The Plant Microbiota: Systems-Level Insights and Perspectives

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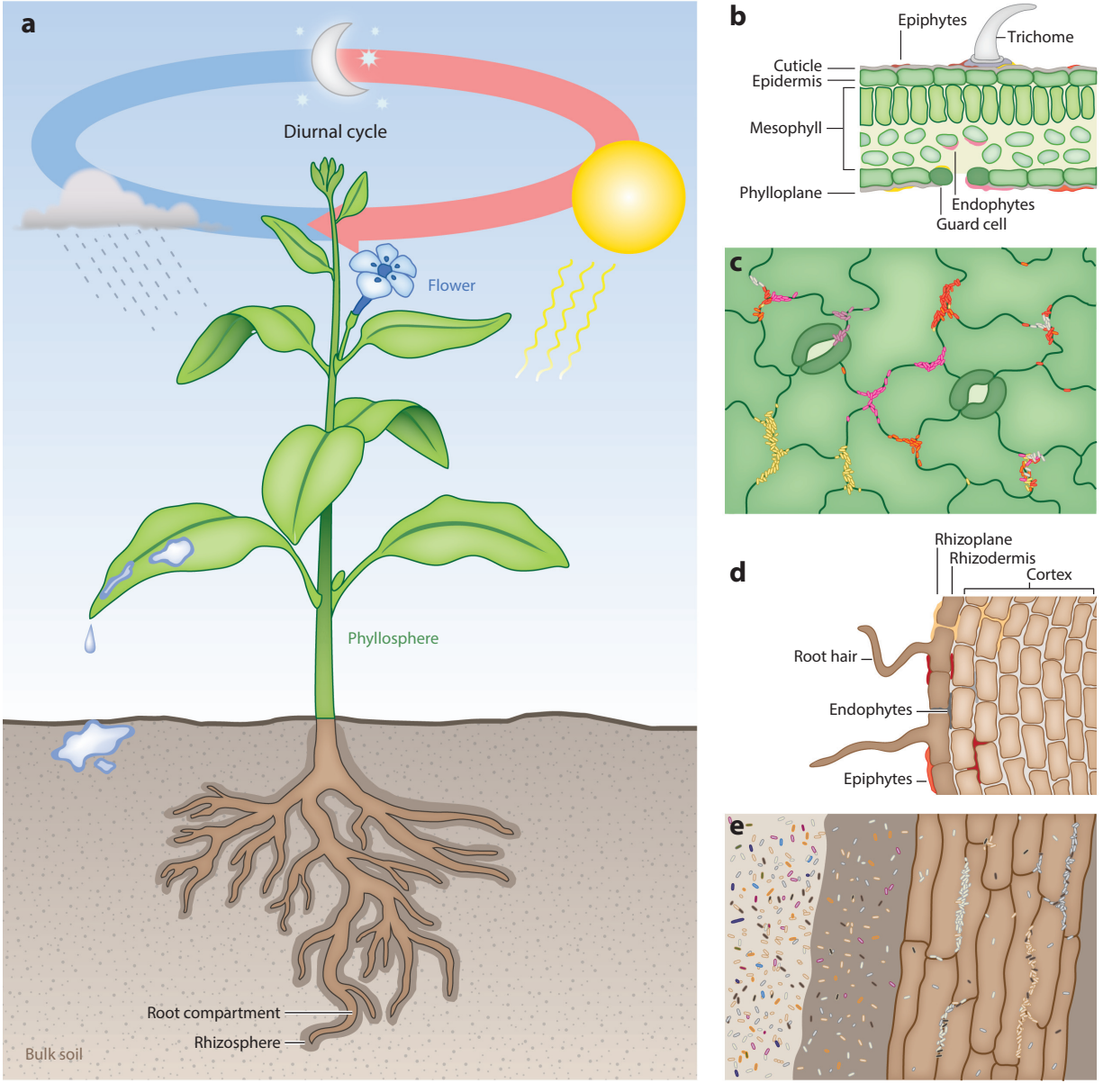
Abstract

Plants do not grow as axenic organisms in nature, but host a diverse community of microorganisms, termed the plant microbiota. There is an increasing awareness that the plant microbiota plays a role in plant growth and can provide protection from invading pathogens. Apart from intense research on crop plants, *Arabidopsis* is emerging as a valuable model system to investigate the drivers shaping stable bacterial communities on leaves and roots and as a tool to decipher the intricate relationship among the host and its colonizing microorganisms. Gnotobiotic experimental systems help establish causal relationships between plant and microbiota genotypes and phenotypes and test hypotheses on biotic and abiotic perturbations in a systematic way. We highlight major recent findings in plant microbiota research using comparative community profiling and omics analyses, and discuss these approaches in light of community establishment and beneficial traits like nutrient acquisition and plant health.

INTRODUCTION

Terrestrial plants are colonized by diverse microorganisms (**Figure 1**) that affect plant health and growth in a beneficial, harmful, or neutral way. Understanding microbially triggered plant diseases helps prevent yield losses of crop plants and stimulated early research on plant-microbe interactions. Molecular studies on plant-pathogen interactions have uncovered an elaborate plant innate immune system that responds to pathogens and their effectors. In addition, a major focus of previous and ongoing research has been on symbiotic nitrogen-fixing rhizobia within root nodules of legumes and the symbiotic association of arbuscular mycorrhizal (AM) fungi with phylogenetically diverse plant species, owing to their role in nutrient uptake and plant growth (93, 134).



In recent years, the plant microbiota has gained great interest, and plant phenotypes are increasingly being recognized as the result of multipartite interactions. Accordingly, a plant in nature constitutes an ecosystem that should be considered from a systems perspective (see also Reference 98). The huge gene pool of the microorganisms living as endophytes and epiphytes in and on plants, respectively, extends the host genome and contributes to its phenotype. As a result, the totality of the genetic information, also referred to as the hologenome (128), may allow adaptation to new or changing environmental conditions as well as the ability to cope with pathogen encounters, which are essential aspects of the sessile lifestyle of plants.

Phylogenetic information about the plant microbiota is rapidly becoming available for an increasing number of host plants; however, fewer studies have addressed the functional capabilities and genomic potential of the indigenous microbiota using metagenome sequencing. Functional microbiome approaches represent valuable tools in the identification of common microbial functions and help elucidate how community properties emerge from functional traits of individual microbial populations. Disentangling the relationships of diverse species is a challenging task in complex systems; however, reductionist approaches using defined synthetic communities (SynComs) facilitate the understanding of microbial interactions within the plant microbiota. Systems-wide omics paired with computational approaches help generate hypotheses and monitor strain interactions at the molecular level. Interesting parallels of the plant microbiota to other host microbe ecosystems, e.g., the human or animal gut, are apparent, and conceptually similar questions on microbiota functions are addressed (e.g., 44, 100), including the evolution of host and microbiota (84, 86, 128). In this review, we highlight recent progress on complementary aspects in plant microbiota research. Although the focus is on bacteria that form structured communities in association with leaves and roots, several interesting aspects of fungi and other eukaryotes are mentioned.

Plant microbiota: all microorganisms, including commensals, symbionts, and opportunistic pathogens, that inhabit the plant host

Endophytes: microorganisms residing inside the different plant organs

Epiphytes: microorganisms inhabiting the plant surface

Synthetic community (SynCom): rationally designed mixture of representative strains

STRUCTURE AND PHYLOGENETIC COMPOSITION OF THE PLANT MICROBIOTA

Healthy plants are host to a taxonomically diverse microbiota (19, 143). One of the key findings of the past decade is that these host-associated microbial communities do not represent random assemblages but show defined phylogenetic structures. Bacteria are highly abundant microorganisms in these communities, but fungi, oomycetes, algae, protozoa, nematodes, and viruses are also important contributors (17, 44, 63, 73, 133). Archaea are apparently not frequent on most

Figure 1

Schematic plant exposed to varying environmental factors and plant microbiota members colonizing niches on and inside plant tissue. (*a*) The aboveground parts of plants, collectively called the phyllosphere, represent an inherently open and variable habitat that is dominated by leaves and is exposed to the diurnal cycle. Microbial inhabitants must cope with natural UV radiation and rapidly changing environmental conditions, including wide temperature gradients and fluctuating water and nutrient availability. In contrast, belowground plant compartments are surrounded by bulk soil, mostly influenced by its edaphic properties. Roots penetrating the soil change local oxygen concentrations and release nutrients by a process called rhizodeposition. (*b*) Schematic leaf cross section showing bacterial colonization patterns on the phylloplane as well as endophytes colonizing the intercellular spaces of the mesophyll. The waxy plant cuticle restricts leaching of photoassimilates to the leaf surface, resulting in an overall oligotrophic environment. Differences in cuticle composition and thickness as well as surface structures such as stomata, trichomes, and hydathodes alter local leaf surface properties, resulting in uneven colonization patterns. (*c*) The abaxial (lower) leaf surface demonstrating preferential colonization along the grooves between epidermal cells and around stomates, with most cells pigmented and arranged in aggregates. (*d*) Schematic root cross section of the root hair zone showing epiphytic colonization of the rhizoplane as well as endophytes migrating into and inhabiting the cortex. (*e*) Schematic longitudinal section of a root. The root surface is inhabited by a distinct microbial community, as compared with the surrounding rhizosphere and bulk soil. Similarly to the leaf surface, heterogeneity of the root surface causes differences in colonization patterns at the root tip, elongation zones, axial cell grooves, and base of lateral roots.

terrestrial plants but contribute key metabolic functions to the belowground carbon cycling of anaerobic habitats, such as rice paddy fields (75, 143).

With the emergence of next-generation sequencing, cultivation-independent analyses have provided deep insights into the community composition of above- and belowground compartments of various host plants, including the widely used model plant *Arabidopsis thaliana* (4, 12, 18, 29, 51, 77) and its close relatives (113), several tree species (62, 102, 116), and relevant crop plants such as barley (17), corn (94), grapevine (157), lettuce (101, 148, 149), potato (55), tomato (91), rice (32, 64), sugarcane (152), and soybean (29, 80), as well as more specialist plants like salt-excreting *Tamarix* trees (37). The use of different sampling protocols, primers, and sequencing pipelines makes it difficult to directly compare the results of these studies, but they have all conclusively demonstrated that the bacterial plant microbiota is composed of only a few dominant phyla, mainly Proteobacteria, Actinobacteria, and Bacteroidetes, and to a lesser extent, Firmicutes (**Figure 2**). The dominance of these phyla was further corroborated by optical approaches targeting the spatial distribution of leaf-colonizing bacteria (105).

Several studies have attempted to identify a core of shared taxa within the plant microbiota below the phylum level. The core community can be defined at various taxonomic ranks and different levels of complexity (138), e.g., the core community of only one plant compartment, the shared core across all studied compartments of one population, or even across different populations or plant species. The core of common taxa is expected to become smaller as the ecological context is extended. However, core microbiota members are seemingly competitive in colonizing different plant compartments or plant species under varying environmental conditions and are prime candidates for the analysis of microbiota functions that might be provided to the plant host.

Various studies have aimed to identify core plant microbiota across phylogenetically distinct plants, multiple accessions of one plant species, or more than one plant compartment. In one of the first comprehensive studies addressing the composition of the leaf microbiota, metagenome sequencing of the bacterial communities on *Arabidopsis*, soybean, and clover leaves revealed a surprising consistency in community composition that translated also to their metaproteomes (see below) (29). The bacterial leaf communities of all three plants were dominated by Proteobacteria, mostly from the Alphaproteobacteria class, Actinobacteria, and Bacteroidetes, with *Methylobacterium*, *Pseudomonas*, and *Sphingomonas* being among the most abundant genera. These genera were also conspicuous and highly abundant on grapevine leaves (157). Kembel and colleagues used 16S rRNA gene profiling to analyze the leaf community of 57 diverse tree species in a neotropical forest and identified a core of 104 operational taxonomic units (OTUs) that was present on nearly all tree species analyzed and made up only 1.4% of the overall diversity but accounted for 73% of all sequencing reads (62). Comparison of the leaf and flower bacterial communities of grapevine plants indicated that the flower community was less diverse and almost exclusively composed of Proteobacteria, mainly the two genera *Pseudomonas* and *Erwinia* (157). Analysis of the apple flower microbiota, however, demonstrated higher bacterial diversity, including taxa of the phyla Deinococcus-Thermus, TM7, Bacteroidetes, Firmicutes, and Proteobacteria (115). Thus, a consensus view of the bacterial flower microbiota composition is not yet possible and requires further analyses; in fact, it may also vary strongly between plant species. Zarraonaindia and coworkers additionally analyzed the grape berry–specific community as well as the belowground compartments, the rhizosphere, roots, and soil, revealing distinct community compositions for all compartments, with roots and flowers being most dissimilar (157).

Two comprehensive studies analyzed bulk soil, the rhizosphere, and the root compartment of *A. thaliana* and found that all three compartments host stable and significantly different communities across multiple environments (18, 77). Species richness was highest in bulk soil and reduced in the rhizosphere and root compartment. Taxa of the Actinobacteria, Bacteroidetes, Firmicutes, and

Next-generation sequencing: collective term for sequencing technologies of the modern genomics era, not relying on Sanger's chain termination method

Cultivation-independent analysis: molecular methods in microbial ecology that do not rely on cultivability of the microbial community

16S rRNA gene profiling: diversity analysis of amplicon libraries by sequencing, denaturing/temperature gradient gel electrophoresis (D/TGGE), or terminal restriction length polymorphism (TRFLP) analysis

Operational taxonomic units (OTUs): diversity units of 16S- or 18S-rDNA sequences sharing identity above a threshold; unless specified, usually 97%

Rhizosphere: layer of soil surrounding and firmly attached to roots that is influenced by plant-derived nutrients and oxygen availability

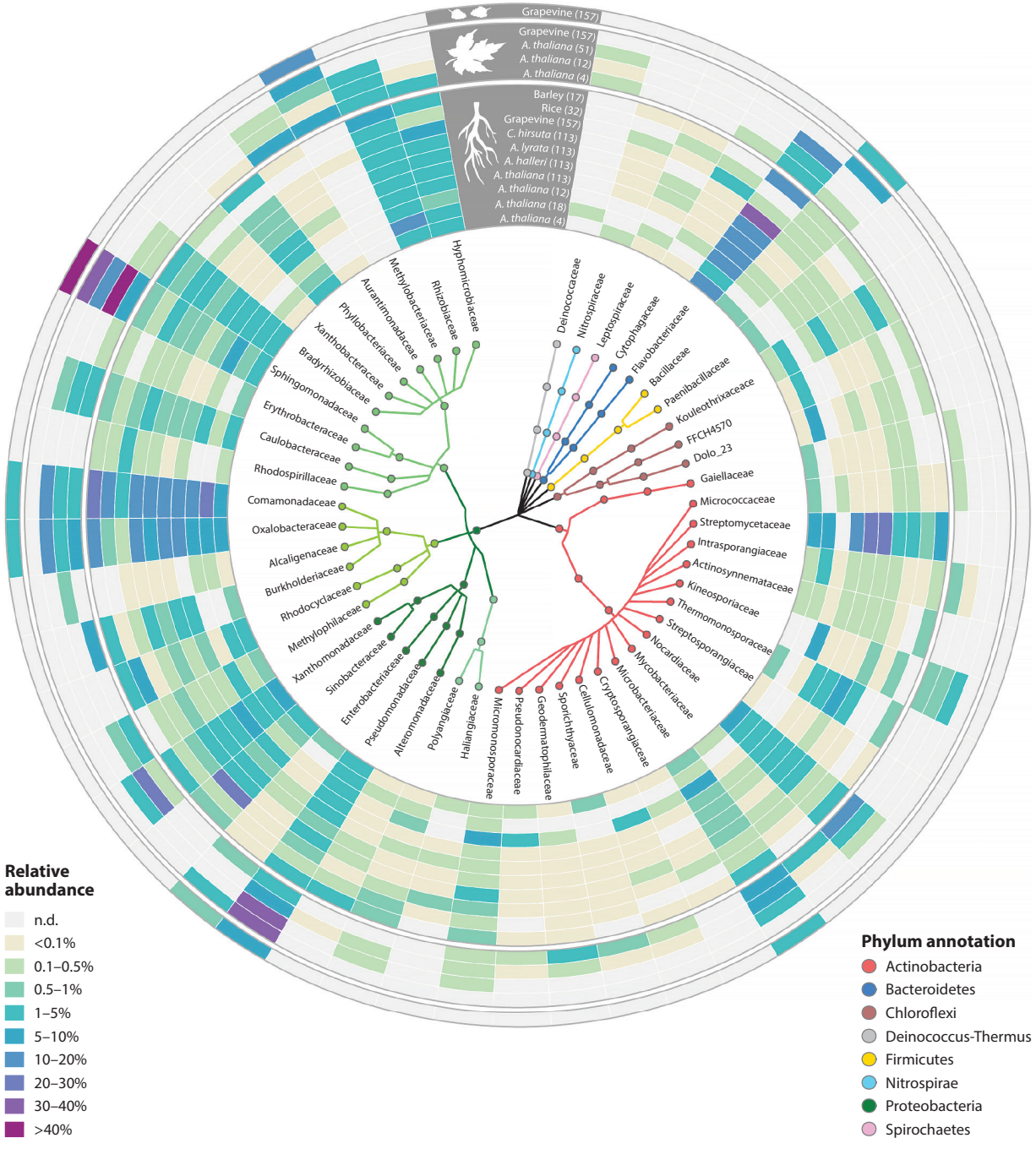


Figure 2

Phylogenetic structure of the plant microbiota. Sequencing data were analyzed using a reference-based operational taxonomic unit (OTU) picking method and were subsequently combined at the family level. Shown are families detected in at least 70% of root and leaf microbiota studies, respectively, with relative abundance ≥0.1% in at least one study. Abbreviation: n.d., not detected.

Proteobacteria (predominantly the class Betaproteobacteria) were enriched in the root compartment compared with bulk soil, whereas Acidobacteria, Verrucomicrobia, and Gemmatimonadetes were depleted. Similar results were obtained with rice plants, confirming that the rhizosphere (weakly), the rhizoplane, and the root endosphere host microbial communities distinct from those in bulk soil (32). The individual microbiota of the different compartments were consistent with a selective gradient from the exterior of the root, across the rhizoplane, to the interior of the root, with the endosphere compartment being most exclusive. Consistently, a weak rhizosphere effect on community composition was also observed in grapevine (157), whereas a more pronounced rhizosphere effect was found in barley (17). Determination of a core set of OTUs enriched in the root endosphere of rice plants grown at several cultivation sites identified 32 commonly shared taxa, with a subset of 11 also being enriched in roots of greenhouse-grown individuals (32). Notably, three of the assigned families (Kineosporiaceae, Rhodocyclaceae, and Myxococcaceae) were also represented by root-enriched OTUs in *A. thaliana*, where 97 OTUs were identified as a root-enriched core community across two different soil types and eight *Arabidopsis* accessions (77). Similar efforts on four *Arabidopsis* relatives, spanning natural and greenhouse-grown populations, identified nine shared root-enriched OTUs of three orders (Actinomycetales, Burkholderiales, and Flavobacteriales), and these OTUs constituted up to half of the root microbiota in all samples tested (113).

Comparison of the culture-dependent diversity of *Arabidopsis* with OTUs detected on leaves and roots of naturally grown plants revealed high conservation, even when samples originated from different continents; additionally, leaf and root isolates showed significant taxonomic overlap, which was substantiated by the corresponding culture-independent data (4). Overall, these studies suggest that generally there are conserved taxa that inhabit a given plant organ across multiple host species and environments.

Rhizosphere effect: a shift in phylogenetic community composition in the rhizosphere compared with surrounding bulk soil

Gnotobiotic systems: host organisms grown under germ-free conditions; can be inoculated with individual strains or a known mixture of strains

SYNTHETIC ECOSYSTEMS: RECONSTITUTION OF THE PLANT MICROBIOTA

Over the past century, genetic and physiological characteristics of numerous bacterial and fungal isolates have been studied in controlled binary interaction model systems with their eukaryotic hosts. In the next step, the design and use of model microbiota systems are crucial in the identification of underlying factors that drive mechanisms of community establishment, community dynamics including resistance and resilience, and microbial physiology in a community context. Representative SynComs of defined complexity allow bottom-up approaches in gnotobiotic systems under controlled and reproducible conditions, as demonstrated by two recent studies on the effects of the host genotype on bacterial community establishment (see below) (11, 70).

To systematically address microbial community structure and functions, SynCom diversity requires comprehensive strain collections that allow mimicking the phylogenetic and functional diversity of the plant microbiota. Several studies indicate that an unexpectedly large fraction of the members of the plant microbiota is cultivable. Thompson and colleagues isolated more than a thousand diverse strains belonging to more than one hundred species, whereas Ercolani isolated 1,701 strains representing well-known plant-associated genera over consecutive growing seasons (34, 130). In addition, efforts directed toward the optically targeted isolation of single cells by fluidic force microscopy (FluidFM) led to the isolation of 70 strains belonging to 23 genera of the four main phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (122). More recently, Bai et al. (4) established extensive culture collections of the *Arabidopsis* leaf and root microbiota, covering the majority of bacterial species that are reproducibly detected by culture-independent profiling. Based on almost 8,000 bacterial isolates, the estimated recovery of taxa was

64% for root-associated OTUs and 47% for leaf-associated OTUs (≥0.1% relative abundance), which covered the majority of bacterial families. Representative sets of 206 root-derived and 224 leaf-derived strains, in addition to a set of bacterial soil isolates, were selected as a core collection, and draft genome sequences were acquired. SynComs composed of both leaf and root isolates formed communities resembling the natural microbiota on their cognate host organ in a gnotobiotic *Arabidopsis* model system. Despite an extensive taxonomic overlap between leaf and root isolates, experiments on the competition of the leaf- and root-derived SynComs suggested specialization and adaptation to their respective niche (4). These recolonization experiments highlight the potential of designed microbiota experiments to disentangle the principles and mechanisms driving community establishment. We speculate that the surprisingly high cultivability of plant-associated bacteria is based on low-complexity food webs, continuous substrate supply by the plant (albeit low in the phyllosphere), and an essentially aerobic environment. Continued isolation of strains from various plants to expand culture collections will improve resemblance of SynComs to natural plant microbiota. Whole-genome sequencing of strains is essential in fully integrating SynCom-derived data, and will be vital in maintaining curated high-quality reference databases for improved data analysis of future culture-independent surveys.

Phyllosphere: all aboveground organs of plants, dominated by leaves, but also including stems, flowers, fruits

Ecological niche: relational position of an organism or population within a given environment, including all biotic and abiotic aspects and how it affects its environment

Plant microbiome: the totality of microbial genomes and genetic information present in the plant habitat

THE PLANT MICROBIOME: FUNCTIONAL ADAPTATION TO THE PLANT ENVIRONMENT

The different plant compartments provide a multitude of different ecological niches for the microorganisms inhabiting them. Microorganisms functionally diversify and adapt in the process of occupying such niches within a certain habitat under competitive conditions, altogether resulting in the coexistence of populations. Therefore, in order to achieve a systems-level understanding of the plant microbiota, it is indispensable to move from population inventories and phylogeny-inferred putative functional traits to actual data on microbiota activities in situ. Over the past decade, large-scale transcriptomics, proteomics, metabolomics, and combinations thereof were applied to plant microbiomes to address environmental adaptation at the community level. The collectivity of microbial genetic information serves as a crucial basis for functional genomics approaches and may be inferred from shotgun metagenome sequencing, pioneered by Handelsman and coworkers (47), or by sequencing entire strain collections (4). In addition, the microbial metagenomes obtained from distinct plant compartments allow for the identification of habitat-specific gene enrichment, potentially underlining functional traits relevant for effective host colonization.

On the basis of these analyses of bacterial communities, a number of traits are emerging as relevant under environmental conditions. For root or rhizosphere communities, genes relating to chemotaxis and motility have been identified as enriched categories in the metagenomes of the respective microbiota of wheat and cucumber (88) and grapevine (157) as well as in the metaproteome of rice (64). Different modes of motility exist and have been shown to be important for migration from soil toward roots and for root colonization. For example, members of the genus *Flavobacterium* (Bacteroidetes) possess unique gliding-motility machinery that is functionally linked to a Bacteroidetes-specific type IX secretion system (67), and twitching motility via type IV pili is essential for migration of endophytic diazotrophic *Azoarcus* into rice roots (14). This indicates that individual taxa have evolved specific strategies to successfully move to favorable locations and persist in these environments. However, transcripts related to motility were underrepresented in a *Burkholderia* strain endophytic in potato plants, indicating that motility might no longer be needed once cells have attached to or entered a plant tissue (117). Similarly, analysis of the transcriptome of a leaf-pathogenic *Pseudomonas* strain revealed high expression of genes related to motility during epiphytic growth, whereas expression was strongly reduced when the bacteria

were colonizing the apoplast (153). Quorum sensing and the second messenger cyclic di-GMP are known to regulate the transition from the motile to the sessile lifestyle, and corresponding genes are abundant in metagenomes of rice root endophytes, potentially reflecting the involvement of these regulatory circuits in the colonization process (114). Ofek-Lalzar and colleagues (88) analyzed microbial adaptation to the rhizoplane by a combinatorial approach of metagenomics and metatranscriptomics. Among other factors, genes and transcripts involved in lipopolysaccharide (LPS) biosynthesis were recognized as enriched compared with those in the surrounding bulk soil (88). LPS is important for binding of bacteria to plant surface glycoproteins (6).

Gene abundance and expression patterns revealed differences in the nutrition of the microbiota of two distinct plant species (88). Enriched expression of genes related to the utilization of C_4 dicarboxylates was characteristic of the wheat root compartment, whereas the cucumber metatranscriptome revealed increased abundance of cell wall–degrading enzymes, potentially reflecting the differences in plant cell wall architecture (88). Indications for cell wall degradation was also obtained from the enrichment of genes involved in the conversion of aromatic compounds in the grapevine root microbiota (157) and of those encoding plant cell wall–degrading enzymes in the maize rhizosphere (72). Interestingly, approximately 40% of *Arabidopsis* root microbiota–enriched OTUs were equally abundant on wood splinters incubated in the same soil type, suggesting that these taxa are capable of growing on the components of lignified cell walls (18). However, the majority (60%) of taxa enriched on *Arabidopsis* roots require metabolically active plant cells to support growth, possibly depending on rhizodeposits, including sugars, amino acids, and organic acids. The latter were mentioned above in the context of the wheat root compartment (88), and genes involved in sugar uptake were shown to be enriched in the microbiota of barley roots compared with those of the respective soil samples (17). Stable isotope probing (SIP) techniques provide an elegant way to trace the directional net carbon flux from the plant and identify metabolically active taxa (46). For example, profiling of ^{13}C-enriched RNA (SIP-RNA) demonstrated the rapid carbon flux and consumption of photoassimilates by AM fungi and further identified bacterial taxa belonging to the Actinobacteria and Proteobacteria as primary consumers of root exudates (137).

Delmotte and coworkers (29) employed community proteogenomics, i.e., a combination of metagenome sequencing and metaproteome analysis, to detect proteins abundant in the leaf microbiota of *A. thaliana*, clover, and soybean plants grown under environmental conditions to infer the physiology of the colonizing microorganisms. Metagenome sequencing significantly improved protein identification, and proteins related to one-carbon metabolism and transport processes were detected in high abundance, suggesting that they are important functional traits of the microbiota of all three plant species (29). Transport proteins comprised TonB-dependent receptors of different specificities, mostly assigned to *Sphingomonas*, β-barrel porins, and ABC transporters for amino acids and mono- as well as disaccharides. Complementary metabolomics approaches confirmed that glucose, fructose, and sucrose are available on *A. thaliana* leaves, and sugars and amino acids (e.g., arginine) were depleted upon colonization by heterotrophic epiphytes (109). Notably, phylloplane metabolite pool sizes varied with the diurnal cycle, indicating that nutrient availability on leaves is temporal and at least partially dependent on the metabolic state of the plant. Methanol is also a common substrate available to leaf bacteria as a function of the diurnal cycle. It is produced by plants in large quantities as a side product of pectin methylesterases during the cell wall remodeling necessary for plant growth (36). Ubiquitously found methanol-consuming bacteria are mostly assigned to the genus *Methylobacterium*, and gain a competitive advantage from methanol utilization during leaf colonization (29, 126).

Besides metabolic capabilities, responses to stress were identified as important functional traits of leaf-colonizing bacteria (29). The phyllosphere is considered a harsh environment with rapidly

changing conditions and exposure to various stresses, including UV radiation, reactive oxygen species (ROS), and desiccation (143). Catalase and superoxide dismutase are enzymes important for the detoxification of ROS, whereas pigmentation prevents and photolyase repairs UV-induced damage of nucleic acids. Extracellular polymeric substances and secretion of bioactive surfactants can increase water permeability and wettability of the plant cuticle, thereby improving epiphytic fitness during conditions of fluctuating humidity (20). Analysis of bacterial adaptation to the leaf surface by proteomics identified a response regulator [phyllosphere-induced regulator (PhyR)] important for epiphytic colonization of *Methylobacterium extorquens* (42) and *Sphingomonas melonis* (59). This protein is a master regulator of the general stress response in Alphaproteobacteria (38). Although root-inhabiting or -associated microorganisms are exposed to stress as well, they are protected from UV stress and environmental conditions are likely to change less frequently. Indeed, stress response was identified as a significantly enriched functional category in grapevine rhizosphere metagenomes compared with surrounding bulk soil (157). Furthermore, transcriptional profiling of endophytic *Burkholderia* of potato plants exposed to drought stress indicated that endophytes are affected by environmental influences, and upregulation of several extracytoplasmic function (ECF) sigma factors potentially involved in stress resistance was observed (117). Besides abiotic stresses, plant microbiota members are exposed to biotic stresses and antimicrobial compounds of both plant and microbial origin. As expected, transporters involved in drug resistance were induced during phyllosphere colonization by *Arthrobacter*, and genes related to detoxification were found enriched in the metagenome of the barley rhizosphere (17, 111). Additionally, genes of the type III secretion system mediating the transmission of effector proteins into host cells, as well as of the type VI secretion system involved in predatory killing, were overrepresented in the barley rhizosphere microbiota, reflecting the exchange of (bio)chemical warfare among microorganisms and with the plant host (17). The importance of interactions with the plant host was further corroborated by enrichment of genes of the type III and type IV secretion systems in bacteria colonizing the rhizoplane of wheat and cucumber (88).

In a comparison of the metaproteomes in the phyllosphere and rhizosphere microbiota on rice plants, Knief and coworkers identified proteins involved in stress response, nutrient uptake systems, and one-carbon metabolism (64). Metagenome comparison indicated the presence of nitrogenase genes both above- and belowground; however, the nitrogenase protein was detected exclusively in the rhizosphere (64). Thus, despite the extensive overlap of encoded functions in the microbiome of different plant compartments, gene expression patterns may vary significantly and to a larger extent than one might deduce from gene enrichments. This conclusion is further supported by the previously mentioned study on the wheat and cucumber rhizoplanes, in which only 3% of the genes involved in the analyzed traits differed in abundance between the metagenomes of the two plant species, whereas 17% of the respective transcripts differed significantly (88).

Taken together, although metagenomic surveys allow the identification of enriched functional categories, complementary methods at the functional level are required to generate insight into gene expression patterns and dynamics. Adaptive responses are likely provoked by changes in environmental conditions, including the diurnal cycle and pathogen encounter. Collectively, environmental factors may affect bacterial physiology in many ways, including carbon availability. Competition for limited resources among microorganisms may lead to a specialization in high affinity uptake systems and may trigger antibiosis. Thus, abiotic and biotic environmental conditions likely have a major impact on the expression of adaptive traits that are crucial for microbe-microbe and microbe-plant interactions. Transcriptome, proteome, and metabolome analyses, alone or combined with stable isotope probing, hold great potential and are, together with spatially structured methods such as matrix-assisted laser desorption ionization (MALDI) imaging,

nanosecondary ion mass spectrometry (SIMS), and single cell Raman spectroscopy, powerful tools in the rapidly developing field of microbial ecology (33, 52, 53, 109).

Seed bank: ecological source of microorganisms colonizing the respective habitat

ESTABLISHMENT OF THE PLANT MICROBIOTA AND DRIVING FACTORS

Microorganisms colonizing the host plant benefit from plant-derived resources and form taxonomically consistent community patterns, as discussed above. In principle, two different, albeit not mutually exclusive, mechanisms might produce such microbiota structures. On the one hand, growing plants provide unoccupied niches to intruding microbial strains capable of exploiting the provided resources, thus resulting in stochastic colonization events. On the other hand, plant-microbe coevolution might provide the basis for a plant-driven selection process, resulting in active recruitment of microbiota members or at least keystone species that provide functions to the plant host. This may subsequently contribute to shaping the ultimate community during plant development. It is inherently difficult to disentangle a system that is as complex as the host microbiota and to distinguish between coevolved interactions and stochastic opportunities (for recent critical discussions, see 84, 128).

Generally, the observed consistency of microbial community patterns supports the notion of underlying principles and forces driving community formation. In addition, community assembly is a dynamic process reflected by shifts in the community composition over time in response to environmental changes and plant development. Although initial bacterial communities are similar to their respective seed banks, including soil and air, they become increasingly plant specific and less diverse as plants grow and develop (22, 27, 32, 78, 118, 125). Many factors, including environmental conditions, plant-derived primary and secondary metabolites, and microbe-microbe and plant-microbe interactions, act on microbial community assembly during all stages of plant growth (**Figure 3**). These factors also determine the overall number of independent colonization events. It is currently unknown whether bacteria establish a founding population early in plant development and then continuously colonize emerging habitats by clonal propagation or whether, alternatively, competitive strains and/or changes in plant developmental processes and environmental conditions favor independent colonization events of newly emerging niches. In addition, invasion and strain replacement may occur by direct interaction and competition for niches that are already occupied.

Sources of Colonizing Bacteria

Different seed banks may contribute to the colonization and microbiota formation on the host plant. A fraction of the plant microbiota may be acquired vertically from seeds and propagate as endophytes (49, 58); however, horizontal transmission is likely to predominate (19, 143). Therefore, microbial biogeography (in this context meaning the distribution of strains competent for host colonization) significantly influences the developing community.

Soil represents an extremely rich microbial reservoir on Earth (40); it is the predominant seed bank for the microbiota of the rhizosphere and the root, and a driver of community formation (10, 18, 77). Pronounced effects of soil on the rhizosphere microbiota have been reported for *Arabidopsis* as well as for various crop plants (15, 18, 32, 56, 77, 80, 94, 157). The microbial diversity declines sequentially from bulk soil to rhizosphere, rhizoplane, and roots, which suggests increasingly stronger competition among microorganisms as the habitat is more tightly defined. Alternatively, and not mutually exclusively, colonization may be described as a multistep selection process of the plant, in which growth of some of the microorganisms is preferentially promoted or inhibited (19, 103).

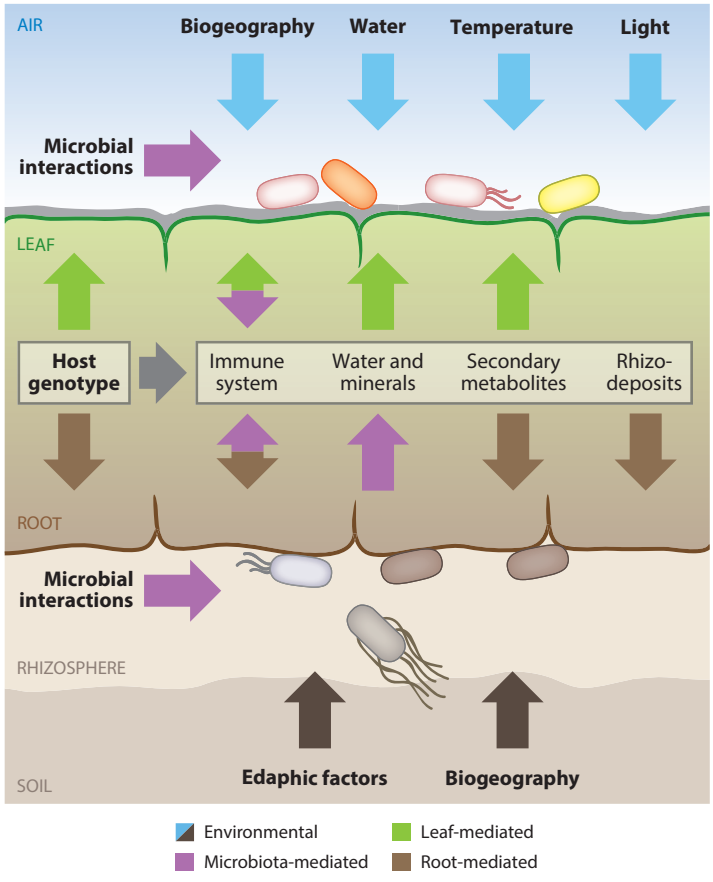


Figure 3

Environmental and soil- and plant-mediated factors influencing the host microbiota. Drivers such as temperature, UV radiation, water availability, biogeography of microorganisms capable of plant colonization, and presence of herbivores and pollinating insects are environmental factors that have an impact on the leaf microbiota. Furthermore, plant-mediated drivers include water and nutrient release, the production of secondary metabolites, and the microbial interplay with the plant innate immune system. The extent to which individual plant-mediated drivers influence the microbiota is dependent on host species and genotype, which is also the basis for important surface properties like cuticle composition and thickness. The root microbiota mostly originates from the surrounding soil, so edaphic factors and biogeography of putative colonizers and herbivores are predominant drivers. Root colonization is primarily fueled by rhizodeposits causing a significant shift in the composition of the root community compared with that of the surrounding rhizosphere and bulk soil. Finally, host-genotype–dependent effects, the plant immune system, and microbial interactions determine the eventual community structure of the plant microbiota.

Aboveground compartments inherently represent more open and fluctuating habitats, and various seed banks (e.g., air, precipitation, and plant and animal vectors), apart from soil or plant seeds, might contribute to the establishment of the microbiota. Notably, phyllosphere communities of annual plants are known to establish themselves in reproducible patterns over consecutive years, arguing for local, site-dependent, consistent sources of colonizers (65). One of these site factors might be the soil, a notion congruent with the observation that the phyllosphere microbiota is strongly influenced by soil at the beginning of the growth season but shifts to leaf-specific taxa as

the season progresses (27). Soil-derived bacteria may colonize leaves by direct (or indirect through rain splash) physical contact of soil with plant parts or by bacterial movement along the plant surface or even within plants, as has been demonstrated for endophytic rhizobia in rice plants (24). The ability of airborne bacteria to form communities on *Arabidopsis* leaves as a result of stochastic events and strong selection processes has been affirmed (78). Reproducible bacterial colonization patterns were observed after both leaf and clay inoculation, demonstrating the ability of bacteria to occupy unexploited niches (4). However, the relative contribution of the inoculum source to the leaf microbiota requires further investigation.

Diazotrophs: organisms capable of using atmospheric molecular nitrogen as sole N source

Environmental Factors

The above-mentioned sources of bacteria act locally at the site where the plant grows, and the same holds true for environmental factors that impact microbial community formation. In a number of studies, various other factors, including water availability, temperature, UV radiation, and macronutrient distribution, have been associated with community changes (13, 16, 27, 60, 61). Regarding macroelement availability, the effects of low nitrogen or phosphate availability on nitrogen-fixing endosymbionts in legume plants or on the association with mycorrhizal fungi have been well described (90, 93). Furthermore, nitrogen availability also affected the rhizosphere communities of *Medicago* (156) and sugarcane (152); however, diazotrophs were not specifically enriched (152). Changes in the composition of the leaf microbiota of maize and soybean in response to nitrogen fertilization have been reported as well (54, 79). Besides abiotic influences, biotic interactions, e.g., herbivore feeding, may have an impact on plant colonization by microorganisms (30, 71, 151).

Host Genetics

Another factor influencing the plant-associated microbiota is plant genotype. Differences among plant species are observed for the rhizosphere (15, 89, 157) and phyllosphere communities (62, 65, 68), which is not surprising given that distinct plants provide different local habitats to microorganisms with regard to root or leaf architecture and nutrient quality and quantity. Several research groups reported that *A. thaliana* ecotypes establish rhizosphere (18, 48, 77) or phyllosphere (1, 51) communities that differ in their composition from each other at a statistically significant level. However, the quantitation of differences in microbiota composition between ecotypes or crop cultivars makes it clear that overall diversity related to genotype is relatively small compared with that causally linked to environmental factors (17, 32, 94). Edwards and colleagues (32) found that a genotype-dependent effect of rice plants on community structure is stronger in the rhizosphere compartment and less pronounced in the root endosphere. Consistently, only 12 out of 778 detectable bacterial OTUs exhibited a pattern of ecotype-dependent enrichment in the endophytic root compartment of *A. thaliana* (77). Interestingly, the root bacterial communities of closely related *Arabidopsis* species differed more than those of *A. thaliana* ecotypes; however, host phylogenetic distance alone could not explain interspecies root microbiota diversity (113).

To exclude site effects and reduce the influence of environmental drivers, a SynCom approach was employed to investigate host genotype-dependent community development. A SynCom of seven members, representing abundant taxonomic groups of the phyllosphere microbiota, revealed genotype effects on the community composition in a small selection of *Arabidopsis* accessions (11). In addition, colonization levels were significantly different between the accessions. In another approach, almost 200 *Arabidopsis* accessions were screened in a hydroponic system to identify those in the presence of which the growth of a beneficial *Pseudomonas* strain was altered (48). In

two accessions, rhizosphere communities were strongly reduced in a subset of Pseudomonadaceae compared to ecotype Columbia (Col-0) in natural soil, which was likely attributed to active growth inhibition by a secreted antimicrobial compound (48).

As a complement to ecotype screening, the community composition of the microbiota of individual plant mutants was analyzed. For example, a mutation of an ABC transporter involved in root exudation (2) or artificial modulation of opine secretion (83) caused changes in the rhizosphere microbiota of *Arabidopsis*. Similarly, silencing of isoflavonoid synthase (IFS) genes altered the composition of the rhizosphere bacterial community of soybean (147). These results are consistent with an important role of root exudates on the establishment of rhizosphere and root microbiota. Regarding the phyllosphere, leaf cuticle mutants strongly affected the composition of the bacterial phyllosphere community (11, 104), as can be expected from the cuticle's function as a diffusion barrier.

Bacterial colonization of plants occurs in light of a sophisticated innate plant immune system, which is capable of detecting a wide range of evolutionarily conserved epitopes (28, 159); however, fundamental questions concerning microbial recognition and immune signaling remain unsolved. *Arabidopsis* leaves, for example, show distinct transcriptional responses to representative phyllosphere commensals in a gnotobiotic system with transcriptional reprogramming and induction of defense genes in plants colonized by *Sphingomonas melonis* but not by *Methylobacterium extorquens* (141). Phytohormones and plant immune signaling pathways are plausible candidates for shaping of the plant-associated microbiota. Using phytohormone mutants of *Arabidopsis* and natural and synthetic bacterial communities, Lebeis et al. (70) showed that salicylic acid (SA) modulates the root-inhabiting bacterial community. Furthermore, the *Arabidopsis myc2* and *med25* mutants deficient in jasmonic acid (JA) signaling harbored root bacterial communities that were distinct from those assembled by wild-type plants (21). In the phyllosphere, induction of SA-mediated defenses reduced endophytic bacterial diversity, whereas epiphytic bacterial diversity increased in plants deficient in JA-mediated defenses (66). In the future, it will be of interest to address how and to what extent the complex interactions between the plant immune system and microorganisms (26) influence plant microbiota composition and whether pattern- and effector-mediated recognition systems are involved in sensing and shaping of the plant microbiota.

Microbial Interactions

The presence of microorganisms per se can have an influence on microbiota establishment. As mentioned above for environmental factors, these effects might be mediated either directly at the level of microbe-microbe interactions or indirectly through interactions with the host plant. The presence of *Rhizoctonia solani*, a soilborne fungal pathogen, caused a significant shift in the composition of a disease-suppressive sugar beet rhizosphere community (23). Oxalobacteraceae, Burkholderiaceae, Sphingobacteriaceae, and Sphingomonadaceae were more abundant in the presence of the pathogen, and stress-related functions were induced in these bacteria. Chapelle et al. (23) proposed a model in which fungal invasion alters rhizosphere structure and, either directly or indirectly, induces stress responses in the community, thus activating antagonistic traits that ultimately lead to control of the pathogen. Differences in bacterial diversity were also reported in the lettuce rhizosphere and phyllosphere after *Rhizoctonia solani* infection (25, 35), as well as in the phyllosphere of maize suffering from leaf blight disease (79). Similarly, powdery mildew infection changed the bacterial community of the cucumber phyllosphere (124), whereas infection of *Arabidopsis* with *Albugo*, a leaf oomycete pathogen, strongly reduced the diversity of the phyllosphere microbiota (1). Taken together, the current data suggest that a combination of microbe-microbe and host-microbe interactions drives microbiota assembly. More detailed

studies are required to examine the effects of bacterial interactions, including antibiosis as an inducible bacterial trait or bacterial interactions mediated by direct interaction (110). In this context, it is interesting to note that in the barley rhizosphere, genes responsible for the type VI secretion system were enriched, which may suggest that microbe-microbe interactions play a role in the establishment of the rhizosphere microbiota (17). Analysis of gene enrichment further identified protein candidates involved in phage-microbe interactions, and analysis of nucleotide polymorphisms indicated that a subset of these proteins is under diversifying selection.

Induced systemic resistance: priming of the entire plant for enhanced resistance against diverse pathogens and herbivores by local stimulation through beneficial microorganisms

IMPORTANCE OF THE MICROBIOTA FOR HOST FITNESS

The plant-associated microbiota can provide benefits to plant growth and health by influencing the nutrient status, by affecting plant-pathogen interactions, and by modifying tolerance to abiotic and biotic stresses (8, 19, 81, 143). The outcome of the respective interactions is context-dependent, and interactions might be beneficial under certain conditions and harmful under others.

Diverse members of the plant microbiota affect the plant nutrient status by providing plants with nutrients, increasing nutrient bioavailability, or enhancing nutrient acquisition capacity in the soil. Symbiotic associations of legumes with nitrogen-fixing rhizobia (134) and of a large number of taxa with mycorrhizal fungi (119) are well-studied examples of how plants gain access to nitrogen and phosphorous, respectively, under limiting conditions. These associations also have implications for ecosystem functions. In a gnotobiotic grassland microcosm, the presence of both symbiotic nitrogen-fixing rhizobacteria and AM fungi synergistically affected composition and function of the plant community in a beneficial way under nutrient-limiting conditions (139). Rhizosphere and endosphere microorganisms other than rhizobia can also fix nitrogen, but the extent to which these diazotrophs contribute to nitrogen input can differ widely in agricultural settings (135, 152). Interestingly, foliar diazotrophs contribute to nitrogen content in a tropical forest ecosystem (39). Other rhizosphere colonizers can mobilize nutrients that are not readily available to plants, such as phosphorous or iron, through solubilization, mineralization, or excretion of siderophores (19, 81). Modulation of plant hormone signaling can also influence plant growth. Auxin, as well as other compounds produced by rhizobacteria, alters root system architecture, thereby indirectly enhancing nutrient acquisition by the roots (5, 120, 121, 155). Furthermore, microorganisms may directly activate nutrient acquisition of plants, as observed for some rhizobacteria that induce the iron acquisition machinery in *Arabidopsis* (154, 158). This response is induced by volatile organic compounds, independently of iron availability in the rhizosphere, and requires a photosynthesis-related signal (154). Interestingly, the plant transcription factor MYB72, which is required for the induction of iron acquisition, is also involved in the activation of induced systemic resistance by these rhizobacteria (154). Another link between nutrient acquisition and plant immunity has recently been found for a fungal endophyte of environmental *Arabidopsis* populations (50). The endophyte *Colletotrichum tofieldiae* provides the plant with phosphorous and alters phosphate translocation, thereby promoting plant growth and fertility under phosphate-deficient conditions. This beneficial interaction is controlled by the phosphate starvation response of the plant, and requires components of the immune system (50). The plant induces defense responses to the endophyte under phosphate-sufficient conditions, whereas root growth and phosphate metabolism are induced under phosphate-limiting conditions, illustrating prioritization of responses dependent on the environmental conditions (45).

The plant microbiota can also increase plant tolerance to abiotic stresses such as flooding, drought, high salinity, extreme temperatures, and heavy metal contamination (31, 41, 150). An enzyme implicated in plant-growth promotion under various stress conditions is 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which converts the ethylene precursor ACC to

α-ketobutyrate and ammonia. Microorganisms with ACC deaminase activity divert ACC, reduce ethylene production via ACC oxidase by the plant, and thereby alleviate ethylene-mediated inhibition of plant growth in response to various stresses (41). The microbiota might also help plants adapt to changing environmental conditions. In a study by Lau & Lennon (69), *Brassica rapa* and associated soil microbial communities were adapted to dry and wet conditions, respectively, for three generations. In a reciprocal transplant experiment, plant fitness was highest when the experimental conditions matched the environmental conditions under which the soil community had previously grown. An influence of the root microbiota on plant fitness and flowering time was also shown in other Brassicaceae (92, 144).

Disease-suppressive soils: natural soils with reduced susceptibility of crop plants to certain diseases

Diverse members of the plant-associated microbiota promote plant growth indirectly by protecting them from biotic stress (8, 9, 76, 81, 132). The mechanisms by which plant-associated microorganisms protect against plant pathogens include competition for niches and nutrients, antibiosis, production of lytic enzymes, inhibition of pathogen virulence, and induction of plant-mediated resistance, and have been reviewed elsewhere for root-associated microorganisms (8, 81, 95, 97). Interactions with other organisms, e.g., herbivores, can be affected by plant-associated microorganisms as well (3, 136). Phyllosphere-colonizing bacteria also contribute to protection against plant pathogens (57, 74, 99, 106, 143). Innerebner et al. (57) observed that several sphingomonads isolated from various plant species protected *Arabidopsis* against foliar bacterial pathogens, whereas isolates from air or water did not. Recent plant transcriptomics and plant mutant analyses revealed a plant-mediated component in protection (141). Furthermore, the identification of several *Sphingomonas* mutants that provide attenuated protection in planta suggested that different mechanisms could contribute incrementally to plant protection (142). Recently, it was also shown that increased resistance of an *Arabidopsis* cuticle mutant to the fungal pathogen *Botrytis cinerea* was conferred by the distinct phyllosphere microbiota harbored by this mutant (106). A *Pseudomonas* species isolated from the phyllosphere of the mutant provided protection against *B. cinerea* in *Arabidopsis* as well as on apple fruits, revealing that a subset of the microbiota might contribute to plant protection under the conditions of this experiment.

Another phenomenon is disease-suppressive soils, in which the control of soilborne pathogens can either be general, because of the overall activity of the microorganisms in the soil, or specific, by relying on the activity of only a subset of microorganisms (8, 43). Mendes et al. (82) compared the rhizosphere microbiota of sugar beet grown in either suppressive or conducive soil. Although the total number of bacterial taxa was similar in the two types of soils, the abundance of certain members of the microbial community differed in suppressive soils. Pseudomonadaceae, Burkholderiaceae, Xanthomonadales, and Lactobacillaceae were associated with disease-suppressive soil irrespective of pathogen presence, whereas Actinobacteria were more abundant in suppressive soil when the pathogen *Rhizoctonia solani* was present. A later study assessed the composition and function of the rhizosphere microbiome of the same disease-suppressive soil (23) and found an increased abundance of several bacterial families and induction of stress-related functions in the presence of the pathogen as discussed above for microbial interactions.

APPLICATIONS

As described above, plant-associated microbial communities can promote plant health and performance under various adverse conditions. With the demand for a more sustainable agriculture, the exploitation of these microbial functions has gained considerable interest from both academia and industry (9, 28, 76, 112, 131).

Various beneficial microorganisms have been commercialized (9); their efficacy has not always been consistent, but using a combination of strains could improve performance. In a recent study,

Phytoremediation: plant-mediated detoxification of environmental pollutants

Wei et al. (145) tested the ability of the plant pathogen *Ralstonia solanacearum* to invade different resident communities of closely related *Ralstonia* strains on the basis of bacterial carbon source competition networks. Resident communities with a clear niche overlap to the pathogen were best at reducing pathogen invasion in microcosms and in plant experiments (145). This is in line both with an earlier study conducted in soil by van Elsas et al. (140) and with conceptual ecological frameworks (127). Moreover, the combination of biocontrol strains that rely on different mechanisms has been found to improve plant protection, provided that the strains were compatible (123).

Engineering of microbiota to optimize specific attributes is a valuable goal (for review, see 85). For example, specific microbiota can be selected to affect flowering time and thus plant development (92). In this study, soil microbiota that stimulated earlier or later flowering times were enriched over 10 generations, and the selected microbiota were able to shift flowering time in other plant species as well. Selection might be performed not only on the microbiota side but also on the plant side. The host genotype has an effect on the microbiota (see above), and even small changes in the microbiota can have strong effects on plant health (48). Manipulation of root exudates to specifically enrich for the presence of certain microorganisms could, for example, improve their colonization competence and ensure continued colonization (83). Benzoxazinoids, for example, improve colonization of maize roots by *Pseudomonas putida* KT2440 (87), whereas malate exuded by *Arabidopsis* when foliarly challenged by *Pseudomonas syringae* promotes root colonization by *Bacillus subtilis* (108).

As beneficial effects of microorganisms on plant growth and health can be context dependent (48, 50, 107, 146), the evaluation of different microbiota needs to be done under conditions relevant for application. A better understanding of the various interactions and processes taking place at a systems level could help improve the use of microbiomes for desired functions. This is also required for the optimization of phytoremediation of contaminated soils, in which plant microbiota are also implicated (7, 96, 129).

SUMMARY POINTS

1. Healthy plants are host to a diverse community of microorganisms, the plant microbiota, that is dominated by bacteria. The communities on above- and belowground organs exhibit a defined taxonomic structure and are consistently composed of a few phyla, mainly Proteobacteria, Actinobacteria, Bacteroidetes, and, to a lesser extent, Firmicutes.
2. Most root-inhabiting bacteria originate from the soil microbiota. The bulk soil, the rhizosphere, and the root host taxonomically distinct bacterial communities and form a selective gradient from the exterior of the root, across the rhizoplane, to the interior of the root, with the root endophytic compartment being most exclusive and home to the least diverse community. Leaves inherently represent a more open habitat, and diverse seed banks contribute to the establishment of the natural microbiota.
3. A high proportion of the plant microbiota is cultivable on standard laboratory media, and inoculation of germ-free plants with SynComs results in reproducible colonization patterns resembling the natural plant microbiota.
4. Members of the plant microbiota are metabolically adapted to the utilization of plant-derived carbon compounds. Apart from utilization of rhizodeposits and low-molecular-weight carbon sources, metabolism of one-carbon compounds and plant cell wall components is frequently found in plant-associated bacteria.

5. The microbiota can promote plant health under various environmental conditions. A better understanding of the various interactions taking place at a systems level can offer advantages for potential applications in sustainable agriculture.

FUTURE ISSUES

1. Several studies have set out to determine the phylogenetic core community on distinct plant organs or host plants. Because phylogenetically diverse taxa might inhabit similar niches on distinct host plants, the identification of functional core characteristics of the plant microbiota will be important.
2. Metagenomics paired with quantitative functional genomic approaches, e.g., proteomics, transcriptomics, and metabolomics, will reveal further insights into microbial interactions.
3. Standardized procedures and protocols for culture-independent surveys would significantly increase data comparability and enhance chances to draw biologically relevant conclusions across studies.
4. Strain collections should be extended to more comprehensively cover the taxonomic diversity of microbiota.
5. Increased diversity of strain collections from different plants, including crops, will improve SynCom resemblance to the natural microbiota and extend the possibilities to draw environmentally relevant conclusions from the results of experiments under controlled laboratory conditions. Furthermore, whole-genome sequencing of pure cultures will allow curation of reference databases for further improvement of data analysis.
6. The plant immune system and the complex interplay of the plant microbiota will require systems-level investigations. It is currently insufficiently clear how the immune system recognizes microbiota members, how it distinguishes between pathogenic bacteria and the majority of nonpathogenic bacteria, and how this might contribute to shaping of the microbiota.

DISCLOSURE STATEMENT

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Errata

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