



Plant–microbiome interactions: from community assembly to plant health

Pankaj Trivedi¹✉, Jan E. Leach¹, Susannah G. Tringe², Tongmin Sa³ and Brajesh K. Singh^{4,5}

Abstract | Healthy plants host diverse but taxonomically structured communities of microorganisms, the plant microbiota, that colonize every accessible plant tissue. Plant-associated microbiomes confer fitness advantages to the plant host, including growth promotion, nutrient uptake, stress tolerance and resistance to pathogens. In this Review, we explore how plant microbiome research has unravelled the complex network of genetic, biochemical, physical and metabolic interactions among the plant, the associated microbial communities and the environment. We also discuss how those interactions shape the assembly of plant-associated microbiomes and modulate their beneficial traits, such as nutrient acquisition and plant health, in addition to highlighting knowledge gaps and future directions.

Holobiont

A plant and the members of its associated microbiota considered as a single entity; this represents the 'unit of selection' at which plant–microbiome interactions have probably co-evolved in order to maintain host functionality and fitness over ecological and even evolutionary timescales.

¹Microbiome Network and Department of Agricultural Biology, Colorado State University, Fort Collins, CO, USA.

²Department of Energy Joint Genome Institute, Berkeley, CA, USA.

³Department of Environmental and Biological Chemistry, Chungbuk National University, Cheongju, Republic of Korea.

⁴Hawkesbury Institute for the Environment, Western Sydney University, Penrith South, NSW, Australia.

⁵Global Centre for Land Based Innovation, Western Sydney University, Penrith South, NSW, Australia.

✉e-mail: Pankaj.trivedi@colostate.edu

<https://doi.org/10.1038/s41579-020-0412-1>

Plants provide a multitude of niches for the growth and proliferation of a diversity of microorganisms, including bacteria, fungi, protists, nematodes and viruses (the plant microbiota). These microorganisms can form complex co-associations with plants and have important roles in promoting the productivity and health of the plant in natural environments. It has been proposed¹ that plants and the associated microbiota form a 'holobiont', whereby evolutionary selection between plants and microorganisms contributes to the overall stability of the system, but this concept has yet to be conclusively demonstrated. Complex plant microbial communities contain taxa from diverse phyla and have deep branching lineages at lower phylogenetic resolution. In recent years, culture-independent high-throughput sequencing has greatly expanded the repertoire of microorganisms known to reside in and on plants as well as in the surrounding environment^{2–19}. Many studies have noted the presence of a subset of microbial lineages, designated the 'core microbiota', that reproducibly associates with a given host across a wide range of environments^{7,12,13,18,20}. Among the plant-associated microbiota, bacteria and to lesser extent fungi are the most dominant forms and are relatively well-studied compared with other members of the community, but other groups — such as archaea, algae, nematodes and protists — also have important roles in plant functioning and health^{21,22}. Genome-wide association studies (GWASs) and metagenome-wide association studies have linked individual microbial taxa and genes to plant colonization, physiology and fitness^{16–18} (BOX 1, Supplementary text S1). Genomics and multi-'omics' have enabled the identification and characterization of genes that govern plant interactions

with the associated microbiomes^{23–26}, thus increasing our understanding of how microorganisms adapt to the plant environment. Numerous genes involved in plant adaptation have been identified that are consistent across phylogenetically diverse bacterial taxa, and a few genes related to plant colonization are even shared with plant-associated eukaryotes²³. The products of these shared genes mimic plant proteins and function as decoys to allow the microbiota to outcompete plant defences for efficient colonization. However, the molecular mechanisms that govern plant–microorganism interactions at a community level are still not well understood.

The members of a plant microbiota comprise beneficial, neutral and pathogenic microorganisms. Microbial communities associated with their hosts have been shown to promote plant growth, nutrient uptake and pathogen resistance^{27–31}. Although individual members of plant-associated microbial communities can possess certain beneficial traits^{30,31}, the manifestation of a trait in the community is an emergent property that cannot be predicted from the individual members. For example, *Pseudomonas* spp. can suppress plant pathogens through antibiosis and competition, yet overall soil disease suppressiveness is an emergent property that depends on multiple factors, including the population dynamics of the pathogen, the genetic backgrounds of both the pathogen and host, biotic and abiotic conditions, and the composition and diversity of the plant microbiota^{32–34}. The benefits conferred by the microorganisms to their host plants can be direct, including transformation and translocation of essential nutrients in the soil to make them available to plants (for example, nitrogen fixation),

Box 1 | Host and environmental factors

Ecological theories suggest that the plant-associated microbiome is shaped by complex interactions among the host, microorganisms and the environment. Genome-wide association studies have shown that the host genome does influence microbiome composition, and many heritable taxa belong to the core microbiome, which suggests positive feedback from the plant microbiome over evolutionary timescales. Several quantitative trait loci associated with biotic and abiotic stress adaptation or morphological and physiological traits have been identified that underlie variation in the microbiome traits, species richness and community structure in roots and phyllosphere. Metabolic diversification of root exudates within the plant kingdom may provide a basis for communication and recognition that directs the assembly and maintenance of a distinct microbiota tailored to the needs of the host^{115,127–129}. Comparative genomics and multi-'omics'-based analysis has demonstrated that metabolic synchronization between plant exudation traits and the microbial substrate's utilization traits results in the predictable temporal patterns of microbial community assembly observed in the rhizospheres of different plant species^{102,127}. Interplay between the microbiome, defence mechanisms (for example, cell wall and cuticle) and multilayered surveillance systems (involving pattern recognition receptors (PRRs) and hormones) has a critical role in maintaining microbial homeostasis, by activating either symbiotic responses that promote microbial colonization or immune responses that limit it. For example, diversification of microorganism-associated molecular patterns (MAMPs) and their PRRs in different hosts can have a fundamental role in the evasion of MAMP-triggered immunity surveillance, thus enabling the assembly of distinct plant-associated microbial communities in natural plant populations in response to local environments^{130,131}. Plants also recruit a 'stress microbiome' that provides protection against various abiotic or biotic stresses. Selective enrichment of the stress-tolerant microbiome is driven by differences in plant metabolism and immune-associated traits^{107,128}. This reinforces the functional links between immunity and nutrition and suggests that plants may repurpose the same stress pathways to effectively respond to both biotic and abiotic stresses. Changes in the microbial seed banks in bulk soil with long-term intensive crop management practices such as heavy fertilization¹³², tillage¹³³ or organic farming¹³⁴ affect the assemblages of the plant-associated microbiome. Agriculture intensification decreases network connectivity and the abundance of keystone taxa, which suggests that intensively farmed systems are less resilient to environmental stresses than are those managed by low-intensity practices such as organic farming^{134,135}. Given the diversity of microorganisms that colonize plants, an emerging focus in the field of plant microbiomes is the role of multikingdom interactions in shaping microbial communities. Although more work is needed to understand how bacterial diversity is influenced by microeukaryotes, data suggest that multitrophic network stability is highly dependent on microbial hubs that transmit effects to the microbial community via microorganism–microorganism interactions^{13,39,62}.

mitigation of environmental stresses (such as drought) and protection from plant pathogens through competition, antibiosis and the production of hydrolytic enzymes^{27,29–31}. By contrast, the benefits can also be indirect, through enhancement of a plant's resistance responses²⁸. Because several plant traits are co-regulated by the plant-associated microbiome, there is an emerging paradigm in which interactions between plants and their associated microbiome should not be considered as inherently either beneficial or deleterious, but rather as means to generate new phenotypes with increased fitness under distinct environmental conditions³⁵. The rational design and application of synthetic communities (SynComs) of microorganisms with broad, persistent and durable plant-growth-promoting traits have the potential to translate basic scientific findings into applications in either greenhouse or field settings (BOX 2, Supplementary text S2)^{14,33,36–39}.

One key finding is that plant-associated microbial communities do not represent random assemblages, but instead are structured by general rules for community assembly and show a defined phylogenetic organization⁴⁰

(BOX 1, Supplementary text S1). Their assembly is governed by complex interactions among microorganisms, their plant host and the environment, although the underlying mechanisms are not fully understood. New insights into these complex interactions will help advance our understanding of the evolutionary and ecological processes that govern community assembly and will guide translational research to improve plant fitness and productivity (BOX 2, BOX 3, Supplementary text S2). To achieve a more comprehensive understanding, it will be important first to characterize the mechanisms that drive the assembly of plant-associated microbiomes (Supplementary text S1). Second, the biochemical and genetic features of metabolic microorganism–microorganism and host–microorganism interactions that result in beneficial ecological outcomes need to be identified. Such data will inform the design and construction of functional microbial systems *de novo* that are based on predictive models of plant–microbiome interactions. Furthermore, the development of microbial inoculants, signalling compounds and other tools will enhance microbiome function in the agricultural setting.

In this Review, we explore the current knowledge of the plant microbiome at the community level. In particular, by drawing from forward genetic approaches and comparative genome and computational analyses of large plant genomic and metagenomic datasets, we discuss the current understanding of the composition, assembly and dynamics of plant-associated microbial communities and the host functions they provide.

The plant microbiome

Composition of plant-associated microbiota. The relative abundance profiles of the major bacterial groups are similar in bulk soil and in the rhizosphere, with a slight increment in the bacteria belonging to phylum Proteobacteria in the rhizosphere^{8–11,13} (FIG. 1). Community composition varies significantly between the rhizosphere, endophytes and the phyllosphere. Plant endophytic communities are frequently enriched in members of the Proteobacteria and Firmicutes (both ~twofold or higher in relative abundance in the endophytic community than in the rhizosphere) and to a lesser extent in Bacteroidetes; they are depleted in members of the Acidobacteria, Planctomycetes, Chloroflexi and Verrucomicrobia (>twofold lower in relative abundance in the endophytic community than in the rhizosphere)^{8–11,13} (FIG. 1). Such enrichment of specific bacterial taxa is consistent even in soils that are dominated by other taxa (for example, in soils dominated by Acidobacteria). The phyllosphere community mainly comprises bacteria belonging to phylum Proteobacteria and to the Bacteroidetes, Firmicutes and Actinomycetes, where members of phylum Proteobacteria constitute ~50% of the community composition (FIG. 1). The vast diversity of fungi that colonize both aboveground and belowground plant tissues mainly belong to the phyla Ascomycota and Basidiomycota^{9–11,13,15,16,19} (FIG. 1). Although arbuscular fungi (phylum Glomeromycota) and ectomycorrhizal fungi are well-studied, these groups represent only minor diversity among fungal communities from roots^{9–11,13}. The 'winnowing' of many bacterial

Core microbiota

The group of microorganisms commonly found within a microbiota; recurrence of an association between microorganisms is used as the criterion to select the microorganisms that potentially provide critical functions within the habitat in which they are found.

Genome-wide association studies

(GWASs). A method to survey entire genomes of the genetic variants of many individuals for genetic polymorphisms, single-nucleotide polymorphisms, that are associated with a particular trait.

Multi-'omics'

An approach (also known as integrative omics) to combine sets of different omic groups — such as the genome, proteome, transcriptome and metabolome — to study biological entities in a concerted way.

Microeukaryotes

Any microscopic eukaryotes, mainly protists, fungi, nematodes and small zooplankton.

Synthetic communities

(SynComs). Communities that comprise individually isolated microorganisms for controlled studies of microbial communities.

and fungal genotypes to only a few successful colonizers could be explained in part by plant–microbiome co-evolution¹²; however, niche adaptation may also have a major role in the selective filtering and recruitment of different microorganisms^{13,14,22,41,42}. These successful colonizers inhabiting the same host niche can either compete for the available resources or form stable co-existing communities through mutual cooperation.

Determinants of the bacterial and fungal community composition at the genus and species levels include host compartment, environmental factors and host genotype^{13,43,44} (BOX 1, Supplementary text S1). The relative importance of genotypes in shaping the microbiota composition in the phyllosphere declines over time, which suggests robust habitat selection of these communities over relatively short plant–host timescales¹⁴. Fungal establishment in the rhizosphere and on plant roots seems to be more affected by stochastic variations and to respond differently to environmental factors than is true for bacteria^{9–11,13}. There are clear differences among the microbial communities in different plant compartments (the rhizosphere, root endosphere, phyllosphere and the bulk and root-zone soils), which indicates that the plant compartment is a major selective force

that shapes the composition of plant-associated microbiota (FIG. 1). A rapid loss of diversity from soil to root and then to shoot and flower indicates the increasing influence of host-specific factors at the root–soil and root–shoot interfaces. The components of the rhizosphere at coarse phylogenetic levels (for example, the phylum and family levels) for various plant species (for example, citrus, barley, maize, sugarcane, *Arabidopsis thaliana* and rice) under field conditions are similar, regardless of their geographical source location^{2–19}. This remarkable similarity suggests that the plant traits that shape the rhizosphere compartment are themselves uncorrelated with host phylogeny. By contrast, root endophytes tend to be phylogenetically clustered, which indicates the co-existence of closely related microorganisms, probably owing to the greater influence of the host plant on the assembly of the endosphere microbiome. Consistent with diversity, microbial network complexity decreases from the soil to endosphere compartments³⁹.

The inoculum source of the aboveground microbiota is more variable, owing to their open nature and rapidly fluctuating environmental conditions. The mode of transmission probably involves transfer by aerosols, insects, soil, pollen and/or migration via other plant tissues^{3,41,42}. Although the assemblies of root-associated bacteria and fungi differ substantially from the aboveground communities, both represent a subset of the microbiota derived from soil communities and enriched in different plant-associated niches, which suggests that soil functions as a common reservoir for both belowground and aboveground plant microbiota^{9–11,13}. However, there is smaller overlap between the fungal community of aerial plant tissues and soil than is true for bacteria, which suggests that other sources are important reservoirs for the phyllosphere fungal community¹³. The host's genetic control of the aboveground microbiota is greater than for the root or rhizosphere microbiota¹⁷; however, the rapidly fluctuating environmental conditions aboveground also have an important role, and only a few microbial groups of the microbiota that have adapted to harsh environments will flourish.

In addition to the role of bacterial and fungal communities, soil and plant processes are directly influenced by other organisms, including viruses, archaea, nematodes and protists (Supplementary text S1). Plant-associated archaeal taxa primarily belong to the phyla Thaumarchaeota, Crenarchaeota and Euryarchaeota^{22,43,45,46}. Although they are consistent members of the plant-associated community, we have little understanding of the contribution of archaeal communities to host performance^{43,45,46}. Recent studies have demonstrated that the plant-associated archaeal community is highly diverse^{46,47} and niche specific (that is, distinct for different parts of the plant)⁴⁷, and that distinct archaeal members are plant specific^{46,47}. Plant-associated archaeal communities possess the ability to degrade glycogen, which is commonly stored and exuded by fungi^{46,47}. This suggests a possible interaction between these different groups in the plant environment. Metagenomic studies have identified functional genes involved in stress tolerance and nutrient cycling in plant-associated archaeal communities^{46,47}.

Box 2 | Microbiome engineering using synthetic microbial communities

Microorganisms have long been applied as inoculants for biocontrol or biostimulation; however, their field efficacy varies with the climate, soil type and other environmental factors. In many cases, these products are single isolates from a different environment that probably cannot compete with the indigenous microbiome. The highly inconsistent performance of microbial inoculants suggests that the functionality and persistence of microorganisms are dependent on interactions with the environment as well as with other microorganisms within a community. To effectively harness the microbiome requires new approaches that recognize that microorganisms living in natural and managed systems typically are members of multispecies communities. Explicit consideration of fundamental ecological processes for the development of complex microbial communities is in its infancy but is critical to the rational design and manipulation of microbiomes in agricultural systems. In recent years, synthetic microbial communities (SynComs) of varied complexity have been constructed using bottom-up combinations and have been applied to plants as a means to study various aspects of plant microbiome interactions, including elucidation of the specific mechanisms that drive community assembly and the interactions among different members⁹⁸. It is clear that there will not be a 'one size fits all' SynComs, and therefore different strategies are being explored in order to select the members of SynComs for reconstitution experiments⁹⁸. Selection for core functional versus core taxonomic microbial members in SynComs has been emphasized¹²⁰. Within this concept, functional keystone species can be predicted through topological networks derived from interactions and through metabolic models. This approach provides a pathway to maximize SynCom persistence and trait expression success in natural settings, by identifying possible points of control for manipulating microbial diversity and microorganism–host interactions. It should be noted that interaction networks and metabolic models are predictions that are often based on correlations and in silico-inferred assumptions. SynComs provide the opportunity to validate these predictions and correlations experimentally and have emerged as an important tool to demonstrate their applicability in an agricultural context. Individual microbial members can be cultured using high-throughput platforms and characterized through genomic, metabolic and physiological analysis. Standardized fabricated ecosystems or microfluidic platforms can be used for reproducible interrogation of beneficial traits. SynComs with different complexities can be designed through predictive modelling that evaluates trait redundancy, dominance, modularity, interactions and assembly. Automated monitoring of the plant response and microbial community structures after the application of SynComs can be achieved by a variety of sensors (including those in drones) together with mobile DNA sequencers. Further integration of microbiome–phenome–environment datasets could forecast microbial dynamics and enable SynCom applications to be scaled up in smart farming systems.

Box 3 | Trait-based framework to understand plant–microbiome interactions

It is intuitive and generally accepted that the functional traits conferred by microbiomes are more informative than taxonomic information, as we are more interested in ‘what they are doing (or can do)’ than in ‘who is there’. However, to date, most plant microbiome studies in the field have focused on community structure rather than function. There are strong indications that plants select for traits rather than taxonomy, as many functions provided by the microbiome are a part of the ‘accessory genome’ and are discontinuously distributed across taxonomies. Phenotyping the functional potential of a microbial community could provide comprehensive information on the possible mechanisms driving plant–microbiome interactions and therefore could be a powerful approach to understanding and modelling the microbial–community functions that support plant growth. The association of genetic variants with specific microbial genes rather than with phylogenetic identity will provide meaningful insight into the possible mechanisms underlying multifaceted plant–microbiome interactions. Defining linkages between plant phenotypes and microbiome functions through genome-wide association studies could help identify host genes that are responsible for the assembly of beneficial microbiomes. In recent years, advances in metagenome-wide association approaches, network analysis, genome-mining methods to identify biosynthetic gene clusters, the reconstruction of transcriptional and regulatory networks, and the integration of modelling and prediction approaches have provided strong foundations for developing a trait-based framework to understand and engineer plant–microbiome interactions. However, whether the functional contributions of the microbiome are the same across different plant species, growth stages and disturbances remains to be determined.

Rhizosphere

The region of soil in the vicinity of plant roots that is influenced by plant-derived nutrients and oxygen availability; it is not a region of definable size or shape, but instead consists of a gradient in chemical, biological and physical properties that change both radially and longitudinally along the root.

Endophytes

The microorganisms residing within plant tissues (the endosphere), such as leaves, roots or stems.

Phyllosphere

All the aboveground organs of plants, including the leaf, flower, stem and fruit.

Hub microorganisms

Microbial groups that are substantially more connected within a co-occurrence network than other groups on the basis of centrality measurements, such as degree, between-ness centrality and closeness centrality.

Keystone species

Highly connected microbial taxa that individually or in a guild show great explanatory power for network structure and functioning, irrespective of their abundance; not all hub species are keystone species, as a high number of direct correlations (a requirement for hubs) is not a requirement for keystone species.

Viruses play a critical part in bacterial community assembly and turnover in the soil, but their function in plant-associated environments is not completely understood⁴⁸. A recent report demonstrated that in soils, viruses affect both the microbiome structure (top-down control, inferred from lysis-dominant microbial members) and function (bottom-up control, inferred from the carriage of auxiliary metabolic genes)⁴⁹. Experimental studies have demonstrated that natural phage communities influence the assembly of phyllosphere communities⁵⁰. Similarly, protists and nematodes greatly contribute to microbiome diversity, and together with other microorganisms, they affect soil–plant processes and ecosystem functioning^{51–53}. Protists may control bacterial and fungal community assembly by modulating predator–prey relationships at different trophic levels⁵⁴. Within protists, most oomycete species that have been described so far are harmful plant pathogens (for example, *Pythium*, *Phytophthora*, *Peronospora* and *Albugo* species)⁵⁵. Although *Albugo* spp. are plant pathogens, members of this obligate biotrophic genus frequently occur on asymptomatic Brassicaceae plants, which suggests a role for environmental factors in triggering the development of symptoms⁵⁶. Also closely related, non-pathogenic oomycetes can have positive effects on plant growth^{57,58}. A study in *A. thaliana* observed that the phenotypic effects of oomycetes vary substantially in the presence and absence of bacterial communities³⁹.

Core and hub microbiota. The plant core microbiota consists of members of the microbial community that are persistent and are ubiquitous in almost all the communities associated with a particular host^{1,59,60}. The core microbiota contains key microbial taxa that carry genes with functions that are essential for host fitness^{1,20,59,60}. Many members of the core microbiota of

different plant species, such as barley, rice, sugarcane, grapevine, citrus, soybean and *A. thaliana*, are common at the genus level (for example, *Pseudomonas*, *Agrobacterium*, *Methylobacterium*, *Sphingomonas*, *Erwinia*, *Cladosporium*, *Coniothyrium*, *Resinicium* and *Fusarium*). This suggests that co-occurring members of the core microbiota are selectively recruited and enriched in parallel and are well adapted to life on and/or inside plant tissues. A core archaeome for plants growing in alpine bog⁴⁶ and for arugula (*Eruca sativa* Mill.)⁴⁷ has recently been reported. Microbial families belonging to Rhizobiales and Pseudomonadales are not only part of the core microbiota (5–17% mean relative abundance)⁶¹ but also represent a universal core plant microbiome. The community composition of these orders remains similar in different plant-associated niches, indicating a conserved adaptation to the plant environment⁶¹. Multiple members affiliated with these core bacterial genera are known to positively influence plant growth through the production of growth-regulating hormones, nutrient mobilization and/or protecting plants from biotic or abiotic stresses^{27–31}. The identification of a co-occurring core of plant-associated microorganisms also provides a useful starting point for studies of how to build SynComs to manipulate plant–microbiome interactions for increased growth and productivity (Supplementary text S2).

Within the core microbiota, a few members, such as ‘hub microorganisms’, can influence the community structure through strong biotic interactions with the host or with other microbial species, rather than simply by their own high abundance^{13,18,62,63}. These hub species may represent keystone species that can exert strong direct and indirect effects on microbiome assembly and that function as mediators between the plant and its associated microbiome. It must be noted that the co-occurrence network approaches generally used to identify hub microorganisms may be insufficient for interpreting species interactions and do not indicate causal relationships⁶⁴. However, elucidating hub microorganisms through co-occurrence networks provides an opportunity to test the causal relationships of hub species with other species and to understand how the ‘keystone behaviour’ of identified hub species can be experimentally validated. Hub microorganisms have a regulatory effect on the network of microbial interactions, as their removal results in the loss of interactions. For example, *Albugo laibachii* and *Dioszegia* spp. have been identified⁶² as highly interactive hubs in the phyllosphere of *A. thaliana*. Through these hubs, host plants selectively influence the structure of their associated microbiota by modulating microorganism–microorganism interactions and changing host fitness. Variation of two hub microorganisms had a significant effect on the assembly of the microbiome, and this influence was independent from external factors such as location and sampling time⁶². Host genomic regions involved in carbohydrate metabolism and stress responses control the abundance of hub microorganisms¹⁸. It has been postulated that hub microorganisms have important roles in organizing the plant-associated microbiome as a network, via selective assembly and recruitment,

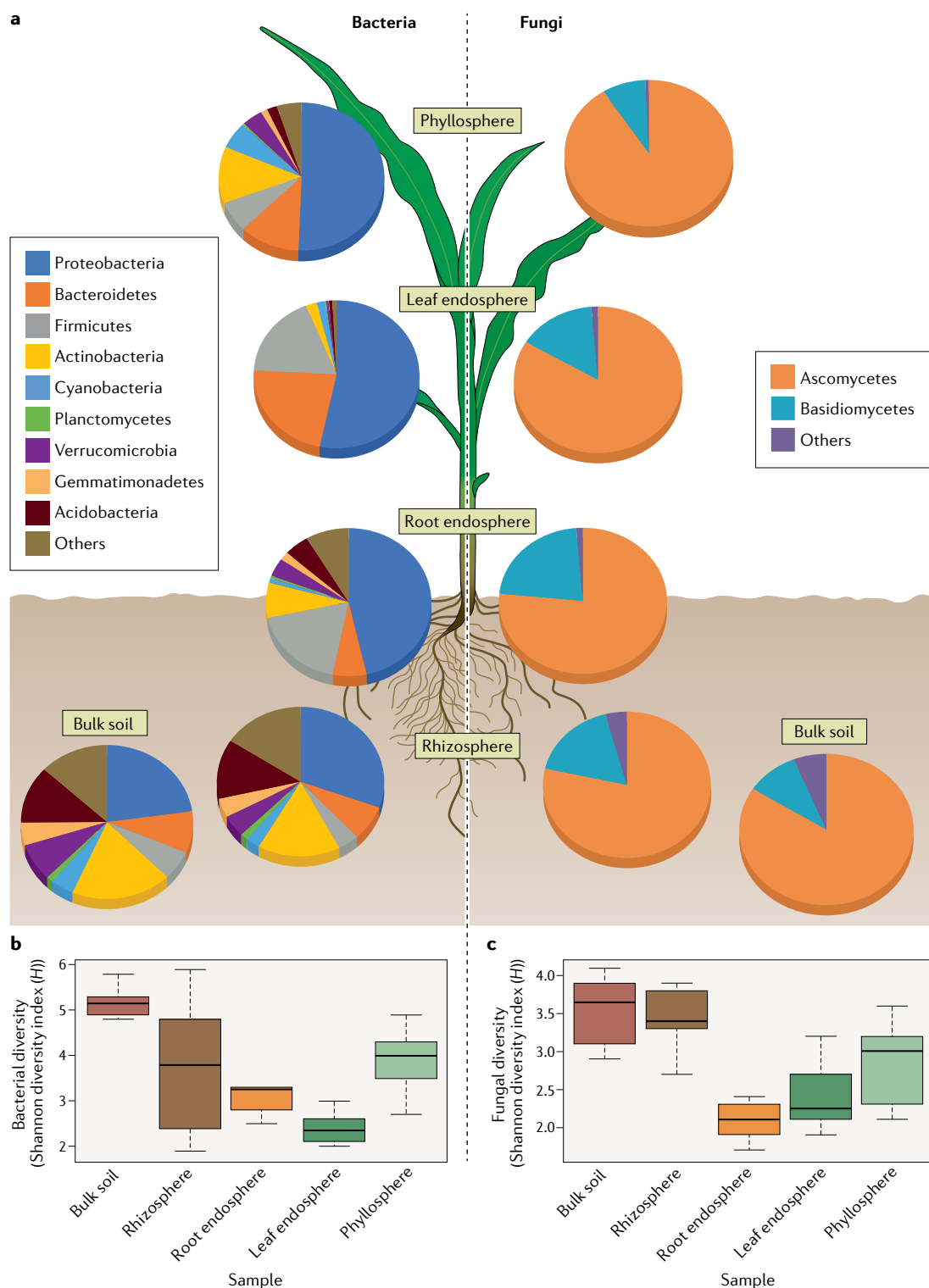


Fig. 1 | General structure of the bacterial and fungal communities from various plant-associated niches.

a | Microbiome composition varies depending on the plant compartment. Pie charts show averages of the relative abundances of the major bacterial (left) and fungal (right) phyla that are detected in the bulk soil, rhizosphere, root endosphere, leaf endosphere and phyllosphere of various plant species (sugarcane^{10,13}, grapes⁹, cactus species (*Myrtillocactus geometrizans* and *Opuntia robusta*)¹¹ and *Agave* species⁹ (*A. tequilana*, *A. salmiana* and *A. deserti*)). Abundances were estimated by marker-gene sequencing (16S rRNA for bacteria, and internal transcribed spacer (ITS) for fungi). Only phyla that represented >0.5% of the total population in at least one sample are included. The studies used high-throughput sequencing to profile the bacterial and fungal communities in different niches in a single study.

b,c | Box plots show bacterial (part **b**) and fungal (part **c**) diversity (in terms of the Shannon diversity index (H)) in the bulk soil, rhizosphere, root endosphere, leaf endosphere and phyllosphere of various plant species (data based on REFS^{8–11,13}).

Chemotaxis

Movement of organisms in response to a chemical stimulus in the direction of a higher concentration of beneficial substances or a lower concentration of toxins.

Plant exudates

Complex mixtures of soluble organic substances — which may contain sugars, amino acids, organic acids, enzymes and other substances — that are secreted by living plants, along with microbially modified products of these substances.

Biofilm

An assemblage of microbial cells that is irreversibly associated with a surface and is enclosed in a matrix of primarily polysaccharide material.

Carbohydrate-active enzymes

Enzymes involved in the biosynthesis, modification, binding and breakdown of carbohydrates.

and thus also have an important role in orchestrating host–microbiome interactions.

Dynamics of the plant-associated microbiome. The assembly of a plant-associated microbiome is a successional, multistep process that is determined by dispersal, species interactions, the environment and the host. Early colonizers could be transmitted vertically, via the parents, through seed transmission pathways⁶⁵. Therefore, these microorganisms may lack features that would otherwise facilitate early colonization — for example, active dispersal — and instead have characteristics selected by the host plants that depend on differences in terms of seed morphology and anatomy. Once seeds germinate, microbiome assembly is likely to be driven by horizontal transfer, where seed-borne microorganisms preferentially become associated with aboveground plant tissues, whereas soil-derived microorganisms are mainly associated with the rhizosphere and roots⁶⁶. The root-associated microbiome is likely to be dynamically recruited and assembled over the life-cycle of its plant host, and the temporal shifts in this microbiome are consistent across geographic locations⁴³. The microbial composition of microbiomes, which can be highly dynamic in the early vegetative phase, begins to converge throughout vegetative growth and stabilizes during the reproductive phase^{43,67}. Repeated sampling to study the structure of plant-associated microbiomes has shown that, although their composition varies with time, a small fraction of microbial taxa belonging to the core microbiota are consistently maintained in high relative abundances throughout plant development^{10,19,43,67}. These microorganisms possess multiple traits, for efficient colonization, stress tolerance and beneficial effects on the hosts^{66,68}. Moreover, traits such as drought tolerance and disease resistance that are conferred by associations with a particular microbial group can be passed from mother plants to their offspring, which indicates the interlinked importance of host genetics and the host-associated microbiome^{69,70}.

Plant colonization and community assembly

Plant sensing and the initiation of colonization. Plant-associated microorganisms use chemotaxis to sense and respond to plant-derived signals, such as organic acids and/or sugars present in plant exudates, and to initiate colonization (FIG. 2). Once a signal is perceived, microorganisms move towards the plant primarily through the use of flagella. Subsequently, microorganisms attach to the root surface and form a biofilm. Genes that encode proteins involved in bacterial chemotaxis, flagella assembly, bacterial motility, biofilm formation, bacterial secretion and two-component regulatory systems are highly abundant in microorganisms and microbial communities found in the root environment^{15,23,38,71,72} as well as in the stem and phyllosphere environments, as compared with the bulk soil^{3,24,25}. A higher number of substrate transporters in the bacterial phyla Proteobacteria and Firmicutes facilitate the increased presence of these bacterial groups in nutrient-rich plant environments. Similarly, a substantially higher fraction of motility genes was observed in the genomes of bacterial isolates derived

from the roots of *A. thaliana* than in bacteria that had been isolated from soil⁴². Combined phylogenomic and phenotypic analyses suggest that such root colonization traits pre-date the acquisition of symbiosis genes in members that belong to the order Rhizobiales, which are part of the core microbiome⁶¹. Polyamines such as arginine and putrescine function as signalling molecules in the root–rhizosphere interface and inform the microbiome of the presence of eukaryotic hosts. Sensing of such molecules triggers a lifestyle switch in order to promote attachment and biofilm formation in many microbial groups^{25,73}. After successful colonization, diverse host processes, such as the activation of plant signalling pathways and/or nutrient-stress-mediated root inhibition that alters the host root architecture, can lead to differential niche colonization patterns among various microbial groups.

Uptake of plant metabolites. Plant metabolism generates chemically distinct environments, and many of the core functional traits that are reported to be over-represented in plant-associated microbiomes correlate with the requirements for microbial growth and survival in such plant environments. Comparative genomic analysis²³ has confirmed the trends of metagenome analysis¹⁵ towards positive selection for genes involved in carbohydrate metabolism and transport and for transcriptional regulators of these genes in the genomes of phylogenetically diverse plant-associated bacteria. Of note, several carbohydrate-active enzymes are shared between plant-associated bacteria and fungi, despite large evolutionary distances⁷⁴. Genomic analysis has shown that fast-growing bacteria, such as the alphaproteobacteria and gammaproteobacteria that are typically enriched in plant environments, have a high number of total transporters — including ATP-binding cassettes, phosphotransferase systems and drug/metabolite transporters — that could import or export a broad range of compounds^{23,75}. The presence of low-affinity transporters in plant-associated microorganisms enables fast growth during periods of feast and endurance of starvation during periods of famine, fluctuating conditions that are typical in the rhizosphere.

Plant hosts provide stable metabolite availability, and this reduces the selective pressure on the associated microbiome to maintain capabilities for the biosynthesis of various essential compounds^{25,26}. A recent study identified mutations in 50 bacterial genes required for amino acid metabolism that confer a fitness advantage to mutants relative to the wild-type strain of *Pseudomonas simiae* WCS417r²⁶. Genes for amino acid biosynthesis were depleted in the global citrus rhizosphere compared with bulk soils¹⁵. The findings indicate that auxotrophy for specific amino acids confers a selective fitness advantage when plant-exuded amino acids are abundant. Interestingly, thiamine auxotrophy of the plant-beneficial endophytic fungus *Serendipita indica* could be satisfied by the ubiquitous soil microorganism *Bacillus subtilis*, but the success of this interaction was only visible when inoculation of *B. subtilis* was separated from that of *S. indica* in either time or space⁷⁶. This highlights the interkingdom cooperation

among microbiomes in order to promote the successful colonization of plant habitats.

Siderophores are important in various ecological phenomena, including iron biogeochemical cycling in soils, pathogen competition, plant growth promotion

and cross-kingdom signalling. Siderophore-producing gene clusters were enriched in the rhizosphere of barley⁶, citrus¹⁵ and grapes⁸ compared with the bulk soil. Metagenomic analysis further revealed enrichment of siderophore-producing genes in the endosphere

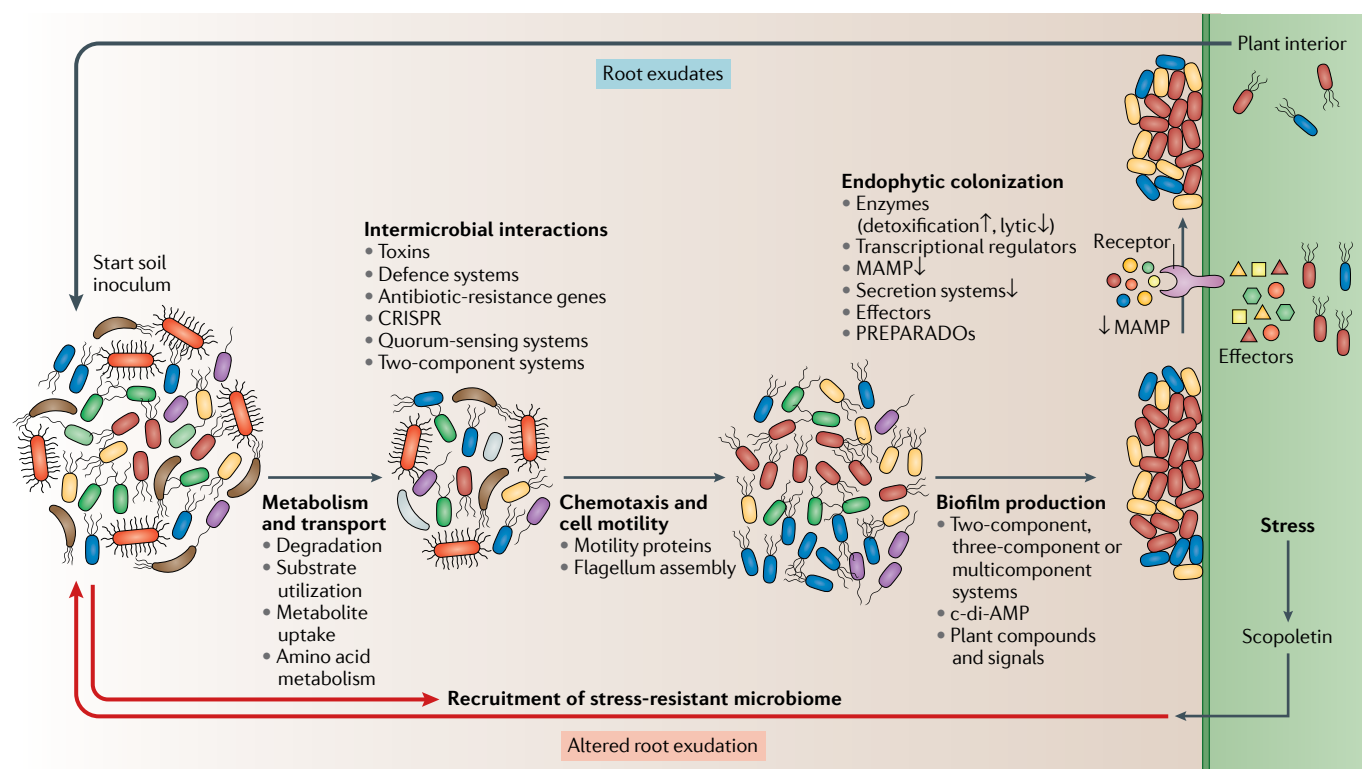


Fig. 2 | Plant colonization and microbiome assembly. Multiple and complex plant–microorganism and microorganism–microorganism interactions are required for the selective assembly of plant-associated microbiomes. Microbial-mediated processes, pathways and proteins that are related to colonization (metabolism and transport; intermicrobial interactions; chemotaxis and cell motility; biofilm production; and effector-mediated colonization and stress responses) are shown. The plant interacts with the microbiome through the release of root exudates (for example, organic acids, sugars and secondary metabolites) throughout its developmental stages. The microorganisms in the bulk soil act as ‘seed banks’ and vary in their genomic potential to degrade, utilize and metabolize distinct metabolite substrates in the root exudate. A rich repertoire of transporters confers selective advantage to a few microorganisms in taking up various plant-derived metabolites. Subsequently, the community is shaped by intense microorganism–microorganism interactions mediated via the strain-specific production and perception of antimicrobial molecules. Signalling events mediated by quorum sensing or other two-component systems have a key role in both intermicrobial and intramicrobial communications among different species. CRISPR-associated genes provide adaptive immunity against bacteriophages and are under strong selection pressure in plant-associated microbiomes. The presence of antibiotic-resistance genes may provide protection against biological and chemical warfare (for example, toxins and defence systems) that occurs during the initial stages of community assembly. Subsequently, the microbial community moves towards the plants through chemotaxis, involving motility proteins and the assembly of flagella. Further colonization is mediated through sophisticated communications between plants and microbial communities, leading to the formation of a biofilm on plant surfaces. Plant-produced compounds and signals not only induce biofilm production but also affect the architecture of the biofilm. Different two-component, three-component or multicomponent sensing and regulatory microbial pathways are involved in the integrated and

coordinated regulation of biofilm formation. The second messenger cyclic di-adenylate monophosphate (c-di-AMP) mediates interbacterial cell–cell communication and the initiation of biofilm formation by influencing the expression of genes involved in biofilm production. Genes involved in motility, chemotaxis, adhesion and biofilm production further contribute to plant colonization and the endophytic lifestyle within the host plant. Suppression of plant-produced reactive oxygen species by microbial detoxification enzymes such as superoxide dismutase, catalase, peroxidase, alkyl hydroperoxide reductase and glutathione-S-transferase facilitates initial endophytic colonization. Entry into plant tissues is facilitated by the production of lytic enzymes such as lysozymes or cell-wall-degrading enzymes. It is postulated that endophytes produce low levels of lytic enzymes as compared with pathogens, thus avoiding triggering the plant immune response. Diversification of microbial-associated molecular patterns (MAMPs) circumvents recognition by the plant immune response and is likely to shape endosphere microbial assemblages in plant populations. Type 3 and type 4 secretion systems that deliver effector proteins are absent or are present in low numbers in the endophytes, thus minimizing the activation of plant defences. However, effector-mediated colonization of the plant interior has been reported for *Rhizobium*-type bacteria or mycorrhizal fungi. Gene products from eukaryotic-like plant-resembling plant-associated and root-associated domains (PREPARADOs) are speculated to mimic the host cell signalling, thus acting as decoys to circumvent plant defences and gain entry into plant tissues. Overall, the interplay between plants and their endophytic microbiota is complex and still far from being fully elucidated. Under biotic and abiotic stress conditions, plants can change their exudation patterns in order to selectively recruit a beneficial ‘stress-tolerant microbiome’. For example, under iron or phosphorus stress conditions, the transcription factor MYB72 and the β -glucosidase BGLU42 (not shown) induce the biosynthesis and exudation of scopoletin, which has antimicrobial activity and selects for beneficial microorganisms that ameliorate stress (altered root exudation).

Siderophores

Low-molecular-weight organic chelators with a very high and specific affinity for ferric iron, which scavenge iron from the environment and make the element available to microbial cells and/or a plant host.

Effector proteins

Proteins produced by plant-associated microorganisms that modify plant functioning in order to promote microbial colonization.

Quorum sensing

Population-density-dependent regulation of gene expression, mediated by the production of signal molecules called autoinducers.

compared with the rhizosphere in *Populus deltoides*⁷⁷ and rice⁷⁸. However, this was not reflected in the comparative genome analysis of bacterial isolates⁷⁷. The prevalence of siderophore gene clusters across multiple biosynthetic pathways suggests that the possession of traits that facilitate competition for limiting resources provides a selective advantage to microorganisms in colonizing plant roots.

Evading plant defences. Evasion or suppression of the plant immune system is not only essential for pathogens to successfully infect plant hosts but also critical for commensals to colonize different plant niches (Supplementary text S1). The plant-associated microbiota must cope with a host immune system that can recognize microorganism-associated molecular patterns (MAMPs), such as flagellin, lipopolysaccharides, chitin and elongation factor Tu-derived peptides⁷⁹. Plant perception of MAMPs involves conserved signal-transduction mechanisms, including the generation of reactive oxygen species, the activation of mitogen-activated protein kinases (MAPKs) and the induction of salicylic acid-signalling and jasmonic acid-signalling pathways⁷⁹. To overcome host immune surveillance, microorganisms secrete effector proteins. Some of these effector proteins emerged from convergent evolution and horizontal transfer of genes encoding protein domains from eukaryotes, such that the effectors mimic plant proteins. Recently it was revealed that plant-resembling plant-associated and root-associated domains (PREPARADOS) were highly abundant in the genomes of plant-associated and root-associated bacteria compared with soil and non-plant-associated bacterial genomes²³. Some PREPARADOS themselves are domains within effectors and are predicted to be secreted by the general secretory pathway or type 3 secretion system (T3SS)⁸⁰. PREPARADOS may represent a ‘camouflage’ strategy to evade MAMP-triggered immunity by binding to extracellular MAMP molecules (for example, mannose) and thereby functioning as a molecular invisibility cloak. A large set of PREPARADO domains are shared between plants, plant-associated fungi, oomycetes and bacteria, which suggests convergent evolution or cross-kingdom horizontal gene transfer between phylogenetically distinct organisms subjected to the shared selective forces of the plant environment. Interestingly, genes encoding proteins involved in the T3SS are higher in nodule-forming symbionts and phytopathogens than in endophytes. By contrast, those genes are found in a substantially larger number of endophytes than in soil bacteria⁸¹. Thus, it will be essential to understand how some taxa exploit virulence mechanisms for symbiotic associations and plant colonization.

Notably, several factors implicated in host–pathogen interactions (such as T3SS, regulation of virulence, invasion and intracellular resistance), microorganism–microorganism interactions (T6SS) and bacteria–phage interactions (bacteriophage integration and transposable elements) were found to be enriched in healthy citrus rhizosphere samples collected from across the globe^{15,71}, as well as in the barley⁶ rhizosphere, relative to bulk soil samples. These signs of positive selection are evidence

of plant–microbiome co-evolution in the rhizosphere and suggest an arms-race co-evolution model in natural communities similar to the model proposed for binary plant–pathogen interactions⁸². Another mechanism of plant defence evasion in plant-associated microorganisms could be the ability to disperse from the initial site of colonization after triggering a plant defence response²⁵. In this respect, genes involved in biofilm production or in the regulation of such genes are important to altering colonization patterns and evading the induction of a host defence.

Microbial interactions. It is not surprising that several microbial genes active and/or enriched in plant environments have a role in cooperative or competitive interactions with other members of the microbiome. Diverse and distinct gene clusters for natural-product biosynthesis from 339 plant-associated bacteria were reported⁷⁷. As the genetic potential of bacteria to synthesize different types of natural products influences microorganism–microorganism and microorganism–plant interactions, a comprehensive understanding of the plant microbiome will require greater knowledge of how these compounds are produced and regulated and of their mechanism of action. The genomes of many plant-associated bacteria encode interspecific and intraspecific bacteria-killing mechanisms (for example, the production of antibiotics), through which they can modulate the distribution, abundance and diversity of other microbial groups within the plant host⁷⁷. Specific functional traits related to pathogen suppression (for example, protein secretion systems and biosynthesis genes for antifungal compounds) are more abundant in the bacterial rhizosphere community of disease-resistant varieties of bean and tomato than in susceptible varieties or in bulk soil^{83–85}. Pathogen-induced activation of chitinase genes and various unknown biosynthetic gene clusters encoding non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) is responsible for disease-suppressive functions in the endophytic root microbiome⁸⁶. Genome mining of phyllosphere bacteria has identified more than 1,000 biosynthetic gene clusters (BGCs) that belong to diverse biosynthetic classes, including ribosomally synthesized and post-translationally modified peptide and terpene systems, NPRSs and *trans*-AT PKSs⁸⁷. These BGCs are postulated to be involved in microorganism–microorganism interactions and niche adaptations. In addition to antibiotic production, genes related to antibiotic resistance were also enriched in the plant environment¹⁵, which suggests an intense microbial arms race that controls the microbial community structure.

Quorum sensing is a well-established mechanism of bacterial cell-to-cell communication that involves the production and sensing of signalling molecules (such as homoserine lactone (HSL))⁸⁸. Different bacterial taxa can generate the same type of signalling molecule, which enables either cooperation or interference (quorum quenching) with other unrelated taxa. Moreover, quorum-sensing molecules also have a role in interkingdom interactions. The perception of HSLs by plants leads to modulation of the plant

Carotenoid

An organic pigment produced by plants, algae and several groups of bacteria and fungi.

metabolism, immune response and root development⁸⁹. Quorum-sensing-mediated swarming of the finger millet endophyte M6 (*Enterobacter* sp.) towards root-invading *Fusarium graminearum*, along with its formation of microcolonies, results in a multilayer root-hair endophyte stack⁹⁰ that forms a physical barrier that prevents entry and/or traps the pathogen, which is subsequently killed. The metagenomic analysis of plant-associated microbiomes¹⁵ has shown enrichment of HSLs in the plant environment compared with bulk soils. About 40–50% of the bacteria isolated from plant environments showed HSL activity^{77,91}. Genes for terpene biosynthesis were present in the largest number of genomes of plant-associated bacteria, with 49% of isolates carrying a gene encoding a terpene synthase⁷⁷. Terpenoids have diverse biological and ecological functions, such as carotenoid production, as well as functioning as chemical defences against herbivores and pathogens⁹². Bacterial terpenes are implicated in interkingdom signalling, as these volatile compounds elicit profound responses from plants⁹³. Specific microbial colonization on the ‘local side’ induces microbiome-reprogrammed systemically induced root exudation of metabolites (SIREM), and microbial colonization on the ‘systemic side’ results in long-distance communication between unshared microhabitats of tomato rhizosphere⁹⁴. For example, inoculation of bacteria that belong to the orders Bacillales or Pseudomonadales on the local side induces the accumulation of bacterial-specific SIREMs. Transportation of these signals through shoots to the unshared area of roots facilitates the assembly and colonization of SIREM-specific microbial populations on the systemic side.

Bacterial T6SSs inhibit competitor cells through a toxic effector and its cognate immunity protein⁹⁵. Microorganisms that can successfully colonize plant environments may use not only host colonization factors but also mechanisms to exclude closely related competitors. Multiple T6SS genes were found to be enriched in the rhizosphere communities of barley⁶, citrus¹⁵, wheat and cucumber⁵. A new family of T6SS effectors, ‘HydE1’, which is specific to *Acidovorax* phytopathogens (absent from *Acidovorax* commensals), is efficient in controlling various leaf bacterial isolates, suggesting a role in interbacterial competition in plants²³. Despite the broad distribution of interbacterial T6SS killing mechanisms, few studies have investigated their ecological roles^{96,97}. Interestingly, some CRISPR-associated proteins have shown positive selection pressure in the root environment⁴⁶, which indicates that microorganisms face intense selection pressures from bacteriophages.

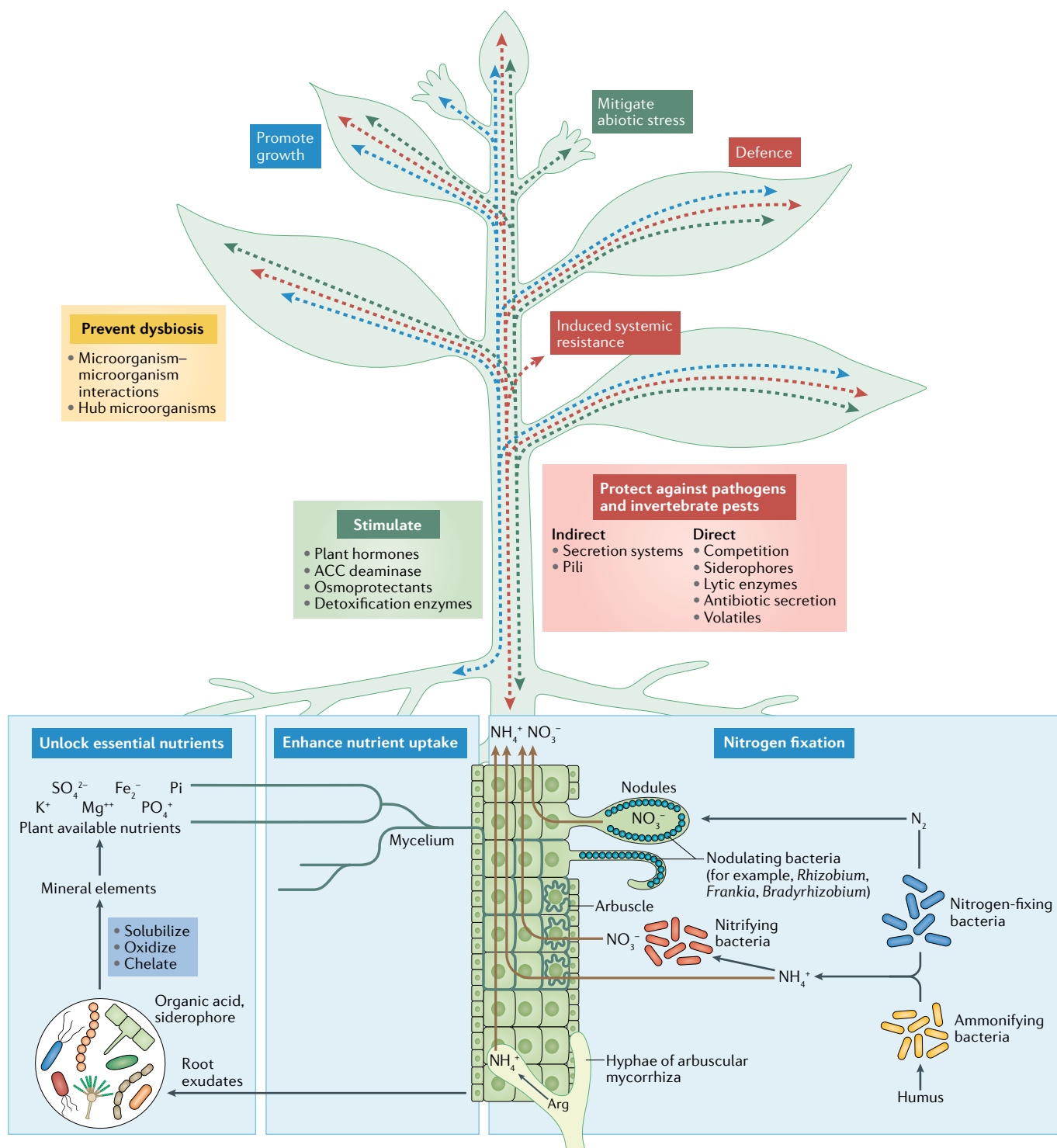
In summary, an extensive repertoire of traits involving resource acquisition, motility, habitat modification and various microorganism–microorganism or plant–microorganism interactions may be needed by members of the plant microbiota in order to colonize the plant environment (FIG. 2). However, most of this knowledge has come from reductionist experiments and does not consider the modular and synergistic interactions under the holobiome concept. Bottom-up experimental approaches are now needed, whereby diverse microbial members from different plant-associated environments

are systematically isolated and functionally characterized, followed by the reconstitution of communities with increasing complexity and gnotobiotic assays in order to quantitatively assess host–microbiome interactions⁹⁸.

Recent research has emphasized the importance of eco-evolutionary processes in the assembly of plant-associated microbiomes, such as dispersal (movement of microorganisms between different niches), selection (biotic and abiotic effects causing fitness differences), ecological drift (stochastic changes in population size that influence the abundance of a particular member) and diversification (processes that generate genetic variation)⁹⁹. Both stochastic colonization and historical contingency may have essential roles in structuring the plant-associated microbiome. Microbial community structure in the rhizosphere⁶⁷ and phyllosphere⁴⁰ is initially determined by stochastic processes. The order and timing of species arrival and dispersal (also known as priority effects) influence all the eco-evolutionary processes that affect assembly of the plant-associated microbiome. Priority effects can cause historical contingency to affect both the structure and function of the plant-associated microbiome and therefore can have long-lasting consequences for plant performance. Although the initial microbiome community is robust, plants can accommodate new species without substantially changing the pre-existing community⁴⁰. Rare taxa are postulated to provide a robust reservoir of ecological functions and could drive priority effects in microbiome assembly during the early stages of plant development, with variable consequences for plant performance at later stages⁶⁷. Determining the principles governing the assembly, dynamics, stability and vulnerability to disturbance of the microbiome, including the roles of biotic and abiotic factors, will lead to a better mechanistic understanding of the influence of the microbiome on both the fitness and function of plant hosts (BOX 1, Supplementary text S1).

Functions of plant-associated microbiomes

Nutrient acquisition. Plant-associated microbiomes have essential functions in improving plant nutrition (FIG. 3). The molecular mechanisms driving nutrient acquisition have been thoroughly studied for plant symbioses with arbuscular mycorrhizal fungi (AMF) and *Rhizobium* bacteria^{27–30}. Moreover, non-symbiotic plant-growth-promoting bacteria can either enhance the bioavailability of insoluble minerals or improve the root system architecture of host plants, thus increasing the exploratory capacity of the root for water and minerals^{27,29}. The functional plant traits related to the acquisition or conservation of nutrients that differentiate exploitative (fast-growing) versus conservative (slow-growing) plant species are contributed by their associated microbiome²⁷. A recent study showed that differences in the nitrogen use efficiency of rice varieties is due to the recruitment of a higher proportion of nitrogen-cycle-related bacteria, leading to more efficient nitrogen transformation processes in the root environment of *Oryza indica* than of *Oryza japonica* varieties³⁸. Notably, a 16-member *indica*-enriched SynCom substantially promoted the growth of an *indica* variety in comparison to a 3-member



japonica-enriched SynCom when organic nitrogen was used as the sole nitrogen source. This suggested that effective transformation of organic nitrogen into nitrate and ammonium by the *indica*-enriched microbiome may contribute to higher nitrogen use efficiency in *indica* rice.

Multipartite microbial synergies between mycorrhizal fungi (which promote nitrogen acquisition but have limited ability to access organic nitrogen) and soil microbial communities (which mineralize organic

nitrogen into bioavailable forms) resulted in a ten-fold increase in the uptake of nitrogen from organic matter in *Brachypodium distachyon* as compared with non-microbial controls¹⁰⁰. It is predicted that these previously unquantified associations may contribute to >70 teragrams (10^{12} g) of annually assimilated plant nitrogen, with a positive impact on net primary productivity¹⁰⁰. Plant traits involved in nutrient use efficiency are related to the differential colonization of plants with two major groups of mycorrhizal fungi¹⁰¹. Plants that are colonized

◀ **Fig. 3 | Beneficial effects of the plant-associated microbiome.** The plant-associated microbiome can provide benefits to the plant through various direct or indirect mechanisms. These benefits include growth promotion (blue), stress control (green) and defence against pathogens and pests (red). Microbiome-mediated benefits can be initiated in any part of a plant (mostly belowground) and can be transmitted to other parts via plant-mediated transport or signals (shown as blue, green and red dashed arrows, representing mechanisms that contribute to plant growth, stress relief and defence, respectively). Direct effects are mediated through nitrogen fixation, through unlocking of essential nutrients from minerals and through enhancing the capability of plants to take up nutrients from the soil. In addition, other direct effects include the stimulation of plant growth via stress alleviation, through the modulation of aminocyclopropane-1-carboxylate (ACC) deaminase expression and the production of plant hormones, detoxification enzymes and osmoprotectants. Benefits can also be indirect, as the plant-associated microbiome protects the plant against pathogens or pests through antagonism or through inducing systemic resistance in plants. Complex microorganism–microorganism and host–microorganism interactions maintain the balance between different members of the microbial community in favour of beneficial microorganisms that contribute to plant health (yellow). Diazotrophic bacteria can fix atmospheric nitrogen (N_2) and might actively transport ammonium (NH_4^+) and nitrate (NO_3^-) to the host. Ammonifying bacteria convert organic N_2 present in the soil to NH_4^+ , which is further converted to NO_3^- by nitrifying bacteria. Leguminous plants develop root nodule symbiosis with N_2 -fixing bacteria. Arbuscular mycorrhizal fungi convert arginine (Arg) to urea and then to NH_4^+ . Microbiomes can unlock essential elements by oxidizing, solubilizing or chelating minerals into plant-available nutrients such as phosphate (Pi), nitrogen (NH_4^+) and potassium (K^+) through the production of organic acids and siderophores. Furthermore, arbuscular mycorrhizal fungi might enhance nutrient availability by long-distance transport through the mycelium and specialized structures called arbuscules (fungal hyphae ensheathed in a modified form of the cortical cell plasma membrane) that transport elements directly to the host cytoplasm. Microbiomes can stimulate plant growth by metabolizing tryptophan and other small molecules in the plant exudates and producing phytohormones that include auxins, gibberellins, cytokinins and phytohormone mimics. Auxins can also induce transcription of the ACC synthase that catalyses the formation of ACC. ACC, the direct precursor of ethylene, is metabolized by bacteria via the enzyme ACC deaminase, thus ameliorating abiotic stress. Members of plant-associated microbiomes produce a range of enzymes that can detoxify reactive oxygen species, thus minimizing plant-induced stress. The plant-associated microbiome protects the plant against pathogens by the production of antibiotics, lytic enzymes, volatiles and siderophores. Various microbial structures — such as secretion systems, flagella and pili — along with proteins such as effector proteins, indirectly contribute to plant defence by triggering an induced systemic resistance response. Siderophore-mediated nutrient competition between commensals and plant pathogens can reduce pathogen titres. Interkingdom and intrakingdom interactions within the microbiome maintain the microbial balance, thus protecting plants from dysbiosis. Furthermore, hub microorganisms can amplify host signals in order to promote the assembly of a microbiome that provides benefits to the plant. Overall, beneficial plant–microbiome interactions improve the growth performance and/or health of plants.

with ectomycorrhizal fungi adopt nutrient conservation strategies, whereas plants that are colonized with AMF are nutrient acquisitive¹⁰¹. Rhizosphere microorganisms that prolong nitrogen bioavailability by nitrification delay flowering time and stimulate plant growth by converting tryptophan to the phytohormone indole acetic acid (IAA), which downregulates genes that trigger flowering¹⁰².

Plant-associated microbiota can mobilize nutrients that are not readily available to plants, such as inorganic phosphate and iron, through solubilization, mineralization or excretion via iron-chelating siderophores (FIG. 3). Under conditions of low inorganic phosphate, plants rely on microbial cooperation partners, including AMF and their symbiotic endophytes, to satisfy needs for this essential nutrient¹⁰³. Under phosphate-limiting conditions, SynComs of different complexity enhanced the transcription of plant genes involved in phosphate starvation responses,

thereby increasing the uptake of inorganic phosphate by plants³⁷. Non-mycorrhizal plants assemble a core inorganic-phosphate-assimilating fungal microbiome that expands the ability of plants to grow in phosphate-depleted soils¹⁰⁴. Phosphate-starvation-mediated repression of the immune response enables a build-up of mutualistic plant–fungal associations that increases plant inorganic-phosphate uptake¹⁰⁵. Such observations suggest that fine-tuned interactions between the nutritional and immune status of plants regulate microbiota-mediated beneficial functions. The *A. thaliana* root bacterial community is shaped by coumarins, plant-derived specialized secondary metabolites that on the one hand facilitate iron mobilization and on the other generate reactive oxygen species that inhibit the proliferation of a relatively abundant *Pseudomonas* species that competes with plants for iron¹⁰⁶. The root-specific transcription factor MYB72 has an important role in both rhizobacteria-mediated induced systemic resistance (ISR) and iron acquisition¹⁰⁷. Interestingly, volatile compounds from ISR-inducing rhizobacteria and *Trichoderma* fungi elicit the expression of MYB72 in order to induce the genetic machinery for enhanced iron uptake by roots¹⁰⁸. Adjustments in local iron homeostasis transmit systemic signals to plant shoots to induce jasmonic-acid-dependent ISR¹⁰⁸. These recent studies have shed light on the vital role of plant-associated microbiomes on plant functioning and open up multiple opportunities to manipulate plant characteristics by using microbial interventions.

Disease resistance. The impact of natural microorganism-based plant defence on plant health is evidenced most clearly in disease-suppressive soils, whereby plant root exudates stimulate, enrich and support soil microorganisms as the first line of defence against soil-borne pathogens³². Community-based analyses of suppressive soils have demonstrated that no single phylum was uniquely associated with disease suppression^{32–34,109,110}. Although the interactions between microbial consortia and a specific pathogen that result in disease suppression are biologically complex, similar mechanisms, such as the production of antifungal metabolites^{32,110} and volatiles^{111,112} by different bacterial genera, are responsible for the build-up of disease-suppressive soils. If the pathogen breaches the first line of rhizosphere-mediated disease resistance, the endophytic microbiome can provide an additional layer of protection through selective enrichment of microbiome members that possess genetic machinery to produce enzymes and secondary metabolites against the pathogen⁸⁶. Interestingly, the MAMP-triggered immunity pathway is induced to higher levels in disease-suppressive soils¹¹³. Management practices such as crop rotation, residue retention and compost additions can induce general or specific disease suppression by affecting microbiome composition driven by the greater availability of carbon¹¹⁴. A recent study³⁴ modelled general disease suppression against *Fusarium oxysporum* and determined that the abundances of bacteria in the phyla Actinobacteria and Firmicutes can be used as predictive markers of disease-suppressive soils at the continental scale. Disease-resistant traits of

Induced systemic resistance (ISR). A physiological 'state of enhanced defensive capacity' of the entire plant against diverse pathogens and herbivores that is induced by local stimulation through beneficial microorganisms.

a tomato variety have been attributed to the selective recruitment of bacteria that can antagonize the wilt pathogen, *Ralstonia solanacearum*⁸⁴. Microbiome-mediated plant protection can be transferred and maintained via soil transplantations^{67,84}. SynComs constructed on the basis of knowledge of the microorganisms in disease-suppressive soils have been reported to control diseases in field conditions^{32,33}.

ISR can be triggered by plant-growth-promoting bacteria and fungi residing in the rhizosphere²⁸. Local suppression of root immune responses is a common feature of ISR-eliciting beneficial microorganisms.²⁸ Pathogen infection causes alterations in root exudation patterns that result in the selective recruitment of ISR-inducing microbiota^{83,113}. The presence of heterogeneous microbial communities living on the surface of the aboveground portion (the episphere) and the endosphere of tomato plants modulates phenylpropanoid metabolism (involved in salicylic acid synthesis), which leads to cell wall fortification that protects the plant from *Fusarium oxysporum* f. sp. *lycopersici*¹¹⁰. Cell wall fortification functions as a barrier not just against pathogen attack but also against abiotic stresses such as salinity, which suggests a direct interplay between stress tolerance and immunity¹¹⁵. The induction of jasmonic acid signalling and plant defences modulated by root-associated bacterial and fungal communities also affects aboveground insect herbivory¹¹⁶. Remarkably, ISR induction by plant-associated microbiomes drives the secretion of antimicrobial compounds such as coumarin¹⁰⁷ and benzoxazinoids¹¹⁷ that further intensify the recruitment of ISR-inducing strains. This suggests that plant immune responses can be manipulated so as to recruit microorganisms that provide stable plant protection over multiple generations. Through plant–soil feedbacks and legacy effects, the selective enrichment of microbial communities in response to both biotic and abiotic stresses can affect plant immunity in subsequent generations¹¹⁸. Engineering plant-associated microbiomes to control plant disease will continue to require a deeper understanding of the plant–microbiome–environment interactions among diverse crops of interest.

Stress tolerance. Plant-associated microorganisms could modify plant evolutionary responses to environmental stress in at least three non-mutually-exclusive pathways: by altering the fitness of individual plant genotypes, the expression of plant traits related to fitness, and the strength or direction of natural selection occurring within populations that experience environmental stress through the microorganisms' effects on reproductive fitness^{12,69,119,120}. Plants generally select a stress-resistance-promoting microbiome under abiotic or biotic stress conditions^{119–124} (BOX 3, Supplementary text S1). The plant traits favoured under adverse conditions, especially drought, may depend on changes in the associated microbiome. Several recent studies have shown that naturally occurring and artificial variation in the microbiome can alter plant flowering time on the order of 1–5 days^{69,119,124}. Drought-induced early flowering has been proposed as a potential mechanism of drought avoidance.

The mechanisms underlying microbiome-induced flowering plasticity remain unknown, but probably they include a combination of direct effects of the microbiome on plant physiology and indirect effects mediated by soil nutrient availability. Drought-mediated production of the plant hormone abscisic acid dampens the plant immune response, thus facilitating large shifts in the root endophytic community¹². These fluctuations mitigate water stress, possibly through the production of plant hormones and/or changes in the biochemical activity of host plants¹². Plants respond to multiple stresses by upregulating the physiologically costly ethylene signalling pathway, which causes trade-offs between stress resistance and growth rate¹²⁵. Both microbial ethylene reduction and gene mutations that disrupt ethylene signalling in plants have strong pleiotropic effects, with increased plant growth, but at the cost of strong stress hypersensitivity³⁵. These findings suggest that plant–microbiome interactions are neither beneficial nor deleterious, but instead function as modulators, generating new phenotypes by reshuffling existing traits. Interestingly, for fungal endophytes, traits related to resource use and stress tolerance predicted 26–53% of the endophyte-mediated effects on plant performance under water stress¹²⁶. The moderately predictive effect of functional microbial traits for plant performance can be used to develop a framework for screening new microorganisms that can increase plant fitness under stress conditions (BOX 3).

Conclusions

In recent years, plant–microbiome research has benefited enormously from cross-disciplinary efforts that have brought concepts from multi-omics, engineering, theory, experimental biology, computational biology and statistics to generate quantitative insights into plant–microbiome interactions. The extensive survey of a few models and of important crops, plants and trees have established a 'parts list' of the major bacterial and fungal associates, although a vast amount of novel diversity remains to be discovered for most plants. A systematic approach to identifying the microbiomes of ecologically and economically important plant species, comparable to human microbiome approaches, will be needed in order to identify core and hub microbiota and the host functions they provide. Although bacterial and fungal lineages contribute the vast majority of the plant-associated microbiome by abundance, there is a critical knowledge gap concerning the shape and drivers of other fractions of the plant microbiome (for example, viruses, archaea, protists and nematodes) that influence bacterial and fungal communities via bottom-up and top-down processes. GWASs and metagenome-wide association studies have identified key drivers that influence the assembly of plant-associated microbiota and have linked individual microbial taxa and genes to plant colonization, plant physiology and traits related to plant fitness. However, they have also revealed that large proportions of the variation in community assembly and the effects of microbiomes on plant fitness remain unexplained. To elucidate these gaps, larger-scale longitudinal studies will be needed to establish baselines

Phytobiome

Plants, their environment and all microorganisms and macroorganisms living in, on or around the plants.

for plant-associated microbiomes, with explicit consideration of temporal dynamics and host age. In recent years, some progress has been made in elucidating multipartite (for example, plant–animal–soil or plant–environment–soil) interactions in the phytobiome; however, we have very little knowledge on the functional properties of the microbiome. The limited but increasing functional understanding of the microbiome is beginning to be translated into accepted practice, in the form of the development and application of SynComs to increase plant fitness and productivity (Supplementary text S2). However, a consensus on the desired microbial endpoints (what is a ‘healthy’ microbiome) has yet to be determined. Similarly to breeding, the plant microbiota may affect the plant phenotype. Although the optimization of SynComs is rapidly advancing, crop-breeding programmes have not yet incorporated the selection of beneficial plant–microorganism interactions in order to breed ‘microorganism-optimized’ plants.

Moving forward, we envision that the next generation of computational and experimental approaches

will be able to resolve some methodological and technological challenges. These include the translation of data into phenotypic links, experimental modelling through co-cultivation or gnotobiotic methods, and multiscale computational simulations. These integrative approaches will combine the skills, methodologies and expertise from a range of disciplines within the sciences, engineering and beyond. Increased understanding of the dynamic plant–microbiome–environment interactions will provide a way forward to engineering complex microbial consortia with predictable behaviour and robust outcomes. By closely coupling modelling with experimental approaches, we expect that scientific advancement will be accelerated, creating a future in which the activities of indigenous microbiomes can be reliably enhanced and in which engineered microbiomes can be deployed safely and effectively in large-scale field settings for improved and sustainable plant production.

Published online 12 August 2020

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Acknowledgements

P.T. and J.E.L. were partially supported by funding from Colorado Agricultural Experiment Station; S.G.T. works at the US Department of Energy Joint Genome Institute and is supported by contract no. DE-AC02-05CH11231; B.K.S. is supported by the Australian Research Council (DP170104634). T.S. is supported by National Research Foundation of Korea (2015R1A2A1A05001885).

Author contributions

P.T. researched data for the article. P.T., J.E.L., S.G.T., T.S. and B.K.S. contributed substantially to the discussion of the content. P.T., J.E.L., S.G.T., T.S. and B.K.S. wrote the article.

Competing interests

The authors declare no competing interests.

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Supplementary information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41579-020-0412-1>.

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