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Opinion

Plant Genetic Networks Shaping Phyllosphere Microbial Community

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Phyllosphere microbial communities inhabit the aerial plant parts, such as leaves and flowers, where they form complex molecular interactions with the host plant. Contrary to the relatively well-studied rhizosphere microbiome, scientists are just starting to understand, and potentially utilize, the phyllosphere microbiome. In this article, we summarize the recent studies that have provided novel insights into the mechanism of the host genotype shaping the phyllosphere microbiome and the possibility to select a stable and well-adapted microbiome. We also discuss the most pressing gaps in our knowledge and identify the most promising research directions and tools for understanding the assembly and function of phyllosphere microbiomes – this understanding is necessary if we are to harness phyllosphere microbiomes for improving plant growth and health in managed systems.

Phyllosphere Microbiome and Its Impacts on Plant Health

The aerial part of terrestrial plants, collectively known as the phyllosphere, is the host to one of the largest and most complex microbial community habitats on earth [1]. The phyllosphere includes leaves, flowers, fruits, buds, and stems, and thus the microorganisms colonizing these surfaces on the outside (epiphytes or phylloplane) or in the inside (endophytes or endosphere) collectively form the phyllosphere microbiome. These microbial communities on healthy leaves consist of bacteria, archaea, fungi, viruses, algae, and occasionally nematodes and protozoa [1]. Bacteria exceed by far the other groups, both in number of cells and diversity of taxonomic groups [2]. Within the bacterial domain, the phylum Proteobacteria, which includes three classes (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria), forms the largest fraction of phyllosphere bacteria, followed by members of the classes Firmicutes, Actinobacteria, and Bacteriodetes [3,4]. A large part of these leaf bacterial communities is locally preserved, for example, in soils, which act as an important reservoir of phyllosphere diversity [5]. However, a small fraction of phyllosphere bacteria is also recruited from the air and other plants [6]. Microbes, aggregated with dust particles, plant debris, and pollens, can travel long distances with wind erosion and rain splashing [7]. For instance, *Massilia* species from Proteobacteria, which is a major contaminant in the air [8], makes 7% of total bacterial population in the spinach leaf microbiome, suggesting its origin from the air [9]. Moreover, animal and insect (especially herbivorous insects) feeding not only transmits the microbes across different plants [10] but also introduces secretions from insect's symbiotic bacteria into plant wounds to modulate plant defense [11]. One study showed higher bacterial diversity (especially *Pseudomonas syringae*) in the herbivore-damaged plants versus undamaged plants [12].

Plants and microbes have evolved together for more than 400 million years, with the phyllosphere as an important platform of their interactions. During this time, complex interactions have developed that significantly influence plant performance, for example, by assisting plants' defense against certain biotic and abiotic stresses [13]. In contrast to the intensively studied

Highlights

Plant immunity networks maintain microbial homeostasis in the phyllosphere, which in turn affects the plant health. Plant exudation and volatiles significantly shape the microbiome structure and composition.

Various environmental stresses shape the complex interaction between phyllosphere microbiome and plant immunity.

Understanding the molecular basis of plant–microbe and microbe–microbe interactions will help elucidate their impact on plant fitness.

Recent advances utilizing synthetic microbial community combined with omics tools (such as metagenomics and metabolomics) provide important insights into the physiology and functionality of the phyllosphere microbiome. An integrated knowledge of multiomics combined with synthetic community approach can help determine the individual as well as community level contribution of phyllosphere microbiome in the host fitness.

Microbiome engineering can reshape the microbial composition in the phyllosphere, and holds potential for large-scale microbiome research and reconfiguration of phyllosphere microbiome with desired traits to fight plant stresses.

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root-colonizing microbiome and its role in plant health, the collective community-level contribution of leaf microbiome to the plant health and development is not well understood [14]. Composition of the leaf microbiome is influenced by intrinsic factors (e.g., genotype, age, and species of plants, microorganisms, etc.) and biotic and abiotic environmental factors (e.g., geographical location, soil type and properties, climate, and insect herbivory, etc.) [1,5,15,16] (Box 1). Among these factors, the extent of plant genetic control over bacterial communities is of great interest to crop breeders and evolutionary biologists. The composition of the phyllosphere microbiome can be influenced by host genotype [15], and a recent ecological study demonstrated that terrestrial ecosystem productivity positively correlates with leaf microbial diversity [17]. However, many questions are still outstanding, for example, around the fundamental mechanisms through which phyllosphere microbiomes are recruited and affect plant health. What is the molecular basis of plant–microbe interactions in the phyllosphere? Do variations in phyllosphere microbiomes contribute to plant health, or is the composition of these microbiomes a consequence of plant health? Moreover, it is unclear whether it is possible to select for a stable and robust microbiome community that positively influences overall plant health. Here, we review recent research to address these fundamental questions, and discuss the potential approaches to understand the molecular mechanisms of phyllosphere microbiome–plant interactions. We identify knowledge gaps and future research directions that need to be addressed if we are to harness phyllosphere microbiomes for improving plant growth and health in managed systems.

Microbiome Assembly under the Control of Plant Genetic Networks

Initial studies have identified plant genotype as an important factor influencing phyllosphere microbial diversity and composition [6,18,19]. For instance, phyllosphere bacterial diversity analysis

Box 1. Environmental Factors Influence the Chemistry of Plant–Phyllosphere Interaction

Plants are constantly exposed to spatial and temporal environmental variations regulated by both abiotic and biotic factors that can influence the phyllosphere microbiome assembly. For instance, soil, an important reservoir of phyllosphere microbial diversity, provides microbes for the phyllosphere through vascular transmission and air dispersal and influences leaf chemistry and microbial assembly [5]. **Climate change** can influence the phylogenetic composition of leaf bacterial microbes by increasing pathogenic bacterial populations but reducing beneficial bacterial abundance [16]. Prominent geographical site effects were recognized on bacterial communities in lettuce plants as well as fungal and bacterial communities of *Tamarix* trees and mustard plants [9,15,88]. Such strong site effects likely correlate with distinct soil pH, nutrient profiles, as well as other environmental factors such as temperature, humidity, and UV radiation that influence plant and soil properties [1]. In this context, a study has shown that phyllosphere bacterial communities differ greatly in urban- and nonurban-grown ivy plants and relative abundance of microbes significantly correlates with exposure to traffic-generated air pollutants in urban locations [89]. Furthermore, agricultural management (foliar spray and tillage) can also affect phyllosphere microbiomes by altering plant and soil traits [1], as evident from a study in which distinct fungal phyllosphere microbial communities were found to be associated with conventional and organic vineyards [90].

Leaf (and soil) bacterial communities differ distinctly from aerial bacterial communities, further supporting the point that plants likely have strong genetic/phenotypic control over the recruitment of these communities, or that airborne bacterial communities are derived from a mixture of soil and leaf bacteria [91]. Biotic factors such as plant development stage drive the phyllosphere microbiome assembly as described by a study in which plants of varied ages harbored differential microbial abundance [15]. Plant nutrient status can also affect the structure of phyllosphere microbial communities [92]. A recent study has reported that the abundance of specific phytopathogens in the leaf increases upon grazing [18]. Not just above-ground grazing, but also the grazing-like feeding on roots, by for example nematodes or root-feeding larvae, can have consequences for the composition of the phyllosphere microbiome. Below-ground interactions of plant roots with harmful, but also with beneficial organisms such as mycorrhizal fungi, influence the concentration of plant defense compounds such as terpenoids and glucosinolates, but they can also affect plant nutrient status [93]. Thus, these below-ground biotic interactions have the potential to directly affect the phyllosphere microbiome, as has been shown in a study in which grass colonized with its native arbuscular mycorrhizal fungus had a distinctly different leaf bacterial community compared to uncolonized individuals [93]. Furthermore, bacteriophages are predicted to maintain bacterial diversity [94,95] and recent work has shown that bacteriophages alter the overall bacterial abundance of dominant community members in the tomato phyllosphere, reducing both bacterial alpha and beta diversity upon colonization to new host plants (N.M. Morella, PhD thesis, University of California, Berkeley, 2019).

identified less bacterial species variability within a plant species as compared to different plant species [19]. A growing body of evidence suggests that plant genetic factors controlling leaf structure (cutin and cuticular wax properties, trichome branching, etc.), leaf physiology (including surface features such as leaf exudates and volatiles), plant defense, and hormone signaling pathways significantly shape the phyllosphere communities and initiate several microbe–microbe interactions [20–24] (Figure 1, Key Figure).

Microbial cells landing on the leaf surface first encounter the leaf cuticle, which is composed of hydrophobic waxy lipid layer is a key interface for microbe–microbe and plant–microbe interactions that greatly influences the leaf microbiome assembly and composition [25]. Genetic factors such as eceriferum (*CER1*, *CER6*, and *CER9*) and resurrection1 (*RST1*) have been identified in

Key Figure

Genetic Networks Controlling Microbial Communities on Leaves

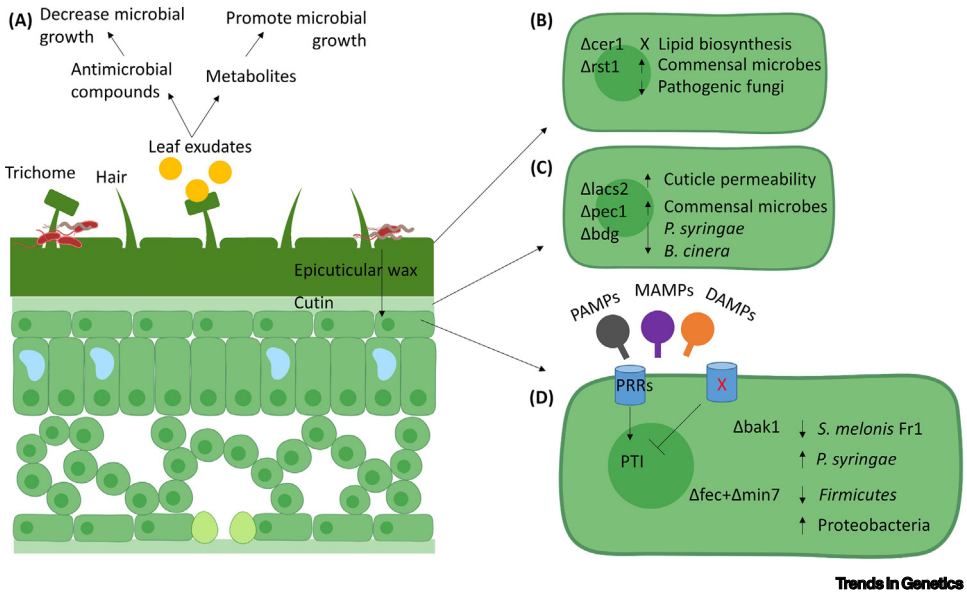

Figure 1. (A) Leaf exudates such as metabolites (sugar and inorganic nutrients) as well as volatile organic compounds released by leaves significantly influence its microbial communities, through its antimicrobial activity or serving as a carbon source [29,99]. (B) Changes in epicuticle wax composition affect the outcome of microbial interactions. For instance, *cer1* and *rst1* mutant plants deficient in lipid biosynthesis is associated with increase abundance of commensal microbes (bacterial and biotrophic fungi) and reduced growth of necrotrophic fungi [26,27]. (C) Plants with altered cutin structure modify epiphytic bacteria. For instance, mutant plants for long-chain acyl-coenzyme A synthetase 2 (*LACS2*), ATP-binding cassette transporter (*PEC1*), and α/β hydrolase (*BDG*) show increased leaf permeability and associated with resistance to fungal/bacterial pathogens [20,28]. Leaf cutin with higher permeability can better penetrate pathogen elicitors, thus inducing plant defense faster and production of reactive oxygen species to kill the pathogenic microbes. (D) Plant maintain microbial homeostasis using its immunity mechanisms. Microbes swimming towards stomata to avoid plant cuticles releases a variety of elicitors [microbe-associated molecular patterns (MAMPs)/pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)]. Plants recognizes these elicitors by interaction with pathogen-recognition receptors (PRRs), thus inducing MAMPs-triggered immunity (MTI). For instance, activation of MTI by phyllosphere commensal bacteria *Sphingomonas melonis* Fr1 (S. Fr1) protects the plant against the leaf pathogen *Pseudomonas syringae* and plants with mutant PRRs (such as *bak1*) lose S. Fr1-mediated protection against *P. syringae* [56]. Similarly, plants defective in three major PRRs (*fls2*, *efr*, and *cerk1*) and vesicle-trafficking pathways (*min7*) display higher abundance of Proteobacteria and limited growth of Firmicutes, thus resulting in leaf necrosis and dysbiosis [21].

Glossary

Climate change: a change in global or regional climate patterns, in particular a change apparent from the mid to late 20th century onwards and attributed largely to the increased levels of atmospheric carbon dioxide produced by the use of fossil fuels.

Induced systemic resistance: a resistance mechanism in plants that is activated by infection. Its mode of action does not depend on direct killing or inhibition of the invading pathogen, but rather on increasing physical or chemical barrier of the host plant.

Gnotobiotic: relating to or denoting an environment for rearing or culturing organisms in which all the microorganisms are either known or excluded.

Metagenomics: the study of microbes in their natural living environment, which involves the complex microbial communities in which they usually exist.

Metabolomics: the large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. Collectively, these small molecules and their interactions within a biological system are known as the metabolome.

Metaproteogenomics: the combination of metagenomics and metaproteomics that studies the whole genome and proteome; in this article this refers to studying the microbial community in order to understand their physiology.

Plant innate immunity: plants immediate defense response that recognizes pathogen-associated molecules and activates physical, chemical and cellular defenses against pathogens.

Rhizosphere: the 0.5–4 mm soil zone surrounding plant roots that is strongly affected by root activities.

Arabidopsis to be involved in cuticle lipid biosynthesis. Mutations in these genes are associated with varied chemical composition of waxes, higher bacterial diversity, and increased proliferation of biotrophic fungal pathogen *Erysiphe cichoracearum* [26,27]. Furthermore, long-chain acyl-coenzyme A synthetase 2 (*LACS2*), ATP-binding cassette (ABC) transporter (*PEC1*), and α/β hydrolase (*BDG*) are involved in the cuticle formation and modulation of the leaf tissue chemistry and surface topology. *Arabidopsis* mutants for these genes are associated with significant microbial community alterations, particularly resistance to *Botrytis cinera* [20,28]. Most of these cuticle mutants overaccumulate reactive oxygen species (ROS) with antimicrobial effects, which might explain their resistance to *B. cinera*.

Leaf exudates also shape phyllosphere microbial assembly that includes primary metabolites such as sugars, carbohydrates, organic acids, and amino acids, and secondary metabolites, such as terpenes, benzenoids, and methanol [22,29]. Leaf exudates are limited sources of carbon nutrients for its microbial inhabitants and competition for nutrients drives microbe–microbe and plant–microbe interactions that ultimately defines the microbial community structure [30,31]. Carbohydrate consumption by leaf colonization by *P. syringae* pv. Tomato and *Sphinogmonas melonis* promotes their growth [32]. Antimicrobial effects of plant volatile organic compounds such as terpenoids, benzenoids, and aldehydes limit the growth of epiphytic microbes in the phyllosphere [33,34]. However, methylotrophic microorganisms such as *Candida boidinii* and *Methylobacterium extorquens* can grow exponentially by efficiently metabolizing plant-emitted carbon compounds such as methanol and chloromethane [4,35] produced by pectin methyl esterase (*PME*) and S-adenosylmethionine and *HOL* (HARMLESS TO OZONE LAYER) genes, respectively [36,37].

Plant exudation is defined by host genotype, age, and abiotic stresses [38–40], and recent studies has shown that all three factors directly influence the phyllosphere microbiome assembly [15,24,41,42] that might be correlated with differential plant exudation patterns. Leaf glandular trichomes and hydrothodes release a variety of exudates in leaves through leaching and guttation, whose formation is controlled by MIXTA like MYB and basic helix-loop-helix (bHLH) transcription factors, respectively [43–45]. Moreover, several plant-encoded transporters are involved in nutrient transport in leaves. For instance, *SWEET17* (for SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTERS17), *ALMT12*, and *ALMT1* (aluminum-activated malate transporters) for sugar and organic acid transport have been characterized [46,47]. Amino acid transport is controlled by LYSINE HISTIDINE TRANSPORTER1 (*LHT1*), bidirectional amino acid transporter 1 (*BAT1*) and Siliques Are Red1 (*SlAR1*) in *Arabidopsis* [48–50]. Several ABC transporters are involved in primary and secondary metabolite transport in *Arabidopsis* [51]. Since the genetic factors involved in synthesis and transport of metabolites in leaves are similar to root exudes, we argue that leaf exudation is also under plant control, similar to root exudation. However, special attention is required to characterize these transporter families with respect to their role in shaping phyllosphere microbiome.

It has been recently identified that plant immunity networks also control the diversity and relative abundance of associated microbes in the phyllosphere through its defense mechanisms. **Plant innate immunity** (see Glossary) targets microbial pathogens either by perceiving microbe-associated molecular patterns (MAMPs) via pattern recognition receptors (PRRs) located on the plant cell surface, known as MAMPs-triggered immunity (MTI), or by recognizing disease-promoting bacterial effectors via nucleotide-binding leucine-rich repeat (NLR) receptors known as effector-triggered immunity (ETI) [52]. However, beneficial microbes have evolved the ability to escape through PRR recognition and MTI [53] as well as ETI, via coding several genes that have domain resemblance with plant NLR domains that can compete with plant

NLRs for effector binding [54]. Despite their escape from immune surveillance systems, beneficial bacteria induce the first layer of defense, MTI, which likely plays a critical role in plant defense against diverse pathogenic stresses [55]. Consistent with this hypothesis, activation of MTI-related defense genes by the phyllosphere commensal bacteria *S. melonis* Fr1 (S. Fr1) provide protection against the leaf pathogen *P. syringae*; whereas the plant's mutant for these PRRs (*BAK1/BKK1*) reveals attenuated S. Fr1-mediated plant protection [56].

Commensal or beneficial bacteria also trigger MAMP-mediated immunity in plants, which raises the question of how they escape or suppress MTI. Although it is possible that MTI functions as a general mechanism to inhibit pathogens and maintain microbial homeostasis by selectively gating microbes in or out upon close contact with the host. Supporting this hypothesis, recent studies have identified the genetic networks through which plant immunity potentially shapes the phyllosphere bacterial community structure and maintains homeostasis. *Arabidopsis min7*, *fls2*, *efr*, and *cerk1* quadruple mutants (*mfec*) impaired in MTI and the *MIN7* vesicle-trafficking pathway, which is the host target for the bacterial effector *HpoM1* to create an aqueous microenvironment for infection, exhibited dyshomeostasis in the phyllosphere endophytic bacterial community, including accumulation of devastating Betaproteobacteria and inhibition of Firmicutes. The altered microbiome alters plant metabolism and gene expression by influencing leaf chemistry and creating a condition suitable for its excessive proliferation that ultimately induces symptoms such as leaf necrosis and chlorosis [21]. The modulatory role of the plant's MTI and *MIN7* vesicle-trafficking pathway for controlled accommodation of endophytic microbial community level and diversity has been characterized, but no direct relation between MTI and epiphytic bacterial community has been identified [21]. Similar mechanisms have been observed for the gut microbiome in humans, where host innate immunity controls the colonization and composition of the gut microbiome and perturbation in the composition of the microbiome can result in immune-related diseases, including inflammatory bowel disease [57,58]. This suggests that plants also control and regulate the commensal microbes' level via their immune systems in order to maintain their health. Taken together, we propose that plant genetics is the most important control over microbiome assembly in the phyllosphere.

Complex Interplay between Plant Immunity, Microbiome, and Environment

Plant immune responses such as jasmonic acid (JA)-, salicylic acid (SA)-, and ethylene (ET)-related signaling pathways are critical in regulating plant defenses and modulation of plant-associated microbiomes. Symbiotic microbes deploy various molecular strategies to evade plant immunity (as mentioned above) but can still induces MTI that triggers the JA-, SA-, and ET-related signaling pathways [59–62]. Plants use such interactions to counteract diverse stresses that consequently influence the microbial diversity and assembly, including the phyllosphere microbiome. Evidently, the role of JA for symbiotic bacterial diversity in the phyllosphere [63] and differential colonization by early nodulation bacteria, *Azoarcus* spp., has been reported in *Arabidopsis* [64,65]. JA signaling also positively regulates specific growth promoting bacteria in the **rhizosphere** [38,66]. Similarly, SA as well as ET signaling pathways are involved in the modulation of commensal bacteria in leaves [63] and roots [20,60,67]. Taken together, this suggests that signaling pathways of the plant immune system play and important role in structuring the reciprocal interplay between plants and their microbiome (Figure 2).

Plant immunity-based reconfiguration of microbial communities is highly influenced by different environmental conditions. Environmental variations such as temperature, nutrient availability, water, and other soil conditions appear to modulate plant immunity through modification of molecular components of MTI and ETI [68–70], crosstalk between defense and stress hormones [71,72], and joint plant response to biotic and abiotic stress due to shared signaling components

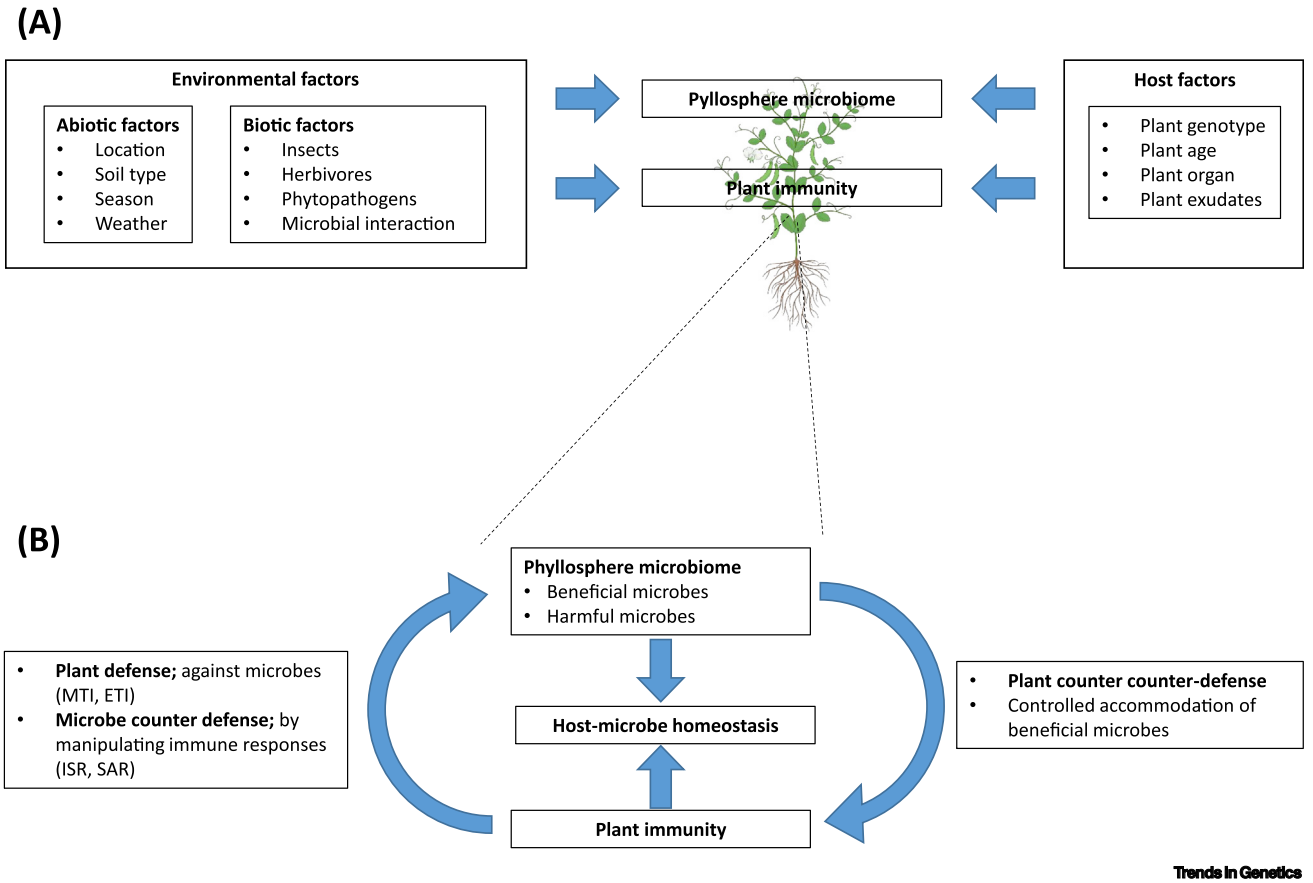

Figure 2. Influence of Environmental Factors and Plant Immunity in Shaping Phyllosphere Microbiome. (A) Plants are continuously exposed to several environmental factors including abiotic factors such as temperature, humidity, weather, soil condition etc., and biotic factors such as insects, herbivores, and phytopathogens. Biotic factors such as herbivory and abiotic factors such as rain and soil introduces new microbes in the phyllosphere that activates plant immunity as well as intermicrobial interactions influencing microbiome assembly [5,7,100]. Apart from environmental factors, several plant factors such as genotype, age, and exudates make a significant influence. For instance, leaf exudation is a limited nutrient source for its microbial inhabitants that promotes microbial growth [22]. Plant-age-dependent variation in microbial assembly might be linked to different exudation pattern in different plant growth stages. (B) These factors, in combination with the phyllosphere microbial community, trigger a cascade of reactions in the plant immune system such as effector-triggered immunity (ETI) and microbe-associated molecular pattern-triggered immunity (MTI); combining to shape plant defense. Plant MTI works in cooperation with commensal/beneficial microbes to maintain microbial homeostasis that maximize plant fitness. Priming of systemic acquired resistance (SAR) and **induced systemic resistance** by pathogenic and commensal/beneficial microbes, respectively, render uninfected plants more resistant to a wide range of plant pathogens. Thus, the plant immune system forms a complex microbial management system that maintains a balance by allowing the beneficial microbes to grow and terminates the harmful microbes. This interplay between plants and microbes helps shape a beneficial phyllosphere microbiome assembly.

[73,74] (Figure 2). Functional links between plant immune systems and the environmental factors that modulate root colonization by commensal and pathogenic bacteria have been described elsewhere [24,75–77]; here, we focus on the phyllosphere. The rhythmic induction of NLR-mediated plant immunity by circadian regulator genes has been reported to control *P. syringae* DC3000 proliferation in *Arabidopsis* leaves [78,79]. Likewise, temperature has been shown to act as a key factor intersecting plant immune response to the *P. syringae* infection, where low temperature favors the secretion of bacterial effectors and activates ETI, while high temperature inhibits the secretion of bacterial effectors that trigger MTI signaling upon enhanced bacterial proliferation [75]. A synchronized interplay between plant immunity and humidity has been reported to control *P. syringae* infection and to fine tune the endophytic commensal communities in the phyllosphere [24]. Under low phosphate conditions, *Arabidopsis* plants recruit the fungus *Colletotrichum tofieldiae* that transfers the macronutrient phosphorus to shoots, which enhances

plant growth and fitness [41]. Moreover, microbes under limiting phosphate conditions activate the expression of phosphate starvation response 1 (*PHR1*) that represses microbial-driven plant immune responses and contributes to a normal root microbiome assembly, thus coordinating the trade-off between defense and nutrition status in *Arabidopsis* plants [73]. Taken together, these studies suggest that microbe-mediated modulation of plant immunity is highly influenced by the environment. Thus, it is critical to understand plant selection for beneficial bacteria and host immune-mediated modulation of associated microbiome under different environmental conditions.

Potential of Omics Approaches and Future of Large-Scale Phyllosphere Research

Recent studies have used omics tools combined with the application of synthetic communities (SynCom) in **gnotobiotic** systems to address the challenges of plant–microbe interactome research [20,80–83]. Synthetic communities can be constructed by mixing microbial strains of interest, manipulated and applied aerially to plants grown under controlled conditions, followed by different omics approaches to determine the underlying mechanisms of plant–microbe interactions at transcriptome, proteome, and metabolome level [83]. These approaches have the potential to provide important insights into the functionality of the microbiome towards plant health and physiology, and establish a causal link between individual microorganisms and plant genotype and/or phenotypes. For instance, a transcriptomic analysis of *Arabidopsis* leaves upon colonization with commensal bacteria, S.Fr1 and *Methylobacterium extorquens* PA1 (M.PA1), lead to the identification of S. Fr1-mediated activation of defense-related genes, protecting the plant against *P. syringae* [56]. Similarly, a synthetic community approach identified host genetic factors involved in cuticle formation and ethylene signaling that shape microbial community structure and abundance in the phyllosphere [20].

A **metagenomics** approach combined with SynCom has unraveled a causal link between plant immunity and phyllosphere bacteria determining plant health [21]. Similarly, the SynCom approach combined with metagenomics and **metabolomics** has been successfully adapted to study microbial interkingdom interactions and for *in vitro* characterization of plant growth-promoting traits activated/produced by the root microbiome [53,80,81]. This combined approach can be applied to compare phyllosphere microbiome assembly under different stress conditions and leaf exudates can be analyzed to identify potential metabolites produced/induced by microbes followed by *in vitro* examination of the plant growth-promoting microbiome and metabolites.

The molecular basis of plant–phyllosphere microbe interactions has also been studied using metabolomics approaches, which identified phyllosphere bacteria-mediated alterations in plant arginine metabolism and phytoalexin biosynthesis [32]. Conversely, the removal of phyllosphere microbes using antibiotics leads to a decreased concentration of several primary and secondary metabolites in plants [84]. Proteomics has been successfully used to study the molecular basis of species-specific adaption mechanisms of two commensal leaf bacteria [85]. Similarly, metagenome and metaproteome analysis of bacterial communities identified functionally convergent set of proteins involved in glycolysis, metabolism, and stress and antioxidant response, which might be important for both bacterial and plant health [86]. Another novel omics approach in this context is **metaproteogenomics**, in which proteins present in complex microbial communities are identified based on their metagenomes. This approach has the potential to double the number of proteins that can be identified compared to protein identification using public databases alone [3,4]. Integrated knowledge from SynCom combined with multiomics tools will provide insights into the functionality of plant microbiome system and help gain a clear and reliable picture of the biological phenomena at work.

Until recently, metagenomics tools have provided insights into the genetics and composition of microbial communities, and their metabolic and physiological potential in improving plant health [87]. However, research to investigate the molecular basis of plant–microbe interactions and microbial community assembly and composition has only just started. Intelligent experimental designs that simultaneously assess microbial community composition, host and microbial gene expression profiles at the transcriptomic and proteome level, and *in situ* quantification of plants' and associated microbial metabolites are key to establish links between plant phenotypes and associated microbiomes. Full control over adjustable factors, including microbial community design, genetic alteration of host and microbial stains, and (biotic and abiotic) growth conditions will facilitate the interpretation of data from such experiments. In addition, quantifying the role of biotic and abiotic environmental factors is essential for understanding these interactions in the real world.

Concluding Remarks

Emerging evidence suggests that phyllosphere microbial assembly is shaped by complex interactions between the abiotic and biotic environment, plant genotype, and microbial communities. The plant genotype is among the most important factors; its detailed study can open new ways to harness the phyllosphere microbiome for plant growth and fitness. Since different accessions of *Arabidopsis* harbor different phyllosphere microbial communities [20], the exploitation of wild accessions offers potential to identify novel genes that influence phyllosphere microbial community composition. Emerging evidence shows that the leaf exudates and volatiles influence phyllosphere microbial community assembly, and this might explain how plant recruitment for specific microbial species plays a fundamental role in defining overall phyllosphere microbial assembly. We argue that accessing the role and molecular mechanisms (including synthesis and transport) of individual leaf-derived molecules towards microbial colonization and plant health is the most promising research direction. Use of modified plant lines with altered leaf structure and enhanced/suppressed expression of specific leaf surface molecules and volatile compounds to determine their respective impact on the phyllosphere microbiome will be the next critical steps. Plant immunity networks not only function to limit pathogen invasion but also maintain microbiome homeostasis for plant health. Engineering plant genomes for selection of a healthy microbiome to protect plants from dysbiosis and other stress conditions is an ambitious strategy. However, we are far from characterizing a healthy plant microbiome and this requires special attention. Contrary to rhizosphere microbiome characterization, the role of individual microorganisms in the phyllosphere, as well as community level contribution towards plant health, needs more research. Recent use of synthetic communities in combination with omics approaches has started to address various aspects of phyllosphere–

Box 2. Phyllosphere Microbiome Engineering for Increased Host Fitness

Microbiome engineering refers to the experimental methods that improve host performance by artificially selecting for microbial communities that positively affect host fitness, and has significant promise to increase overall productivity and resilience to perturbations in agricultural systems [96]. Engineering of the phyllosphere microbiome can be performed by applying multigenerational artificial selection upon plants that vary in their phyllosphere microbiome with the aim to alter plant traits. However, this is a challenging task and, to date, there are only a limited number of studies on the phyllosphere engineering [97]. A major challenge for phyllosphere engineering is the fact that phyllosphere microbial diversity varies with minor variations in environmental biotic or abiotic factors. Furthermore, a slight change in the genotype of the host plant can also shape the selective phyllosphere [20]. Thus, it is necessary to take into account the various factors that influence not only how the microbiome interacts with the plants but also how the plant shapes the microbiome. Recently, microbiome engineering was performed to manipulate the tomato phyllosphere microbiome using an experimental evolution approach. An initial diverse microbial inoculum was sprayed onto plants and the resulting microbial community was collected from the leaves, a process known as passaging. After four such passaging events, the diversity of the phyllosphere microbial community decreased, and when the community resulting from the fourth passaging was combined with the initial microbial inoculum and applied to the leaf, the resulting community strongly resembled the fourth passaging community. This suggests that the selected microbes were adapted to the local leaf environment [98]. Approaches like this enhance our tools to reconfigure the plant phyllosphere microbiome with desired traits to fight plant stresses, as well as to study large-scale microbial interactions in the natural fields.

Outstanding Questions

How much do plant genotype and interactions of plant genotype with the local environment contribute to phyllosphere microbiome variation?

How do plant molecular mechanisms differentiate between pathogenic and beneficial microbes?

Soil acts as a reservoir for phyllosphere diversity; how do host plant selection, environment filtering and intermicrobe interactions function to recruit microbial communities from soil?

Do phyllosphere microbes interact with the rhizosphere microbiome?

It is known that plants attract beneficial microbes in the rhizosphere in the time of stress known as the 'cry for help' strategy. To what extent does this strategy work in the phyllosphere? If such interactions occur, what are the signaling cues that a plant uses to attract phyllosphere microbes?

How and to what degree plant modulate leaf exudes to interact with specific microbes to shape phyllosphere microbiome?

How does the phyllosphere microbiome reassemble and interact with plant defense mechanisms under different stress conditions, such as insect herbivory?

How do changes in plant physiology and defense conditions affect the protective behavior of microbes in the phyllosphere?

How does the phyllosphere microbiome influence plant metabolic and defense pathways?

Can we identify and characterize plant growth promoting phyllosphere microbiomes or metabolites?

plant interactions, but key knowledge gaps remain, especially regarding the molecular basis of host–microbe and microbe–microbe interaction for shaping and maintaining microbial community assembly (see Outstanding Questions). Understanding the diverse bipartite (e.g., plant–microbe and microbe–microbe) and tripartite (e.g., plant–animal–microbes and plant–environment–microbe) interactions will help us to characterize plant microbiome taxonomy and their function in plant health. With the changing climate, it is crucial to understand and harness the beneficial microbiome, to develop the climate resilient future crops (Box 2). Characterization of the microbiome community assembly under different stress conditions, its molecular level interaction with plants and other microbes, and *in vitro* characterization of growth-promoting phyllosphere microbes and their compounds will be critical next steps that have the potential to improve plant productivity and environmental sustainability based on biological solutions.

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