Translating plant microbiome research to successful field interventions: deep discovery and difficult deployment

Dor Russ [1,2,#], Connor R. Fitzpatrick[1,2,#], Paulo J. P. L. Teixeira[3,#], Jeffery L. Dangl[1,2,#,*]

[1] Department of Biology, University of North Carolina at Chapel Hill, USA
[2] Howard Hughes Medical Institute, University of North Carolina at Chapel Hill, USA
[3] Department of Biological Sciences, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil.
[#] Contributed equally

* Lead author for correspondence dangl@email.unc.edu

Abstract

Plant-associated microbiota can extend plant immune system function, improve nutrient acquisition and availability, and alleviate abiotic stresses. Thus, naturally beneficial microbial therapeutics are enticing tools to improve plant productivity. Basic definition of plant microbiota across species and ecosystems, combined with the development of reductionist experimental models and manipulation of plant phenotypes with microbes has fueled interest in translation to agriculture. However, the great majority of microbes exhibiting plant productivity traits in the lab and greenhouse fail in the field. Therapeutic microbes must reach détente with the plant immune system, invade heterogeneous pre-established plant-associated communities and persist in a new and potentially re-modeled community. Environmental conditions can alter community structure and thus impact the engraftment of therapeutic microbes. We survey recent breakthroughs, challenges and opportunities in translating beneficial microbes from the lab to the field.

Introduction

Plants host communities of viruses, bacteria, fungi, oomycetes, archaea, and algae in both epiphytic and endophytic habitats, collectively referred to as the plant microbiota[1]. Plant-associated microbiota represent a unique subset of the microbial diversity found in free-living habitats[2–5]. The diversity, composition, and abundance of these microbial communities vary among habitats within the plant microbiome (e.g. leaves versus roots or epiphytic versus endophytic), plant populations and species, and environmental conditions[6]. The importance of plant-microbe interactions for plant physiological, ecological, and evolutionary processes has long been recognized[7]. An explosion of research in the past twenty years (Figure 1A) reveals how plants, microbiota, and the environment shape a complex chemical dialogue that collectively orchestrates the assembly and function of the plant microbiome[2,8].

Interactions with their associated microbiota were required by plants in their migration to the terrestrial environment[9] and continue to drive contemporary plant ecology and evolution[10]. Although the precise mechanisms through which microorganisms influence plant phenotypes are not well understood, numerous studies identified specific microbial species that enhance plant growth by mobilizing nutrients to plant roots, modulating hormonal signaling, producing antibiotics, and engaging in interactions with the plant immune system[1,3,11,12]. As such, plant microbiome research has high translational potential to address urgent global concerns related to food and fiber production in the face of climate change and the growing human population[13–16]. Plant productivity is increasingly compromised in agriculture and silviculture due to the combined effects of climate change[17], soil degradation, and increasing pressure from pathogens, parasites, herbivores, and plant competitors, both introduced and native[18]. Traditional mitigation approaches are accompanied by high monetary, energy, and environmental costs, and exhibit diminishing returns[19]. Deploying individual strains, microbial consortia, or managing existing communities to

enhance or buffer plant productivity are potential interventions due to the microbial potential to modify plant phenotypes and mitigate abiotic and biotic stressors[13,14,20–22].

Using Brazil[23] and the USA as case studies, the commercial use of microbes in agriculture has risen since their introduction in the middle of the last century (Figure 1B). Microbial products are used to inhibit plant pathogens, nematodes, herbivorous insects, and to fortify plant nutrition across a range of environmental conditions. For example, deployment of *Bacillus thuringiensis* strains is widely adopted around the world and is remarkably successful at reducing the negative impacts of herbivorous insects and traditional insecticides[24]. Similarly, products based on nitrogen-fixing bacteria are largely used in the cultivation of legumes, such as soybean[25].

However, relative to the pace of basic plant microbiome research, translation into viable microbial interventions in plant production is lagging. This discrepancy is due, in part, to an incomplete understanding of the processes leading to successful colonization and persistence in the plant microbiome. From high diversity communities in the surrounding environment, microorganisms are either attracted or deterred from plant epiphytic and endophytic habitats due to the unique combination of chemical and physical properties on and surrounding plant root and shoot surfaces. After navigating this novel chemical milieu, a microorganism must then contend with the plant immune system, which can act as both a ‘carrot and a stick’ during colonization depending on the presence of additional molecular signals. Once a microbe reaches epiphytic habitats, it then competes for space and resources with other hopeful microbial colonists on the plant. At this point, unique habitat features governed by plant organ development and cell-type specific immune function also structure the fine-scale biogeography of plant microbiota. Microbial expansion in the plant endophytic habitat requires further détente with the plant immune system and consideration of the host plant organ’s developmental and cell type-specific differentiation stages. Finally, environmental conditions can drastically alter the rules governing successful colonization throughout this process resulting in the fine-tuning of microbiota (Figure 2). Addressing knowledge gaps throughout this process of successful microbial invasion, in addition

to improving the identification and application of plant beneficial microbes will narrow the chasm between basic science effort and translational success.

Progressive spatial winnowing determines habitat-specific community structure

Microbial communities that associate with plants are highly diverse and dynamic systems selected from soil communities that vary across environments, individuals, and time[2–5]. Microbiota intimately associated with plant organs are mainly derived from highly complex soil communities by progressive winnowing. Following the initial high-throughput surveys that characterized microbiota composition across different plant species, tissues, and environments, researchers directed efforts toward unraveling the molecular mechanisms that govern the structures and functions of microbial communities in plants[4,5,26–29]. Microbial diversity progressively decreases from the soil environment to the rhizosphere and further to the root endophytic compartment, reflecting a gradient of decreasing species richness and increasing specialization within the plant microbiome[4,5,30]. During that winnowing, members of the phyla Planctomyces and Acidobacteria, which are highly abundant in the soil, are depleted from the plants, and Proteobacteria and Actinobacteria are highly enriched in root epiphytic and endophytic tissues[4,5]. Similarly, the phyllosphere, which refers to the aerial parts of plants such as leaves, stems, and flowers, generally exhibits lower microbial diversity compared to both the soil and rhizosphere. The limited nutrient availability, fluctuating environmental conditions, and physical barriers posed by the leaf surface contribute to the establishment of a relatively specialized microbial phyllosphere community, consisting of microbes adapted to survive and thrive under these unique conditions[11,31]. Interestingly, although the community composition of above- and below-ground tissues are different[32,33], large similarities are found in the functional capabilities of those communities[28].

The reduction of diversity observed in the plant microbiome relative to the surrounding environment suggests that plants exert selective pressure on microbial communities. Within these communities, beneficial, neutral, and pathogenic members coexist in homeostasis and exert context-dependent effects on plant health and development[34]. Selective pressure arises from the ability to actively recruit and favor certain microbial taxa that are better adapted to colonize and interact with plant tissues. Through complex chemical signals and root exudates, plants create a specific microenvironment that can support the growth of beneficial microbes while deterring or excluding pathogens. In a very specific mutualistic symbiosis, legumes produce specific flavones to attract nitrogen-fixing symbiont Rhizobial strains[35]. Expanding to less specific interactions, the plant hormone strigolactone is secreted from plant roots and promotes the common symbiotic arbuscular mycorrhizal fungi (AMF)[36]. Alternatively, antagonist exudates like benzoxazinoids[37], coumarins[38], and triterpenes[39] can selectively exclude community members; mutant plants compromised in the biosynthetic pathways for those antagonists assemble altered communities. The plant immune system is a major player gating microbes into plant tissues. The reduced diversity in the plant microbiome compared to the surrounding environment thus signifies a finely tuned co-evolutionary process, highlighting the plant's role as an active participant in shaping its microbial partners.

Plant organs provide developmental and immune-gated micro-niches. For example, while the receptor for the flagellin 22 peptide (flg22) immuno-epitope, FLS2, is expressed in all leaf mesophyll cells, its expression is restricted to specific cell types in the root[40]. This restriction is crucial for proper plant development[41]. The plant also partitions metabolite exudation, likely based on developmental and cell type-specific differentiation cues. For example, glucose secretion is higher from the root base than from the root tip[42]. Developmental, immune-restricted, and metabolite-specific micro-niches likely drive variability in localized micro-communities that

colonize the root. Indeed, sampling of the root at a millimeter scale revealed high variability across the bacterial communities that inhabit different patches sampled from the same root[43].

A therapeutic microbe needs to be targeted to the micro niche where its function contributes the most to plant productivity. While it seems obvious that a direct antagonist of a leaf pathogen should be directed to the leaf, and a nitrogen-fixing bacteria should be targeted to roots hairs on the rhizoplane, we remain largely ignorant of how communities form into spatially restricted microcolonies in different plant tissues. There is an urgent need for further refinement to micrometer resolution spatial mapping of strains on these plant organs[44–46] and cell-resolved spatial transcriptomics of both host and community members to learn the rules that will allow deployment of focal strains to specific micro-niches.

Invasion and persistence of therapeutical microbes into existing microbial communities

Resident community members prevent microbial invasion by diverse mechanisms. The winnowing of the soil community, as it approaches the plant tissue, is also associated with increased bacterial density[47]. The increased density and reduced diversity promote strong competition for resources. An invading therapeutic strain will face multiple obstacles when infiltrating such an existing microbial community, from niche availability to direct antagonism by community members to locally distributed phages.

Every habitat offers different resources, and the resident homeostatic microbial community likely exhausts the available niches in that resource space[48]. For instance, root microbiome members exploit the multitude of compounds exuded from the plant to the rhizosphere, and access to the inner cell types of the root is winnowed by these compounds and by root architecture[49]. To infiltrate into an assembled community, an invading microbe can find an available niche by exploiting a previously unused nutrient (Figure 3A). For example, strains of the

genus *Variovorax* are prevalent plant colonizers capable of invading a pre-established community[50]. *Variovorax's* ability to assimilate auxin may open a specialized niche in the root microbiome[51]. Alternatively, an invading bacteria can cooperate with the plant to create a new niche for itself, as in the case of the legume-rhizobium symbiosis[52] or with resident community members to extend their collective nutrient use. *Sphingomonas* and *Rhizobium* strains capture the same ecological niche when colonizing Arabidopsis plants in isolation, but modulate each other's proteome to extend their niches and assimilate non-overlapping carbon sources when co-inoculated[53]. If no niche is available for the invading microbe, it may deploy molecular tools to create one by attacking a competing strain to open a niche (Figure 3B).

Many microbes produce antagonistic agents to extend their niche and improve fitness in a diverse microbial community[54]**.** The antimicrobial agents produced in microbial warfare are the source of most known commercial antibiotics[55]. Members of both the root[56] and the shoot[57] microbiome produce antimicrobial compounds (Figure 3B). While the specific compounds that mediate microbe-microbe interactions in the phyllosphere are mostly unknown, a recent study found that Non-Ribosomal Peptide production is enriched among antimicrobial producers in the root. Specifically, the iron chelator pyoverdine and the antimicrobial 2,4-diacetylphloroglucinol (DAPG) were found to explain the majority of inhibitory interactions of the root colonizer *Pseudomonas brassicacearum*[56]. While stable natural communities are composed of both resistant and sensitive strains in addition to the producing strain, antagonistic compounds have an essential role in shaping the plant microbiome[58,59]. The plethora of antimicrobials produced by any homeostatic plant-associated microbial community can be seen as a chemical barrier that protects the community from the invasion of new strains[60]. Since the diversity of the plant microbiome is high, and even the same crop presents different but overlapping microbiomes across its taxonomic core through time and space, it is a great challenge to tailor a bacterial therapy that will be able to invade any community at every location.

Phages are abundant in natural microbial communities and can play an important role in community assembly[61]. Phages can limit the growth of highly abundant species according to the "kill the winner" hypothesis[62], and alter bacteria-bacteria competition and bacterial evolution[63]. Members of a local community can escape phage-derived killing by either resistance or spatial separation. However, new immigrant bacteria can be rapidly attacked by local phages (Figure 3B). Interestingly, the plant environment adds a constraint to the evolution of phage resistance. The evolutionary trajectory of phage resistance in planta is different from evolution in rich media[64]. Additionally, potassium availability limits phage evolution in planta[65]. The diverse mechanisms by which stable natural communities prevent the invasion of new species is a major hurdle for the development of bacterial therapeutics and further investigation is required to develop novel approaches for improving and delivering the right treatment.

Microbes take different avenues to invade an existing community. The conflict between the natural community and the invading species is not unilateral, and an invading strain can create a niche by attacking members of the preexisting microbial community. Inhibition of closely related bacteria can open a new niche for colonization[50]. An invader may deploy bacteriocins that specifically inhibit bacteria that are similar to the producer and benefit host colonization[66,67]. Attacking only related bacteria can open a niche for the invader while minimizing the collateral damage to the community structure. An invader can deploy contact-dependent bacterial secretion systems to focus on nearby bacteria. The type six secretion system (T6SS) is composed of a contractile tail used to inject effectors into neighboring bacteria to clear space for colonization. T6SS genes are highly prevalent among proteobacteria and are enriched among plant-associated bacteria where the community is denser[68]. For example, T6SS helps *Pseudomonas chlororaphis* to invade a resident wheat-associated community which improves colonization and persistence in the wheat rhizosphere[69]. In addition to improving its own colonization, the *Pseudomonas putida* T6SS can also inhibit the growth of the phytopathogen *Xanthomonas campestris* in planta and

reduce disease-associated necrosis on *Nicotiana benthamiana* leaves[70]. Similarly, Type 4b Secretion Systems (T4BSS), can have similar functions. The T4BSS translocates effectors into neighboring cells using specialized pili and may even be more effective than T6SS for bacterial competition. In a competition assay between two strains of *Pseudomonas putida*, the T4BSS-expressing cells kill T6SS-expressing cells, infiltrate into an existing *Arabidopsis* microbial community and inhibit the phytopathogen *Ralstonia solanacearum* to improve plant fitness[71]. These examples highlight the increasing number of defined mechanisms evolved to enhance bacterial invasion. We anticipate more will be discovered as research on invasion and persistence expands.

A foreign, potentially therapeutic, strain trying to colonize a plant must invade the appropriate niche after delivery and contend with an established plant microbial community on the target organ. In the lab, one can use synthetic communities (SynComs) to study and model invasion into natural communities in a controlled system[71–73]. To date, invading simple, less diverse, communities with a focal strain is experimentally tractable, but natural microbial communities are potentially more resilient to invasion than synthetic lab communities[74]. SynComs of increasing diversity that more accurately represent real-world conditions are experimentally difficult to assemble but are required for realistic tests of invasion and persistence. This is an area ripe for the development of in-field monitoring devices of beneficial strains and of AI-mediated development of combinatorial communities that can represent the diversity of plant-associated microbial communities under field conditions.

Maintaining a healthy microbiome

A healthy microbiome is important for host fitness. Community diversity as a whole is an established sign of a healthy microbiome and consequent host fitness[75]. Dysbiosis, an imbalanced microbiome that has negative effects on the host, can result from the loss of a beneficial strain, loss of diversity, or the proliferation of a pathogen[76] (Figure 3C-D). In mammals,

dysbiosis can be caused by antibiotic treatment or by diet and is characterized by either a bloom of a pathogen or by an imbalanced microbiome[77]. A balanced microbiome is required for proper immune training, and dysbiosis can cause diverse gastrointestinal diseases and is even linked to neurodegenerative diseases[76]. In plants, dysbiosis is often manifested as an imbalanced equilibrium between bacteria and fungi, which leads to fungal-derived disease[78,79], or expansion of a bacterial pathogen that increases the total bacterial load[80]. The plant immune system is important for maintaining a balanced microbial community. Dysbiotic communities can be transferred from sick plants and cause disease symptoms in healthy plants[81,82]. The plant microbiome plays a role in age-dependent immune maturation and hypersensitivity to pathogens by unknown mechanisms[81]. Overall, the maintenance of a balanced microbiome is important for plant health and performance.

An invading species can alter the natural microbiome**.** While natural microbial communities are generally stable, strong perturbations can alter community assembly. As noted above, strong perturbations are often external and include antibiotic treatment, changes in the available nutrient, or altered environmental conditions[83,84]. Application of a high dose of a functional focal therapeutic strain might affect community composition by direct antagonism of community members or by interfering with the network of interactions between other community members. For example, a pathogen can lead to a change in the profile of compounds that the plant secretes and to an altered microbial community[85–87]. The addition of a focal therapeutic strain to an established community can drive community assembly into a new steady state even if the invader doesn’t survive that transition[88]. A new strain that invades the plant microbiome may also inhibit a beneficial strain or lower community diversity (Figure 3C-D). These collateral alterations may hinder the therapeutic strain's beneficial effect.

Plant immunity gates microbiome assembly and influences microbiome manipulation

The plant immune system plays a pivotal role in safeguarding plants against invaders by orchestrating a sophisticated array of biochemical responses triggered upon the detection of non-self or modified-self molecules[89,90]. Over the past three decades, research unveiled the intricate interplay between the plant immune system and pathogenic microorganisms, shedding light on the strategies employed by harmful microbes to suppress or evade defense responses during disease. This accumulated knowledge has been successfully translated into practical applications, as exemplified by the development of disease-resistant plants through genetic engineering of immune receptors or susceptibility genes[91]. In contrast, the understanding of how non-pathogenic commensal microorganisms engage with plant immune components and how plants maintain microbiota homeostasis in the face of various stresses recently emerged as a dynamic area of investigation[92–95]. Thus, translational endeavors targeting microbiome manipulation are comparatively less advanced.

The field of plant-microbe interactions witnessed recent remarkable advances regarding the interplay between the plant immune system and the microbiota (Figure 4). Progress has led to the emergence of novel concepts, including the role of the microbiota in enhancing plant defense responses, the significance of plant-microbe and microbe-microbe interactions in shaping microbiota composition, or the influence of abiotic factors on plant-microbe interactions. In this section, we synthesize these recent advances into three fundamental frameworks: (1) the plant immune system controls microbiota homeostasis, which is fundamental for plant health; (2) the microbiota modulates plant immunity; and (3) the microbiota provides an additional layer of protection against diseases, extending the plant immune system. By integrating these perspectives, we aim to provide a comprehensive overview of the current understanding of the interaction between the plant immune system and the microbiota.

The plant immune system controls microbiota homeostasis. Building upon the knowledge gained from the study of plant-pathogen interactions, Arabidopsis mutants with defects in different sectors of the plant immune system were evaluated for alterations in microbiota composition. For instance, screens employing mutants with compromised hormonal signaling revealed that the phytohormones salicylic acid (SA), ethylene (ET) and jasmonic acid (SA), which orchestrate defense responses against pathogens, are also required for the assembly of normal bacterial communities in both roots and leaves[72,96–98]. Furthermore, exogenous application of these phytohormones can lead to alterations in the structure of plant-associated microbiota, indicating that the regulatory circuits that regulate interactions with pathogens also control the interaction with commensals. However, defense phytohormones appear to serve functions beyond immune response regulation. Certain bacteria exhibit reduced abundance in mutants deficient in salicylic acid, suggesting that they can metabolize this hormone as a growth signal or carbon source[72]. Thus, some commensal microbes appear to benefit from the immune responses in their host, challenging the conventional notion that the immune system serves to terminate microbial growth.

The participation of the plant immune system in regulating the microbiome is further underscored by the fact that loss of function mutants of specific immune receptors can lead to significant alterations in plant-associated microbial communities[82,99,100]. Plant immune receptors encompass two mutually reinforcing layers: the first layer consists of Pattern Recognition Receptors (PRRs), which are cell membrane receptors responsible for detecting extracellular molecules, such as microbe-associated molecular patterns (MAMPs). In contrast, the second layer comprises intracellular receptors from the NLR family that monitor the interior environment of plant cells[89,90,101]. While the involvement of NLRs in plant-microbiota interactions remains unconfirmed, cell surface receptors were implicated in maintaining microbiota homeostasis. Notably, pioneering studies revealed that immunocompromised mutants with impaired MAMP recognition and displaying an abnormal apoplastic microenvironment show spontaneous leaf

lesions reminiscent of disease symptoms, particularly under high humidity conditions[82,100]. These lesions were attributed to the over-proliferation of specific groups of commensal bacteria in the leaf interior, providing the first evidence of dysbiosis in plants. Importantly, experiments utilizing a gnotobiotic system and microbiome transplantation assays conclusively established that the altered microbiota was the cause of the disease-like lesions, rather than a consequence of unidentified abnormalities in the mutants[82]. The significance of the immune system in microbiota assembly is further supported by findings demonstrating that mis-localization of immune receptors in root cells affects the colonization of commensals[41] and that full immune function is not unleashed until localized damage to plant cells is sensed in the presence of immunogenic microbial patterns[102].

Upon activation, cell surface receptors initiate a series of biochemical responses collectively known as MAMP-triggered immunity (MTI). These responses encompass a wide range of biochemical alterations, including the activation of phosphorylation cascades, production of reactive oxygen species (ROS), calcium influx, transcriptional reprogramming, and the synthesis of antimicrobial proteins and secondary metabolites[103]. Given the pivotal role of PRRs in both pathogenic and nonpathogenic interactions, it is reasonable to assume that at least part of these downstream responses affects the plant microbiota. Supporting this notion, the Feronia receptor kinase controls the abundance of pseudomonads in the rhizosphere by inducing ROS production[99]. The involvement of ROS in maintaining microbial homeostasis was also reported in the phyllosphere. A screen using immunocompromised mutants demonstrated that the absence of RBOHD, an NADPH oxidase that is responsible for extracellular ROS production during immune responses, results in significant alterations in the bacterial community of Arabidopsis leaves[104]. Particularly, the *rbohD* mutant allows the proliferation of opportunistic *Xanthomonas* strains that normally grow asymptomatically in wild-type plants but cause disease in the mutant. Interestingly, the transition from commensalism to pathogenicity of opportunistic *Xanthomonas* is

prevented by ROS, which suppresses the secretion of hydrolytic enzymes by the bacterial type-two secretion system (T2SS)[105,106]. Furthermore, the dysbiosis observed in the plant *rbohD* mutant is primarily driven by the over-proliferation of *Xanthomonas*, with changes in the abundance of other bacteria being indirect consequences of niche alterations caused by the opportunistic strain[106]. These findings highlight a major role for ROS in regulating microbiota homeostasis and illustrate how loss of immune function can allow the transition of a commensal strain into a potentially harmful pathogen. Yet, ROS production may favor specific microbes, as a recent study found that ROS stimulates the growth and colonization capacity of a beneficial strain of *Bacillus velezensis*[107]. Thus, the precise effect of immune responses on plant-associated microbes depends on the interacting partners.

The production of secondary metabolites with antimicrobial activity can also play a role in microbiota homeostasis. An Arabidopsis mutant lacking the ability to produce tryptophan-derived metabolites exhibits compromised health and increased fungal loads in the root when colonized with a multikingdom microbial synthetic community, indicating a dysbiotic phenotype[79]. Interestingly, both plant-derived tryptophan metabolism and bacterial commensals are necessary to prevent excessive fungal growth[78,79], highlighting the significance of plant-microbe and microbe-microbe interactions for the maintenance of a healthy microbiota. Taken together, these examples illustrate the emerging role of the plant immune system in preserving microbiota homeostasis within plant tissues.

Microbiota modulate plant immunity. Despite the existence of efficient mechanisms to detect and fight off invaders, plant tissues harbor highly complex and dynamic microbial communities, raising the question of whether and how plants distinguish pathogenic from nonpathogenic microorganisms. This fundamental question has guided much of the research in the past few years, yielding new concepts. For instance, although pathogens have long been known for carrying molecules that elicit immune response in plants (e.g., MAMPs), it is now widely

accepted that such molecules are not exclusive to pathogens[93,108]. Furthermore, while the ability to suppress defense responses is a hallmark of successful pathogens, new studies revealed that nonpathogenic microbes that naturally coexist with plants also possess the capability to modulate or escape immune responses[105,108–112].

Screens of microbial collections reveal that immunosuppressive bacteria are common in the plant microbiota, constituting up to 65% of the evaluated strains[109–111]. Moreover, immune suppression capabilities were observed across various taxonomic groups, indicating an independent evolution of multiple mechanisms. Yet, specific examples of the molecular mechanisms of immuno-suppression by commensals remain limited. One was the demonstration that beneficial *Pseudomonas* spp. colonizing the rhizosphere secrete gluconic acid to acidify the extracellular environment and, consequently, impair the detection of MAMPs by cell surface immune receptors[111]. However, immunomodulation by other suppressive commensals occurs independently of extracellular acidification and, thus, is achieved by different mechanisms[110]. For instance, *Dyella japonica* MF79 requires the type-2-secretion system (T2SS) to suppress the immune response triggered by flg22 in Arabidopsis roots. Interestingly, this strain carries genes for the assembly of the type-3-secretion system (T3SS), but these are not required for the suppression ability displayed by this commensal. Similar independence of the T3SS for immunomodulation has been reported for other root commensals[109]. Since the T3SS is often required for the virulence of bacterial pathogens, this suggests that pathogens and commensals may rely on different tools to manipulate the immune system of their hosts. While pathogens usually utilize highly specialized effector molecules that function inside the plant cell, commensals may employ less specific extracellular strategies. Further investigation into additional suppression mechanisms employed by commensals is required to validate this hypothesis.

Immune evasion is another strategy employed by nonpathogenic microbes to overcome plant defenses. The small peptide flg22, derived from the flagellin protein FliC found in bacterial

flagella, is a potent antigen capable of triggering immune responses in most plant species[113]. Remarkably, commensal bacteria exhibit substantial diversity in the amino acid sequence of this MAMP, often enabling their flagellum to evade recognition by plant receptors[108,114]. Interestingly, some microbes produce variations of the flg22 peptide that competitively inhibit plant receptors, thereby preventing the recognition of their immunogenic counterparts[108,114]. Additional mechanisms employed by nonpathogenic microbes to evade plant immunity include the modification of MAMPs, such as chitin deacetylation by fungi[115], sequestration of MAMPs by specialized proteins to render them unavailable to plant receptors[116,117], and the downregulation of MAMP expression during plant colonization[118]. Many of these evasion mechanisms have also been described in pathogens[119,120], implying that pathogenic and nonpathogenic microbes evolved similar evasion solutions to counter the barriers imposed by the plant immune system.

Given that roots grow in a microbial-rich environment, plants must exert tight control over their immune system to prevent overstimulation by the wealth of microbial molecules that is prevalent in the rhizosphere. It is likely that the suppression and evasion strategies employed by commensal microorganisms contribute to this regulation. However, plant intrinsic mechanisms also appear to play a role and aid in the distinction between pathogenic and nonpathogenic microbes. Notably, the simultaneous presence of MAMPs and the occurrence of tissue damage is required for the activation of potent immune responses in roots[102]. By integrating these two signals, root cells are thought to selectively initiate defense responses in the presence of harmful pathogens, thereby facilitating the accommodation of commensal and beneficial microbes.

The microbiota provides an additional layer of protection against diseases. Although plant diseases are traditionally studied as binary interactions between a host and a pathogen, the resident microbiota in plant tissues exert a significant impact on the outcome of plant-pathogen interactions[92]. Recently, an elegant screen using a collection of bacteria isolated from the Arabidopsis phyllosphere revealed that approximately 20% of the evaluated strains could prevent

or mitigate disease caused by the pathogen *Pseudomonas syringae* pv. tomato DC3000[60]. Numerous other studies have identified microbes that confer protection against pathogens in different plant species, with some of them even constituting bioprotective commercial products[121,122]. However, the molecular mechanisms underlying the protective roles are often unknown, posing challenges to the efficacy and durability of strategies reliant on bioproducts.

Disease protection mediated by plant microbiota can be either direct or indirect[93] (Figure 5). Direct protection results from pathogen inhibition due to microbe-microbe interactions. For instance, plant-associated microorganisms may produce antimicrobial molecules or compete with pathogens for essential resources, impeding their growth and survival[57,123–126]. In contrast, indirect protection occurs when the microbiota modulates the plant immune system or metabolism, enhancing the host's ability to combat subsequent pathogen infections[60,127,128]. Interestingly, a majority of the plant microbiota members seem to induce the expression of defense-related genes to some extent when in mono-association with the host[110,129]. Moreover, phylogenetically diverse bacteria activate a convergent set of plant genes involved in the biosynthesis of tryptophan-derived secondary metabolites, many of which are required for resistance against pathogens[129].

Plant-associated microbial communities exhibit dynamic changes in response to various environmental stimuli, including biotic stresses (Figure 5). In Arabidopsis, infection of leaves by the oomycete *Hyaloperonospora arabidopsidis* triggers the recruitment of protective microbes in the roots[85]. Remarkably, these beneficial microbes can persist in the soil as a legacy and confer enhanced disease resistance to the subsequent generation of plants. Similar reshaping of plant-associated communities and recruitment of protective microbes have been observed in different plant species as a response to fungi, bacteria, and herbivores[130–134]. In this context, modification of surrounding environments through the secretion of primary and secondary metabolites appears to represent a major strategy used by plants to recruit beneficial microbes during stress responses. This process can be viewed as a strategic "cry-for-help" mechanism employed by

plants to establish symbiotic relationships that confer stress tolerance[86,135]. Understanding such mechanisms should support the deployment of microbial communities that make plants resilient to infection and abiotic stresses. A well-known protection mechanism mediated by the microbiome is Induced Systemic Resistance (ISR), which is characterized by the promotion of disease resistance in the aboveground plant organs by microorganisms that colonize the roots[128]. In ISR, sensing of some root microbes activates the root-specific transcription factor MYB72, which in turn promotes the expression of the beta-Glucosidase BGLU42[136,137]. ISR is activated and propagated in the plant in a jasmonic acid- and ethylene-response dependent manner[138]. Plants colonized by microorganisms that promote ISR display stronger and faster immune responses specifically when challenged with pathogens or pests.

Since the microbiome extends the plant immune system, it is not surprising that pathogens evolved strategies to manipulate the composition of the microbial communities that live in association with their hosts, thus facilitating plant colonization. This was initially demonstrated for the fungus *Verticillium dahliae*, which produces a set of effectors that possess selective antimicrobial activity against specific groups of bacteria or other fungi[139–141]. More recently, effectors with antimicrobial activity were identified in another fungal pathogen[142], suggesting that the manipulation of the plant microbiota may be a strategy employed by several other phytopathogens. These findings add an important layer to the interactions that result in plant disease. Understanding the mechanisms used by pathogens to modulate the microbiota of their hosts will be important for the development of disease-protective microbial communities that are resistant to pathogen manipulation.

Environmental heterogeneity alters the assembly rules of plant microbiomes and the outcome of plant-microbe interactions.

Since their invasion of terrestrial Earth, plants have faced a complex and dynamic environment[143]. The environment can vary in temperature, precipitation, nutrient availability, soil properties, and the presence of interacting organisms ranging from pathogens to mutualists. This heterogeneity has led to the evolution of complex and coordinated molecular, physiological, and anatomical plant responses to environmental variation (e.g. abscisic acid pathway evolution [144]). Importantly, microorganisms accompanied plants throughout this evolutionary process and resulting in an integration of environmental cues with appropriate immune responses in order to maintain health and nutrition in changing environments[9,49]. This integration of plant responses to environmental variation and microbiota poses both a challenge and an opportunity for the successful deployment of plant-associated microorganisms in managed settings. Environmental heterogeneity can change the determinants of successful microbial colonization, invasion, and persistence in the plant microbiome[145]. Changing environments can also render host plants more vulnerable to microbial pathogens and parasites[15,126,146,147]. However, interactions with microorganisms present a potential solution to some of the stresses plants face in changing environmental conditions including nutrient limitations, osmotic stress, and attack from pathogens[15,148].

Environmental heterogeneity can alter the rules of assembly either directly or indirectly via plant responses. The plant microbiome is populated by microorganisms in the surrounding environment. Therefore, environmental heterogeneity can alter the identity and frequency of microbial colonists of plant habitats through effects on microbial population growth, survival, and dispersal in the surrounding environment[149]. However, most research to date shows that the effects of environmental heterogeneity on the assembly of the plant microbiome occurs indirectly through plant responses[29,150,151]. Environmental heterogeneity can alter host plant biology from

molecular to morphological plant responses, potentially altering the suitability of the plant host as a habitat for microorganisms[38,152,153]. There are likely many environmental factors eliciting changes in the plant microbiome, however, the best studied to date are drought, and limitations in iron, and phosphate.

During drought, the microbial community in plant roots undergoes a drastic compositional shift, typified by the enrichment of actinobacteria, predominantly *Streptomyces*[154,155] (Figure 6A). This shift is conserved across major lineages of flowering plants and requires living plant roots[29,156]. To date, the precise mechanisms underlying this enrichment are not completely understood but likely include changes in the resources available for microbes in the root during drought, including plant-derived metabolites and essential micronutrients[153,157]. For example, *Sorghum bicolor* suppresses its iron uptake during drought by downregulating the biosynthesis and transport of the phytosiderophore mugineic acid[153]. Host plant suppression of iron uptake was accompanied by an enrichment in bacterial genes associated with iron metabolism in corresponding rhizosphere metagenomes. This indicates that competition for iron increased in the root microbiome during drought and contributed to the observed enrichment of members of the actinobacteria[153].

Iron limitation in soils and corresponding plant and bacterial responses to bio-available iron are emerging as major drivers of plant-microbe dynamics[158]. Iron is an essential micronutrient for all life due to its activity in numerous fundamental processes and although highly abundant in the Earth's crust, iron availability is low due to its insolubility in most soils[159]. During iron stress, plants activate a coordinated molecular and physiological response to scavenge scarce iron from soil[160]. Across angiosperms two iron uptake strategies have been identified. In strategy I under acidic conditions, iron is reduced at the root surface via a ferric reductase oxidase and transported into the plant. Under alkaline conditions strategy I plants excrete phenolic compounds, of which coumarins are the most well-studied[158,161], that improve the phytoavailability of iron by both

mobilization and reduction[162,163,159]. Strategy II is restricted to the true grasses and involves the production of iron chelating compounds termed phytosiderophores, which are transported back into roots after binding to iron in the soil.

Key genes in both iron uptake strategies appear to contribute to the composition of root microbial communities[38,153,164,165] (Figure 6A) Due to their ability to generate reactive oxygen species, coumarins can have direct antagonistic activity against diverse root-inhabiting microorganisms including commensal bacteria and fungal pathogens[38,164,165]. However, under iron-limited conditions, bacteria can also benefit from the iron bound to plant-derived compounds, including coumarins[166–168]. Additionally, commensal bacteria can induce iron leakage from roots to facilitate colonization[169]. Microbially derived siderophores can also be potent drivers of both root microbiome assembly and the success of invading phytopathogens, implicating iron as a key node in nutritional dynamics and community structure in plant-microbe systems[56,124]. The production of the bacterial siderophores, Pyoverdines, strongly inhibits co-occurring root bacteria and is required for peak abundance of a prominent pseudomonad in a root but not soil bacterial community[56]. Evidence from a large-scale metagenomic study supports the notion that competition for essential nutrients which vary across environments, including iron, is a widespread feature in the plant microbiome[170].

Phosphate (Pi) is another abundant essential nutrient that has low availability in soil depending on environmental conditions and is a central component of plant-microbiota interactions (Figure 6A). Plants deploy a phosphate starvation response (PSR) that includes an increase in lateral root formation and the accumulation of H^+-coupled Pi transporters of the PHOSPHATETRANSPORTER1 (PHT1) family at the plasma membrane of root epidermal cells[171]. In Arabidopsis, mutants impaired in PSR assemble irregular root microbiota in the absence of Pi limitation[150,152]. This is explained by the finding that the PSR transcriptional regulator, PHOSPHATE STARVATION RESPONSE 1 (PHR), jointly regulates plant responses

to Pi limitation and suppresses a large sector of plant immunity[152]. PHT mediated Pi uptake is suppressed by direct phosphorylation by the PTI activated BOTRYTIS-INDUCED KINASE 1 (BIK1)[172]. Arabidopsis does not engage in symbioses with arbuscular mycorrhizal fungi to meet Pi needs like many other plants, but was recently shown to suppress plant immunity under low Pi[172–174]. This enables colonization by the beneficial fungal endophyte *Colletotrichum tofieldiae*, which provides Pi to the plant[174]. In rice, PHRs promote the expression of arbuscular mycorrhizal symbiosis genes under Pi limitation, while under conditions of high Pi this expression is suppressed[175].

Crosstalk with plant immune and symbiosis pathways is emerging as a common theme among abiotic stress responses. Different environments can directly alter plant immunity through the expression of MTI and ETI-associated genes[176]. For example, elevated temperature leads to reduced formation of the transcriptional complex required for the expression of master immune transcription factors[146] (Figure 6B). Plant responses to various forms of abiotic stress also often lead to complex antagonistic effects on plant immunity through the suppression of the jasmonic acid and salicylic acid defense pathways[177,178]. Berens et al. recently showed that the antagonistic effects of salinity and ABA signaling on SA-mediated plant immunity were dependent on leaf age via AVRPPHB SUSCEPTIBLE 3 (PBS3) [177] (Figure 6B). Additionally, such interactions can span multiple plant organs; low photosynthetically active radiation (PAR) sensed in leaves leads to altered bacterial communities in roots, which rescue plant performance under suboptimal PAR in a manner dependent, in part, on JA signaling[179] (Figure 6B). Finally, plant responses to environmental stress can share signaling components with plant immune pathways[180,181], leading to coordinated plant immune and abiotic stress outputs. Such regulation of plant-microbial interactions via direct integration of environmental responses with plant immune and symbiosis pathways allows for fine-tuning of associated microbiota, presumably to satisfy nutritional demands and activate appropriate defense responses in a changed environment[49,95].

Microorganisms may enhance the maintenance of plant health and nutrition under various forms of abiotic stress[13–15,20,21,148]. There are two broad categories of studies that investigate the effects of microorganisms on plant performance during environmental stress. In the first category, researchers screen microbial isolate collections from either targeted or untargeted localities (e.g. locations with high occurrence of environmental stress or not) for plant growth promoting traits and beneficial plant-effects[182]. These studies defined remarkable microbial abilities to rescue plant performance under abiotic stress. However, these studies can be limited in that the colonization ability of the tested strains under stress conditions in wild soil is unknown. This is an important consideration given the above examples of how environmental heterogeneity can alter the invasion success of plant microbiome members. In the second category, researchers focus on microbes that are uniquely enriched in the plant microbiome under stress conditions and test their ability to rescue plant performance[183–185]. These studies typically identify enriched microbes in the context of a wild soil inoculum and thus start from the vantage point of successful invasion under environmental heterogeneity and standing community complexity. However, the magnitude or even presence of a plant benefit of these naturally, stress-enriched microorganisms is not guaranteed[150,164,186]. There are countless explanations for such an outcome but the simplest is that the plant microbiome represents a microbial niche and environmental variation alters it. Exploitation of the altered niche may have little or even negative consequences for plant health. These two broad approaches yield complementary insight into the mechanisms underpinning microbial dynamics and corresponding plant effects in the plant microbiome across environments. Environmental variability is increasing worldwide, including variability in soil quality, temperature, precipitation, and the occurrence of extreme weather events[17,187]. Therefore, greater effort is required to understand how environmental heterogeneity will impact the assembly and function of plant microbiota.

Summary and future directions.

The last decade of plant microbiome research has led to remarkable insight into the mechanistic interplay between plants, microbiota, and the environment and the resultant assembly and function of plant microbiota. While our knowledge is growing exponentially (Figure 1), there is much to learn before the promise of rational design in the plant microbiome for improved plant growth is realized. What additional plant performance-promoting traits are there? How to improve invasion and colonization while minimizing deleterious effects on the resident microbiome? Can treatment be tailored for specific soils or environmental conditions? How can we engineer microbial communities to enhance plant immunity against pathogens without compromising plant productivity? Given that the rate of climate change is rapidly outpacing the rate of plant evolution, can we engineer the required adaptation to abiotic stresses using microbes?

High-throughput assays are commonly employed to screen for microorganisms exhibiting desirable traits such as nutrient solubilization, plant hormone production and degradation, and antimicrobial activity against pathogens. However, these assays are usually conducted *in vitro*, and the beneficial traits displayed by individual strains under laboratory conditions rarely manifest in the context of microbial communities *in planta*. Furthermore, these screenings focus on a limited set of well-established traits, limiting the exploration of new mechanisms that could enhance plant health. These discrepancies present challenges for translational research, as large-scale evaluations of plant-microbiome interactions under field conditions or even in controlled environments are significantly more difficult. A more complete mechanistic understanding of the successful colonization of diverse microorganisms into plant habitats during diverse environmental conditions will yield new traits of interest to screen for in microbial culture collections. Furthermore, a broad understanding of how microbes of interest interact with plant immunity is fundamental for the efficient manipulation of microbiomes in agricultural contexts.

While current products are usually composed of one strain, consortia of multiple strains can have many advantages (Figure 7A). The function of consortia members can be redundant, increasing the likelihood that they will perform that function upon successful colonization. Since invasion and persistence is a major hurdle, consortia might also be composed of a focal plant growth-promoting strain accompanied by helper strains that promote ideal conditions for its colonization. Finally, the functions of consortia members can be additive or synergistic, where the cumulative effect is higher than that of any single strain, or complementary, where consortia members are acting in unison to promote plant growth. Yang et al. identified a cooperative mechanism, where drought protecting biofilm emerges only when consortia members are applied together[188]. Designing successful consortia could include assembly of functional redundancy for a plant-productivity trait of interest that is provided by diverse taxa to increase the likelihood of invasion and persistence. Alternatively, consortia could be built from functionally diverse members of related taxa in the hopes of creating a stable sub-niche of these that delivers multiple plant phenotypes. Functional consortia add complexity and thus require more knowledge and deeper mechanistic understanding of each system. And, while there might be advantages to development of consortia, there are still immense challenges to large scale fermentation and formulation of such products at scale[189–191].

An alternative approach is to combine traits instead of combining strains (Figure 7B). While environmental regulation is a major barrier for the release of genetically engineered strains, increased understanding of microbiota systems and advances in molecular biology and gene editing tools will hasten strain engineering. In this approach, gene clusters from different strains are collected into one domesticated “trait delivery strain” that can perform all the desirable functions. Deeper mechanistic understanding of plant productivity-promoting strains and culture independent approaches will ultimately enable genome writing to produce de-novo packages of traits in engineered strains.

Acknowledgements

This work was supported by HHMI funding and NSF grant IOS-1917270 to J.L.D. J.L.D. is an Investigator of the Howard Hughes Medical Institute. D.R. was supported by EMBO (EMBO long-term fellowship, ALTF 743–2019). C.R.F was supported by a Natural Sciences and Engineering Research Council of Canada postdoctoral fellowship (532852-2019). P.J.P.L.T is funded by the International Centre for Genetic Engineering and Biotechnology (ICGEB; grant CRP/BRA19-05_EC), Serrapilheira Institute (grant G-1811-25705), Aprosoja (grant CTR-0064-2020), and the São Paulo Research Foundation (FAPESP; grant 2018/24432-0). We thank Dr. Sarah R. Grant for critical reading and comments.

Author Contributions

Conceptualization: All authors. Initial draft: D.R., C.F and P.J.P.L.P. Editing: All Authors.

Declaration of Interests

J.L.D. is a co-founder of, and shareholder in, AgBiome LLC, a corporation whose goal is to use plant-associated microbes to improve plant productivity.

References

1. Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., and Singh, B.K. (2020). Plant-microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. *18*, 607–621. 10.1038/s41579-020-0412-1.

2. Fitzpatrick, C.R., Salas-González, I., Conway, J.M., Finkel, O.M., Gilbert, S., Russ, D., Teixeira, P.J.P.L., and Dangl, J.L. (2020). The Plant Microbiome: From Ecology to Reductionism and Beyond. Annu. Rev. Microbiol. *74*, 81–100. 10.1146/annurev-micro-022620-014327.

3. Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. *64*, 807–838. 10.1146/annurev-arplant-050312-120106.

4. Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T.G., et al. (2012). Defining the core Arabidopsis thaliana root microbiome. Nature *488*, 86–90. 10.1038/nature11237.

5. Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., et al. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature *488*, 91–95. 10.1038/nature11336.

6. Cordovez, V., Dini-Andreote, F., Carrión, V.J., and Raaijmakers, J.M. (2019). Ecology and Evolution of Plant Microbiomes. Annu. Rev. Microbiol. *73*, 69–88. 10.1146/annurev-micro-090817-062524.

7. Hartmann, A., Rothballer, M., and Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil *312*, 7–14. 10.1007/s11104-007-9514-z.

8. Teixeira, P.J.P., Colaianni, N.R., Fitzpatrick, C.R., and Dangl, J.L. (2019). Beyond pathogens: microbiota interactions with the plant immune system. Curr. Opin. Microbiol. *49*, 7–17. 10.1016/j.mib.2019.08.003.

9. Delaux, P.-M., and Schornack, S. (2021). Plant evolution driven by interactions with symbiotic and pathogenic microbes. Science *371*, eaba6605. 10.1126/science.aba6605.

10. Hawkes, C.V., Bull, J.J., and Lau, J.A. (2020). Symbiosis and stress: how plant microbiomes affect host evolution. Philos. Trans. R. Soc. B Biol. Sci. *375*, 20190590. 10.1098/rstb.2019.0590.

11. Sohrabi, R., Paasch, B.C., Liber, J.A., and He, S.Y. (2023). Phyllosphere Microbiome. Annu. Rev. Plant Biol. *74*, 539–568. 10.1146/annurev-arplant-102820-032704.

12. Eichmann, R., Richards, L., and Schäfer, P. (2021). Hormones as go-betweens in plant microbiome assembly. Plant J. Cell Mol. Biol. *105*, 518–541. 10.1111/tpj.15135.

13. Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., Morsy, M., Eisen, J.A., Leach, J.E., and Dangl, J.L. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. PLOS Biol. *15*, e2001793. 10.1371/journal.pbio.2001793.

14. Jurburg, S.D., Eisenhauer, N., Buscot, F., Chatzinotas, A., Chaudhari, N.M., Heintz-Buschart, A., Kallies, R., Küsel, K., Litchman, E., Macdonald, C.A., et al. (2022). Potential of microbiome-based solutions for agrifood systems. Nat. Food *3*, 557–560. 10.1038/s43016-022-00576-x.

15. Trivedi, P., Batista, B.D., Bazany, K.E., and Singh, B.K. (2022). Plant–microbiome interactions under a changing world: responses, consequences and perspectives. New Phytol. *234*, 1951–1959. 10.1111/nph.18016.

16. Liu, H., Brettell, L.E., Qiu, Z., and Singh, B.K. (2020). Microbiome-Mediated Stress Resistance in Plants. Trends Plant Sci. *25*, 733–743. 10.1016/j.tplants.2020.03.014.

17. Lesk, C., Rowhani, P., and Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. Nature *529*, 84–87. 10.1038/nature16467.

18. Delgado-Baquerizo, M., Guerra, C.A., Cano-Díaz, C., Egidi, E., Wang, J.-T., Eisenhauer, N., Singh, B.K., and Maestre, F.T. (2020). The proportion of soil-borne pathogens increases with warming at the global scale. Nat. Clim. Change *10*, 550–554. 10.1038/s41558-020-0759-3.

19. Singh, B.K., Trivedi, P., Egidi, E., Macdonald, C.A., and Delgado-Baquerizo, M. (2020). Crop microbiome and sustainable agriculture. Nat. Rev. Microbiol. *18*, 601–602. 10.1038/s41579-020-00446-y.

20. Friesen, M.L., Porter, S.S., Stark, S.C., Von Wettberg, E.J., Sachs, J.L., and Martinez-Romero, E. (2011). Microbially Mediated Plant Functional Traits. Annu. Rev. Ecol. Evol. Syst. *42*, 23–46. 10.1146/annurev-ecolsys-102710-145039.

21. Giauque, H., Connor, E.W., and Hawkes, C.V. (2019). Endophyte traits relevant to stress tolerance, resource use and habitat of origin predict effects on host plants. New Phytol. *221*, 2239–2249. 10.1111/nph.15504.

22. Hawkes, C.V., Kjøller, R., Raaijmakers, J.M., Riber, L., Christensen, S., Rasmussen, S., Christensen, J.H., Dahl, A.B., Westergaard, J.C., Nielsen, M., et al. (2021). Extension of Plant Phenotypes by the Foliar Microbiome. Annu. Rev. Plant Biol. *72*, 823–846. 10.1146/annurev-arplant-080620-114342.

23. MEYER, M.C., BUENO, A. de F., MAZARO, S.M., and Da SILVA, J.C. (2022). Bioinsumos na cultura da soja. In (Embrapa Soja).

24. Sanahuja, G., Banakar, R., Twyman, R.M., Capell, T., and Christou, P. (2011). Bacillus thuringiensis: a century of research, development and commercial applications: A century of Bacillus thuringiensis. Plant Biotechnol. J. *9*, 283–300. 10.1111/j.1467-7652.2011.00595.x.

25. de Souza, G.K., Sampaio, J., Longoni, L., Ferreira, S., Alvarenga, S., and Beneduzi, A. (2018). Soybean inoculants in Brazil: an overview of quality control. Braz. J. Microbiol. *50*, 205–211. 10.1007/s42770-018-0028-z.

26. Bulgarelli, D., Garrido-Oter, R., Münch, P.C., Weiman, A., Dröge, J., Pan, Y., McHardy, A.C., and Schulze-Lefert, P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe *17*, 392–403. 10.1016/j.chom.2015.01.011.

27. Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A., and Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. U. S. A. *112*, E911-920. 10.1073/pnas.1414592112.

28. Bai, Y., Müller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N., Münch, P.C., Spaepen, S., Remus-Emsermann, M., et al. (2015). Functional overlap of the Arabidopsis leaf and root microbiota. Nature *528*, 364–369. 10.1038/nature16192.

29. Fitzpatrick, C.R., Copeland, J., Wang, P.W., Guttman, D.S., Kotanen, P.M., and Johnson, M.T.J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. Proc. Natl. Acad. Sci. *115*. 10.1073/pnas.1717617115.

30. Thiergart, T., Durán, P., Ellis, T., Vannier, N., Garrido-Oter, R., Kemen, E., Roux, F., Alonso-Blanco, C., Ågren, J., Schulze-Lefert, P., et al. (2020). Root microbiota assembly and adaptive differentiation among European Arabidopsis populations. Nat. Ecol. Evol. *4*, 122–131. 10.1038/s41559-019-1063-3.

31. Vorholt, J.A. (2012). Microbial life in the phyllosphere. Nat. Rev. Microbiol. *10*, 828–840. 10.1038/nrmicro2910.

32. Bodenhausen, N., Horton, M.W., and Bergelson, J. (2013). Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. PLOS ONE *8*, e56329. 10.1371/journal.pone.0056329.

33. Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., and Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat. Commun. *7*, 12151. 10.1038/ncomms12151.

34. Paasch, B.C., and He, S.Y. (2021). Toward understanding microbiota homeostasis in the plant kingdom. PLOS Pathog. *17*, e1009472. 10.1371/journal.ppat.1009472.

35. Peters, N.K., Frost, J.W., and Long, S.R. (1986). A Plant Flavone, Luteolin, Induces Expression of Rhizobium meliloti Nodulation Genes. Science *233*, 977–980. 10.1126/science.3738520.

36. Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature *435*, 824–827. 10.1038/nature03608.

37. Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat. Commun. *9*, 2738. 10.1038/s41467-018-05122-7.

38. Stringlis, I.A., Yu, K., Feussner, K., De Jonge, R., Van Bentum, S., Van Verk, M.C., Berendsen, R.L., Bakker, P.A.H.M., Feussner, I., and Pieterse, C.M.J. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. Proc. Natl. Acad. Sci. *115*. 10.1073/pnas.1722335115.

39. Huang, A.C., Jiang, T., Liu, Y.-X., Bai, Y.-C., Reed, J., Qu, B., Goossens, A., Nützmann, H.-W., Bai, Y., and Osbourn, A. (2019). A specialized metabolic network selectively modulates Arabidopsis root microbiota. Science *364*, eaau6389. 10.1126/science.aau6389.

40. Wyrsch, I., Domínguez-Ferreras, A., Geldner, N., and Boller, T. (2015). Tissue-specific FLAGELLIN-SENSING 2 (FLS2) expression in roots restores immune responses in Arabidopsis fls2 mutants. New Phytol. *206*, 774–784. 10.1111/nph.13280.

41. Emonet, A., Zhou, F., Vacheron, J., Heiman, C.M., Tendon, V.D., Ma, K.-W., Schulze-Lefert, P., Keel, C., and Geldner, N. (2021). Spatially Restricted Immune Responses Are

Required for Maintaining Root Meristematic Activity upon Detection of Bacteria. Curr. Biol. *31*, 1012-1028.e7. https://doi.org/10.1016/j.cub.2020.12.048.

42. Voothuluru, P., Braun, D.M., and Boyer, J.S. (2018). An in Vivo Imaging Assay Detects Spatial Variability in Glucose Release from Plant Roots. Plant Physiol. *178*, 1002–1010. 10.1104/pp.18.00614.

43. Attia, S., Russel, J., Mortensen, M.S., Madsen, J.S., and Sørensen, S.J. (2022). Unexpected diversity among small-scale sample replicates of defined plant root compartments. ISME J. *16*, 997–1003. 10.1038/s41396-021-01094-7.

44. Nobori, T., Oliva, M., Lister, R., and Ecker, J.R. (2022). PHYTOMap: Multiplexed single-cell 3D spatial gene expression analysis in plant tissue. 2022.07.28.501915. 10.1101/2022.07.28.501915.

45. Cao, Z., Zuo, W., Wang, L., Chen, J., Qu, Z., Jin, F., and Dai, L. (2023). Spatial profiling of microbial communities by sequential FISH with error-robust encoding. Nat. Commun. *14*, 1477. 10.1038/s41467-023-37188-3.

46. Saarenpää, S., Shalev, O., Ashkenazy, H., Oliveira-Carlos, V. de, Lundberg, D.S., Weigel, D., and Giacomello, S. (2022). Spatially resolved host-bacteria-fungi interactomes via spatial metatranscriptomics. 2022.07.18.496977. 10.1101/2022.07.18.496977.

47. Wang, X., Wang, M., Xie, X., Guo, S., Zhou, Y., Zhang, X., Yu, N., and Wang, E. (2020). An amplification-selection model for quantified rhizosphere microbiota assembly. Sci. Bull. *65*, 983–986. 10.1016/j.scib.2020.03.005.

48. Pocheville, A. (2015). The Ecological Niche: History and Recent Controversies. In Handbook of Evolutionary Thinking in the Sciences, T. Heams, P. Huneman, G. Lecointre, and M. Silberstein, eds. (Springer Netherlands), pp. 547–586. 10.1007/978-94-017-9014-7_26.

49. Salas-González, I., Reyt, G., Flis, P., Custódio, V., Gopaulchan, D., Bakhoum, N., Dew, T.P., Suresh, K., Franke, R.B., Dangl, J.L., et al. (2021). Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis. Science *371*, eabd0695. 10.1126/science.abd0695.

50. Carlström, C.I., Field, C.M., Bortfeld-Miller, M., Müller, B., Sunagawa, S., and Vorholt, J.A. (2019). Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. Nat. Ecol. Evol. *3*, 1445–1454. 10.1038/s41559-019-0994-z.

51. Finkel, O.M., Salas-González, I., Castrillo, G., Conway, J.M., Law, T.F., Teixeira, P.J.P.L., Wilson, E.D., Fitzpatrick, C.R., Jones, C.D., and Dangl, J.L. (2020). A single bacterial genus maintains root growth in a complex microbiome. Nature. 10.1038/s41586-020-2778-7.

52. Oldroyd, G.E.D., Murray, J.D., Poole, P.S., and Downie, J.A. (2011). The Rules of Engagement in the Legume-Rhizobial Symbiosis. Annu. Rev. Genet. *45*, 119–144. 10.1146/annurev-genet-110410-132549.

53. Hemmerle, L., Maier, B.A., Bortfeld-Miller, M., Ryback, B., Gäbelein, C.G., Ackermann, M., and Vorholt, J.A. (2022). Dynamic character displacement among a pair of bacterial phyllosphere commensals in situ. Nat. Commun. *13*, 2836. 10.1038/s41467-022-30469-3.

54. Granato, E.T., Meiller-Legrand, T.A., and Foster, K.R. (2019). The Evolution and Ecology of Bacterial Warfare. Curr. Biol. *29*, R521–R537. 10.1016/j.cub.2019.04.024.

55. Clardy, J., Fischbach, M., and Currie, C. (2009). The natural history of antibiotics. Curr. Biol. CB *19*, R437–R441. 10.1016/j.cub.2009.04.001.

56. Getzke, F., Hassani, M.A., Crüsemann, M., Malisic, M., Zhang, P., Ishigaki, Y., Böhringer, N., Jiménez Fernández, A., Wang, L., Ordon, J., et al. (2023). Cofunctioning of bacterial exometabolites drives root microbiota establishment. Proc. Natl. Acad. Sci. *120*, e2221508120. 10.1073/pnas.2221508120.

57. Helfrich, E.J.N., Vogel, C.M., Ueoka, R., Schäfer, M., Ryffel, F., Müller, D.B., Probst, S., Kreuzer, M., Piel, J., and Vorholt, J.A. (2018). Bipartite interactions, antibiotic production and biosynthetic potential of the Arabidopsis leaf microbiome. Nat. Microbiol. *3*, 909–919. 10.1038/s41564-018-0200-0.

58. Ratzke, C., Barrere, J., and Gore, J. (2020). Strength of species interactions determines biodiversity and stability in microbial communities. Nat. Ecol. Evol. *4*, 376–383. 10.1038/s41559-020-1099-4.

59. Schäfer, M., Vogel, C.M., Bortfeld-Miller, M., Mittelviefhaus, M., and Vorholt, J.A. (2022). Mapping phyllosphere microbiota interactions in planta to establish genotype–phenotype relationships. Nat. Microbiol. *7*, 856–867. 10.1038/s41564-022-01132-w.

60. Vogel, C.M., Potthoff, D.B., Schäfer, M., Barandun, N., and Vorholt, J.A. (2021). Protective role of the Arabidopsis leaf microbiota against a bacterial pathogen. Nat. Microbiol. *6*, 1537–1548. 10.1038/s41564-021-00997-7.

61. Koskella, B., and Brockhurst, M.A. (2014). Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS Microbiol. Rev. *38*, 916–931. 10.1111/1574-6976.12072.

62. Thingstad, T.F. (2000). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol. Oceanogr. *45*, 1320–1328. 10.4319/lo.2000.45.6.1320.

63. Brockhurst, M.A., Fenton, A., Roulston, B., and Rainey, P.B. (2006). The impact of phages on interspecific competition in experimental populations of bacteria. BMC Ecol. *6*, 19. 10.1186/1472-6785-6-19.

64. Hernandez, C.A., and Koskella, B. (2019). Phage resistance evolution in vitro is not reflective of in vivo outcome in a plant-bacteria-phage system*. Evolution *73*, 2461–2475. 10.1111/evo.13833.

65. Tzipilevich, E., and Benfey, P.N. (2021). Phage-Resistant Bacteria Reveal a Role for Potassium in Root Colonization. mBio *12*, 10.1128/mbio.01403-21. 10.1128/mbio.01403-21.

66. Brown, S.P., Fredrik Inglis, R., and Taddei, F. (2009). Evolutionary ecology of microbial wars: within-host competition and (incidental) virulence. Evol. Appl. *2*, 32–39. 10.1111/j.1752-4571.2008.00059.x.

67. Riley, M.A. (2011). Bacteriocin-Mediated Competitive Interactions of Bacterial Populations and Communities. In Prokaryotic Antimicrobial Peptides: From Genes to Applications, D. Drider and S. Rebuffat, eds. (Springer), pp. 13–26. 10.1007/978-1-4419-7692-5_2.

68. Geller, A.M., Zlotkin, D., and Levy, A. (2021). Large-scale discovery of candidate type VI secretion effectors with antibacterial activity. 2021.10.07.463556. 10.1101/2021.10.07.463556.

69. Boak, E.N., Kirolos, S., Pan, H., Pierson, L.S., and Pierson, E.A. (2022). The Type VI Secretion Systems in Plant-Beneficial Bacteria Modulate Prokaryotic and Eukaryotic Interactions in the Rhizosphere. Front. Microbiol. *13*.

70. Bernal, P., Allsopp, L.P., Filloux, A., and Llamas, M.A. (2017). The Pseudomonas putida T6SS is a plant warden against phytopathogens. ISME J. *11*, 972–987. 10.1038/ismej.2016.169.

71. Purtschert-Montenegro, G., Cárcamo-Oyarce, G., Pinto-Carbó, M., Agnoli, K., Bailly, A., and Eberl, L. (2022). Pseudomonas putida mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. Nat. Microbiol. *7*, 1547–1557. 10.1038/s41564-022-01209-6.

72. Lebeis, S.L., Paredes, S.H., Lundberg, D.S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Glavina del Rio, T., Jones, C.D., Tringe, S.G., et al. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science *349*, 860–864. 10.1126/science.aaa8764.

73. Vorholt, J.A., Vogel, C., Carlström, C.I., and Müller, D.B. (2017). Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host Microbe *22*, 142–155. 10.1016/j.chom.2017.07.004.

74. Van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottovā, D., Krištůfek, V., and Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc. Natl. Acad. Sci. *109*, 1159–1164. 10.1073/pnas.1109326109.

75. Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., and Van Der Heijden, M.G.A. (2019). Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat. Commun. *10*, 4841. 10.1038/s41467-019-12798-y.

76. Petersen, C., and Round, J.L. (2014). Defining dysbiosis and its influence on host immunity and disease. Cell. Microbiol. *16*, 1024–1033. 10.1111/cmi.12308.

77. Bergogne-Bérézin, E. (2000). Treatment and prevention of antibiotic associated diarrhea. Int. J. Antimicrob. Agents *16*, 521–526. 10.1016/S0924-8579(00)00293-4.

78. Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., and Hacquard, S. (2018). Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. Cell *175*, 973-983.e14. 10.1016/j.cell.2018.10.020.

79. Wolinska, K.W., Vannier, N., Thiergart, T., Pickel, B., Gremmen, S., Piasecka, A., Piślewska-Bednarek, M., Nakano, R.T., Belkhadir, Y., Bednarek, P., et al. (2021). Tryptophan metabolism and bacterial commensals prevent fungal dysbiosis in *Arabidopsis* roots. Proc. Natl. Acad. Sci. *118*, e2111521118. 10.1073/pnas.2111521118.

80. Karasov, T.L., Neumann, M., Duque-Jaramillo, A., Kersten, S., Bezrukov, I., Schröppel, B., Symeonidi, E., Lundberg, D.S., Regalado, J., Shirsekar, G., et al. (2020). The relationship between microbial population size and disease in the Arabidopsis thaliana phyllosphere. 828814. 10.1101/828814.

81. Paasch, B.C., Sohrabi, R., Kremer, J.M., Nomura, K., Martz, J., Kvitko, B., Tiedje, J.M., and He, S.Y. (2023). A critical role of a eubiotic microbiota in gating proper immunocompetence in Arabidopsis. bioRxiv, 2023–03.

82. Chen, T., Nomura, K., Wang, X., Sohrabi, R., Xu, J., Yao, L., Paasch, B.C., Ma, L., Kremer, J., Cheng, Y., et al. (2020). A plant genetic network for preventing dysbiosis in the phyllosphere. Nature *580*, 653–657. 10.1038/s41586-020-2185-0.

83. Modi, S.R., Collins, J.J., and Relman, D.A. (2014). Antibiotics and the gut microbiota. J. Clin. Invest. *124*, 4212–4218. 10.1172/JCI72333.

84. Zogg, G.P., Zak, D.R., Ringelberg, D.B., White, D.C., MacDonald, N.W., and Pregitzer, K.S. (1997). Compositional and Functional Shifts in Microbial Communities Due to Soil Warming. Soil Sci. Soc. Am. J. *61*, 475–481. 10.2136/sssaj1997.03615995006100020015x.

85. Berendsen, R.L., Vismans, G., Yu, K., Song, Y., de Jonge, R., Burgman, W.P., Burmølle, M., Herschend, J., Bakker, P.A.H.M., and Pieterse, C.M.J. (2018). Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J. *12*, 1496–1507. 10.1038/s41396-018-0093-1.

86. Rolfe, S.A., Griffiths, J., and Ton, J. (2019). Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Curr. Opin. Microbiol. *49*, 73–82. 10.1016/j.mib.2019.10.003.

87. Goossens, P., Spooren, J., Baremans, K.C.M., Andel, A., Lapin, D., Echobardo, N., Pieterse, C.M.J., Van Den Ackerveken, G., and Berendsen, R.L. (2023). Congruent downy mildew-associated microbiomes reduce plant disease and function as transferable resistobiomes (Microbiology) 10.1101/2023.03.14.532520.

88. Amor, D.R., Ratzke, C., and Gore, J. (2020). Transient invaders can induce shifts between alternative stable states of microbial communities. Sci. Adv. *6*, eaay8676. 10.1126/sciadv.aay8676.

89. Zhou, J.-M., and Zhang, Y. (2020). Plant Immunity: Danger Perception and Signaling. Cell *181*, 978–989. 10.1016/j.cell.2020.04.028.

90. Jones, J.D.G., and Dangl, J.L. (2006). The plant immune system. Nature *444*, 323–329. 10.1038/nature05286.

91. van Esse, H.P., Reuber, T.L., and van der Does, D. (2020). Genetic modification to improve disease resistance in crops. New Phytol. *225*, 70–86. 10.1111/nph.15967.

92. Pereira, L.B., Thomazella, D.P.T., and Teixeira, P.J.P.L. (2023). Plant-microbiome crosstalk and disease development. Curr. Opin. Plant Biol. *72*, 102351. 10.1016/j.pbi.2023.102351.

93. Teixeira, P.J.P., Colaianni, N.R., Fitzpatrick, C.R., and Dangl, J.L. (2019). Beyond pathogens: microbiota interactions with the plant immune system. Curr. Opin. Microbiol. *49*, 7–17. 10.1016/j.mib.2019.08.003.

94. Vannier, N., Agler, M., and Hacquard, S. (2019). Microbiota-mediated disease resistance in plants. PLoS Pathog. *15*, e1007740. 10.1371/journal.ppat.1007740.

95. Hacquard, S., Spaepen, S., Garrido-Oter, R., and Schulze-Lefert, P. (2017). Interplay Between Innate Immunity and the Plant Microbiota. Annu. Rev. Phytopathol. *55*, 565–589. 10.1146/annurev-phyto-080516-035623.

96. Carvalhais, L.C., Dennis, P.G., Badri, D.V., Kidd, B.N., Vivanco, J.M., and Schenk, P.M. (2015). Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. Mol. Plant-Microbe Interact. MPMI *28*, 1049–1058. 10.1094/MPMI-01-15-0016-R.

97. Doornbos, R.F., Geraats, B.P.J., Kuramae, E.E., Van Loon, L.C., and Bakker, P.A.H.M. (2011). Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of Arabidopsis thaliana. Mol. Plant-Microbe Interact. MPMI *24*, 395–407. 10.1094/MPMI-05-10-0115.

98. Kniskern, J.M., Traw, M.B., and Bergelson, J. (2007). Salicylic Acid and Jasmonic Acid Signaling Defense Pathways Reduce Natural Bacterial Diversity on *Arabidopsis thaliana*. Mol. Plant. Microbe Interact. *20*, 1512–1522. 10.1094/MPMI-20-12-1512.

99. Song, Y., Wilson, A.J., Zhang, X.-C., Thoms, D., Sohrabi, R., Song, S., Geissmann, Q., Liu, Y., Walgren, L., He, S.Y., et al. (2021). FERONIA restricts Pseudomonas in the rhizosphere microbiome via regulation of reactive oxygen species. Nat. Plants *7*, 644–654. 10.1038/s41477-021-00914-0.

100. Xin, X.-F., Nomura, K., Aung, K., Velásquez, A.C., Yao, J., Boutrot, F., Chang, J.H., Zipfel, C., and He, S.Y. (2016). Bacteria establish an aqueous living space in plants crucial for virulence. Nature *539*, 524–529. 10.1038/nature20166.

101. Couto, D., and Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. Nat. Rev. Immunol. *16*, 537–552. 10.1038/nri.2016.77.

102. Zhou, F., Emonet, A., Dénervaud Tendon, V., Marhavy, P., Wu, D., Lahaye, T., and Geldner, N. (2020). Co-incidence of Damage and Microbial Patterns Controls Localized Immune Responses in Roots. Cell *180*, 440-453.e18. 10.1016/j.cell.2020.01.013.

103. DeFalco, T.A., and Zipfel, C. (2021). Molecular mechanisms of early plant pattern-triggered immune signaling. Mol. Cell *81*, 4346. 10.1016/j.molcel.2021.09.028.

104. Pfeilmeier, S., Petti, G.C., Bortfeld-Miller, M., Daniel, B., Field, C.M., Sunagawa, S., and Vorholt, J.A. (2021). The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. Nat. Microbiol. *6*, 852–864. 10.1038/s41564-021-00929-5.

105. Entila, F., Han, X., Mine, A., Schulze-Lefert, P., and Tsuda, K. (2023). Commensal lifestyle regulated by a negative feedback loop between Arabidopsis ROS and the bacterial T2SS. bioRxiv, 2023–05.

106. Pfeilmeier, S., Werz, A., Ote, M., Bortfeld-Miller, M., Kirner, P., Keppler, A., Hemmerle, L., Gaebelein, C.G., Pestalozzi, C.M., and Vorholt, J.A. (2023). Dysbiosis of a leaf microbiome is caused by enzyme secretion of opportunistic Xanthomonas strains. bioRxiv, 2023–05.

107. Tzipilevich, E., Russ, D., Dangl, J.L., and Benfey, P.N. (2021). Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria. Cell Host Microbe *29*, 1507-1520.e4. 10.1016/j.chom.2021.09.005.

108. Colaianni, N.R., Parys, K., Lee, H.-S., Conway, J.M., Kim, N.H., Edelbacher, N., Mucyn, T.S., Madalinski, M., Law, T.F., Jones, C.D., et al. (2021). A complex immune response to flagellin epitope variation in commensal communities. Cell Host Microbe *29*, 635-649.e9. 10.1016/j.chom.2021.02.006.

109. Ma, K.-W., Niu, Y., Jia, Y., Ordon, J., Copeland, C., Emonet, A., Geldner, N., Guan, R., Stolze, S.C., Nakagami, H., et al. (2021). Coordination of microbe-host homeostasis by crosstalk with plant innate immunity. Nat. Plants *7*, 814–825. 10.1038/s41477-021-00920-2.

110. Teixeira, P.J.P.L., Colaianni, N.R., Law, T.F., Conway, J.M., Gilbert, S., Li, H., Salas-González, I., Panda, D., Del Risco, N.M., Finkel, O.M., et al. (2021). Specific modulation of the root immune system by a community of commensal bacteria. Proc. Natl. Acad. Sci. U. S. A. *118*, e2100678118. 10.1073/pnas.2100678118.

111. Yu, K., Liu, Y., Tichelaar, R., Savant, N., Lagendijk, E., van Kuijk, S.J.L., Stringlis, I.A., van Dijken, A.J.H., Pieterse, C.M.J., Bakker, P.A.H.M., et al. (2019). Rhizosphere-Associated *Pseudomonas* Suppress Local Root Immune Responses by Gluconic Acid-Mediated Lowering of Environmental pH. Curr. Biol. *29*, 3913-3920.e4. 10.1016/j.cub.2019.09.015.

112. Garrido-Oter, R., Nakano, R.T., Dombrowski, N., Ma, K.-W., AgBiome Team, McHardy, A.C., and Schulze-Lefert, P. (2018). Modular Traits of the Rhizobiales Root Microbiota and Their Evolutionary Relationship with Symbiotic Rhizobia. Cell Host Microbe *24*, 155-167.e5. 10.1016/j.chom.2018.06.006.

113. Felix, G., Duran, J.D., Volko, S., and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. Cell Mol. Biol. *18*, 265–276. 10.1046/j.1365-313x.1999.00265.x.

114. Parys, K., Colaianni, N.R., Lee, H.-S., Hohmann, U., Edelbacher, N., Trgovcevic, A., Blahovska, Z., Lee, D., Mechtler, A., Muhari-Portik, Z., et al. (2021). Signatures of antagonistic pleiotropy in a bacterial flagellin epitope. Cell Host Microbe *29*, 620-634.e9. 10.1016/j.chom.2021.02.008.

115. Cord-Landwehr, S., Melcher, R.L.J., Kolkenbrock, S., and Moerschbacher, B.M. (2016). A chitin deacetylase from the endophytic fungus Pestalotiopsis sp. efficiently inactivates the elicitor activity of chitin oligomers in rice cells. Sci. Rep. *6*, 38018. 10.1038/srep38018.

116. Zeng, T., Rodriguez-Moreno, L., Mansurkhodzaev, A., Wang, P., van den Berg, W., Gasciolli, V., Cottaz, S., Fort, S., Thomma, B.P.H.J., Bono, J.-J., et al. (2020). A lysin motif effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis. New Phytol. *225*, 448–460. 10.1111/nph.16245.

117. Deng, Y., Chen, H., Li, C., Xu, J., Qi, Q., Xu, Y., Zhu, Y., Zheng, J., Peng, D., Ruan, L., et al. (2019). Endophyte Bacillus subtilis evade plant defense by producing lantibiotic subtilomycin to mask self-produced flagellin. Commun. Biol. *2*, 368. 10.1038/s42003-019-0614-0.

118. Pfeilmeier, S., Saur, I.M.-L., Rathjen, J.P., Zipfel, C., and Malone, J.G. (2016). High levels of cyclic-di-GMP in plant-associated Pseudomonas correlate with evasion of plant immunity. Mol. Plant Pathol. *17*, 521–531. 10.1111/mpp.12297.

119. Sanguankiattichai, N., Buscaill, P., and Preston, G.M. (2022). How bacteria overcome flagellin pattern recognition in plants. Curr. Opin. Plant Biol. *67*, 102224. 10.1016/j.pbi.2022.102224.

120. Buscaill, P., and van der Hoorn, R.A.L. (2021). Defeated by the nines: nine extracellular strategies to avoid microbe-associated molecular patterns recognition in plants. Plant Cell *33*, 2116–2130. 10.1093/plcell/koab109.

121. Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., and Montesinos, E. (2022). Bacteria as Biological Control Agents of Plant Diseases. Microorganisms *10*, 1759. 10.3390/microorganisms10091759.

122. Köhl, J., Kolnaar, R., and Ravensberg, W.J. (2019). Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. Front. Plant Sci. *10*.

123. Wang, N.R., Wiesmann, C.L., Melnyk, R.A., Hossain, S.S., Chi, M.-H., Martens, K., Craven, K., and Haney, C.H. (2022). Commensal *Pseudomonas fluorescens* Strains Protect Arabidopsis from Closely Related *Pseudomonas* Pathogens in a Colonization-Dependent Manner. mBio *13*, e0289221. 10.1128/mbio.02892-21.

124. Gu, S., Wei, Z., Shao, Z., Friman, V.-P., Cao, K., Yang, T., Kramer, J., Wang, X., Li, M., Mei, X., et al. (2020). Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. Nat. Microbiol. *5*, 1002–1010. 10.1038/s41564-020-0719-8.

125. Chen, Y., Wang, J., Yang, N., Wen, Z., Sun, X., Chai, Y., and Ma, Z. (2018). Wheat microbiome bacteria can reduce virulence of a plant pathogenic fungus by altering histone acetylation. Nat. Commun. *9*, 3429. 10.1038/s41467-018-05683-7.

126. Berg, M., and Koskella, B. (2018). Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen. Curr. Biol. *28*, 2487-2492.e3. 10.1016/j.cub.2018.05.085.

127. Vogel, C., Bodenhausen, N., Gruissem, W., and Vorholt, J.A. (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 Phytol. *212*, 192–207. 10.1111/nph.14036.

128. Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., and Bakker, P.A.H.M. (2014). Induced systemic resistance by beneficial microbes. Annu. Rev. Phytopathol. *52*, 347–375. 10.1146/annurev-phyto-082712-102340.

129. Maier, B.A., Kiefer, P., Field, C.M., Hemmerle, L., Bortfeld-Miller, M., Emmenegger, B., Schäfer, M., Pfeilmeier, S., Sunagawa, S., Vogel, C.M., et al. (2021). A general non-self response as part of plant immunity. Nat. Plants *7*, 696–705. 10.1038/s41477-021-00913-1.

130. Friman, J., Karssemeijer, P.N., Haller, J., de Kreek, K., van Loon, J.J.A., and Dicke, M. (2021). Shoot and root insect herbivory change the plant rhizosphere microbiome and affects cabbage-insect interactions through plant-soil feedback. New Phytol. *232*, 2475–2490. 10.1111/nph.17746.

131. Kong, H.G., Kim, B.K., Song, G.C., Lee, S., and Ryu, C.-M. (2016). Aboveground Whitefly Infestation-Mediated Reshaping of the Root Microbiota. Front. Microbiol. *7*, 1314. 10.3389/fmicb.2016.01314.

132. Lee, B., Lee, S., and Ryu, C.-M. (2012). Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non-pathogenic bacteria in pepper. Ann. Bot. *110*, 281–290. 10.1093/aob/mcs055.

133. Yang, J.W., Yi, H.-S., Kim, H., Lee, B., Lee, S., Ghim, S.-Y., and Ryu, C.-M. (2011). Whitefly infestation of pepper plants elicits defence responses against bacterial pathogens in leaves and roots and changes the below-ground microflora. J. Ecol. *99*, 46–56.

134. Rudrappa, T., Czymmek, K.J., Paré, P.W., and Bais, H.P. (2008). Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol. *148*, 1547–1556. 10.1104/pp.108.127613.

135. Bakker, P.A.H.M., Pieterse, C.M.J., de Jonge, R., and Berendsen, R.L. (2018). The Soil-Borne Legacy. Cell *172*, 1178–1180. 10.1016/j.cell.2018.02.024.

136. Van der Ent, S., Verhagen, B.W.M., Van Doorn, R., Bakker, D., Verlaan, M.G., Pel, M.J.C., Joosten, R.G., Proveniers, M.C.G., Van Loon, L.C., Ton, J., et al. (2008). MYB72 Is Required in Early Signaling Steps of Rhizobacteria-Induced Systemic Resistance in Arabidopsis. Plant Physiol. *146*, 1293–1304. 10.1104/pp.107.113829.

137. Zamioudis, C., Hanson, J., and Pieterse, C.M.J. (2014). β-Glucosidase BGLU42 is a MYB72-dependent key regulator of rhizobacteria-induced systemic resistance and modulates iron deficiency responses in Arabidopsis roots. New Phytol. *204*, 368–379. 10.1111/nph.12980.

138. Pieterse, C.M.J., van Wees, S.C.M., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., and van Loon, L.C. (1998). A Novel Signaling Pathway Controlling Induced Systemic Resistance in Arabidopsis. Plant Cell *10*, 1571–1580. 10.1105/tpc.10.9.1571.

139. Snelders, N.C., Boshoven, J.C., Song, Y., Schmitz, N., Fiorin, G.L., Rovenich, H., van den Berg, G.C.M., Torres, D.E., Petti, G.C., Prockl, Z., et al. (2022). A highly polymorphic effector protein promotes fungal virulence through suppression of plant-associated Actinobacteria. New Phytol. 10.1111/nph.18576.

140. Snelders, N.C., Petti, G.C., van den Berg, G.C.M., Seidl, M.F., and Thomma, B.P.H.J. (2021). An ancient antimicrobial protein co-opted by a fungal plant pathogen for in planta mycobiome manipulation. Proc. Natl. Acad. Sci. U. S. A. *118*, e2110968118. 10.1073/pnas.2110968118.

141. Snelders, N.C., Rovenich, H., Petti, G.C., Rocafort, M., van den Berg, G.C.M., Vorholt, J.A., Mesters, J.R., Seidl, M.F., Nijland, R., and Thomma, B.P.H.J. (2020). Microbiome manipulation by a soil-borne fungal plant pathogen using effector proteins. Nat. Plants *6*, 1365–1374. 10.1038/s41477-020-00799-5.

142. Chavarro, E., Snelders, N., Torres, D., Kraege, A., Lopez-Moral, A., Petti, G., Punt, W., Wieneke, J., Garcia-Velasco, R., Lopez-Herrera, C., et al. (2023). The soil-borne white root rot pathogen Rosellinia necatrix expresses antimicrobial proteins during host colonization. bioRxiv, 2023–04.

143. De Vries, J., and Archibald, J.M. (2018). Plant evolution: landmarks on the path to terrestrial life. New Phytol. *217*, 1428–1434. 10.1111/nph.14975.

144. Komatsu, K., Takezawa, D., and Sakata, Y. (2020). Decoding ABA and osmostress signalling in plants from an evolutionary point of view. Plant Cell Environ. *43*, 2894–2911. 10.1111/pce.13869.

145. Zhu, Y., Xiong, C., Wei, Z., Chen, Q., Ma, B., Zhou, S., Tan, J., Zhang, L., Cui, H., and Duan, G. (2022). Impacts of global change on the phyllosphere microbiome. New Phytol. *234*, 1977–1986. 10.1111/nph.17928.

146. Kim, J.H., Castroverde, C.D.M., Huang, S., Li, C., Hilleary, R., Seroka, A., Sohrabi, R., Medina-Yerena, D., Huot, B., Wang, J., et al. (2022). Increasing the resilience of plant immunity to a warming climate. Nature *607*, 339–344. 10.1038/s41586-022-04902-y.

147. Velásquez, A.C., Castroverde, C.D.M., and He, S.Y. (2018). Plant–Pathogen Warfare under Changing Climate Conditions. Curr. Biol. *28*, R619–R634. 10.1016/j.cub.2018.03.054.

148. Cheng, Y.T., Zhang, L., and He, S.Y. (2019). Plant-Microbe Interactions Facing Environmental Challenge. Cell Host Microbe *26*, 183–192. 10.1016/j.chom.2019.07.009.

149. Jansson, J.K., and Hofmockel, K.S. (2020). Soil microbiomes and climate change. Nat. Rev. Microbiol. *18*, 35–46. 10.1038/s41579-019-0265-7.

150. Finkel, O.M., Salas-González, I., Castrillo, G., Spaepen, S., Law, T.F., Teixeira, P.J.P.L., Jones, C.D., and Dangl, J.L. (2019). The effects of soil phosphorus content on plant microbiota are driven by the plant phosphate starvation response. PLOS Biol. *17*, e3000534. 10.1371/journal.pbio.3000534.

151. Ramirez-Villacis, D.X., Finkel, O.M., Salas-González, I., Fitzpatrick, C.R., Dangl, J.L., Jones, C.D., and Leon-Reyes, A. (2020). Root Microbiome Modulates Plant Growth Promotion Induced by Low Doses of Glyphosate. mSphere *5*, e00484-20. 10.1128/mSphere.00484-20.

152. Castrillo, G., Teixeira, P.J.P.L., Paredes, S.H., Law, T.F., De Lorenzo, L., Feltcher, M.E., Finkel, O.M., Breakfield, N.W., Mieczkowski, P., Jones, C.D., et al. (2017). Root microbiota drive direct integration of phosphate stress and immunity. Nature *543*, 513–518. 10.1038/nature21417.

153. Xu, L., Dong, Z., Chiniquy, D., Pierroz, G., Deng, S., Gao, C., Diamond, S., Simmons, T., Wipf, H.M.-L., Caddell, D., et al. (2021). Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. Nat. Commun. *12*, 3209. 10.1038/s41467-021-23553-7.

154. Naylor, D., DeGraaf, S., Purdom, E., and Coleman-Derr, D. (2017). Drought and host selection influence bacterial community dynamics in the grass root microbiome. ISME J. *11*, 2691–2704. 10.1038/ismej.2017.118.

155. Santos-Medellín, C., Edwards, J., Liechty, Z., Nguyen, B., and Sundaresan, V. (2017). Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes. mBio *8*, e00764-17. 10.1128/mBio.00764-17.

156. Simmons, T., Styer, A.B., Pierroz, G., Gonçalves, A.P., Pasricha, R., Hazra, A.B., Bubner, P., and Coleman-Derr, D. (2020). Drought Drives Spatial Variation in the Millet Root Microbiome. Front. Plant Sci. *11*, 599. 10.3389/fpls.2020.00599.

157. Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K.K., Kim, Y.-M., Zink, E.M., Engbrecht, K.M., Wang, Y., et al. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proc. Natl. Acad. Sci. *115*. 10.1073/pnas.1717308115.

158. Stassen, M.J.J., Hsu, S.-H., Pieterse, C.M.J., and Stringlis, I.A. (2021). Coumarin Communication Along the Microbiome–Root–Shoot Axis. Trends Plant Sci. *26*, 169–183. 10.1016/j.tplants.2020.09.008.

159. Vélez-Bermúdez, I.C., and Schmidt, W. (2023). Plant strategies to mine iron from alkaline substrates. Plant Soil *483*, 1–25. 10.1007/s11104-022-05746-1.

160. Riaz, N., and Guerinot, M.L. (2021). All together now: regulation of the iron deficiency response. J. Exp. Bot. *72*, 2045–2055. 10.1093/jxb/erab003.

161. Robe, K., Izquierdo, E., Vignols, F., Rouached, H., and Dubos, C. (2021). The Coumarins: Secondary Metabolites Playing a Primary Role in Plant Nutrition and Health. Trends Plant Sci. *26*, 248–259. 10.1016/j.tplants.2020.10.008.

162. Schmid, N.B., Giehl, R.F.H., Doll, S., Mock, H.-P., Strehmel, N., Scheel, D., Kong, X., Hider, R.C., and Von Wiren, N. (2014). Feruloyl-CoA 6'-Hydroxylase1-Dependent Coumarins Mediate Iron Acquisition from Alkaline Substrates in Arabidopsis. PLANT Physiol. *164*, 160–172. 10.1104/pp.113.228544.

163. Rajniak, J., Giehl, R.F.H., Chang, E., Murgia, I., Von Wirén, N., and Sattely, E.S. (2018). Biosynthesis of redox-active metabolites in response to iron deficiency in plants. Nat. Chem. Biol. *14*, 442–450. 10.1038/s41589-018-0019-2.

164. Harbort, C.J., Hashimoto, M., Inoue, H., Niu, Y., Guan, R., Rombolà, A.D., Kopriva, S., Voges, M.J.E.E.E., Sattely, E.S., Garrido-Oter, R., et al. (2020). Root-Secreted Coumarins and the Microbiota Interact to Improve Iron Nutrition in Arabidopsis. Cell Host Microbe *28*, 825-837.e6. 10.1016/j.chom.2020.09.006.

165. Voges, M.J.E.E.E., Bai, Y., Schulze-Lefert, P., and Sattely, E.S. (2019). Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. Proc. Natl. Acad. Sci. *116*, 12558–12565. 10.1073/pnas.1820691116.

166. Boiteau, R.M., Markillie, L.M., Hoyt, D.W., Hu, D., Chu, R.K., Mitchell, H.D., Pasa-Tolic, L., Jansson, J.K., and Jansson, C. (2021). Metabolic Interactions between *Brachypodium* and Pseudomonas fluorescens under Controlled Iron-Limited Conditions. mSystems *6*, e00580-20. 10.1128/mSystems.00580-20.

167. Jurkevitch, E., Hadar, Y., Chen, Y., Chino, M., and Mori, S. (1993). Indirect utilization of the phytosiderophore mugineic acid as an iron source to rhizosphere fluorescent Pseudomonas. Biometals *6*. 10.1007/BF00140113.

168. McRose, D.L., Li, J., and Newman, D.K. (2023). The chemical ecology of coumarins and phenazines affects iron acquisition by pseudomonads. Proc. Natl. Acad. Sci. *120*, e2217951120. 10.1073/pnas.2217951120.

169. Liu, Y., Shu, X., Chen, L., Zhang, H., Feng, H., Sun, X., Xiong, Q., Li, G., Xun, W., Xu, Z., et al. (2023). Plant commensal type VII secretion system causes iron leakage from roots to promote colonization. Nat. Microbiol., 1–16. 10.1038/s41564-023-01402-1.

170. Dar, D., Thomashow, L.S., Weller, D.M., and Newman, D.K. (2020). Global landscape of phenazine biosynthesis and biodegradation reveals species-specific colonization patterns in agricultural soils and crop microbiomes. eLife *9*, e59726. 10.7554/eLife.59726.

171. Chiou, T.-J., and Lin, S.-I. (2011). Signaling Network in Sensing Phosphate Availability in Plants. Annu. Rev. Plant Biol. *62*, 185–206. 10.1146/annurev-arplant-042110-103849.

172. Dindas, J., DeFalco, T.A., Yu, G., Zhang, L., David, P., Bjornson, M., Thibaud, M.-C., Custódio, V., Castrillo, G., Nussaume, L., et al. (2022). Direct inhibition of phosphate transport by immune signaling in Arabidopsis. Curr. Biol. *32*, 488-495.e5. 10.1016/j.cub.2021.11.063.

173. Hacquard, S., Kracher, B., Hiruma, K., Münch, P.C., Garrido-Oter, R., Thon, M.R., Weimann, A., Damm, U., Dallery, J.-F., Hainaut, M., et al. (2016). Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. Nat. Commun. *7*, 11362. 10.1038/ncomms11362.

174. Hiruma, K., Gerlach, N., Sacristán, S., Nakano, R.T., Hacquard, S., Kracher, B., Neumann, U., Ramírez, D., Bucher, M., O'Connell, R.J., et al. (2016). Root Endophyte Colletotrichum tofieldiae Confers Plant Fitness Benefits that Are Phosphate Status Dependent. Cell *165*, 464–474. 10.1016/j.cell.2016.02.028.

175. Shi, J., Zhao, B., Zheng, S., Zhang, X., Wang, X., Dong, W., Xie, Q., Wang, G., Xiao, Y., Chen, F., et al. (2021). A phosphate starvation response-centered network regulates mycorrhizal symbiosis. Cell *184*, 5527-5540.e18. 10.1016/j.cell.2021.09.030.

176. MacQueen, A., and Bergelson, J. (2016). Modulation of R-gene expression across environments. J. Exp. Bot. *67*, 2093–2105. 10.1093/jxb/erv530.

177. Berens, M.L., Wolinska, K.W., Spaepen, S., Ziegler, J., Nobori, T., Nair, A., Krüler, V., Winkelmüller, T.M., Wang, Y., Mine, A., et al. (2019). Balancing trade-offs between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk. Proc. Natl. Acad. Sci. *116*, 2364–2373. 10.1073/pnas.1817233116.

178. Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. *28*, 489–521. 10.1146/annurev-cellbio-092910-154055.

179. Hou, S., Thiergart, T., Vannier, N., Mesny, F., Ziegler, J., Pickel, B., and Hacquard, S. (2021). A microbiota–root–shoot circuit favours Arabidopsis growth over defence under suboptimal light. Nat. Plants *7*, 1078–1092. 10.1038/s41477-021-00956-4.

180. Loo, E.P.-I., Tajima, Y., Yamada, K., Kido, S., Hirase, T., Ariga, H., Fujiwara, T., Tanaka, K., Taji, T., Somssich, I.E., et al. (2022). Recognition of Microbe- and Damage-Associated Molecular Patterns by Leucine-Rich Repeat Pattern Recognition Receptor Kinases Confers Salt Tolerance in Plants. Mol. Plant-Microbe Interactions® *35*, 554–566. 10.1094/MPMI-07-21-0185-FI.

181. Espinoza, C., Liang, Y., and Stacey, G. (2017). Chitin receptor CERK1 links salt stress and chitin-triggered innate immunity in Arabidopsis. Plant J. Cell Mol. Biol. *89*, 984–995. 10.1111/tpj.13437.

182. Finkel, O.M., Castrillo, G., Herrera Paredes, S., Salas González, I., and Dangl, J.L. (2017). Understanding and exploiting plant beneficial microbes. Curr. Opin. Plant Biol. *38*, 155–163. 10.1016/j.pbi.2017.04.018.

183. Li, H., La, S., Zhang, X., Gao, L., and Tian, Y. (2021). Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. ISME J. *15*, 2865–2882. 10.1038/s41396-021-00974-2.

184. He, D., Singh, S.K., Peng, L., Kaushal, R., Vílchez, J.I., Shao, C., Wu, X., Zheng, S., Morcillo, R.J.L., Paré, P.W., et al. (2022). Flavonoid-attracted Aeromonas sp. from the Arabidopsis root microbiome enhances plant dehydration resistance. ISME J. *16*, 2622–2632. 10.1038/s41396-022-01288-7.

185. Carrión, V.J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., De Hollander, M., Ruiz-Buck, D., Mendes, L.W., Van Ijcken, W.F.J., Gomez-Exposito, R., Elsayed, S.S., et al. (2019). Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science *366*, 606–612. 10.1126/science.aaw9285.

186. Santos-Medellín, C., Liechty, Z., Edwards, J., Nguyen, B., Huang, B., Weimer, B.C., and Sundaresan, V. (2021). Prolonged drought imparts lasting compositional changes to the rice root microbiome. Nat. Plants *7*, 1065–1077. 10.1038/s41477-021-00967-1.

187. Satoh, Y., Yoshimura, K., Pokhrel, Y., Kim, H., Shiogama, H., Yokohata, T., Hanasaki, N., Wada, Y., Burek, P., Byers, E., et al. (2022). The timing of unprecedented hydrological drought under climate change. Nat. Commun. *13*, 3287. 10.1038/s41467-022-30729-2.

188. Yang, N., Nesme, J., Røder, H.L., Li, X., Zuo, Z., Petersen, M., Burmølle, M., and Sørensen, S.J. (2021). Emergent bacterial community properties induce enhanced drought tolerance in Arabidopsis. Npj Biofilms Microbiomes *7*, 82. 10.1038/s41522-021-00253-0.

189. Sessitsch, A., Pfaffenbichler, N., and Mitter, B. (2019). Microbiome Applications from Lab to Field: Facing Complexity. Trends Plant Sci. *24*, 194–198. 10.1016/j.tplants.2018.12.004.

190. Xia, J., Wang, G., Lin, J., Wang, Y., Chu, J., Zhuang, Y., and Zhang, S. (2016). Advances and Practices of Bioprocess Scale-up. In Bioreactor Engineering Research and Industrial Applications II Advances in Biochemical Engineering/Biotechnology., J. Bao, Q. Ye, and J.-J. Zhong, eds. (Springer), pp. 137–151. 10.1007/10_2014_293.

191. Berninger, T., González López, Ó., Bejarano, A., Preininger, C., and Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. Microb. Biotechnol. *11*, 277–301. 10.1111/1751-7915.12880.

Figures

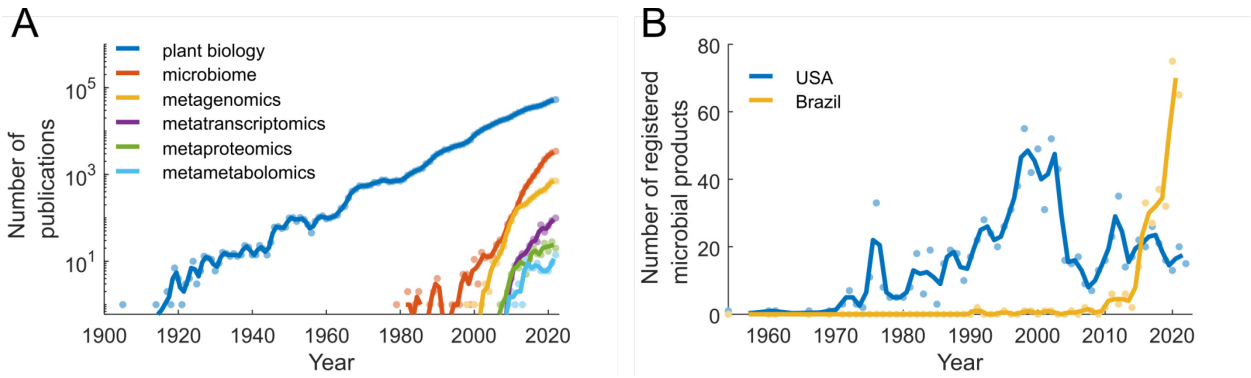

Figure 1. The pace of basic plant microbiome research far exceeds that of translation into registered microbial products in agriculture. (A) The number of articles over time among different categories of plant research in the PubMed database. **(B)** The number of actively registered microbial products per year in Brazil and the USA. Data (dots) are smoothed with a sliding window of two data points (solid line). Data for Brazil obtained from Meyer et al., 2022 [23]. Data for the USA obtained from the United States Environmental Protection Agency Active Pesticide Product Registration Informational Listing. https://ordspub.epa.gov/ords/pesticides/f?p=APPRIL_PUBLIC:2

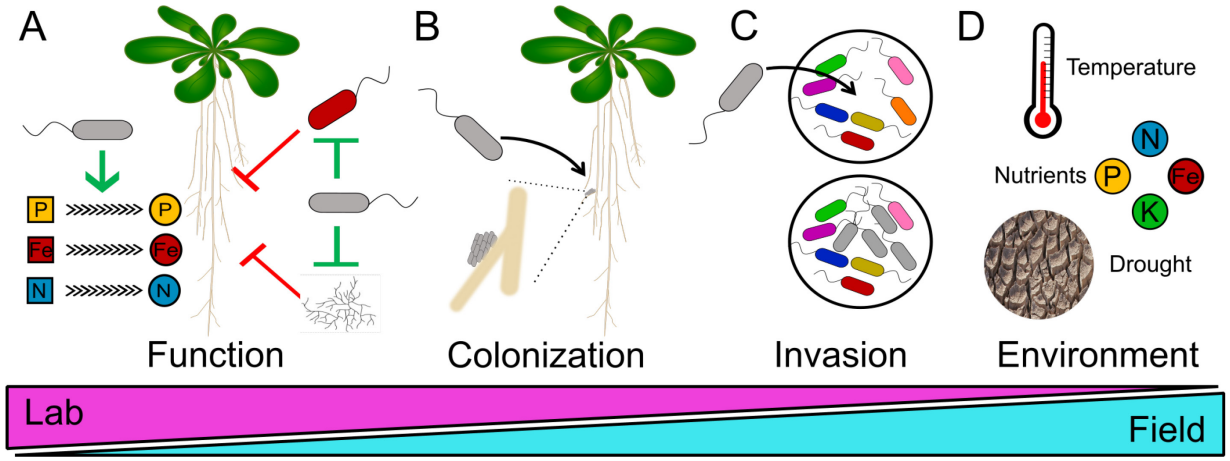

Figure 2. Requirements of microbes for improved plant productivity. For a microbe (focal strain, gray) to be used to enhance plant productivity, it must satisfy a few demands: **(A)** It must have a beneficial *function*, for example, direct or indirect inhibition of a pathogen or provision of a nutrient like iron (Fe), Phosphate (P), or Nitrogen (N), available to the plant; **(B)** it needs to *colonize* the right plant organ and tissue; **(C)** it must *invade*, at least temporarily, the pre-established heterogeneous microbial community; **(D)** and finally, it must do all of this while exposed to a potentially unstable *environment*. While some of those demands can be screened for and tested in the laboratory, all traits destined for deployment ultimately need to be tested under field conditions.

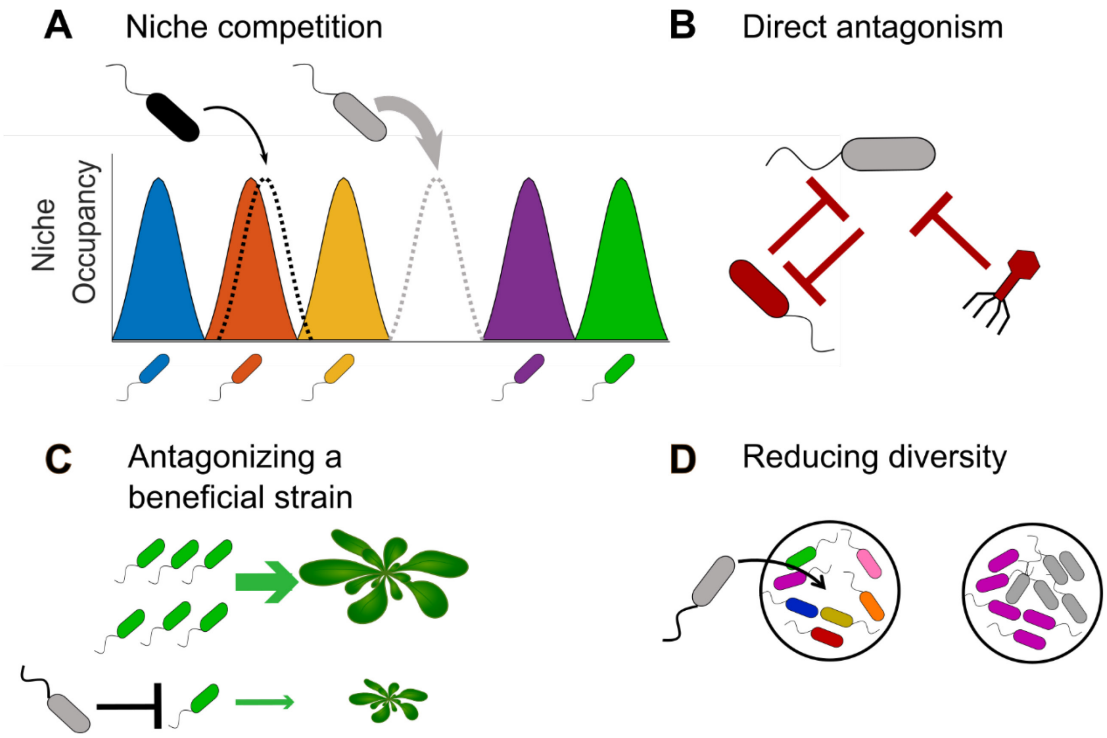

Figure 3. Invading a pre-established community, bacteria face two major types of challenges. (A) Most metabolic niches are pre-occupied by community members (colored Gaussian). It is easier for an invading bacterial strain (gray) that has a low overlap with occupied niches (dashed, gray) to invade and persist than it is for an invader (black) with a high overlap (dashed, black). **(B)** Upon infiltration into a pre-established community, an invading bacterium (gray) is attacked by both resident phages and bacteria (red). The invading microbe can create a niche for itself using diverse mechanisms. **(C)** An invading bacteria (gray) may antagonize a pre-existing beneficial taxon (green). That antagonism can reduce the abundance of the beneficial strain and lead to an overall reduction in plant performance. **(D)** A new invader (gray) into a pre-established microbial community (colored bacteria, left) can alter community assembly and lead to reduced diversity (right). That reduced diversity often has a deleterious effect on microbiome function.

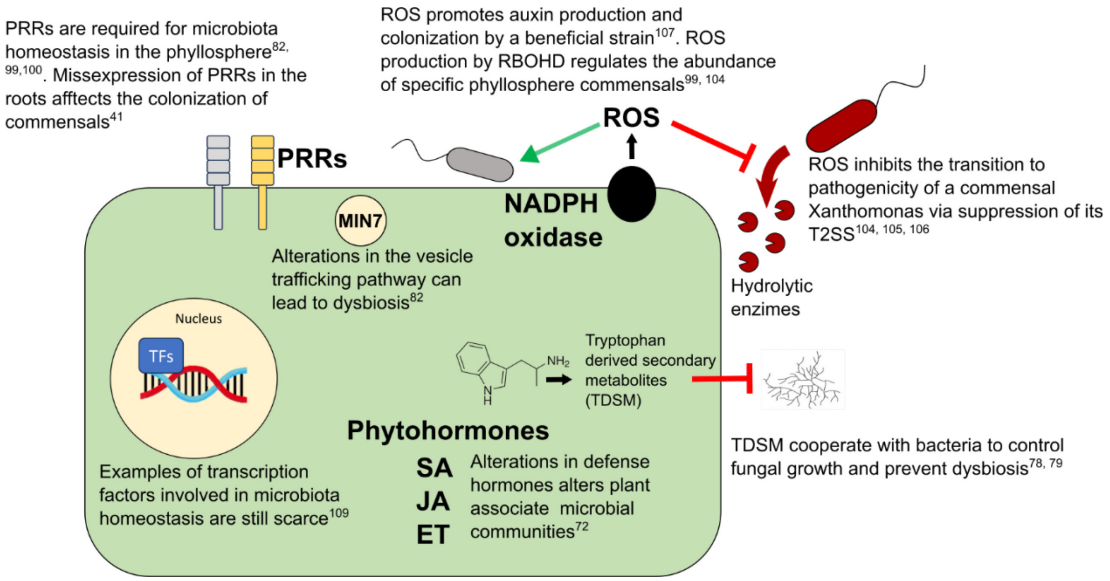

Figure 4. The plant immune system controls microbiome composition. Different components of the plant immune system have been shown to interfere with the composition of plant-associated microbial communities through different mechanisms.

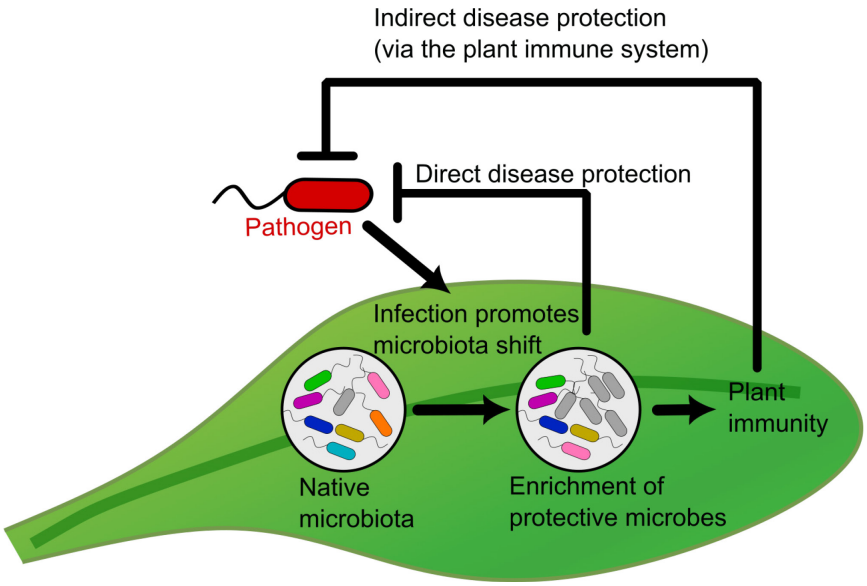

Figure 5. The microbiome can be an extension of the plant immune system. Infection of plant tissues by an invading pathogen often change the composition of the resident microbiota. Recruitment of protective microbes can occur, mitigating the impact of disease. Root exudates play a major role in re-shaping the rhizosphere microbiome during stresses. The molecular mechanisms that modulate the phyllosphere microbiome during an infection are still largely unknown. Beneficial microorganisms can protect the plant from diseases directly via microbe-microbe interactions (e.g., niche competition or production of antibiotics), or indirectly by modulating plant immunity.

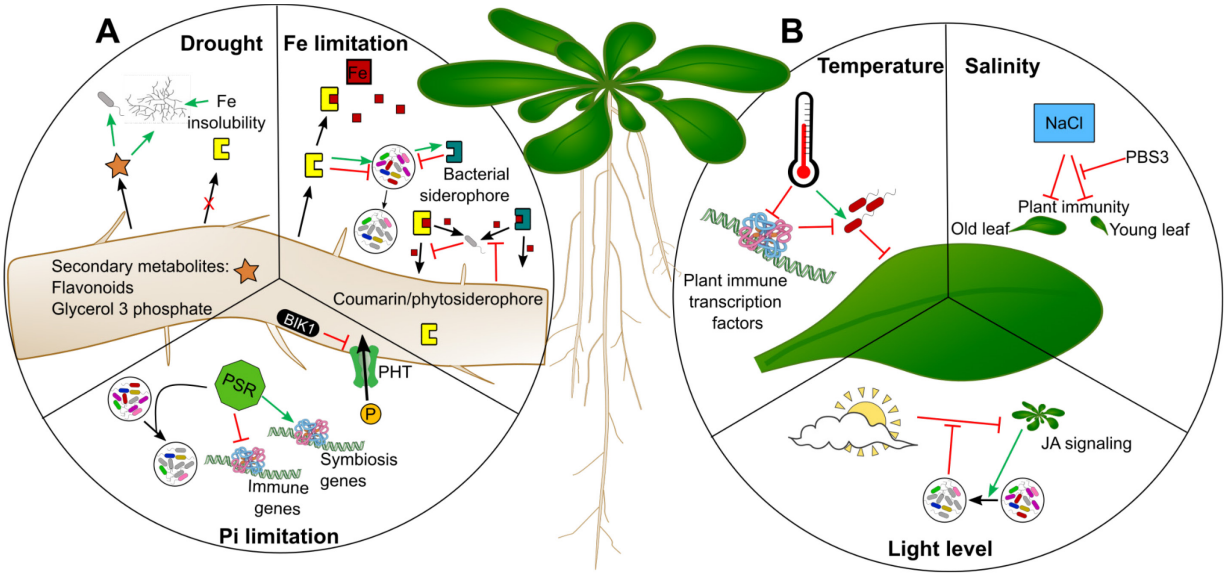

Figure 6. The environment, host plant, and microbiota interact to shape microbiome assembly and function. (A) Drought, iron limitation, and phosphate limitation influence the assembly of the root microbiome. During drought, plant excretion of secondary metabolites and the downregulation of iron uptake pathways lead to shifts in root bacterial communities, typified by enrichment of members of the phylum Actinobacteria. Plants secrete iron mobilizing compounds during iron limitation, which have mixed effects on microbial community members in the rhizosphere. Bacterial siderophores can also have large effects on the composition of root microbiota. Iron bound to plant-derived compounds can be stolen by bacteria and iron bound to bacterial siderophores can be stolen by plants. The plant phosphate starvation response (PSR) downregulates genes involved in plant immunity and upregulates genes involved in symbiosis. Mutants impaired in PSR exhibit altered root microbiota. Phosphate transporters (PHT) at the plasma membrane of root epidermal cells are directly suppressed via phosphorylation by the plant immune coreceptor BIK1. **(B)** Variation in temperature, salinity, and photosynthetically active radiation (PAR) have diverse effects on plant-microbe interactions in foliar tissue. Elevated temperatures can impair the expression of central plant immune transcription factors and increase the virulence of pathogenic bacteria. Salinity stress dampens plant immunity in old but not young leaves in a PBS3 dependent manner. Low PAR sensed in leaves alters root bacterial communities via JA signaling, which can mitigate the negative growth effects of suboptimal light levels.

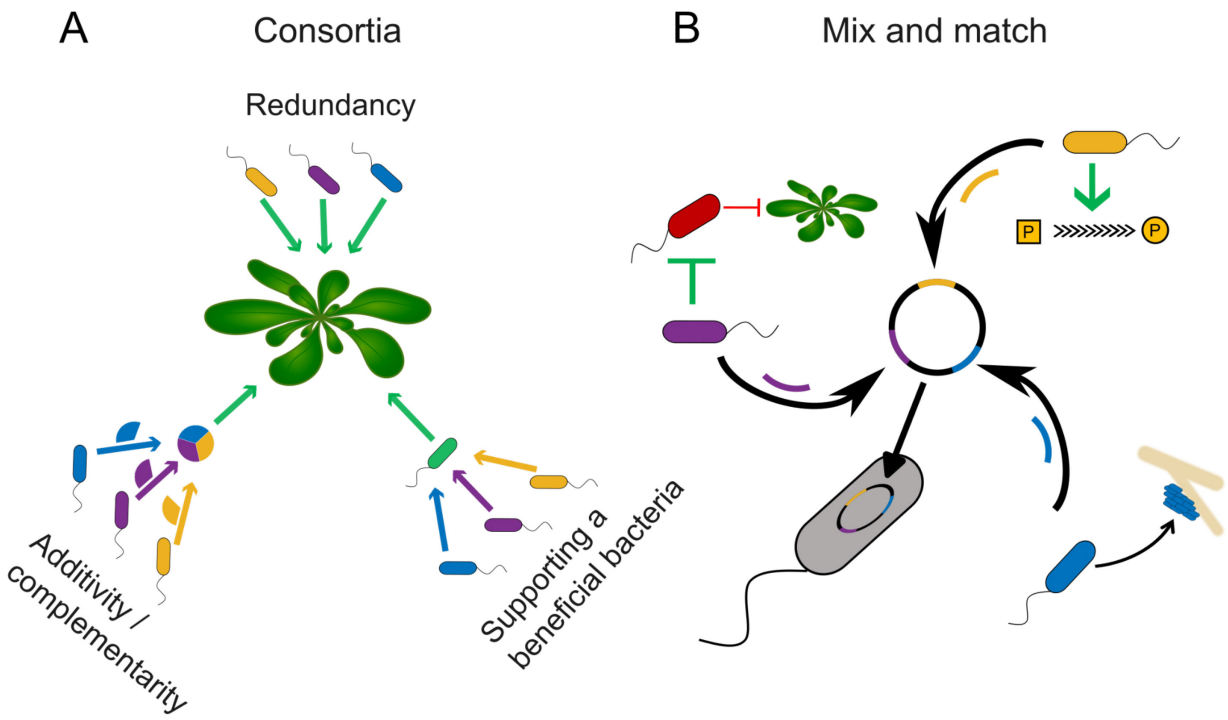

Figure 7. Future approaches to improve plant microbiome therapy. (A) Instead of inoculating plants with a single strain, treat plants with a consortium of multiple strains. Members of the consortium can have a redundant plant growth-promoting function (top); functions of consortium members can add on one another or complement each other (left); the consortia can be composed of a focal beneficial strain (green) with additional strains that support its invasion and persistence. **(B)** Engineering an optimal plant growth-promoting bacteria by mixing and matching traits from different sources. Here, a scaffold bacterium (gray) is supplemented by genes from other bacteria: pathogen antagonism (purple), phosphate solubilization (yellow), and improved root colonization (blue). The supplemented strain will perform all those tasks in one inoculant.