Shaping the leaf microbiota: plant–microbe–microbe interactions

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Received 1 May 2020; Editorial decision 2 September 2020; Accepted 7 September 2020

Editor: Stanislav Kopriva, University of Cologne, Germany

Abstract

The aerial portion of a plant, namely the leaf, is inhabited by pathogenic and non-pathogenic microbes. The leaf's physical and chemical properties, combined with fluctuating and often challenging environmental factors, create surfaces that require a high degree of adaptation for microbial colonization. As a consequence, specific interactive processes have evolved to establish a plant leaf niche. Little is known about the impact of the host immune system on phyllosphere colonization by non-pathogenic microbes. These organisms can trigger plant basal defenses and benefit the host by priming for enhanced resistance to pathogens. In most disease resistance responses, microbial signals are recognized by extra- or intracellular receptors. The interactions tend to be species specific and it is unclear how they shape leaf microbial communities. In natural habitats, microbe–microbe interactions are also important for shaping leaf communities. To protect resources, plant colonizers have developed direct antagonistic or host manipulation strategies to fight competitors. Phyllosphere-colonizing microbes respond to abiotic and biotic fluctuations and are therefore an important resource for adaptive and protective traits. Understanding the complex regulatory host–microbe–microbe networks is needed to transfer current knowledge to biotechnological applications such as plant-protective probiotics.

Keywords: Biofilm, innate immunity, microbe–microbe interaction, microbial colonization, phyllosphere, quorum sensing.

Introduction

This review examines how aerial parts of plants, particularly leaves, are colonized by microbes. The first section (’Colonizing leaf surfaces’) dissects biotic and abiotic factors that shape leaf microbial communities and determine the quality and quantity of colonization. We discuss pre-formed barriers, such as the cuticle, that restrict plant host colonization, and environmental conditions that enhance selection pressures. As a consequence of the extreme conditions on leaves, the properties and
generation of biofilms through quorum sensing (QS) are considered. Leaf colonization by microbes is not only impacted by host and environmental factors but also by resident microbes, including pathogens that can severely perturb microbial communities. The second section (‘Microbe–microbe–host interactions’) discusses effects of microbial communities on host susceptibility to pathogens and the impact of plant pathogens and endophytes on microbial host colonization and community composition. A particular focus is on microbe–microbe interactions that are often mediated via the plant host. Individual plant cells also have the capacity to steer microbial activities by pre-formed or induced structures and compounds that influence microbial growth on the leaf surface. The third section (‘Role of the plant immune system in shaping the phyllosphere microbiome’) considers the role of the plant immune system on microbial host interactions with a focus on plant leaf colonization, leaf–microbe outputs, and microbial diversity.

**Colonizing leaf surfaces**

*The leaf environment*

All terrestrial plants are inhabited by diverse, complex, and interactive communities of microorganisms. With this intimate association, the host plant and its associated microbiota are regarded as a close knit entity and are collectively defined as the holobiont. The holobiont concept implies that evolutionary selection takes place between the host and its associated microbes, and within microbe–microbe members (Vandenkoornhuyse et al., 2015; Hassani et al., 2018; Teixeira et al., 2019). The phenotype of a plant host is the collective outcome of numerous interactions with its microbiota in a particular environment at a time (Vorholt et al., 2017). The ‘phyllosphere’ is referred to as the above-ground portion of plants, dominated by leaves. Its surface represents one of the most abundant habitats on earth (Lindow and Brandl, 2003). Leaves create a fluctuating and unstable environment exposed to multiple stresses and relatively devoid of nutrient sources (Bringel and Couée, 2015). The study of microbial communities inhabiting this stressful leaf habitat and their collective contribution to plant growth, development, and protection has gained intense interest over the last decade. The leaf harbors diverse microorganisms that inhabit the surface and the interior, and are known as epiphytes and endophytes, respectively (Beattie and Lindow, 1999; Lindow and Brandl, 2003). These microorganisms include bacteria as the most common inhabitants, followed by filamentous fungi and yeast strains (Stone et al., 2018), protists (Sapp et al., 2018), and bacteriophages (Balogh et al., 2018). The bacterial titer accounts for \(10^6\)–\(10^7\) cells cm\(^{-2}\) of leaf area (Lindow and Brandl, 2003), whereas a typical yeast titer ranges from \(10^4\) to \(10^5\) cells cm\(^{-2}\) of leaf (Shivas and Brown, 1984). The origin of leaf microbial communities is not restricted to a single source. Microbes can colonize the plant leaf vertically through seeds or pollen and horizontally from the air, soil, and insects (Vorholt, 2012; Bodenhauser et al., 2013; Maignien et al., 2014; Bai et al., 2015; Frank et al., 2017).

A stressed and nutrient-poor condition of the leaf surface makes this environment selective to certain microorganisms. Hence, different microbial mechanisms such as ability to extract nutrients, produce hormones and surfactants, as well as motility and biofilm formation can be key to colonization success (Nadakuduti et al., 2012; Ueda et al., 2018; Leveau, 2019; Oso et al., 2019; Streletskaia et al., 2019). Most epiphytes survive on the leaf surface by forming large aggregates which help them to cope with the surrounding milieu and maintain a hydrated surface by production of extracellular polymeric substances (EPS) (Morris and Kinkel, 2002; Lindow and Brandl, 2003; Baldotto and Olivares, 2008; Vorholt, 2012). Other microbes, which are not considered as part of common leaf microbiota, are human commensal or pathogenic bacteria. These can survive and proliferate on the plant leaf, as documented by numerous outbreak studies of human infections on leafy vegetables (Beuchat, 2002; Lindow and Brandl, 2003; Naami et al., 2003; Islam et al., 2004; Melotto et al., 2006; Munther et al., 2020). Such microbes are able to colonize and survive in an unfavorable leaf environment if they are teamed up with aggregates of pre-colonized leaf microbiota (Monier and Lindow, 2005).

Host-adapted microbial colonizers are more tolerant to abiotic stresses such as harmful UV radiation (Kamo et al., 2018), oxidative stress, and desiccation (Vorholt, 2012), and can utilize nutrients (Crombie et al., 2018) and vitamins (Yoshida et al., 2019) available on the leaf surface. By mitigating biotic and/or abiotic stress(es) and influencing plant growth and fitness, microbes develop adaptive traits and intimate associations with leaves (Vorholt, 2012; Hellrich et al., 2018). Plant host–microbiota interactions are built on the transfer of molecular and genetic information. Important colonization factors, such as secondary metabolites, QS systems, biofilm formation, and cell signaling, are responsible for this exchange of information (Braga et al., 2016; Leveau, 2019; Flores-Núñez et al., 2020). Leaf microbial communities influence plant fitness by modulating the host plant immune system and promoting plant growth in above-ground tissues (Stone et al., 2018).

**Leaf surface structure and chemistry relevant to microbiota assembly**

Leaf structure and its surface chemistry create a peculiar micro-environment. During evolution, the formation of a leaf cuticle layer was a prerequisite for land plants to survive out of water. The composition and function of the cuticle is summarized in a review (Müller and Riederer, 2005). The cuticle also covers preferential sites for microbiota colonization, such as the surface of leaf epidermal cells, stomata, and trichomes (Teplitski...
et al., 2011; Peredo and Simmons, 2018). The cuticle layer is composed of structurally and chemically heterogeneous compounds primarily made of biopolyester cutin, wax, and more minor compounds such as phenolics, cutan, and polysaccharides (Gniwotta et al., 2005; Nawrath et al., 2013). Under constant exposure to abiotic and biotic factors, the epidermal layer of leaf tissues performs its primary function as a protective barrier by preventing seepage of water from the leaf surface as well as external water and solutes from entering the plant. Moreover, the cuticle plays a critical role in mediating interactions with leaf microbiota, including commensal, beneficial, and pathogenic microorganisms (Schönherr, 2006; Vorholt, 2012; Vacher et al., 2016).

Leaf microbiota utilize a number of strategies to enter and penetrate the leaf cuticle. A major route is through natural stomatal openings and wounds resulting from lytic enzymes and osmotic pressure (Frank et al., 2017). Stomata are enclosed by two guard cells to regulate gas exchange and transpiration from the leaf epidermis. Movement of microbes between the external and internal parts of the phyllosphere via stomata has been generally regarded as a passive process, in which the microorganism and plant leaf do not engage in active dialog to permit and/or restrict microbe entry (Underwood et al., 2007). Studies have demonstrated the role of signal transduction cascades in bacterial regulation of stomatal aperture (Zeng et al., 2010; Zheng et al., 2012).

Stomatal aperture is regulated by biotic and abiotic environmental conditions. In general, successful microbial colonization of the leaf depends on stomatal aperture (Ou et al., 2014). Decades of research have shown that phytopathogenic bacteria and fungi exploit stomata as a point of entry for invasion. To breach surface barriers via stomata, host-adapted bacteria subvert plant abiotic stress signaling to suppress stomatal closure during infection (Melotto et al., 2006; Okamoto et al., 2009; Zeng et al., 2010; Xin et al., 2018). To counter pathogen invasion, stomatal guard cells recognize diverse pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs) such as flagellin, chitin, and chitosan (Arnaud and Hwang, 2015). These recognitions and downstream signaling processes lead to closing of stomatal pores and hence prevent bacterial entry as part of the plant immune response. Suppressing the stomatal defense system is an important adaptation mechanism for switching from an epiphytic to an endophytic lifestyle, leading to bacterial disease (Melotto et al., 2017).

Structural and chemical heterogeneity of the leaf cuticle is detected within and between plant genotypes, organs, and even developmental stages (Müller and Riederer, 2005). A role for leaf surface microbiota, together with leaf cuticle mechanisms, was observed in Arabidopsis thaliana in resistance against Botrytis cinerea, a broad host-range necrotrophic fungal pathogen (Ripitapkhong et al., 2016). Analyses reveal important effects of variation in cuticle chemical and physical composition modulating associations between plants and microbiota, including beneficial and pathogenic microorganisms (Aragón et al., 2017).

Apoplastic spaces inside leaves are large intercellular spaces which mediate gas exchange between cells and are essential for most plant species to achieve efficient photosynthesis (Chen et al., 2020). Humidity controls occupancy of pathogens in apoplastic spaces and is an important initial determinant of leaf colonization (Xin et al., 2016, 2018). Indeed, water availability in the leaf apoplast is a key factor determining successful colonization by neutral and beneficial, but also pathogenic microbes that compete with the host for water (Aung et al., 2018; Chen et al., 2020). It is therefore not surprising that the leaf apoplast has emerged as a decisive environment for host–microbe communication during colonization and for the mobilization of active defense mechanisms to counter pathogen infection.

Diversity of leaf-colonizing microbiota

Distinct microbiota interactions are found in the leaf compartment which influence the plant host, shaping the microbial community and colonization success. The microbiota in the leaf is not composed of a single species but rather intraspecies, interspecies, and cross-kingdom microbial assemblages of bacteria, yeast, fungi, and protists, establishing the leaf environment (Harold et al., 2015). The establishment and abundance of these leaf microbial communities and their distinct effects on the host plant—whether this is commensal, beneficial, or detrimental—are the outcome of numerous interactive processes. These processes are, in turn, influenced by incoming and outgoing microorganisms to and from the leaf habitat and their rate of multiplication, dispersal, and decline in a particular niche (Vorholt, 2012; Wagner et al., 2014, 2016; Lebeis et al., 2015; Vacher et al., 2016; Remus-Emsermann and Schlechter, 2018; Stone et al., 2018; Laforest-Lapointe and Whitaker, 2019).

To gain a better understanding of plant leaf–microbe interactions and outcomes, it is crucial to identify and characterize the microbial community that has evolved and adapted to the leaf environment. There are now ample studies describing the diversity and community structure of leaf-associated microbes, their characterization by next-generation sequencing, culture-independent and culture-dependent methods based on taxonomic markers, and their roles in host development and protection against stress (Romero et al., 2014; Harsonowati et al., 2017; Wallace et al., 2018; Dong et al., 2019). Notably, the leaf-associated microbial community in plants such as common bean (Phaseolus vulgaris), lettuce (Lactuca sativa), and neotropical forest and poplar trees consists of four major bacterial phyla, namely Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (de Oliveira Costa et al., 2012; Rastogi et al., 2012; Kembel et al., 2014; Durand et al., 2018). Rastogi et al (2012) demonstrated variability in bacterial community profiles on field-grown lettuce leaves with respect to time, space, and environment. In characterizing a tropical tree microbiome, Kembel et al. (2014) showed that leaf bacterial communities are dominated by Actinobacteria, Alpha-, Beta-, and Gammaproteobacteria, and Sphingobacteria. The Kembel study also identified microbial
correlations with host growth, mortality, and function. In *A. thaliana*, there is a taxonomic and functional overlap between bacterial communities in the leaves and roots, and evidence that soil is the main driver of leaf bacterial community structure (Bai et al., 2015). Phyla belonging to *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were found to be most abundant in *A. thaliana*, common ash (*Fraxinus excelsior*), and other tree leaves (Redford et al., 2010; Bodenhausen et al., 2013; Bai et al., 2015; Griffiths et al., 2020; Ulrich et al., 2020).

Redford et al. (2010) in a study of leaves of 56 tree species also concluded that interspecies variation is more prevalent than intraspecies variation and that there is a correlation between tree phylogeny and bacterial community composition. Using 16S rRNA, ammonia oxidation (*amoA*), and nitrogen fixation (*nifH*) gene markers, Bao et al. (2020) characterized phyllosphere bacteria and established differences in the diversity and composition of bacteria, including diazotrophic communities, over two seasons in three different tree species. An abundance of season-specific bacterial genera highlighted that there might be particular mechanisms of leaf adaptation in different seasons (Bao et al., 2020). However, *Methylobacterium* and *Sphingomonas* species were highly abundant in the plant leaf environment of three species, namely *A. thaliana*, *Trifolium repens*, and *Glycine max* (Delmote et al., 2009). Furthermore, Durand et al. (2018) reported bacterial community members belonging to *Methylobacterium*, *Kineococcus*, *Sphingomonas*, and *Hymenobacter* on the leaf surface of poplar trees. Apart from these, members of the genus *Pseudomonas* are also predominantly found in the phyllosphere of a wide range of plant species (Rastogi et al., 2013).

Numerous studies revealed the association of diverse leaf epiphytic and endophytic filamentous fungi and yeasts with plant host (Arnold et al., 2007; Kharwar et al., 2010; Porras-Alfaro and Bayman, 2011; Sun et al., 2014; Wang et al., 2016; Qian et al., 2018; Yao et al., 2019; Into et al., 2020). Huge diversity, spatial structure, and host association were observed among leaf endophytes and a role in protecting the plant against the devastating foliar oomycete pathogen, *Phytophthora sp.* (Arnold et al., 2003). Qian et al. (2018) found that *Dothideomycetes* and *Eurotiomycetes* are dominant members in *Mussaenda pubescens* and identified intraspecific host genetics as primary drivers in shaping regional phyllosphere fungal communities. Yao et al. (2019) reported that *Dothideomycetes* and *Tremellomycetes* are dominant members in the mangrove ecosystem in six mangrove species, namely *Avicennia marina*, *Rhizophora stylosa*, *Aegiceras corniculatum*, and *Excoecaria agallocha*, and obtain ecosystem insights for species co-existence and community stability. Using a culture-independent approach, Agler et al. (2016) extracted yeast genera belonging to *Protomyces*, *Dioiszea*, *Leucosporidium*, and *Rhodotorula* in the phyllosphere of wild *A. thaliana* populations from Germany. In another study, Dhayanithy et al. (2019) characterized fungal endophytes from the leaves and stems of *Catharanthus roseus* and reported *Colletotrichum*, *Alternaria*, and *Chaetomium* genera as common members. *Cladosporium* and *Alternaria* filamentous fungi, *Cryptococcus* and *Sporobolomyces* yeasts, and *Pseudomonas* spp. and *Erwinia herbicola* bacteria were commonly found colonizing leaves of *Beta vulgaris* (Thompson et al., 1993). In another study, by Glushakova and Chernov (2004), changes in epiphytic yeast populations were observed over the year in evergreen common wood sorrel *Oxalis acetosella* L., revealing that species diversity was high in autumn and low in spring. In contrast, *Rhodotorula glutinis* and *Sporobolomyces roseus* species were abundant throughout the year. Interestingly, leaves also harbor certain suppressive bacteria that can restrict phyllosphere bacterial diversity and increase resistance, for example in maize, to Southern leaf blight (SLB) fungal infection (Balint-Kurti et al., 2010). Numerous studies showed the diversity and abundance of yeast in the leaf environment; however, there is a need for more in-depth understanding of the biological mechanisms that showed their role towards host growth and protection.

Sometimes, serious human pathogens such as *Salmonella enterica* serovar *Typhimurium* 14028s (*S. typhimurium* 14028s) and *Escherichia coli* O157:H7 (*E.coli*157) colonize fresh leafy vegetables such as lettuce (*Lactuca sativa*) via damaged leaf tissue and can cause food-borne disease outbreaks (Saldaña et al., 2011; Roy et al., 2013). At the site of injury, lettuce leaf tissue provides substrates for proliferation, and choline which helps the pathogen combat osmotic stress (Scott et al., 2017). It is not clearly understood how human pathogenic/commensal bacteria survive in the extreme environmental conditions encountered by plants. Nevertheless, human pathogens can stay as persister cells also known as cells in a transient dormant state on the plant and cause disease once they encounter a new environment (Munther et al., 2020). In a recent study, Jacob and Melotto (2020) discovered genetic diversity among lettuce genotypes and resident human pathogenic *S. Typhimurium* 14028s and *E. coli* O157:H7, and found a link between genetic diversity and differences in plant immune responses to these bacteria. However, in comparison with human pathogens, less is studied on persister cells in phytopathogen associations with the leaf (Martins et al., 2018). Mechanisms preventing invasion by plant pathogenic microorganisms and plant-induced defense responses are discussed below.

Role of metabolites in leaf preferential colonization by microbiota

Although present as epiphytes on plant hosts, not all microbes are able to colonize and establish themselves inside leaves. Initial colonization and entry of microbes as a community into a leaf is not a random process in which arbitrary communities adhere and grow, but an organized series of events. Steps involve attachment, movement, and cellular interactions. These steps are facilitated by the leaf surface structure (see above) which regulates colonization as an important priming event in microbial community interactions with the plant (Lebeis
Biofilm formation by leaf microbiota

Microbes colonize leaves as complex multicellular communities. Long-term co-evolution of communities that have co-adapted and specialized results in distinct associations which further facilitate mutualistic, symbiotic, competitive, antagonistic, and indeed pathogenic microbial lifestyles with the host (Braga et al., 2016). Association between communities starts with initial adhesion to the leaf surface and ends with a complex network of interactions. Most research on leaf microbiota has focused on bacterial communities which assemble in aggregates of up to 10^6 cells (Monier and Lindow, 2004). These bacterial clusters are the result of aggregation between multiple cell types or clonal reproduction of a single cell (Tecon and Leveau, 2012). Bacterial surfaces play a critical role in aggregation, biofilm formation, adherence, and survival on leaf surfaces. As a part of a survival strategy, human pathogens also formed aggregates with other bacteria on the leaf, probably affording some protection during their limited survival span (Brandl and Mandrell, 2002).

Biofilms are aggregates of microbial communities in which cells adhere to each other and to a surface enveloped in a matrix of extracellular polymeric compounds, which protects the community under adverse conditions (Davies and O’toole, 2000). In nature, ~70% of bacteria on leaves are found in aggregates which confer a survival and colonization selective advantage over solitary cells on leaf surfaces (Morris and Kinkel, 2002; Monier and Lindow, 2003). Bacterial cell aggregates need to reach a minimum size to gain protection in unfavorable environments. For instance, Monier and Lindow (2003) showed that aggregates of ~100 or more cells are essential for protection against desiccation on plant leaf surfaces. A large pool of microbial communities on the leaf is protected in stress-tolerant aggregates, and dispersal of single cells leads to new microcolonies (Danhorn and Fuqua, 2007). From the attachment of cells to generation of mature biofilms, specific traits such as motility and adhesion are necessary to move and disperse on the leaf, for optimal resilience to biotic and abiotic stresses (Grinberg et al., 2019).

Using atomic force microscopy (AFM), Mittelvielhaus et al. (2019) quantified high magnitude differences in adhesion forces of leaf bacteria. Biofilm formation can also be observed in symbiotic and pathogenic lifestyles on plants, and linked with the disease cycle of phytopathogenic bacteria (Bogino et al., 2013). A role for the biofilm in colonization, disease development, and biocontrol activity was established in different studies for Xanthomonas axonopodis pv. citri, Xanthomonas vesicatoria, and Bacillus amyloliquefaciens (Malamud et al., 2011; Felipe et al., 2018; Salvatierra-Martinez et al., 2018). Nevertheless, the precise mechanism(s) by which plants regulate biofilm-associated communities are unclear (Ference et al., 2018; Kyrkou et al., 2018). The phytopathogen X. axonopodis pv. citri forms biofilms on leaves of citrus species during the development of citrus canker disease (Brunings and Gabriel, 2003; Rigano et al., 2007; Malamud et al., 2011). Notably, microbial biofilms are also important for pathogenesis by Xylella fastidiosa, the causal agent of devastating Pierce’s disease of grapes, olives, and citrus fruits, which culminates in blockage of the host vascular system (Hopkins, 1989; Marques et al., 2002; Thorne et al., 2006; Rudrappa et al., 2008; Kyrkou et al., 2018). Bacterial
brown spot disease of bean leaves caused by *P. syringae* pv. *syringae* was also found to require biofilm formation (Monier and Lindow, 2004). Similarly, motility in biofilm formation was essential for host colonization by phytopathogens such as *Ralstonia solanacearum*, *Pantocea stewartii*, and *Dickeya dadantii* (Tans–Kersten et al., 2001; Herrera et al., 2008; Jahn et al., 2008). Much biofilm research has concentrated on specific groups of microorganisms with emphasis on bacteria. These studies emphasize the importance of biofilms in the survival and colonization of leaves by both damaging and potentially beneficial microbe communities under unfavorable conditions.

**Quorum sensing in leaf microbiota**

Microbial colonization to plants is regulated by the density-dependent QS phenomenon, a strategy to survive in the challenging leaf habitat. QS mechanisms involve intra- and also interspecies bacterial communication to share information and regulate their physiological activities and coordinate gene expression of factors such as motility, biofilm, host colonization, and virulence (Ng and Bassler, 2009; Elias and Banin, 2012). Different bacterial groups synthesize and use particular chemical signals or QS molecules for communication. For example, Gram-negative bacteria employ N-acyl-l-homoserine lactone [AHL; also called autoinducer-1 (AI-1)] and quinolones as QS molecules, whereas modified oligopeptides (autoinducer peptides, AIPs) are commonly used by Gram-positive bacteria for communication between cells (Taga and Bassler, 2003). Other autoinducers belonging to boron furan-derived QS molecules or AI-2 are specifically for interspecies communication (Federle, 2009). Diffusible signal factor (DSF) is another family of conserved QS signals utilized for the regulation of virulence factor in numerous Gram-negative bacterial pathogens (Li et al., 2019).

Interestingly, these QS molecules can also be recognized by cells of eukaryotes, including plants and fungi (Dudler and Eberl, 2006; Irie and Parsek, 2008). QS signal information during initial leaf surface colonization is highly localized and the quorum area can be as low as 10 cells (Gantner et al., 2006; Dulla and Lindow, 2008). AHL QS signaling molecules occur naturally in the leaf environment and might impact leaf–bacterium interactions (Enya et al., 2007). Lv et al. (2012) screened a number of AHLs produced by Gram-negative *Proteobacteria* as QS signals in the tobacco phyllosphere and monitored bacterial community composition. Here, *Pseudomonas* and other AHL-producing *Gamma*-proteobacteria were found to use QS signals for survival and protection against other epiphytic members in the nutrient-limited phyllosphere environment. It is therefore likely that AHL QS signaling can also limit pathogenic microbes on leaves. On the other hand, in some phytopathogens, intraspecies QS was studied; for instance in *Xanthomonas* associated with grapevines, QS molecules control the expression of virulence factor as well as biofilm formation (Danhorn and Fuqua, 2007). For *Pseudomonas syringae* in tobacco and bean interaction, QS mediated control of motility and exopolysaccharide synthesis was observed for their role in biofilm formation and colonization of bacteria on leaf (Quiñones et al., 2005).

In this section, we covered microbial community composition and diversity, and colonization, survival, and adaptation in a leaf habitat. There are still significant gaps in knowledge of the types of microbial interaction, and mechanisms of competition and cooperation between leaf microbiota members, that facilitate microbial community stability and structure.

**Microbe–microbe–host interactions**

**Communication between pathogenic microbes**

Infection by pathogens can have a significant impact on the resident leaf microbial community. For example, severe SLB disease was correlated with reduced species richness in the epiphytic bacterial population of maize (Manching et al., 2014). Pathogenic microbes can also increase the susceptibility of their host plant to colonization by other microbes, which would not normally be invasive. For example, *Albugo candida* (white rust) enhanced susceptibility of various *Brassicaceae* species to fungal mildew pathogens (Cooper et al., 2002, 2008). In turn, white rust disease symptoms caused by *A. candida* in *Brassica juncea* were elevated by subsequent inoculation with the downy mildew pathogen *Hyaloperonospora parasitica*, which normally colonizes *B. juncea* asymptptomatically and thereby increases its susceptibility towards white rust (Kaur et al., 2011). A similar mutual infectivity relationship was found in *A. thalana*, in which an adapted oomycete pathogen, *Albugo laibachii*, induced susceptibility to the non-host pathogen *Phytophthora infestans* (Belhaj et al., 2017). Therefore, *Albigo* infections in *Brassicaceae* in some way promote a host jump by certain pathogens (Thines, 2014).

While several reports highlight the importance of interspecies microbial communication in disease development, it should be emphasized that different cells of a single pathogenic microorganism behave distinctly to orchestrate successful colonization of the host. This was shown for hyphal cells of the fungal pathogen *Sclerotinia sclerotiorum*, which have differential gene expression patterns and metabolic heterogeneity during successful colonization of host plants (Peyraud et al., 2019).

QS between pathogenic microbes leads to an increase in virulence and pathogenicity in the host plant. QS-based systems of the *Pseudomonas savastanoi* pv. *savastanoi* (olive knot pathogen) and *Erwinia toletana* (olive knot cooperator) stabilize the community and exchange QS signals, and this cooperation results in a more aggressive disease on olive plants (*Olea europaea*) (Cabalino et al., 2018). An intriguing example of QS mediating pathogen infection in eukaryotes was reported for the oomycete pathogen *Phytophthora nicotiana*. 
Zoopore-derived extracellular fluids contain QS components which induced zoospore aggregation that increased pathogen infectivity (Kong et al., 2010).

**Interactions of foliar pathogens with endophytes**

Endophytes are microorganisms which colonize the internal organs of the plant without causing visible symptoms. In several cases, endophytic microbes were reported to impact plant stress protection and development. Plant pathogens can be inhibited by a number of mechanisms, for example hyperparasitism, competition, and/or antibiosis (Busby et al., 2016). Fungal endophytes promoted induction of phenolic compounds in perennial ryegrass, thereby providing resistance against pathogenic growth (Païka et al., 2013). Direct interactions are also observed between endophytes and pathogens. Fungal endophytes of oak tree were found to be possible antagonists of *Erysiphe alphioides*, the causal agent of powdery mildew disease (Jakuschkin et al., 2016). *Metarhizium robertsi* colonizes insect larvae present in the plant root tissue and transfers nutrients from the insect to the host (Branine et al., 2019). Biosynthetic gene clusters including non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes were identified in endophytes (Miller et al., 2012; Ludlow et al., 2019), which might contribute to their biocontrol potential.

Busby et al. (2015) reported that disease modification is an ecological function shared by common foliar fungi of *Populus trichocarpa*. Species of *Cladosporium* and *Trichoderma* were identified to be antagonists of *Melampsora* rust pathogen in wild *P. trichocarpa* populations. These results differ from previous studies by Raghavendra and Newcombe (2013) where Stachybrotys sp., *Trichoderma atroviride*, Ulocladium atrum, and *Truncatella angustata* have been reported to induce quantitative disease resistance in *P. trichocarpa* against *Melampsora* rust pathogen under controlled experimental conditions. On the contrary, the above fungi were found to be quite rare in wild *P. trichocarpa* (Busby et al., 2015) and this hints at the disparity between disease-modifying action of foliar fungi under wild and experimental conditions.

Endophytes utilize QS to act against pathogenic microbes by expressing QS inhibitors (QSIs) to attenuate the activity of AIs, or quorum quenching (QQ) enzymes to disrupt signaling molecules. For example, AHL lactonase enzyme (a potent quorum quencher) present in endophytic bacteria has been reported to inhibit the plant pathogens *Envinia carotovora* (Dong et al., 2000, 2001), *Bacillus* sp., subspecies of *Bacillus thuringiensis* (Lee et al., 2002; Ulrich, 2004), and *Enterobacter asburiae* (Rajesh and Ravishankar Rai, 2014). Ma et al. (2013) explored the diversity of tobacco (*Nicotiana tabacum*) leaf-associated strains with QQ activity for disruption of AHL-mediated QS, by using the biosensor reference strain *Chromobacterium violaceum* CV1026. These bacterial quorum quenchers can be used as effective biocontrol agents against plant pathogens (Ma et al., 2013). More research is needed to understand how these interactive chemical processes impact plant microbiota community structure and function on plant hosts, and their consequences for plant health.

**Microbial succession in host interactions**

There is a constant struggle between different microorganisms residing inside the plant for nutrients, space, and survival. In this arena, the order of arrival of microbes can be a decisive factor between host disease resistance and facilitation. In planta experiments on *Phaseolus lunatus* have shown that, if a pathogen was introduced to the plant on the same day or before inoculation with an endophyte, disease resistance was more strongly reduced than when the endophyte had already colonized the host (Adame-Alvarez et al., 2014).

The situation is reversed in the case of the biotrophic maize smut fungus *Ustilago maydis* which is inhibited by the endophyte *Fusarium verticillioides* when both organisms are co-inoculated to the plant. Pre-inoculation with the endophyte had no impact on disease severity, whereas post-inoculation caused greater disease progression and decreased plant growth (Lee et al., 2009). This result suggests that *F. verticillioides* can inhibit *U. maydis* by direct interaction and not by induction of host defense responses. In line with this, the presence of *U. maydis* does not result in a significant difference in the diversity of the endophytic community, causing small localized differences in the community structure because of infection (Pan et al., 2008). Also, variation of the endophytic community does not correlate with levels of resistance to *U. maydis* in different maize lines (Pan et al., 2008).

**Microbial lifestyle in host interactions**

While so far there is no direct evidence for modulation of *U. maydis* infection with endophytic microbes, the group of smut fungi themselves represents an interesting example of organisms which exhibit different lifestyles in different niches. Generally, basidiomycete yeasts are present abundantly in the leaf microbial community of *A. thaliana*, along with other endophytic bacteria, as well as oomycetes (Agler et al., 2016). The anamorphic yeast *Moesziomyces albigenus* was recently found to antagonize *Albugo laibachii* infection and reduce disease development on plants (Etzen et al., 2020, Preprint). This happens to differ from previous studies by Agler et al. (2016), where the presence of the basidiomycete yeast, *Dioszegia* sp., was positively correlated with the oomycete, *A. laibachii*.

*Moesziomyces* sp. (classified as *Pseudozyma* sp. until phylogenetic reconstruction by Wang et al., 2015) belong to the order of *Ustilaginales*, and have been reported to act as biocontrol agents in a number of cases (Barda et al., 2014; Gafni et al., 2015). Comparative transcriptomics identified a secreted hydrolase of *M. albigenus* being induced on the Arabidopsis leaf surface in the presence of *A. laibachii*, and reverse genetics demonstrated...
that the antagonism of M. albugensis towards A. laibachii depends on the expression of this enzyme (Eitzen et al., 2020, Preprint).

Such insights into the functional basis of microbial interactions of the Ustilaginales, where members of the same species can either be plant pathogens or beneficial epiphytes, show us that there might be no clearly demarcated barrier between organisms which behave as a pathogen and a plant-protecting microbe (i.e. a pathogen’s antagonist). Similarly, different strains of the pathogenic fungus Fusarium oxysporum can act as microbial antagonists against other F. oxysporum strains (van Dam et al., 2016). The differences in their lifestyle have traced back to the effector repertoire, with the epi-/endophytic strains having fewer or no host-specific effectors (de Lamo and Takken, 2020). Moeziomyces sp., however, encodes a fully equipped set of effector genes (Eitzen et al., 2020, Preprint), including a functional homolog of the U. maydis core virulence effector Pep1 (Sharma et al., 2019). This evidence suggests that anamorphic Ustilaginales yeasts have the potential to form infectious filamentous structures (Kruse et al., 2017) and at the same time raise the question of which factors drive the adaptation of these organisms to either a pathogenic or an epiphytic lifestyle.

Knowledge of the roles of microbe–microbe–host interactions in determining microbial invasiveness will aid understanding of the cross-domain interactions in pathogenicity. Nevertheless, more fundamental research is needed to disentangle microbe–microbe and microbe–host interactions at the level of individual strains to determine what underpins functional microbial assemblies in nature.

Role of the plant immune system in shaping the leaf microbiome

The plant innate immune system comprises a large repertoire of plasma membrane-localized (surface) and intracellular receptors which recognize microbial or modified host molecular signatures and retain plant health and secure plant propagation. Surface immune receptors (often referred to as pattern recognition receptors, or PRRs) are members of a diverse family of ligand-binding proteins that sense microbial, environmental, developmental, and nutritional cues (Saijo et al., 2018; Cheng et al., 2019). In terms of shaping microbial communities, it is the PRR activities that are thought to gate microbial entry into leaf tissues, and effectively ward off colonization by host non-adapted strains (Boutrot and Zipfel, 2017). The intracellular receptor panels [consisting mostly of nucleotide-binding/leucine-rich repeat (NLR) proteins] are similarly diverse and are selected as triggers of strain-specific resistance to host-adapted pathogens (Eitas and Dangl, 2010; Jones et al., 2016; Wu et al., 2017; Burdett et al., 2019; de Weyer et al., 2019).

The activation of plant immune responses by mobilizing a network of defense and stress hormone pathways has been extensively characterized in binary plant–pathogen interactions (Noman et al., 2019; Zhao et al., 2019). Little is known about the impact of plant immunity signaling networks on host–microbe interactions in leaf microbi communities (Fig. 1D). High-throughput DNA and RNA sequencing of leaf samples from natural environments have enabled examination of complex microbial communities in plant-specific niches in time and space (Agler et al., 2016). Analysis of microbial metadata and their integration with experimental testing should provide a clearer picture of the role of plant immunity signaling in shaping leaf microbial community structure and, in turn, how resident microbes influence host immunity.

In this section, we consider evidence that abiotic and biotic stress responses modulate microbial consortia on leaves and discuss the consequences for plant fitness. It is becoming clear that microbial community structure throughout a plant host’s life cycle is dynamic and modulated by the innate immune system, which itself is tuned to environmental changes.

The role of pattern-triggered immunity in shaping the leaf microbiota

Most microorganisms on plant leaves are non-pathogenic. However, a broad range of microbes are able to prime innate plant immunity to counter subsequent pathogen attacks (Ritpittakphong et al., 2016; Vogel et al., 2016). Many microbes are recognized by terrestrial plants through their MAMPs initiating pattern-triggered immunity (PTI) responses. PTI is an induced and often low-level but broadly effective resistance response involving phytohormone signaling, secretion of antimicrobial compounds, generation of ROS and mitogen-activated proteinc (MAP) kinase cascades, and stomatal closure (Bigard et al., 2015; Bi and Zhou, 2017). Notably, the phytohormone ethylene is required for ROS production in PTI, for example in Arabidopsis resistance to P. syringae bacteria and rice resistance to the rice blast fungus, Magnapothe oryzae (Mersmann et al., 2010; Guan et al., 2015; Helliwell et al., 2016; Yang et al., 2017). In Arabidopsis, an ethylene-insensitive2 (ein2) mutant displayed an altered bacterial leaf community compared with wild-type plants, suggesting that ethylene signaling is important for modulating the leaf microbiota (Bodenhausen et al., 2014; Nascimento et al., 2018).

A recent study by Chen et al. (2020) provided experimental evidence that PTI signaling controls the diversity of endophytic leaf microbiota in microorganism-rich environments. An Arabidopsis quadruple mutant [min7 fbs2 efr cerk1 (mfec)] that is defective in PTI and the MIN7 vesicle trafficking pathway (affecting the aqueous apoplastic microenvironment) and a constitutively activated cell death1 (cad1) mutant had altered endophytic bacterial leaf diversity (Chen et al., 2020). In particular, the relative abundance of the bacterial phyla Firmicutes was significantly reduced, whereas Proteobacteria became the dominating bacterial community members in the mutant
plants. The occurrence of PTI components MIN7 and CAD1 across major plant lineages suggests that a number of common pathways might govern endophytic microbial proliferation of certain taxa in leaves.

Further research has revealed the importance of resident *Pseudomonas* sp. (*Proteobacteria*) in protecting Arabidopsis against infection by a fungal necrotrophic pathogen, *B. cinerea* (*Ritpitakphong et al., 2016*). Notably, prominent bacterial clades from soil microbiota such as filamentous *Actinobacteria* (*Streptomycetes* sp.) are able to activate plant biosynthesis of salicylic acid (SA) and promote leaf defense responses against fungal pathogens (*Vergnes et al., 2020*). These findings highlight...
actions of soil-borne microbial inocula of leaves on immunity (Bakker et al., 2013; Haney et al., 2018; Vannier et al., 2019).

The above studies emphasize the role of both commensal and pathogenic microbes in priming PTI as a barrier to colonization of the leaf compartment by host non- or poorly adapted pathogens. Nevertheless, these host–microbe interactions were examined mostly under controlled laboratory conditions. Further research is needed to gain an understanding of how PTI shapes plant immune responses and microbiota communities in nature.

Leaf effector-triggered immunity as a potential microbial gateway

Strain-specific resistance, known as effector–triggered immunity (ETI), is often mediated by intracellular NLR receptors which recognize certain pathogen-delivered virulence factors (effectors) to induce immunity (Monteiro and Nishimura, 2018; Seong et al., 2019; Feehan et al., 2020). Pathogen effector-activated NLRs accelerate and amplify many PTI responses, often resulting in host-localized cell death (a hypersensitive response) and rapid pathogen containment (Peng et al., 2018). Expressed NLR genes in roots are observed in dicot plant species such as the legume Lotus (Lai and Eulgem, 2018). This in contrast to tested Brassicaceae species including A. thaliana and the crop oilseed rape (Brassica napus), which favor NLR expression in the phyllosphere (Munch et al., 2018). Although NLR activation and downstream signaling mechanisms are becoming resolved, the extent to which this layer of protection against pathogens shapes plant microbial communities is hardly understood.

Diverse microbial communities in leaves can be controlled directly through pathogen colonization on the host or indirectly by host–microbe interactions involving the innate immunity network (Agler et al., 2016). Thus, pathogenic microbes can act as highly interconnected community members (so-called ‘hub microbes’) that dominate microbial community assemblies. For example, the causal agent of white rust on Arabidopsis, Albugo sp., appears to act as a hub which alters epiphytic and endophytic bacterial colonization of leaves (Agler et al., 2016; Ruhe et al., 2016). Perturbations of microbial communities by host-adapted biotrophic pathogens such as Albugo and Hyaloperonospora arabidopsidis (Hpa) reduce microbial diversity within leaf habitats and stabilize microbial communities among wild plants (Karasov et al., 2019, Preprint). Hence, microbial diversity can be used as an indicator for microbial community imbalance (Chen et al., 2020).

Whether ETI reactions directly lead to defense priming is not well studied, although in Arabidopsis one important ETI branch leads to a reinforcement and spread of pathogen resistance (so-called basal immunity) in leaf tissues (Lapin et al., 2020). A recent study by Levy et al. (2018) analyzed >3800 genomes of plant-associated (pathogenic and non-pathogenic) bacteria. The analysis identified plant-mimicking protein domains (named PREPARADOS) that carry non-canonical ‘embedded’ NLR domains. An increasing number of NLR-fused domains are related to authentic effector targets. PREPARADOS are highly abundant in the bacterial families Bacteroides and Xanthomonadaceae (Frank, 2019). These findings point to potential interactions between commensal and or pathogenic bacteria with intracellular receptors in host plants. Additional studies are needed to test this hypothesis and dissect functional relationships between NLR panels and the leaf microbiota.

Stability of microbial consortia against pathogen perturbation

The plant and its associated microbiota is not a static environment but is altered by numerous factors including host genotype, environmental fluctuations, surrounding macro- and microorganisms, and geographical location and associated local variables such as climate (Laforest-Lapointe et al., 2016; Poudel et al., 2016; Wagner et al., 2016; Singh et al., 2018). The stability of a leaf microbial community is measured as the ability to maintain a stable equilibrium state (homeostasis) under biotic or abiotic perturbations (Thébault and Fontaine, 2010). Generally, higher community complexity in a network reflects a more stable community structure (Mougi and Kondoh, 2012). Stable microbial communities or consortia have greater ability to resist perturbation (Ives et al., 2000; Luo et al., 2019; Morella et al., 2020). Studies using culture-independent DNA sequencing revealed similar microbial community patterns in successive year samplings (Copeland et al., 2015). In the phyllosphere, microbial communities can often undergo drastic changes and establish a distinctive and less diverse community (Manching et al., 2014; Copeland et al., 2015). Different computational and experiment-based approaches have been used to capture microbial community homeostasis or deviations over time. Computational microbial network analysis and mining of core microbes are valuable in understanding the factors underlying microbial resilience to controlled perturbations (Astudillo-García et al., 2017; Lemanceau et al., 2017). Much less is known about the dynamics and stability of leaf microorganisms in the field since there is a lack of high-resolution experimental data linked to plant disease and health with respect to time, space, and environmental scale. In recent studies, leaf diseases were linked to disruption of microbial community network stability, resulting in ecosystem dysfunc tion (Kerdraon et al., 2019; Luo et al., 2019; Leopold and Busby, 2020, Preprint). Understanding how a microbial community corrects itself under conditions of environmental stress is crucial to harness its potential in probiotic applications against aggressive plant pathogens and to track plant-associated human pathogen outbreaks.

Does immunity priming affect microbial leaf communities?

Various abiotic and biotic factors impact dynamic changes on microbial leaf communities as depicted in the modes of
### Table 1. Summary of important studies associated with the leaf microbiome

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<th>Host plant</th>
<th>Leaf microbiota/leaf microbe under study</th>
<th>Perturbation</th>
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<td><strong>Microbial colonization</strong></td>
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<tr>
<td>Arabidopsis thaliana</td>
<td>Bacteria</td>
<td>–</td>
<td>Phyllosphere community profile of <em>A. thaliana</em> wild-type Landsberg erecta (Ler) and ecerinum (cer) mutants (cer1, cer6, cer9, and cer16) involved in cuticle biosynthesis. Plant cuticular wax composition affects the phyllosphere bacterial community.</td>
<td>Reisberg et al. (2013)</td>
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<tr>
<td>Faba bean (Vicia faba L.) and Arabidopsis thaliana</td>
<td>Pseudomonas syringae DC3118, a coronatine-deficient mutant of <em>Pseudomonas syringae</em> DC3000</td>
<td>–</td>
<td>In a specific environmental setting, leaf surface colonization by bacteria correlated with stomatal aperture regulation.</td>
<td>Ou et al. (2014)</td>
</tr>
<tr>
<td>Bean (Phaseolus vulgaris L.)</td>
<td><em>P. syringae</em> pv. syringae B728a</td>
<td>–</td>
<td>Biosurfactant, syringafactin, produced by <em>P. syringae</em> pv. syringae B728a on leaves adsorbed on waxy leaf cuticle surface. Provide benefit to bacteria by attracting moisture and aid in nutrient availability.</td>
<td>Burch et al. (2014)</td>
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<tr>
<td>Arabidopsis thaliana</td>
<td><em>Pseudomonas syringae</em> DC3000</td>
<td>–</td>
<td>Humidity-controlled, pathogen-guided establishment of an aqueous intercellular space (apoplast) as an important step in leaf bacterial infection.</td>
<td>Xin et al. (2016)</td>
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<td><strong>Microbial composition and diversity</strong></td>
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<tr>
<td>Sugar beet (<em>Beta vulgaris</em>)</td>
<td>Bacteria, yeasts, and filamentous fungi</td>
<td>–</td>
<td>Seasonal dynamics over a growing season. Fungi: <em>Cladosporium</em> and <em>Alternaria</em> sp. Yeast: <em>Cryptococcus</em> and <em>Sporobolomyces</em> <em>Bacteria: Pseudomonas</em> sp. and <em>Enxinia herbicola</em></td>
<td>Thompson et al. (1993)</td>
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<td>Loblolly pine (<em>Pinus taeda</em>)</td>
<td>Fungi (endophytes)</td>
<td>–</td>
<td>High diversity of foliar fungal endophytes.</td>
<td>Arnold et al. (2007)</td>
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<tr>
<td>Arabidopsis thaliana, <em>Trifolium repens</em>, and <em>Glycine max</em></td>
<td>Bacteria</td>
<td>–</td>
<td>Metaproteogenomic analysis found consistency in three plant species.</td>
<td>Delmotte et al. (2009)</td>
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<td></td>
<td></td>
<td></td>
<td>High abundance of <em>Sphingomonas</em> sp. and <em>Methylobacterium</em> sp. Important role of the one-carbon metabolism and transport processes in the microbiota.</td>
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<tr>
<td>Tree species</td>
<td>Bacteria (epiphytes)</td>
<td>–</td>
<td>In trees, interspecies variation is more than intraspecies variation in bacterial communities. Correlation between tree phylogeny and bacterial community composition.</td>
<td>Redford et al. (2010)</td>
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<td>Maize</td>
<td>Bacteria (epiphytes)</td>
<td>Southern leaf blight (SLB)</td>
<td>A specific set of epiphytic bacteria can restrict phyllosphere bacterial diversity and increase resistance to Southern leaf blight (SLB) fungal infection.</td>
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<td>Eucalyptus citriodora Hook</td>
<td>Fungi (epiphytes and edophytes)</td>
<td>–</td>
<td>Total 33 fungal species assigned to 33 taxa (endophytes, 20; epiphytes, 22). Difference in frequency of colonization. Antagonism against human and plant pathogen.</td>
<td>Kharwar et al. (2010)</td>
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<td>Lettuce</td>
<td>Bacteria</td>
<td>–</td>
<td>Bacterial community composition by pyrosequencing. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria—most abundant phyla. Insights on variability in bacterial community profile with respect to time, space, and environment.</td>
<td>Rastogi et al. (2012)</td>
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<tr>
<td><strong>Tobacco (Nicotiana L.)</strong></td>
<td>Bacteria (endophytes)</td>
<td>–</td>
<td>158 culturable endophytic bacteria. Phyla distribution 36.7% Proteobacteria, 32.9% Firmicutes, 29.7% Actinobacteria, and 0.6% Bacteroidetes</td>
<td>de Oliveira Costa et al. (2012)</td>
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<tr>
<td><strong>Arabidopsis thaliana</strong></td>
<td>Bacteria (epiphytes and endophytes)</td>
<td>–</td>
<td>Proteobacteria, Actinobacteria, and Bacteroidetes were found most abundant. Massilia and Flavobacterium are prevalent genera</td>
<td>Bodenerhausen et al. (2013)</td>
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<tr>
<td><strong>Tomato (Solanum lycopersicum L.)</strong></td>
<td>Bacteria (epiphytes)</td>
<td>–</td>
<td>Members of endophytic bacterial communities of tomato leaves exert multiple effects on growth and health of tomato plants.</td>
<td>Romero et al. (2014)</td>
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<td><strong>Rice (Oryza sativa L.)</strong></td>
<td>Actinomycetes (syn. Magnaporthe oryzae)</td>
<td>Pyricularia oryzae</td>
<td>Rice phyllosphere-associated actinomycetes produce bioactive compounds and control leaf blast disease caused by <em>Pyricularia oryzae</em>.</td>
<td>Harsonowati et al. (2017)</td>
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<tr>
<td><strong>Sugar maple (Acer saccharum)</strong></td>
<td>Fungi (epiphytes and endophytes)</td>
<td>–</td>
<td>Microbial communities at the edge of the species’ elevational range differ from those within the natural range.</td>
<td>Wallace et al. (2018)</td>
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<td><strong>Poplar tree</strong></td>
<td>Fungi</td>
<td>Mercury</td>
<td>Methylobacterium, Kineococcus, Sphingomonas, and Hymenobacter on the leaf surface.</td>
<td>Durand et al. (2018)</td>
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<td><strong>Mussaenda pubescens var. alba</strong></td>
<td>Bacteria (endophytes)</td>
<td>–</td>
<td>Dothideomycetes and Eurotiomycetes are dominant members. Intraspecific host genetic identity, primary driver in shaping regional phyllosphere fungal communities.</td>
<td>Qian et al. (2018)</td>
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<tr>
<td><strong>Arabidopsis thaliana</strong></td>
<td>Bacteria (epiphytes)</td>
<td>–</td>
<td>Determined biosynthetic potential of 224 bacterial strains from <em>Arabidopsis</em> leaf microbiome. Phyllosphere as a valuable resource for the identification and characterization of antibiotics and natural products.</td>
<td>Heflich et al. (2018)</td>
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<td><strong>Tomato (Solanum lycopersicum L.)</strong></td>
<td>Fungi (epiphytes and endophytes)</td>
<td>–</td>
<td>Comprehensive view of the tomato-associated bacterial community. Isolation of beneficial bacterial for future functional studies.</td>
<td>Dong et al. (2019)</td>
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<td><strong>Mangrove</strong></td>
<td>Fungi (epiphytes and endophytes)</td>
<td>–</td>
<td><em>Dothideomycetes</em> and <em>Tremellomycetes</em> are dominant members. Plant identity significantly affects endophytic but not epiphytic fungi.</td>
<td>Yao et al. (2019)</td>
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<td><strong>Catharanthus roseus</strong></td>
<td>Fungi (Endophytes)</td>
<td>–</td>
<td><em>Clotetotrichium</em>, <em>Alternaria</em>, and <em>Chaetomorpha</em> are common genera.</td>
<td>Dhayanithy et al. (2019)</td>
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<td><strong>Biofilm</strong></td>
<td>P. syringae pv. syringae</td>
<td>–</td>
<td>Cause of brown spot disease of bean leaves was the result of biofilm formation of <em>P. syringae</em>.</td>
<td>Monier and Lindow (2004)</td>
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<td><strong>Citrus limon ‘Eureka’</strong></td>
<td>Xanthomonas axonopodis pv. citri</td>
<td>–</td>
<td>Motility and role of flagellum is required for mature biofilm and canker development.</td>
<td>Malamud et al. (2011)</td>
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<td><strong>Tomato (Solanum lycopersicum L.)</strong></td>
<td>Xanthomonas vesicatoria</td>
<td>–</td>
<td>Aggressiveness of Xv strains correlated with their ability to move by flagella or type IV pili, adherence to leaves and form well-developed biofilms, help in improved phyllosphere colonization.</td>
<td>Felipe et al. (2018)</td>
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<td><strong>Tomato (Solanum lycopersicum L.)</strong></td>
<td>Bacillus amyloliquefaciens</td>
<td>Botrytis cinerea</td>
<td>Reduction of biocontrol of BBC 023 on leaves due to its limited ability to generate robust biofilms and colonization in the phylloplane.</td>
<td>Salvatierra-Martinez et al. (2018)</td>
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<td><strong>Quorum sensing</strong></td>
<td>Bacteria</td>
<td>–</td>
<td>Culturable leaf-associated bacteria community with BCA activity against tomato disease have the ability to produce AHL and IAA. AHLs induced variation in the bacterial community composition.</td>
<td>Enya et al. (2007)</td>
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<td><strong>Tobacco (Nicotiana tabacum)</strong></td>
<td>Epiphytes</td>
<td>–</td>
<td>Pseudomonas and other AHL-producing Gammaproteobacteria use QS signals for their survival and protection.</td>
<td>Lv et al. (2012)</td>
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<th>Host plant</th>
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<th>Perturbation</th>
<th>Key findings</th>
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<td>Tobacco (Nicotiana tabacum), common bean (Phaseolus vulgaris)</td>
<td>Pseudomonas syringae</td>
<td>–</td>
<td>QS-mediated control of motility and exopolysaccharide synthesis was observed for their role in biofilm formation and colonization of bacteria on leaf.</td>
<td>Quiñones et al. (2005)</td>
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<td>Quercus robur L.</td>
<td>Foliage fungi and bacteria</td>
<td>Erysiphe alphitoides</td>
<td>Direct interaction between E. alphitoides and 13 fungal and bacterial operational taxonomic units (OTUs). Fungal endophytes Mycosphaerella punctiformis and Monochaetia kansensis could be possible antagonists of E. alphitoides.</td>
<td>Jakuschkin et al. (2016)</td>
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<td>Phaseolus lunatus</td>
<td>Endophytic fungi for e.g. Rhizopus, Fusarium, Penicillium, Cochliobolus, and Artomyces spp.</td>
<td>Pseudomonas syringae pv. syringae, Enterobacter sp. strain FCB1, and the fungus Colletotrichum lindemuthianum</td>
<td>Order of arrival of fungal endophytes and pathogens on the plant surface can determine disease resistance or facilitation.</td>
<td>Adame-Alvarez et al. (2014)</td>
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<td>Zea mays</td>
<td>Endophyte Fusarium verticillioides</td>
<td>Ustilago maydis</td>
<td>F. verticillioides can inhibit U. maydis disease progression by direct interaction.</td>
<td>Lee et al. (2009)</td>
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<td>Olive plants (Olea europaea)</td>
<td>Pseudomonas savastanoi pv. savastanoi (olive knot pathogen) and Envinia toletana (olive knot cooperator).</td>
<td></td>
<td>The bacteria stabilize the community, exchange QS signals, and this cooperation results in disease aggression.</td>
<td>Caballo-Ponce et al. (2018)</td>
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<td>Arabidopsis thaliana</td>
<td>Basidiomycete yeast, Dioszegia sp.</td>
<td>Albugo laibachii</td>
<td>Construction of an extensive phyllosphere microbial network encompassing bacterial, fungal, and oomycetal communities. Presence of Dioszegia sp. is positively correlated with that of A. laibachii.</td>
<td>Agler et al. (2016)</td>
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<td>Arabidopsis thaliana</td>
<td>Basidiomycete yeast, Moeziomyces albigenis</td>
<td>Albugo laibachii</td>
<td>Moeziomyces albigenis antagonizes A. laibachii on the host leaf surface.</td>
<td>Eitzen et al. (2020)</td>
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<td>Innate immunity interaction</td>
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<td>Arabidopsis thaliana</td>
<td>Bacteria</td>
<td>–</td>
<td>The author showed evidence of ethylene signaling (ein2) affecting the abundance of Variovorax.</td>
<td>Bodenhausen et al., (2014)</td>
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<tr>
<td>Arabidopsis thaliana</td>
<td>Bacteria</td>
<td>–</td>
<td>Affected diversity of Firmicutes sp. and Proteobacteria sp. in min7 fts2 eff cerk1 (mfec) and constitutively activated cell death1 (cat1) mutants (involving PTI, MIN7 vesicle trafficking, or cell death pathways).</td>
<td>Chen et al. (2020)</td>
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<td>Arabidopsis thaliana</td>
<td>Streptomyces AgN23. Alternaria brassicicola</td>
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<td>The bacteria Streptomyces induces defense responses, which prevents Alternaria infection.</td>
<td>Vergnes et al. (2020)</td>
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</tbody>
</table>
microbial colonization, microbe–microbe, and microbe–host interactions (see Fig. 1 and Table 1). Nevertheless, fundamental mechanisms of microbial community assembly remain barely understood. One major goal of current microbiome research is to understand how microbial consortia in nature secure plant protection during pathogen perturbation. Immunity priming (IP) effects through abiotic (applied chemical compounds) and biotic (biocontrol agents) stimuli seem to play an important role in managing abiotic stress tolerance and disease resistance (Kumar and Verma, 2018). IP has been described as a ‘positive cost–benefit balance in times of stress’ (Martinez-Medina et al., 2016). Another interesting IP compound, (R)-β-homoserine (RBH), primes ethylene and JA pathways and is effective against necrotrophic pathogens such as Botrytis cinerea in tomato (RBH), primes ethylene and JA pathways and is effective against necrotrophic pathogens such as Botrytis cinerea in tomato (Pst) to Arabidopsis roots attracted Bacillus subtilis and led to IP upon Pst infection (Rudrappa et al., 2008; Vannier et al., 2019). In contrast, applying P. syringae pathovar tomato (Pst) to Arabidopsis roots attracted Bacillus subtilis and led to IP upon Pst infection (Rudrappa et al., 2008; Vannier et al., 2019). The ecological impact of BCAs on the leaf microbiome while controlling disease resistance remains an open research question. Current reports emphasize a linkage between certain bacterial taxa (Bacillus, Pantoea, Sphingomonas, Pseudomonas, and Trichoderma) affecting microbial diversity (Zhang et al., 2008; Bruisson et al., 2019; Ulrich et al., 2014; Ritpitakphong et al., 2016; Qin et al., 2019). In particular, highly diverse leaf communities are negatively correlated with pathogen invasion and colonization, and vice versa (Purahong et al., 2018; Qin et al., 2019). Other reports describe difficulties encountered in the application of biocontrol agents such as B. subtilis, which did not alter the microbial leaf community under rainy field conditions (Wei et al., 2016). Thus, use of biocontrol agents under natural conditions might be challenging and require further analysis. However, BCAs and IP-inducing compounds can potentially be used to monitor disease control to improve crop yield and production in new biological breeding strategies. There is clearly a need to increase efforts in this research field to explore the effects and underlying mechanisms of abiotic and biotic stress on IP and how they are transmitted to microbial leaf communities.

## Table 1. Continued

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Leaf microbiota/leaf microbe under study</th>
<th>Perturbation</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (Solanum lycopersicum, Solanum pimpinellifolium)</td>
<td>Bacteria</td>
<td>–</td>
<td>Host resistance shapes leaf microbiota under environmental fluctuations and is time dependent.</td>
<td>Morella et al. (2020)</td>
</tr>
<tr>
<td>Cucumber Cucumis sativus (Suyan 10)</td>
<td>Bacteria and fungi</td>
<td>Pseudomonas syringae pv. Lachrymans</td>
<td>Plant-specific microbes such as Sphingomonas, Methylbacterium, Pseudomonas, and Alternaria are significantly affected by the causal agent of angular leaf-spot of cucumber at different infection stages.</td>
<td>Luo et al. (2019)</td>
</tr>
<tr>
<td>Pepper (Capsicum annuum L.)</td>
<td>Bacillus thuringiensis</td>
<td>–</td>
<td>Significant changes of phyllosphere microbiota in Firmicutes and Gammaproteobacteria.</td>
<td>Zhang et al. (2008)</td>
</tr>
<tr>
<td>Grapevine (Vitis vinifera)</td>
<td>Bacteria</td>
<td>Botrytis cinerea, Phytophthora infestans</td>
<td>Potential biocontrol agents (Bacillus, Variovorax, Pantoea, Staphylococcus, Herbaspirillum, Sphingomonas) from leaf microbiome acting against phytopathogens.</td>
<td>Bruisson et al. (2019)</td>
</tr>
<tr>
<td>Wheat (Triticum aestivum)</td>
<td>Bacteria and fungi</td>
<td>Zymoseptoria tritici</td>
<td>Microbial dynamics upon infection.</td>
<td>Kendraoen et al. (2019)</td>
</tr>
<tr>
<td>Tobacco (Nicotiana sp.)</td>
<td>Bacteria</td>
<td>Pseudomonas syringae pv. tabaci</td>
<td>The application of two BCAs changed the bacterial phyllosphere community and decreased bacterial wildfire outbreak.</td>
<td>Qin et al. (2019)</td>
</tr>
</tbody>
</table>

Effects of biocontrol agents (BCAs) on crops such as potato against biotrophic (P. infestans) and grapevine against necrotrophic (B. cinerea) fungi have been studied extensively in vitro (Bailly and Weiszkopf, 2017; De Vrieze et al., 2018; Bruisson et al., 2019). In contrast, applying P. syringae pathovar tomato (Pst) to Arabidopsis roots attracted Bacillus subtilis and led to IP upon Pst infection (Rudrappa et al., 2008; Vannier et al., 2019). The ecological impact of BCAs on the leaf microbiome while controlling disease resistance remains an open research question. Current reports emphasize a linkage between certain bacterial taxa (Bacillus, Pantoea, Sphingomonas, Pseudomonas, and Trichoderma) affecting microbial diversity (Zhang et al., 2008; Bruisson et al., 2019; Ulrich et al., 2020) and IP induction on leaves (Cawoy et al., 2014; Ritpitakphong et al., 2016; Qin et al., 2019). In particular, highly diverse leaf communities are negatively correlated with pathogen invasion and colonization, and vice versa (Purahong et al., 2018; Qin et al., 2019). Other reports describe difficulties encountered in the application of biocontrol agents such as B. subtilis, which did not alter the microbial leaf community under rainy field conditions (Wei et al., 2016). Thus, use of biocontrol agents under natural conditions might be challenging and require further analysis. However, BCAs and IP-inducing compounds can potentially be used to monitor disease control to improve crop yield and production in new biological breeding strategies. There is clearly a need to increase efforts in this research field to explore the effects and underlying mechanisms of abiotic and biotic stress on IP and how they are transmitted to microbial leaf communities.
Conclusion and outlook

The plant phyllosphere is a highly competitive and challenging habitat for microbes to colonize. Pre-formed barriers such as the hydrophobic cuticle, stomata, or cell wall structures require specific adaptation for the microbes, and persistence of microbes strongly depends on their ability to interact with others. Thus understanding microbiota assembly and persistence in the plant phyllosphere requires investigating ecological factors that shape pre-formed plant structures and therefore directly act on host-microbe and indirectly microbe-microbe interactions. Microbe-microbe interactions in turn not only impact microbial behavior but can also impact host fitness by antagonizing plant pathogens. Pathogen invasion generally has a significant negative effect on host fitness caused by tissue damage, nutrient loss to invaders, and reallocation of resources to immune activation. Successful pathogenesis on the other hand is a complex process that requires multiple steps of host colonization and reproduction, and is generally the result of long-term co-evolution (Hall et al., 2017). Considering the enormous gene pool and diversity of all non-pathogenic microbes that are associated with the phyllosphere and other parts of the plant, and considering this pool under constant selection to benefit plant fitness directly or indirectly, we can expect an enormous unexploited pool of beneficial microbes to antagonize pathogenicity processes. In addition, phyllosphere-colonizing microbes are highly adapted to abiotic and biotic fluctuations and are therefore an enormous pool for new adaptive traits.

The downside is, however, that this pool is highly dynamic and probably requires a stable co-existence of different microbial species in one habitat in order to express beneficial traits. Interconnected networks between organisms can be an important element in providing a buffer against perturbations since such links help to recruit microbes to fulfill specific functions in cases where another organism that, for example, provides important antimicrobial compounds or enzymes within the network is lost.

A main goal to develop strategies to protect the phyllosphere from pathogen invasion, such as wheat from rusts, is to identify probiotics that can either stabilize the natural community or become stable on its own. One major effort to develop such probiotics is therefore understanding the multistep process of establishing a niche and defending this niche. Only once we know how to combine traits for stability with our desired traits such as plant protection will we be able to develop products that can replace the majority of our current agrochemicals.

Acknowledgements

VC gratefully acknowledges support from the Alexander von Humboldt Foundation for a Humboldt Research Fellowship (2018–2020). PR acknowledges the financial support from the European Research Council (ERC) under the DeCoCt research program (grant agreement: ERC-2018-COG 820124). PS would like to acknowledge the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy EXC-2048/1, Project ID 390686111, and the DFG priority program SPP2125 ‘DECryPT’, for their financial support. We thank the two anonymous reviewers for their suggestions and constructive comments.

Conflict of interest

The authors declare that they have no conflict of interest.

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