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A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome

Johan HJ Leveau

The plant leaf surface, or phyllosphere, represents a unique and challenging microbial biome with a diverse and dynamic community of commensal, parasitic, and mutualistic agents of microscopic proportions. This mini-review offers a digest of recently published research dedicated to the study of phyllosphere microbiota, framed in the context of processes and outcomes of microbial community assembly, structure, and (inter)activity in the phyllosphere, with particular focus on the contributions of environment, plant, and microbe, and on the potential benefits of interrogating those contributions at finer resolutions.

Address
Department of Plant Pathology, University of California, Davis, CA 95616, USA

Corresponding author: Leveau, Johan HJ (jleveau@ucdavis.edu)

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Introduction

The purpose of this mini-review is to provide a short status report on the field of phyllosphere microbiology by assimilating recently published research into an updated narrative that aims to convey where the field stands currently and where it may be headed in the future. In line with this purpose, source materials were limited to a selection of papers that appeared in print or online in 2018 or early 2019. These papers include an impressive collection of excellent review articles that are dedicated in full or in part to phyllosphere microbiology (Table 1). Collectively, they represent a body of knowledge that this mini-review draws from, further adds to, and provides perspective on.

The term 'phyllosphere' is often introduced or used [3,6,12,13,16] to broadly include not only plant leaves ('phyllo-') but also other above-ground plant parts such as flowers [17,18], pollen [19], fruit [20,21], stalks [22], and bark [23]; not only leaf surface colonizers (known as epiphytic, epiphyllous or foliicolous [24,25] microorganisms), but also endophytes that are found within leaf tissues [26,27]; and not only terrestrial, but also submerged plants [28,29]. Here, however, the term 'phyllosphere' will be used to exclusively refer to aerial plant leaf surfaces. Such a narrower focus allows this mini-review to 1) highlight the features and challenges unique to the leaf surface as a microbial biome or habitat, 2) consider other plant compartments as potential sources and sinks of leaf surface microbes and as connected parts of a larger living entity (i.e. the plant) that influences, and is influenced by, leaf surface-associated microbiota, and 3) provoke thinking about the leaf surface itself as consisting of linked but discrete smaller compartments, such as the phylloplane, that is, leaf surface landscape, and phyllotelma, that is, leaf surface waterscape [11••,16].

The phyllosphere is colonized by a wide variety of microorganisms, including most commonly and abundantly bacteria, fungi, and yeast [13], as well as protists [30•,31], algae [24], and bacteriophages [32]. The foliar presence and abundance of these microorganisms, whether they be parasitic, commensal, or mutualistic, are a function of multiple concurrent processes that include immigration and emigration to and from the phyllosphere, as well as microbial multiplication, dispersal, and death within the phyllosphere (Figure 1). Factors that influence each of these processes include 1) the environment to which the plant and its leaves are exposed, 2) the plant genotype and phenotype, and 3) the ability of microbial colonizers to exploit phyllosphere-specific resources, to tolerate or avoid phyllosphere-specific stresses, and to interact with each other and their host. This environment-plant-microbe triad (Figure 1) will be used here as a scaffold to present some of the latest findings in phyllosphere microbiology and to highlight discoveries and insights that are novel and most likely to point the field into new directions of scientific exploration.

Environment

Plants growing in different environments (i.e. in different locations or under different conditions) typically carry different microbiota on their leaves. This has been documented most recently for plants grown in urban versus sylvan environments [33], in vineyards versus native forests [34,35], at different elevations [36], in different weather conditions [37,38•], or under different management regimes [15,35]. Although several of the studies

Table 1

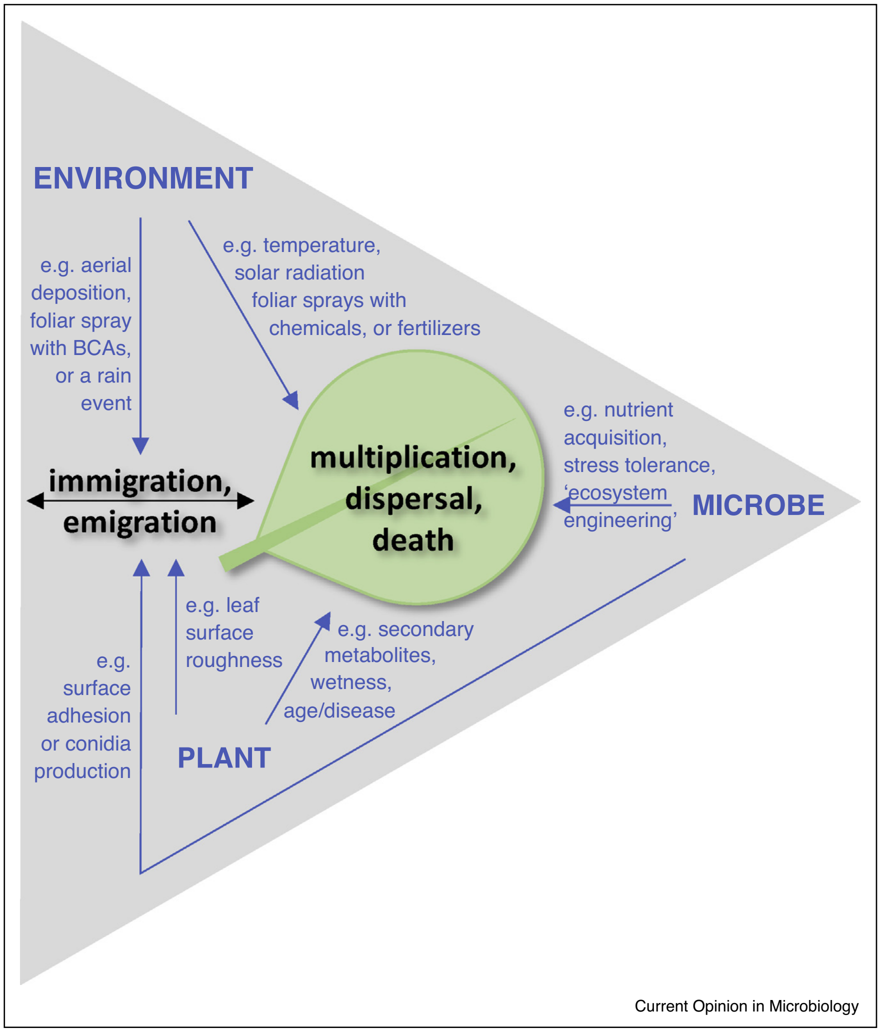
Recent review articles on topics related to phyllosphere microbiology

Authors	Year	Title	Topic	Reference
Ab Rahman *et al.*	2018	Emerging microbial biocontrol strategies for plant pathogens	Synopsis of existing and new microbial biocontrol options, including plant breeding, biologicals, and microbiome engineering.	[1]
Beilsmith *et al.*	2019	Genome-wide association studies on the phyllosphere microbiome: embracing complexity in host-microbe interactions	Use of GWAS to link genetic variation in plants to leaf-associated microbial community composition and to answer questions about mechanisms, evolution, and ecology of plant-microbe interactions.	[2]
Carvalho and Castillo	2018	Influence of light on plant-phyllosphere interaction	Direct and plant-mediated effects of light on bacteria and fungi (pathogenic or commensal) and on their interactions with the plant.	[3]
Hassani *et al.*	2018	Microbial interactions within the plant holobiont	Contribution of microbe-microbe interactions to microbial community structure and plant health.	[4]
Hoagland *et al.*	2018	Foodborne pathogens in horticultural production systems: ecology and mitigation	Mechanisms and management of prolonged survival by enteric pathogens on leaf greens.	[5]
Laforest-Lapointe and Whitaker	2019	Decrypting the phyllosphere microbiota: progress and challenges	Role for phyllosphere microbiota in driving ecosystem function and plant community dynamics.	[6]
Leveau	2018	Microbial communities in the phyllosphere	Recently re-published primer from 2011 on microbial assembly, interactions, and survival strategies on leaf surfaces.	[7]
Orozco-Mosqueda *et al.*	2018	Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms	Overview of approaches for selecting or manipulating microbial communities with plant-beneficial properties.	[8]
Panstruga and Kuhn	2019	Mutual interplay between phytopathogenic powdery mildew fungi and other microorganisms	Case study on the interactions between a foliar plant pathogen and other members of the plant (leaf) microbiota and their impact on disease progression.	[9]
Preininger *et al.*	2018	Concepts and applications of foliar spray for microbial inoculants	Practical considerations for the foliar introduction and commercialization of microorganisms with plant-beneficial properties	[10]
Remus-Emsermann and Schlechter	2018	Phyllosphere microbiology: at the interface between microbial individuals and the plant host	Insights into the phyllosphere microbiome from the perspective of its inhabitants: promises and challenges of single-cell approaches.	[11••]
Schlechter *et al.*	2019	Driving factors of epiphytic bacterial communities: a review	Plea to study leaf physicochemistry, microbial competition and cooperation, and plant-microbe interactions at micrometer resolution.	[12]
Stone *et al.*	2018	The role of the phyllosphere microbiome in plant health and function	Comprehensive synthesis of past and current discoveries and developments in the area of phyllosphere microbiology.	[13]
Terhonen *et al.*	2019	Forest tree microbiomes and associated fungal endophytes: functional roles and impact on forest health	Focus on microbiota associated with foliage from forest trees in context of forest management.	[14]
Thapa and Prasanna	2018	Prospecting the characteristics and significance of the phyllosphere microbiome	Description and implication of spatial and temporal changes in phyllosphere microbial communities.	[15]

referenced here also involved different host species so that a contribution of plant genotype (see below) cannot be ruled out, these observations are at least consistent with a role of the plant environment as a source of microbial immigrants to the phyllosphere [39••]. Microorganisms may arrive through a variety of processes, including but not limited to, aerial deposition [40], from other plant compartments or from soil [39••], by irrigation [37] or throughfall [41], or by intentional introduction, for example by spraying crops with biological control agents (BCAs) for the control of foliar pathogens [42••].

Another way in which the environment can alter the size and structure of microbial communities in the phyllosphere is through impact on microorganisms already present on leaf surfaces. For example, environmentally imposed events such as rain in forest canopies [41] or sanitation washes of leafy greens [43] can facilitate the removal (i.e. emigration) of leaf surface-colonizing microorganisms. Environmental factors such as temperature [44] and solar radiation [37] also have been implicated in altering microbial community, as have foliar sprays with chemical pollutants [45], fertilizers [46] or

Figure 1



Simplified scheme of the processes (in black font) that underlie the microbial community composition in the phyllosphere. The terms immigration and emigration refer to the arrival and departure of microorganisms to and from the leaf surface, respectively. On the leaf itself, depending on prevailing conditions, microorganisms may find or craft conditions to survive and produce offspring, disperse to other parts of the leaf, or die. As drivers of microbial community composition, these processes are influenced by one or more factors of the environment-plant-microbe triad (in blue). Some examples of such influencing are provided in the figure, but more are given in the text.

biostimulants [47] and exposure to gamma-irradiation [43] or ozone [48].

When pointing at the environment as a factor that drives phyllosphere microbial community composition, it is important to consider the potentially confounding nature of such environmental effects. For example, are microorganisms on the leaf surface directly affected by temperature, or is the effect indirect, that is, in response to temperature-induced changes in plant leaf biology? Or are there alternative (not necessarily mutually exclusive) explanations? For example, does temperature impact the microbial diversity in the environment that serves as a source of microbial immigrants to the phyllosphere? Another confounder is the environmental effect referred to as 'season', which blends multiple factors, including not only temperature but also time. For example, to what extent do microbiotic changes observed later in the season stem from longer exposure of the leaves to the environment as a source of microbial immigrants, from changes in leaf phenotype as plants age, or from changes in temperature and other weather-related or management-related phenomena? More studies that systematically and quantitatively assess the relative contributions of individual environmental factors, especially in natural settings, will help better explain and predict the assembly and dynamics of leaf surface microbial communities.

Plant

Another key factor in shaping microbial community structure in the phyllosphere is the plant itself, more specifically the plant genotype and phenotype [12]. In a recent study, trees belonging to six different spruce species but grown under identical environmental conditions differed significantly in the microbial diversity on their foliage [49]. These differences correlated with a number of leaf phenotypes, such as stomatal conductance and tissue chemistry. Common garden trials like these are key in phyllosphere microbiology because they minimize the confounding impact of environmental factors that are likely to mask plant-driven effects on the leaf microbiota. Clues to the mechanisms underlying such effects continue to come from a variety of approaches, including genome-wide association studies [2] and analyses of leaf surface properties. Among twelve genotypes of silver birch, the quantities of plant-derived metabolites on the leaf surface varied substantially [50]. Plant metabolites such as leaf-emitted isoprenes can be utilized as sources of carbon and energy by representatives of some taxa of the phyllosphere microbial community but not others [51]. These examples illustrate possible mechanisms for the generally accepted principle that different plant genotypes generate different leaf surface phenotypes that in turn select for a subset of plant leaf immigrants or colonizers, thus contributing to differences in microbial community composition.

Considering that disease resistance has a genetic basis in plants, the observation that resistant and susceptible varieties of cacao plants differed significantly in the microbiota on their leaves [52] further supports the notion that genotype can shape phyllosphere microbial communities. However, this particular study also claimed that the leaves of the resistant variety featured greater microbial diversity and a higher relative abundance of microbes with antagonistic activity against plant pathogens. This raises interesting questions of causality: is the resistant genotype a driver of both disease suppression and microbial community structure, or is disease suppression achieved as an extended phenotype, that is, by a plant genotype that selects for a protective leaf microbiota? The latter explanation offers the fascinating prospect of breeding plants for their ability to carry beneficial microbiota. Efforts towards such a goal will benefit from genome-wide association approaches to identify plant genetic loci that control microbial diversity on leaf surfaces [2].

While different compartments of the same plant (e.g. leaves, fruits, and flowers) represent the same genotype, they are very different manifestations of that genotype, that is, very different phenotypes. The observation that leaf surfaces harbor microbial communities that are distinctively different from other above-ground plant compartments on the same plant, for example grape leaves versus berries [53] or kiwi leaves versus flowers [54], further underscores the role of plant phenotype as a driver of microbial diversity. Higher levels of leaf surface roughness (another plant phenotype) have been implicated in greater foliar retention of microorganisms [55], offering an example of plant 'control' over microbial emigration. Diseased plants express phenotypes that are different from those of their healthy counterparts and that select for different microbiota in the phyllosphere [56]. Citrus trees diagnosed with Huanglongbing (HLB) were shown to carry a significantly different microbial community structure on their leaves than healthy-looking trees [57]. Whereas in this particular case cause and effect were compartmentally separated (i.e. the HLB-pathogen *Candidatus* Liberibacter asiaticus resides in the phloem, whereas changes were observed in microbial community composition on leaf surfaces), disease-induced changes in phyllosphere microbiota have also been reported for pathogens that colonize leaves [54]. Such changes are likely to be a combined result of pathogen-induced modification of plant phenotype and of direct interactions of the pathogen with the native leaf microbiota. Microbe-microbe interactions as drivers of phyllosphere microbial community composition will be discussed in the next section.

Microbe

The phyllosphere represents a habitat with a unique set of challenges to its microscopic occupants. Variation among microbial taxa or functional guilds in their ability to deal with these challenges contributes to inter-species or intra-species differences in phyllospheric fitness. In turn, such differences in fitness can be major drivers of microbial community composition on plant leaf surfaces. Successful colonizers are more likely to be tolerant of phyllosphere-specific stresses such as harmful UV [58••] and oxidative stress [59], and capable of exploiting resources that the plant provides on its leaf surface, such as nutrients [51] and vitamins [60]. The relatively oligotrophic nature of the phyllosphere is prone to select for microorganisms that can 'engineer' the leaf surface to gain access to nutrients, for example by thinning the leaf cuticle [61], secreting plant hormones [62] or producing surfactants [63]. Surface motility [64], surface adhesion [65] and biofilm formation [66] are also considered contributors to phyllospheric survival, as means to disperse to unexplored areas of the same leaf, to prevent removal from the foliage, or to avoid desiccation, respectively. Some adaptations facilitate movement within or between canopies: in an original study that combined comparative genomics with phyllosphere fitness, the L morphotype of the fungal species *Aspergillus flavus* was shown to produce more conidia than the S morphotype, suggesting superior capability for emigration and dissemination [67••].

Some phyllosphere-specific adaptations have collateral consequences for the plant host, which tends to change our categorization of microorganisms with such adaptations from commensal to parasitic or mutualistic. The epiphytic fitness of certain plant pathogens like *Xanthomonas* is proportionally related to their chances of internalizing and proliferating endophytically [64]. A *Bacillus* strain isolated from the rice phyllosphere was shown to not only be drought-tolerant itself but also confer this property onto its host [68]. It remains unknown if this is related to the production of acetoin and 2,3-butanediol, which has been reported for other *Bacillus* species and which is known to induce stomatal closure [69]. Bacterial isolates from plant leaves in the Antarctic have ice recrystallization inhibition activity [70], which provides frost protection to the plant and presumably to the bacteria as well.

An additional contributor to microbial community dynamics in the phyllosphere is the direct or indirect interaction among microorganisms [12]. Certain species of phyllosphere yeasts produce esterases that render the tomato leaf cuticle more permeable to plant-derived nutrients; however, the same activity makes the plants more susceptible to pathogenic *Botrytis* [61]. These yeasts are not considered pathogenic themselves, but their activity facilitates the establishment of microbes that are. Another example of such facilitation is the interaction of otherwise harmless bacteria of the genus *Erwinia* with the foliar pathogen *Pseudomonas savastanoi*, resulting in more aggressive disease [71•]. Microorganisms in the phyllosphere may also do the opposite and mitigate the effects of pathogens, for example by competing for limited space and resources, producing antimicrobial compounds, or triggering a plant immune response [12]. Microorganisms with such properties have potential as BCAs, and are subject of much research. The phyllosphere microbiome is a rich source for BCA-type microorganisms [10], which can be tested for pathogen-antagonistic activity in the lab [72] and prioritized for further study in greenhouse or field. BCAs include various types of agents of biological origin, not only bacteria [66,73,74] and fungi [75], but also bacteriophages [32] and tailocins [76••]. Individual BCAs may have one or more modes-of-action, including antibiosis [77] and parasitism [78]. Foliar introduction of BCAs can impact the activity and relative abundance of not only the target pathogen, but also other members of the bacterial or fungal community [79]. Mixtures of BCAs can be more efficient against a pathogen than individual BCA strains [80], either as a result of additive, independent modes of action, or as a property emerging from synergistic microbe-microbe interactions.

Studies that employ genome sequencing of individual phyllosphere isolates [67••,72,81] or entire communities [82] are revealing a tremendous functional diversity, including a largely untapped source of natural products [72] for which a role in phyllosphere fitness remains mostly unknown. Approaches that employ comparative genomics of plant-associated microorganisms from different plant compartments [83] offer great promise to reveal what it takes to be a successful colonizer of the phyllosphere. While such approaches will undoubtedly inform ongoing and future efforts to select for better foliar BCAs, they also are beginning to expose our relative ignorance about the sources and importance of genome-level diversity. In a key contribution to the field, an investigation of leaf-associated *Pseudomonas* populations on wild *Arabidopsis* [81] showed that the operational taxonomic unit that was found most commonly on (and in) leaves (called OTU5, based on 99% identity at 16S rRNA gene level) in fact represented an astonishing 165 different strains or genomes (>99.9% sequence identity cutoff). The existence of such diversity offers an exciting point-of-departure for future inquiries into the significance of such diversity as it relates to the ecology and evolution of phyllosphere-associated microbial species, populations, and communities. Whereas most isolates belonging to OTU5 were able to cause disease symptoms on *Arabidopsis*, not all isolates were able to do so. Surprisingly, all OTU5 strains lacked genes for the biosynthesis of coronatine and syringomycin/syringopeptin. These phytotoxins are well recognized as virulence factors for plant-pathogenic *Pseudomonas* species. In fact, production of syringomycin was recently shown to contribute to endophytic but not epiphytic fitness of *Pseudomonas* [84]. The mismatch between these two observations calls for further experimental interrogation that focuses on finding a mutually inclusive explanation and draws from concepts of ‘division of labor’ [85] and ‘microscale heterogeneity’ [86] as drivers behind the observed genomic microdiversity within OTU5. Division of labor was elegantly demonstrated to contribute to the colonization of *Arabidopsis thaliana* leaves by cells of the fungal pathogen *Sclerotinia sclerotiorum* [87], and microscale heterogeneity of the leaf surface has been put forward as an underexplored and underappreciated factor in phyllosphere microbiology [11••].

Scales of interrogation

The narrative of phyllosphere microbiology is rich with complexity: it involves multiple concurrent processes and multiple, oftentimes interdependent, factors that impact those processes (Figure 1). Continued efforts to reveal not only correlations but also causations that underlie this complexity are going to be crucial for an improved understanding of the rules and exceptions that govern phyllospheric life, in particular as they pertain to plant health and productivity. Such efforts will benefit greatly from the experimental malleability of the individual processes (immigration, multiplication, dispersal, death, emigration) and factors (environment, plant, microbe) discussed here. Arguably, such efforts also stand to gain from interrogation of these processes and factors at finer resolution. A powerful example is the aforementioned study of *Pseudomonas* populations on *Arabidopsis* [81] which revealed a considerably greater diversity at whole-genome resolution than at the level of 16S rRNA sequence similarity. Incidentally, this finding not only invites further scrutiny into the significance of such genome-level diversity; it also cautions more generally against absolute statements about microbial diversity and community similarity without providing context about the level of taxonomic resolution at which microbial communities were analyzed and compared. Another case in point of finer-scale investigation pertains to the factor 'environment' which is relatively well studied as a driver of phyllospheric microbial community structure at the landscape or even canopy level, but much less appreciated at the single-leaf or subleaf level. It is noteworthy that at the scale at which epiphytic microorganisms operate and interact, the factors 'environment' and 'plant' are essentially synonymous. Under the microscope, plant leaves differ dramatically in terms of lateral surface topography [88,89] and surface chemistry [90]. The impact of this 'micro-environmental' heterogeneity on the interactions of phyllosphere colonizers and on the outcomes of those interactions is still poorly understood but may represent a bottom-up, mechanistic explanation for coarser scale observations and a means to convert correlation into causation. Consider the co-occurrence of two microbial taxa in a phyllosphere community profile: does this signify a spurious association (linked to some confounding variable) or is it a true interaction, and if so, can such an interaction be experimentally demonstrated? What about leaf wetness: at finer resolution, what does the waterscape or phyllotelma look like on leaf surfaces, and to what extent does the fragmented nature of the phyllotelma facilitate or constrain interactions between microorganisms? These types of questions call for the adoption of novel experimental approaches that explain microbial community structure in terms of micrometer spatial organization [92] and microbial interactions at the single cell level [11••] and that allow manipulation of microbes in their natural environment at scales that matter to them. An exciting recent example of the latter is a study that showed differences between pathogenic and non-pathogenic fungi in their interaction with individual trichomes of tomato and the outcomes of these interactions [91••]. Such approaches, complementary to the ones that have become more standard tools in the hands of phyllosphere microbiologists, and in combination with agent-based modeling [65], hold significant promise in pursuit of a mechanistic appreciation for microscopic life [93] in order to predict, prevent or beget phyllosphere phenomena that matter at macroscopic scales.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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