

Review

Deep discovery informs difficult deployment in plant microbiome science

Dor Russ,^{1,2,4} Connor R. Fitzpatrick,^{1,2,4} Paulo J.P.L. Teixeira,^{3,4} and Jeffery L. Dangl^{1,2,4,*}¹Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA²Howard Hughes Medical Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA³Department of Biological Sciences, “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil⁴These authors contributed equally*Correspondence: dangl@email.unc.edu<https://doi.org/10.1016/j.cell.2023.08.035>

SUMMARY

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. The basic definition of plant microbiota across species and ecosystems, combined with the development of reductionist experimental models and the manipulation of plant phenotypes with microbes, has fueled interest in its 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, the establishment of uneasy homeostasis, with the plant immune system, invade heterogeneous pre-established plant-associated communities, and persist in a new and potentially remodeled 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 (organ and tissue surfaces, often restricted to above-ground organs, with rhizoplane referring specifically to root surfaces) and endophytic (inter-cellular spaces of any organ) 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.^{2–4} The diversity, composition, and abundance of microbial communities vary among these habitats, across individual plants and populations or species, and environmental conditions.⁵ The importance of plant-microbe interactions for plant physiological, ecological, and evolutionary processes has long been recognized.⁶ A complex chemical dialogue between plant and microbial functions shapes colonization and microbiota assembly^{2,7} (Figure 1). Invasion of useful microbes into standing heterogeneous communities and continued delivery of phenotypic effects on plant productivity across changing environments ultimately determine microbial therapeutic efficacy (Figure 1).

Interactions with their associated microbiota were required by plants in their migration to the terrestrial environment⁸ and continue to drive contemporary plant ecology and evolution.⁹ 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.^{1,10} 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.^{11,12} Plant productivity is increasingly compromised in agriculture and silviculture due to the combined effects of climate change,¹³ soil degradation, and increasing pressure from pathogens, parasites, herbivores, and plant competitors, both introduced and native.¹⁴ Traditional mitigation approaches are accompanied by high monetary, energy, and environmental costs, and exhibit diminishing returns.¹⁵ Deploying individual strains, microbial consortia, or managing existing communities to enhance or buffer plant productivity are potential interventions due to the ability of some microbes to modify plant phenotypes and mitigate abiotic and biotic stressors.^{11,16,17}

There has been an explosion of plant microbiome research in the past twenty years. Using Brazil¹⁸ and the USA (https://ordspub.epa.gov/ords/pesticides/f?p=APPRIL_PUBLIC:2) as large agronomic case studies for which data are readily available, the commercial use of microbes in agriculture has risen since their introduction in the middle of the last century (Figure 2B). Microbial products are used to inhibit plant pathogens, nematodes, and 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.¹⁹ Similarly, products based on nitrogen-fixing bacteria are largely used in

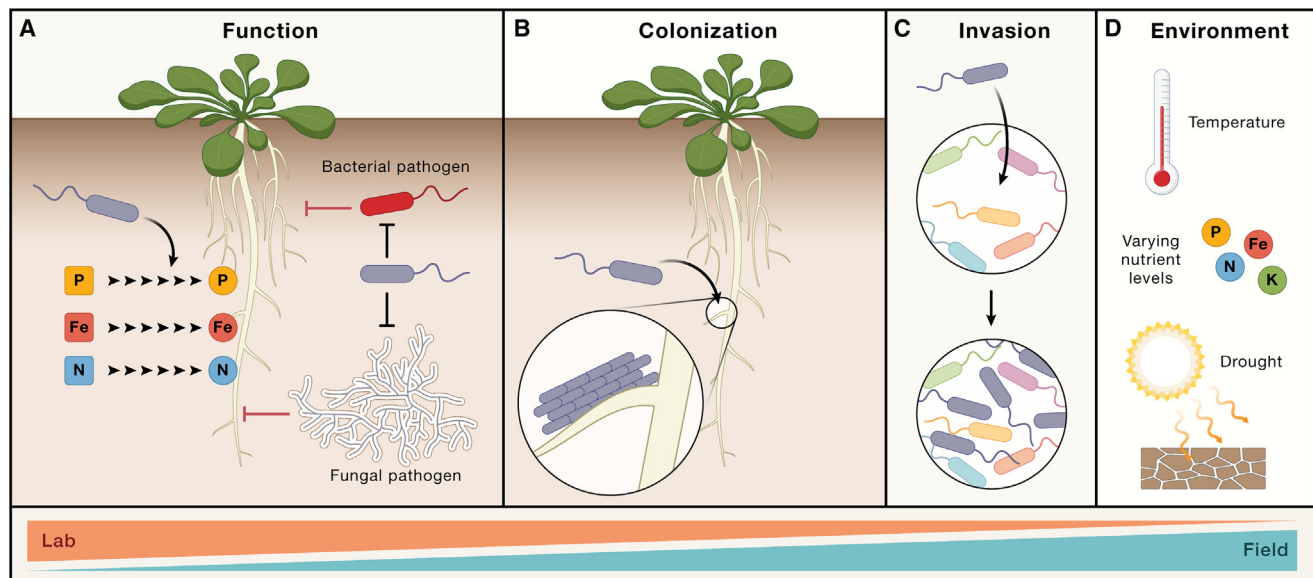


Figure 1. Requirements of microbes for improved plant productivity

(A–D) For a microbe (focal strain, gray) to enhance plant productivity, it must satisfy a few demands: (A) it must have a beneficial *function*, for example, provision of a nutrient like iron (Fe), phosphate (P), or nitrogen (N), available to the plant, or direct or indirect inhibition of a pathogen; (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*. Although some of those demands can be screened for and tested in the laboratory (function and, to some extent, colonization), others emerge only in the field (to some extent, invasion and response to the environment). Eventually, all traits destined for deployment need to be tested under field conditions.

the cultivation of legumes, such as soybeans.²⁰ A basic metric of successful deployment would be a microbe or consortium of microbes that increases plant productivity or enhances pest resistance to an extent that it could replace, in part or in whole, current chemical intervention and be economically viable as a product.

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 to, or deterred from, colonizing the plant epiphytic and, subsequently, endophytic habitats. This is due to the unique combination of chemical and physical properties surrounding, on, and within plant roots and leaves. Although navigating this novel chemical and structural milieu, a microorganism must then contend with the plant immune system, which can both limit and promote colonization in different circumstances. Once a microbe reaches an epiphytic habitat, it then competes for space and resources with other hopeful microbial colonists. 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 into 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 1). 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 efforts and translational success.²¹

PROGRESSIVE SPATIAL WINNOWING AND 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.^{2–4} Microbiota intimately associated with plant organs are mainly derived from highly complex soil communities by progressive winnowing, as best defined in roots^{3,4} but also observed in leaves.^{22,23} Following the initial high-throughput surveys that characterized microbiota composition across different plant species, organs, tissues, and environments, researchers directed efforts toward unraveling the molecular mechanisms that govern the structures and functions of microbial communities in plants.^{3,4,24,25} Microbial diversity progressively decreases from the soil environment to the rhizosphere surrounding the root and further to the root endophytic compartment, reflecting a gradient of decreasing species richness and increasing specialization within the root microbiome.^{3,4,26} During that winnowing, members of the phyla Planctomycetes and Acidobacteria, which are highly abundant in the soil, are depleted from the plants, and Proteobacteria and Actinobacteria are highly enriched in root epiphytic (rhizoplane) and endophytic tissues.^{3,4} Similarly, the phyllosphere, which refers to the aerial parts of plants such as leaves, stems, flowers,

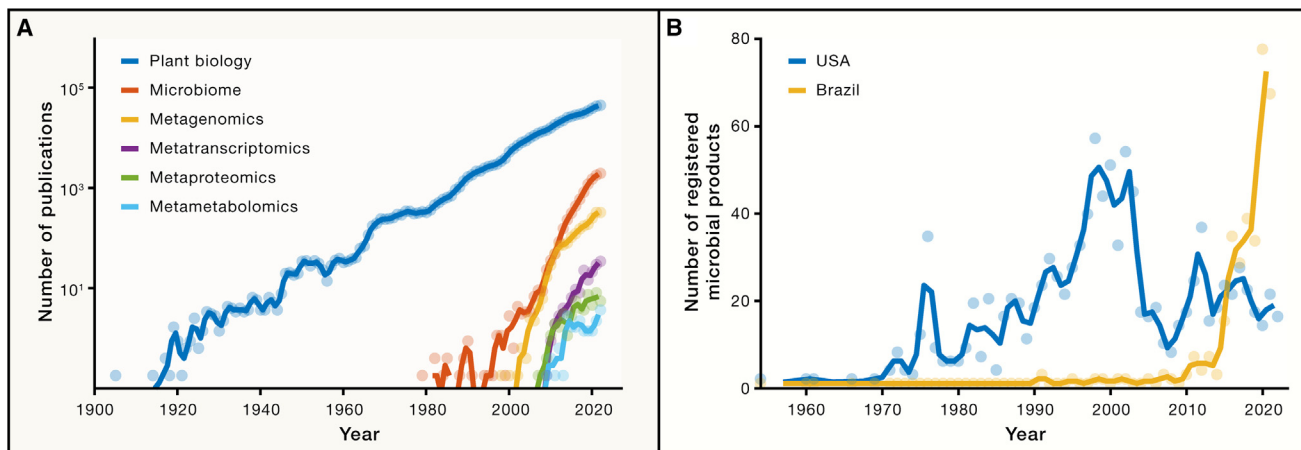


Figure 2. The pace of basic plant microbiome research far exceeds that of translation into registered microbial products in agriculture

(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 was obtained from Meyer et al.¹⁸ Data for the USA was obtained from the United States Environmental Protection Agency Active Pesticide Product Registration Informational Listing. https://ordspub.epa.gov/ords/pesticides/f?p=APRIL_PUBLIC:2.

and fruits, generally exhibits lower microbial diversity compared with 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.^{10,27} Interestingly, although the community composition of above- and below-ground tissues is different,^{28,29} large similarities are found in the functional capabilities of those communities.²⁴

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.³⁰ 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.³¹ Expanding to less specific interactions, the plant hormone strigolactone is secreted from plant roots and promotes the common symbiotic arbuscular mycorrhizal fungi (AMF).³² Alternatively, antagonist exudates like benzoxazinoids,³³ coumarins,³⁴ and triterpenes³⁵ can selectively exclude community members, and 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 with the surrounding environment thus signifies a finely tuned selection 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, although the receptor for the flagellin 22 (flg22) peptide immuno-epitope, FLS2, is expressed in all leaf mesophyll cells, its expression is restricted to specific cell types in the root.³⁶ This restriction is crucial for proper plant development.³⁷ The plant also partitions metabolite exudation, likely based on developmental and cell type-specific differentiation cues.^{38–40} For example, glucose secretion is higher from the root base than from the root tip.⁴¹ 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.⁴²

A therapeutic microbe needs to be targeted to the micro niche where its function contributes the most to plant productivity. Although it seems obvious that a direct antagonist of a leaf pathogen should be directed to the leaf and a nitrogen-fixing bacterium should be targeted to root hairs on the rhizoplane, we remain largely ignorant of how communities form into spatially restricted microcolonies in different plant tissues. Notably, spatially separated plant Sugars Will Eventually be Exported Transporters (SWEETs) and metabolites along the length of the root do drive some spatial organization of the associated bacterial microbiota.⁴⁰ There is an unmet need for further refinement to micrometer-resolution spatial mapping of strains on these plant organs^{43–45} 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 THERAPEUTIC 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

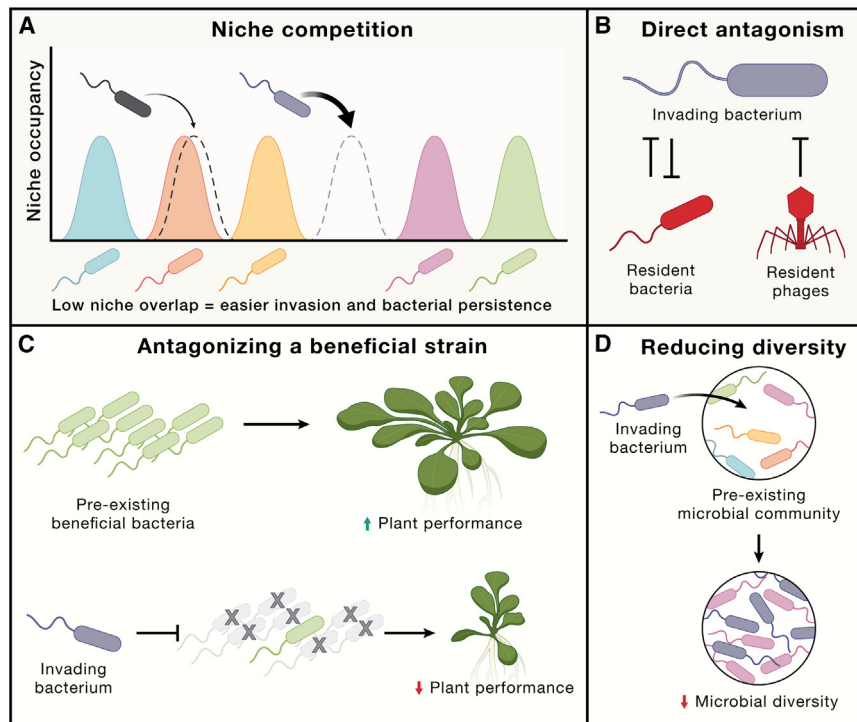


Figure 3. Invading a pre-established community, bacteria face two major types of challenges

(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.

bacterial density.⁴⁶ 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.⁴⁷ 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.⁴⁸ To infiltrate into an assembled community, an invading microbe can find an available niche by exploiting a previously unused nutrient^{49,50} (Figure 3A). For example, strains of the genus *Variovorax* are prevalent plant colonizers capable of invading a pre-established community.⁵¹ *Variovorax* assimilate auxin to exploit a specialized niche in the root microbiome.⁵² 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,⁵³ 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.⁵⁴ 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).

Antagonistic mechanisms can limit microbial invasion

Many microbes produce antagonistic agents to extend their niche and improve fitness in a diverse microbial community.⁵⁵

The antimicrobial agents produced in microbiome warfare are the source of most known commercial antibiotics.⁵⁶ Members of both the root⁵⁷ and the shoot⁵⁸ microbiome produce antimicrobial compounds (Figure 3B). Although the specific compounds that mediate microbe-microbe interactions 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*.⁵⁷ Although 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.^{59,60} 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.⁶¹ 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.⁶² Phages can limit the growth of highly abundant species, according to the “kill the winner” hypothesis,⁶³ and alter bacteria-bacteria competition and bacterial evolution.⁶⁴ 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 presumably adds a constraint to the evolution of phage resistance. The evolutionary trajectory of phage resistance *in planta* is different from evolution in rich media.⁶⁵ Additionally, potassium availability limits phage evolution *in planta*.⁶⁶

The diverse mechanisms by which stable natural communities prevent the invasion of new species are 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.

Different mechanisms are deployed 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 pre-existing microbial community. Inhibition of closely related bacteria, which often share similar capabilities, can open a new niche for colonization.⁵¹ An invader may deploy bacteriocins that specifically inhibit bacteria that are similar to the producer and benefit host colonization.^{67,68} 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 6 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.⁶⁹ For example, T6SS helps *Pseudomonas chlororaphis* to invade a resident wheat-associated community, which improves colonization and persistence in the wheat rhizosphere.⁷⁰ 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.⁷¹ Similarly, type 4b secretion systems (T4BSSs), 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.⁷² These examples highlight the increasing number of defined mechanisms that have 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 to study and model invasion into natural communities in a controlled system.^{51,72–74} 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.⁷⁵ Synthetic community diversity that more accurately represents real-world conditions is experimentally difficult to assemble but is 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 artificial intelligence (AI)-mediated development of combinatorial communities that can represent

the diversity of plant-associated microbial communities under field conditions.

MAINTENANCE OF A HEALTHY MICROBIOME

Community diversity as a whole is an established sign of a healthy microbiome and consequent host fitness.⁷⁶ 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 pathogen⁷⁷ (Figures 3C and 3D). 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.⁷⁸ In plants, dysbiosis is often manifested as an imbalanced equilibrium between bacteria and fungi, which leads to fungal disease,^{79,80} or the expansion of a bacterial pathogen that increases the total bacterial load.⁸¹ The plant immune system is important for maintaining a balanced microbial community. Preliminary evidence suggests that dysbiotic communities can be transferred from sick plants and cause disease symptoms in healthy plants.^{82,83} The plant microbiome plays a role in age-dependent immune maturation and hypersensitivity to pathogens by unknown mechanisms.⁸² Overall, the maintenance of a balanced microbiome is important for plant health and performance.

An invading species can alter the natural microbiome. Although 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 nutrients, or altered environmental conditions.^{84,85} 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.^{86,87} The addition of a focal therapeutic strain to an established community can drive community assembly into a new steady state, even if the invader does not survive that transition.⁸⁸ A new strain that invades the plant microbiome may also inhibit a beneficial strain or lower community diversity (Figures 3C and 3D). These collateral alterations may hinder the therapeutic strain's beneficial effect.

PLANT IMMUNITY IN MICROBIOME ASSEMBLY AND MANIPULATION

The plant immune system plays a pivotal role in safeguarding plants against invaders by orchestrating a sophisticated array of transcriptional and biochemical responses triggered upon the detection of non-self or modified-self molecules.^{89,90} Over the past three decades, research has 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

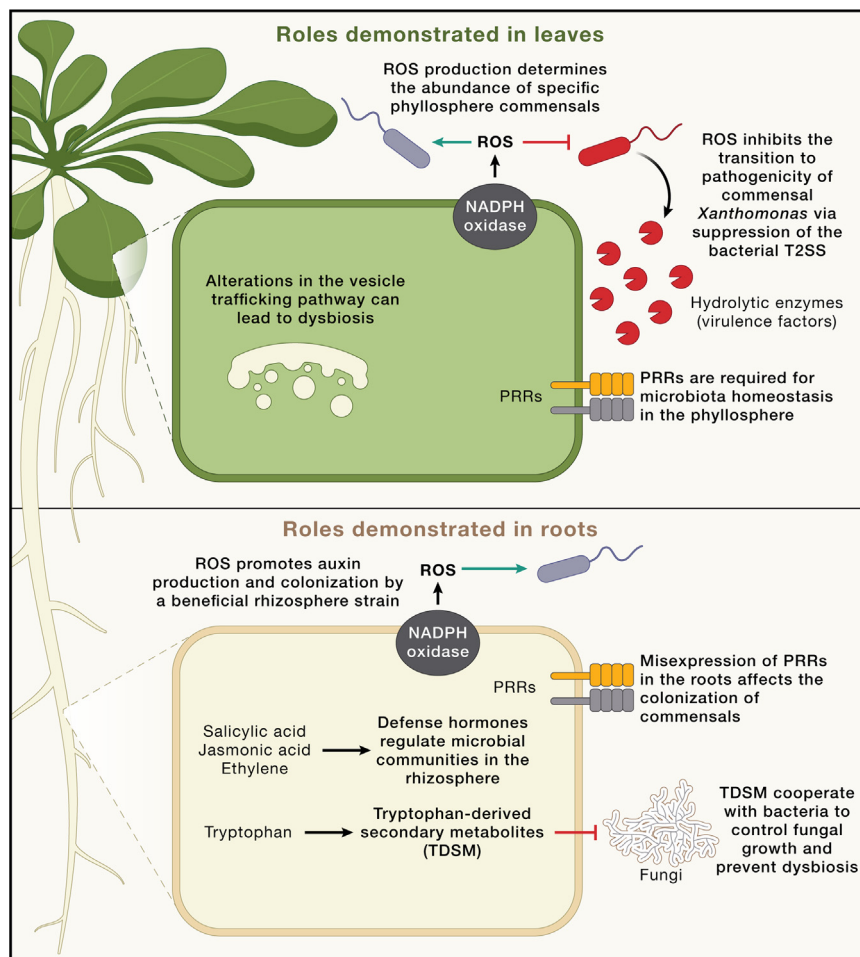


Figure 4. The plant immune system controls microbiota composition

Different components of the plant immune system in either the shoots (left) or roots (right) have been shown to influence the composition of plant-associated microbial communities. Most studies focused on a single tissue thus far, and it remains unknown whether microbiota homeostasis is maintained through the same or different mechanisms in different plant tissues. PRRs, pattern recognition receptors; ROS, reactive oxygen species; T2SS, type 2 secretion system.

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 (JA), which orchestrate defense responses against pathogens, are also required for the assembly of normal bacterial communities in both roots and leaves.^{73,95,96} 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 SA, suggesting that they can metabolize this hormone as a growth signal or carbon source.⁷³ 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.^{83,97,98}

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). By contrast, the second layer comprises intracellular receptors from the Nucleotide-Binding Leucine-Rich repeat (NLR) family that monitor the interior environment of plant cells.^{89,90,99} Although 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.^{83,98} These lesions were

The field of plant-microbe interactions has also witnessed recent remarkable advances regarding the interplay between the plant immune system and the microbiota (Figure 4).^{7,92–94} 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

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.⁸³ The significance of the immune system in microbiota assembly is further supported by findings demonstrating that mislocalization of immune receptors in root cells affects the colonization of commensals³⁷ and that full immune function is not unleashed until localized damage to plant cells is sensed in the presence of immunogenic microbial patterns.¹⁰⁰

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.⁹⁹ 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.⁹⁷ The involvement of ROS in maintaining microbial homeostasis was also reported in the phyllosphere. A screen using immunocompromised mutants demonstrated that the absence of Respiratory Burst Oxidase Homolog D (RBOHD), a Nicotinamide-Adenine Dinucleotide Phosphate (NADPH) oxidase that is responsible for extracellular ROS production during immune responses, results in significant alterations in the bacterial community within *Arabidopsis* leaves.¹⁰¹ Particularly, the *rbohD* mutant allows the proliferation of opportunistic *Xanthomonas* strains that normally grow asymptotically in wild-type plants but cause disease in the mutant. Interestingly, preliminary evidence suggests that the transition from commensalism to pathogenicity of opportunistic *Xanthomonas* is potentially prevented by ROS, which suppresses the secretion of hydrolytic enzymes by the bacterial type 2 secretion system (T2SS).^{102,103} 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.¹⁰³ 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*.¹⁰⁴ 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.⁸⁰ Interestingly, both plant-derived tryptophan metabolism and bacterial commensals are necessary to prevent excessive fungal growth,^{79,80} 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.

The microbiota modulates 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.^{7,105} Furthermore, although 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.^{102,105–109}

Screens of microbial collections reveal that immunosuppressive bacteria are common in the plant microbiota, constituting up to 65% of the evaluated strains.^{106–108} 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.¹⁰⁸ However, immunomodulation by other suppressive commensals occurs independently of extracellular acidification and, thus, is achieved by different mechanisms.¹⁰⁷ For instance, *Dyella japonica* MF79 requires the 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.¹⁰⁶ 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. Although 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 FlhC found in bacterial flagella, is a potent antigen capable of triggering immune responses in most plant species. Remarkably, commensal

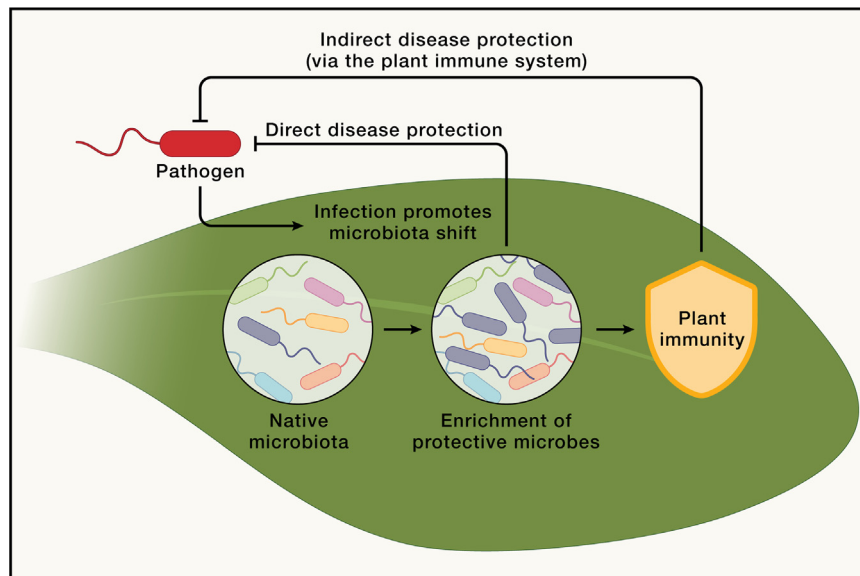


Figure 5. The microbiome can be an extension of the plant immune system

Infection of plant tissues, here a leaf, by an invading pathogen often changes the composition of the resident microbiota. Recruitment of protective microbes can occur, mitigating the impact of disease. Exudates play a major role in reshaping the 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.

plant-pathogen interactions.⁹² Recently, a 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

bacteria exhibit substantial diversity in the amino acid sequence of this MAMP, often enabling their flagellum to evade recognition by plant receptors.^{105,110} Interestingly, some microbes produce variations of the flg22 peptide that competitively inhibit plant receptors, thereby preventing the recognition of their immunogenic counterparts.^{105,110} Additional mechanisms employed by nonpathogenic microbes to evade plant immunity include the modification of MAMPs, such as chitin deacetylation by fungi,¹¹¹ sequestration of MAMPs by specialized proteins to render them unavailable to plant receptors,^{112,113} and the downregulation of MAMP expression during plant colonization.¹¹⁴ Many of these evasion mechanisms have also been described in pathogens,^{115,116} 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 systems to prevent overstimulation by the wealth of microbial molecules that are 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 are required for the activation of potent immune responses in roots.¹⁰⁰ 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

DC3000.⁶¹ Numerous other studies have identified microbes that confer protection against pathogens in different plant species, with some of them even constituting bioprotective commercial products.¹¹⁷ 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 indirect⁷ (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.^{58,118–121} By contrast, indirect protection occurs when the microbiota modulates the plant immune system or metabolism, enhancing the host's ability to combat subsequent pathogen infections.^{61,122,123} 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.^{107,124} 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.¹²⁴

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.⁸⁷ 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.^{125,126} 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.^{86,127} 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 above-ground plant organs by microorganisms that colonize the roots.¹²³ 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.^{128,129} ISR is activated and propagated in the plant in a JA- and ET-response-dependent manner.¹²³ 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.^{130–132} More recently, effectors with antimicrobial activity were preliminarily described¹³³ or identified in other fungal and oomycete pathogens,¹³⁴ suggesting that the manipulation of the plant microbiota may be a strategy commonly employed by 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.

INFLUENCE OF ENVIRONMENTAL HETEROGENEITY ON MICROBIOME ASSEMBLY RULES AND PLANT-MICROBE INTERACTIONS

Since their invasion of terrestrial Earth, plants have faced a complex and dynamic environment.¹³⁵ 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 evolution¹³⁶). Importantly, microorganisms accompanied plants throughout this evolutionary process, resulting in an integration of environmental cues with appropriate immune responses in order to maintain health and nutrition in changing environments.^{8,48} 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.¹³⁷ Changing environments can also render host plants more vulnerable to microbial pathogens and para-

sites.^{12,138} 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.¹²

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.¹³⁹ However, most research to date shows that the effects of environmental heterogeneity on the assembly of the plant microbiome occur indirectly through plant responses.^{25,140,141} 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.^{34,142,143} 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*¹⁴⁴ (Figure 6B). This shift is conserved across major lineages of flowering plants and requires living plant roots.²⁵ 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.^{143,145} For example, *Sorghum bicolor* suppresses its iron uptake during drought by downregulating the biosynthesis and transport of the phytosiderophore mugineic acid.¹⁴³ 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.¹⁴³

Iron limitation in soils and corresponding plant and bacterial responses to bio-available iron are emerging as major drivers of plant-microbe dynamics.¹⁴⁶ 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.¹⁴⁷ During iron stress, plants activate a coordinated molecular and physiological response to scavenge scarce iron from soil.¹⁴⁸ 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, that improve the phytoavailability of iron by both mobilization and reduction.¹⁴⁹ 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^{34,143,150,151}

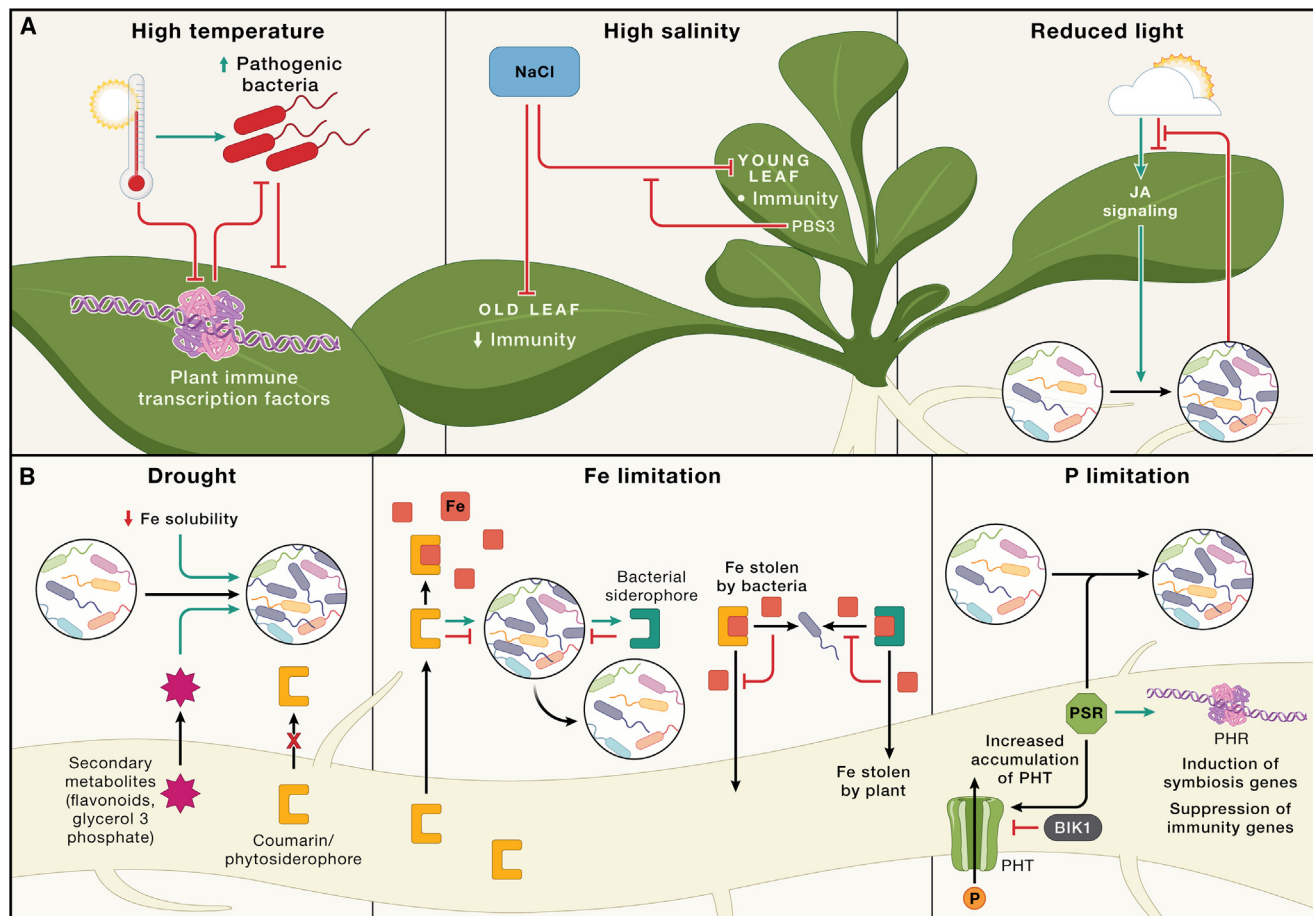


Figure 6. The environment, host plant, and microbiota interact to shape microbiome assembly and function

(A) Variations 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.

(B) 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 mobilization and 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 (PHTs) at the plasma membrane of root epidermal cells are directly suppressed via phosphorylation by the plant immune coreceptor BIK1.

(Figure 6B). Due to their ability to generate ROS, coumarins can have direct antagonistic activity against diverse root-inhabiting microorganisms, including commensal bacteria and fungal pathogens.^{34,150,151} However, under iron-limited conditions, bacteria can also benefit from the iron bound to plant-derived compounds, including coumarins.^{152,153} Additionally, commensal bacteria can induce iron leakage from roots to facilitate colonization.¹⁵⁴ 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.^{57,119} 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.⁵⁷ Evidence from a large-scale metagenomic study supports the notion that competition for essential nutrients that vary across environments, including iron, is a widespread feature in the plant microbiome.¹⁵⁵

Phosphate (P) 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 6B). Plants deploy a phosphate starvation response (PSR) that includes an increase in lateral root formation and the accumulation of H⁺-coupled phosphate transporters of the Phosphate Transporter 1 (PHT1) family at the plasma membrane of root epidermal cells.¹⁵⁶ In *Arabidopsis*, mutants impaired in PSR assemble irregular root microbiota in the absence of

phosphate limitation.^{140,142} This is explained by the finding that the PSR transcriptional regulator, Phosphate Starvation Response 1 (PHR), jointly regulates plant responses to phosphate limitation and suppresses a large sector of plant immunity.¹⁴² PHT-mediated phosphate uptake is suppressed by direct phosphorylation by the MTI-activated Botrytis-Induced Kinase 1 (BIK1).¹⁵⁷ *Arabidopsis* does not engage in symbioses with AMF to meet phosphate needs like many other plants, but was recently shown to suppress plant immunity under low phosphate levels.^{157–159} This enables colonization by the beneficial fungal endophyte *Colletotrichum tofieldiae*, which provides phosphate to the plant.¹⁵⁹ In rice, PHRs promote the expression of arbuscular mycorrhizal symbiosis genes under phosphate limitation, whereas under conditions of high phosphate, this expression is suppressed.¹⁶⁰

Crosstalk modulates plant immune system and abiotic stress

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 Effector triggered immunity (ETI)-associated genes.¹⁶¹ For example, elevated temperature leads to reduced formation of the transcriptional complex required for the expression of master immune transcription factors¹³⁸ (Figure 6A). Plant responses to various forms of abiotic stress also often lead to complex antagonistic effects on plant immunity through the suppression of the JA and SA defense pathways.^{162,163} For example, the antagonistic effects of salinity and abscisic acid (ABA) signaling on SA-mediated plant immunity are dependent on leaf age via AvrPphB Susceptible 3 (PBS3)¹⁶² (Figure 6A). Additionally, such interactions can span multiple plant organs, and 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 signaling¹⁶⁴ (Figure 6A). Finally, plant responses to environmental stress can share signaling components with plant immune pathways,^{165,166} 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.^{48,94}

Microorganisms can deliver stress relief to plants

Microorganisms may enhance the maintenance of plant health and nutrition under various forms of abiotic stress.^{11,12} 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.¹⁶⁷ 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 impor-

tant 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.^{168–170} 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 stress-enriched microorganisms is not guaranteed.^{140,171} There are many potential explanations for such an outcome, but the simplest is that the plant microbiome represents a collection of microbial niches that can each be idiosyncratically altered by environmental variation. Microbial exploitation of any given 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.^{13,172} Therefore, greater effort is required to understand how environmental heterogeneity will impact the assembly and function of plant microbiota.

CONCLUSIONS 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. Although our knowledge is growing exponentially (Figure 2), there is much to learn before the promise of rational design in the plant microbiome for improved plant growth is realized.²¹ What additional plant performance-promoting traits are there to be discovered? How can we 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

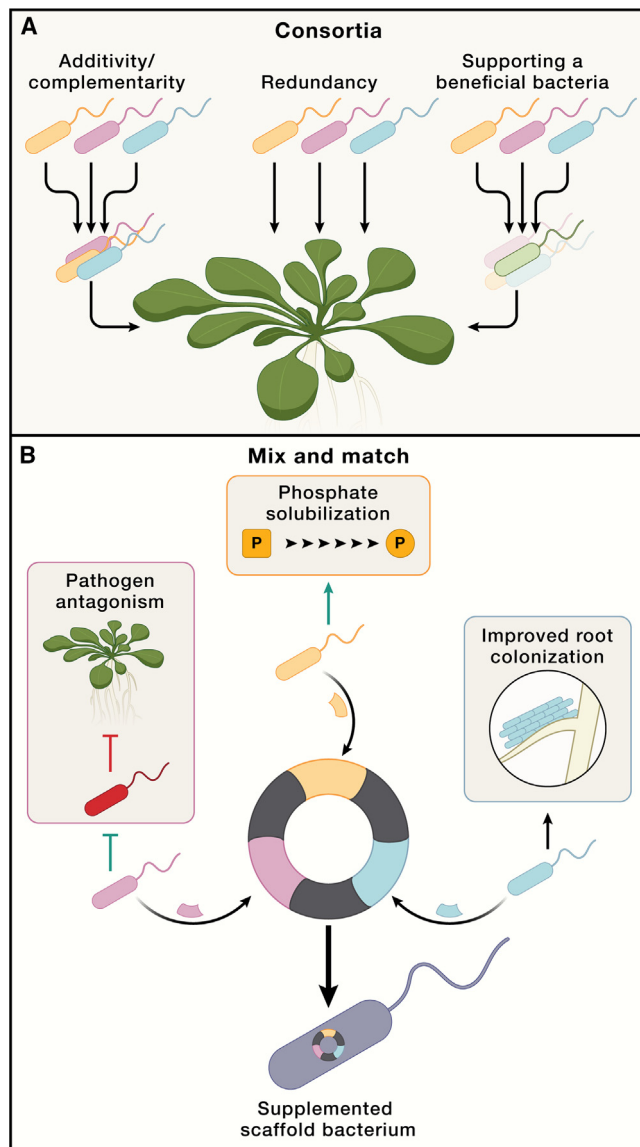


Figure 7. Future approaches to improve plant microbiome therapy
(A) Instead of inoculating plants with a single strain, plants could be treated with a consortium of multiple strains. Members of the consortium can have a redundant plant growth-promoting function (top), the functions of consortium members can add on one another or complement each other (left), and 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 bacterium 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.

microorganisms into plant habitats during diverse environmental conditions will yield new traits of interest to screen across microbial culture collections but in ecologically more realistic experimental conditions. Furthermore, a broad understanding of how microbes of interest interact with plant immunity is fundamental for the efficient manipulation of microbiomes in agricultural contexts.

Although current products are usually composed of one strain, consortia of multiple strains could have advantages (Figure 7A). Designing successful consortia could include the 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 invading a stable sub-niche of these that delivers multiple plant-beneficial phenotypes. Because invasion and persistence are major hurdles, consortia might also be composed of a focal plant growth-promoting strain accompanied by helper strains that promote ideal conditions for its colonization. The desired functions of consortia members could 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. In one example, drought-protecting biofilm emerges only when consortia members are applied together.¹⁷³ A roadmap for how to define candidate strains for such small consortia based on nutrient utilization profiles in combination with strain-based functional profiling was recently published¹⁷⁴ Functional consortia add complexity and thus require more knowledge and a deeper mechanistic understanding of each system. But, although there might be advantages to the development of consortia, there are still immense challenges to registration and large-scale fermentation and formulation of such products at scale.^{175–177}

An alternative approach is to combine traits instead of combining strains (Figure 7B). Although environmental regulation is a major barrier for the release of genetically engineered strains, an 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. A 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.

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AUTHOR CONTRIBUTIONS

Conceptualization, all authors; initial draft, D.R., C.R.F., and P.J.P.L.T.; 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.

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