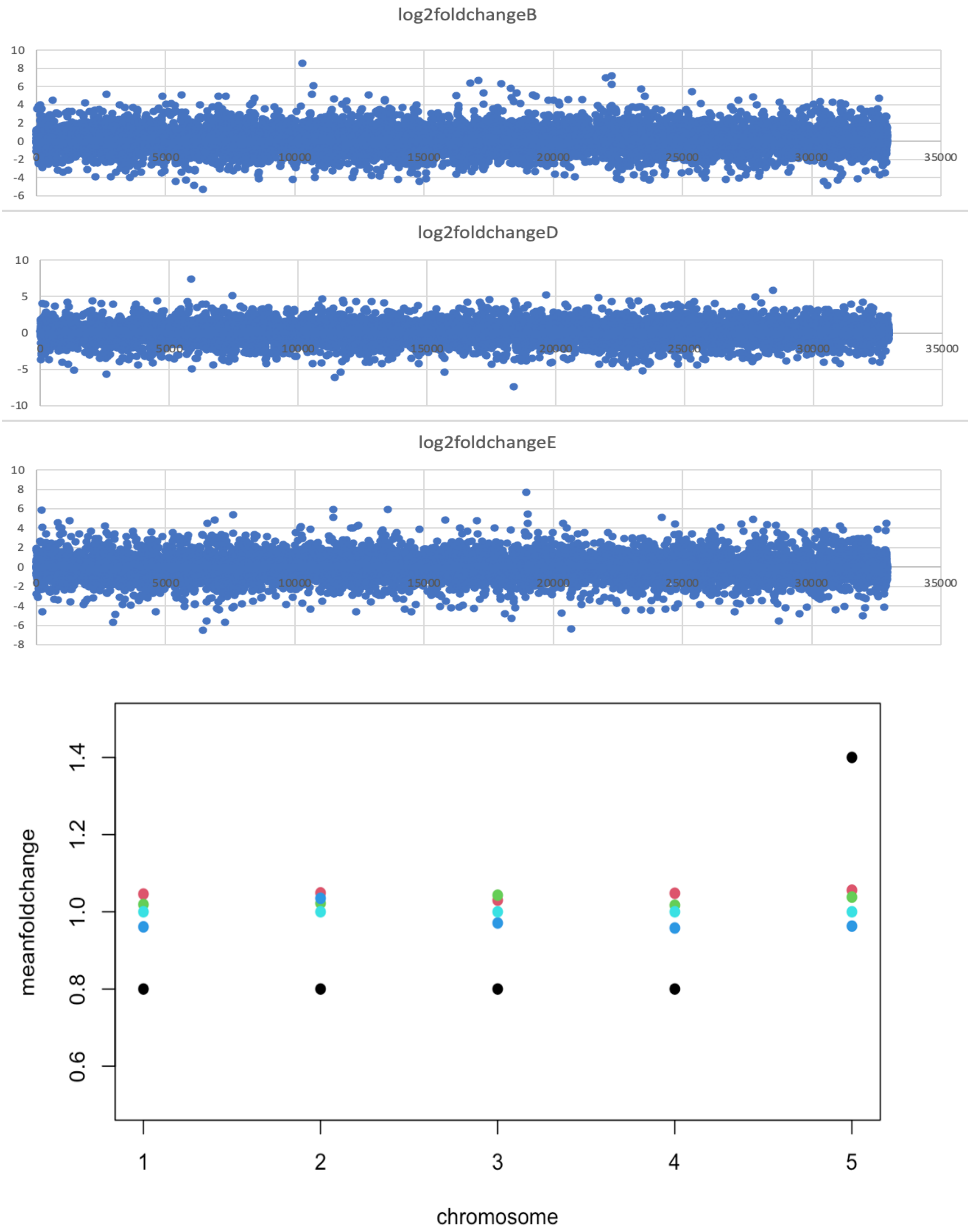
Supplemental Table 1: Post hoc Tukey HSD (emmeans) for the Diploid Linear Mixed Effects Model.

Genus	**Species**
Brevibacterium	*frigoritolerans*
Bacillus	*wiedmannii*
Curtobacterium	*herbarum*
Curtobacterium	*pusillum*
Erwinia	*tasmaniensis*
Exiguobacterium	*sibiricum*
Frigoribacterium	*endophyticum*
Microbacterium	*oleivorans*
Pantoea	*aurea*
Pantoea	*agglomerans*
Pantoea	*allii*
Pseudomonas	*asturiensis*
Pseudomonas	*rhizosphaerae*
Pseudomonas	*rhodesiae*
Pseudomonas	*moraviensis*
Rathayibacter	*festucae*

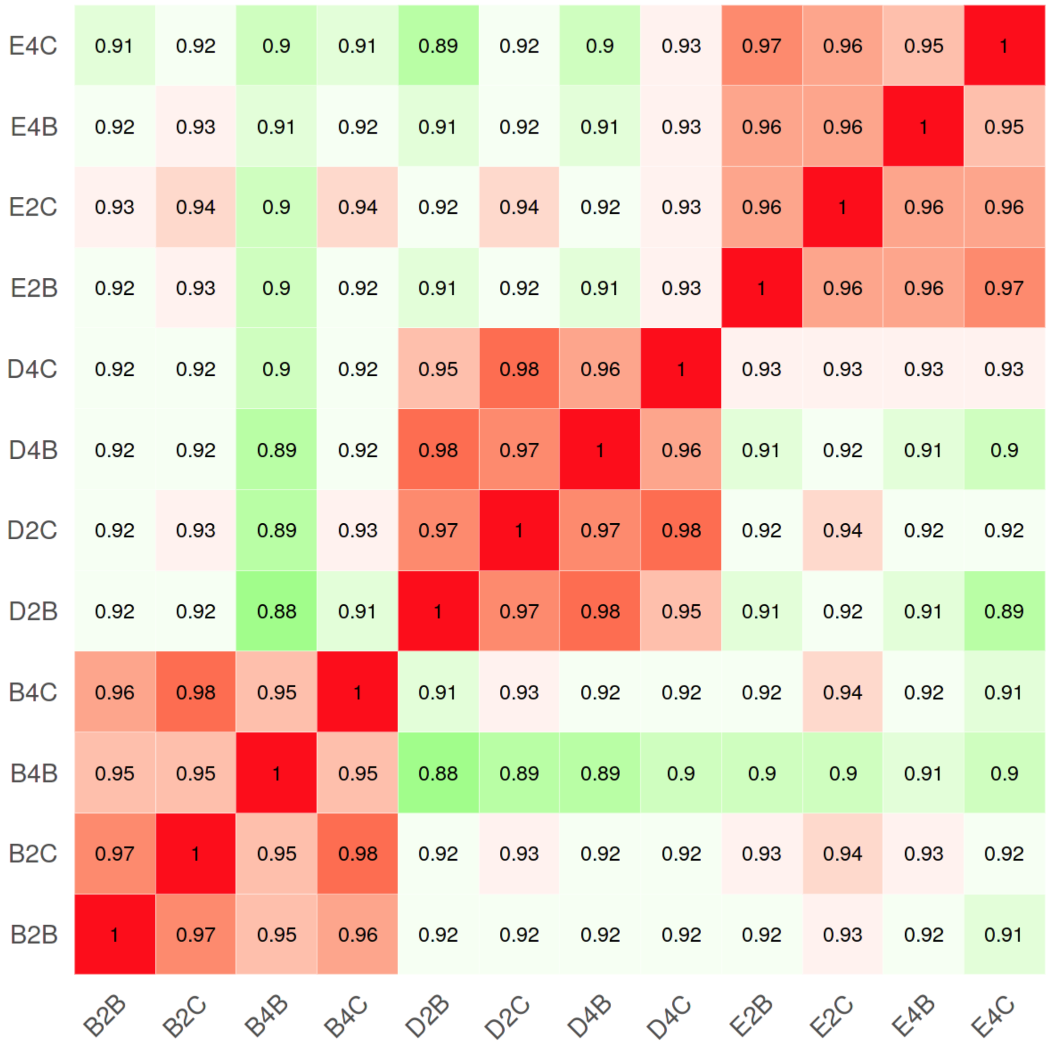
Supplemental Table 2. Taxa List of Synthetic Community Members

Name	Sequence (5'-3')	Tm (C)	GC%	Length (bp)
Forward	GACCAAGGATGCAGCAGAAA	61	50	187
Reverse	GCCGTTACGGATATCAACGA	60	50	

Supplemental Table 3. *Pseudomonas syringae* pv. tomato DC3000 specific primer used for ddPCR amplification

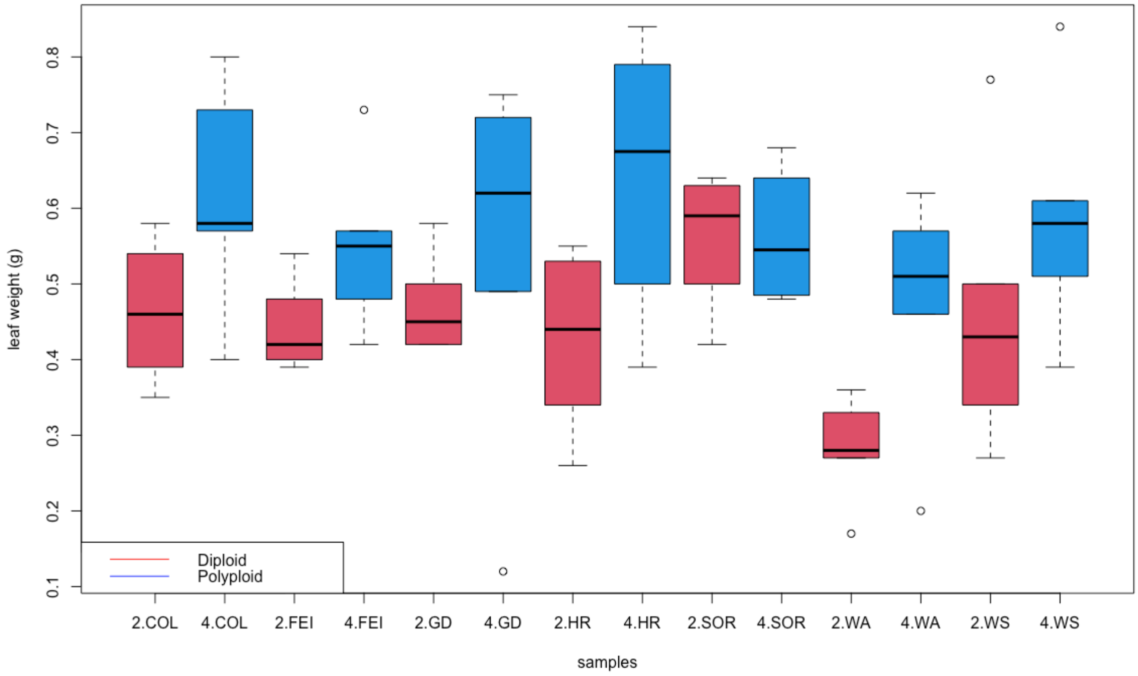


Supplemental Figure 1. Assessment of polyploid lines for aneuploidy. Top: Transcripts per million (TPM) per gene among the biological replicates of an accession (Col-0 (top), Ws-2 (middle), or Sorbo (bottom)) and plotted along the length of all five chromosomes. If any one showed a stretch (or whole chromosome) of elevated or lowered TPM relative to any of the others it would suggest aneuploidy (chromosomal or segmental). Bottom: Blue, green, and red dots represent the mean fold change per gene per chromosome for Col-0, Sorbo, and Ws-2, respectively. Cyan dots represent the expected pattern for an euploid (all 1.0) and black dots represent the expected pattern for an aneuploid where there is a coordinated transcriptional increase due to a segmental or chromosome duplication.



	B2B	B2C	B4B	B4C	D2B	D2C	D4B	D4C	E2B	E2C	E4B	E4C
E4C	0.91	0.92	0.9	0.91	0.89	0.92	0.9	0.93	0.97	0.96	0.95	1
E4B	0.92	0.93	0.91	0.92	0.91	0.92	0.91	0.93	0.96	0.96	1	0.95
E2C	0.93	0.94	0.9	0.94	0.92	0.94	0.92	0.93	0.96	1	0.96	0.96
E2B	0.92	0.93	0.9	0.92	0.91	0.92	0.91	0.93	1	0.96	0.96	0.97
D4C	0.92	0.92	0.9	0.92	0.95	0.98	0.96	1	0.93	0.93	0.93	0.93
D4B	0.92	0.92	0.89	0.92	0.98	0.97	1	0.96	0.91	0.92	0.91	0.9
D2C	0.92	0.93	0.89	0.93	0.97	1	0.97	0.98	0.92	0.94	0.92	0.92
D2B	0.92	0.92	0.88	0.91	1	0.97	0.98	0.95	0.91	0.92	0.91	0.89
B4C	0.96	0.98	0.95	1	0.91	0.93	0.92	0.92	0.92	0.94	0.92	0.91
B4B	0.95	0.95	1	0.95	0.88	0.89	0.89	0.9	0.9	0.9	0.91	0.9
B2C	0.97	1	0.95	0.98	0.92	0.93	0.92	0.92	0.93	0.94	0.93	0.92
B2B	1	0.97	0.95	0.96	0.92	0.92	0.92	0.92	0.92	0.93	0.92	0.91

Supplemental Figure 2. RNA seq heatmap of the sample-to-sample distances for Col-0 (B), Ws-2 (D), and Sorbo (E) accessions for treated (B) and untreated (C) plant samples.



Supplemental Figure 3. Leaf weights for diploid (red) and polyploid (blue) plants.

Concluding Remarks

Throughout this dissertation I highlight my work developing and utilizing the PhylloStart SynCom to understand the importance of the phyllosphere associated microbiota to its plant host. Of the conclusions that can be drawn from these studies, there is one point I would like to emphasize, which is that the environment, host condition, and community composition are critically important to determining the functions that the community can perform. For example, in Chapter 2, I describe an experiment where *P. agglomerans*, which I had previously established to provide protective benefits to the plant, is paired with other members of the PhylloStart community. When designing this experiment, I predicted that certain species would show an additive protective effect, and so that the addition of *B. wiedmanni*, which also showed a trend in decreased pathogen abundance, would be more protective than *P. agglomerans* alone. This hypothesis was correct, but interestingly, I also found a species, *E. tasmiensis*, that when added with *P. agglomerans* decreased the former's protective effects. This highlights the importance of understanding the dynamics within a microbial community when trying to predict how they will function, or how a certain species could be used for protection. Indeed, biocontrol strains often fail in real world scenarios (Handelsman & Stabb, 1996), with these non-focal species interactions potentially playing a role.

Another example of the context dependent benefits of microbial associations can be found in Chapter 3, where I show that amendment with the PhylloStart community leads to a significant increase in fruit production in the greenhouse, but not in the field. There are two primary explanations for this result, firstly, as is described in the chapter, it could be that the plants were colonized by a more diverse consortia of phyllosphere associated bacteria while in the field, and so the growth promoting benefits of an additional early exposure to the PhylloStart bacteria were not evident when compared to the broad exposure they received after transplantation. Alternatively, exposure to a much larger pool of colonizing microbes could have led to the PhylloStart community being outcompeted and replaced on the plants, preventing it from providing its growth promoting functions. After all, the plants that were moved to the field showed a significantly lower abundance of the PhylloStart associated bacteria than those that remained in the greenhouse. Regardless of which, or what combination, of these two led to the results we saw, this again shows the importance of the conditions under which the host is developing. This point is further established in another experiment in Chapter 3, where we find that, repeating an experiment previously describe by our lab (Berg & Koskella, 2018), the pathogen protective effect of the community is greater when the plant is under nutritional stress, paralleling the stress gradient hypothesis (Bertness & Callaway, 1994; David *et al.*, 2020). Combined with the growth promoting effects we see under other stressful conditions in the greenhouse, this shows that cooperation between the host and its microbial communities may become more important when the host is experiencing stressful conditions.

Emphasizing how dynamic these systems can be, Chapter 4 highlights research showing that stressful conditions do not always lead to a greater reliance on the microbiome. Using an Arabidopsis polyploidy model, we show that only diploids rely on their phyllosphere bacterial communities for pathogen protection, while the polyploids are broadly protected regardless of their exposure to the PhylloStart community or not. This is especially interesting for two reasons, firstly, that we do not see a difference in the communities that these plants assemble, regardless of what their ploidy is, which shows that under differing conditions plants can respond differently to the same microbial communities. Secondly, polyploidy is often seen as an evolutionary stress response (Thébault *et al.*, 2011; King *et al.*, 2012), quickly introducing a large amount of genetic variation on which selection can act, potentially buffering against changing environmental conditions. While the resulting changes in microbial reliance could simply be a byproduct of gene dosage changes, it is important to point out that, regardless of its cause, this reduced reliance on the microbiome is potentially adaptive. This chapter also provides useful information on the plant transcriptional responses to phyllosphere commensal species, which has, to my knowledge, only been done in two other studies (Vogel *et al.*, 2016; Maier *et al.*, 2021). In Vogel *et al*. they find a large set of genes that are associated with phyllosphere colonization by a commensal *Sphingomonas* species, many of which are associated with response to abiotic or biotic stimulus and response to stress. Likewise, we see that many of the genes that are differentially expressed in response to PhylloStart presence are associated with defense response and various other biotic and abiotic stresses. These results indicate that, not only do plants respond robustly to leaf colonization by certain commensal species, but that despite, or perhaps as a cause of, the beneficial effects that these communities provide, the experience in and of itself subjects the plant to additional stressors, likely due, at least in part, to epitopes shared with pathogenic bacterial species.

For example, it has been shown in several systems that a reliance on the microbiome can lead to a constrained ability to adapt to novel environments (Wernegreen, 2012; Nougué *et al.*, 2015). Further, while the microbiome can be leveraged to offload costly functions, such as the insulin signaling regulation in fruit flies, this means that they are now dependent on their microbes to survive (Shin *et al.*, 2011). Likewise, the diploid plants show a much lower baseline expression of defense associated genes when they are not in the presence of a phyllosphere community, and only upregulate defense expression when exposed to these bacteria. This also comes at a cost, because, without the microbial associations, they fare worse when challenged with a pathogen. So, it makes sense that one of the consequences of polyploidy, a response to change or stress in the external environment, could be to reclaim the functions that were previously outsourced to the microbiome. After all, when faced with these changing conditions, is it safe to rely on species that may not be available to recruit?

While it should now be clear that context is important in understanding host-microbial interactions, further work must be done to understand the mechanisms underlying these effects. Herein lies one of the greatest benefits of the SynCom approach, once an effect is measured, the same community can be leveraged for future studies. For example, is the decrease in *P. agglomerans* protection with the addition of *E. tasmiensis* due to interactions between the two bacteria, between the *E. tasmiensis* and the pathogen, or between the *E. tasmiensis* and the plant? Experiments can be designed to test these hypotheses (indeed, preliminary results indicate that *E. tasmiensis* alone may have a negative effect on plant outcomes). Likewise, I show that the phyllosphere community provides important reproductive benefits to its host, but it remains unclear how these effects are conveyed. These effects could potentially be mediated through the production of auxin, as previously mentioned, which several members of the SynCom have genes for, or through regulation of ethylene, which is a promoter of flower abscission (Botton & Ruperti, 2019), drawing from data in chapter 4 where diploid Arabidopsis respond to PhylloStart presence by upregulating several ethylene-responsive transcription factors. This process is likely somewhat complicated, as some of these factors are activators and others are inhibitors. These hypotheses can be tested through screening each member for auxin and applying members individually to the plant to determine the importance of each species in modulating auxin and ethylene production. Finally, while we were able to uncover the mechanism that influenced differing reliance on the microbiome in diploid and polyploid lines; differences in defense pathways activation, this community could be further leveraged to study variation among individual lines in terms of microbiome reliance, or to understand what other microbial functions change after whole genome duplications.

In conclusion, this dissertation shows that the phyllosphere microbiome provides important benefits to its host, such as disease protection and increasing reproductive success, but that these functions are highly dependent on host and environmental context. The PhylloStart community also represents an important resource for understanding and unraveling these, and other complex interactions between host and phyllosphere microbiome. This research has important implications for understanding microbial ecology and host-microbial interactions, but also for agricultural practices and applied approaches to crop production.

References

Abadi VAJM, Sepehri M, Rahmani HA, Zarei M, Ronaghi A, Taghavi SM, Shamshiripour M. **2020**. Role of Dominant Phyllosphere Bacteria with Plant Growth–Promoting Characteristics on Growth and Nutrition of Maize (Zea mays L.). *Journal of Soil Science and Plant Nutrition* **20**: 2348–2363.

Adesemoye A o., Torbert H a., Kloepper J w. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* **54**: 876–886.

Ali B, Sabri A n., 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.

Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, Dombrowski N, Münch PC, Spaepen S, Remus-Emsermann M, *et al.* 2015. Functional overlap of the Arabidopsis leaf and root microbiota. *Nature* **528**: 364–369.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, *et al.* 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* **19**: 455–477.

Bashan Y, Holguin G. **1998**. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* **30**: 1225–1228.

Baskins S, Bond J, Minor T. **2019**. Unpacking the Growth in Per Capita Availability of Fresh Market Tomatoes. : 17.

Beattie GA. **2011**. Water relations in the interaction of foliar bacterial pathogens with plants. *Annual Review of Phytopathology* **49**: 533–555.

Beneduzi A, Ambrosini A, Passaglia LMP. **2012**. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* **35**: 1044–1051.

Bent AF. **1992**. Disease Development in Ethylene-Insensitive *Arabidopsis thaliana* Infected with Virulent and Avirulent *Pseudomonas* and *Xanthomonas* Pathogens. *Molecular Plant-Microbe Interactions* **5**: 372.

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

Berg M, Koskella B. **2018**. Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen. *Current Biology* **28**: 2487-2492.e3.

Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, *et al.* 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* **8**: 103.

Berrin J-G, Pierrugues O, Brutesco C, Alonso B, Montillet J-L, Roby D, Kazmaier M. **2005**. Stress induces the expression of AtNADK-1, a gene encoding a NAD(H) kinase in Arabidopsis thaliana. *Molecular genetics and genomics: MGG* **273**: 10–19.

Bertness MD, Callaway R. **1994**. Positive interactions in communities. *Trends in Ecology & Evolution* **9**: 191–193.

Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC. **2011**. Defence responses of Arabidopsis thaliana to infection by Pseudomonas syringae are regulated by the circadian clock. *PloS One* **6**: e26968.

Bhattacharyya PN, Jha DK. **2012**. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* **28**: 1327–1350.

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. **2014**. A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota (U Hentschel, Ed.). *PLoS Genetics* **10**: e1004283.

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

Bolger AM, Lohse M, Usadel B. **2014**. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England)* **30**: 2114–2120.

Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S. **2006**. Infection with virulent and avirulent P. syringae strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. *Planta* **225**: 1–12.

Bordenstein SR, Theis KR. **2015**. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLOS Biology* **13**: e1002226.

Botton A, Ruperti B. **2019**. The Yes and No of the Ethylene Involvement in Abscission. *Plants* **8**: 187.

Bowers RM, McLetchie S, Knight R, Fierer N. **2011**. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *The ISME Journal* **5**: 601–612.

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlach J. **2001**. Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. *The Plant Cell* **13**: 1499–1510.

Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, Stern DL. **2003**. Developmental Origin and Evolution of Bacteriocytes in the Aphid–Buchnera Symbiosis. *PLOS Biology* **1**: e21.

Brandl MT, Lindow SE. **1998**. Contribution of Indole-3-Acetic Acid Production to the Epiphytic Fitness of Erwinia herbicola. *Applied and Environmental Microbiology* **64**: 3256–3263.

Brandl MT, Quiñones B, Lindow SE. **2001**. Heterogeneous transcription of an indoleacetic acid biosynthetic gene in Erwinia herbicola on plant surfaces. *Proceedings of the National Academy of Sciences* **98**: 3454–3459.

Brockhurst MA, Fenton A, Roulston B, Rainey PB. **2006**. The impact of phages on interspecific competition in experimental populations of bacteria. *BMC Ecology* **6**: 19.

Browne PD, Nielsen TK, Kot W, Aggerholm A, Gilbert MTP, Puetz L, Rasmussen M, Zervas A, Hansen LH. **2020**. GC bias affects genomic and metagenomic reconstructions, underrepresenting GC-poor organisms. *GigaScience* **9**: giaa008.

Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. **2013**. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807–838.

Burch AY, Do PT, Sbodio A, Suslow TV, Lindow SE. **2016**. High-Level Culturability of Epiphytic Bacteria and Frequency of Biosurfactant Producers on Leaves. *Applied and Environmental Microbiology* **82**: 5997–6009.

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

Carlström CI, Field CM, Bortfeld-Miller M, Müller B, Sunagawa S, Vorholt JA. **2019**. Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. *Nature Ecology & Evolution* **3**: 1445–1454.

Castrillo G, Teixeira PJPL, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, *et al.* 2017. Root microbiota drive direct integration of phosphate stress and immunity. *Nature* **543**: 513–518.

Chen ZJ. **2010**. Molecular mechanisms of polyploidy and hybrid vigor. *Trends in Plant Science* **15**: 57–71.

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. **2009**. Glucosinolate metabolites required for an Arabidopsis innate immune response. *Science (New York, N.Y.)* **323**: 95–101.

Coate JE, Luciano AK, Seralathan V, Minchew KJ, Owens TG, Doyle JJ. **2012**. Anatomical, biochemical, and photosynthetic responses to recent allopolyploidy in Glycine dolichocarpa (Fabaceae). *American Journal of Botany* **99**: 55–67.

Colaianni NR, Parys K, Lee H-S, Conway JM, Kim NH, Edelbacher N, Mucyn TS, Madalinski M, Law TF, Jones CD, *et al.* 2021. A complex immune response to flagellin epitope variation in commensal communities. *Cell Host & Microbe* **29**: 635-649.e9.

Compant S, Clément C, Sessitsch A. **2010**. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* **42**: 669–678.

Conrath U, Pieterse CMJ, Mauch-Mani B. **2002**. Priming in plant–pathogen interactions. *Trends in Plant Science* **7**: 210–216.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal Community Succession of the Phyllosphere Microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cuevas DA, Edirisinghe J, Henry CS, Overbeek R, O'Connell TG, Edwards RA. **2016**. From DNA to FBA: How to Build Your Own Genome-Scale Metabolic Model. *Frontiers in Microbiology* **7**.

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.

David AS, Thapa-Magar KB, Menges ES, Searcy CA, Afkhami ME. **2020**. Do plant–microbe interactions support the Stress Gradient Hypothesis? *Ecology* **101**: e03081.

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

Dehal P, Boore JL. **2005**. Two Rounds of Whole Genome Duplication in the Ancestral Vertebrate. *PLOS Biology* **3**: e314.

Derakhshani H, Bernier SP, Marko VA, Surette MG. **2020**. Completion of draft bacterial genomes by long-read sequencing of synthetic genomic pools. *BMC Genomics* **21**: 519.

Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. **2019**. Role of the microbiome in human development. *Gut* **68**: 1108–1114.

Douglas AE. **1998**. Nutritional Interactions in Insect-Microbial Symbioses: Aphids and Their Symbiotic Bacteria *Buchnera*. *Annual Review of Entomology* **43**: 17–37.

Ecker JR, Davis RW. **1987**. Plant defense genes are regulated by ethylene. *Proceedings of the National Academy of Sciences of the United States of America* **84**: 5202–5206.

Esİtken A, Ercİslİ S, Karlİdag H, Sahİn F. **2005**. Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. *Proceedings of the international scientific conference: Environmentally friendly fruit growing, Polli, Estonia, 7-9 September, 2005*: 90–97.

Etesami H, Alikhani HA, Hosseini HM. **2015**. Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX* **2**: 72–78.

Eulgem T, Somssich IE. **2007**. Networks of WRKY transcription factors in defense signaling. *Current Opinion in Plant Biology* **10**: 366–371.

Faith JJ, McNulty NP, Rey FE, Gordon JI. **2011**. Predicting a Human Gut Microbiota's Response to Diet in Gnotobiotic Mice. *Science* **333**: 101–104.

Felix G, Duran JD, Volko S, Boller T. **1999**. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *The Plant Journal: For Cell and Molecular Biology* **18**: 265–276.

Finkel OM, Castrillo G, Herrera Paredes S, Salas González I, Dangl JL. **2017**. Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology* **38**: 155–163.

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. **2017**. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**: 43–51.

Fukao T, Bailey-Serres J. **2004**. Plant responses to hypoxia--is survival a balancing act? *Trends in Plant Science* **9**: 449–456.

Galland M, Gamet L, Varoquaux F, Touraine B, Touraine B, Desbrosses G. **2012**. The ethylene pathway contributes to root hair elongation induced by the beneficial bacteria Phyllobacterium brassicacearum STM196. *Plant Science* **190**: 74–81.

Garbeva P, Silby MW, Raaijmakers JM, Levy SB, Boer W de. **2011**. Transcriptional and antagonistic responses of Pseudomonas fluorescens Pf0-1 to phylogenetically different bacterial competitors. *The ISME Journal* **5**: 973–985.

Glandorf DCM, Brand I, Bakker PAHM, Schippers B. **1992**. Stability of rifampicin resistance as a marker for root colonization studies of Pseudomonas putida in the field. *Plant and Soil* **147**: 135–142.

Glick BR. **2012**. Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica* **2012**: 1–15.

Goldford JE, Lu N, Bajić D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segrè D, Mehta P, Sanchez A. **2018**. Emergent simplicity in microbial community assembly. *Science* **361**: 469–474.

Goldstein BP. **2014**. Resistance to rifampicin: a review. *The Journal of Antibiotics* **67**: 625–630.

Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, Ludington WB. **2018**. Microbiome interactions shape host fitness. *Proceedings of the National Academy of Sciences* **115**: E11951–E11960.

Großkopf T, Soyer OS. **2014**. Synthetic microbial communities. *Current Opinion in Microbiology* **18**: 72–77.

Guan R, Su J, Meng X, Li S, Liu Y, Xu J, Zhang S. **2015**. Multilayered Regulation of Ethylene Induction Plays a Positive Role in Arabidopsis Resistance against *Pseudomonas syringae*. *Plant Physiology* **169**: 299–312.

Handelsman J, Stabb E. **1996**. Biocontrol of Soilborne Plant Pathogens. *The Plant Cell* **8**: 1855–1869.

Helfrich EJN, Vogel CM, Ueoka R, Schäfer M, Ryffel F, Müller DB, Probst S, Kreuzer M, Piel J, Vorholt JA. **2018**. Bipartite interactions, antibiotic production and biosynthetic potential of the Arabidopsis leaf microbiome. *Nature Microbiology* **3**: 909–919.

Holtan HE, Bandong S, Marion CM, Adam L, Tiwari S, Shen Y, Maloof JN, Maszle DR, Ohto M, Preuss S, *et al.* **2011**. BBX32, an Arabidopsis B-Box Protein, Functions in Light Signaling by Suppressing HY5-Regulated Gene Expression and Interacting with STH2/BBX21[W][OA]. *Plant Physiology* **156**: 2109–2123.

Hu Y, Dong Q, Yu D. **2012**. Arabidopsis WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen Pseudomonas syringae. *Plant Science: An International Journal of Experimental Plant Biology* **185–186**: 288–297.

Hu J, Wei Z, Friman V-P, Gu S, Wang X, Eisenhauer N, Yang T, Ma J, Shen Q, Xu Y, *et al.* **2016**. Probiotic Diversity Enhances Rhizosphere Microbiome Function and Plant Disease Suppression. *mBio* **7**: e01790-16.

Huang M-L, Deng X-P, Zhao Y-Z, Zhou S-L, Inanaga S, Yamada S, Tanaka K. **2007**. Water and Nutrient Use Efficiency in Diploid, Tetraploid and Hexaploid Wheats. *Journal of Integrative Plant Biology* **49**: 706–715.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System ▿. *Applied and Environmental Microbiology* **77**: 3202–3210.

Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. **2017**. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. *Frontiers in Plant Science* **8**.

Jacobs JL, Carroll TL, Sundin GW. **2005**. The role of pigmentation, ultraviolet radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. *Microbial Ecology* **49**: 104–113.

Jin DJ, Gross CA. **1989**. Characterization of the pleiotropic phenotypes of rifampin-resistant rpoB mutants of Escherichia coli. *Journal of Bacteriology* **171**: 5229–5231.

K DA, Sabarinathan KG, Gomathy M, Kannan R, Balachandar D. **2020**. Mitigation of drought stress in rice crop with plant growth-promoting abiotic stress-tolerant rice phyllosphere bacteria. *Journal of Basic Microbiology* **60**: 768–786.

Karasov TL, Chae E, Herman JJ, Bergelson J. **2017**. Mechanisms to Mitigate the Trade-Off between Growth and Defense. *The Plant Cell* **29**: 666–680.

Kassambara A. **2021**. *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*.

Ke J, Wang B, Yoshikuni Y. **2021**. Microbiome Engineering: Synthetic Biology of Plant-Associated Microbiomes in Sustainable Agriculture. *Trends in Biotechnology* **39**: 244–261.

Kim D, Langmead B, Salzberg SL. **2015**. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods* **12**: 357–360.

King KC, Bonsall MB. **2017**. The evolutionary and coevolutionary consequences of defensive microbes for host-parasite interactions. *BMC evolutionary biology* **17**: 190.

King KC, Seppälä O, Neiman M. **2012**. Is more better? Polyploidy and parasite resistance. *Biology Letters* **8**: 598–600.

King EO, Ward MK, Raney DE. **1954**. Two simple media for the demonstration of pyocyanin and fluorescin. *The Journal of Laboratory and Clinical Medicine* **44**: 301–307.

Kloepper JW, Leong J, Teintze M, Schroth MN. **1980**. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**: 885–886.

Knief C, Ramette A, Frances L, Alonso-Blanco C, Vorholt JA. **2010**. Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. *The ISME Journal* **4**: 719–728.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. **2013**. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental Microbiology* **79**: 5112–5120.

Kurkjian HM, Akbari MJ, Momeni B. **2021**. The impact of interactions on invasion and colonization resistance in microbial communities. *PLoS Computational Biology* **17**: e1008643.

Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, Sasidharan R, Muller R, Dreher K, Alexander DL, Garcia-Hernandez M, *et al.* **2012**. The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Research* **40**: D1202-1210.

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Rio TG del, Jones CD, Tringe SG, *et al.* **2015**. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*.

Lee K-H, Munusamy M, Kim C-W, Lee H-S, Selvaraj P, Sa T. **2004**. Isolation and Characterization of the IAA Producing Methylotrophic Bacteria from Phyllosphere of Rice Cultivars(Oryza sativa L.). *Korean Journal of Soil Science and Fertilizer* **37**: 235–244.

Leggat W, Ainsworth T, Bythell J, Dove S, Gates R, Hoegh-Guldberg O, Iglesias-Prieto R, Yellowlees D. **2007**. The hologenome theory disregards the coral holobiont. *Nature Reviews Microbiology* **5**: 826–826.

Leitch IJ, Bennett MD. **1997**. Polyploidy in angiosperms. *Trends in Plant Science* **2**: 470–476.

Leopold DR, Busby PE. **2020**. Host Genotype and Colonist Arrival Order Jointly Govern Plant Microbiome Composition and Function. *Current Biology* **30**: 3260-3266.e5.

Leveau JHJ, Lindow SE. **2001**. Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences* **98**: 3446–3453.

Levin DA. **1983**. Polyploidy and Novelty in Flowering Plants. *The American Naturalist* **122**: 1–25.

Li M, Xu G, Xia X, Wang M, Yin X, Zhang B, Zhang X, Cui Y. **2017**. Deciphering the physiological and molecular mechanisms for copper tolerance in autotetraploid Arabidopsis. *Plant Cell Reports* **36**: 1585–1597.

Liang W, Li C, Liu F, Jiang H, Li S, Sun J, Wu X, Li C. **2009**. The Arabidopsis homologs of CCR4-associated factor 1 show mRNA deadenylation activity and play a role in plant defence responses. *Cell Research* **19**: 307–316.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Lindow SE, Desurmont C, Elkins R, McGourty G, Clark E, Brandl MT. **1998**. Occurrence of Indole-3-Acetic Acid-Producing Bacteria on Pear Trees and Their Association with Fruit Russet. *Phytopathology®* **88**: 1149–1157.

Love MI, Huber W, Anders S. **2014**. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Rio TG del, *et al.* **2012**. Defining the core Arabidopsis thaliana root microbiome. *Nature* **488**: 86–90.

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

Lymperopoulou DS, Adams RI, Lindow SE. **2016**. Contribution of Vegetation to the Microbial Composition of Nearby Outdoor Air. *Applied and Environmental Microbiology* **82**: 3822–3833.

Madden LV, Hughes G Bosch, Frank van den. **2017**. The study of plant disease epidemics.

Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, Kolesnikov A, Lopez R. **2022**. Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic acids research*: gkac240.

Madhaiyan M, Poonguzhali S, Sundaram SP, Sa T. **2006**. A new insight into foliar applied methanol influencing phylloplane methylotrophic dynamics and growth promotion of cotton (Gossypium hirsutum L.) and sugarcane (Saccharum officinarum L.). *Environmental and Experimental Botany* **57**: 168–176.

Maier BA, Kiefer P, Field CM, Hemmerle L, Bortfeld-Miller M, Emmenegger B, Schäfer M, Pfeilmeier S, Sunagawa S, Vogel CM, *et al.* 2021. A general non-self response as part of plant immunity. *Nature Plants* **7**: 696–705.

Maignien L, DeForce EA, Chafee ME, Eren AM, Simmons SL. **2014**. Ecological Succession and Stochastic Variation in the Assembly of Arabidopsis thaliana Phyllosphere Communities. *mBio* **5**.

Malard F, Dore J, Gaugler B, Mohty M. **2021**. Introduction to host microbiome symbiosis in health and disease. *Mucosal Immunology* **14**: 547–554.

Mauch-Mani B, Baccelli I, Luna E, Flors V. **2017**. Defense Priming: An Adaptive Part of Induced Resistance. *Annual Review of Plant Biology* **68**: 485–512.

McLaren MR, Callahan BJ. **2020**. Pathogen resistance may be the principal evolutionary advantage provided by the microbiome. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**: 20190592.

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

McNulty NP, Yatsunenko T, Hsiao A, Faith JJ, Muegge BD, Goodman AL, Henrissat B, Oozeer R, Cools-Portier S, Gobert G, *et al.* 2011. The Impact of a Consortium of Fermented Milk Strains on the Gut Microbiome of Gnotobiotic Mice and Monozygotic Twins. *Science Translational Medicine* **3**: 106ra106-106ra106.

Mehlferber EC, McCue KF, Ferrel JE, Koskella B, Khanna R. **2022**. Temporally Selective Modification of the Tomato Rhizosphere and Root Microbiome by Volcanic Ash Fertilizer Containing Micronutrients (JD Semrau, Ed.). *Applied and Environmental Microbiology* **88**: e00049-22.

Meir S, Philosoph-Hadas S, Sundaresan S, Selvaraj KSV, Burd S, Ophir R, Kochanek B, Reid MS, Jiang C-Z, Lers A. **2010**. Microarray Analysis of the Abscission-Related Transcriptome in the Tomato Flower Abscission Zone in Response to Auxin Depletion. *Plant Physiology* **154**: 1929–1956.

Melotto M, Underwood W, Koczan J, Nomura K, He SY. **2006**. Plant stomata function in innate immunity against bacterial invasion. *Cell* **126**: 969–980.

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, *et al.* 2011. Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* **332**: 1097–1100.

Mercier J, Lindow SE. **2001**. Field Performance of Antagonistic Bacteria Identified in a Novel Laboratory Assay for Biological Control of Fire Blight of Pear. *Biological Control* **22**: 66–71.

Metcalf CJE, Henry LP, Rebolleda-Gómez M, Koskella B. **2019**. Why Evolve Reliance on the Microbiome for Timing of Ontogeny? *mBio* **10**: e01496-19.

Metcalf CJE, Koskella B. **2019**. Protective microbiomes can limit the evolution of host pathogen defense. *Evolution Letters* **3**: 534–543.

Meyer KM, Leveau JHJ. **2012**. Microbiology of the phyllosphere: a playground for testing ecological concepts. *Oecologia* **168**: 621–629.

Meyer KM, Porch R, Muscettola IE, Vasconcelos ALS, Sherman JK, Metcalf CJE, Lindow SE, Koskella B. **2022**. Plant neighborhood shapes diversity and reduces interspecific variation of the phyllosphere microbiome. *The ISME Journal* **16**: 1376–1387.

Monier J-M, Lindow SE. **2005**. Spatial Organization of Dual-Species Bacterial Aggregates on Leaf Surfaces. *Applied and Environmental Microbiology* **71**: 5484–5493.

Moran NA, Sloan DB. **2015**. The Hologenome Concept: Helpful or Hollow? *PLOS Biology* **13**: e1002311.

Morella NM, Gomez AL, Wang G, Leung MS, Koskella B. **2018a**. The impact of bacteriophages on phyllosphere bacterial abundance and composition. *Molecular Ecology*.

Morella NM, Weng FC-H, Joubert PM, Metcalf CJE, Lindow S, Koskella B. **2020**. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. *Proceedings of the National Academy of Sciences* **117**: 1148–1159.

Morella NM, Yang SC, Hernandez CA, Koskella B. **2018b**. Rapid quantification of bacteriophages and their bacterial hosts in vitro and in vivo using droplet digital PCR. *Journal of Virological Methods* **259**: 18–24.

Morella NM, Zhang X, Koskella B. **2019**. Tomato Seed-Associated Bacteria Confer Protection of Seedlings Against Foliar Disease Caused by Pseudomonas syringae. *Phytobiomes Journal* **3**: 177–190.

Morrissey EM, Mau RL, Schwartz E, Caporaso JG, Dijkstra P, van Gestel N, Koch BJ, Liu CM, Hayer M, McHugh TA, *et al.* 2016. Phylogenetic organization of bacterial activity. *The ISME Journal* **10**: 2336–2340.

Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu M-H, Oldroyd GED, Poole PS, *et al.* 2016. Symbiotic Nitrogen Fixation and the Challenges to Its Extension to Nonlegumes. *Applied and Environmental Microbiology* **82**: 3698–3710.

Mwajita MR, Murage H, Tani A, Kahangi EM. **2013**. Evaluation of rhizosphere, rhizoplane and phyllosphere bacteria and fungi isolated from rice in Kenya for plant growth promoters. *SpringerPlus* **2**: 606.

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

Ng DW-K, Zhang C, Miller M, Shen Z, Briggs SP, Chen ZJ. **2012**. Proteomic divergence in Arabidopsis autopolyploids and allopolyploids and their progenitors. *Heredity* **108**: 419–430.

Ni Z, Kim E-D, Ha M, Lackey E, Liu J, Zhang Y, Sun Q, Chen ZJ. **2009**. Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. *Nature* **457**: 327–331.

Niu D-D, Liu H-X, Jiang C-H, Wang Y-P, Wang Q-Y, Jin H-L, Guo J-H. **2011**. The plant growth-promoting rhizobacterium Bacillus cereus AR156 induces systemic resistance in Arabidopsis thaliana by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. *Molecular plant-microbe interactions: MPMI* **24**: 533–542.

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

Noorani Azad H, Hassan Poor A, Bakhshikhaniki GR, Ebrahimi MA. **2016**. Physiological responses of two tomato (Lycopersicun esculentum M.) cultivars to Azomite fertilizer under drought stress. *Iranian Journal of Plant Physiology* **6**: 1833–1843.

Nougué O, Gallet R, Chevin L-M, Lenormand T. **2015**. Niche Limits of Symbiotic Gut Microbiota Constrain the Salinity Tolerance of Brine Shrimp. *The American Naturalist* **186**: 390–403.

Nuismer SL, Thompson JN. **2001**. Plant polyploidy and non-uniform effects on insect herbivores. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **268**: 1937–1940.

O'Brien EJ, Monk JM, Palsson BO. **2015**. Using Genome-Scale Models to Predict Biological Capabilities. *Cell* **161**: 971–987.

Okada H, Kuhn C, Feillet H, Bach J-F. **2010**. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clinical and Experimental Immunology* **160**: 1–9.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, *et al.* 2020. *vegan: Community Ecology Package.*

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, *et al.* 2022. vegan: Community Ecology Package.

Oswald BP, Nuismer SL. **2007**. Neopolyploidy and pathogen resistance. *Proceedings of the Royal Society B: Biological Sciences* **274**: 2393–2397.

Ottesen AR, González Peña A, White JR, Pettengill JB, Li C, Allard S, Rideout S, Allard M, Hill T, Evans P, *et al.* 2013. Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC Microbiology* **13**: 114.

Ottesen AR, Gorham S, Reed E, Newell MJ, Ramachandran P, Canida T, Allard M, Evans P, Brown E, White JR. **2016**. Using a Control to Better Understand Phyllosphere Microbiota (M-J Virolle, Ed.). *PLOS ONE* **11**: e0163482.

Overmyer K, Brosché M, Kangasjärvi J. **2003**. Reactive oxygen species and hormonal control of cell death. *Trends in Plant Science* **8**: 335–342.

Oyola SO, Otto TD, Gu Y, Maslen G, Manske M, Campino S, Turner DJ, MacInnis B, Kwiatkowski DP, Swerdlow HP, *et al.* 2012. Optimizing illumina next-generation sequencing library preparation for extremely at-biased genomes. *BMC Genomics* **13**: 1.

Pacey EK, Maherali H, Husband BC. **2020**. The influence of experimentally induced polyploidy on the relationships between endopolyploidy and plant function in *Arabidopsis thaliana*. *Ecology and Evolution* **10**: 198–216.

Parangan-Smith A, Lindow S. **2013**. Contribution of Nitrate Assimilation to the Fitness of Pseudomonas syringae pv. syringae B728a on Plants. *Applied and Environmental Microbiology* **79**: 678–687.

Pati BR, Chandra AK. **1981**. Effect of spraying nitrogen-fixing phyllospheric bacterial isolates on wheat plants. *Plant and Soil* **61**: 419–427.

Paul D, Nair S. **2008**. Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *Journal of Basic Microbiology* **48**: 378–384.

Penrose DM, Glick BR. **2003**. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum* **118**: 10–15.

Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. **2016**. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protocols* **11**: 1650–1667.

Phillippy AM, Schatz MC, Pop M. **2008**. Genome assembly forensics: finding the elusive mis-assembly. *Genome Biology* **9**: R55.

Pinheiro J, Bates D, DebRoy S, Sarkar D, Heisterkamp S, Van Willigen B, Ranke J. **2022**. nlme: Linear and Nonlinear Mixed Effects Models.

Pogorelko GV, Mokryakova M, Fursova OV, Abdeeva I, Piruzian ES, Bruskin SA. **2014**. Characterization of three Arabidopsis thaliana immunophilin genes involved in the plant defense response against Pseudomonas syringae. *Gene* **538**: 12–22.

Ponsford JCB, Hubbard CJ, Harrison JG, Maignien L, Buerkle CA, Weinig C. **2022**. Whole-Genome Duplication and Host Genotype Affect Rhizosphere Microbial Communities (D Bulgarelli, Ed.). *mSystems* **7**: e00973-21.

Preuss SB, Meister R, Xu Q, Urwin CP, Tripodi FA, Screen SE, Anil VS, Zhu S, Morrell JA, Liu G, *et al.* 2012. Expression of the Arabidopsis thaliana BBX32 gene in soybean increases grain yield. *PloS One* **7**: e30717.

Qin C, Tao J, Liu T, Liu Y, Xiao N, Li T, Gu Y, Yin H, Meng D. **2019**. Responses of phyllosphere microbiota and plant health to application of two different biocontrol agents. *AMB Express* **9**: 42.

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.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves: Biogeography of phyllosphere bacterial communities. *Environmental Microbiology* **12**: 2885–2893.

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, *et al.* 2008. Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (Phaseolus vulgaris L.). *Plant and Soil* **302**: 149–161.

Remus-Emsermann MNP, Lücker S, Müller DB, Potthoff E, Daims H, Vorholt JA. **2014**. Spatial distribution analyses of natural phyllosphere-colonizing bacteria on Arabidopsis thaliana revealed by fluorescence in situ hybridization: Bacterial distribution on Arabidopsis phylloplanes. *Environmental Microbiology* **16**: 2329–2340.

Remus-Emsermann MNP, Schlechter RO. **2018**. Phyllosphere microbiology: at the interface between microbial individuals and the plant host. *New Phytologist* **218**: 1327–1333.

Remus-Emsermann MNP, Tecon R, Kowalchuk GA, Leveau JHJ. **2012**. Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere. *The ISME Journal* **6**: 756–765.

RUINEN J. **1965**. THE PHYLLOSPHERE: III. NITROGEN FIXATION IN THE PHYLLOSPHERE. *Plant and Soil* **22**: 375–394.

Russel J, Røder HL, Madsen JS, Burmølle M, Sørensen SJ. **2017**. Antagonism correlates with metabolic similarity in diverse bacteria. *Proceedings of the National Academy of Sciences* **114**: 10684–10688.

Saei A, Hoeata K, Krebs A, Sutton P, Herrick J, Wood M, Gea L. **2018**. The status of Pseudomonas syringae pv. actinidiae (Psa) in the New Zealand kiwifruit breeding programme in relation to ploidy level. *Acta Horticulturae*: 293–298.

Šantl-Temkiv T, Gosewinkel U, Starnawski P, Lever M, Finster K. **2018**. Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states. *FEMS Microbiology Ecology* **94**: fiy031.

Sanyal SK, Kanwar P, Fernandes JL, Mahiwal S, Yadav AK, Samtani H, Srivastava AK, Suprasanna P, Pandey GK. **2020**. Arabidopsis Mitochondrial Voltage-Dependent Anion Channels Are Involved in Maintaining Reactive Oxygen Species Homeostasis, Oxidative and Salt Stress Tolerance in Yeast. *Frontiers in Plant Science* **11**: 50.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al.* 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schoen DJ, Burdon JJ, Brown AHD. **1992**. Resistance of Glycine tomentella to soybean leaf rust Phakopsora pachyrhizi in relation to ploidy level and geographic distribution. *Theoretical and Applied Genetics* **83**: 827–832.

Seemann T. **2014**. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**: 2068–2069.

Segerman B. **2020**. The Most Frequently Used Sequencing Technologies and Assembly Methods in Different Time Segments of the Bacterial Surveillance and RefSeq Genome Databases. *Frontiers in Cellular and Infection Microbiology* **10**.

Selosse M-A, Bessis A, Pozo MJ. **2014**. Microbial priming of plant and animal immunity: symbionts as developmental signals. *Trends in Microbiology* **22**: 607–613.

Sexton R, Roberts JA. **1982**. Cell Biology of Abscission. *Annual Review of Plant Physiology* **33**: 133–162.

Sgro GG, Oka GU, Souza DP, Cenens W, Bayer-Santos E, Matsuyama BY, Bueno NF, dos Santos TR, Alvarez-Martinez CE, Salinas RK, *et al.* 2019. Bacteria-Killing Type IV Secretion Systems. *Frontiers in Microbiology* **10**.

Sharma A, Johri BN, Sharma AK, Glick BR. **2003**. Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (Vigna radiata L. Wilzeck). *Soil Biology and Biochemistry* **35**: 887–894.

Shi H, Liu W, Yao Y, Wei Y, Chan Z. **2017**. Alcohol dehydrogenase 1 (ADH1) confers both abiotic and biotic stress resistance in Arabidopsis. *Plant Science: An International Journal of Experimental Plant Biology* **262**: 24–31.

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, Yoon J-H, Ryu J-H, Lee W-J. **2011**. Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science (New York, N.Y.)* **334**: 670–674.

Smits THM. **2019**. The importance of genome sequence quality to microbial comparative genomics. *BMC Genomics* **20**: 662.

Sonenshein AL, Cami B, Brevet J, Cote R. **1974**. Isolation and Characterization of Rifampin-Resistant and Streptolydigin-Resistant Mutants of Bacillus subtilis with Altered Sporulation Properties. *Journal of Bacteriology* **120**: 253–265.

Song MJ, Potter BI, Doyle JJ, Coate JE. **2020**. Gene Balance Predicts Transcriptional Responses Immediately Following Ploidy Change in Arabidopsis thaliana. *The Plant Cell* **32**: 1434–1448.

de Souza RSC, Armanhi JSL, Arruda P. **2020**. From Microbiome to Traits: Designing Synthetic Microbial Communities for Improved Crop Resiliency. *Frontiers in Plant Science* **11**.

Stappenbeck TS, Virgin HW. **2016**. Accounting for reciprocal host–microbiome interactions in experimental science. *Nature* **534**: 191–199.

Stone BWG, Weingarten EA, Jackson CR. **2018**. The Role of the Phyllosphere Microbiome in Plant Health and Function. In: Annual Plant Reviews online. American Cancer Society, 533–556.

Sturz AV, Christie BR. **2003**. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil and Tillage Research* **72**: 107–123.

Teixeira PJP, Colaianni NR, Fitzpatrick CR, Dangl JL. **2019**. Beyond pathogens: microbiota interactions with the plant immune system. *Current Opinion in Microbiology* **49**: 7–17.

Thébault A, Gillet F, Müller-Schärer H, Buttler A. **2011**. Polyploidy and invasion success: trait trade-offs in native and introduced cytotypes of two Asteraceae species. *Plant Ecology* **212**: 315–325.

Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, Cryan JF, Gilbert SF, Goodnight CJ, Lloyd EA, *et al.* **2016**. Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *mSystems* **1**: e00028-16.

Ton J, Flors V, Mauch-Mani B. **2009**. The multifaceted role of ABA in disease resistance. *Trends in Plant Science* **14**: 310–317.

Trivedi P, Mattupalli C, Eversole K, Leach JE. **2021**. Enabling sustainable agriculture through understanding and enhancement of microbiomes. *New Phytologist* **230**: 2129–2147.

Truernit E, Schmid J, Epple P, Illig J, Sauer N. **1996**. The sink-specific and stress-regulated Arabidopsis STP4 gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge. *The Plant Cell* **8**: 2169–2182.

Tukey HB. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

UniProt Consortium. **2019**. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research* **47**: D506–D515.

Valeri MC, Novi G, Weits DA, Mensuali A, Perata P, Loreti E. **2021**. *Botrytis cinerea* induces local hypoxia in Arabidopsis leaves. *New Phytologist* **229**: 173–185.

Van de Peer Y, Mizrachi E, Marchal K. **2017**. The evolutionary significance of polyploidy. *Nature Reviews Genetics* **18**: 411–424.

Vannier N, Agler M, Hacquard S. **2019**. Microbiota-mediated disease resistance in plants (C Zipfel, Ed.). *PLOS Pathogens* **15**: e1007740.

Violle C, Nemergut DR, Pu Z, Jiang L. **2011**. Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters* **14**: 782–787.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New Phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. **2014**. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative. *Ecology Letters* **17**: 717–726.

Wang L, Cao S, Wang P, Lu K, Song Q, Zhao F-J, Chen ZJ. **2021**. DNA hypomethylation in tetraploid rice potentiates stress-responsive gene expression for salt tolerance. *Proceedings of the National Academy of Sciences* **118**: e2023981118.

Wang W, He Y, Cao Z, Deng Z. **2018**. Induction of Tetraploids in Impatiens (Impatiens walleriana) and Characterization of Their Changes in Morphology and Resistance to Downy Mildew. *HortScience* **53**: 925–931.

Watanabe N, Lam E. **2011**. Arabidopsis metacaspase 2d is a positive mediator of cell death induced during biotic and abiotic stresses. *The Plant Journal: For Cell and Molecular Biology* **66**: 969–982.

Wei Z, Gu Y, Friman V-P, Kowalchuk GA, Xu Y, Shen Q, Jousset A. **2019**. Initial soil microbiome composition and functioning predetermine future plant health. *Science Advances*.

Wernegreen JJ. **2012**. Mutualism meltdown in insects: bacteria constrain thermal adaptation. *Current Opinion in Microbiology* **15**: 255–262.

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

Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim Y-M, Zink EM, Engbrecht KM, Wang Y, *et al.* 2018. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences*: 201717308.

Yasuda M, Ishikawa A, Jikumaru Y, Seki M, Umezawa T, Asami T, Maruyama-Nakashita A, Kudo T, Shinozaki K, Yoshida S, *et al.* 2008. Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. *The Plant Cell* **20**: 1678–1692.

Ye Y, Doak TG. **2009**. A Parsimony Approach to Biological Pathway Reconstruction/Inference for Genomes and Metagenomes. *PLOS Computational Biology* **5**: e1000465.

Yoshida T, Christmann A, Yamaguchi-Shinozaki K, Grill E, Fernie AR. **2019**. Revisiting the Basal Role of ABA – Roles Outside of Stress. *Trends in Plant Science* **24**: 625–635.

Zhang X-Y, Hu C-G, Yao J-L. **2010**. Tetraploidization of diploid Dioscorea results in activation of the antioxidant defense system and increased heat tolerance. *Journal of Plant Physiology* **167**: 88–94.

Zilber-Rosenberg I, Rosenberg E. **2008**. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–735.

Bibliography

Abadi VAJM, Sepehri M, Rahmani HA, Zarei M, Ronaghi A, Taghavi SM, Shamshiripour M. **2020**. Role of Dominant Phyllosphere Bacteria with Plant Growth–Promoting Characteristics on Growth and Nutrition of Maize (Zea mays L.). *Journal of Soil Science and Plant Nutrition* **20**: 2348–2363.

Abawi GS, Widmer TL. **2000**. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* **15**: 37–47.

Abdelfattah A, Wisniewski M, Schena L, Tack AJM. Experimental evidence of microbial inheritance in plants and transmission routes from seed to phyllosphere and root. *Environmental Microbiology* **n/a**.

Adamowicz EM, Flynn J, Hunter RC, Harcombe WR. **2018**. Cross-feeding modulates antibiotic tolerance in bacterial communities. *The ISME Journal* **12**: 2723–2735.

Adesemoye AO, Kloepper JW. **2009**. Plant–microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology* **85**: 1–12.

Adesemoye A o., Torbert H a., Kloepper J w. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* **54**: 876–886.

Adler PB, HilleRisLambers J, Levine JM. **2007**. A niche for neutrality. *Ecology Letters* **10**: 95–104.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, Kemen EM. **2016**. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation (MK Waldor, Ed.). *PLOS Biology* **14**: e1002352.

Ai C, Liang G, Sun J, Wang X, He P, Zhou W, He X. **2015**. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. *Soil Biology and Biochemistry* **80**: 70–78.

Alba R. **2005**. Transcriptome and Selected Metabolite Analyses Reveal Multiple Points of Ethylene Control during Tomato Fruit Development. *THE PLANT CELL ONLINE* **17**: 2954–2965.

Amaral DOJ do, Lima MM de A, Resende LV, Silva MV da. **2008**. Differential gene expression, induced by salicylic acid and Fusarium oxysporum f. sp. lycopersici infection, in tomato. *Pesquisa Agropecuária Brasileira* **43**: 1017–1023.

Anderson B, Johnson SD. **2007**. THE GEOGRAPHICAL MOSAIC OF COEVOLUTION IN A PLANT-POLLINATOR MUTUALISM: COEVOLUTION BETWEEN MUTUALISTS. *Evolution* **62**: 220–225.

Armstrong RA. **1976**. Fugitive Species: Experiments with Fungi and Some Theoretical Considerations. *Ecology* **57**: 953–963.

Ashby B, Watkins E, Lourenço J, Gupta S, Foster KR. **2017**. Competing species leave many potential niches unfilled. *Nature Ecology & Evolution* **1**: 1495–1501.

Bacterial Endophyte Colonization and Distribution within Plants. **2017**. *Microorganisms* **5**: E77.

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

Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, Dombrowski N, Münch PC, Spaepen S, Remus-Emsermann M, *et al.* 2015. Functional overlap of the Arabidopsis leaf and root microbiota. *Nature* **528**: 364–369.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. **2006**. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annual Review of Plant Biology* **57**: 233–266.

Balmer A, Pastor V, Gamir J, Flors V, Mauch-Mani B. **2015**. The 'prime-ome': towards a holistic approach to priming. *Trends in Plant Science* **20**: 443–452.

Basak BB, Sarkar B, Naidu R. **2021**. Environmentally safe release of plant available potassium and micronutrients from organically amended rock mineral powder. *Environmental Geochemistry and Health* **43**: 3273–3286.

Baskins S, Bond J, Minor T. **2019**. Unpacking the Growth in Per Capita Availability of Fresh Market Tomatoes. : 17.

Beattie GA. **2011**. Water relations in the interaction of foliar bacterial pathogens with plants. *Annual Review of Phytopathology* **49**: 533–555.

Beneduzi A, Ambrosini A, Passaglia LMP. **2012**. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* **35**: 1044–1051.

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

Berg M, Koskella B. **2018**. Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen. *Current Biology* **28**: 2487-2492.e3.

Bergelson J. **2001**. Evolutionary Dynamics of Plant R-Genes. *Science* **292**: 2281–2285.

Bernays E, Graham M. **1988**. On the Evolution of Host Specificity in Phytophagous Arthropods. *Ecology* **69**: 886–892.

Bertness MD, Callaway R. **1994**. Positive interactions in communities. *Trends in Ecology & Evolution* **9**: 191–193.

Bhattacharyya PN, Jha DK. **2012**. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* **28**: 1327–1350.

Biswas S, McDonald M, Lundberg DS, Dangl JL, Jojic V. **2015**. Learning Microbial Interaction Networks from Metagenomic Count Data. In: Przytycka TM, ed. Research in Computational Molecular Biology. Cham: Springer International Publishing, 32–43.

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. **2014**. A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota (U Hentschel, Ed.). *PLoS Genetics* **10**: e1004283.

Boltz TP, Ferrel J, Bowen KM, Harding KL, Ayres VE, Moritz JS. **2021**. The effect of a dacitic tuff breccia (Azomite) in corn, soybean, and dried distillers grains with solubles–based diets that vary in inorganic phosphate source on pellet mill production rate and pellet quality. *Journal of Applied Poultry Research* **30**: 100147.

Boots M, White A, Best A, Bowers R. **2012**. The importance of who infects whom: the evolution of diversity in host resistance to infectious disease (P Thrall, Ed.). *Ecology Letters* **15**: 1104–1111.

Boots M, White A, Best A, Bowers R. **2014**. How Specificity and Epidemiology Drive the Coevolution of Static Trait Diversity in Hosts and Parasites: Diversity in Hosts and Parasites. *Evolution* **68**: 1594–1606.

Bottini R, Cassán F, Piccoli P. **2004**. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Applied Microbiology and Biotechnology* **65**: 497–503.

Brachi B, Filiault D, Darme P, Le Mentec M, Kerdaffrec E, Rabanal F, Anastasio A, Box M, Duncan S, Morton T, *et al.* **2017**. Plant genes influence microbial hubs that shape beneficial leaf communities.

Brandl MT, Lindow SE. **1998**. Contribution of Indole-3-Acetic Acid Production to the Epiphytic Fitness of Erwinia herbicola. *Applied and Environmental Microbiology* **64**: 3256–3263.

Brandl MT, Quiñones B, Lindow SE. **2001**. Heterogeneous transcription of an indoleacetic acid biosynthetic gene in Erwinia herbicola on plant surfaces. *Proceedings of the National Academy of Sciences* **98**: 3454–3459.

Braun-Kiewnick A, Jacobsen BJ, Sands DC. **2000**. Biological Control of Pseudomonas syringae pv. syringae, the Causal Agent of Basal Kernel Blight of Barley, by Antagonistic Pantoea agglomerans. *Phytopathology®* **90**: 368–375.

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

Burg SP, Burg EA. **1962**. Role of Ethylene in Fruit Ripening. *Plant Physiology* **37**: 179–189.

Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T. **2011**. Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences* **108**: 14288–14293.

Buss LW, Jackson JBC. **1979**. Competitive Networks: Nontransitive Competitive Relationships in Cryptic Coral Reef Environments. *The American Naturalist* **113**: 223–234.

Caddell DF, Deng S, Coleman-Derr D. **2019**. Role of the Plant Root Microbiome in Abiotic Stress Tolerance. In: Verma SK, White J James Francis, eds. Seed Endophytes: Biology and Biotechnology. Cham: Springer International Publishing, 273–311.

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

Castrillo G, Teixeira PJPL, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, *et al.* **2017**. Root microbiota drive direct integration of phosphate stress and immunity. *Nature* **543**: 513–518.

Cernava T, Erlacher A, Soh J, Sensen CW, Grube M, Berg G. **2019**. Enterobacteriaceae dominate the core microbiome and contribute to the resistome of arugula (Eruca sativa Mill.). *Microbiome* **7**.

Chaudhari D, Rangappa K, Das A, Layek J, Basavaraj S, Kandpal BK, Shouche Y, Rahi P. **2020**. Pea (Pisum sativum l.) Plant Shapes Its Rhizosphere Microbiome for Nutrient Uptake and Stress Amelioration in Acidic Soils of the North-East Region of India. *Frontiers in Microbiology* **11**: 968.

Cherrad S, Charnay A, Hernandez C, Steva H, Belbahri L, Vacher S. **2018**. Emergence of boscalid-resistant strains of Erysiphe necator in French vineyards. *Microbiological Research* **216**: 79–84.

Chesson P. **1992a**. A Need for Niches?

Chesson P. **1992b**. Multi-species Competition in Variable Environments.

Chesson P. **2000a**. General Theory of Competitive Coexistence in Spatially-Varying Environments. *Theoretical Population Biology* **58**: 211–237.

Chesson P. **2000b**. Mechanisms of Maintenance of Species Diversity. *Annual Review of Ecology and Systematics* **31**: 343–366.

Chinnadurai C, Balachandar D, Sundaram SP. **2009**. Characterization of 1-aminocyclopropane-1-carboxylate deaminase producing methylobacteria from phyllosphere of rice and their role in ethylene regulation. *World Journal of Microbiology and Biotechnology* **25**: 1403–1411.

Compant S, Clément C, Sessitsch A. **2010**. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* **42**: 669–678.

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* **19**: 29–37.

Connell J. **1978**. Diversity in Tropical Rain Forests and Coral Reefs.

Conrath U. **2011**. Molecular aspects of defence priming. *Trends in Plant Science* **16**: 524–531.

Conrath U, Pieterse CMJ, Mauch-Mani B. **2002**. Priming in plant–pathogen interactions. *Trends in Plant Science* **7**: 210–216.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal Community Succession of the Phyllosphere Microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cui Z, Huntley RB, Schultes NP, Steven B, Zeng Q. **2020**. Inoculation of Stigma-Colonizing Microbes to Apple Stigmas Alters Microbiome Structure and Reduces the Occurrence of Fire Blight Disease. *Phytobiomes Journal*: PBIOMES-04-20-0035-R.

Cui Z, Huntley RB, Zeng Q, Steven B. **2021**. Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen Erwinia amylovora. *The ISME Journal* **15**: 318–329.

Cunniffe NJ, Gilligan CA. **2011**. A theoretical framework for biological control of soil-borne plant pathogens: Identifying effective strategies. *Journal of Theoretical Biology* **278**: 32–43.

Dai Z, Liu G, Chen H, Chen C, Wang J, Ai S, Wei D, Li D, Ma B, Tang C, *et al.* 2020. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *The ISME Journal* **14**: 757–770.

Dai Z, Su W, Chen H, Barberán A, Zhao H, Yu M, Yu L, Brookes PC, Schadt CW, Chang SX, *et al.* 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Global Change Biology* **24**: 3452–3461.

Dangl JL, Horvath DM, Staskawicz BJ. **2013**. Pivoting the plant immune system from dissection to deployment. *Science (New York, N.Y.)* **341**: 746–751.

Datnoff LE, Elmer WH, Huber DM. **2007**. *Mineral nutrition and plant disease.* St. Paul: Amer Phytopathological Society.

David AS, Thapa-Magar KB, Menges ES, Searcy CA, Afkhami ME. **2020**. Do plant–microbe interactions support the Stress Gradient Hypothesis? *Ecology* **101**: e03081.

De Vleesschauwer D, Höfte M. **2009**. Chapter 6 Rhizobacteria-Induced Systemic Resistance. In: Advances in Botanical Research. Advances in Botanical Research. Academic Press, 223–281.

De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Métraux J-P, Van Loon LC, Dicke M, *et al.* 2005. Signal Signature and Transcriptome Changes of *Arabidopsis* During Pathogen and Insect Attack. *Molecular Plant-Microbe Interactions* **18**: 923–937.

Deng S, Wipf HM-L, Pierroz G, Raab TK, Khanna R, Coleman-Derr D. **2019**. A Plant Growth-Promoting Microbial Soil Amendment Dynamically Alters the Strawberry Root Bacterial Microbiome. *Scientific Reports* **9**: 17677.

Dey R, Pal KK, Bhatt DM, Chauhan SM. **2004**. Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research* **159**: 371–394.

Dulla GFJ, Krasileva KV, Lindow SE. **2010**. Interference of quorum sensing in Pseudomonas syringae by bacterial epiphytes that limit iron availability: Interference of quorum sensing in Pseudomonas syringae. *Environmental Microbiology* **12**: 1762–1774.

Durán P. Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. : 26.

Edwards JA, Santos-Medellín CM, Liechty ZS, Nguyen B, Lurie E, Eason S, Phillips G, Sundaresan V. **2018**. Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice (J Gore, Ed.). *PLOS Biology* **16**: e2003862.

Ehrlich PR, Raven PH. **1964**. Butterflies and Plants: A Study in Coevolution. : 24.

Eisa AI, Taha MB, Abdalla AM. **2011**. AMENDMENT OF SOIL FERTILITY AND AUGMENTATION OF THE QUANTITY AND QUALITY OF SOYBEAN CROP BY USING PHOSPHORUS AND MICRONUTRIENTS. *INTERNATIONAL JOURNAL Of ACADEMIC RESEARCH* **3**: 10.

Elser J, Bennett E. **2011**. A broken biogeochemical cycle. *Nature* **478**: 29–31.

Enya J, Shinohara H, Yoshida S, Tsukiboshi T, Negishi H, Suyama K, Tsushima S. **2007**. Culturable Leaf-Associated Bacteria on Tomato Plants and Their Potential as Biological Control Agents. *Microbial Ecology* **53**: 524–536.

Esİtken A, Ercİslİ S, Karlİdag H, Sahİn F. **2005**. Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. *Proceedings of the international scientific conference: Environmentally friendly fruit growing, Polli, Estonia, 7-9 September, 2005*: 90–97.

Etesami H, Emami S, Alikhani HA. **2017**. Potassium solubilizing bacteria (KSB):: Mechanisms, promotion of plant growth, and future prospects A review. *Journal of soil science and plant nutrition* **17**: 897–911.

Evans S, Martiny JBH, Allison SD. **2017**. Effects of dispersal and selection on stochastic assembly in microbial communities. *The ISME Journal* **11**: 176–185.

Fatima U, Senthil-Kumar M. **2015**. Plant and pathogen nutrient acquisition strategies. *Frontiers in Plant Science* **6**.

Fiala V, Glad C, Martin M, Jolivet E, Derridj S. **1990**. Occurrence of soluble carbohydrates on the phylloplane of maize (Zea mays L.): variations in relation to leaf heterogeneity and position on the plant. *New Phytologist* **115**: 609–615.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. **2012**. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME journal* **6**: 1007–1017.

Finkel OM, Castrillo G, Herrera Paredes S, Salas González I, Dangl JL. **2017**. Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology* **38**: 155–163.

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

Flor HH. **1956**. The Complementary Genic Systems in Flax and Flax Rust. : 26.

Foster KR, Bell T. **2012**. Competition, Not Cooperation, Dominates Interactions among Culturable Microbial Species. *Current Biology* **22**: 1845–1850.

Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E. **2011**. Competitive and cooperative metabolic interactions in bacterial communities. *Nature Communications* **2**: 589.

Friman V-P, Hiltunen T, Laakso J, Kaitala V. **2008**. Availability of prey resources drives evolution of predator-prey interaction. *Proceedings of the Royal Society B: Biological Sciences* **275**: 1625–1633.

Fukami T. **2015**. Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. *Annual Review of Ecology, Evolution, and Systematics* **46**: 1–23.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. **2008**. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *The ISME Journal* **2**: 561–570.

Gaby JC, Buckley DH. **2012**. A Comprehensive Evaluation of PCR Primers to Amplify the nifH Gene of Nitrogenase (JL Balcazar, Ed.). *PLoS ONE* **7**: e42149.

Gahan J, Schmalenberger A. 2014. The role of bacteria and mycorrhiza in plant sulfur supply. *Frontiers in Plant Science* **5**: 723.

Galland M, Gamet L, Varoquaux F, Touraine B, Touraine B, Desbrosses G. 2012. The ethylene pathway contributes to root hair elongation induced by the beneficial bacteria Phyllobacterium brassicacearum STM196. *Plant Science* **190**: 74–81.

Garbeva P, van Veen JA, van Elsas JD. 2004. MICROBIAL DIVERSITY IN SOIL: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology* **42**: 243–270.

Garcia K, Doidy J, Zimmermann SD, Wipf D, Courty P-E. 2016. Take a Trip Through the Plant and Fungal Transportome of Mycorrhiza. *Trends in Plant Science* **21**: 937–950.

García-Fraile P, Menéndez E, Rivas R, García-Fraile P, Menéndez E, Rivas R. 2015. Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioengineering* **2**: 183–205.

Gill US, Lee S, Mysore KS. 2015. Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals. *Phytopathology* **105**: 580–587.

Glick BR, Bashan Y. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. *Biotechnology Advances* **15**: 353–378.

Goh C-H, Veliz Vallejos DF, Nicotra AB, Mathesius U. 2013. The Impact of Beneficial Plant-Associated Microbes on Plant Phenotypic Plasticity. *Journal of Chemical Ecology* **39**: 826–839.

Goldford JE, Lu N, Bajic D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segre D, Mehta P, Sanchez A. 2017. Emergent Simplicity in Microbial Community Assembly.

Grinnell J. 1917. The Niche-Relationships of the California Thrasher. *The Auk* **34**: 427–433.

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KWT, Vitousek PM, Zhang FS. 2010. Significant Acidification in Major Chinese Croplands. *Science* **327**: 1008–1010.

Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, *et al.* 2015. Microbiota and Host Nutrition across Plant and Animal Kingdoms. *Cell Host & Microbe* **17**: 603–616.

Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P. 2017. Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology* **55**: 565–589.

Hajishengallis G, Darveau RP, Curtis MA. 2012. The keystone-pathogen hypothesis. *Nature Reviews Microbiology* **10**: 717–725.

Hassan JA, Zhou YJ, Lewis JD. 2017. A Rapid Seedling Resistance Assay Identifies Wild Tomato Lines That Are Resistant to *Pseudomonas syringae* pv. *tomato* Race 1. *Molecular Plant-Microbe Interactions* **30**: 701–709.

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

Heil M, Hilpert A, Kaiser W, Linsenmair KE. **2000**. Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? *Journal of Ecology* **88**: 645–654.

Helfrich EJN, Vogel CM, Ueoka R, Schäfer M, Ryffel F, Müller DB, Probst S, Kreuzer M, Piel J, Vorholt JA. **2018**. Bipartite interactions, antibiotic production and biosynthetic potential of the Arabidopsis leaf microbiome. *Nature Microbiology* **3**: 909–919.

Henry PM, Gebben SJ, Tech JJ, Yip JL, Leveau JHJ. **2016**. Inhibition of Xanthomonas fragariae, Causative Agent of Angular Leaf Spot of Strawberry, through Iron Deprivation. *Frontiers in Microbiology* **7**.

Herrera Paredes S, Gao T, Law TF, Finkel OM, Mucyn T, Teixeira PJPL, Salas González I, Feltcher ME, Powers MJ, Shank EA, *et al.* **2018**. Design of synthetic bacterial communities for predictable plant phenotypes (E Kemen, Ed.). *PLOS Biology* **16**: e2003962.

Hestrin R, Hammer EC, Mueller CW, Lehmann J. **2019**. Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. *Communications Biology* **2**: 1–9.

Heuvelink E. **2018**. *Tomatoes, 2nd Edition*. CABI.

Hinze M, Köhl L, Kunz S, Weißhaupt S, Ernst M, Schmid A, Voegele RT. **2016**. Real-time PCR detection of Erwinia amylovora on blossoms correlates with subsequent fire blight incidence. *Plant Pathology* **65**: 462–469.

Hirano SS, Nordheim EV, Arny DC, Upper CD. **1982**. Lognormal Distribution of Epiphytic Bacterial Populations on Leaf Surfaces. **44**: 6.

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell RJ, *et al.* **2016**. Root Endophyte Colletotrichum tofieldiae Confers Plant Fitness Benefits that Are Phosphate Status Dependent. *Cell* **165**: 464–474.

Hood ME. **2003**. Dynamics of Multiple Infection and Within-Host Competition by the Anther-Smut Pathogen. *The American Naturalist* **162**: 122–133.

Howden SM, Soussana J-F, Tubiello FN, Chhetri N, Dunlop M, Meinke H. **2007**. Adapting agriculture to climate change. *Proceedings of the National Academy of Sciences* **104**: 19691–19696.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der Heijden MGA, *et al.* **2018**. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* **9**.

Hu J, Wei Z, Friman V-P, Gu S, Wang X, Eisenhauer N, Yang T, Ma J, Shen Q, Xu Y, *et al.* 2016. Probiotic Diversity Enhances Rhizosphere Microbiome Function and Plant Disease Suppression. *mBio* **7**: e01790-16.

Hu J, Wei Z, Weidner S, Friman V-P, Xu Y-C, Shen Q-R, Jousset A. **2017**. Probiotic Pseudomonas communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. *Soil Biology and Biochemistry* **113**: 122–129.

Hubbell SP. **2005**. Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology* **19**: 166–172.

Hurlbert SH. **1984**. Pseudoreplication and the Design of Ecological Field Experiments. *Ecological Monographs* **54**: 187–211.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System. *Applied and Environmental Microbiology* **77**: 3202–3210.

Jacob C, Panchal S, Melotto M. **2017**. Surface Inoculation and Quantification of Pseudomonas syringae Population in the Arabidopsis Leaf Apoplast. *BIO-PROTOCOL* **7**.

Jacobs JL, Carroll TL, Sundin GW. **2005**. The role of pigmentation, ultraviolet radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. *Microbial Ecology* **49**: 104–113.

Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S. **2017**. The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Frontiers in Plant Science* **8**.

Janz N. **2011**. Ehrlich and Raven Revisited: Mechanisms Underlying Codiversification of Plants and Enemies. *Annual Review of Ecology, Evolution, and Systematics* **42**: 71–89.

Janzen DH. **1970**. Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist* **104**: 501–528.

Janzen DH. **1980**. When is it Coevolution?

du Jardin P. **2015**. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* **196**: 3–14.

Ji P, Wilson M. **2002**. Assessment of the Importance of Similarity in Carbon Source Utilization Profiles between the Biological Control Agent and the Pathogen in Biological Control of Bacterial Speck of Tomato. *Applied and Environmental Microbiology* **68**: 4383–4389.

Jones JDG, Dangl JL. **2006**. The plant immune system. *Nature* **444**: 323–329.

K DA, Sabarinathan KG, Gomathy M, Kannan R, Balachandar D. **2020**. Mitigation of drought stress in rice crop with plant growth-promoting abiotic stress-tolerant rice phyllosphere bacteria. *Journal of Basic Microbiology* **60**: 768–786.

Kamel L, Keller-Pearson M, Roux C, Ané J-M. **2017**. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist* **213**: 531–536.

Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B. **2005**. Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology* **7**: 1809–1817.

Kanehisa M. **2019**. Toward understanding the origin and evolution of cellular organisms. *Protein Science: A Publication of the Protein Society* **28**: 1947–1951.

Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. **2021**. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Research* **49**: D545–D551.

Kanehisa M, Goto S. **2000**. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* **28**: 27–30.

Karlova R, Rosin FM, Busscher-Lange J, Parapunova V, Do PT, Fernie AR, Fraser PD, Baxter C, Angenent GC, de Maagd RA. **2011**. Transcriptome and Metabolite Profiling Show That APETALA2a Is a Major Regulator of Tomato Fruit Ripening. *The Plant Cell* **23**: 923–941.

Kearns LP, Hale CN. **1995**. Incidence of bacteria inhibitory to Erwinia amylovora from blossoms in New Zealand apple orchards. *Plant Pathology* **44**: 918–924.

KeÇpczyński J, KeÇpczyńska E. **1997**. Ethylene in seed dormancy and germination. *Physiologia Plantarum* **101**: 720–726.

Khare A, Tavazoie S. **2015**. Multifactorial Competition and Resistance in a Two-Species Bacterial System. *PLOS Genetics* **11**: e1005715.

KINKEL LL, LINDOW SE. **1993**. Invasion and Exclusion among Coexisting Pseudomonas syringae Strains on Leaves. *APPL. ENvIRON. MICROBIOL.* **59**: 8.

Kloepper JW, Leong J, Teintze M, Schroth MN. **1980**. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**: 885–886.

Kneitel JM, Chase JM. **2004**. Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecology Letters* **7**: 69–80.

Knief C, Frances L, Cantet F, Vorholt JA. **2008**. Cultivation-Independent Characterization of Methylobacterium Populations in the Plant Phyllosphere by Automated Ribosomal Intergenic Spacer Analysis. *Applied and Environmental Microbiology* **74**: 2218–2228.

Knief C, Ramette A, Frances L, Alonso-Blanco C, Vorholt JA. **2010**. Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. *The ISME Journal* **4**: 719–728.

Korenblum E, Dong Y, Szymanski J, Panda S, Jozwiak A, Massalha H, Meir S, Rogachev I, Aharoni A. **2020**. Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proceedings of the National Academy of Sciences* **117**: 3874–3883.

Kosina SM, Danielewicz MA, Mohammed M, Ray J, Suh Y, Yilmaz S, Singh AK, Arkin AP, Deutschbauer AM, Northen TR. **2016**. Exometabolomics Assisted Design and Validation of Synthetic Obligate Mutualism. *ACS Synthetic Biology* **5**: 569–576.

Koskella B, Giraud T, Hood ME. **2006**. Pathogen Relatedness Affects the Prevalence of Within-Host Competition. *The American Naturalist* **168**: 121–126.

Kozik EU, Sobiczewski P. ASSESSMENT OF INOCULATION TECHNIQUES SUITABILITY FOR DETERMINATION OF TOMATO PLANTS RESISTANCE TO BACTERIAL SPECK (PSEUDOMONAS SYRINGAE PV. TOMATO). : 10.

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM. **2015**. Community assembly, coexistence and the environmental filtering metaphor (J Fox, Ed.). *Functional Ecology* **29**: 592–599.

Krashevska V, Sandmann D, Maraun M, Scheu S. **2014**. Moderate changes in nutrient input alter tropical microbial and protist communities and belowground linkages. *The ISME Journal* **8**: 1126–1134.

Kremer JM, Paasch BC, Rhodes D, Thireault C, Froehlich JE, Schulze-Lefert P, Tiedje JM, He SY. **2018**. FlowPot axenic plant growth system for microbiota research.

Kurkcuoglu S, Degenhardt J, Lensing J, Al-Masri AN, Gau AE. **2006**. Identification of differentially expressed genes in Malus domestica after application of the non-pathogenic bacterium Pseudomonas fluorescens Bk3 to the phyllosphere. *Journal of Experimental Botany* **58**: 733–741.

Kwak M-J, Kong HG, Choi K, Kwon S-K, Song JY, Lee J, Lee PA, Choi SY, Seo M, Lee HJ, *et al.* 2018. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology* **36**: 1100–1109.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, *et al.* 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* **31**: 814–821.

Layeghifard M, Hwang DM, Guttman DS. **2017**. Disentangling Interactions in the Microbiome: A Network Perspective. *Trends in Microbiology* **25**: 217–228.

Leach JE, Triplett LR, Argueso CT, Trivedi P. **2017**. Communication in the Phytobiome. *Cell* **169**: 587–596.

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Glavina del Rio T, Jones CD, Tringe SG, *et al.* 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* **349**: 860–864.

Lee K-H, Munusamy M, Kim C-W, Lee H-S, Selvaraj P, Sa T. **2004**. Isolation and Characterization of the IAA Producing Methylotrophic Bacteria from Phyllosphere of Rice Cultivars(Oryza sativa L.). *Korean Journal of Soil Science and Fertilizer* **37**: 235–244.

Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C. **2017**. Plant Communication With Associated Microbiota in the Spermosphere, Rhizosphere and Phyllosphere. In: Advances in Botanical Research. Elsevier, 101–133.

Levine JM, Bascompte J, Adler PB, Allesina S. **2017**. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature* **546**: 56–64.

Li Y, Shipley B, Price JN, Dantas V de L, Tamme R, Westoby M, Siefert A, Schamp BS, Spasojevic MJ, Jung V, *et al.* 2018. Habitat filtering determines the functional niche occupancy of plant communities worldwide (P Vesk, Ed.). *Journal of Ecology* **106**: 1001–1009.

Liddycoat SM, Greenberg BM, Wolyn DJ. **2009**. The effect of plant growth-promoting rhizobacteria on asparagus seedlings and germinating seeds subjected to water stress under greenhouse conditions. *Canadian Journal of Microbiology* **55**: 388–394.

Lind K, Lafer G, Schloffer G, Innerhoffer H, Meister H. **2003**. *Organic Fruit Growing*. CABI.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Lindow SE, Leveau JHJ. **2002**. Phyllosphere microbiology. *Current Opinion in Biotechnology* **13**: 238–243.

Liu X, Sheng H, Jiang S, Yuan Z, Zhang C, Elser JJ. **2016**. Intensification of phosphorus cycling in China since the 1600s. *Proceedings of the National Academy of Sciences* **113**: 2609–2614.

Livingston G, Matias M, Calcagno V, Barbera C, Combe M, Leibold MA, Mouquet N. **2012**. Competition–colonization dynamics in experimental bacterial metacommunities. *Nature Communications* **3**.

Lucy M, Reed E, Glick BR. **2004**. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek* **86**: 1–25.

Lu-Irving P, Harencˇár JG, Sounart H, Welles SR, Swope SM, Baltrus DA, Dlugosch KM. **2019**. Native and Invading Yellow Starthistle (Centaurea solstitialis) Microbiomes Differ in Composition and Diversity of Bacteria. **4**: 16.

Luna E, Bruce TJA, Roberts MR, Flors V, Ton J. **2012**. Next-Generation Systemic Acquired Resistance. *PLANT PHYSIOLOGY* **158**: 844–853.

MacArthur R. **1957**. On the Relative Abundance of Bird Species.

Macarthur R, Levins R. **1967**. The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist* **101**: 377–385.

Maignien L, DeForce EA, Chafee ME, Eren AM, Simmons SL. **2014**. Ecological Succession and Stochastic Variation in the Assembly of Arabidopsis thaliana Phyllosphere Communities. *mBio* **5**.

Manzoni S, Trofymow JA, Jackson RB, Porporato A. **2010**. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecological Monographs* **80**: 89–106.

Marschner P, Crowley D, Yang CH. **2004**. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil* **261**: 199–208.

Marschner P, Rengel Z. **2012**. Chapter 12 - Nutrient Availability in Soils. In: Marschner P, ed. Marschner's Mineral Nutrition of Higher Plants (Third Edition). San Diego: Academic Press, 315–330.

Marusina AI, Boulygina ES, Kuznetsov BB, Tourova TP, Kravchenko IK, Gal'chenko VF. **2001**. A System of Oligonucleotide Primers for the Amplification of nifH Genes of Different Taxonomic Groups of Prokaryotes. **70**: 6.

Mauch-Mani B, Baccelli I, Luna E, Flors V. **2017**. Defense Priming: An Adaptive Part of Induced Resistance. *Annual Review of Plant Biology* **68**: 485–512.

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

McMurdie PJ, Holmes S. **2014**. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible (AC McHardy, Ed.). *PLoS Computational Biology* **10**: e1003531.

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.

Mercier J, Lindow SE. **2000**. Role of Leaf Surface Sugars in Colonization of Plants by Bacterial Epiphytes. *Applied and Environmental Microbiology* **66**: 369–374.

Mercier J, Lindow SE. **2001**. Field Performance of Antagonistic Bacteria Identified in a Novel Laboratory Assay for Biological Control of Fire Blight of Pear. *Biological Control* **22**: 66–71.

Metcalf CJE, Henry LP, Rebolleda-Gómez M, Koskella B. **2019**. Why Evolve Reliance on the Microbiome for Timing of Ontogeny? *mBio* **10**: e01496-19.

Mishina Tatiana E., Zeier Jürgen. **2007**. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. *The Plant Journal* **50**: 500–513.

Mitchell PJ, Simpson AJ, Soong R, Schurman JS, Thomas SC, Simpson MJ. **2016**. Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition. *Biogeochemistry* **130**: 227–245.

Monier J-M, Lindow SE. **2004**. Frequency, Size, and Localization of Bacterial Aggregates on Bean Leaf Surfaces. *Applied and Environmental Microbiology* **70**: 346–355.

Monier J-M, Lindow SE. **2005**. Spatial Organization of Dual-Species Bacterial Aggregates on Leaf Surfaces. *Applied and Environmental Microbiology* **71**: 5484–5493.

Moran NA, Munson MA, Baumann P, Ishikawa H. **1993**. A Molecular Clock in Endosymbiotic Bacteria is Calibrated Using the Insect Hosts. *Proceedings: Biological Sciences* **253**: 167–171.

Moroenyane I, Mendes L, Tremblay J, Tripathi B, Yergeau É. **2021**. Plant Compartments and Developmental Stages Modulate the Balance between Niche-Based and Neutral Processes in Soybean Microbiome. *Microbial Ecology*.

Morris BEL, Henneberger R, Huber H, Moissl-Eichinger C. **2013**. Microbial syntrophy: interaction for the common good. *FEMS microbiology reviews* **37**: 384–406.

van de Mortel JE, de Vos RCH, Dekkers E, Pineda A, Guillod L, Bouwmeester K, van Loon JJA, Dicke M, Raaijmakers JM. **2012**. Metabolic and Transcriptomic Changes Induced in Arabidopsis by the Rhizobacterium Pseudomonas fluorescens SS101. *PLANT PHYSIOLOGY* **160**: 2173–2188.

Muller R, Morant M, Jarmer H, Nilsson L, Nielsen TH. **2006**. Genome-Wide Analysis of the Arabidopsis Leaf Transcriptome Reveals Interaction of Phosphate and Sugar Metabolism. *PLANT PHYSIOLOGY* **143**: 156–171.

Musthafa MS, Ali ARJ, Mohamed MJ, Jaleel MMA, Kumar MSA, Rani KU, Vasanth K, Arockiaraj J, Preetham E, Balasundaram C, *et al.* **2016**. Protective efficacy of Azomite enriched diet in Oreochromis mossambicus against Aeromonas hydrophila. *Aquaculture* **451**: 310–315.

Mwajita MR, Murage H, Tani A, Kahangi EM. **2013**. Evaluation of rhizosphere, rhizoplane and phyllosphere bacteria and fungi isolated from rice in Kenya for plant growth promoters. *SpringerPlus* **2**: 606.

Nakano S, Kobayashi T, Funabiki K, Matsumura A, Nagao Y, Yamada T. **2003**. Development of a PCR Assay for Detection of Enterobacteriaceae in Foods. *Journal of Food Protection* **66**: 1798–1804.

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

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

Noorani Azad H, Hassan Poor A, Bakhshikhaniki GR, Ebrahimi MA. **2016**. Physiological responses of two tomato (Lycopersicun esculentum M.) cultivars to Azomite fertilizer under drought stress. *Iranian Journal of Plant Physiology* **6**: 1833–1843.

O'connell PF. **1992**. Sustainable Agriculture-a Valid Alternative. *Outlook on Agriculture* **21**: 5–12.

Ortiz-Álvarez R, Fierer N, de los Ríos A, Casamayor EO, Barberán A. **2018**. Consistent changes in the taxonomic structure and functional attributes of bacterial communities during primary succession. *The ISME Journal*.

Ottesen AR, González Peña A, White JR, Pettengill JB, Li C, Allard S, Rideout S, Allard M, Hill T, Evans P, *et al.* 2013. Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC Microbiology* **13**: 114.

Ottesen AR, Gorham S, Reed E, Newell MJ, Ramachandran P, Canida T, Allard M, Evans P, Brown E, White JR. **2016**. Using a Control to Better Understand Phyllosphere Microbiota (M-J Virolle, Ed.). *PLOS ONE* **11**: e0163482.

Paradis E, Claude J, Strimmer K. **2004**. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**: 289–290.

Pascale A, Proietti S, Pantelides IS, Stringlis IA. **2020**. Modulation of the Root Microbiome by Plant Molecules: The Basis for Targeted Disease Suppression and Plant Growth Promotion. *Frontiers in Plant Science* **10**: 1741.

Pati BR, Chandra AK. **1981**. Effect of spraying nitrogen-fixing phyllospheric bacterial isolates on wheat plants. *Plant and Soil* **61**: 419–427.

Pati BR, Sengupta S, Chandra AK. **1995**. Impact of selected phyllospheric diazotrophs on the growth of wheat seedlings and assay of the growth substances produced by the diazotrophs. *Microbiological Research* **150**: 121–127.

Paul D, Nair S. **2008**. Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *Journal of Basic Microbiology* **48**: 378–384.

Pauw A, Stofberg J, Waterman RJ. **2009**. FLIES AND FLOWERS IN DARWIN'S RACE. *Evolution* **63**: 268–279.

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.

Peñuelas J, Terradas J. **2014**. The foliar microbiome. *Trends in Plant Science* **19**: 278–280.

Peredo EL, Simmons SL. **2018**. Leaf-FISH: Microscale Imaging of Bacterial Taxa on Phyllosphere. *Frontiers in Microbiology* **8**.

Pieterse CM, van Wees SC, van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, van Loon LC. **1998**. A novel signaling pathway controlling induced systemic resistance in Arabidopsis. *The Plant Cell* **10**: 1571–1580.

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.

Potvin C, Tardif S. **1988**. Sources of Variability and Experimental Designs in Growth Chambers. *Functional Ecology* **2**: 123.

Poudel R, Jumpponen A, Schlatter DC, Paulitz TC, Gardener BBM, Kinkel LL, Garrett KA. **2016**. Microbiome Networks: A Systems Framework for Identifying Candidate Microbial Assemblages for Disease Management. *Phytopathology* **106**: 1083–1096.

Preuss SB, Meister R, Xu Q, Urwin CP, Tripodi FA, Screen SE, Anil VS, Zhu S, Morrell JA, Liu G, *et al.* **2012**. Expression of the Arabidopsis thaliana BBX32 gene in soybean increases grain yield. *PloS One* **7**: e30717.

Pusey PL, Stockwell VO, Reardon CL, Smits THM, Duffy B. **2011**. Antibiosis activity of Pantoea agglomerans biocontrol strain E325 against Erwinia amylovora on apple flower stigmas. *Phytopathology* **101**: 1234–1241.

Qin C, Tao J, Liu T, Liu Y, Xiao N, Li T, Gu Y, Yin H, Meng D. **2019**. Responses of phyllosphere microbiota and plant health to application of two different biocontrol agents. *AMB Express* **9**: 42.

Ramirez KS, Lauber CL, Knight R, Bradford MA, Fierer N. **2010**. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* **91**: 3463–3470.

Rastogi G, Coaker GL, Leveau JHJ. **2013**. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Rastogi G, Sbodio A, Tech JJ, Suslow TV, Coaker GL, Leveau JHJ. **2012**. Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. *The ISME Journal* **6**: 1812–1822.

Ratzke C, Barrere J, Gore J. **2019**. Strength of species interactions determines biodiversity and stability in microbial communities. *bioRxiv*: 671008.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves: Biogeography of phyllosphere bacterial communities. *Environmental Microbiology* **12**: 2885–2893.

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, *et al.* 2008. Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (Phaseolus vulgaris L.). *Plant and Soil* **302**: 149–161.

Remus-Emsermann MNP, Lücker S, Müller DB, Potthoff E, Daims H, Vorholt JA. **2014**. Spatial distribution analyses of natural phyllosphere-colonizing bacteria on Arabidopsis thaliana revealed by fluorescence in situ hybridization: Bacterial distribution on Arabidopsis phylloplanes. *Environmental Microbiology* **16**: 2329–2340.

Remus-Emsermann MNP, Schlechter RO. **2018**. Phyllosphere microbiology: at the interface between microbial individuals and the plant host. *New Phytologist* **218**: 1327–1333.

Remus-Emsermann MNP, Tecon R, Kowalchuk GA, Leveau JHJ. **2012**. Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere. *The ISME Journal* **6**: 756–765.

Rengel Z. **1997**. Root exudation and microflora populations in rhizosphere of crop genotypes differing in tolerance to micronutrient deficiency. In: Ando T, Fujita K, Mae T, Matsumoto H, Mori S, Sekiya J, eds. Developments in Plant and Soil Sciences. Plant Nutrition for Sustainable Food Production and Environment: Proceedings of the XIII International Plant Nutrition Colloquium, 13–19 September 1997, Tokyo, Japan. Dordrecht: Springer Netherlands, 243–248.

Robertson GP, Vitousek PM. **2009**. Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. *Annual Review of Environment and Resources* **34**: 97–125.

Rodríguez H, Fraga R. **1999**. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* **17**: 319–339.

RUINEN J. **1965**. THE PHYLLOSPHERE: III. NITROGEN FIXATION IN THE PHYLLOSPHERE. *Plant and Soil* **22**: 375–394.

Russel J, Røder HL, Madsen JS, Burmølle M, Sørensen SJ. **2017**. Antagonism correlates with metabolic similarity in diverse bacteria. *Proceedings of the National Academy of Sciences* **114**: 10684–10688.

Saavedra S, Rohr RP, Bascompte J, Godoy O, Kraft NJB, Levine JM. **2017**. A structural approach for understanding multispecies coexistence. *Ecological Monographs* **87**: 470–486.

Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N. **2014**. Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. *Protoplasma* **251**: 511–523.

Sainju UM, Dris R, Singh B. Mineral nutrition of tomato. : 9.

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

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

Schlechter RO, Miebach M, Remus-Emsermann MNP. **2019**. Driving factors of epiphytic bacterial communities: A review. *Journal of Advanced Research*: S2090123219300694.

Selosse M-A, Bessis A, Pozo MJ. **2014**. Microbial priming of plant and animal immunity: symbionts as developmental signals. *Trends in Microbiology* **22**: 607–613.

Shade A, McManus PS, Handelsman J. **2013**. Unexpected Diversity during Community Succession in the Apple Flower Microbiome. *mBio* **4**.

Shea K. **2002**. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* **17**: 170–176.

Shoji S, Takahashi T. Environmental and Agricultural Significance of Volcanic Ash Soils. : 24.

Siddiqui S, Alamri SA, Alrumman SA, Meghvansi MK, Chaudhary KK, Kilany M, Prasad K. **2015**. Role of Soil Amendment with Micronutrients in Suppression of Certain Soilborne Plant Fungal Diseases: A Review. In: Meghvansi MK, Varma A, eds. Soil Biology. Organic Amendments and Soil Suppressiveness in Plant Disease Management. Cham: Springer International Publishing, 363–380.

Siepielski AM, McPeek MA. **2010**. On the evidence for species coexistence: a critique of the coexistence program. *Ecology* **91**: 3153–3164.

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

Simon A. Levin. **1970**. Community Equilibria and Stability, and an Extension of the Competitive Exclusion Principle. *The American Naturalist* **104**: 413–423.

Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B. **2012**. Descendants of Primed Arabidopsis Plants Exhibit Resistance to Biotic Stress. *PLANT PHYSIOLOGY* **158**: 835–843.

Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP. **2006**. Quantifying the roles of immigration and chance in shaping prokaryote community structure. *Environmental Microbiology* **8**: 732–740.

Sloan DB, Warren JM, Williams AM, Wu Z, Abdel-Ghany SE, Chicco AJ, Havird JC. **2018**. Cytonuclear integration and co-evolution. *Nature Reviews Genetics* **19**: 635–648.

Sloan WT, Woodcock S, Lunn M, Head IM, Curtis TP. **2007**. Modeling Taxa-Abundance Distributions in Microbial Communities using Environmental Sequence Data. *Microbial Ecology* **53**: 443–455.

Song GC, Choi HK, Kim YS, Choi JS, Ryu C-M. **2017**. Seed defense biopriming with bacterial cyclodipeptides triggers immunity in cucumber and pepper. *Scientific Reports* **7**.

Spinelli F, Ciampolini F, Cresti M, Geider K, Costa G. **2005**. Influence of Stigmatic Morphology on Flower Colonization by Erwinia amylovora and Pantoea agglomerans. *European Journal of Plant Pathology* **113**: 395–405.

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. **1999**. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. *Nature* **400**: 667–671.

Stone BWG, Weingarten EA, Jackson CR. **2018**. The Role of the Phyllosphere Microbiome in Plant Health and Function. In: Annual Plant Reviews online. American Cancer Society, 533–556.

Stubbendieck RM, Vargas-Bautista C, Straight PD. **2016**. Bacterial Communities: Interactions to Scale. *Frontiers in Microbiology* **7**.

Tanaka T, Kawasaki K, Daimon S, Kitagawa W, Yamamoto K, Tamaki H, Tanaka M, Nakatsu CH, Kamagata Y. **2014**. A Hidden Pitfall in the Preparation of Agar Media Undermines Microorganism Cultivability. *Applied and Environmental Microbiology* **80**: 7659–7666.

Teale WD, Paponov IA, Palme K. **2006**. Auxin in action: signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology* **7**: 847–859.

Tejera N, Lluch C, Martìnez-Toledo MV, Gonzàlez-López J. **2005**. Isolation and characterization of Azotobacter and Azospirillum strains from the sugarcane rhizosphere. *Plant and Soil* **270**: 223–232.

Tenenbaum D. **2021**. KEGGREST: Client-side REST access to the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Tilman D. **2004**. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences* **101**: 10854–10861.

Tilman D, Balzer C, Hill J, Befort BL. **2011**. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences* **108**: 20260–20264.

Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, Fukuda S, Ushio M, Nakaoka S, Onoda Y, *et al.* 2018. Core microbiomes for sustainable agroecosystems. *Nature Plants* **4**: 247–257.

Trivedi P, Delgado-Baquerizo M, Jeffries TC, Trivedi C, Anderson IC, Lai K, McNee M, Flower K, Singh BP, Minkey D, *et al.* 2017. Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. *Environmental Microbiology* **19**: 3070–3086.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. **2020**. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* **18**: 607–621.

Tsuchiya Y, Nishio N, Nagai S. **1980**. Medium optimization for a methanol utilizing bacterium based on chemostat theory. *European Journal of Applied Microbiology and Biotechnology* **9**: 121–127.

Tukey HB. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, *et al.* 2009. A core gut microbiome in obese and lean twins. *Nature* **457**: 480–484.

Turner TR, James EK, Poole PS. **2013**. The plant microbiome. *Genome Biology* **14**.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016**. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Van Der Heijden MGA, Bardgett RD, Van Straalen NM. **2008**. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Venturelli OS, Carr AC, Fisher G, Hsu RH, Lau R, Bowen BP, Hromada S, Northen T, Arkin AP. **2018**. Deciphering microbial interactions in synthetic human gut microbiome communities. *Molecular Systems Biology* **14**: e8157.

Verbon EH, Liberman LM. **2016**. Beneficial Microbes Affect Endogenous Mechanisms Controlling Root Development. *Trends in Plant Science* **21**: 218–229.

Verster KI, Wisecaver JH, Duncan RP, Karageorgi M, Gloss AD, Armstrong E, Price DK, Menon AR, Ali ZM, Whiteman NK. **2019**. Horizontal transfer of prokaryotic cytolethal distending toxin B genes to eukaryotes. *bioRxiv*.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New Phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

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.

Wade MJ. **2007**. The co-evolutionary genetics of ecological communities. *Nature Reviews Genetics* **8**: 185–195.

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

Wakelin SA, Macdonald LM, Rogers SL, Gregg AL, Bolger TP, Baldock JA. **2008**. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. *Soil Biology and Biochemistry* **40**: 803–813.

Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, González-Peña A, Peiffer J, Koren O, Shi Q, *et al.* 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of Sciences* **115**: 7368–7373.

Warner RR, Chesson PL. **1985**. Coexistence Mediated by Recruitment Fluctuations: A Field Guide to the Storage Effect. *The American Naturalist* **125**: 769–787.

Wei Z, Yang T, Friman V-P, Xu Y, Shen Q, Jousset A. **2015**. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nature Communications* **6**.

Weibull J, Ronquist F, Brishammar S. **1990**. Free Amino Acid Composition of Leaf Exudates and Phloem Sap : A Comparative Study in Oats and Barley. *PLANT PHYSIOLOGY* **92**: 222–226.

Williams TR, Marco ML. **2014**. Phyllosphere Microbiota Composition and Microbial Community Transplantation on Lettuce Plants Grown Indoors. *mBio* **5**.

WILSON M, LINDOW SE. **2017**. Coexistence among Epiphytic Bacterial Populations Mediated through Nutritional Resource Partitioning. *APPL. ENVIRON. MICROBIOL.* **60**: 10.

Wise MJ, Rausher MD. **2013**. EVOLUTION OF RESISTANCE TO A MULTIPLE-HERBIVORE COMMUNITY: GENETIC CORRELATIONS, DIFFUSE COEVOLUTION, AND CONSTRAINTS ON THE PLANT'S RESPONSE TO SELECTION: MULTIPLE HERBIVORES CONSTRAIN HOST-PLANT EVOLUTION. *Evolution* **67**: 1767–1779.

Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, Taylor JE, Paul ND, Roberts MR. **2012**. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytologist* **193**: 770–778.

Wright JS. **2002**. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**: 1–14.

Xin X-F, Kvitko B, He SY. **2018**. Pseudomonas syringae: what it takes to be a pathogen. *Nature Reviews Microbiology* **16**: 316–328.

Xin X-F, Nomura K, Aung K, Velásquez AC, Yao J, Boutrot F, Chang JH, Zipfel C, He SY. **2016**. Bacteria establish an aqueous living space in plants crucial for virulence. *Nature* **539**: 524–529.

Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim Y-M, Zink EM, Engbrecht KM, Wang Y, *et al.* **2018**. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences*: 201717308.

Yang Y-W, Chen M-K, Yang B-Y, Huang X-J, Zhang X-R, He L-Q, Zhang J, Hua Z-C. **2015**. Use of 16S rRNA Gene-Targeted Group-Specific Primers for Real-Time PCR Analysis of Predominant Bacteria in Mouse Feces (MW Griffiths, Ed.). *Applied and Environmental Microbiology* **81**: 6749–6756.

Yang T, Wei Z, Friman V-P, Xu Y, Shen Q, Kowalchuk GA, Jousset A. **2017**. Resource availability modulates biodiversity-invasion relationships by altering competitive interactions: Resource availability modulates biodiversity. *Environmental Microbiology* **19**: 2984–2991.

Yeoh YK, Paungfoo-Lonhienne C, Dennis PG, Robinson N, Ragan MA, Schmidt S, Hugenholtz P. **2016**. The core root microbiome of sugarcanes cultivated under varying nitrogen fertilizer application: N fertilizer and sugarcane root microbiota. *Environmental Microbiology* **18**: 1338–1351.

Yuan Z-S, Liu F, Liu Z-Y, Huang Q-L, Zhang G-F, Pan H. **2021**. Structural variability and differentiation of niches in the rhizosphere and endosphere bacterial microbiome of moso bamboo (Phyllostachys edulis). *Scientific Reports* **11**: 1574.

Zamioudis C, Pieterse CMJ. **2011**. Modulation of Host Immunity by Beneficial Microbes. *Molecular Plant-Microbe Interactions®* **25**: 139–150.

Zaneveld JR, McMinds R, Vega Thurber R. **2017**. Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology* **2**.

Zeng J, Liu X, Song L, Lin X, Zhang H, Shen C, Chu H. **2016**. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry* **92**: 41–49.

Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP, *et al.* 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology* **3**: 470–480.

Zhang J, Gao Q, Zhang Q, Wang T, Yue H, Wu L, Shi J, Qin Z, Zhou J, Zuo J, *et al.* 2017. Bacteriophage–prokaryote dynamics and interaction within anaerobic digestion processes across time and space. *Microbiome* **5**.

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.