The Phyllosphere: Microbial Jungle at the Plant–Climate Interface

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Abstract

The surface of plant leaves, or the phyllosphere, harbors hyperdiverse microbial communities. These communities mediate foliar functional traits, influence plant fitness, and contribute to several ecosystem functions, including nutrient and water cycling. In this review, we briefly recall the history of phyllosphere research and present the features of this microbial habitat. Adopting a recent framework for evolutionary community ecology, we then review evidence for each of the four major processes shaping phyllosphere microbial communities: dispersal, evolutionary diversification, selection, and drift. We show how these processes are influenced by the host plant, the surrounding atmospheric conditions, and microbial interactions. Rapidly growing evidence indicates that phyllosphere microbial communities are altered by global change, with potential cascading effects on plant performance, plant evolution, and ecosystem functioning. We propose future avenues for phyllosphere research aimed at improving plant adaptation and ecosystem resilience to environmental changes.

1. INTRODUCTION

Almost all plant tissues harbor microorganisms. Plant leaves do not escape the rule and interact with a multitude of microbial species. Microbiologists and plant pathologists have studied this microbial habitat, termed the phyllosphere, since the mid-1950s, mostly because some microorganisms are pathogens and threaten plant health whereas others improve plant performance. The emergence of meta-omics during the past decade has fostered the study of nonculturable microorganisms, opening the door to new discoveries and a better understanding of phyllosphere microbial communities (PMCs). A large body of evidence showing that PMCs exert significant effects on plant fitness and ecosystem functions now exists. Despite these discoveries, PMCs have rarely been integrated into studies of plant ecology and evolution. In this review, we provide an evolutionary community ecology framework for the phyllosphere based on a comprehensive overview of the leaf and its microbial inhabitants. This framework should help foster the integration of PMCs into the global change research agenda.

Meta-omics: a set of methods providing information on all the genes, transcripts, proteins, and metabolites present in an environmental sample

Community: a group of organisms belonging to multiple species that live in a specified place and time

Fitness: the relative number of offspring contributed to the next generation by an individual

Ecosystem functions: ecological processes that control the fluxes of energy, nutrients, and organic matter through an environment

Global change: planetary-scale changes in the Earth system, including climate change, land use change, pollution, and biological invasions

PMC: phyllosphere microbial community

1.1. A Brief Historical Overview of Phyllosphere Research

The term phyllosphere was first introduced by the plant pathologist F.T. Last in 1955. The aim of his work was to measure seasonal variations in the abundance of "pink yeasts" (*Sporobolomyces* sp.) on cereal leaves and to assess their relationship with infection by powdery mildew (*Erysiphe graminis*). By doing so, he isolated several other fungal species. Drawing an analogy with the rhizosphere, he concluded that "leaves have a 'phyllosphere,' with a characteristic microflora that may contain many species" (Last 1955, p. 221). The microbiologist J. Ruinen drew the same analogy at nearly the same time (Ruinen 1956). Five years later, she wrote: "The external surface of the leaf, as an environment for micro-organisms, has been termed the 'phyllosphere' by analogy with the rhizosphere of roots . . . [and] the term has been accepted" (Ruinen 1961, p. 82). Other pioneering studies gave the first insight into the composition of PMCs. For instance, they showed that the fungal communities growing on the leaves of pasture plants (di Menna 1959) and tropical trees (Ruinen 1963) are dominated by basidiomycetous yeasts belonging to the genus *Cryptococcus*, followed by *Rhodotorula* and *Sporobolomyces*. They also revealed that yeast communities of the phyllosphere and soil are not driven by the same environmental factors and are taxonomically distinct (di Menna 1959).

Studies soon pointed out the influence of PMCs on plant health and performance. In particular, the importance of PMCs for disease protection has been a mainstream topic since the beginning of phyllosphere research. Leben (1965) acknowledged that foliar pathogens are part of a complex, fluctuating microbial system comprising two compartments: the residential community and the casual community. Residential microorganisms multiply on the surface of healthy, living leaves without noticeably affecting the plant. Casual microorganisms are inactive and may be present on the plant accidentally. Leben and other plant pathologists hypothesized that residents may hinder disease development through direct interactions (e.g., production of antibacterial or antifungal compounds) and indirect interactions (e.g., competition for foliar nutrients or alteration of plant physiology) with the pathogen (Last & Deighton 1965, Leben 1965). The influence of PMCs on ecosystem functions was also acknowledged early. For instance, Ruinen (1965) proposed that the atmospheric dinitrogen fixed by phyllosphere bacteria of the genera *Beijerinckia* and *Azotobacter* could substantially contribute to plant growth in the tropics by being directly taken up by the leaves or mobilized by rain and made available to the roots. A few years later, Jones reached the same conclusion for a temperate tree species (Jones 1970).

The growing evidence for the functional importance of PMCs has coincided with an increasing awareness of their response to anthropogenic perturbations. An early example comes from the work of Hislop & Cox (1969), who assessed the impact of fungicide sprays on PMCs in apple orchards.

In the 1980s, the focus switched from the impact of agricultural practices to that of air pollution (Khanna 1986), particularly acid rains (Helander et al. 1993). The impact of climate change on PMCs has become a central topic since the 1990s (Magan & Baxter 1996, Runion et al. 1994).

Questions regarding global change promoted a change in the research framework for the phyllosphere, raising interest in the dynamics of PMCs from a purely ecological perspective. Thus, Kinkel et al. (1987) tested the equilibrium theory of island biogeography, assuming that individual leaves form discrete habitat patches for microbes analogous to oceanic islands for macroorganisms. They found that, contrary to theory, species number at equilibrium is not related to leaf size. This study encouraged the search for drivers of phyllosphere microbial diversity (Kinkel 1997) and, more recently, comparisons of diversity patterns between microorganisms and macroorganisms to assess the generality of ecological theories (Fierer et al. 2011, Meyer & Leveau 2012).

Phyllosphere research entered a new era in the 2000s when the development of sequencing technologies facilitated the study of nonculturable microorganisms (Rastogi et al. 2013). Fingerprinting approaches first revealed that PMCs are far more complex than previously thought (Yang et al. 2001). Newly emerging meta-omic approaches then confirmed these findings (Jumpponen & Jones 2009) and started to unveil the functional potential of PMCs (Delmotte et al. 2009). Now, after a decade of high-throughput sequencing, many methodological issues linked with the production and analysis of these "big data" have been overcome. The challenge now is to incorporate meta-omic data into a conceptual framework that links observed patterns with the underlying ecological and evolutionary processes.

1.2. Academic and Empirical Delimitations of the Phyllosphere

The term phyllosphere initially referred to "the external surface of the leaf, as an environment for micro-organisms" (Ruinen 1961, p. 82). This definition was recently refined by Doan & Leveau (2015), who considered "the leaf surface ('phyllosphere') as a habitat that features two intimately connected but very different compartments, i.e., the leaf surface landscape ('phylloplane') and the leaf surface waterscape ('phyllotelma') . . . . 'Phyllosphere' microorganisms . . . include all the cuticle-attached microbes in addition to those that are present in the waterscape" (pp. 1036, 1040). Morris (2002) extended the phyllosphere environment further inside and outside the leaf (**Figure 1*a***), considering that "the phyllosphere is the microenvironment extending from the leaf surface outward to the outer edge of the boundary layer surrounding the leaf and inward into the leaf tissues" (p. 1). No clear-cut border exists between the leaf surface and internal tissues (**Figure 2**), as "natural openings in the leaf tissue, such as stomata and hydathodes, allow ready passage of microorganisms between the external and internal domains of the phyllosphere" (p. 1). There is also a continuum between the leaf surface and the surface of other above-ground plant parts, such as the cauloplane (surface of stems), the anthoplane (surface of flowers), and the carpoplane (surface of fruits). These continuums explain why phyllosphere delimitations in empirical studies often differ from the academic definitions. In this review, we mainly focus on microbial communities of the leaf surface, without excluding studies that have not distinguished between the surface and the internal tissues of the leaf.

2. PHYLLOSPHERE PLAYGROUND: THE LEAF

2.1. Leaf Anatomy

Had bacteria eyes, their view of the leaf surface would not be smooth at all. It would look like a jungle in which epicuticular wax crystals form a rough terrain (**Figure 1*b*,*c***), veins are grooves,

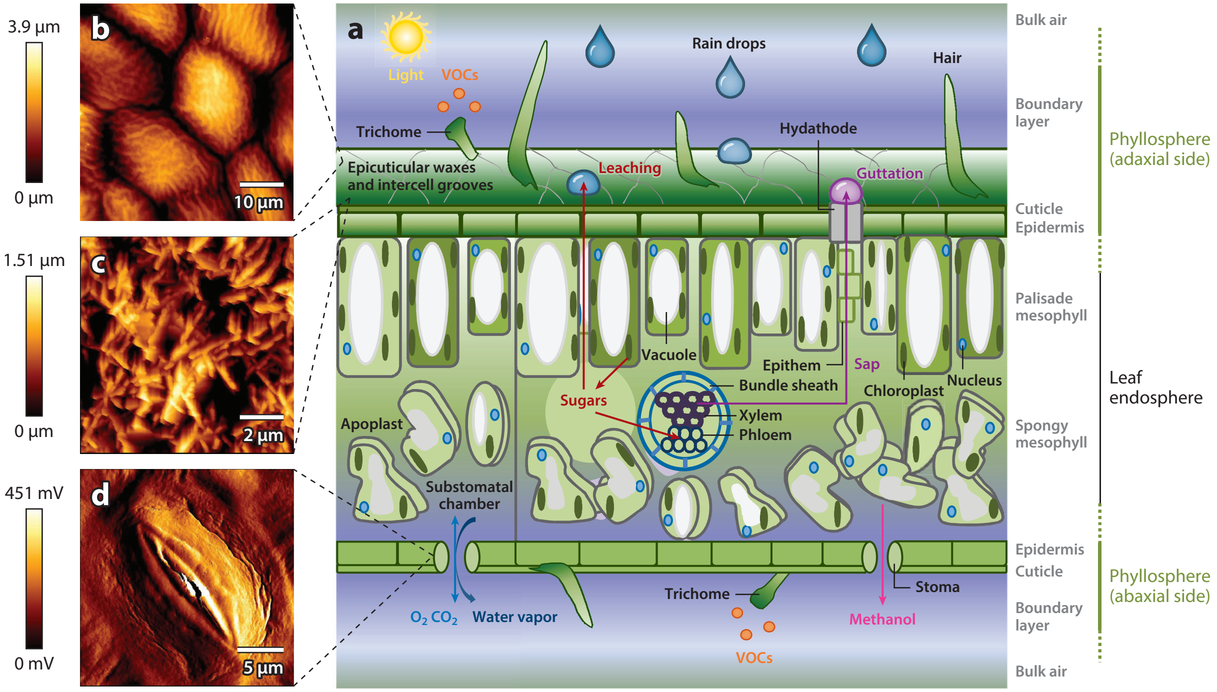

Figure 1

Illustration of some structural and functional attributes of the phyllosphere. (*a*) The phyllosphere extends from the leaf surface outward to the outer edge of the boundary layer and inward into the leaf tissues (Morris 2002). It is the habitat for a wide range of microbes (Lindow & Brandl 2003, Vorholt 2012). The leaf cross section diagram shows the flow of several metabolites used as nutrients by phyllosphere microorganisms. These microorganisms use the sugars and inorganic nutrients exported to the leaf surface through leaching (Van Der Wal & Leveau 2011) and guttation (Singh 2014) and can also use volatile organic compounds (VOCs) emitted by the leaf, such as methanol (Knief et al. 2012). Phyllosphere microorganisms are in contact with the waxy layer covering the leaf, the cuticle, on both the upper (adaxial) and the lower (abaxial) leaf surface. Stomata and hydathodes are major points of entry for microorganisms into internal leaf tissues. (*b*–*d*) These attributes of the phyllosphere habitat are revealed by atomic force microscopy: (*b*) cuticular striae on the upper leaf surface of grapevine (*Vitis vinifera* cv. Zweigelt), (*c*) wax rosettes on the upper leaf surface of pedunculate oak (*Quercus robur*), and (*d*) a stoma on the lower leaf surface of grapevine (*V. vinifera* cv. Zweigelt).

stomata and hydathodes are cracks and craters (**Figure 1*d***), and trichomes and fungal hyphae are trees and vines (**Figure 2**).

Microbial cells and spores landing on the leaf surface first contact the cuticle, a hydrophobic layer that protects the plant against desiccation and other stresses. Its outer part is mainly formed of waxes whose architecture and composition varies substantially among environmental conditions and plant species (**Figure 1*b*,*c***). These epicuticular waxes may form films, giving a glossy appearance to leaves, or crystals, resulting in a dull, glaucous appearance (Yeats & Rose 2013). Trichomes are epidermal outgrowths that give relief to the phyllosphere landscape (**Figure 2*b***). They help control the leaf temperature, protect the leaf against UV light, and secrete a variety of secondary metabolites that can deter herbivores and inhibit pathogen development. Stomata and hydathodes punctuate the leaf surface with numerous openings (**Figure 2*b*,*d***). A stoma consists of two symmetrically opposed cells (termed guard cells) that can shrink and swell in response to environmental conditions (**Figure 1*d***). Guard cell movements open or close the central pore, controlling the plant gas exchange and water loss. Hydathodes, also known as water stomata or water

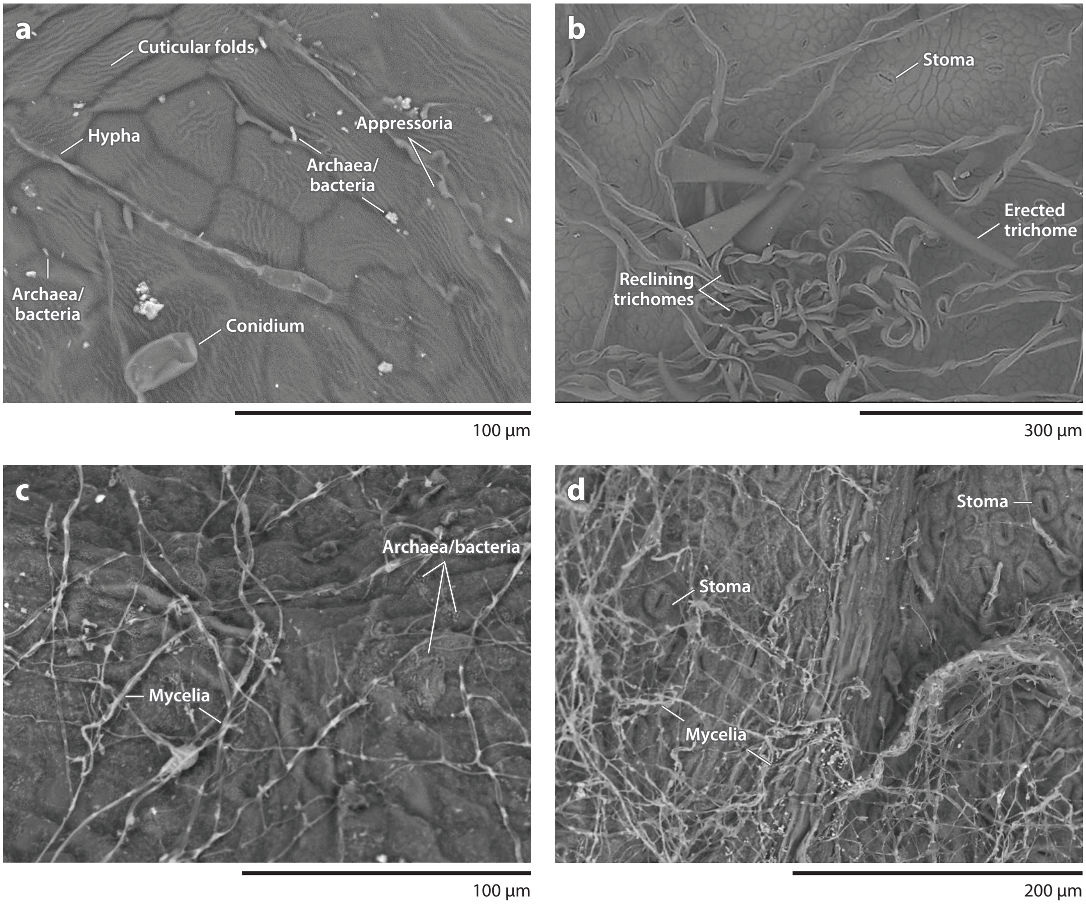

Figure 2

Three-dimensional structure of the phyllosphere revealed by scanning electron microscopy. (*a*) Upper surface of a grapevine leaf (*Vitis vinifera* cv. Neuburger) colonized by powdery mildew (*Erysiphe necator*). (*b*) Lower surface of a grapevine leaf (*V. vinifera* cv. Welschriesling) showing stomata and two types of trichomes. (*c*) Upper surface of an oak leaf (*Quercus robur*) colonized by powdery mildew (*Erysiphe* sp.). (*d*) Lower surface of an oak leaf (*Q. robur*) showing numerous stomata partially covered with mycelia.

pores, are positioned at the leaf margins and are permanently open. They mediate the process of guttation, the exudation of a liquid containing a number of organic and inorganic compounds (Singh 2014).

In most angiosperm species, the leaf structure differs between the upper (adaxial) and the lower (abaxial) side (**Figure 1*a***). The adaxial domain of the leaf comprises epidermal cells with a relatively thick cuticle, beneath which a densely packed layer of palisade mesophyll cells optimizes light capture. The abaxial domain of the leaf specializes in gas exchange and the regulation of transpiration. It consists of an epidermis with abundant stomata, beneath which there are loosely packed cells termed the spongy mesophyll. The microorganisms entering the inner leaf tissues through stomata first colonize the intercell spaces of the spongy mesophyll. Thus, they get closer to the minor leaf veins where sugars are uploaded into the phloem sieve elements (Rennie & Turgeon 2009).

2.2. Leaf Physiology

Living conditions in and on the leaf depend on plant physiology. Photosynthesis, in particular, controls the availability of nutrients for microorganisms on the leaf surface and in the leaf tissues. Photosynthesis occurs primarily in the palisade mesophyll cells and produces carbohydrates (sugars) that are exported to other plant organs through the phloem (**Figure 1*a***). Carbohydrates can be transported from the mesophyll cells to the phloem sieve elements via two pathways: the symplastic pathway and the apoplastic pathway. The symplastic pathway relies on the movement of carbohydrates from the cytoplasm of one cell to that of the other. The apoplastic pathway relies on the movement of sucrose in the extracellular space, the apoplast. Sucrose is first secreted by the mesophyll cells, then it diffuses in cell walls and spaces between cells, and finally it is uploaded into phloem sieve elements by active pumping (Rennie & Turgeon 2009). However, some sugars present in the apoplast are not uploaded into the phloem but diffuse through the cuticle (**Figure 1*a***). This phenomenon, termed leaching, concerns not only sugars but also many other organic and inorganic nutrients (Tukey 1970). The presence of liquid water on the outside of the cuticle is necessary for leaching to occur. Leaching tends to increase with leaf age, probably owing to the higher wettability of older leaves (Tukey 1970). The leaching flow is a function of sugar concentrations in the apoplast and in the water on the leaf surface as well as the permeability of the cuticle (Van Der Wal & Leveau 2011). The cuticle permeability can be increased by up to 50% by the biosurfactants (biologically produced surface active agents) synthesized by phyllosphere bacteria, for example, the syringafactin produced by *Pseudomonas syringae* (Burch et al. 2014, Schreiber et al. 2005). Overall, however, carbohydrate concentration is much lower on the leaf surface than in the inner tissues: in the range of a few milligrams versus hundreds to thousands of milligrams per square meter of leaf (Fiala et al. 1990). Carbohydrate composition also differs between the leaf surface and the inner tissues. Sucrose predominates in the inner tissues, whereas smaller sugars such as glucose and fructose predominate in leachates (Fiala et al. 1990) and guttation fluid (Goatley & Lewis 1966).

ROS: reactive oxygen species

PAMP (or MAMP): pathogen-associated molecular pattern (also termed microbe-associated molecular pattern)

VOCs: volatile organic compounds

Living conditions in and on the leaf are also shaped by the fluxes of CO_2, O_2, and water vapor resulting from photosynthesis, respiration, and evapotranspiration (**Figure 1*a***). Water vapor diffusing through stomata condenses and creates microscopic water films on the leaf surface (Burkhardt & Hunsche 2013). O_2 can transform into reactive oxygen species (ROS). ROS can be formed by the leakage of electrons onto O_2 from the electron transport activities of chloroplasts and mitochondria. They are also produced in other compartments of the plant, including cell walls and the apoplast, especially under stressful conditions. At low concentration, ROS act as signaling molecules. At high concentration, they induce damage to proteins, lipids, and DNA (Sharma et al. 2012). Apoplastic ROS are typically produced upon recognition of microorganisms by the plant. Plant cell-surface receptors can recognize some highly conserved microbial molecules, termed pathogen-associated molecular patterns (PAMPs). The recognition triggers an oxidative burst that regulates the interactions between the plant and pathogenic microorganisms and may also regulate the leaf colonization by beneficial microorganisms (Torres 2010). Plant–pathogen interactions can also be regulated by stomata. The guard cells have the ability to sense PAMPs and to close the stomatal pore in response to infection. In turn, some bacterial strains have the ability to counteract this line of defense. They produce a virulence factor, termed coronatine, that reopens the stomata (Melotto et al. 2008) and again permits pathogen entry and gas exchanges.

In addition to CO_2, O_2, and water vapor, plant leaves emit a wide spectrum of volatile organic compounds (VOCs) (**Figure 1*a***), either constitutively or in response to biotic or abiotic stresses. Isoprene and monoterpenes are by far the most abundant compounds and may protect the plant against high temperatures (Loreto & Schnitzler 2010). VOCs also mediate plant–plant interactions, plant–insect interactions, and plant–microorganism interactions (Junker & Tholl 2013). Methanol, for instance, is primarily released through the stomata (Abanda-Nkpwatt et al. 2006) as a by-product of cell growth (Galbally & Kirstine 2002) and forms a carbon source for some phyllosphere microorganisms (**Figure 1*a***).

2.3. Leaf Microclimate

The leaf microclimate can be described by numerous parameters, including leaf surface temperature and water availability, spectral irradiance, and features of the air around the leaf such as humidity and wind speed (Chelle 2005). Variation in leaf microclimate is uncoupled from that of ambient air by the boundary layer surrounding the leaf (**Figure 1*a***), an air layer of <1 mm to 1 cm in which wind speed is reduced by surface friction (Morris 2002).

Temperature on the leaf surface directly impacts microorganism development (Bernard et al. 2013). It typically exceeds air temperature, although substantial variation exists among plant species and among leaves within the same plant. For example, at an air temperature of 25°C, the average canopy temperatures of adjacent trees of larch (*Larix decidua*), sessile oak (*Quercus petraea*), and hornbeam (*Carpinus betulus*) were 25°C, 27.5°C, and 30°C, respectively. At the same air temperature, some shaded leaves of a large-leaved linden tree (*Tilia platyphyllos*) were 25.1°C, whereas other leaves reached 37°C (Leuzinger & Körner 2007). Temperature also varies by several degrees across a single leaf. The leaf center is generally warmer than the edges (Stokes et al. 2006), and herbivore damage can create even warmer areas (Pincebourde & Woods 2012). Finally, leaf temperature exhibits strong temporal variation as a consequence of diurnal cycles, meteorology, and transient phenomena such as sunflecks (Chelle 2005).

The quantity and distribution of water on the leaf is another highly dynamic parameter of the leaf microclimate that drastically influences microorganism development (Morris 2002). This water can include microscopic leaf wetness, thin water films <1 μm maintained by the condensation of transpired water vapor, and macroscopic leaf wetness, thicker water films and water droplets that form temporarily during and after rain events and at night with dew (Burkhardt & Hunsche 2013). These water films give place to chemical reactions between compounds dissolved in the rain or dew water and those leaching from the leaf. These reactions, in turn, impact phyllosphere microorganisms by modifying the water pH and the availability of nutrients (Morris 2002).

3. PHYLLOSPHERE PLAYERS: THE MICROORGANISMS

3.1. General Composition

The heterogeneous environment formed by the phyllosphere favors the coexistence of a wide range of microorganisms including prokaryotes (bacteria and, less frequently, archaea) and eukaryotes (fungi, oomycetes, and, less frequently, nematodes), as well as viruses (Koskella 2013, Lindow & Brandl 2003, Vorholt 2012) (**Figure 3**). Bacterial communities are generally dominated by Proteobacteria. α-Proteobacteria (e.g., *Methylobacterium*, *Sphingomonas*) are often most abundant, although γ-Proteobacteria (e.g., *Pseudomonas*) may sometimes reach high levels of frequency (Bodenhausen et al. 2013, Delmotte et al. 2009, Fierer et al. 2011, Kembel et al. 2014, Redford & Fierer 2009, Redford et al. 2010). Fungal communities typically are dominated by Ascomycota (Coince et al. 2014; Jumpponen & Jones 2009, 2010; Kembel & Mueller 2014). Within Ascomycota, the genera *Aureobasidium*, *Cladosporium*, and *Taphrina* are often among the most common. Basidiomycetous yeasts belonging to the genera *Cryptococcus* and *Sporobolomyces* are generally also very abundant (Cordier et al. 2012b; Jumpponen & Jones 2009, 2010; Ottesen et al. 2013). The

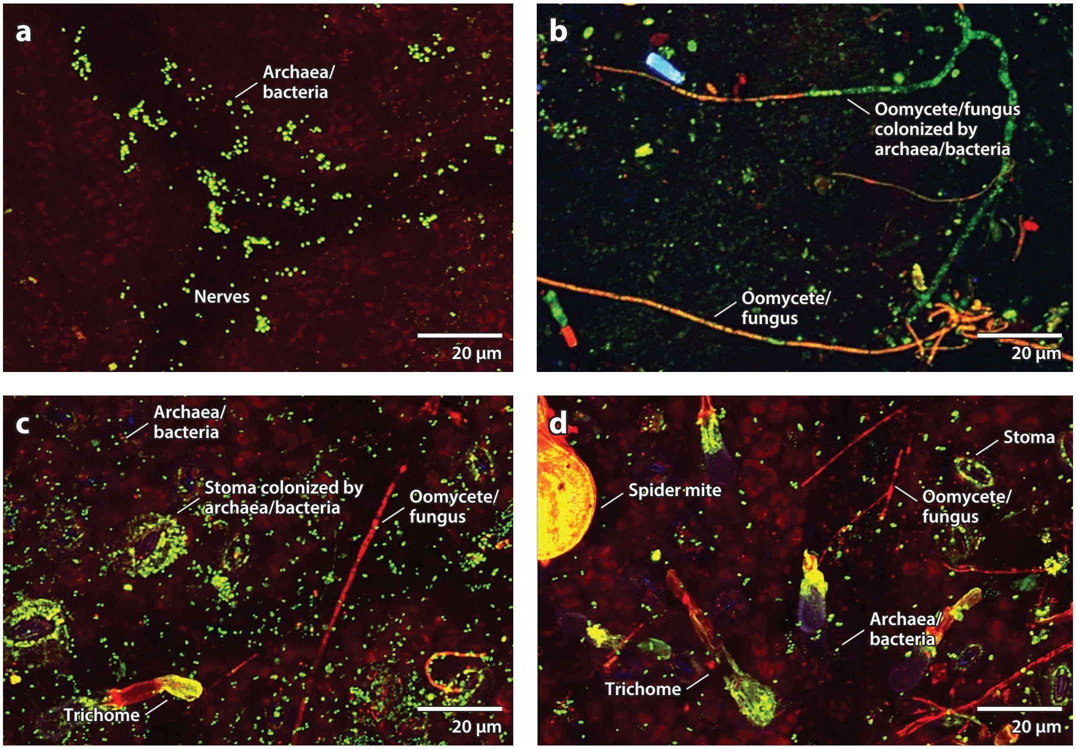

Figure 3

Phyllosphere microorganisms revealed by live staining. Images were taken of the upper (*a*–*b*) and the lower (*c*–*d*) surface of oak leaves (*Quercus robur*) colonized by powdery mildew (*Erysiphe* sp.) using SYTO9 and propidium iodide. Living bacteria and archaea appear in green, dead bacteria and archaea appear in yellow, and fungi and oomycetes appear in red. The dark red background corresponds to the leaf chlorophyll. These images reveal that bacteria (*a*) tend to aggregate along the leaf nerves on the upper leaf surface and (*b*) can colonize fungal hyphae. On the lower leaf surface, (*c*) bacteria can be present at high density and tend to aggregate around stomata. They also (*d*) colonize trichomes.

community composition and diversity are seasonally dynamic, for both bacteria (Copeland et al. 2015, Peñuelas et al. 2012, Redford & Fierer 2009) and fungi (Cordier et al. 2012b, Jumpponen & Jones 2010, Peñuelas et al. 2012).

3.2. Bacteria

Our knowledge about phyllosphere inhabitants hardly extends to the precise identity and function of most species. Only a few species and genera have been thoroughly studied. The most notable exception is *Pseudomonas syringae*, a bacterial pathogen that causes diseases of economic importance to a wide range of plant species (Mansfield et al. 2012). *P. syringae* is able to grow epiphytically and to enter inner leaf tissues through stomata or wounds, causing disease through multiplication in intercellular spaces. It has been widely used to elucidate the mechanisms of plant–pathogen interactions (e.g., Melotto et al. 2008), to understand adaptations to the epiphytic lifestyle (e.g., Burch et al. 2014), and to decipher microbial interactions in the phyllosphere (e.g., Innerebner et al. 2011). *P. syringae* is also present in nonplant habitats, including rain, snow, streams, lakes, and clouds. Bacteria growing as epiphytes on plant leaves are indeed regularly swept up into the atmosphere. They are believed to have the potential to induce precipitation, by catalyzing ice formation in clouds (Morris et al. 2014a). This capacity to catalyze ice formation, conferred by a highly conserved gene referred to as *ice* or *ina*, also leads to frost injury on above-ground plant parts (Hirano & Upper 2000). It has been argued that ice nucleation activity might confer a selective advantage to the bacteria, by facilitating access to nutrients in damaged leaves and by favoring redeposition in suitable habitats after airborne transfer (Morris et al. 2013).

Methylotrophic bacteria have also received a fair amount of attention (Iguchi et al. 2015). These bacteria use methanol (CH_3OH) or methane (CH_4) as a carbon source. Methanol-using bacteria, such as the genus *Methylobacterium*, are often quite abundant in the phyllosphere and can benefit the plant by promoting its growth (Abanda-Nkpwatt et al. 2006). Proteins involved in methanol-based methylotrophy, such as methanol dehydrogenase, prevail in the phyllosphere (Knief et al. 2012). Methane-using bacteria are also present in the phyllosphere (Iguchi et al. 2015); however, their contribution to the methane cycle is suspected to be very low because enzymes involved in methanotrophy have not been detected in the phyllosphere (Knief et al. 2012).

Several studies have also focused on phyllosphere diazotrophic bacteria (Freiberg 1998, Fürnkranz et al. 2008, Rico et al. 2014, Ruinen 1965). These microorganisms can use atmospheric dinitrogen (N_2) as a source of nitrogen and include both Proteobacteria (e.g., *Beijerinckia*, *Azotobacter*, and *Klebsiella*) and Cyanobacteria (e.g., *Nostoc*, *Scytonema*, and *Stigonema*). N_2 is fixed by the nitrogenase enzyme encoded by *nif* genes, and the gene *nifH* is commonly used to assess their community structure (Fürnkranz et al. 2008, Rico et al. 2014). The richness of N_2-fixing bacteria has been shown to increase with drought, suggesting that their recruitment may extend the capacity of the plant to adapt to the environment (Rico et al. 2014).

3.3. Fungi

In addition to bacteria, the leaf surface is massively colonized by yeasts and filamentous fungi (**Figure 3**). Yeast density may be as high as 500 cells/cm^2 but is highly variable both within individual plants and across plant species (Inácio et al. 2010). The yeast-like fungus *Aureobasidium pullulans* is usually very abundant in the phyllosphere (e.g., Cordier et al. 2012b) as well as on fruit surfaces (e.g., Setati et al. 2012). It can be used in biological control because of its high ability to outcompete pathogenic microorganisms. Alongside yeasts, numerous fungal pathogen species elongate their mycelium on the leaf surface. This is the case of the powdery mildews (e.g., *Erysiphe*, *Podosphaera*, *Phyllactinia*, *Blumeria*), some of the world's most common plant pathogens. These species derive resources from the plant cells by penetrating the cuticle and then breaching the cell wall with specialized structures termed appressoria (**Figure 2*a***). After infection, mycelia grow superficially (**Figure 2*c*,*d***), covering leaves and fruits with a white or gray coat (Glawe 2008).

Many fungal species also develop within leaf tissues without causing visible symptoms. Many of these endophytic fungi can be transmitted horizontally (i.e., from one adult plant to another) and thus have an epiphytic stage before entering into the leaf tissues (Rodriguez et al. 2009). Some of them protect the host plant against stresses, including pathogens, herbivores, and drought. These beneficial effects have been extensively reviewed elsewhere (Porras-Alfaro & Bayman 2011, Rodriguez et al. 2009). Other endophytic fungi are latent pathogens. For instance, in the tropical palm tree *Iriartea deltoidea*, strong light triggers the production of ROS by the endophytic fungus *Diplodia mutila* and converts it to a pathogen, thus constraining seedling survival to shaded areas (Álvarez-Loayza et al. 2011).

Finally, a considerable overlap between fungal communities of living leaves and those of leaf litter indicates that some phyllosphere fungal species are dormant saprotrophs (Osono 2006, Unterseher et al. 2013, Voříšková & Baldrian 2013). These species are involved in the decomposition of cellulose in senescent leaves and young litter. They are then replaced with new colonizers with a higher capacity to decompose lignin; these colonizers mostly belong to the Basidiomycota (Voříšková & Baldrian 2013). In riparian ecosystems, the process of decomposition is driven by a particular group of fungi termed aquatic hyphomycetes (or Ingoldian fungi). This polyphyletic group of fungi plays a key role in the functioning of aquatic food webs, because its activity improves the palatability of leaves to invertebrates (Bärlocher 2016). Interestingly, aquatic hyphomycetes have also been found in the phyllosphere of more than 50 plant species (Chauvet et al. 2016). Phyllosphere fungal communities therefore play an active role in nutrient cycling and in the functional coupling of terrestrial and aquatic ecosystems. Hence, identifying the processes that shape these communities and assessing their response to global change is important.

Horizontal gene transfer (HGT): transmission of genetic material between species

4. AN EVOLUTIONARY COMMUNITY ECOLOGY FRAMEWORK FOR THE PHYLLOSPHERE

Adopting the view that the phyllosphere represents an ecological interface between the plant and its environment (**Figure 4**), we interpret the processes that shape PMCs using the framework proposed by Vellend (2010). According to this framework, ecological communities are shaped by four processes: dispersal, speciation, selection, and drift. Dispersal moves organisms across space, speciation creates new species, selection reflects deterministic fitness differences among species, and drift represents stochastic changes in species abundance (Vellend 2010). These processes are analogues to the evolutionary processes that contribute to phenotypic variation within and among populations of a species. Applying these processes at the community level requires viewing the community as a population, whose individuals belong to different co-occurring species, and considering species identity as a categorical phenotype with almost perfect heritability (i.e., except when speciation occurs). Selection can favor one species over another depending on its relative fitness, with species fitness defined as the mean fitness across all individuals of a given species in the community (Vellend 2010).

A few adjustments, however, are required to successfully apply Vellend's framework to PMCs. A particularity of microorganisms is that within-species phenotypic variation is often large, whereas among-species variation can be blurred by horizontal gene transfer (HGT), both within bacteria and fungi and between them (Koonin et al. 2001, Soanes & Richards 2014). Thus, to analyze the selective pressures shaping PMCs, a functional definition of the community is more effective than a taxonomic definition. The structure of the community can actually be described by the relative frequency of functional traits present (**Figure 4**), with functional traits being any trait affecting, directly or indirectly, individual performance (Violle et al. 2012). Examples of such traits would be the ability of bacteria to use the methanol produced by the plant (Delmotte et al. 2009, Knief et al. 2012) or their ability to produce biosurfactants to enhance the leaching of nutrients (Burch et al. 2014). For this reason, Nemergut et al. (2013) suggested replacing the term speciation in Vellend's framework with that of evolutionary diversification (**Figure 4**). Evolutionary diversification is the result of both genomic changes from within (mutation, deletion, duplication, or transposition) and genomic changes from without (incorporation of foreign DNA) (Koskella & Vos 2015). It creates new phenotypic variants that harbor new sets of functional traits on which selection can act. According to Violle et al. (2012), these selective pressures can be of two types (**Figure 4**). On the one hand, external selective pressures such as climatic constraints sort individuals from a regional pool by selecting those with optimal trait values. On the other hand, internal selective pressures that originate from the biotic interactions within the community regulate the local coexistence of individuals.

Finally, the evolution of the host plant must be taken into account if we wish to fully understand the structure of PMCs. The plant host is a living being whose phenotype and fitness are shaped

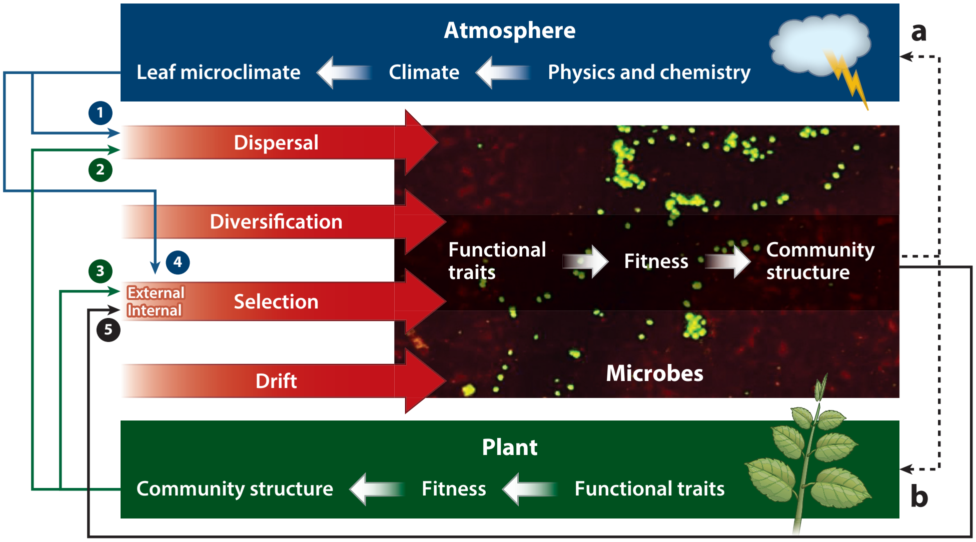

Figure 4

An evolutionary community ecology framework for the phyllosphere. Phyllosphere microbial communities are at the interface between the plant and the atmosphere. We propose a conceptual framework (after Nemergut et al. 2013, Vellend 2010), in which they are shaped by four eco-evolutionary processes depicted here by large red arrows: dispersal, evolutionary diversification, selection, and drift. Dispersal and evolutionary diversification shape the functional diversity of the microorganisms that arrive on plant leaves. External selection favors those that are most adapted to the local conditions (i.e., leaf morphology, chemistry, and microclimate). The selected microorganisms then grow and reproduce. Internal selection through biotic interactions such as competition or parasitism further regulates the structure of the microbial community. Drift causes stochastic changes in the community structure. The structure of the microbial community thus results from the influence of the plant, the atmosphere, and the community itself. Thin solid lines show their most likely effects. Dispersal can be influenced by ❶ meteorological conditions (Galès et al. 2014) and ❷ vegetation structure (Bailey et al. 2014). Selection can be exerted by ❸ foliar traits (Kembel & Mueller 2014, Kembel et al. 2014), ❹ climate conditions (Bálint et al. 2015, Cordier et al. 2012b), and ❺ biotic interactions occurring on the leaf (Innerebner et al. 2011, Koskella 2013). Phyllosphere microbial communities can in turn influence the functioning of the plant and the atmosphere. Feedback effects *a* and *b* are represented by thin dotted arrows. Phyllosphere microbial communities can (*a*) impact atmospheric physics and chemistry (Morris et al. 2014a) and (*b*) mediate plant functional traits by bringing novel metabolic or physiological capabilities or by altering existing ones (Friesen et al. 2011).

by its environment, including its microbial communities. The Vellend framework captures the selective pressure exerted by the host plant on PMCs but not the effect that PMCs can exert on the traits and fitness of their host. This feedback effect is better captured by the hologenome theory of evolution, which posits that the holobiont is a unit of selection in evolution (Zilber-Rosenberg & Rosenberg 2008). According to this theory, the host and the associated microbial genomes act as a consortium that copes with environmental change by evolving as a whole. During periods of rapid environmental change, a diverse microbial community can thus aid the holobiont in surviving, multiplying, and buying the time necessary for the host genome to evolve (Zilber-Rosenberg & Rosenberg 2008). To account for the effect of PMCs on the fitness of their plant host, we use the concept of microbially mediated plant functional trait (Friesen et al. 2011), defined as a novel metabolic or physiological capability given to the plant by microorganisms or a change in an existing trait caused by microorganisms (**Figure 4**).

Holobiont: the plant or animal with all of its associated microorganisms

5. ECO-EVOLUTIONARY PROCESSES SHAPING PHYLLOSPHERE MICROBIAL COMMUNITIES

Bioaerosol: fine particles of biological origin in suspension in the air

According to the proposed framework (**Figure 4**), four eco-evolutionary processes—dispersal, evolutionary diversification, selection, and drift—shape PMCs, ultimately contributing to the fitness of the host plant. In this section, we review current knowledge of these processes.

5.1. Dispersal

The microorganisms that colonize the leaf surface can come from at least five types of sources and can arrive at different stages of the plant ontogeny. The earliest potential source is the seed tissue and the germination environment (Barret et al. 2015). Once the seedling has emerged, bioaerosols become a major source of leaf colonization. These can be of very diverse origin, ranging from marine and soil to plant and animal sources (Bulgarelli et al. 2013). Microorganisms can also arrive on leaves via rainfall and subsequent splashing of raindrops or irrigation water (Morris 2002). The transmission of microorganisms by animals (herbivorous insects in particular) represents a fourth route of colonization. Finally, in the case of perennial plants, leaves can be colonized at bud burst by microorganisms that have spent the winter in buds or on twigs (Osono 2014).

Bioaerosols contain bacteria in the form of single cells, aggregates of cells, or spores and fungi in the form of spores and fragments of hyphae. Microorganisms can also be aggregated with dust particles, small plant debris, or pollen. The dispersal of phyllosphere microorganisms via bioaerosols occurs in three steps (Morris et al. 2014b): escape from the source (aerosolization), transport in air flows, and deposition. Aerosolization of foliar microorganisms and of fine leaf particles involves passive processes such as wind erosion and rain splashing but can also involve active spore release (Morris et al. 2014b). The ejection of particles above the canopy depends on its density and heterogeneity (Bailey et al. 2014). Particles can remain within the canopy and be deposited on adjacent plants, or they can travel over more or less long distances. Their trajectory depends, among other factors, on the height of release, the atmospheric conditions, and the particle size and specific density. Atmospheric circulation models suggest that particles less than 20 μm in diameter or with equivalent specific density can easily move between continents during the course of a year (Wilkinson et al. 2012). This is the case with most bacteria cells (typically 0.25–8 μm in diameter) and many fungal spores (typically 1–30 μm in diameter) (Jones & Harrison 2004).

Dispersal limitation is expected to generate a decay in microbial community similarity with geographic distance (Hanson et al. 2012). Geographical trends in phyllosphere bacterial communities of *Tamarix* trees along a 500-km transect supported this expectation (Finkel et al. 2012). Similarly, Galès et al. (2014) found that the deposition of a bacterial species on pine needles decreased with the distance to the source and that it was influenced by wind direction (**Figure 4**, effect ❶). The existence of dispersal limitation implies that the composition of the local plant community and the vegetation structure can influence PMCs to a larger or smaller extent (**Figure 4**, effect ❷).

5.2. Evolutionary Diversification

The rate of evolutionary diversification of phyllosphere microorganisms is likely to be high. Phyllosphere microorganisms are subject to intense abiotic stresses and some stresses are expected to increase mutation rates, such as ultraviolet radiations or ROS (Vorholt 2012). Moreover, the reservoir of genetic information that can be mobilized through HGT is likely to be large. As most microbial communities, PMCs typically comprise a few very abundant taxa and a huge number of rare taxa (Unterseher et al. 2011). These rare taxa, termed the rare biosphere, represent "a nearly inexhaustible source of genomic innovation" (Sogin et al. 2006, p. 12115). Current data suggest that HGTs have shaped several functional traits of plant pathogenic fungi, including the crucial ability to degrade and penetrate the host cell wall (Soanes & Richards 2014).

5.3. Selection

PMCs live at the plant–climate interface (**Figure 4**). External selection shapes them locally by favoring microbial functional traits that confer higher fitness under the local conditions defined by the leaf surface and the microclimate. Internal selection occurs when microorganisms interact. It acts on the microbial traits that condition interaction outcomes.

5.3.1. External selection. The ability of a microorganism to establish, thrive, and reproduce on the leaf surface depends on several microbial functional traits, such as the abilities to attach to the cuticle and to use the foliar nutrients (**Figure 1*a***). Leaf morphology, chemistry, and physiology differ among plant genotypes and species. Therefore, selective pressures exerted by the plant upon PMCs (**Figure 4**, effect ❸) vary from one host plant to the other. This variation has been inferred by numerous studies showing that the composition of PMCs varies among plant genotypes (Bálint et al. 2013; Balint-Kurti et al. 2010; Bodenhausen et al. 2014; Cordier et al. 2012a; Horton et al. 2014; Hunter et al. 2010, 2015; but see Mason et al. 2015) and plant species (Inácio et al. 2010, Kembel & Mueller 2014, Kembel et al. 2014, Kim et al. 2012, Lambais et al. 2014, Redford et al. 2010, Sapkota et al. 2015). A particularly elegant test of selection was performed by Maignien et al. (2014), who exposed microbe-free *Arabidopsis thaliana* to airborne communities and showed that phyllosphere bacterial communities initially mirrored airborne communities but gradually developed a distinct composition. The plant traits exerting selective pressures on PMCs, and the plant genes governing these traits, have mainly been studied in *A. thaliana*. Mutant lines have been used to show that the cuticular wax composition influences the composition of phyllosphere bacterial communities (Bodenhausen et al. 2014, Reisberg et al. 2013), whereas the presence of trichomes does not (Reisberg et al. 2012). Horton et al. (2014) used genome-wide association mapping to show that plant loci responsible for defense against pathogens drive variation in PMCs. These results parallel those obtained in maize (Balint-Kurti et al. 2010). For perennial plants, Yadav et al. (2005) showed that leaf water content is the primary driver of phyllosphere bacteria abundance in Mediterranean trees and shrubs, followed by the leaf phosphorus content. Leaf phosphorus content was also found to influence bacterial community structure in tropical trees (Kembel et al. 2014), whereas leaf aluminum content was more influential for fungi (Kembel & Mueller 2014).

The ability of a microorganism to establish, thrive, and reproduce in the phyllosphere also depends on its ability to withstand the leaf microclimate. For instance, microbial resistance to desiccation and UV radiations is increased by the ability to form cell aggregates and to produce pigments, respectively (Vorholt 2012). The leaf microclimate varies with regional climate and exhibits fine-scale variations due to landscape, vegetation, and canopy structure. This variation in selective pressures (**Figure 4**, effect ❹) has been assessed by comparing PMCs of leaves from different parts of the same tree canopy (Cordier et al. 2012a, Leff et al. 2015, Osono 2014), by using altitudinal climatic gradients (Coince et al. 2014, Cordier et al. 2012b), and by manipulating climate in common garden experiments (Bálint et al. 2015). These studies all suggest significant effects of climate and microclimate on phyllosphere fungal and bacterial communities.

Future research needs to address the relative effect of selective pressures exerted by the host plant versus selective pressures exerted by climate and microclimate. To do that, the structure of

PMCs can be considered a phenotypic trait of the plant (Whitham et al. 2006) and the genetic architecture and phenotypic plasticity of the trait can be studied, using the framework developed by plant quantitative geneticists (Des Marais et al. 2013).

Genetic architecture: genetic basis of a phenotype, including the number of genes, their allelic effects, and interactions among loci and the environment

Phenotypic plasticity: the ability of a single genotype to produce multiple phenotypes in response to the environment

Ecological interaction network: a representation of the biotic interactions (network links) between taxa (network nodes) in a specified place and time

5.3.2. Internal selection. Once established on the leaf, microorganisms start to interact with each other (**Figure 3*b***). Microbial interactions span the whole range of interactions known for macroorganisms, including competition, parasitism, predation, mutualism, commensalism, and amensalism (Faust & Raes 2012). Interactions can select for specific microbial traits. A notable example concerns interactions between phyllosphere bacteria and phages in the horse chestnut (*Aesculus hippocastanum*), in which bacterial resistance against phages increases along the vegetative season (Koskella 2013). Interactions also influence the fitness of phyllosphere microorganisms. Competition for nutrients was proposed to be the foundation of the observation by Remus-Emsermann et al. (2012) that the reproductive success of a bacterial cell landing on the leaf surface decreases with the increased local density of cells already present. Through their effects on microbial traits and fitness, interactions govern the trajectory of PMC assembly (**Figure 4**, effect ❺). The microbial taxa that first colonize the phyllosphere can determine the range of microbial taxa that are subsequently able to colonize it. These effects, termed priority effects, have been observed by Maignien et al. (2014) in *A. thaliana* plants belonging to the same genotype and growing in the same climate chamber. The community assembly followed divergent trajectories, probably owing to different initial colonization events. Microbial interactions in the phyllosphere can be represented as ecological interaction networks. A current challenge is to decipher these complex and dynamic networks that—contrary to more conventional trophic networks for which a well-developed theory exists—contain various types of interactions among microorganisms from various kingdoms. Another challenge is to understand how these interactions regulate the growth rate and fitness of plant pathogens and to use these findings to develop biological control strategies (Agler et al. 2016, Jakuschkin et al. 2016, Vacher et al. 2016).

5.4. Drift

Ecological drift structures PMCs by inducing stochastic changes in the abundance of microbial taxa. As mentioned above, PMCs comprise few very abundant taxa and a huge number of rare taxa (Unterseher et al. 2011). Drift primarily affects these rare taxa, because small changes in their abundance can result in their extinction on a local scale (Nemergut et al. 2013). Drift is also expected to cause stochasticity in the success of early colonization events, which can then affect the trajectory of the whole community (Maignien et al. 2014).

5.5. Feedback Effects on the Plant and the Atmosphere

PMCs are shaped by external selective pressures exerted by both climatic conditions and the plant host. In turn, they influence atmospheric processes (**Figure 4**, feedback effect *a*) [reviewed by Morris et al. (2014a)] and the foliar functional traits of their plant host (**Figure 4**, feedback effect *b*). For instance, the colonization of maize seedlings by the bacterial strain *Burkholderia phytofirmans* PsJN influences the number of leaves per plant, leaf area, stomatal conductance, the efficiency of photosystem II, leaf chlorophyll content, leaf water content, and the production of foliar ROS (Naveed et al. 2014). The infection of oak leaves by the fungal pathogen *Erysiphe alphitoides* alters foliar traits including the net CO_2 assimilation rate, stomatal conductance, and the emission of VOCs (Copolovici et al. 2015). PMCs also modulate leaf susceptibility to infection. Sterile leaves are usually much more susceptible, indicating that PMCs can protect the plant against foliar diseases (Innerebner et al. 2011, Ritpitakphong et al. 2016). PMCs can thus increase or decrease the fitness of their host plant, depending on the microbial taxa involved and the local environmental conditions. Current challenges are to understand which properties of PMCs (e.g., taxonomic diversity, functional diversity, community composition, microbial network structure) will be beneficial under future environmental conditions and to foster these properties in crop plants to sustain the productivity and resilience of agricultural systems.

6. PHYLLOSPHERE MICROBIAL COMMUNITIES IN A FUTURE WORLD

The impact of global change on the relationship between plants and their PMCs is a salient question. The hologenome theory of evolution (Zilber-Rosenberg & Rosenberg 2008) predicts that the fast evolution of microorganisms could help the plant to cope with environmental change. Yet rapid anthropogenic environmental changes could also disrupt the plant–PMCs relationship, diminishing the protective ability of PMCs and exacerbating antagonistic interactions. In this section, we review current knowledge of the impact of climatic stresses and other anthropogenic disturbances on PMCs.

6.1. Response to Climatic Stressors

Few experiments have to date manipulated climatic conditions (e.g., air and soil temperature, soil water content, or air CO_2 concentration) to assess their effects on above-ground microbial communities. In comparison, the effects of climatic stressors on below-ground microbial communities have received more attention (Compant et al. 2010). The available evidence indicates that phyllosphere bacterial communities are particularly influenced by drought. For instance, rain exclusion altered the composition of phyllosphere bacterial communities in Holm oak (*Quercus ilex*) with a notably increased richness of epiphytic communities in the summer (Peñuelas et al. 2012, Rico et al. 2014). Warming the surface soil in a rice paddy by about 2°C altered the composition of bacterial endophytic communities on only the lower plant leaves and did not affect epiphytic bacterial communities (Ren et al. 2015). Likewise, CO_2 enrichment did not alter the composition of bacterial epiphytic and endophytic communities in either rice (Ren et al. 2015) or cotton (Runion et al. 1994). In addition, current knowledge indicates that phyllosphere fungal communities respond strongly to warming. For instance, increasing both the air and soil temperature by approximately 2°C during a whole growing season altered the composition of foliar fungal endophytic communities in Balsam poplar (*Populus balsamifera*). It also decreased the diversity of endophytic communities and increased the abundance of plausible pathogens (Bálint et al. 2015). Significant variations in the composition of foliar fungal communities of European beech (*Fagus sylvatica*) along elevation gradients parallel this finding (Coince et al. 2014, Cordier et al. 2012b). Drought did not alter the composition of foliar fungal communities in cotton (Runion et al. 1994) but influenced the seasonal variation of fungal diversity in Holm oak (Peñuelas et al. 2012). CO_2 enrichment only influenced the abundance of a few fungal genera in cotton and winter wheat (Magan & Baxter 1996, Runion et al. 1994).

6.2. Response to Pesticides

The effects of pesticides on PMCs have been much more thoroughly studied. Significant effects have been found in some cases (Bertelsen et al. 2001, Ottesen et al. 2015, Zhang et al. 2009), but in general PMCs tend to be very resilient to pesticide applications. For instance, 10 years of streptomycin application in apple orchards did not influence the composition of phyllosphere bacterial communities (Yashiro & McManus 2012). Similarly, the application of penconazole to grapevine did not influence the diversity and composition of PMCs (Perazzolli et al. 2014), whereas the application of metalaxyl and imidacloprid to pepper caused only minor changes (Moulas et al. 2013). The application of captan on apple leaves reduced the abundance of phyllosphere fungi but only temporarily. One month after the last application, phyllosphere fungal communities again resembled those of unsprayed trees (Hislop & Cox 1969). Interestingly, some evidence indicates that pesticide-mediated alterations of PMC structure can affect leaf physiology. Thus, applying azoxystrobin and epoxiconazole in winter wheat significantly reduced the mycelial growth of some saprophytic foliar fungal species (*Alternaria alternata* and *Cladosporium macrocarpum*), causing a delay in leaf senescence (Bertelsen et al. 2001).

6.3. Response to Air Pollutants

Plant leaves also are exposed to gaseous air pollutants (sulfur dioxide, nitrogen dioxide, ozone) and particulate matter (heavy metals, black carbon, polycyclic aromatic hydrocarbons) emitted by human activities. These pollutants alter foliar traits, including cuticle properties (Rashidi et al. 2012), leaf chemistry (Jumpponen & Jones 2010), and phenology (Jochner et al. 2015). Moreover, some of the pollutants can be used by phyllosphere bacteria as a carbon source (Sandhu et al. 2007). The effects of air pollutants on both foliar traits and bacterial activity could explain the marked differences reported between urban and nonurban environments in the diversity and composition of PMCs (Brighigna et al. 2000, Jumpponen & Jones 2010).

6.4. Response to Invasive Species

Biological invasions can alter the functioning of whole ecosystems (Simberloff et al. 2013), but their effect on PMCs remains virtually unknown. One study has investigated the impact of the invasive spruce bud scale (*Physokermes piceae*) on phyllosphere fungal communities of Norway spruce (*Picea abies*). The insect weakened trees by sucking sap of needles and young shoots and covering needles with honeydew, causing significant changes in the diversity and composition of phyllosphere fungal communities (Menkis et al. 2015).

7. CONCLUSION

After more than 50 years of microbiological, plant pathological, and ecological research, the phyllosphere can no longer be considered "an ecologically neglected milieu" (Ruinen 1961, p. 81). Meta-omic approaches have recently fostered empirical research on this microbial habitat. The concomitant development of conceptual frameworks rooted in community ecology and evolutionary ecology (Vellend 2010, Zilber-Rosenberg & Rosenberg 2008) represents a further necessary step toward a proper understanding of the structure and function of PMCs. These frameworks allow us to go beyond classical approaches that focus on specific plant–microbe interactions. They allow us to explore real-world situations in which plants interact with a myriad of microorganisms that themselves interact with each other (Hacquard & Schadt 2015, Vandenkoornhuyse et al. 2015). This holistic view of plant–microbe interactions opens new questions that can be formulated in the context of community and ecosystem ecology (Mushegian & Ebert 2016, Peñuelas & Terradas 2014, Whitham et al. 2006). Which properties of PMCs improve the functioning of both their plant host and the ecosystem? How do they relate to the resilience of plants and ecosystems to environmental change? What is the genetic architecture of these beneficial properties? Does

PMC evolution foster the adaptation of their plant host to novel environmental conditions? These and many more questions can now be addressed by integrating observational and experimental research on PMCs, plant genotypes, and environments. The findings will be of great value for improving plant adaptation and ecosystem resilience to ongoing and impending anthropogenic environmental changes.

SUMMARY POINTS

1. The surfaces of plant leaves harbor hyperdiverse microbial communities of prokaryotes, eukaryotes, and viruses that interact with each other and with the host plant.
2. Research on this microbial habitat, termed the phyllosphere, started in the mid-1950s and bloomed in the 2000s with the emergence of meta-omic approaches.
3. PMCs mediate foliar functional traits and affect plant fitness. They also influence ecosystem functions such as nutrient and water cycling.
4. PMCs can be seen as entities shaped by four eco-evolutionary processes: dispersal, evolutionary diversification, selection, and drift.
5. Evolutionary diversification is the result of changes in microbial genomes. It creates new microbial variants that harbor new sets of traits on which selection can act.
6. PMCs are subject to external selection exerted by the host plant and the surrounding atmosphere and to internal selection arising from within-community biotic interactions.
7. There is increasing evidence that global change alters the structure of PMCs, with potential cascading effects on plant performance, plant evolution, and ecosystem functioning.

FUTURE ISSUES

1. We are far from a full understanding of the diverse microbial interactions in the phyllosphere. Future research needs to decipher these complex and dynamic interaction networks and assess their role in plant health.
2. We are also far from a full understanding of the physiology of phyllosphere microorganisms. Meta-omic approaches should be used to better understand the function of PMCs (what they do) in addition to their taxonomic composition (who they are).
3. The relationships among the diversity of PMCs, plant performance, and ecosystem functioning has hardly been studied. Future research needs to identify exactly which whole-community properties of PMCs mediate plant performance and ecosystem functions.
4. Surveys and experiments across a wide range of spatial and temporal scales should be performed to assess the relative roles of dispersal, evolutionary diversification, selection, and drift in shaping PMCs.
5. The relationship between the dynamics and evolution of PMCs and plant adaptation to environmental change is far from being understood. The influence of environmental change on the diversification of PMCs, and the subsequent effects on plant fitness, should be investigated.

6. Research of the plant traits that shape PMCs and the underlying genes should be pursued in model and nonmodel species. These findings should be integrated into plant breeding programs to foster agricultural productivity and resilience.
7. The global biogeography of PMCs is mostly unknown. Global surveys should be performed and used to predict changes in the distribution of PMCs and their cascading effects on plant communities and biogeochemical cycles under climate change scenarios.

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