

1 Spotlight

2 **Linking the phyllosphere microbiome to plant health**

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12 **Keywords**

13 phyllosphere; microbiome; plant health; ecosystem; dysbiosis

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15

16 **Abstract**

17 The phyllosphere harbors diverse microbial communities that influence ecosystem functioning.
18 Emerging evidence suggests that plants impaired in genetic networks harbor an altered
19 microbiome and develop dysbiosis in the phyllosphere, which pinpoints plant genetics as a
20 key driver of the phyllosphere microbiome assembly and links the phyllosphere microbiome
21 to plant health.

22

23 **Phyllosphere microbiome at the interface of multitrophic interactions**

24 The phyllosphere refers to the collective microbial environments of all aboveground portions
25 of a plant. These include stems, leaves, flowers (including pollen and nectar) and fruits, and
26 their colonizing epiphytes (surface colonizers) and endophytes (within tissue colonizers). The
27 phyllosphere is an open environment, which is subject to diverse biotic (e.g., pollinator and
28 insect herbivore visitations) and abiotic (e.g., ultraviolet radiation, rainfall and heat) stimuli as
29 well as anthropogenic pressures (e.g., agricultural practices) [1]. This makes the phyllosphere
30 a dynamic and heterogeneous environment. Leaf and stem make up the bulk of the
31 aboveground plant, but their surfaces are often considered relatively oligotrophic
32 environments, as they are being disconnected from soil and subject to rainfall leaching.
33 Evolutionary experiments indicate that the phyllosphere can filter microbes from the broader
34 environments and allow particular phylotypes to colonize on/in the plant [2], which in turn
35 serves host and ecosystem functions [3]. Microbial phylotypes are remarkably consistent at
36 phylum level across plant species and geographic locations and are dominated by
37 Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes (**Figure 1A**) [2].

38

39 The phyllosphere microbiome intimately interacts with the plant and influences multiple
40 processes, from the individual plant level to influencing productivity of the terrestrial
41 ecosystem [3]. The microbes residing in/on pollen and nectar (e.g., *Lactobacillus* spp.) play a
42 noticeable role in plant-pollinator interactions [4]. They can be consumed by pollinators and
43 influence their gut microbiome assembly and health. In turn, healthy pollinators are pivotal to

44 ensure successful pollination and plant productivity [4]. The phyllosphere mediates
45 transmission of beneficial (e.g., *Streptomyces* endophytes) and pathogenic (e.g., viruses and
46 fungal pathogens) microbes between plants via insects, which also have significant
47 consequences for plant health [4]. Within the phyllosphere, the majority of studies have
48 focused on the leaf, which evidently plays a critical role in the diversification and adaptation
49 of the plant, and influences terrestrial ecosystem functions such as productivity and
50 atmospheric nitrogen fixation [5].

51

52 A vast number of microbes (averaging 10^6 - 10^7 cells cm^{-2}) reside on the leaf surface [1]. These
53 can competitively balance the wide leaf surface microbiome, preventing the outgrowth of
54 bacterial and fungal pathogens [1, 6]. However, mechanistic knowledge on their interactions
55 with plant immunity and the consequences for host plant growth and defense are largely
56 unknown. In an era of unprecedented anthropogenic pressure and climate change,
57 understanding the role of the phyllosphere microbiome in plant health can be a novel pathway
58 for enhancing ecosystem health and productivity.

59

60 **Factors governing the phyllosphere microbiome assembly**

61 The main drivers, which may cause synergistic impacts on the microbiome can include three
62 main aspects (**Figure 1A**). (i) Spatial and temporal environments the plant is exposed to. These
63 regulatory factors may be abiotic (e.g. climate, soil type and properties), which affect the
64 physiochemical properties of the leaf, or biotic (e.g. attack by insects and pathogens), which
65 can affect plant immunity and biology, and therefore the plant-associated microbial taxa [1,
66 7]. Insect and pathogen invasions also directly influence the phyllosphere microbiome by
67 introducing additional microbes. (ii) the genetic traits of the plant (genotype and phenotype)
68 [8]. Plant genetic traits mediate leaf tissue chemistry and the lateral surface topology (e.g.,
69 roughness) that influences microbial immigration and emigration. And, (iii) disease-induced
70 changes in the phyllosphere microbiome [9]. Diseased plants may harbor altered leaf microbial
71 communities compared with healthy plants [9], which consequently affect the plant-microbe
72 and microbe-microbe interactions in the phyllosphere. While we know these factors affect the
73 phyllosphere microbiome, it remains a challenge to identify plant exudates *in situ* and the
74 immunity-related regulatory mechanisms for controlling the phyllosphere microbial ecology.

75

76 **Linking the phyllosphere microbiome to plant health**

77 Dysbiosis is a microbial imbalance due to an overgrowth of pathobionts (potentially
78 pathogenic members) and/or loss of microbial diversity, which causally links to potential
79 health issues of animals [10]. A recent study demonstrated that dysbiosis can occur to
80 arabidopsis (*Arabidopsis thaliana*) leaf [11], a phenomenon analogous to that seen in the
81 human gastrointestinal tract environment in inflammatory bowel disease patients. In the study,
82 a quadruple arabidopsis mutant (*min7 fls2 efr cerk1*, abbreviated as *mfec*) that is defective in
83 certain pattern triggered immunity and cell surface component structuring genes assembled a
84 numerically larger (100-fold) but less diverse bacterial community in the leaf endosphere
85 relative to the arabidopsis Col-0 (wild type), and induced disease symptoms (leaf chlorosis
86 and necrosis) in the phyllosphere. Notably, accumulation of the devastating

87 Betaproteobacteria that can inhibit the growth of other species was observed, while the relative
88 abundance of Firmicutes declined in the *mfec* mutant endosphere. No pathogenic species were
89 detected in the *mfec* mutant. Even more interestingly, re-introduction of a synthesized
90 community reconstructed using 52 bacteria isolated from the *mfec* mutant to a new Col-0 host
91 generated the same disease phenotype as the *mfec* mutant. This indicates that the microbiome
92 can transfer a plant phenotype to a new host. However, whether restoration of the reduced
93 Firmicutes can reverse the dysbiosis-associated phenotypes was not investigated in the study
94 [11].

95
96 Overall, this study demonstrated that an impaired genetic network can affect the leaf
97 endophytic microbial composition and the generated microbial variation correlates with leaf
98 phenotypes. Bacterial diversity also seems critical to maintaining plant health, likely due to
99 high bacterial diversity supporting more mutualistic microbial interactions with plant immune
100 system to avoid arising of pathobionts. Similarly, another study found that genes-associated
101 with defense (e.g., ethylene signaling) and cuticle formation also drive the assembly of
102 particular bacterial groups in the leaf [8]. Despite no symptom of dysbiosis being depicted in
103 mutants *lacs2*, *pec1* and *ein2*, distinct microbial community composition and/ or higher
104 bacterial abundance were found in these mutants compared with the wild-type plants. These
105 two studies demonstrate the importance of the plant immune system and genetic networks in
106 the assemblage of health-promoting microbes in the leaf (**Figure 1B**). Without the eukaryotic
107 genes, the phyllosphere microbiome becomes abnormal and a dysbiosis symptom can occur.

108
109 Pattern-triggered immunity and constitutively activated cell death 1 (CAD1) that have been
110 investigated in the study by Chen et al. are broadly employed by plants to cope with biotic and
111 abiotic stresses. As such, plants monitoring their bacterial endophytes in the phyllosphere is
112 likely a conserved feature across the plant kingdom as a consequence of millions of years
113 coevolution with microbes. Previous findings have demonstrated that plant defense signaling
114 pathways could regulate the structure and function of the root-associated microbiome, such as
115 salicylic acid and jasmonic acid signaling pathways [12]. Therefore, there is the possibility to
116 regulate plant genetic pathways to attract microbiota for mutualistic interactions with plants
117 in both the rhizosphere and phyllosphere. However, significant knowledge gaps remain
118 regarding the genetic control of microbiome assembly in the phyllosphere and how this affects
119 plant health, such as (i) what metabolites and molecular mechanisms mediate the microbial
120 changes in the phyllosphere, (ii) whether the dysbiotic phenotype is merely a product of a
121 genotype that selects for a dysbiotic microbiota or is caused by both the genotype and the
122 microbiome. If it is the former, it offers a scientific rationale for breeding microbe-optimized
123 crops by engineering plant genetic pathways, and (iii) how the reshaped phyllosphere
124 microbiome changes in metabolites, and how it and the metabolites modulate epigenetics and
125 gene expression in the plant. These are critical knowledge gaps that need to be systematically
126 addressed to advance the fundamental understanding of plant-microbe interactions and in turn,
127 be harnessed to increase ecosystem productivity and sustainability.

128
129 **Concluding remarks**

130 The intimate interactions of the phyllosphere microbiota with the plant itself determine the
131 plant responses to changing environments, and form a regulatory feedback loop that underpins
132 a co-evolutionary mutualistic relationship between the two ecosystem components. Defects in
133 innate immunity to effectively cope with biotic and abiotic stresses are likely a common
134 challenge for the plant kingdom; modulating key host genetic networks to prevent dysbiosis
135 is likely a novel pathway to leverage the native phyllosphere microbiome to improve the
136 natural and agricultural plant performance.

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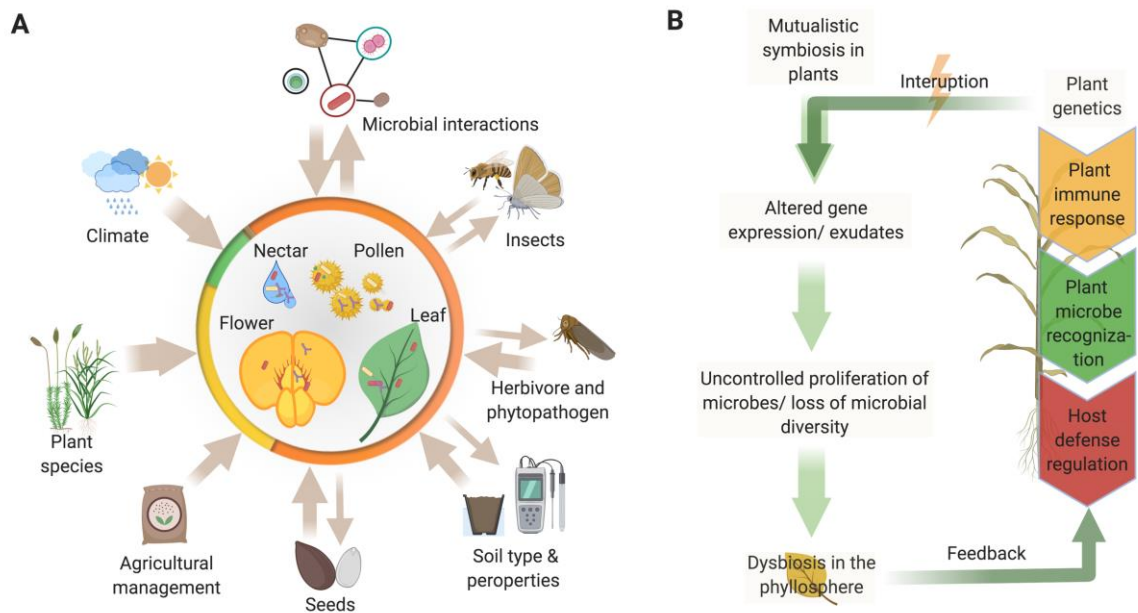
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Assembly and dysbiosis of the phyllosphere microbiome



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Figure caption

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Figure 1 Microbiome assembly and plant health in the phyllosphere. (A) Multiple factors

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can influence the assembly of the phyllosphere microbiome. Soil, a reservoir of the

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phyllosphere microbial diversity, interacts with plant nutrition and growth, providing microbes

172

for the phyllosphere (via vascular transmission and air dispersal) and influences leaf chemistry.

173

Agricultural management (e.g., tillage, foliar agrochemical application) and climate (e.g.,

174

sunlight, rainfall) also influence the phyllosphere microbiome via alteration of soil and plant

175

traits [1]. Biotic factors including plant genotype and development stage, insect herbivore

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attack, pollinator visitation, pathogen infection as well as microbial competition and

177

cooperation also drive the microbiome assembly in the phyllosphere [8, 9]. Many mechanisms

178

associated with these factors are putatively mediated by plant genetic traits and metabolism.

179

The inserted circle represents the microbial composition at phylum level in the phyllosphere.

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Orange, Proteobacteria; yellow, Actinobacteria; green, Bacteroidetes; red, Firmicutes. (B)

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Occurrence of dysbiosis and its impact on plant-microbe interactions in the phyllosphere.

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Impaired plant genetic networks and disruptions to homeostasis alter plant gene expression

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and metabolism, which consequently impact leaf chemistry and lead to the occurrence of

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dysbiosis [11]. The altered microbiome can in turn interact with the plant host and create a

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condition that supports their proliferation (e.g., the beta-proteobacteria observed by Chen et

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al., 2020), forming a feedback loop whereby the microbiome inside the phyllosphere and the

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plant genetics cross-regulate each other.