Trends in Plant Science

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REVIEWS

Opinion

Plant Symbionts Are Engineers of the Plant-Associated Microbiome

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Plants interact throughout their lives with environmental microorganisms. These interactions determine plant development, nutrition, and fitness in a dynamic and stressful environment, forming the basis for the holobiont concept in which plants and plant-associated microbes are not considered as independent entities but as a single evolutionary unit. A primary open question concerns whether holobiont structure is shaped by its microbial members or solely by the plant. Current knowledge of plant–microbe interactions argues that the establishment of symbiosis directly and indirectly conditions the plant-associated microbiome. We propose to define the impact of the symbiont on the plant microbiome as the 'symbiosis cascade effect', in which the symbionts and their plant host jointly shape the plant microbiome.

Highlights

Plants are associated with an enormous diversity of microorganisms, some of which are symbiotic.

Symbiont establishment is accompanied by structural and physiological changes in the host plant, including qualitative and quantitative changes in root exudates.

Studies on plants impaired in their ability to enter symbiosis, or after controlled inoculation with symbionts, demonstrate that symbionts play an important role in the taxonomic and functional structuring of the phytomicrobiome.

Plant symbionts drive the composition of the phytomicrobiome; hence, plant symbionts are ecological engineers of the holobiont.

Plant Symbionts as Ecological Engineers of the Phytobiome

Microorganisms play a crucial role in environmental geochemical cycles and in plant nutrition and development. Some microorganisms have evolved the ability to establish symbiotic interactions with their host, be they mutualists (positive impact), commensals (no visible impact), or detrimental (negative impact). Many of these microorganisms are recruited from the plant environment, whereas others are vertically transferred – such as **endophytes** (see Glossary) contained within seeds. Symbioses play a key role in plant life, potentially affecting even plant speciation [1,2]. Most of these symbiotic interactions have been considered only from a single angle, such as the **symbiont**, the plant host, or the interaction between the two. We have rarely considered how the establishment of the symbiont and the response of the plant influence the recruitment of the environmentally recruited, plant-associated **microbiota** (the **phytomicrobiome**) and its **functioning**. This is not surprising because the importance of the phytomicrobiome to plant health has only recently been demonstrated, and that the composition of the plant **microbiome** is mainly determined by **extrinsic factors** (e.g., soil conditions, climate, culture management practices [3]), although **intrinsic factors** (e.g., vertical transfer through seeds, plant characteristics, plant organs, and plant–microbe interactions [4–7]) also play a role (Figure 1). Nonetheless, the driving factors (e.g., keystone species, metabolites) that underlie the assembly and composition of the phytomicrobiome remain uncertain, and their identification is a key issue in understanding **holobiont** dynamics.

What is the role of symbionts? Although symbionts are members of the phytomicrobiome, are they intrinsic or extrinsic drivers of the composition of the phytomicrobiome and **phytobiome**? How do symbiotic interactions and the dynamics of their establishment impact on the rules of phytomicrobiome assembly? Symbionts strongly modify plant ecophysiological traits, colonize plant tissues, and modify local soil properties. Symbioses are also known to modify plant signaling molecules (e.g., strigolactone), hormones (e.g., auxin), the immune system [e.g., the jasmonate (JA) signaling pathway], and exudate compositions (e.g., trehalose, glucosamine derivatives). In this Opinion we describe how molecular dialog between the symbionts shapes the taxonomic

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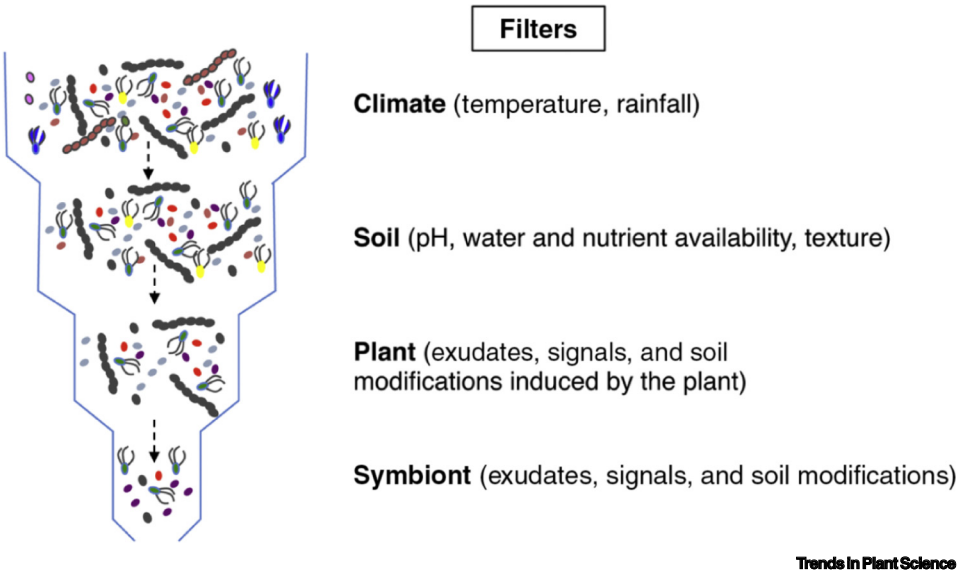

Figure 1. Known or Suspected Environmental Drivers of the Taxonomic and Functional Structure of the Phytomicrobiome. Different environmental filters (and related factors) that are suspected to drive the structure of the plant-associated microbiota are presented. The last filter presented corresponds to the symbiont effect discussed in this manuscript. The different forms represent different microorganisms whose composition is modified by the different filters at each step from top to bottom.

and functional **structure** of the phytomicrobiome, as well as its functioning, thereby defining symbiotic organisms as ecological engineers of the phytomicrobiome. To support this concept we have taken examples from the best-documented symbioses, the **endospheric symbioses**, either mutualistic or detrimental, because these are the only symbioses whose impact on the phytomicrobiome has been tested experimentally.

Plant–Symbiont Interactions: Reprogramming the Plant

What Is Symbiosis?

Symbiosis means 'living together', and is understood here to encompass all close long-term interactions between plants and microorganisms. In symbiosis, interaction is the key notion. Symbionts exert influence on one another, and enter into a reciprocal dialogue which eventually (but not necessarily) leads to modification of the partners. In this view, the notion of symbiosis *de facto* excludes organisms whose presence in the vicinity of the plant is due solely to chance and their spatial distribution in the environment, and which display no interactions with the plant – in the same way that a bird resting on a telegraph pole cannot be considered as a symbiont of the pole, whereas a bird nesting in, or feeding from, a tree might well be. The most emblematic and ultimate symbioses remain the (chloro)plasts and mitochondria, which correspond to long-term coevolutionary relationships between eukaryotic cells and symbiotic bacteria. *Per se*, symbioses are not necessarily beneficial to the host. For example, *Agrobacterium tumefaciens*, the causal agent of **crown gall** disease, illustrates the fuzzy limits between beneficial and detrimental symbionts. Although this pathogen uses horizontal gene transfer to engineer the plant and create its own ecological niche, this process usually only marginally impairs plant growth. Numerous cases of beneficial plant symbiosis have been documented in depth, such as the nitrogen-fixing symbioses (e.g., *Rhizobium*/legumes) and the mutualistic association between **mycorrhizal** fungi [e.g., **ectomycorrhizal** (EM) and **arbuscular mycorrhizal** (AM) fungi of grasses and trees].

Glossary

Arbuscular mycorrhiza: from *myco*, 'fungus', and *rhiza*, 'root', the symbiotic association between the roots of 85% of land plants and fungi belonging to the Glomeromycota division. These symbiotic fungi penetrate the cortical cells of the root and form arbuscules, 'tree-like' fungal structures that develop within plant cortical cells in arbuscular mycorrhizal (AM) symbiosis.
Calcium/calmodulin-dependent protein kinase (CCaMK): this kinase is central to bacterial infection and nodule organogenesis, as well as to arbuscular mycorrhizal symbiosis.
Crown gall: a disease induced by *Agrobacterium tumefaciens* which is characterized by tumoral growth. Apart from hairy root disease, crown gall is the only known example of natural genetic transformation; development of this system has allowed the creation of genetically engineered plants.
Ectomycorrhiza: the symbiotic association between roots of trees/shrubs and fungi belonging to the Ascomycota and Basidiomycota phyla. Fungi form a **symbiotic interface** encompassing plant cortical cells in ectomycorrhizal symbiosis. Described for the first time by Robert Hartig, and therefore termed the Hartig network.
Endophyte: microorganisms residing in plant tissues.
Endosphere: internal regions of plant tissues that can be colonized by microorganisms.
Endospheric symbiosis: refers to a symbiotic association in which the symbiont colonizes the inside of the plant (e.g., the endosphere). This term contrasts with exosymbiosis, a symbiotic association in which the symbiont does not enter plant tissues.
Extrinsic factors: factors related to the environment.
Functioning: in complex assembly systems (e.g., microbial communities and/or plant–microbe interactions) this term refers to the global phenotype observed, which results from the sum of all the functions of the members of the complex assembly.
Holobiont: the assemblage of different species that form an ecological unit [55]. We limit our definition here to the plant and all its symbiotic microbiota. The holobiont is an ecosystem in which the host is the biotope and microorganisms are the biocenosis.

How Does Symbiosis Affect Plant and Symbiont Partners?

From the plant perspective, interactions with symbionts modify intracellular and intercellular communication, the expression of hundreds of genes (Box 1), and the diversity and quantities of exudated metabolites [8,9], as well as cell and tissue structures. These aspects have been described in depth for endospheric symbioses. The modifications begin with an increased intracellular calcium levels a few seconds to minutes after the initial interaction with symbionts. Novel or mixed organs can be formed, as in the nodules generated by *Rhizobium* and the mycorrhizal roots formed by symbiotic fungi. In these hybrid structures the mycelium forms a specific network in the apoplastic space, allowing nutrient exchange between the host plant and the fungus, as well as metabolic reorientation such as decreased starch and sucrose levels, increased trehalose and mannitol production, and increased respiration [10] or the accumulation of oxalate around mycorrhizal roots. Metabolic reprogramming is also characteristic of gall-forming *A. tumefaciens* infection which leads not only to the production of *Agrobacterium*-specific amino acid derivatives, the opines, but also to major remodeling of plant resource allocation (translocation of nutrients and water) to the benefit of the tumor, and the accumulation of a dozen other carbon sources [11–13]. Following *Rhizobium* and *Frankia* infection, root cells differentiate to form nodules in which low-oxygen and carbon-rich conditions occur. (See Box 2.)

From the microbial side, cellular and genomic differentiation can take place. Upon induction of symbiosis, the bacterial cells undergo multiple rearrangements to create specialized cells. In plant root nodules colonized by nitrogen-fixing rhizobia, bacteria form immobile, larger cells with increased nitrogenase activity (i.e., bacteroids) [14]. Similarly, *Frankia* cells form larger cells with diazovesicles and nitrogenase activity [15]. During the plant/*Agrobacterium* interaction, no major morphological modifications occur, but the symbiosis provokes genomic rearrangements of the microbial community via the dissemination of pathogenic plasmids. Last, obligate symbionts such as mitochondria, plasts, and mollicutes, for example, display both morphological and extensive genomic optimization.

In most cases, plants associated with symbionts such as mycorrhizal fungi or nodule-forming rhizobacteria exhibit higher biomass, hence the general terminology of plant growth-promoting rhizobacteria (PGPR). The biomass of *Medicago truncatula* $Nod^{-}Myc^{-}$ mutants that are unable to form mycorrhizae and nodules can be reduced by up to tenfold relative to colonized $Nod^{+}Myc^{+}$ plants [16]. Interestingly, the host plants seem to be able to select the most effective symbionts, for example rhizobia with higher nitrogenase activity, although effectiveness-driven selection remains to be confirmed [17,18]. Similarly, during AM symbiosis, the plant and the AM fungi establish a reciprocal 'fair trade' [19], but this textbook picture is highly variable and probably depends on the plant species, the plant genotype, and the AM fungal species [20,21].

From an evolutionary perspective, we are far from knowing all the cellular modifications induced by the **endosphere** symbiotic association including both recent symbionts, such as mycorrhiza, and ancient symbionts, such as mitochondria/chloroplasts. Our current knowledge points to changes in hormone production (auxin, strigolactone) and exudate composition, immune system adjustment (salicylate, JA), and volatiles – in other words changes in molecules that are all potentially involved in the complex dialogue with the phytomicrobiome. The diverse modifications in metabolite production induced following *Agrobacterium* infection offer a clear example of how subtle modifications in metabolite or hormone balance can lead to important modifications of the **metabolome** and **signalome** of the host plant, and therefore of its **interactome**. In addition, symbiosis establishment also leads to modification of the physicochemical properties of the soil (e.g., pH changes, increased content of nitrogen or trehalose, soil aggregation).

Interactome: all interactions between organisms within a functional community.
Intrinsic factors: in the present context, this term is used to refer to the ensemble of all plant characteristics (species, genotype), plant organs (stem, root), and plant–microbe interactions.
Metabolome: the entire biochemical complement of an organism. Metabolic change is a major feature of plant genetic modification and of plant interactions with pathogens, pests, symbionts, free-living microbiota, and the environment.
Microbiome: the microorganisms and their genetic material (genome, plasmids and mobile elements) that associate in the short-term or long-term with a particular environment. The microbiomes of individual plants can be extremely diverse, and even within a plant there can be extensive variation in the composition of the microbiome (e.g., phyllospheric or rhizosphere microbiomes).
Microbiota: the community of microorganisms (bacteria, Archaea, fungi, viruses, protists, and other microeukaryota) that are associated with an organism, here a plant.
Mycorrhiza: specialized soil fungi that form an intimate association with plant roots. There are seven types of mycorrhiza, but ectomycorrhiza and arbuscular mycorrhiza are the most common.
Phytobiome: according to the Phytobiomes Alliance, the phytobiome comprises the plant, its environment, the associated microorganisms (e.g., the phytomicrobiome), and all the environmental modifications induced by these interactions.
Phytomicrobiome: diverse interacting microscopic organisms that are associated with a plant living in its environment.
Rhizosphere: the volume of soil around living plant roots that is influenced by root activity
Signalome: signaling molecules produced within an organism or during interaction between organisms.
Structure: in the field of phytomicrobiome analysis, this term encompasses not only the composition of the taxa and/or functions encountered in the community but also a quantitative view (e.g., their relative abundance).
Symbiont: an organism that establishes a close and long-term interaction with its host (here the plant).

Impact of Symbiosis on the Phytomicrobiome

It was recently predicted that plant endospheric symbionts may be keystone organisms that are capable of modifying their environment (i.e., the phytobiome) [22], but without experimental demonstration. No symbiont-free plants exist in nature, and naturalistic approaches are therefore ill suited to study the impact of symbiosis. However, comparative analyses of plants impaired in their ability to enter symbiosis, in the presence or absence of symbionts, or colonized by different symbionts, can help to decipher the relative roles of endospheric symbionts in modulating the composition of the phytomicrobiome and the evolution of the holobiont.

What Can We Learn from Plants Impaired in Their Ability To Enter Symbiosis?

One elegant way to address the impact of symbionts on the phytomicrobiome is to use plants impaired in their ability to associate with symbionts. Several plants incapable of forming symbiotic

The interaction can be obligate, as in the case of the endosymbiosis.

Symbiotic interface: synonymous with 'symbiotic apoplast', this describes the cellular space between the plant and fungal membranes that delimits the site of reciprocal nutrient exchange between the partners.

Box 1. Gene Locks Acting on the Establishment of Symbiosis, and Effects on Symbionts of the Main Molecules Produced by Plants

Gene regulation differs (i) between plants colonized by the same EM fungus (i.e., *Populus trichocarpa* and *Pseudotsuga menziesii* colonized by *Laccaria bicolor* [56]), (ii) between plant tissues (the Hartig net vs the mantle in *Tuber melanosporum-Coryllus avellana* [57]), and (iii) for the same EM fungus when colonizing two distinct plants [56]. The main steps in the interactions between symbionts and the host plant are presented in Figure I. The formation of AM symbiosis and nodules starts similarly, through the common symbiotic signaling pathway (CSSP). A subset of these genes are essential for either the generation or decoding of calcium spiking, including a nuclear **calcium/calmodulin-dependent protein kinase** (CCaMK), DMI3 [58,59]. These genes control transcription factors including Nodulation Signaling Pathway 1 (NSP1) and Required for Arbuscular Mycorrhization 1 (RAM1) that are involved in nodulation and mycorrhization, respectively [60,61].

Although rhizobia and AM fungi share the same pathway, they have specific features [62,63]. The development and spread of AM fungi within the root are predominantly under the control of the host plant, and depend on its developmental and physiological status. Notably, DIS, RAM1, BCP1, RAM2, and PT4 are required for arbuscule development, whereas a cysteine protease (CP3) is necessary for arbuscule degeneration [64]. In EM symbiosis, root hairs can be colonized by different fungi (brown and blue cells). The set of genes involved in the formation/degeneration of arbuscules or nodulation are well known (blue arrows), except the steps before the CSSP in AM symbiosis (blue broken arrow). The red locks correspond to genes in which mutation blocks symbiotic organ formation. The main molecules produced by the plants (purple arrows) could act as a physiological hub. All these regulatory mechanisms are potential drivers of the structure of the phytomicrobiome, and may act directly or indirectly on the phytomicrobiome (green arrow). Interestingly, the effect of dysregulation of some of these pathways on the phytomicrobiome has already been tested (see Table 1 in main text).

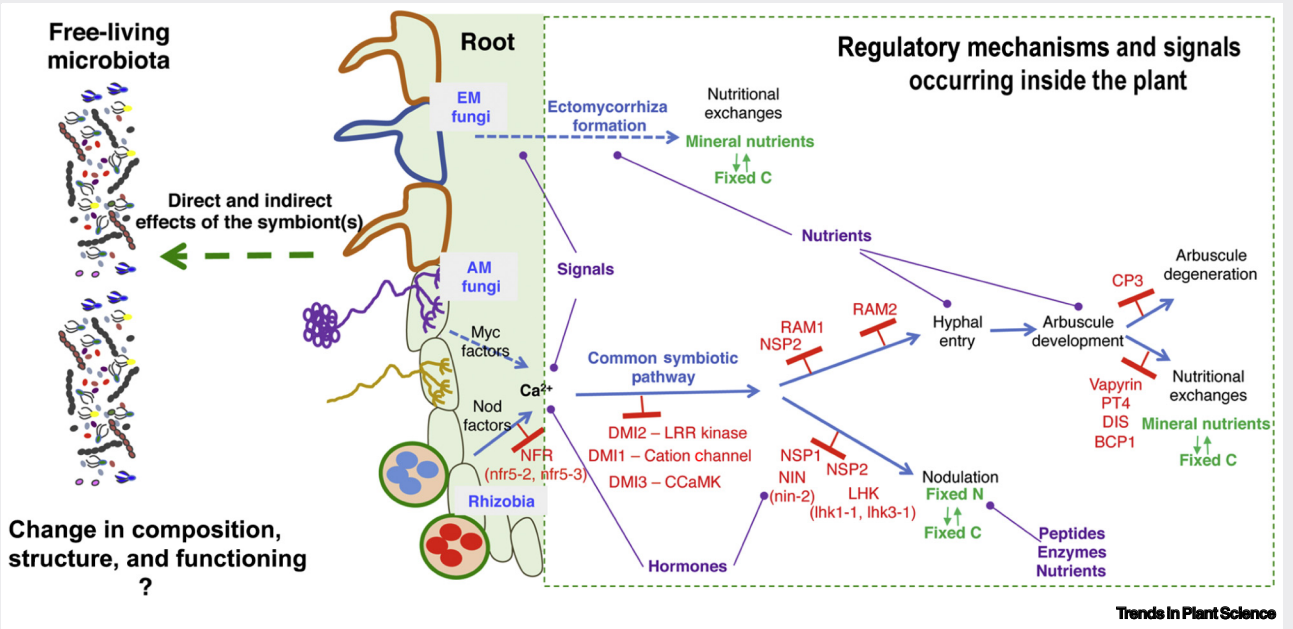

Figure I. Regulatory Pathways inside the Plant during Plant–Symbiont Interactions.

Box 2. 'Symbiotic Cascade Effects' or How Symbiont Establishment Affects and Drives the Phytomicrobiome

We present here the cascade of events which shape the structure of the plant-associated microbiome as well as the main molecules differing between plants associated or not with symbionts. The microbiota can be affected at each of the different steps of the plant–symbiont interaction. (i) As soon as symbionts interact with the host plant (e.g., at the presymbiotic stage or at the seed germination stage for seed endophytes) physiological changes are induced in the plant through signaling molecules and physical contact, and competition occurs between the plant tissues and the free-living microbiota. (ii) During symbiont establishment, the physiological changes in the plant are amplified and structural changes can appear (e.g., nodule or mycorrhiza formation). (iii) During symbiosis, the metabolites (carbohydrates, hormones, signals, and volatiles; Table I) produced by the plant and potentially exudated are modified quantitatively and/or qualitatively (e.g., new metabolites are produced due to the symbiont), and the plant defense response may also be affected. (iv) The impact of the plant on soil parameters differs between non-associated and symbiont-associated plants. All these modifications impact on the taxonomic and functional structure and composition of the phytomicrobiome as well as on its functioning, and eventually on plant fitness (Table I).

Table I. Main Molecules Produced by the Plant with and without Symbionts Which Could Drive Modifications of the Phytomicrobiome[a]

Molecule type	EM and AM molecules	Nodule molecules	Crown gall molecules
Nutrients (carbohydrates, amino acids, and derivatives)	Trehalose, mannitol, chitin, and derivatives	Nitrogen	Opines, proline, 3-caffeoylquinate, glucosinolate-2, pipecolate, pyruvate, dopamine, salicylate, calystegine B4, nicotinate, *trans*-ferulate, gulonate, 4-hydroxyproline, nicotianamine, melezitose, spermidine, lactobionate
Signals	Calcium, ethylene, JA, sesquiterpene	Flavoinoids, phenolic acids	Acyl homoserine lactone (AHL)
Hormones	Hypaphorin (tryptophan betain)	Auxin	N.d.[b]
Peptides	Mycorrhiza-induced secreted proteins (MISPs)	Nodule-specific cysteine-rich rhizobial factors	N.d.
Enzyme		1-aminocyclopropane-1-carboxylate (ACC) deaminase (acdS)	N.d.

[a]The table is a non-exhaustive list of host plant and/or symbiont metabolites that modulate the structure of the phytomicrobiome. The listed metabolites are primarily produced only in presence of the symbionts or their concentrations change notably during symbiosis.
[b]N.d., not determined.

associations with nodules and/or mycorrhiza-forming symbionts are currently available (*Glycine max*, *Lotus japonicus*, *Lycopersicon esculentum*, *Medicago truncatula*, *Nicotiana attenuata*, *Phaseolus vulgaris*, *Pisum sativum*, and *Vicia faba* [23]). Only a few of these plants have been used to assay the impact of this phenotype on the phytomicrobiome. Furthermore, these studies have mainly focused on taxonomic composition, for example the taxa in the phytomicrobiome, or taxonomic structure, such as the relative abundance of these taxa (Table 1). Among these, the most extensive study was performed in *M. truncatula* Gaertn. cv. Jemalong line J5 [wild type (WT), Myc^+Nod^+] and its symbiosis-defective mutants TRV48 (Myc^+Nod^-; affected in the gene *Mtsym15*) and TRV25 (Myc^-Nod^-; affected in the gene *DMI3*). Investigation of *M. truncatula* plants impaired in their ability to form one or both nodule or mycorrhizal symbioses revealed a strong impact of the presence/absence of the symbiont(s) on the taxonomic and functional structure of the phytomicrobiome [16,24,25]. Both **rhizosphere** and endophytic microbiota were affected by the absence of the symbionts in the double Myc^-Nod^- mutant, but this effect was not visible with the Myc^+Nod^- mutant, suggesting a differential impact of nodule-forming

Table 1. Studies Analyzing the Effects of the Presence/Absence of Symbionts on the Endophytic and Rhizospheric Microbiota[a,b]

Plant	Comparison	Approach	Effect observed	Refs
Plants impaired in their ability to enter symbiosis				
Nicotiana attenuata	WT plants and three mutated lines silenced in the expression of CCaMK	Endophytic microbiota analyzed by 16S and ITS sequencing	No visible effect on the fungal communities Stronger effects on the bacterial microbiota for the irCCaMK3 mutant	[65]
Oriza sativa	WT and two OsCCaMK mutants	Endophytic and epiphytic microbiota analyzed by 16S rRNA sequencing	Enrichment of Actinobacteria and Chloroflexi in the mutated lines relative to the WT Decrease of α- and β-Proteobacteria in the mutated lines relative to the WT	[66]
		Root and rhizosphere microbiota; gas measurements; *pmoA* and *mcrA* quantification	Significantly more methanotrophic bacteria in the root and rhizosphere soil of the mutant plant than for the WT Significantly higher CH_4 emission with the mutant plant than with the WT Similar methanotroph community composition between WT and mutant plants	[67]
Medicago truncatula Gaertn. cv. Jemalong	WT line J5 (Myc^+Nod^+) and its symbiosis-defective mutants TRV48 (Myc^+Nod^-; affected in gene *Mtsym15*) and TRV25 (Myc^-Nod^-; affected in gene *DMI3*)	ARISA analysis of endophytic and rhizosphere bacteria	Significant effects of the absence of symbionts on the taxonomic structure of the rhizosphere and endophytic microbiota in the Myc^- versus Myc^+ plants No effect visible when comparing Myc^+/Nod^- mutant plants and WT	[16]
		Culture-dependent approach and 16S rRNA genotyping on rhizosphere microbiota	Preferential association of the Comamonadaceae, Oxalobacteraceae (i.e., *Collimonas* spp.), and *Rubrivivax* spp. in Myc^+ plants compared to Myc^- plants	[24]
		Culture-dependent approach targeting *Pseudomonas*/T3SS genes	Significant enrichment of type III secretion system (T3SS)-carrying *Pseudomonas* spp. in the rhizosphere of mycorrhizal plants (Myc^+) than in non-mycorrhizal plants (Myc^-) or in the surrounding bulk soil	[25]
Glycine max [L.] Merr	WT line (Nod^+) and hypernodulated (Nod^{++}) and non-nodulated (Nod^-) lines	ARISA and cloning/sequencing of stem and rhizosphere microbiota	No visible effect on the stem microbiota Visible effect on the rhizosphere microbiota *Pseudomonas fluorescens* was exclusively found on Nod^+ plants, whereas *Micromonospora echinospora* and Sphingomonadaceae (α-Proteobacteria assigned to the genera *Sphingomonas* and *Novosphingobium*) were specific for Nod^- plants *Exidia saccharina* was enriched on Nod^- plants, whereas *Fusarium solani* was detected only on Nod^+ plants	[31]
		Culture-dependent approach and 16S rRNA genotyping on endophytic microbiota	Increased Rhizobiaceae and Sphingomonadaceae on Nod^- plants relative to Nod^+ plants Increased *Pseudomonas* spp. on Nod^+ versus Nod^- plants	[68]
Lotus japonicus	WT (ecotype Gifu B-129) and its symbiosis-defective mutants (Nod^-; 4 mutated lines: *nfr5-2*, *nfr5-3*, *nin-2*, and *lhk1-1*)	Rhizosphere, endosphere, nodule, and bulk soil microbiota analyzed by 16S rRNA sequencing	No differences between the microbiota associated with the different mutant lines Flavobacteriales, Myxococcales, Pseudomonales, Rhizobiales, and Sphingomondales appeared to be decreased in relative abundance in the symbiosis-defective mutants compared to the WT	[26]
	WT (ecotype Gifu B-129) and its symbiosis-defective mutants (mutated lines: *nfr5-2*, *ram1-2*, *symrk-3*, and *ccamk-13)*	Rhizosphere, endosphere, nodule, and bulk soil microbiota analyzed by 16S rRNA and ITS sequencing	Significant differences for both bacteria and fungi between the WT and symRK and ccamk lines Depletion of Glomeromycota-related taxa in the AM mutant lines	[27]

Table 1. (continued)

Plant	Comparison	Approach	Effect observed	Refs
Plant inoculated or not with a symbiont				
Alfalfa	WT plants inoculated or not with *Trichoderma harzianum*	Rhizosphere microbiota analyzed by 16S rRNA and ITS sequencing	Increased proportions of Ascomycota, *Pseudomonas*, *Kaitobacter*, and *Lysobacter* spp. following inoculation	[69]
Soybean	Two cultivars with or without inoculation of *Rhizobium* spp.	Rhizosphere and bulk soil microbiota analyzed by 16S rRNA sequencing	Changed microbial community structure following inoculation Increased proliferation of potential beneficial microbes following inoculation	[70]
Salvia officinalis L., *Lavandula dentata* L., and *Thymus vulgaris* L.	Plants inoculated or not with *Rhizophagus irregularis*	Rhizosphere microbiota analyzed by 16S rRNA sequencing	Modification of the bacterial communities Increased *Bacillus* spp. in presence of the symbiont Decreased Gemmatimonadetes in the non-inoculated rhizosphere	[71]
Dalbergia odorifera	Plants inoculated or not with *Bradyrhizobium elkanii* H255, *Rhizobium multihospitium*-like HT221, or *Burkholderia pyrrocinia*-like H022238	Rhizosphere and nodule microbiota analyzed by 16S rRNA sequencing	Significant alteration of the bacterial communities in the rhizospheres and nodules following symbiont treatment Increased *Lactococcus*, *Bacillus*, and *Pseudomonas* spp. in the rhizosphere of the symbiont-inoculated plants	[72]
Maize (Zea mays L. cv Cherif)	Plants inoculated or not with *Glomus mosseae* (BEG 107) or *Glomus intraradices* (BEG 110)	Soil and rhizosphere microbiota analyzed by 16S rRNA DGGE and measurements of global AP activity	Higher AP activity following symbiont inoculation Community structure modified in the rhizosphere and soil following symbiont inoculation Higher effect on the community structure when the two symbionts were coinoculated	[73]
Robinia pseudacacia	Plants inoculated or not with *Rhizobium* spp.	Rhizosphere and bulk soil microbiota analyzed by 16S rRNA sequencing	Increased proportion of the genera *Mesorhizobium, Variovorax*, *Streptomyces*, and *Rhodococcus spp. following inoculation* Increased number of genes encoding ATP-binding cassette transporters in the rhizosphere following inoculation Reduced number of genes related to sulfur/nitrogen metabolism in the rhizosphere following inoculation	[74]
Plant inoculated or not with a mycorrhizal helper bacteria				
Medicago truncatula	T3SS$^+$ mycorrhiza helper bacterium *Pseudomonas fluorescens* (C7R12) or a T3SS mutant of the strain.	Rhizosphere microbiota 16S rRNA and ITS sequencing	Increased root mycorrhization (especially Claroidoglomeraceae) following inoculation with the T3SS$^+$ strain Changed bacterial community structure following inoculation with the T3SS$^+$ strain	[30]

[a]The table lists studies dealing with the effects of the absence/presence of symbionts based on (i) experiments with plants impaired in their ability to form symbiosis, (ii) experiments where the symbiont was inoculated or not, and (iii) experiments where a mycorrhizal helper bacteria strain was inoculated or not. The observed effect of the treatment on the plant-associated microbiota is presented in each case.
[b]Abbreviations: AP, alkaline phosphatase; ARISA, automated ribosomal intergenic spacer analysis; DGGE, denaturing gradient gel electrophoresis; ITS, internal transcribed spacer.

symbiosis. Mycorrhizal plants displayed a preferential association with Comamonadaceae, Oxalobacteraceae (i.e., *Collimonas* spp.), and *Rubrivivax* spp., as well as an enrichment of type III secretion system (T3SS)-carrying *Pseudomonas* spp., relative to non-mycorrhizal plants [25]. Similarly, studies on mutant lines of *Lotus japonicus* impaired in different stages of nodulation showed that the level of perturbation of nodulation did not impact on the taxonomic structure and composition of the bacterial communities associated with the different mutant plants [26]. However, their phytomicrobiomes differed significantly from those of the WT (Table 1), and this was attributed to symbiosis-related metabolic changes between the WT and mutant plants as

alternative drivers of phytomicrobiome differentiation [26]. Further work confirmed the stronger impact on the phytomicrobiome for mutant lines affected in their ability to establish both mycorrhizal and nodule symbioses [27]. Although both mycorrhization and nodulation seem to impact on the phytomicrobiome, the differences reported suggest that these two compartments (i.e., mycorrhizae and nodule) do not impact on the phytomicrobiome in the same way or intensity. These results demonstrate that the absence of a single member of the phytomicrobiome (i.e., mycorrhizal symbiont) can strongly reshape the holobiont, affecting both the composition and function of the phytomicrobiome as well as plant growth [25]. Interestingly, work on *M. truncatula* suggests that mycorrhizal symbiosis has a stronger impact on the phytomicrobiome than does nodulation [16,24]. One may explain this difference by the fact that mycorrhizal fungi exert a stronger influence on the surrounding plant environment through the direct effects of the fungal mantle formed around the roots, which modifies soil properties and metabolites around the roots, and consequently the recruitment of bacteria to the hyphal network (e.g., fungal highway). Consistent with this view, the functional characterization of the taxonomic groups enriched in fungal environments demonstrated their ability to hydrolyze chitin, utilize oxalate, glycerol, or trehalose, or carry genes encoding T3SS, features that are poorly encountered in bulk soil bacterial communities [25,28]. Interestingly, T3SS genes, that are usually associated with pathogenic bacteria, were also found in non-pathogenic bacteria, and were demonstrated to play a role in fungal interactions and more especially in plant ectomycorrhizal or arbuscular mycorrhization [29,30]. A last important point relates to differences between the endophytic and rhizosphere microbiota in the presence/absence of the endospheric symbiont. Although many studies have reported that absence of the endospheric symbiont affects both the endophytic and rhizosphere microbiota (Table 1), this was not the case in other studies where only the rhizosphere microbiota was affected [31], suggesting that subtle regulatory effects differently drive the endophytic and rhizosphere microbiota.

What Can We Learn from Comparative Analyses of Natural and Inoculated Systems?

Another way to assess the impact of symbiosis on the phytomicrobiome is to analyze plants colonized by different symbiont species, and that are capable of entering symbiosis with more than one type of symbiont, some to acquire nitrogen based on nodule-forming bacteria (i.e., *Rhizobium* or *Frankia*), and some to acquire other inorganic nutrients (i.e., AM or EM fungi). Although AM fungi are able to colonize root nodules under laboratory conditions, such colonization was rarely observed *in situ*. Considering that different plants (*Lotus*, *Trifolium*, and *Ononis* spp.) grow naturally on sand dunes, Scheublin *et al.* [32] reported that AM fungal communities differed between roots with and without nodules. One hypothesis is that an overlap between signals associated with AM fungi and *Rhizobium* spp. symbioses prevents the later establishment of AM fungi [33]. Another may be related to the induction of plant defenses upon rhizobial infection, which could block further AM fungal colonization. Last, a priority effect may occur between the two symbionts, thereby determining community succession [34,35] in the root system on a 'first come, first served' basis. This is the case of *Frankia* spp. and EM fungi that compete for the roots of *Alnus* spp. trees, where actinorhizal nodules are formed before the establishment of EM fungi [34,35]. We observe that the community structure of EM fungi is a function not only of the age of *Alnus* trees [36] but also of the density of actinorhizal nodules on the root system. Because of variable primary symbiont colonization, this competition subsequently leads to diverging phytomicrobiomes, as revealed by comparison of the phytomicrobiomes associated with the root systems of the same plant colonized by different EM species [34–39]. For instance, young *Pinus sylvestris* seedlings grown in pots harbor specific phytomicrobiomes according to the EM fungal species (i.e., roots associated with *Russula* and *Piloderma* spp., *Meliniomyces variabilis*, and *Paxillus involutus*) which comprise common (i.e., *Burkholderia*) and EM

species-specific (i.e., *Actinospica*) bacterial genera [39]. Experiments based on controlled inoculation of plants with/without a specific microorganism such as a symbiont or a mycorrhizal helper bacterial strain are another means to determine the relative effects of the presence of the symbiont on the plant microbiota without potential bias related to genetic modification of the host plant (Table 1). Similarly, it is possible that endophytes can affect the phytomicrobiome. Indeed, some endophytes are vertically transferred, whereas others are acquired from the plant environment. Although most do not provoke apparent cell differentiation in the plant, several studies have pointed to a role in plant development and fitness [40]. Comparing poplars inoculated or not with endophytes (i.e., *Mortierella elongate* or *Ilyonectria europaea*), Liao *et al*. [41] reported that inoculated plants displayed better plant growth, transcriptional changes in poplar tissues, and different compositions of their phytomicrobiome relative to non-inoculated plants. Together, these comparisons highlight that the dynamics of root system colonization by symbionts (including endophytes) is important, and that the type of symbiont and/or the species strongly condition the taxonomic composition, and thus the function, of the phytomicrobiome.

Agrobacterium Tumors: A Molecular Demonstration of How Symbiosis Impacts on the Phytomicrobiome

The *Agrobacterium*/plant interaction is a very interesting system in which the plant cellular factory is reprogrammed to produce novel substrates, the opines [42], thereby creating a specific ecological niche for the pathogen (the opine concept [43]). Plant cell reprogramming in *Agrobacterium* tumors also involves major remodeling of the metabolome, with increased production in the tumor of more than 20 organic compounds, such as pyruvate and gluconate, whose production is increased by a factor of up to 5.10^5 relative to tumor-free plants [44], as well as the accumulation of signaling molecules, including plant hormones and bacterial signaling molecules such as *N*-acyl homoserine lactone produced by *Agrobacterium* spp., which diffuse in the surrounding environment of the plant and may impact on the surrounding phytomicrobiome. The reprogramming of the cell results from integration into the plant genome of only a few genes for the synthesis of plant hormones, leading to unlimited plant cell growth and the production of novel substrates. Interestingly, because this symbiosis is based on gene transfer into the genome of the plant, and not on the pathogen itself, it can be easily manipulated to generate axenic plants to assay the impact of *Agrobacterium*-induced plant reprogramming on the phytomicrobiome. Opines confer a fitness advantage *in vitro* and *in vivo* on bacteria that are able to metabolize these molecules [45], and a clear reshaping of the phytomicrobiome can be observed irrespective of which specific opine is used [45–47]. The modifications impact on community composition, and moreover on its functional structure, because specific microorganisms are selected and increase significantly in abundance [45,47]. These only partly correspond to bacteria that are able to utilize opines newly produced by the host plant. In the field, the microbiome of the crown gall tumor also differs significantly from that of the healthy plant in composition, richness, and dynamics [48]. Thus, by directly and indirectly modifying the capacity of the plant cell to produce organic molecules and to secrete them into the extracellular space, this endospheric interaction illustrates how the establishment of symbiosis (here a detrimental symbiosis) can reshape the phytomicrobiome by modifying plant signals and/or reprogramming cell exudates. We describe this cascade of effects in the plant and the symbiont as 'symbiotic cascade effects' (Box 2), in which the symbiont reshapes the phytomicrobiome through direct and indirect effects on the plant. Of course, the mechanisms involved (gene regulation, metabolites, signals) may strongly differ from one symbiont to another, and differ according to the host plant. Whether and how this is controlled by, or affects, the health of the plant remains open question.

Concluding Remarks and Future Perspectives

For decades, the ability of plants to grow and adapt to extreme and dynamic conditions has been attributed to their functional versatility. It is now clear that this depends on the ability of the plant to establish interactions (sometimes symbiotic) with specific bacteria and/or fungi recruited from their environment or vertically transferred (e.g., from seeds), and possibly also Archaea, as well as on interactions between microorganisms [49]. We propose here a new paradigm that we term 'symbiotic cascade effects', which proposes that the plant and its environment are not the only engineers of the phytomicrobiome, and that members of the phytomicrobiome such as the symbionts also play a major role (Box 2 and Table 1). Recent findings suggest that these symbiotic cascade effects may be extended to other microorganisms such as endophytes [41]. Modifications of the plant microbiota can result from direct actions of the symbionts through priority effects, competition for the same ecological niche, or the production of signaling molecules, new metabolites, or the modulation of plant signaling. The priority effect – the sequential arrival of microbial populations in the vicinity of the root system – is a strong driver of phytomicrobiome structure and composition that has been demonstrated in several plant systems. However, it is also clear that a plant impaired in its ability to enter symbiosis does not react in the same way to the presence of bacteria in its vicinity. This is visible in the transcriptomic response of the plant, where several signal transduction pathway genes are expressed in the WT, but only one is expressed in $Myc^{-}Nod^{-}$ mutants [50], suggesting attenuation of the plant response in the absence of symbionts. This has strong implications for our understanding of the holobiont because it means that the presence/absence of a symbiont conditions the holobiont. Similarly, mycorrhizal establishment is known to modify the balance of immune molecules. In this view, JA is strongly suspected to be a key molecule driving selection of the phytomicrobiome [51–54]. Indeed, although addition of JA to soil microcosms planted with *Arabidopsis thaliana* significantly impacts on the establishment of rhizosphere communities, JA has no effect on the microbiota in the surrounding bulk soil. JA, salicylate, and nitrite oxide also induce important modifications in the metabolite composition of plant rhizosphere exudates, and specific molecules such as kaempferol-3-O-β-d-glucopyranoside-7-O-α-l-rhamnoside have been reported [51]. In addition to JA, many other signals and metabolites are produced during microbe–microbe and microbe–plant interactions that may be involved in symbiotic cascade effects [50]. Their identities and relative roles remain to be determined. Last, the impact of symbionts on the phytomicrobiome can also be indirect, for example via environmental changes. Indeed, mycorrhizal fungi are known to increase soil aggregation around roots, leading to improved stability of the soil matrix and physicochemical changes (e.g., resource depletion), and nodules are known to enrich the surrounding bulk soil in nitrogen. Experiments on symbiosis-deficient versus WT plants have demonstrated that a complex cascade of events takes place in response to symbiosis, leading to modifications of the taxonomic and functional structure of the phytomicrobiome. The question is now to identify the mechanisms by which these modifications are driven (see Outstanding Questions). Discussion has mainly focused on the effects of endospheric symbionts colonizing the root system because this is so far the only system in which experimental data are available. However, during the establishment of a microbial community at the plant/environment interface a molecular dialogue takes place between the plant and the newcomers. The depth of the dialogue will depend not only on the types of organisms but also on the duration of the interaction (i.e., short or long term). This dialogue triggers modifications in the plant and/or the phytomicrobiome, which in turn can impact on the relationships of the plant with its phytomicrobiome. Further studies combining environmental genomics and microbiology, plant physiology, and metabolomics will be necessary to advance in this direction. Progress in this field would open new perspectives in understanding and engineering the phytomicrobiome and its performance (see Outstanding Questions).

Outstanding Questions

Are endospheric symbionts the keystone or hub species that drive the rest of the plant-associated microbial communities? Does this role to extend to all plant symbionts?

Are phytomicrobiome modifications induced by the symbiont mainly explained by direct or indirect effects on (i) the architectural modification of the roots, (ii) competition for a specific niche, (iii) modification of the soil physicochemical properties, (iv) the production of new metabolites and signals, or (v) activation of plant immune and defense system (i.e., ethylene, JA, salicylate)? How can all these potential effects be disentangled?

Do different plant species and symbionts employ common mechanisms to shape the plant-associated microbiome (i.e., the phytomicrobiome)? Are these mechanisms adapted according to nutrient availability?

Although endophytic microorganisms represent a low biomass relative to the symbionts and free-living microbiota colonizing the rhizosphere, do they play a role in the symbiotic cascade effects proposed here?

There have been several initiatives to use rhizosphere microorganisms to improve plant productivity. Can we take advantage of symbiotic cascade effects to (i) increase plant production, (ii) decrease the agronomic use of chemical supplements, or (iii) improve soil health? Can we predict the consequences of symbiotic cascade effects on the phytomicrobiome and plant productivity?

The evolution of eukaryotes is intimately linked to the development of symbiosis. Deciphering the molecular bases of symbiotic cascade effects will permit better understanding of the relationships between plant and microbes. Can we build on this intimacy to engineer novel obligate symbionts to improve plant health and growth?

Plants impaired in their ability to enter symbiosis represent a very promising tool to better understand the relationship between symbionts and the phytomicrobiome. However, we need to better understand the effect(s) of

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such mutations not only on plant physiology but also on their interactions with the soil microbiota. Are these mutant lines affected in their susceptibility to pathogens?

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