Plant Symbionts Are Engineers of the Plant-Associated Microbiome

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Abstract:

Throughout their life plants interact with environmental microorganisms. The realization that these interactions determine plant development, nutrition and fitness in a dynamic and stressing environment is the basis for the holobiont concept, in which plants and plant-associated microbes are not considered as independent entities, but as a whole evolutionary unit. The main question remaining open concerns the dynamic of this holobiont and whether it is modeled by microbial members of this holobiont or solely by the plant. Interpreting our current knowledge of plant-microbe interaction, and especially the plant endosphere, we can show that the establishment of symbiosis directly and indirectly conditions the plant-associated microbiome, e.g. that microbes within the holobiont shape the structure and composition of the phytomicrobiome, and thus shape the structure of the holobiont. We propose to define the impact of the symbiont on the plant microbiome as the 'symbiosis cascade effect', in which symbionts and their plant host jointly shape the plant microbiome.

Key words: symbiosis, endosphere, mutualistic, parasitic, plant, microbiome, phytomicrobiome, interaction, ecological engineer, cascade effect, phytobiome

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 mutualistic (positive impact), commensalistic (no visible impact), or parasitic (negative impact). Many of these microorganisms are recruited from the plant environment, while others are vertically transferred such as the endophytes contained into the 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 both. Rarely have we considered how the establishment of the symbiont and response of the plant influence the recruitment of the environmentally recruited, plant-associated microbiota (the phytomicrobiome) and its functioning. This is no surprise considering that the importance of the phytomicrobiome on plant health has only been demonstrated recently and that phytomicrobiome composition is mainly determined by extrinsic factors (*e.g.* soil conditions, climate, culture management practices; [3]), but also by intrinsic factors (*e.g.* vertical transfer through seeds, plant characteristics, plant organs and plant-microbial interactions; [4-7])(**Figure 1**). Nonetheless, identifying the driving-factors (*e.g.* keystone species, metabolites) explaining the assembly and composition of the phytomicrobiome is still a challenge and a key question to understand the holobiont dynamic.

What is the role of symbionts? Although symbionts are members of the phytomicrobiome, are they intrinsic or extrinsic drivers of phytomicrobiome composition and of the phytobiome? How do symbiont interactions and the dynamic of their establishment impact the rules of assembly the phytomicrobiome? Symbionts strongly modify plant ecophysiological traits, colonize part of the plant tissues and modify the local soil properties. Furthermore, symbioses are known to modify plant signals (*e.g.* strigolactone), hormones (*e.g.* auxin), the immune system (*e.g.* jasmonate signaling pathway) and exudates compositions (*e.g.* trehalose, glucosamine derivatives). In this opinion paper, we describe how the molecular dialog between the symbionts shapes the taxonomic and functional structures and the functioning of the phytomicrobiome, defining symbiotic organisms as ecological engineers of the phytomicrobiome. To exemplify this concept, we have taken examples from the best-documented symbioses, *e.g.* the endospheric symbioses, either mutualistic or detrimental, since they are the only ones which 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 as all close and long term interactions between plants and microorganisms. In symbiosis, interaction is the key notion. Symbionts exert influence on one another, and express a reciprocal dialog, which eventually leads to modifications of the partners, but not necessarily. In this view, the notion of symbiosis *de facto* excludes organisms which presence in the vicinity of the plant is due solely to hazard and their spatial repartition in the environment, which display no interactions with the plant, in the same way that a bird resting on a telegraph pole cannot be considered a symbiont of the pole, but a bird nesting in, or feeding from, a tree could be. The most emblematic and ultimate symbioses remain the (chloro)plasts and mitochondria, which correspond to long co-evolution processes between the eukaryotic cell and symbiotic bacteria. *Per se*, symbioses are not necessarily beneficial to the host. Amongst plant-pathogen relationships, the example of *Agrobacterium tumefaciens*, the causal agent of crown gall disease, is emblematic of 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) or the mutualistic association between mycorrhizal fungi (*e.g.*, ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) fungi and grass or trees).

How does symbiosis affect plant and symbiont partners?

From the plant perspective, interactions with symbionts modify intracellular and intercellular communications, expression of hundred of genes (**Box 1**), and diversity and quantities of exudated metabolites [8,9], but also cell and tissue structure. These aspects have been described in depth for endospheric symbioses. The modifications begin by an increase of intracellular calcium few seconds to minutes after the interaction with symbionts. Formation of novel or mixed organs can be observed as in the nodules formed by *Rhizobium* or in the mycorrhizal roots formed by symbiotic fungi. In these hybrid structures, the mycelium forms a specific network in the apoplastic space, allowing for nutritive exchanges between the host plant and the fungus and some metabolic reorientation, *i.e.* a decrease of starch and sucrose, and an increase of trehalose and mannitol production, as well as an increase in respiration [10] or the accumulation of oxalate around mycorrhizal roots. Metabolic reprogramming is also emblematic of the gall-forming *A. tumefaciens* infection which leads to the production of *Agrobacterium* specific amino-acid derivatives, the opines, but also to a large remodeling of the plant resource allocation (translocation of nutrients and water) to the benefit of the tumor, and the accumulation

of dozen other carbon sources [11-13]. During the *Rhizobium* and *Frankia* infection, root cells are differentiated to form nodules, in which low-oxygen and carbon rich conditions occur.

From the microbial side, cellular and genomic differentiation can occur. Upon induction of symbiosis, the bacterial cell will undergo multiple rearrangements to create specialized cells. In plant root nodules, colonized by nitrogen-fixing rhizobia, bacteria form immobile, larger cells with higher nitrogenase activity (*i.e.*, the 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 the pathogenic plasmids. Last, obligate symbionts such as mitochondria, plasts and mollicutes for examples have both morphological and extensive genomic optimizations.

In most cases, plants associated with symbionts such as mycorrhizal fungi or nodule forming rhizobacteria present a higher biomass, hence the general terminology of plant growth promoting rhizobacteria (PGPR). *Medicago truncatula* Nod^-Myc^- mutants incapable of forming mycorrhizae and nodules, have a loss of biomass that can reach up to 90% compared to the Nod^+Myc^+ colonized plants [16]. Interestingly, the host-plants seem capable of selecting the most effective symbionts, *e.g.* rhizobia with the higher nitrogenase activity, although the effectiveness-driven selection remains to be confirmed [17,18]. Similarly, during the AM symbiosis, plant and AM fungi set up a reciprocal **"** fair trade**"** [19], but this text book picture is highly variable and probably depends on the plant species, the plant genotype and the AM fungal species [20,21].

In an evolutionary perspective, we are far from knowing all the cellular modifications induced during the endosphere symbiotic association whether recent, such as mycorrhiza, or ancient, such as mitochondria/chloroplasts. Our current knowledge points out at changes in hormone production (auxin, strigolactone) and exudate composition, immune system adjustment (salicylate, jasmonate), volatiles, *i.e.* all compounds potentially involved in the complex dialogue with the phytomicrobiome. The extent of modifications in metabolite production induced during the *Agrobacterium* infection is a clear example that subtle modifications in the metabolite or hormone balance can lead to important modifications of the metabolome and signalome of the plant, and therefore of its interactome. In addition, the symbiosis establishment also yields modifications of the physico-chemical properties of the soil (*i.e.*, variation of pH, higher content of N or trehalose, soil aggregation…).

Impact of symbiosis on the phytomicrobiome

The possibility that plant endospheric symbionts may be keystone organisms capable of modifying their environment (*i.e.*, the phytobiome) has recently been predicted [**22**], but without experimental demonstration. In nature, no symbiont-free plants exist. Hence, naturalistic approaches are ill suited to study the impact of the onset of symbiosis. However, experimental comparative analyses of plants impaired or not in their symbiotic capabilities, in presence or absence of symbionts or colonized by different symbionts can help decipher the relative role of endospheric symbionts on the phytomicrobiome and on the evolution of the holobiont.

What can we learn from plants impaired in their ability to form symbiosis?

One elegant way to observe the potential impact of symbionts on the phytomicrobiome is to use plants impaired in their ability to associate with symbionts. To do so, several plants incapable of forming symbiotic association with nodule and/or mycorrhizal forming symbionts are currently available (*Glycine max*, *Lotus japonicus, Lycopersicon esculentum, M. trunculata, Nicotiana attenuata, Phaseolus vulgaris, Pisum sativum or 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 mainly focused on the taxonomic composition, *e.g.* the taxa in the phytomicrobiome, or structure, *e.g.* the relative abundance of these taxa (**Table 1**). Amongst these, the most extended study was performed in the *M. truncatula* Gaertn. cv. Jemalong line J5 (Wild type (WT), Myc^+ Nod^+) and its symbiosis-defective mutants TRV48 (Myc^+Nod^-; affected on the gene Mtsym15) and TRV25 (Myc^-Nod^-; affected on the gene DMI3). The monitoring of *Medicago truncatula* plants impaired in their ability to form one or both nodule- or mycorrhizal symbioses showed a strong impact of the presence/absence of the symbiont(s) on the taxonomic and functional structures of the phytomicrobiome [16,24,25]. These studies demonstrated that 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 symbiosis. Mycorrhizal plants were characterized by a preferential association with *Comamonadaceae*, *Oxalobacteraceae* (*i.e. Collimonas*) and *Rubrivivax* and an enrichment of type III secretion system (T3SS) carrying *Pseudomonas* in comparison to the non-mycorrhizal plants [25]. Similarly, the monitoring of mutant lines of *Lotus japonicus* impaired at different stage of nodulation, showed that the level of perturbation of nodulation did not impact the taxonomic structure and composition of the bacterial communities associated to the different mutant plants [26]. However, their phytomicrobiome differed significantly from that of the WT (**Table 1**), which was attributed to symbiosis-related metabolic changes between the WT and mutant genotypes 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 (http://dx.doi.org/10.1101/547687). While mycorrhization and nodulation seem to impact the phytomicrobiome, the differences reported suggest that these two compartments (*i.e.*, mycorrhizae and nodule) do not impact the phytomicrobiome in the same way or intensity. These results demonstrate how 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 and the plant growth. [25]. Interestingly, the work done on *M. truncatula* suggests that mycorrhizal symbiosis has a stronger impact on the phytomicrobiome than 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 effect of the fungal mantle formed around the roots, which modifies soil properties and metabolites found around the roots, and consequently the recruitment of bacteria by the hyphal network (*e.g.*, fungal highway). Consistent with this view, the functional characterization of the taxonomic groups enriched in fungal environments evidenced their ability to hydrolyze chitin, utilize oxalate, glycerol or trehalose, or carry T3SS genes, abilities poorly encountered in bulk soil bacterial communities [25,27]. Interestingly, the T3SS gene, usually associated with pathogenic bacteria, was also found in non-pathogenic bacteria and was demonstrated to have a role in fungal interactions and more especially in plant ectomycorrhizal or arbuscular mycorrhization [28,29]. A last important point is related to the differences observed between the endophytic and rhizosphere microbiota in presence/absence of the endospheric symbiont. While many studies reported an effect of the absence of the endospheric symbiont on both the endophytic and rhizosphere microbiota (**Table 1**), it was not the case in others where only the rhizosphere microbiota was affected [30], suggesting that subtle regulations drive differently the endophytic and rhizosphere microbiota.

What can we learn from comparative analyses of natural and inoculated systems?

Another way to determine the impact of symbiosis on the phytomicrobiome is to analyze plants colonized by different symbionts species and capable of forming 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. While AM fungi are able to colonize root nodules in laboratory conditions, such colonization was rarely observed *in situ*. Considering different plants (*Lotus, Trifolium* and *Ononis*) growing naturally in sand dunes, Scheublin *et al.* [31] reported that the AM fungal communities varied between roots with and without nodules. One hypothesis is that the overlap existing between the signals of the

AM fungi and *Rhizobium* symbiosis prevents the later establishment of AM fungi [32]. Another one may be related to the induction of plant defenses upon rhizobial infection, which blocks further AM fungal colonization. Last, a priority effect may occur between the two symbionts determining community succession [33,34] on the root system in *a* 'first come, first served' basis. This is for example the case of *Frankia* and mycorrhizal fungi that compete for the roots of *Alnus* trees. Actinorhizal nodules are formed prior to mycorrhizal fungi establishment [33,34]. We observe that the community structure of EM fungi is function of the age of *Alnus* trees [35], but also of the density of actinorhizal nodules on the root system. Leading to a variable primary symbiont colonization, this competition subsequently leads to diverging phytomicrobiomes as evidenced in comparisons of the phytomicrobiomes associated to the root system of the same plant, but colonized by different ectomycorrhizal species [33-38]. For instance, young *Pinus sylvestris* seedlings grown in pots harbor specific phytomicrobiomes according to the EM fungal species (*i.e*., roots associated to *Russula* sp., *Piloderma* spp., *Meliniomyces variabilis* and *Paxillus involutus*) which comprise common (*i.e*., *Burkholderia*) and EM species-specific (*i.e., Actinospica*) bacterial genera [38]. Experiments based on controlled inoculation of plants with/without a specific microorganism such as a symbiont or a mycorrhizal helper bacterial strains represent another alternative to determine the relative effect of the presence of the symbiont on the plant microbiota without the potential bias related to the genetic modification of the host-plant (**Table 1**). Similarly, we can wonder if endophytes can affect the phytomicrobiome. Indeed, some of these endophytes are vertically transferred, while others are acquired from the plant environment. Although most of them do not provoke apparent cell differentiation in the plant, several studies pointed out their role in plant development and fitness [39]. Comparing poplar inoculated or not with an endophyte (*i.e*., *Mortierella elongate* or *Ilyonectria europaea*), Liao *et al*. [40] reported that plants, inoculated with the endophyte presented a better plant growth, transcriptional changes in the poplar tissues and different compositions of their phytomicrobiome than non-inoculated plants. Altogether, these comparisons highlight that the dynamic of colonization of the root system by symbionts (even 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 tumor : a molecular demonstration of how a symbiosis impacts 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 [41], creating a specific ecological niche for the pathogen (the opine concept, [42]). Plant cell reprogramming in the *Agrobacterium tumor* also implies a larger remodeling

of the metabolome, with the increased production in the tumor of more than 20 other carbon compounds, such as pyruvate, gluconate, which production is increased up to 5.10^5 times compared to the plant without tumor [43], and the accumulation of signal molecules, plant hormones and bacterial signaling molecules, such as N-acyl homoserine lactone produced by *Agrobacterium*, which diffuse in the surrounding environment of the plant and may impact the surrounding phytomicrobiome. The short-circuiting of the cell results from the integration into the plant genome of only a few genes for the synthesis of plant hormones, which leads to an unlimited growth of the plant cell, and for the production of the novel substrates. Interestingly, as this symbiosis is based on gene transfer into the genome of the plant but 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 give a fitness advantage *in vitro* and *in vivo* to the bacteria capable of metabolizing these molecules [44]. One can observe a clear reshaping of the phytomicrobiome whichever opine is used [44-46]. The modifications impact the community composition, but moreover its functional structure, since specific microorganisms are selected and increase significantly in proportion [44,46]. These correspond only in part to bacteria able to utilize opines newly produced by the plants. In the field, the microbiome of the crown gall tumor also differs significantly from that of the healthy plant in both composition, richness and dynamics [47]. Thus, by directly and indirectly modifying the capacity of the plant cell to produce carbon molecules and to secrete them in the extracellular space, this endospheric interaction perfectly illustrates the possibility that exists during the establishment of a symbiosis (here a detrimental symbiosis) to reshape the phytomicrobiome, through the modification of plant signals and/or reprogramming of cell exudates. This cascade of effects in the plant and the symbiont is what we termed the ‘symbiotic cascade effects' (**Box2**), in which the symbiont through its direct and indirect effects on the plant reshapes the phytomicrobiome. Of course, the mechanisms involved (gene regulation, metabolites, signals) may strongly differ from a symbiont to another or depending of the host-plant. Whether and how, this is controlled by, or affects, the plant health *in fine* is still an open question.

Concluding remarks and future perspectives:

For decades, the ability of plants to grow and adapt to extreme and dynamic conditions has been linked to their functional versatility. Today, it is obvious that it largely depends on their ability to establish interactions (sometimes symbiotic) with specific bacteria and/or fungi recruited from their environment or vertically transferred (*e.g.*, from the seeds), and possibly archaea and on the interactions between microorganisms [62]. Here, we propose a new paradigm that we termed the ‘symbiotic cascade effects’, which proposes to consider

not only the plant and its environment as engineers of the phytomicrobiome, but also members of the phytomicrobiome such as the symbionts (**Box 2, Table 1**). Recent findings suggest that these symbiotic cascade effects may be extended to other microorganisms such as endophytes [40]. Modifications of the plant microbiota can be due to a direct action of the symbionts, through a priority effect, a competition for the same ecological niche or through the production of signal molecules, new metabolites or the modulation of plant signaling. The priority effect, *e.g.* the sequential arrival of microbial populations in the vicinity of the root system, is a strong driver of phytomicrobiome structuration and composition which has been demonstrated in several plant systems. However, it is also clear that a plant impaired in its ability to form a symbiosis does not react in the same way to the presence of bacteria in its vicinity. This is already visible in the plant transcriptomic response of the plant which expresses several genes of the signal transduction pathway in the WT, but only one in the Myc^-Nod^- mutants [48], suggesting an attenuation of the reaction of the plant when symbionts are absents. This observation has strong implications on our understanding of the holobiont, as 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, jasmonic acid (JA) is strongly suspected to be a key molecule driving the selection of the phytomicrobiome [49-52]. Indeed, while JA addition in soil microcosms planted with *Arabidopsis thaliana* significantly impacts the rhizosphere communities, no effect is observed on those from the surrounding bulk soil. JA, salicylate, or nitrite oxide also induce important modifications of the metabolites exudated in the rhizosphere of plants, with the presence of specific substrates such as for example kaempferol-3-O-β-d-glucopyranoside-7-O-α-l-rhamnoside [49]. Beside JA, many other signals and metabolites produced during microbe-microbe or microbes-plants interactions may be involved in the symbiotic cascade effect [48]. Their identity and their relative role remain to be determined. Last, the impact of symbionts on the phytomicrobiome can also be indirect through environmental changes. Indeed, mycorrhizal fungi are known to increase soil aggregation around roots allowing for a better stability of the soil matrix and physico-chemical changes (*e.g*. resource depletion), while nodules are known to enrich the surrounding bulk soil in nitrogen. Based on the experiments done with plants impaired in their ability to form symbiosis compared to their wild-type relatives, we know that a complex cascade of events occurs allowing for modifications of the taxonomic and functional structures of the phytomicrobiome. The question is now to identify the mechanisms by which these modifications are driven (see Outstanding questions). The demonstration provided here is mainly focused on the effect on the phytomicrobiome of endosphere symbionts colonizing the root system because they are the only systems for which some data exists. However, during the establishment of any microbial community at the plant/

environment interface a molecular dialog will take place between the plant and the new comers. The depth of the dialog will depend on the type of organisms, but also on the duration of the interaction (*i.e.,* short or long term). This dialogue will trigger modifications in the plant and/or the phytomicrobiome, which in turn will potentially impact the relationships of the plant with its phytomicrobiome. Whatever the symbiont and the plant compartment, more studies combining environmental genomics and microbiology, plant physiology, metabolomics are required to progress in this direction. Progressing in this field would permit to open new perspectives in the prediction and engineering of the phytomicrobiome and its performances.

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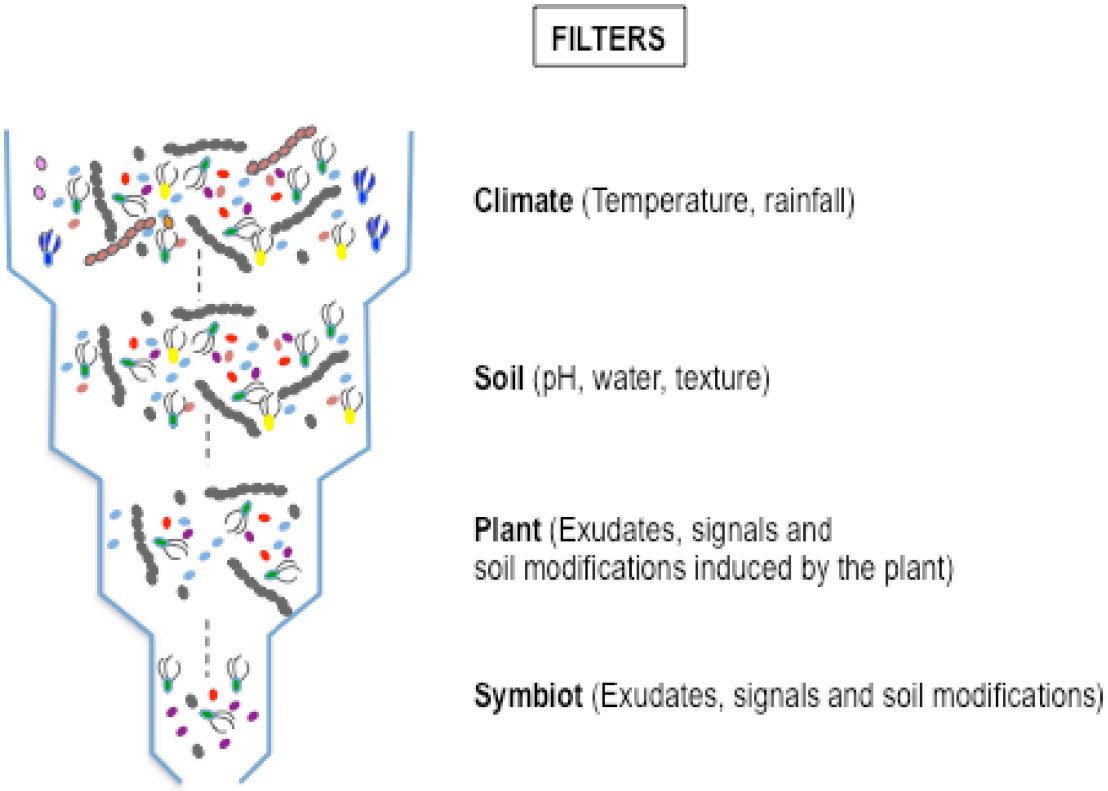
FIGURES, TABLES AND BOX

Figure 1: Known or suspected environmental drivers of the taxonomic and functional structures of the phytomicrobiome. Here are presented the different environmental filters (and the related factors) highly suspected to drive the structuration of the plant-associated microbiota. The last filter presented corresponds to the symbiont effect discussed in this manuscript. The different forms visible represents different microorganisms which composition is modified by the different filters and at each step from the top to the bottom.



Box 1. Gene locks acting on the establishment of symbiosis and effects on the main molecules produced by plants on symbionts.

Gene regulation is (i) different between plants when colonized by the same EM fungus (i.e., *Populus trichocarpa* and *Pseudotsuga menziesii* colonized by *Laccaria bicolor*; [53]), (ii) different between plant tissues (the Hartig net vs the mantle in *Tuber melanosporum-Coryllus avellana*; [54]), and (iii) different for the same EM fungus when colonizing two distinct plants [53]. The main steps of the interactions between symbionts and the host plant are presented in **Figure I**. The formation of AM symbiosis and nodules starts similarly, trough the common symbiotic signaling pathway (CSSP). A subset of these genes is essential for either the generation or the decoding of calcium-spiking: a nuclear calcium and calmodulin-dependent protein kinase called DMI3 [55,56]. These genes control transcriptions factors including Nodulation Signaling Pathway 1 (NSP1) and Required for Arbuscular mycorrhization 1 (RAM1) involved in nodulation and mycorrhization, respectively [57,58].

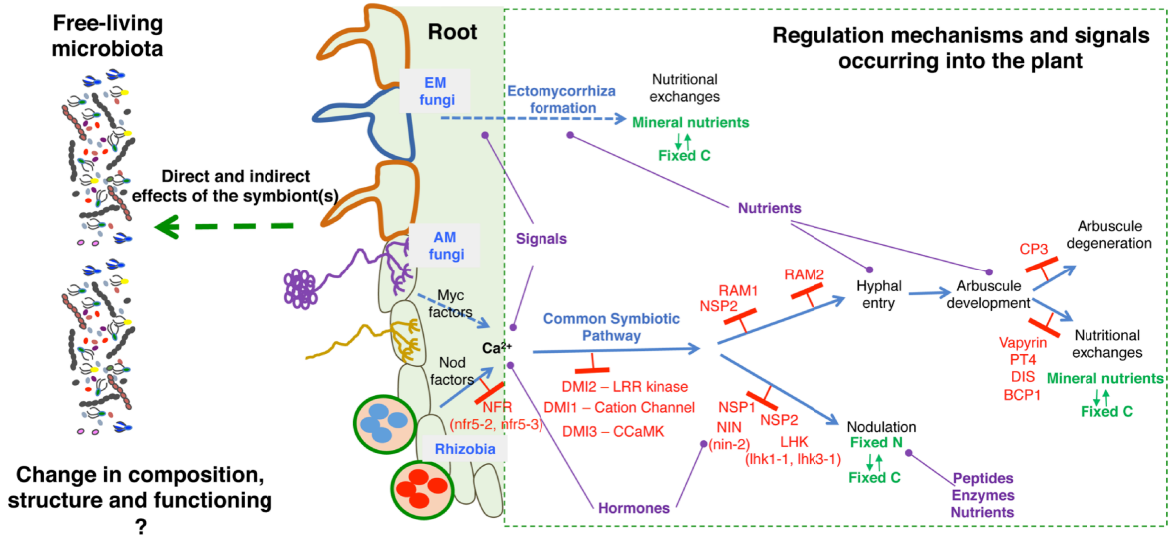


Figure I. Regulation pathways involved in plant/symbiont interactions

Although rhizobia and AM fungi share the same pathway, they harbor specific features [59, 60]. The development and spread of AM fungi within the root is predominantly under the control of the host plant and depends on its developmental and physiological status. Notably, DIS, RAM1, BCP1, RAM2 and PT4 are required for arbuscule development, whereas a Cysteine Protease (CP3) is required for arbuscule degeneration [61]. In EM symbiosis, root hairs could be colonized by different fungi (brown and blue cell). The set of genes involved in the formation/degeneration of arbuscules or nodulation are well known and characterized (blue arrow), except the steps before the CSSP for AM symbiosis (blue dashed arrow). The red locks correspond to genes for which mutation leads to the way-out of the symbiotic organ formation. The main molecules produced by the plants (purple arrow) could act on physiological hub. All these known regulation mechanisms represent potential drivers of the structuration of the phytomicrobiome. Interestingly, the effect of the deregulation of some of these pathways on the phytomicrobiome has already been tested (Table 1).

Box 2: The 'symbiotic cascade effects' or how symbiont establishment affects and drives the phytomicrobiome.

Here, we present the cascade of events, which allows the structuration of the plant-associated microbiome. At each of the different steps of the plant-symbiont interaction, the microbiota can be impacted. **1)** As soon as symbionts interact with the host-plant (*i.e.*, at the pre-symbiotic stage or at the seed germination stage for seed endophytes) through signal molecules and physical contact, physiological changes are induced in the plant and competition occur for the plant tissues with the free-living microbiota. **2)** During symbiont establishment, the physiological changes in the plant are amplified and structural changes can appear (*i.e.*, nodule or mycorrhiza formation). **3)** During symbiosis, the metabolites (carbohydrates, hormones, signals, volatiles) produced by the plant and potentially exudated are modified quantitatively and/or qualitatively (*i.e.*, new metabolites are produced due to the symbiont) as well as the plant defense response. **4)** The impact of the plant on the soil parameters differs from the plant non-associated to symbionts. All these modifications impact the taxonomic and functional structuration of the phytomicrobiome as well as its functioning, and *in fine* the plant fitness.

Table I: Main molecules produced by the plant with and without symbionts, which could drive modifications of the phytomicrobiome. The table is a non-exhaustive list of metabolites involved in the structuration of the phytobiome by the host-plant and/or the symbionts. Here, we mainly listed the metabolites produced only in presence of the symbionts or which concentrations change notably.

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 Ferulate-trans Gulonate 4-hydroxyproline Nicotianamine Melezitose Spermidine Lactobionate
Signals	Calcium, ethylene Jasmonate Sesquiterpene	Flavoinoids, Phenolic acids	- Acyl homoserine lactone (AHL)
Hormones	Hypaphorin (Tryptophan betain)		
Peptides	Mycorrhizal induced secreted proteins (MISP)	Nodule specific cysteine rich Rhizobial factors	
Enzyme		1-aminocyclopropane-1-carboxylate (ACC) deaminase (*acdS*)	

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Table 1: Studies analyzing the effect of the presence/absence of the symbionts on the endophytic and rhizospheric microbiota.

The table lists studies dealing with the effect 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. In each case the observed effect of the treatment on the plant-associated microbiota is presented.

Plant	Comparison done	Approach	Effect observed	References
Plant impaired in their ability to form symbiosis				
Nicotiana attenuata	WT plants and 3 mutated lines silenced in the expression of **CCaMK**	Endophytic microbiota analyzed by 16S and ITS sequencing	- No visible effect on the fungal communities - Stronger effect on the bacterial microbiota for the irCCaMK3 mutant	[63]
Oriza sativa	WT and **2 OsCCaMK** mutants	Endophytic and epiphytic microbiota analyzed by 16S rRNA sequencing	- Enrichment of Actinobacteria and Chloroflexi in the mutated lines vs WT - Decrease of α- and β-Proteobacteria in the mutated lines vs the WT plant.	[64]
		Root and rhizosphere microbiota; Gaz measurement; *pmoA* and *mcrA* quantification	- Significantly more methanotrophic bacteria in the root and in the rhizosphere soil of the mutant plant than with the WT plant - Significantly higher CH_4 emission with the mutant plant than with the WT plant - Similar methanotroph community composition between WT and mutant plants.	[65]
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 approach on endophytic and rhizosphere bacteria	- Significant effect of the absence of the symbionts on the taxonomic structure of the rhizosphere and endophytic microbiota in the Myc^- plant vs Myc^+ plant - No effect visible when comparing the Myc^+/Nod^- mutant plant and the WT plant	[16]
		Culture-dependent approach and 16S rRNA genotyping on rhizosphere microbiota	Preferential association of the *Comamonadaceae*, *Oxalobacteraceae* (i.e. *Collimonas*) and *Rubrivivax* in the Myc^+ plant compared to the Myc^- plant	[24]

		Culture-dependent approach targeting *Pseudomonas* / T3SS gene	- Significant enrichment of type III secretion system (T3SS) carrying *Pseudomonas* in the rhizosphere of mycorrhizal-plant (Myc^+) than in non-mycorrhizal plants (Myc^-) or in the surrounding bulk soil.	[25]
Glycine max [L.] Merr	WT line (Nod^+) and hyper-nodulated (Nod^{++}) and non-nodulated (**Nod^-**) lines	ARISA and cloning/sequencing on stem and rhizosphere microbiota	- No visible effect on the stem microbiota -Visible effect on the rhizosphere microbiota - *Pseudomonas fluorescens* exclusively found on nod+ plant, while *Micromonospora echinospora* and *Sphingomonadaceae* (α-proteobacteria assigned to the genus *Sphingomonas* and *Novosphingobium*) appeared specific of the nod- plants - *Exidia saccharina* enriched on nod- plants, while *Fusarium solani* detected only on nod+ plants	[30]
		Culture-dependent approach and 16S rRNA genotyping on endophytic microbiota	- Increase of *Rhizobiaceae* and *Sphingomonadaceae* on nod- plants vs nod+ plant - Increase of *Pseudomonas* on the nod+ plant vs nod- plant	[66]
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 analysed by 16S rRNA sequencing	- No differences between the microbiota associated to the different mutant lines - *Flavobacteriales*, *Myxococcales*, *Pseudomonales*, *Rhizobiales* and *Sphingomondales* appeared 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*	Rhizosphere, endosphere, nodule and bulk soil microbiota analysed by 16S rRNA and ITS sequencing	- Significant difference for both the bacteria and fungi between the WT and symRK and ccamk lines. - Depletion of Glomeromycota related	dx.doi.org/10.1101/547687

	and ccamk-13)		taxa in the AM mutant lines	
Plant inoculated or not with a symbiont				
Alfalfa	WT inoculated or not with *Trichoderma harzianum*	Rhizosphere microbiota analysed by 16S rRNA and ITS sequencing	- Increase of the proportion of Ascomycota and *Pseudomonas*, *Kaitobacter* and *Lysobacter* in the inoculated treatment.	[67]
Soybean	2 cultivars with or without inoculation of *Rhizobium*	Rhizosphere and bulk soil microbiota analysed by 16S rRNA sequencing	- Change of the microbial community structure when inoculated. - Increase of the proliferation of potential beneficial microbes when inoculated.	[68]
Salvia officinalis L., *Lavandula dentata* L., and *Thymus vulgaris* L.	Plant inoculated or not with *Rhizophagus irregularis*	Rhizosphere microbiota analysed by 16S rRNA sequencing	- Modification of the bacterial communities - Increase of *Bacillus* in presence of the symbiont - Decrease of the *Gemmatimonadetes*, in the non-inoculated rhizosphere	[69]
Dalbergia odorifera	Plant 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 in the symbiont treatment - Increase of *Lactococcus*, *Bacillus*, and *Pseudomonas* in the rhizosphere of symbiont inoculated plants	[70]
Maize *(Zea mays L.* cv Cherif*)*	Plant inoculated or not with *Glomus mosseae* (BEG 107) or with *Glomus intraradices* (BEG 110)	Soil and rhizosphere microbiota analyzed by 16S rRNA DGGE and measure of global alkaline phosphatase (AP) activity	- Higher AP activity in the treatment inoculated with the symbionts. - Community structure modified in the rhizosphere and soil of the treatments inoculated with the symbionts - Higher effect on the community structure when the two symbionts were co-inoculated	[71]
Robinia pseudacacia	Plant inoculated or not with *Rhizobium*	Rhizosphere and bulk soil microbiota analyzed by 16S rRNA sequencing	- Increase of the proportion of the genera *Mesorhizobium, Variovorax*, *Streptomyces*, and *Rhodococcus* in the inoculated treatment	[71]

			- Increase of the number of genes encoding ATP-binding cassette transporters in the rhizosphere of the inoculated treatment - Reduction of the number of genes related to sulfur/nitrogen metabolism in the rhizosphere of the inoculated treatment.	
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 analyzed 16S rRNA and ITS sequencing	- Increase of root mycorrhization (especially *Claroidoglomeraceae*) in the treatment inoculated with the T3SS+ strain - Change of the bacterial community structure in the treatment inoculated with the T3SS+ strain	[29]

GLOSSARY :

Arbuscular mycorrhiza: from *myco*, fungus; and *rhiza*, root, the symbiotic association between roots of 85% of land plants and fungi belonging to the Glomeromycota division. Symbiotic fungi that penetrate inside the cortical cells of the root and form arbuscules, the 'tree-like' fungal structure developing within plant cortical cells in arbuscular mycorrhizal symbiosis.

CCaMK: Refers to the Calcium/calmodulin-dependent protein kinase, which is central for bacterial infection and nodule organogenesis as well as for arbuscular mycorrhizal symbiosis.

Crown gall: Disease provoked by *Agrobacterium tumefaciens* which is characterized by tumoral growth. Along with the hairy root disease, crown gall is the only known example of natural genetic transformation which development allowed the creation of genetically engineered plants.

Ectomycorrhiza : the symbiotic association between roots from trees and 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 termed as the Hartig network.

Endophyte: Microorganisms residing in the plant tissues.

Endosphere: Internal regions of plant tissues that can be colonized by microorganisms.

Endospheric symbiosis: Refer to a symbiotic association where the symbiont colonizes the inside of the plant (i.e., the endosphere). This term is to oppose to exosymbiosis, which corresponds to a symbiotic association, where the symbiont does not colonize the plant tissues.

Extrinsic factors: Factors related to the environment

Functioning: In complex assembly systems (e.g., microbial communities and/or plant-microbe interaction) this term refers to the global phenotype observed, which results from the relative sum of all the functions of the members of this complex assembly.

Holobiont: Assemblage of different species that form an ecological unit (see ref. [73]). Here, we limit our definition to the plant and all its symbiotic microbiota. Holobiont is an ecosystem where the host is the biotope and microorganisms are the biocenosis.

Interactome: All interactions between organisms within a functional community and their cascade.

Intrinsic factors: In the context of the current demonstration, this term is to be understood as the ensemble of all plant characteristics (species, genotype), plant organs (stem, root) and plant-microbe interactions.

Metabolome: The entire biochemical complement present within an organism. Metabolic change is a major feature of plant genetic modification and plant interactions with pathogens, pests, symbionts, free-living microbiota and their environment.

Microbiome: Microorganisms and their genetic material (genome, plasmids and mobile elements), short-term or long-term interacting with a particular environment. The diversity in microbiomes between individual plants is huge, and even within a plant there can be extensive variation in their microbiome makeup (*i.e.* phyllospheric or rhizospheric microbiome).

Microbiota : Community of microorganisms (bacteria, archaea, fungi, viruses, protists and other microeukaryota) associated with an organism, here a plant.

Mycorrhiza: Intimate association of plant roots with specialized soil fungi. Seven types of mycorrhiza exist, but ectomycorrhiza and arbuscular mycorrhiza are the most common.

Phytobiome : According to the Phytobiomes Alliance, it consists in the plant, its environment, the associated microorganisms (*i.e.*, the phytomicrobiome) and all the environment modifications induced by these interactions.

Phytomicrobiome: Diverse interacting microscopic organisms associated with a plant living in its environment.

Rhizosphere : The volume of soil around living plant roots that is influenced by root activity

Signalome: Signal molecules produced withing an organism or during interaction between organisms.

Structure: In the field of analysis of the phytomicrobiome, this term provides not only the composition of the taxa and/or functions encountered in the community, but also a quantitative view (*i.e*., the relative abundance).

Symbiont : Organism establishing a close and long-term interaction with its host (here the plant). This interaction can be obligate as in the case of the endosymbiosis.

Symbiotic interface (synonym, symbiotic apoplast) : The cellular space between the plant and fungal membranes, delimiting the site of reciprocal nutrient exchanges between the partners.