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The foliar microbiome

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Proficient performance in plants is strongly associated with distinct microbial communities that live in and on their organs. We comment here on the current knowledge of the composition of the foliar microbiome, highlight its importance for plants, ecosystemic functioning, and crop yields, and propose tools and experiments to overcome the current knowledge gap.

Microbial communities

Humans have millions of microbial organisms in and on their bodies. The human genome contains only 21 000–23 000 genes, but the gut microbial metagenome alone contains over three million genes. This microbiota plays a key role in human health and in the functioning of all animals [1]. Similarly in plants, the importance of distinct microbial communities hosted in and on various organs is also recognized, and the root microbiome has recently been defined [2,3]. Less is known, however, about the composition and the physiological and ecological roles of microbiotas in and on leaves [4–7], which is somewhat surprising given that the foliar surface alone represents the largest biological surface on Earth, with an estimated area exceeding 10^8 km^2 globally. Each km^2 is estimated to harbor 10^{16}–10^{18} bacteria [4,7], outnumbering the cells of the plants themselves, in addition to an unknown number of invertebrates, fungi, and other microorganisms. We have an incomplete understanding of how and why microbiotic composition varies across spatial and temporal scales, which genetic adaptations enable the survival of microorganisms in the phyllosphere, and how the microbiotas interact among themselves and with their hosts [4–7]. We also have even more serious gaps in our understanding of microbiotic functions and the actual implications for plants, ecosystems, and agriculture. Here we highlight the need for more research on these microbiotas and their contributions to plant and ecosystem functioning and crop yields, and discuss what tools are available to address the knowledge gap.

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The foliar microbiome

The microbiotas of the phyllosphere (in foliar tissues and on foliar surfaces) are known to be abundant, and are assumed to play crucial roles in protecting plants from diseases and in promoting their growth by various mechanisms (Figure 1), but they are generally not well characterized and little is known about their functions, except for some N-fixing symbionts and for pathogens causing epidemic diseases in agriculture or silviculture [4–7]. The availability of high-throughput and cost-effective molecular technologies is now enabling us to begin to address the compositions and functions of these microbiotas across a large number of samples with high, in-depth coverage [5–7]. The research on these two questions – composition and role – using a combination of metagenomics, metabolomics, and laboratory and field experiments, will allow us to make significant steps forward in a wide range of fields of plant science, from basic ecology to agricultural applications.

Composition

The application of these molecular tools, complemented by microscopic techniques, to reveal the patterns of microbiotic colonization in the phyllosphere is beginning to establish the rules of assembly of foliar microbiotas *in situ*, including the host factors that shape the compositions of the microbial communities. These tools show that the phyllospheres of various plants harbor diverse and complex communities of microorganisms [8]. The diversity of phyllospheric bacteria appears to be as high as that of the bacteria of roots or of the human gut [9]. Diverse epifoliar and endophytic fungi also occur in and on the leaves of all major lineages of land plants from the arctic to the tropics [10]. As in roots [2,3], phyllospheric bacteria and fungi vary quantitatively among plants of different developmental stage and genotype [9–11]. A recent study on root microbiome characterization [2,3] showed that the rhizosphere immediately surrounding the root and the microbiota in the root are most strongly influenced by soil type, but also by host genotype, although to a lesser degree. The available data indicate that most of the microorganisms of the foliar microbiota can also randomly emerge from neighboring environmental ecosystems, but their survival and ultimately their presence are generally regulated by the plant [9]. Interestingly, several studies have shown that some of these microorganisms of the foliar microbiota are transferred across generations of plants, and not only through

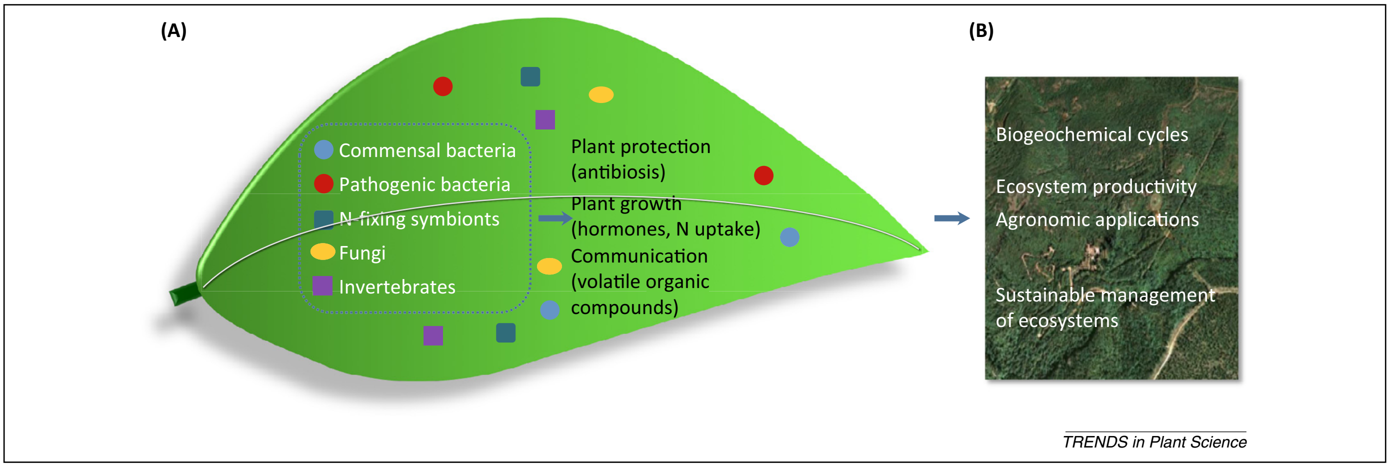

Figure 1. The phyllosphere and associated microbiotas, illustrating their roles **(A)** and the ecological and agronomic implications **(B)**.

environmental exchange [12]. For example, bacteriosomes have been observed in the parenchymal cells of leaves – and also of roots and rhizomes – of axenic peach palms (*Bactris gasipaes*) [13]. This study demonstrated that even embryos excised from seeds of the peach palm contained endosymbionts, but we still do not know how they were acquired by the host or what their beneficial effects are. Colonization of the leaves, as happens with the colonization of the root, occurs despite a sophisticated plant immune system [14], suggesting a finely tuned discrimination of mutualists and commensals from pathogens. The mechanisms involved in this discrimination are mostly unknown.

Physiological and ecological roles

These foliar microbiotas can have positive, neutral, or negative influences on their hosts [4–7] (Figure 1), and the activities of the microbial communities in and on leaves are therefore very likely to influence plant health and growth significantly [4–7], and therefore ecosystemic productivity. The fixation of nitrogen is one of the best-studied functions of the foliar microbiota. Some authors have even suggested that nitrogen fixation in the phyllosphere is the main mechanism for adding nitrogen in humid tropical ecosystems [15]. The establishment of phyllospheric populations of nitrogen-fixing bacteria has been observed mainly in plants of tropical rainforests, but these organisms also occur in temperate-forest ecosystems where their abundance and diversity vary depending on the availability of water [11]. Foliar microorganisms can also affect plant growth by the production of growth hormones [16]. Internal and external foliar microbiotas can have many other functions, including indirect protection against pathogens, through the interaction of commensal bacteria with the foliar plant pathogen [5,6,17], or communication, through their contribution to different types and quantities of emissions of volatile organic compounds [5,6].

Concluding remarks and future perspectives

The internal and external foliar microbiota, and their likely key roles in plant performance, growth, and health (Figure 1), warrant study to understand better plant functioning and its responses and effects in a changing world. To fulfill this aim, first, robust observational systems based on high-throughput deep sequencing in a controlled design are needed. Second, in those systems the host genes that potentially contribute to the phenotypes of microbial association should be identified. Such plant genes would constitute major agronomic targets. Third, the knockdown of those genes, or if still unidentified, even much simpler approaches, such as eliminating the foliar bacteria with antibiotics, is necessary to discern the consequences on plant performance of losing the foliar microbiome. The combination of multiple 'omic' technologies, such as metagenomics, proteomics, and metabolomics, both observational and experimental, will lead to a system-level understanding of the composition of phyllospheric microbial communities and of their physiological and ecological roles in the global biogeochemical cycles and the biosphere. Specifically, ecological theory (island biogeography, macroecology, and landscape ecology) will benefit from the large range of spatial scales offered by the foliar surface [18]. In addition, novel sustainable practices of agricultural and ecosystemic management will be possible in agriculture: for example, foliar microbiotas could be managed to reduce the use of fertilizers and pesticides or to control plant growth.

As the medical community is doing with the human microbiome, the physiological, ecological, environmental, and agricultural scientific communities studying plants should also begin to focus on the foliar microbiome and, in fact, on the entire plant–microbe system, and not only on the plants alone.

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