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The plant microbiota: composition, functions, and engineering

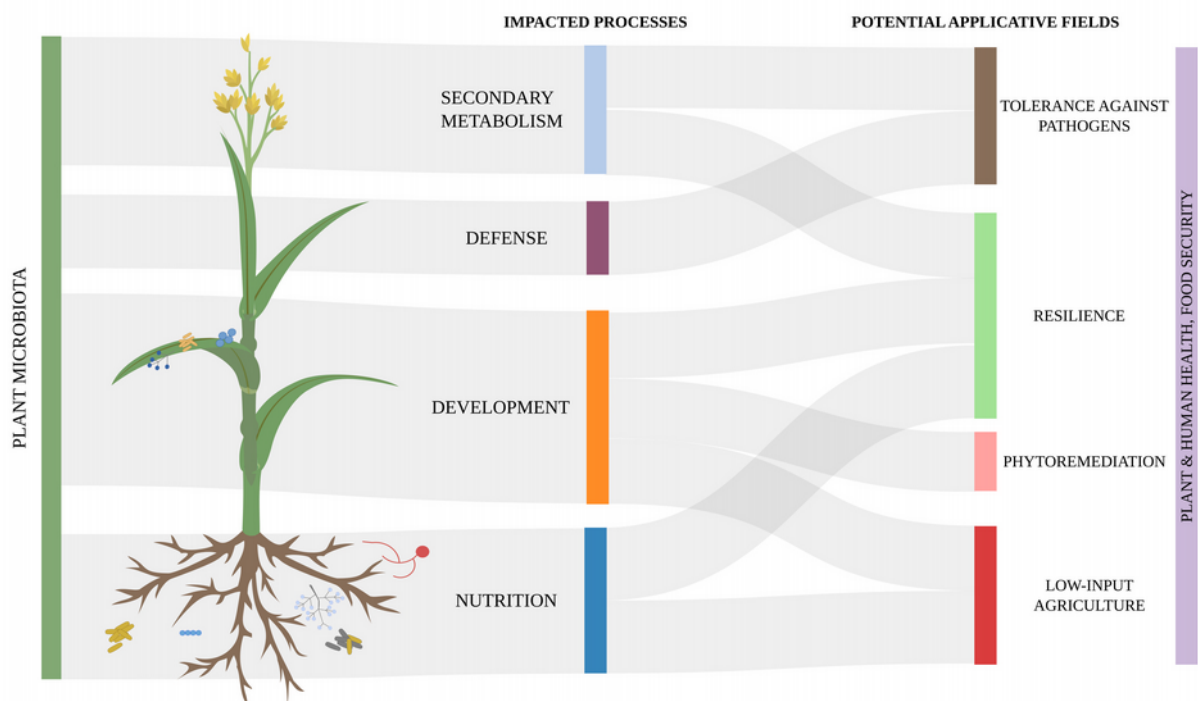
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Graphical Abstract



Abstract

Plants growing in nature live in association with beneficial, commensal, and pathogenic microbes, which make up the plant microbiota. The close interaction between plants and their microbiotas has raised fundamental questions about plant responses to these microbes and the identity of the main factors driving microbiota structure, diversity, and function in bulk soil, in the rhizosphere, and in the plant organs. Beneficial microorganisms have long been used as inoculants for crops; the current development of synthetic microbial communities and the identification of plant traits that respond to the microbiota form the basis for rational engineering of the plant microbiota to improve sustainable agriculture.

Introduction

Since the first phototrophs conquered land over 400 million years ago, they have established intimate relationships with surrounding microorganisms that conferred them additional functions. Fossil and molecular evidence strongly suggests that the first plant ancestors, which lacked a root apparatus, were aided during land colonization by the establishment of symbioses with mycorrhizal fungi; to this day more than 90% of vascular plants rely on mycorrhizal fungi for nutrient and water uptake [1,2].

In nature, plants mostly live as super-organisms in association with microbial communities. This concept has permeated plant sciences in the last decade, steering research trends across disciplines from microbiology to evolutionary biology and agronomy [3,4]. In this review, we first summarize the major recent findings on plant-associated microbes and their effect on plant biology. We then review potential biotechnological applications, with a focus on crop plants in the context of the United Nations 2030 Agenda for Sustainable Development.

Plant microbiota research comes of age

Plants growing in natural ecosystems live in close association with a multitude of microorganisms (virus-like particles, bacteria, fungi, and oomycetes), collectively known as the microbiota. Some of these microbes promote plant growth and others are commensals or pathogenic, but – as with the human microbiota – a balance among them is essential to guarantee plant health.

The plant microbiota emerged as a hot topic for research only recently, when the August 2012 issue of *Nature* dedicated a commentary and its cover to two papers describing the bacterial microbiota inhabiting the *Arabidopsis* (*Arabidopsis thaliana*) root [5,6]. These studies defined the methodological standards for future research, *i.e.* high-throughput amplicon sequencing of marker genes (metabarcoding) such as bacterial 16S ribosomal RNA. Both articles illustrated the dominance of Proteobacteria, Bacteroidetes, and Actinobacteria, and showed how soil features strongly influence microbiota composition. Moreover, these studies raised fundamental questions about the regulation of plant–microbe interactions in the context of the plant immune system, plant nutrition and development, and the factors that shape microbiota diversity. The blueprint established by these seminal works has blossomed into a multitude of published articles (>99% of papers on this topic were published after 2012 [7]) describing the microbiotas associated with specific plants living in the most diverse biomes, from natural environments to intensively managed ecosystems.

Building on sequencing, modern microbiome analysis integrates omics data for the host and microbes to recreate the complex phenotypes emerging from their interplay [8]. For example, metabarcoding analysis generates a huge mass of information about the identity of plant-associated microbes, regardless of their cultivability, as well as the possible local-scale environmental and host-related determinants. Metagenomics, *i.e.* the description of all DNA sequences in environmental samples, reveals the genomic landscape of microbial communities living in specific ecological niches, and transcriptomics, proteomics, and metabolomics studies highlight the active metabolic pathways underlying the interaction between plants and their associated microbial communities.

Assessing plant microbiota diversity and its drivers: from local to global inventories

Plants are hosts to different microbiotas on various tissues (roots, leaves, seeds, stems, flowers), which represent specific niches characterized by distinct chemical and physical properties. Initial studies focused on belowground organs, as the root–soil interface represents the largest contact surface between plants and their environment, with striking implications for plant health and nutrition. Indeed, different compartments form a continuum from the soil to the rhizosphere/rhizoplane and the root endosphere [9]. Plants acquire their root microbiota from the reservoir of microorganisms in the soil, which is one of the most diverse niches on Earth, through a

multi-step selection process [10]. The plant rhizosphere behaves like free-living soil microbiotas in terms of diversity [11] but is consistently enriched in specific phyla across different plants, including Firmicutes, Actinobacteria, Cyanobacteria, Acidobacteria, Bacteroidetes, Verrucomicrobia, and Proteobacteria for bacteria and Ascomycota and Basidiomycota for fungi [12]. These bacterial phyla are quantitatively more abundant in the rhizosphere than in the soil, as revealed by quantitative microbiota profiling [10]. This amplification of bacteria may occur in response to root exudates and rhizodeposits (phenolics, organic acids, phytohormones, and other metabolites), and might be linked to specific microbial features including nutrient use efficiency and growth rate. The assembly of the root-associated microbiota also depends on host physiology and nutrient availability in the soil, which are connected. For instance, the phosphate starvation response drives shifts in specific microorganisms such as *Burkholderia* from commensalism to parasitism [13]. Moreover, the root microbiota may be locally influenced by plant emissions of volatile organic compounds in concert with phytohormones [14].

A subset of rhizosphere microbes (fungi and bacteria) also dwells as endophytes inside the root endosphere, whose lower diversity relative to the rhizosphere is under genotype-dependent selection by the host plant. For example, the barrel clover (*Medicago truncatula*) root endophytes Alphaproteobacteria, Bacteroidetes, Betaproteobacteria, Firmicutes, and Deltaproteobacteria were more abundant than in the rhizosphere [10].

Although roots caught the eye of microbiologists, the phyllosphere (the exterior leaf surface) and leaf endosphere also play crucial roles in plants [15]. These niches host bacterial communities dominated by Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, as well as still overlooked fungal communities [15]. Notably, leaf bacterial communities exhibit low specialization in plants grown in the wild, as they originate from passive transport through the atmosphere from water, pollen, and other particles, or from the soil [16].

The soil is the major source of microbes colonizing plants, but the microbiota as a whole or as specific microbial cohorts may be inherited vertically across generations, for example through the seed microbiota inhabiting the so-called spermosphere and seed endosphere [17]. Seeds may indeed host highly specialized microbial communities determined by environmental factors that comprise beneficial and/or pathogenic microbes, as in chili (*Capsicum* spp.) and rice (*Oryza sativa*) plants [18,19], and may thus have biotechnological applications in agriculture. In clonal plants, vegetative reproduction may also transmit microbes, *e.g.* through stolons, as in ground ivy (*Glechoma hederacea*) [20].

After a descriptive phase, scientific interest is now moving towards understanding the environmental factors that shape plant-associated communities at larger macro-ecological scales, such as landscapes or along continental/global environmental gradients in a biogeographical context [21]. Indeed, while local-scale evidence is now emerging, there are few worldwide large-scale analyses. In one such example, *Arabidopsis* root microbiotas were recently investigated in Europe across 17 sampling sites over three years, showing an overall convergence of root microbial communities, despite contrasting soil and climatic features [22], highlighting how drivers of plant-associated microbiota differ from those identified in soil, for which geography plays a major role. These factors deserve further attention, as genotype-environment interactions influence plant-associated microbiotas [23] and can globally hinder our understanding of microbiota dynamics.

From diversity to function: how the microbiota leads to an extended plant phenotype

In analogy to the microbiota–gut–brain axis [24] proposed in animals, recent data support a microbiota–root–shoot axis in plants that links belowground and aboveground microbial communities in response to environmental stimuli [25]. Indeed, microbes that live in symbiosis with roots have long been recognized as being beneficial to plants, long before the omics era, as with mycorrhizal fungi and nitrogen-fixing bacteria (reviewed extensively in [1,26]). In addition, a

plethora of plant growth-promoting bacteria improve plant nutrition and growth under limiting conditions [27] due to their capacity to mobilize nutrients (phosphorus, potassium, zinc), fix nitrogen symbiotically, release siderophores, and interfere with plant hormone metabolism or produce phytohormones [28].

The microbiota modulates regulatory networks involved in nutrient acquisition, plant development, and immune responses. Root microbial communities mediate plant adaptation to nutrient starvation, such as iron, nitrogen, and phosphorus. For iron, root-exuded coumarins, whose biosynthesis is controlled by the transcription factor MYB72, select microorganisms to help mobilize iron and make it available to the host [27]. For nitrogen, variation in the nitrate transporter NITRATE TRANSPORTER 1.1B (NRT1.1B) between *indica* and *japonica* rice varieties modulates the recruitment of genera involved in ammonification [29], thus promoting plant growth.

Better nutrition affects plant development and plant phenotypes, as beautifully illustrated by mycorrhizal fungi. These effects rely on reprogramming of plant metabolism at local and systemic levels. By affecting primary and secondary (lignin, pigments) metabolism, mycorrhizal fungi stimulate plant growth as well as seed phenotypes (grain yield) [30]. Plant phenotypes may also be influenced by the application of a mixture of selected microbes whose few key components can provide an advantage to their host. For instance, bacterial genus *Variovorax* can reverse the severe inhibition of root growth induced by other bacterial strains via modulating phytohormone levels [31]. In another example, microbes can reduce endodermal suberization at the Casparian strip, which controls mineral transport across the cell plasma membrane, thus optimizing mineral nutrient homeostasis [32].

An obvious question arose alongside the description of the diversity in the plant microbiota: how do they cope with plant innate immunity? Since plants detect highly conserved microbe-associated molecular patterns (MAMPs) to initiate defense responses, how do plants decide when to fight and when to tolerate microbes? The last ten years have demonstrated that the plant microbiota modulate plant immunity by activating different strategies. Indeed, Rhizobiales and Sphingomonadales strains suppress MAMP-triggered immunity (MTI) [33]. In addition, taxonomically diverse MTI-suppressor strains were identified and described as efficient root colonizers, through a type 2 secretion system [34]. These findings showed that the suppression of MTI is independent of growth promotion, and was likely acquired independently across bacterial phylogeny. This observation suggests that commensals in the plant microbiota can evade host immune responses, while the plant immune system actively shapes the microbial community [35]. As previously described for arbuscular mycorrhizal (AM) fungi, a number of root-inhabiting microbes leads to induced systemic resistance (ISR), which forms a positive feedback loop to increase tolerance to pathogens [36]. Other evidence shows that microbe–microbe interactions are equally important in maintaining plant health, as bacterial root commensals can limit the growth of filamentous eukaryotes, including pathogens, thus maintaining an interkingdom balance [37].

Intriguingly, nutrient acquisition and immunity are often coordinately modulated. For example, plants modulate defense and nutrient acquisition through a complex network that is activated by the presence of root microbiota and is mediated by the key regulator PHOSPHATE STARVATION RESPONSE 1 (PHR1) [38]. Similarly, the transcription factor MYB72 regulates both ISR and iron acquisition [27].

In summary, the plant microbiota affords an extended phenotype to its host, enhancing mineral nutrition, changing developmental plans, and improving defenses against pathogens.

The plant microbiota at the crossroads of plant and human health

The plant microbiome may be considered an accessory genome that extends plant functions, thus representing a natural, sustainable tool to increase plant plasticity in the face of fluctuating

environmental conditions due to global climate change. The plant microbiota may thus contribute to improving yields in low-input agriculture [4]. In addition, microbes can help in phytoremediating polluted soils [39] and be reservoirs of bioactive secondary metabolites for bioprospecting [40]. Therefore, the plant microbiota can contribute to soil security and water sanitation, which are explicit sustainable development goals of the United Nations. Moreover, the plant microbiota has been suggested to exert an indirect but crucial influence on human health as a close link with the gut microbiota could occur [41].

Companies are exploring microbe-based alternatives to agrochemicals, stressing the increased global awareness of the plant microbiota. Indeed, the biostimulants and biopesticides market is expanding and may be particularly relevant for small- and medium-size ventures and those active in less developed countries [42]. For example, *Azospirillum*-based formulations with plant growth-promoting activities have been successfully deployed in South America to increase yield in maize (*Zea mays*), wheat (*Triticum aestivum*), and soybean (*Glycine max*) [43]. However, the success of field application of microbial inoculants largely depends on complex interplays occurring between soil features, including the resident native microorganisms, plant genotype and field management practices [44]. A high level colonization of plant tissues is usually a desirable trait and it can be achieved by some highly competitive microbial inoculants in a wide range of environmental condition and hosts, such as the well studied *Variovorax paradoxus* C5-2 isolate [44]. However, in some cases microbes-induced resilience can be dependent on the plant genotype [44], pointing to the need to identify best plant-microbe combinations.

Another growing market is the production of inocula containing AM fungi [45]. Similarly, the use of AM fungi in agriculture poses some technical challenges. Their obligate biotrophy can be a major limitation to large-scale inoculum production [46]. The recent finding that myristate (C14:0) can feed AM fungi under asymbiotic conditions paves the way for the scaling up of pure inocula production [47]. Another challenge is related to the application and the persistence under field conditions [46]. Nowadays, field inoculation has been scarcely used in crop industry and very few studies tracked persistence in soil of specific inoculants [48]. Concerns about the consequences on the introduction of exotic AM fungal species on plants and soil microbial communities point to the preferential use of AM isolates from local soils, which may be even more adapted to specific environmental conditions [46, 48].

The development of tailored microbial inoculums is a promising strategy to boost crop production and quality; however, these efforts should be coordinated with high-throughput cultivation methods [49] and with the establishment of large-scale microbial collections covering the different domains of microbial diversity. Artificially constructed communities, called synthetic communities (SynComs), are being tested to better understand plant-microbe interactions [50]. These studies allow the selection of strains based on functional traits (*e.g.* production of phytohormones, volatiles, antimicrobial compounds, nutrient solubilization, colonization efficiency) rather than on microbial abundance. SynComs will thus potentially improve plant yield and disease resistance and, possibly, show increased resilience, especially in complex open field environments [51]. However, we anticipate that the design of appropriate formulations of inoculants, in the form of lyophilized powder applied to soils, seed coats [52], or reproductive organs [53], will require significant investment. Plant-microbe communication within the rhizosphere also represents a promising line of investigation, as root exudates play a role in the assembly of rhizosphere and root microbial communities and therefore could offer tools for a more tailored and technologically advanced applications of beneficial microbes [54].

Conclusions and perspectives

Ten years of research allowed the identification of factors that drive the assembly of plant microbiota mostly at local scales, but global-scale studies are largely lacking. In other fields, large-

scale collaborative projects have generated huge amounts of data (e.g. the Earth Microbiome Project, the Global Water Microbiome Consortium, the Human Microbiome Project). Such efforts have yet to be attempted for plant-associated microorganisms outside of AM fungi [55].

Drawing attention to the knowledge and conservation of soil- and plant-associated microorganisms is now a priority based on the One Health vision from the World Health Organization whereby human health is connected to animal and ecosystem health, which was brought to the fore by the current COVID-19 pandemic. More attention should be given to conserving soil biodiversity to sustain terrestrial organisms and processes (food production) [41], as these tasks are not covered by recent calls addressing nature conservation [56].

The microbiota affects critical plant functions such as nutrition, water use efficiency, and pathogen resistance. When moving from ecological studies to reductionistic approaches [57], it will be a priority to decipher how microbiota exert their influence on crops. Joint efforts from scientists, farmers, agro-biotechnological companies, and governments are needed to successfully use the plant microbiota for sustainable agriculture and as an opportunity for nature-based solutions.

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Author contributions

MC, LL, PB, Conceptualization; PB, LL, Funding acquisition; MC, Visualization; MC, LL, PB, Writing - original draft; MC, LL, PB, Writing - review & editing.

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Figures

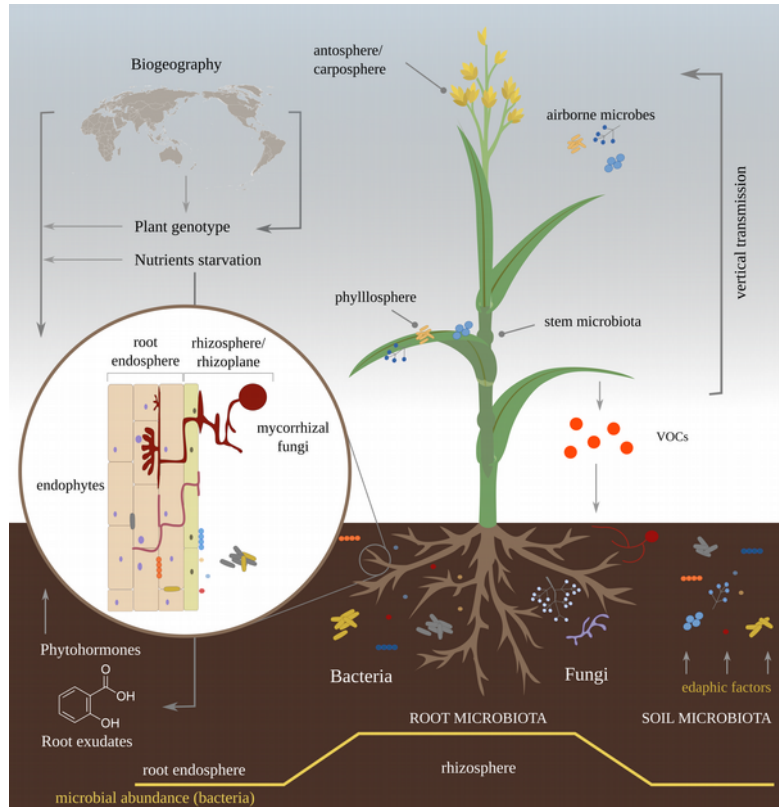


Figure 1. Scheme summarizing the main factors shaping plant-associated microbiota along the different host compartments. Plant-associated microbes are mainly recruited from the soil, by vertical transmission through seeds and by airborne microbes. VOCs. Volatile Organic Compounds.

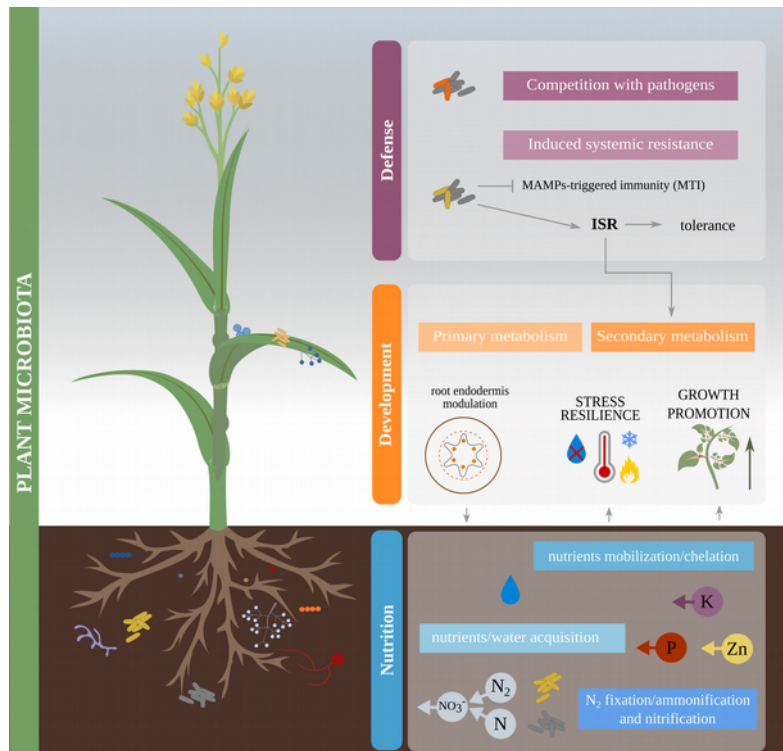


Figure 2. Beneficial effects of microbiota on plant nutrition, development and defense. ISR, Induced Systemic Resistance.