Trends in Plant Science

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Feature Review

Feed Your Friends: Do Plant Exudates Shape the Root Microbiome?

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Plant health in natural environments depends on interactions with complex and dynamic communities comprising macro- and microorganisms. While many studies have provided insights into the composition of rhizosphere microbiomes (rhizobiomes), little is known about whether plants shape their rhizobiomes. Here, we discuss physiological factors of plants that may govern plant–microbe interactions, focusing on root physiology and the role of root exudates. Given that only a few plant transport proteins are known to be involved in root metabolite export, we suggest novel families putatively involved in this process. Finally, building off of the features discussed in this review, and in analogy to well-known symbioses, we elaborate on a possible sequence of events governing rhizobiome assembly.

The Root Microbiome (Rhizobiome)

Plant growth and yield in natural environments depend on a plethora of interactions with bacteria and fungi [1] (one example is discussed in Box 1). The microbial community associated with roots was proposed to be assembled in two steps: first, the **rhizosphere** (see Glossary) is colonized by a subset of the bulk soil community and, second, the **rhizoplane** and the **endosphere** are colonized by a subset of the rhizosphere community [2,3]. Intriguingly, a set of recurring plant-associated microbes has emerged (core microbiome) [2,4]. This review focuses on how plants shape their **rhizobiome**. On the one hand, common factors among plants likely lead to the assembly of the core microbiome. On the other hand, factors specific to certain plants result in an association with microbes that are not members of the core microbiome. Here, we discuss evidence that plant genetic factors, specifically root morphology and root exudation, shape rhizobiomes.

Initial evidence for an influence of plant genotype on rhizobiome composition was that similar rhizobiomes assembled in association with arabidopsis (*Arabidopsis thaliana*) and barley (*Hordeum vulgare*) grown in the same experimental conditions, although they displayed different relative abundances and some specific taxonomic groups [5]. A correlation between phylogenetic host distance and rhizobiome clustering was described for Poaceae species [6], distant relatives of arabidopsis [7], rice varieties [3], and maize lines (*Zea mays*) [6], but not for closely related arabidopsis species and ecotypes [7]. Distinct rhizobiomes were also described for domesticated plants, such as barley, maize, agave (*Agave sp.*), beet (*Beta vulgaris*), and lettuce (*Lactuca sp.*), compared with their respective wild relatives [5,8–11]. Interestingly, not all plants have a rhizobiome distinct from bulk soil: some species, such as maize and lotus (*Lotus japonicus*) [12–15], have assembled a distinct rhizobiome, whereas other species, such as arabidopsis and rice, assembled a rhizobiome similar to bulk soil [3,5,7]. The former species display a strong, and the latter a weak **rhizosphere effect** (Figure 1, Key Figure). The cause of

Trends

Recent advances in sequencing technology have been enabling high-throughput characterization of highly complex plant-associated microbial communities, which are relevant for plant health.

Metagenomic approaches have been identifying the metabolic potential of microbes, and are starting to reveal functional groups of microbes, reducing the overall complexity of rhizobiomes.

Metabolomic studies are uncovering differential root exudation in various environments and plant developmental stages, as well as consumption of specific exometabolites by microbes.

Genome-wide association studies and other comparative genomic approaches have been revealing a link between plant genetic factors, exudation profiles, and microbiome compositions.

Molecular studies have been characterizing plant transport proteins necessary for interactions with specific microbes, although the transport of most nutrients and signaling molecules remains unexplored.

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Box 1. Phytoremediation: Interplay of Exudates, Border Cells, and Rhizobiomes

Plants grown on soils contaminated with heavy metals and organic pollutants (phytoremediation) assemble a rhizobiome that is distinct from that of plants grown on non-contaminated rhizosphere, or bulk soils [111–113] supporting plant growth [114,115] and higher heavy metal uptake [116]. Consequently, efforts have aimed at increasing the phytoremediation potential of heavy metal accumulators by combining them with specific microbial communities. However, due to limited understanding of the plant–microbe–environment interplay, these endeavors have had limited success so far [111,112]. Below, we discuss both, the response of plants and microbes to contaminated soils.

Plants display distinct responses to contaminated soils: legumes exhibited a systemic response, whereas grasses exhibited a more local response [117]. Various wheat cultivars displayed varying degrees of heavy metal tolerance, which were associated not only with distinct rhizobiomes [118], but also with the ability to exude organic acids [75]. Tolerant lines increased the expression of specific genes, such as the malate transporter ALMT1 (see Box 2 and Table 1 in the main text) and organic acid exporters of the MATE family [119]. Organic acid exudation has two main effects. First, (heavy) metal ions are chelated, and, second, they can act as anion exchanger and release tightly bound phosphate, supporting plant growth [111,112]. Increased organic acid exudation could also lead to increased nutrient availability for the microbial community, and have signaling functions. A second physiological response to protect the root from heavy metals is an increased production and shedding of border cells accumulating heavy metals [120]. The interplay of border cell production and differential exudation by alteration of transporter abundance not only determines the performance of the plant on contaminated soils, but also the environment for the microbial community.

Similar to plants, microbes in contaminated soils are under selective pressure from several sides: they have to tolerate the toxic environment, grow on root exudates, and compete for niches and resources [111,114]. Thus, it is no surprise that rhizobiomes of contaminated soils were found to be generally less diverse compared with other environments [114]. The addition of a substrate mix or the supply of plants with distinct exudation patterns to contaminated soils could increase of the growth of specifically engineered or native microbes, leading to an increase in the functional traits of the rhizobiome, and the phytoremediation potential of the microbial community. In addition, a tritrophic bacteria–fungi–plant interactions on contaminated soil was reported recently: microbes simultaneously increased arsenic tolerance in rice and resistance against disease [115]. Overall, further investigations of the roles of exudates, transporters, border cells, and bacterial and fungal communities will contribute to deciphering the effects of contaminated soils on plants, and lead to more- efficient phytoremediation procedures.

this phenomenon is currently unknown. The strength of the rhizosphere effect varies with the developmental stage of the plant [16–18]. Similarly, root exudation [16] and microbial communities were found to change with the age of the plant.

Furthermore, distinct rhizobiomes were associated with different developmental stages of arabidopsis [16,19], rice [3], and *Avena fatua* grown during two consecutive seasons [20]. Pioneering studies demonstrated the ability of microbes to alter plant development. Overall, it appears evident that host genotype, domestication, and plant development influence the composition of rhizobiomes. As an alternative to plant developmental stage, residence time of plants in soil was discussed as a hypothesis for successive microbiomes [21]. These contrasting results might be partially explained by differing environmental influences, host plants, or soils, and additional work is needed to resolve these questions.

In this review, we discuss root morphology and root exudates as two genetic factors shaping plant–microbiome interactions, and we examine the following aspects: (i) how root morphology and **border cells** affect rhizobiomes; (ii) how plant exudates shape the rhizobiome; and (iii) possible plant transport proteins involved in exudation. Figure 1 provides a general overview of **exometabolite** networks in the rhizosphere, and Box 1 illustrates the interplay between root exudates, border cells, and rhizobiomes in **phytoremediation**. We conclude by integrating these ideas into a possible scenario of rhizobiome assembly.

Root Physiological Features Shape Rhizobiomes and Exudation

Rhizobiomes are influenced by their spatial orientation towards roots in two ways. First, the radial proximity of microbial communities to roots defines community complexity and composition, as described in recent publications [3,19,22], and as outlined by the two-step model of

Glossary

Antiporter: a transporter utilizing a proton gradient to shuttle protons and substrates in opposite directions across a membrane.
Apoplasm: intercellular space between plasma membranes of plant tissue.
Border cells: root cap cells released into the rhizosphere with a distinct transcriptome, releasing mucilage, distinct proteins, and extracellular DNA.
Casparian strip: suberin-based connection between endodermal root cells, blocking the passive apoplasmic flow of liquids and compounds.
Endophyte: microbe living within plant tissue in the endosphere.
Endosphere: all endophytes of a plant. Relevant to this review are microbes living in root tissues.
Epiphyte: microbe living on plant tissue.
Exometabolites: compounds released by roots or microbes into the rhizosphere, often acting either as nutrients or signaling molecules.
Isolate: a microbial strain isolated from a natural environment, such as the rhizosphere, to be used in a laboratory setting.
Mucilage: matrix of high-molecular-weight compounds released by border cells and root tip.
Phytoremediation: accumulation of heavy metals or organic pollutants of contaminated soils in plant tissue, with the aim to clean soils.
Rhizobiome: the rhizosphere microbiome, comprising microbes associated with plant roots.
Rhizoplane: root surface including tightly adhered microbes.
Rhizosphere: 1–3-mm zone around root shaped by roots, and exudates.
Rhizosphere effect: plants with a strong rhizosphere effect have a rhizosphere microbiome distinct from bulk soil. Developmental stage and the type of plant species both influence the strength of the rhizosphere effect.
Symporter: a transporter utilizing a proton gradient to shuttle protons and substrates in the same direction across a membrane.
Uniporter: a transporter binding one substrate molecule at a time, facilitating diffusion across a membrane.

Key Figure

Plant and Microbial Exometabolite Networks

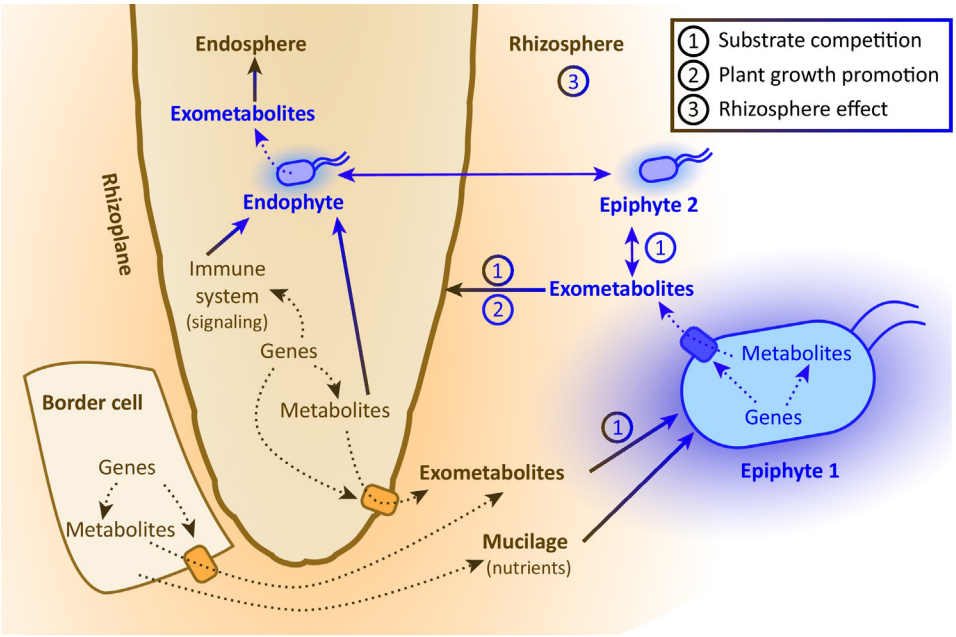

Figure 1. Plant roots and border cells (brown) and microbes (blue) synthesize metabolites and transporters (boxes), and export certain metabolites into the rhizosphere. This network is depicted by broken arrows. Exometabolites can have nutritional value and signaling functions (unbroken arrows indicate direction; if the metabolite has only a nutrient or signaling function, the role is specified in brackets). Some microbial epiphytes can migrate from the rhizosphere into the rhizoplane and into the root, where they become endophytes. Plant–microbe and microbe–microbe exometabolite interactions are displayed with numbers: (1) substrate competition between microbes, or between microbes and roots; (2) plant growth promotion by microbial compounds; and (3) rhizosphere effect, likely influenced by the presence of exometabolites. Plant and microbial exudates are displayed as gradients. Organisms and cells are not to scale.

microbial root colonization mentioned above [2]. Second, the lateral position of microbes along a root shapes the community, as exemplified by early studies [23,24]. Importantly, recent microbiome studies take into consideration the former, but not the latter aspect. In this section, we discuss specific microbial associations with various root regions, and the role of spatially distinct root exudation.

Root tips are the first tissues that make contact with bulk soil: root tips are associated with the highest numbers of active bacteria compared with other root tissues, and likely select microbes in an active manner [23]. The root elongation zone is specifically colonized by *Bacillus subtilis,* which suggests a particular role of this zone in plant–microbe interactions [25]. Mature root zones feature a microbial community distinct from root tips [25]. Their community includes decomposers [11,24], which could be involved in the degradation of dead cells shedding from old root parts [26]. Similarly, lateral roots are associated with distinct microbial communities, differing between tips and bases, as well as between different types of lateral root [14].

One trait influencing the differential microbial colonization of root tissues could be the differential exudation profiles of the distinct root parts. This is illustrated in the following example. Cluster

roots are densely packed lateral roots formed by some plants growing on extremely nutrient-poor soils; these roots exude high amounts of organic acids and, in some cases, protons, to solubilize phosphate [27]. The low pH and carboxylate-rich rhizosphere of cluster roots is associated with a specialized rhizobiome, dominated by *Burkholderia* species that metabolize citrate and oxalate [28]. Besides organic acids, mature cluster roots also exude isoflavonoids and fungal cell wall-degrading enzymes, leading to a decrease in bacterial abundance, as well as fungal sporulation [29]. Taken together, cluster root exudates not only solubilize phosphate, but also regulate microbes in such a way that they do not interfere with phosphate uptake. Beyond this example, spatial patterns of metabolite exudation are largely unexplored. We hypothesize that such patterns exist in all root systems for the following reasons: (i) spatially distinct organic acid exudation is a trait of all root systems (Table 1 and Box 1); (ii) spatially distinct exudation was similarly detected for strigolactones, amino acids, and sugars (Table 1) [30,31]; and (iii) root nutrient uptake, which is sometimes coupled with proton transport, can also exhibit spatial patterns (Table 1). Overall, spatially defined metabolite exudation by distinct root parts is likely an important factor in structuring the rhizobiome. Future studies should aim at characterizing spatially distinct rhizobiomes and their functional traits, and at investigating spatially distinct root exudation.

Root Border Cells and Mucilage Shape Plant–Microbe Interactions

Root tips are not only associated with high numbers of bacteria ([24], see above), but also produce border cells and **mucilage** (Figure 1), crucial for plant–microbe interactions. Depending on the root meristem organization, border cells are released into the rhizosphere either as single cells or as border-like cells (which remain attached to each other). Residence time in the soil is different for the two types of border cell. Single maize border cells stayed alive in soil for months, likely due to the presence of starch deposits [32], whereas arabidopsis border-like cells survived for only 2 weeks [33]. Border cells have a transcriptional profile distinct from root tip wells, with overall lower primary and higher secondary metabolism [32]. ABC transporters constitute a large fraction of differentially expressed genes, which is consistent with transport of secondary metabolites [32,34]. Secondary metabolites are likely central to the role of border cells in defense against pathogens [35–37].

Pathogen attack can result not only in higher border cell production and release [35–37], but also in higher mucilage production by border cells and root tip cells. Mucilage contains proteins with antimicrobial functions [35,38,39], as well as extracellular DNA involved in defense against fungi [40] and certain bacteria [41]. Importantly, mucilage is also produced under nonpathogenic conditions, serving as a lubricant for the root environment and stabilizing soil particles [42]. Interestingly, mucilage also provides distinct carbon sources for microbes, thus influencing rhizobiome composition [43,44].

Border cells similarly interact with nonpathogenic microbes (Figure 2): they release flavonoids that attract rhizobia, uncharacterized compounds that induce branching of mycorrhizal hyphae, and arabinogalactans that trigger biofilm formation of specific beneficial bacteria (Box 2) [32,33,45,46]. The full extent of how border cells and mucilage shape root–microbe interactions remains unclear. It is tempting to speculate that the specialized metabolism of the border cells results in a distinct exudation profile of not only proteins and mucilage, but also low-molecular-weight compounds that could serve as microbial nutrients or as signaling compounds. Further research should focus on the genetic and physiological differences between border cells and border-like cells, as well as on the transport proteins involved in exudation of low-molecular-weight compounds, DNA, and proteins.

How Microbial Communities Interact, and the Influence of Plant Exudates

Plant–microbe interactions are not only defined by plant root morphology and plant-derived exudates, but also by microbe–microbe interactions (Figure 1). Thus, we focus further here on

Table 1. Transporters for Metabolite Uptake and Release[a,b,c]

Transport mode	Metabolite examples		
	Transporter family	Description and localization	Refs
Sugars (glucose, fructose, sucrose, arabinose, xylose, mannose, maltose, ribose, galactose, galactinol, glycerol)			
Import	MFS (SUC)	Sucrose: H^+ symporter, PM	[137]
	MFS (STP, PMT)	Hexose: H^+ symporter, PM	[81,82,138]
Export	SWEETS	Mono- and disaccharides; indirect, vacuolar	[83,84]
	MFS (ESL)	*Uniporter? Vacuolar*	[139]
	MFS family	*Sugar: H^+ antiporter, sugar uniporter*	[85]
Sugar alcohols (inositol, myo-inositol, threitol, xylitol, erythritol, ribitol)			
Import	MFS (INT)	Inositol: H^+ symporter, PM	[138,140]
	MFS (PMT)	Polyol: H^+ symporter, PM	[138,141,142]
Export	MFS (INT)	Inositol: H^+ symporter, indirect, vacuolar	[138,142]
	MFS family	*H^+ antiporter, uniporter*	
Sugar phosphate (glucose-6-phosphate, glucose-1-phosphate)			
Amino acids (glutamic acid, aspartic acid, alanine, threonine, serine, asparagine, glutamine, valine, glycine, isoleucine, homoserine, histidine, lysine, arginine, leucine, proline, phenylalanine, 4-aminobutyric acid, methionine, ornithine, tryptophan, tyrosine)			
Import	APC (LHT, AAP, ProT, ANT)	Neutral, acidic, basic amino acids, PM	[143–145]
Export	GDU	Glutamine, vasculature, PM	[93]
	BAT1	*Bidirectional amino acids in yeast, PM*	[146]
	SIAR2	*Bidirectional amino acids in yeast, PM*	[147]
	CAT	*Gamma-aminobutyric acid, bidirectional? Vacuolar*	[148]
	UmamiT	*Phloem, amino acid export, PM*	[92]
Organic acids (succinic, malic, tartaric, lactic, formic, butyric, acetic, propionic, gluconic, oxalic, citric, pyruvic, formic, malonic, α-ketoglutaric, fumaric, *trans*-aconitic, aspartic, benzoic, glyceric acid)			
Export	ALMT	Malate, some aluminium, pathogen activated, PM	[80,115,149]
	MATE	Citrate, aluminium, iron activated. PM	[150,151]
Nucleotides (adenosine, guanosine, cytidine, thymine)			
Import	Heterocyclic nitrogen	Allantoin: H^+ symporter, PM	[95]
	PUP	Purine: H^+ symporter, PM	[94]
	ENT	Nucleoside, nucleotide, some H^+ symporters, PM	[152]
Export	P-type ATPase	Extracellular ATP degradation, indirect, PM	[97]
	ANT	*Nucleotide transport,* Escherichia coli, *PM*	[153]
	MDR (ABC)	*Nucleotide transport, PM*	[96,97]
Peptides			
Import	*OPT*	*Oligopeptide: H^+ symporter, glutathione, phytochelatins, PM*	[154,155]
	PTR	*Di-, tripeptide transporter, PM*	[132,155,156]

Table 1. (continued)

Transport mode	Metabolite examples		
	Transporter family	Description and localization	Refs
Export	*MDR (ABC)*	*Peptides, PM*	[34]
Fatty acids (linoleic, oleic, palmitic, stearic)			
Import	P4-ATPase (ALA)	ATP-dependent flippase, PM	[157]
Export	ABC (PDR, WBC)	Lipids for cutin, sterols, mycorrhizal fungi, PM	[98,99,158–160]
Inorganics (nitrate, phosphate, sulfate, potassium)			
Import	NRT1, NRT2	NO^{3-}/H^{+} symporter, high/low affinity, PM	[155]
	AMT	NH^{4+}, PM?	[132]
	MFS (PHT)	Phosphorus/H^{+} symporter, PM	[132,161]
	SULTR	Sulfate, PM	[132]
	KUP	Potassium, PM	[132]
Export	ATPase	H^{+}, ATP dependent, PM	[162]
Secondary metabolites, hormones (Coumarins: esculetin, esculin, scopoletin, scopolin, 4-methylumbelliferone; Sterols: campesterol, cholesterol, sitosterol, stigmasterol; Flavonoids: hormones, glucosinolates)			
Import	ABC (PDR)	Hormones, PM	[163,164]
	AUX/LAX	Auxin, PM	[165]
	NRT	Hormones, glucosinolates, PM	[163,166]
Export	ABC (PDR, MRP, MDR)	Hormones, heavy metals, ATP dependent, PM	[31,34,101,103]
	MATE	Flavonoids, anthocyanins, xenobiotics, phenolics, PM	[134,167,168]

[a]An overview of metabolite classes with examples frequently detected in root exudates, with transporter families involved in metabolite import or export. The transporter function is given in the description section, with localization of the family in roots when not at the plasma membrane.
[b]Text in italics: additional families likely involved in export of metabolites without experimental validation.
[c]Abbreviation: PM, plasma membrane.

microbial communities. Specifically, we discuss: (i) how plant exudates influence microbial diversity; (ii) how plant-responsive microbes are identified; (iii) how microbes interact and (iv) how mycorrhizal fungi influence root–bacteria interactions.

The rhizosphere serves as carbon-rich niche for the establishment of microbial communities, in contrast to bulk soil, which is rapidly depleted in carbon and other nutrients by heterotrophic microbes. Given that the ability of microbes to metabolize plant-derived exometabolites might determine their success in the microbial community, several studies have investigated whether the diversity of plant exudates correlates with microbial diversity. Some studies found higher plant diversity was associated with higher microbial diversity [47,48], and that the addition of a diverse exudate mix to plant monocultures increased microbial diversity [49]. Interestingly, **isolates** from soils with a diverse plant community consistently exhibited less-narrow niches and displayed less resource competition than did isolates from low plant diversity environments [50,51]. Although on a global scale, environmental factors had a larger impact on microbial diversity than did plant diversity [48], we can conclude that, on a local scale, high plant diversity likely promotes a diverse microbial community.

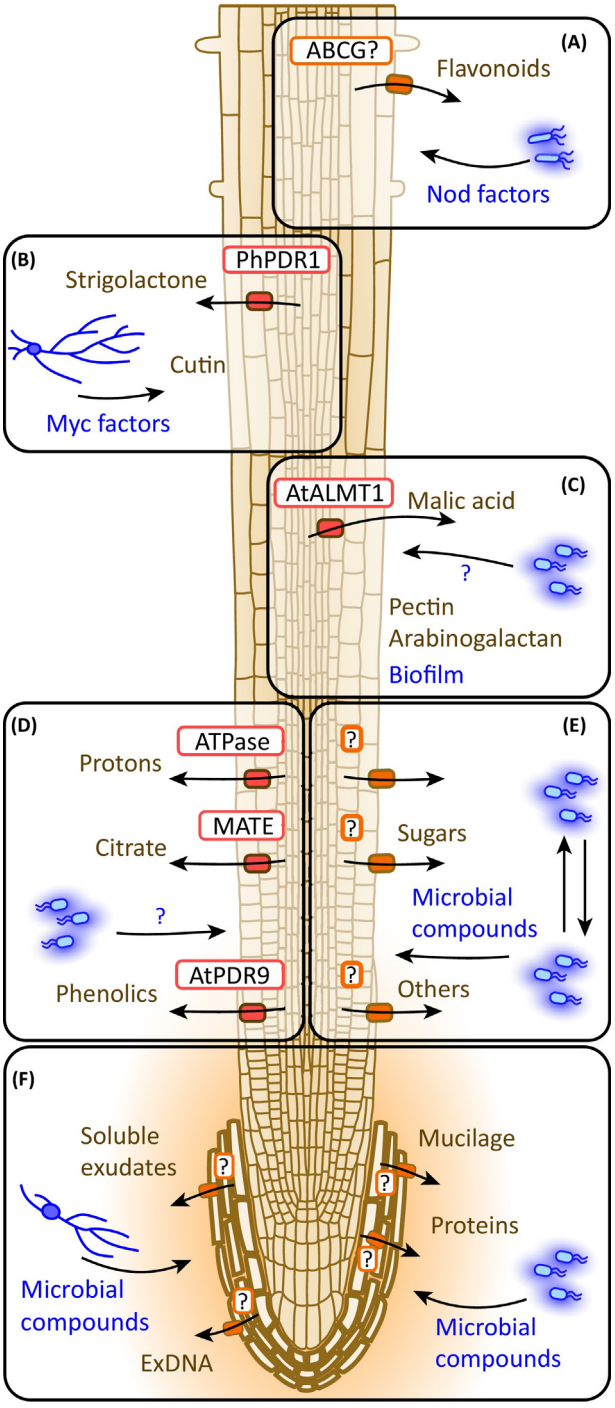

Figure 2. Metabolite Exchange Networks in the Rhizosphere. (A) Flavonoids are exuded, likely by an ABCG-type transporter [122], and sensed by rhizobia that in turn produce Nod factors. Rhizobacteria enter the root via root hairs or cracks between epidermal cells [133]. (B) Strigolactones are exuded by ABCG-type *Petunia hybrida* PDR1 localized in the subepidermal layer of the root maturation zone [31], and sensed by Glomeromycota that in turn produce Myc factors. Chitin has a role in hyphal attachment to the root. (C) At Aluminium-Activated Malate Transporter 1 (ALMT1) is located in the cortex of the elongation zone, and is involved in malic acid exudation in *Pseudomonas*-infected *Arabidopsis thaliana*, which attracts *Bacillus subtilis* [80]. *B. subtilis* forms biofilms on roots, a process dependent on root pectin and arabinogalactan [45]. (D) ATPases exude protons altering rhizospheric pH, enabling proton-dependent transport processes. Multidrug and Toxic Compound Extrusion (MATE) transporters exude citrate [119], which can be metabolized by microbes, and AtPDR9 transports phenolic compounds [134]. The signaling function and potential crosstalk with microbes are currently unknown. (E) Involvement of transporters in metabolite exudation is generally poorly understood [26,91,92]. Microbes exude compounds that are utilized by other microbes [59,135] and sensed by plants. (F) Border cells produce mucilage (red gradient), exude proteins, extracellular DNA, as well as metabolites, all of which impact the microbial community [33,40,136]. Currently, the mode of transport of these compounds is not characterized. Key: blue, microbial components; brown, plant components; red transporters, characterized; orange transporters, uncharacterized. Abbreviation: exDNA, extracellular DNA.

The large diversity of microbial communities is a current challenge for plant–microbe research, because it is impractical to study questions such as how members of a community interact, and what specific traits a microbial community has. Therefore, many studies currently aim at identifying the subset of microbes responsive to plants. Strikingly, only 7% of bulk soil microbes increased in abundance in the rhizosphere compared with bulk soil [24], which reduces the

number of taxa to investigate from thousands to hundreds. Other approaches to identifying plant-responsive microbes have focused on transcriptional profiling. Compared with soil-abundant microbes, plant-associated microbes exhibited distinct transcriptional responses to plant exudates [52,53] and, intriguingly, displayed distinct phylogenetic clustering [18,52]. Network analyses further revealed that rhizosphere microbes displayed higher levels of interactions than did bulk soil microbes [54]. These studies illustrate the potential for the identification of a distinct set of plant-responsive microbes.

The above points highlight how plants influence microbial communities. However, the members of microbial communities also interact with each other. Compellingly, it is still unclear whether microbe–microbe interactions are predominantly positive or negative. Network analyses reported predominantly positive intrakingdom interactions [54,55]. By contrast, laboratory growth assays identified competition as the major factor in shaping isolate communities, and cooperation could only be detected for 6–10% of the isolates [56–58]. One major difference between the two experimental approaches is that the former investigates a natural system, whereas the latter is based on the ability to culture microbes. Isolation of microbes introduces a bias, since it can select against cooperators, precluding obligate syntrophs. Further evidence that at least some microbes avoid competition was provided by co-cultivation experiments. Environmental isolates: (i) displayed high substrate specialization [59]; (ii) did not necessarily take up the compound with the highest energy [60]; and (iii) diverged in substrate use when cultivated for several generations [50,57]. In addition, some metabolites exuded by microbes could be metabolized by others [59], suggesting potential cross-feeding between community members. The above findings suggest complex interactions of microbes. It remains to be resolved in which situation competition or cooperation dominates communities. However, it is evident that microbial interactions are based on altered gene expression. Microbes responded to competing bacteria [61] or even close relatives [62] by differentially regulating genes involved

Box 2. Is there a Common Theme of Symbiosis?

The establishment of symbioses between plants and mycorrhiza or rhizobia is detailed in the literature, but the assembly of plant-associated microbiomes remains unclear. Here, we present a hypothesis on for the assembly of a complex microbial community in the rhizosphere that is based on the mechanism reported for the aforementioned symbioses (Figure I).

Plants induce symbioses with mycorrhiza and rhizobia in nutrient- poor soils. The symbionts are attracted by strigolactones exported by an ABCG- type transporter located in a specific root zone, or by flavonoids likely exported by a transporter of the same family (see Figure 2A,B) [31,121,122]. Signaling molecules leading to the assembly of rhizobiomes are largely uncharacterized, but one example illustrates a symbiotic interaction with a beneficial microbe (see Figure 2C in the main text): pathogen-infected or elicitor- treated arabidopsis plants increased ALMT1 expression and malic acid exudation (see Table 1 in the main text), which lead to specific attraction and root colonization of the biocontrol agent *Bacillus subtilis*, [80]. Interestingly, *B. subtilis* root colonization was not malic acid dependent [80], suggesting the presence of additional signaling compounds.

Signaling compounds are similarly exuded by mycorrhiza and rhizobia, and: ILipochitooligosaccharides (LCOSs) are required for the induction of symbiosis [123,124]. Mycorrhiza further require plant-derived cutin to attach to the root surface [125]. Some rhizosphere microbes produce N-acyl homoserine lactones (AHL) and volatile organic compounds (VOCs) that are sensed by plants [64]. Biofilm-derived exopolysaccharides similarly elicit plant transcriptional responses [126]. These compounds could be part of the plant – microbe crosstalk. Furthermore, plant-derived cell wall polysaccharides and other signals [45] were shown to initiate microbial root colonization and biofilm formation [22].

In a next step, plants respond to rhizobia and mycorrhiza by initiating the common symbiosis pathway (SYM), altering gene expression and root morphology [127]. The response of the immune system is distinct, with mycorrhiza eliciting, and rhizobia suppressing, an initial pathogen response [128,129]. The immune system is also important in microbiome establishment (see Figure 1 in the main text): for example, the phytohormone salicylic acid is not only involved in responses to microbial pathogens, but is also required for assembly of a typical microbiome [130]. Also, the genetic network for phosphate starvation signaling was found to influence the structure of microbiomes [131]. Nevertheless, the exact mechanism of how the plant immune system shapes microbiome formation remains to be determined.

After the successful establishment of symbiosis with rhizobia and mycorrhiza, specifically expressed transporters translocate nutrients between the partners [132]. Plants deliver sugars, organic acids, and lipids, and, in return, receive phosphate, nitrogen, and other nutrients provided by the microbes [99,127]. Compound exchange between plants and rhizobiomes, remains uncharacterized, and we propose plant transporters that could be involved in the process (see Table 1 and the discussion in the main text).

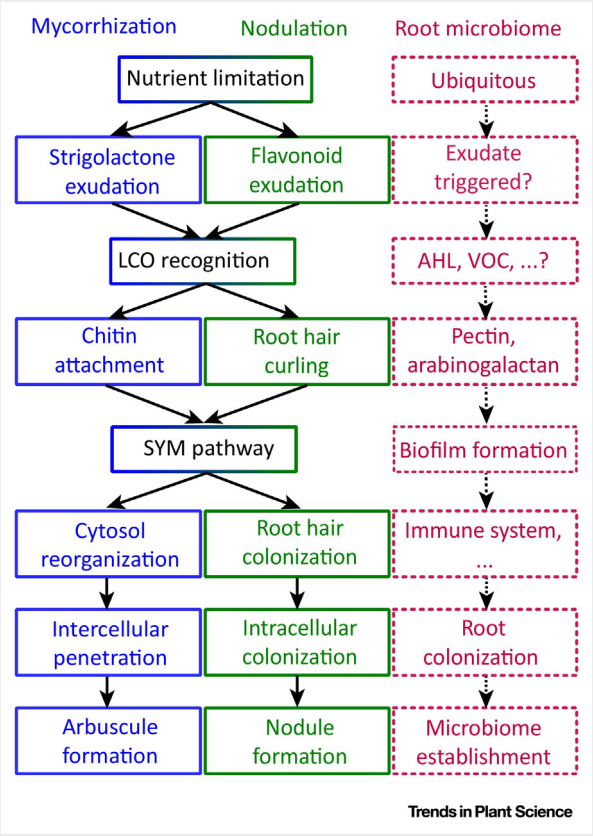

Figure I. Comparison of Symbiosis Establishment in Mycorrhization and Nodulation with Root Microbiome Formation.

in metabolite exudation and transport processes [61,63], making the study of microbial transporters a compelling topic for future studies. Thus, metabolite uptake, release, and sensing are important factors in shaping microbial communities.

Metabolite turnover in soil is influenced not only by plants, but also by functionally diverse bacteria, fungi, and animals [64]. Plant–fungal and plant–animal interactions in the rhizosphere go beyond the scope of this review, and are discussed elsewhere [64–66]. Here, we provide a few brief examples focusing on the impacts of mycorrhiza on rhizobiomes and exometabolite turnover. Endomycorrhizal fungi receive a significant fraction of the carbon fixed by plants (Box 2). Interestingly, these fungi also exude sugars [67], shaping a distinct bacterial community [67,68]. Likewise, Ectomycorrhiza receive carbon from plants, and form a dynamic bacterial community [69]; they even participate in plant-to-plant carbon transport [70]. The field of fungal microbiomes is nascent: if and how fungi control exudation, whether fungal microbiomes have beneficial functions, and how plant and fungal microbiomes influence each other are all unknowns. Although many questions remain, these recent findings already suggest that a holistic view of rhizosphere nutrient cycling and signaling exchange via exometabolites requires a whole-community approach including all domains of life.

Exudates Are Diverse and Dynamic

Plant exudates shape microbial communities. Overall, plants exude up to 20% of fixed carbon and 15% of nitrogen [65,71], which includes an array of simple molecules, such as sugars, organic acids, and secondary metabolites, as well as complex polymers, such as mucilage (Table 1 and Figures 1 and 2). Although every plant produces exudates, the amount and composition of root exudates varies. First, exudation is defined by the genotype of the host, as observed in the distinct exudation patterns of 19 arabidopsis accessions [72]. Strikingly, the amount of variation between the accessions depended on the metabolite class investigated. Glucosinolates displayed most, flavonoids medium, and phenylpropanoids low variability [73]. Second, exudation changes with plant developmental stage: with increasing age, arabidopsis sugar exudation decreased, and amino acid and phenolic exudation increased [16]. Third, exudation is modulated by abiotic stresses: the amounts of exuded amino acids, sugars, and organic acids changed in maize grown in phosphate-, iron-, nitrogen-, or potassium-deficient conditions [53]. In addition, phosphate-deficient arabidopsis plants increased coumarin and oligolignol exudation [74], heavy metal-treated poplar (*Populus tremula*) induced organic acid exudation [75], and zinc-deficient wheat increased phytosiderophore exudation [76]. Differential exudation is a plausible mechanism by which plants could modulate their interaction with microbes, as exemplified by the correlation between exudation patterns and rhizobiome variation reported for eight arabidopsis accessions [77]. Differential exudation modulated by transport proteins is discussed below.

Characterized and Putative Plant Transporters for Exudation

Plant-derived exometabolites need to cross at least one membrane to transit from the cytoplasm of root cells into the rhizosphere. There is considerable discussion as to what degree plants are able to regulate this transport. In general, different modes of transport could be envisioned. First, small, hydrophilic compounds could diffuse from the root into the rhizosphere, driven by the large concentration gradient [26,78]. Second, channel proteins could facilitate such diffusion. Third, active (ATP-driven) or secondary active (proton gradient driven) transporters could shuttle compounds across membranes against a concentration gradient. Diffusion of compounds can only be relevant in young root tissue, which is still devoid of **Casparian strips** or suberized endodermis that both block **apoplasmic** flow in adult tissues. Transport proteins involved in exudation are mostly elusive. From a conceptual point of view, plasma membrane-localized exporters likely have a direct, and vacuolar transporters an indirect effect on exudation. The vacuole is a major storage organelle for many metabolites detected in exudates, such as sugars, organic acids, and secondary metabolites [79]. Alteration of vacuolar transporter levels impacts vacuolar and cytosolic concentrations and, thus, can influence metabolite exudation into the rhizosphere.

The few characterized transporters involved in exudation are essential for the transport of specific compounds (Figure 2 and Box 2) [31,80], and are presented in Table 1. Since only a few transporters involved in exudation have been characterized, we suggest additional families that might be involved in the process. To complete the picture of metabolite exchange between roots and soil, Table 1 additionally contains a few important plasma membrane-localized metabolite uptake systems. Below, we discuss the evidence for transport processes involved in the import and exudation of compounds detected in root exudates, such as sugars, organic acids, and secondary metabolites.

Sugars

Sugars constitute a significant fraction of exudates, and are a main carbon source for microbes [14,42]. Interestingly, many more sugar uptake than release systems have been described. Sugar Transport Proteins (STPs) utilize high extracellular proton levels to import sugars, and mutation of STPs leads to higher external sugar levels [81,82]. Sugars Will Eventually Be

Exported Transporters (SWEETs) are sugar **uniporters**, and all root-expressed members localize to the vacuole [83,84]. Due to an alteration of root sugar homeostasis, SWEET mutant plants exhibited higher sugar export from roots compared with wild-type plants, and were more susceptible to disease [85]. Intriguingly, no transporters directly exporting sugars into the rhizosphere have been characterized so far, and it is debated whether sugar exudation is a transport-driven process at all [26]. Potential evidence for passive sugar efflux was supported by the observation of higher sucrose concentrations around young, permeable root tissue than around older, less-permeable root tissue [30]. However, because sugars are synthesized in leaves, they still need to be unloaded either from phloem or from root cells to be exuded into the rhizosphere, a process likely depending on transporters due to the hydrophilic nature of sugars. A further indication of the presence of elusive transporters is the differential sugar exudation in various environments, as shown, for example, for maize grown in potassium-, phosphate-, or iron-deficient conditions [85–88].

Sugar Alcohols and Phosphates

Sugar alcohols are imported by secondary active proteins with broad substrate specificity (Table 1), whereas the modes of export are enigmatic. Sugar phosphates are involved in intracellular carbohydrate metabolism, and plastid-localized sugar–phosphate co-transporters have been reported in several species [89]. Although sugar phosphates are detected in exudates, neither import nor export mechanisms are currently characterized.

Amino Acids

Amino acids are recognized by microbial chemoreceptors crucial for the early steps of root colonization [90], making amino acids an important fraction of exudates. Modulation of amino acid transport could be either a means of communication with microbes, or a response to microbial presence. Amino acid uptake is mediated by several transporter families with broad substrate specificity (Table 1) [91]. Amino acid exudation is affected by several transporters expressed in vascular tissue: mutation of phloem-localized UmamiTs resulted in lower amino acid exudation [92], whereas mutation of xylem-localized Glutamine Dumpers (GDUs) caused increased exudation [93]. Although no plasma membrane-localized amino acid exporters have been characterized so far, several lines of evidence suggest their presence. First, higher tryptophan exudation from older root zones than younger parts [30] suggests the involvement of transport proteins in exudation, due to the fully formed Casparian strips and thick cell walls in mature root parts interfering with diffusion. Second, concentration differences between amino acids in root exudates and root extracts are not the same for all the amino acids [91], suggesting the selective transport of at least some amino acids. Third, various transporter families exhibit bidirectional amino acid transport characteristics in heterologous systems (Table 1), and could be involved in amino acid exudation.

Organic Acids

Organic acids constitute a large fraction of exudates, and are microbial nutrients. No importers have been characterized so far, but the release of malate and citrate by Aluminium-Activated Malate Transporters (ALMT) and Multidrug and Toxic Compound Extrusion (MATE) families are among the few well-understood examples of transporters involved in exudation (Table 1 and Figure 2). Activity of members of both families is often modulated by metal ions (Box 1) and microbes (Box 2). Uncharacterized ALMT and MATE family members are primary candidates for exporters of other organic acids due to their similarity to already-characterized members, their plasma membrane localization, and their function as proton **antiporters**.

Nucleotides and Peptides

Nucleotides are imported by secondary active transporters, but their exudation remains elusive (Table 1) [94,95]. It is well established that extracellular ATP has a signaling function, and ABC

transporters were proposed to mediate cellular export [97,98]. Peptide uptake is transporter mediated in heterologous systems, and a role of ABC transporters in peptide exudation has been suggested (Table 1).

Fatty Acid

Fatty acid transport is necessary for mycorrhizal symbiosis: mycorrhizal fungi depended on their hosts for the synthesis of certain fatty acids [98], and the current model includes transport of lipids by ABCG proteins in the symbiotic membrane [98,99]. One ABCG member, STR, was previously shown to be required for mycorrhization [100]. Interestingly, arabidopsis ABCG transporters were similarly shown to export fatty acids for cutin synthesis in aboveground tissues (Table 1). Lipid transport was required not only for symbiotic interactions, but also for pathogen colonization [98]. Fatty acids are detected in root exudates (Table 1), but the mode of lipid exudation into the rhizosphere has yet to be discovered. A role in lipid exudation could be envisioned for root-expressed ABCG members (Table 1 and Figure 2).

Secondary Metabolites

Secondary metabolites are ubiquitous in root exudates, and ABC transporters are likely candidates for specialized metabolite transport into the rhizosphere. A distinct exudation profile was described for seven ABC mutants [101], and one mutant line displayed an altered microbial community [102]. Although the causal metabolites could not be identified, the authors noted transport of the same compound by various proteins, and possible broad substrate specificity for some transporters [101]. In a later study, exudates of arabidopsis ABCG37/PDR9 mutant lines were found to be deficient in several phenylpropanoids [103] (Figure 2D). Arabidopsis PDR9 was previously characterized as auxin precursor transporter [104], which suggests a broad substrate specificity for PDR9. Interestingly, a PDR9 homolog was highly expressed in cluster roots of white lupin devoid of phosphate [105], illustrating PDR9 involvement in response to various abiotic stresses. These studies illustrate the potential for the discovery of novel transporter functions in the ABC family, an excellent target for future studies investigating root exudation. In addition, MATE proteins transport secondary metabolites into the vacuole, and plasma membrane-localized members could also be involved in secondary metabolite exudation.

In summary, more transport proteins involved in metabolite import into roots than in export from roots have been reported so far (Table 1). The characterization of additional transport families involved in exudation will enable the generation of mutant lines that are devoid of the exudation of specific metabolites. Such lines could be used to investigate the correlation of exudation profiles and microbial communities.

How Do Rhizobiomes Assemble?

Plant-derived transporters and exometabolites are intrinsic to plant–mycorrhizal and –rhizobial symbioses (Box 2). We speculate that, although there is paucity of evidence, plants analogously select for a beneficial rhizobiome. Given that plants evolved in the presence of microbes, a subset of which benefits plant growth, we hypothesize that, over millennia, plant exudation via active transport processes evolved with the substrate specificity of plant-associated bacteria. In Box 2, we discuss exudates and other steps involved in root microbiome assembly, analogously to the establishment of plant–mycorrhizal and –rhizobial symbioses. However, intense future research is needed to reveal the precise mechanisms governing plant microbiome assembly, and the possible beneficial functions of the microbial community.

The major mechanisms by which plants are thought to modulate microbial interactions currently include: (i) modulation of their exudate profiles (alteration of biosynthesis and/or transport of microbial substrates and signaling molecules); (ii) root morphology (number

and length of roots, and root surface); and (iii) regulation of immune system activities (tolerance or avoidance). In turn, mechanisms for successful rhizosphere colonization by soil microbes require that they: (i) are metabolically active (catabolism of exudates); (ii) sense the plant (receptors for exudates); (iii) move towards the root (chemotaxis and mobility) and (iv) successfully compete with other microbes for root niches (physical colonization, substrate competition, and defense against toxins). In addition, for successful colonization of the rhizoplane or root tissue, microbes must be able to (v) attach to the surface (cell wall sensing or biofilm formation) or (vi) enter root tissue (evasion and/or manipulation of immune system).

Despite apparent parallels between plant microbiomes and the aforementioned symbioses, plant microbiomes have some specific characteristics. First, microbiomes are detected in all environmental conditions, whereas mycorrhizal and rhizobial symbioses are induced in specific circumstances. Second, microbiomes occur on various tissues, whereas rhizobia and mycorrhiza interface with roots only. Third, microbiomes comprise many members, whereas the aforementioned symbioses persist between two predominant partners. Fourth, although most members of the microbiome originate from the environment [2,4,106] similar to rhizobia and mycorrhiza, there is evidence that some endophytes may be vertically transmitted via seeds [107–110]. Future research should focus on the factors involved in microbiome assembly, the relative contribution of epi- and endophytes to microbiomes, and the signaling crosstalk between plants and microbial communities.

Concluding Remarks and Future Directions

Rhizobiome assembly and the involvement of the plant in this process are currently enigmatic. Here, we have discussed multiple factors shaping the rhizobiome, including host genotype and development, root morphology, border cells and mucilage, and root exudates. Root exudation is a dynamic process, likely dependent on a plethora or transporters that are mostly uncharacterized. Spatially defined exudation likely results in distinct microbial communities that are observed to be associated with specific root parts. The success of microbial colonization of the rhizosphere depends on several aspects, such as chemotaxis, substrate specificity, competitiveness, and cooperativeness. Furthermore, endophytes likely form biofilms on the root surface, and encounter the plant immune system. Although some factors shaping root microbiomes emerge, many open questions remain (see Outstanding Questions).

One major challenge will be to analyze root exudation in natural settings. Due to the chemical complexity of soil, exudation is traditionally analyzed in hydroponic culture [14,16,72,88], an environment distant from the more natural settings of plant microbiome studies. Furthermore, novel technologies enabling high-throughput screening of putative transporters against possible substrates are needed to reveal the impact of the respective substrates on the rhizobiome and, in turn, on plant health. An increased understanding of root morphology, exudation, and involved transporters will likely enable the engineering or breeding of plants with altered abilities to interact with specific beneficial microbes or pathogens. This needs to be complemented with an improved understanding of the substrate preferences of plant-associated microbes, their interactions, and the mechanisms through which they benefit the plant. A holistic understanding of the functions of a healthy plant rhizobiome would enable the directed design of customized microbial communities. With this, specific plants in a given environment could be tailored to a specific purpose, such as phytoremediation, stress resistance, altered plant development, or increased yield.

Outstanding Questions

Can we define standardized laboratory plant–soil–rhizobiome relationships to decipher the mechanisms of plant and microbial nutrient exchange and other beneficial activities by controlling confounding environmental variables?

How and to what degree do plants control exudation to specifically interact with microbes, shaping the rhizobiome? Special attention should be given to transporter families expressed in the root epidermis and root tip.

How can novel, high-throughput techniques be utilized to identify key nutrients and signaling molecules exchanged between plants and microbes, as well as the transport proteins involved in the process?

What are the distinct aspects of the microbial communities associated with root tips, lateral roots, mature root parts, and border cells? How are these communities influenced by exometabolites and nutrient uptake by the plant?

Are there functional classes and potentially crucial taxa common to plant rhizobiomes that can be used to design customized rhizobiomes persisting in a given environment, supporting host plant growth?

How are bacterial communities associated with mycorrhizal hyphae assembled, and how do they interact with rhizobiomes? Are microbes transferred between roots and hyphae?

How do reciprocal interactions between the rhizobiome and plant alter the trajectory of plant development, stress responses, as well as microbial community succession and activities?

How can we alter the host genotype to more efficiently select a beneficial rhizobiome that increases plant health and yield?

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