Metabolic interactions in beneficial microbe recruitment by plants

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During millions of years of evolution, land plants and microorganisms have established elaborate partnerships. Microbes play essential roles in plant fitness and help plants cope with environmental challenges. *Vice versa*, plants provide the microbes with a niche and food. In the soil, a complex network of interactions mediated by metabolic signals drives the relationship between plants and microbes. Here, we review the roles of metabolic signaling in the plant–microbiome interaction. We discuss how plant-produced small molecules are involved in the recruitment of the microbiome. Also the microbial partners in this relationship use small molecules, such as quorum sensing molecules and volatiles for intra-species and inter-species communication. We give an overview of the regulation of the biosynthesis, secretion and perception of both plant and microbial small molecules and discuss the examples of biotechnological approaches to engineer the plant–microbiome interaction by targeting these metabolic dialogues. Ultimately, an improved understanding of the plant–microbiome interaction and engineering possibilities will pave the way to a more sustainable agriculture.

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Introduction

Plants have evolved a diverse range of mechanisms to deal with changing environmental conditions. One of these mechanisms consists of the recruitment of beneficial microbes from the soil to mitigate environmental challenges [1,2]. Plant beneficial microbes are involved in a range of processes from improving nutrient availability, providing growth hormones, and modulating the abiotic stresses response in plants to mitigating biotic stresses by inducing resistance and synthesizing antibiotics targeting pathogens [1,3–5]. To facilitate this beneficial interaction, plants and their microbial partners have evolved a sophisticated chemical dialogue. Using small molecules, plants communicate with and modify the microbiome composition in their rhizosphere [2]. *Vice versa*, microbial interspecies and intraspecies signalling molecules can be perceived by plants and elicit a response [6••]. Moreover, plants sometimes mimic these microbial signalling molecules, hence affecting microbiome assembly [7–9]. Taken together, a highly convoluted metabolic dialogue goes on in the rhizosphere. In this review, we will discuss how small, plant and microbial, molecules play a role in shaping the plant-rhizosphere microbial community interaction, paying particular attention to the biosynthesis and regulation of the production of these signalling molecules under environmental stresses. Although it is clear that this metabolic interaction is essential for the plant–microbe interaction, the perception of these signaling molecules by the signal-receiving organisms and how the signaling is relayed are still an enigma. Finally, we will discuss studies on the use of biotechnological approaches to study these metabolic interactions and how a better understanding of the mechanisms underlying beneficial microbe recruitment may be used to create a more sustainable agriculture.

Plant metabolites recruit beneficial microbes in the rhizosphere

Plants invest up to 20% of photosynthesized carbon in the production of metabolites that are secreted into the rhizosphere. However, for just a fraction of these metabolites, a role in (beneficial) microbe recruitment has been demonstrated [1,10]. Strigolactones (SL), a class of carotenoid-derived phytohormones, play dual roles as plant developmental regulators and rhizosphere signaling molecules [11,12•]. Upon exposure to phosphorus deficiency, the accumulation of rhizosphere SLs is strongly increased, this is due to the upregulation of SL biosynthetic and transporter genes [11] (Figure 1b). Overexpression of the *Petunia hybrida* SL-transporter PLEIOTROPIC DRUG RESISTANCE 1 (PaPDR1), which results in a higher

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Figure 1
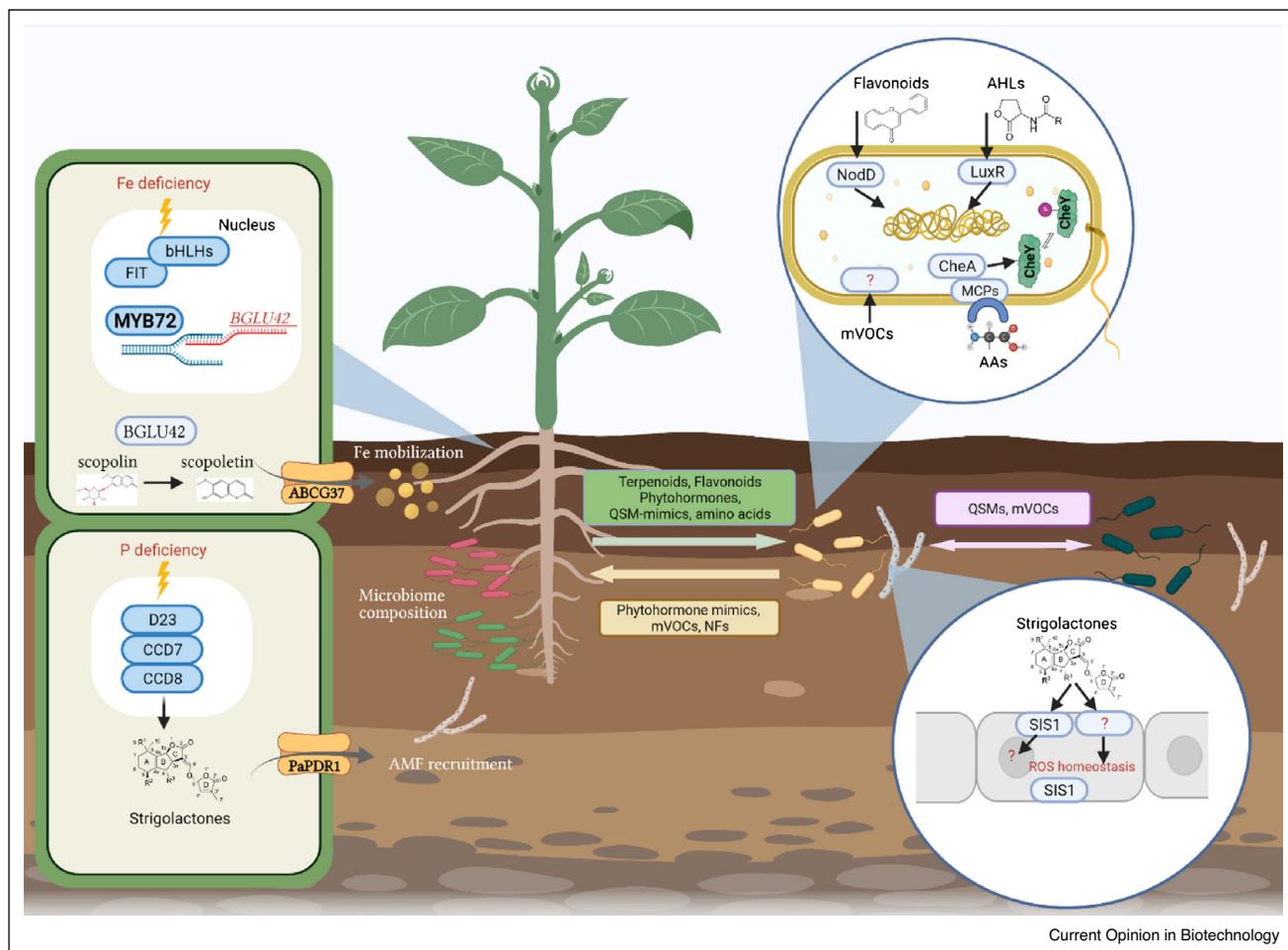
Metabolic interactions in the soil. **(a)** Under Fe scarcity, the activity of several transcription factors such as bHLHs and FIT, upregulate the expression of *MYB72*, which results in upregulation of coumarin biosynthesis. Hydrolysis of scopolin by β-glucosidase *BGLU42* results in secretion of coumarin through the ABCG37/PDR9 transporter. Secreted coumarins not only facilitate Fe uptake but also affect bacterial communities. **(b)** Strigolactones (SLs) are produced under low phosphate conditions and are excreted via the PaPDR1 ATP-binding cassette-transporter. The exuded coumarin and SLs can improve Fe mobilization and P uptake, respectively. Both alter the microbial community in the surrounding soil. **(c)** Flavonoids, which are exuded under nitrogen deficiency, bind the NODULATION (Nod)D regulator in Rhizobium, which induces *nod* gene expression resulting in Nod factor (NF) production. Although *N*-acyl-homoserine lactones (AHLs) are mainly produced by bacteria as quorum sensing molecules (QSMs), plants also release QSM mimics, which can bind to the bacterial LuxR receptor and alter bacterial communities. Amino acids (AAs) are perceived by methyl-accepting chemotaxis proteins (MCPs) that increase the histidine kinase CheA autophosphorylation rate. CheA transfers a phosphate group to the CheY response regulator, which changes the rotation of the flagella from counterclockwise to clockwise and thereby the direction and speed of the bacterial movement. **(d)** SLs potentially interact with the SL-INDUCED PUTATIVE SECRETED PROTEIN 1 (SIS1) in fungi and their perception regulates the expression of genes involved in reactive oxygen species homeostasis. However, it is not known if other receptors exist and what their target genes are.
This figure was created in BioRender (https://biorender.com/).

secretion of SLs into the rhizosphere, increased AM fungi colonization in *Medicago truncatula* [13], showing that plants employ signaling to recruit beneficial microbes to mitigate phosphate starvation stress. In addition to AM fungi, SLs also seem to affect rhizosphere bacteria. For example, the abundance of beneficial bacterial taxa, including Nitrosomonadaceae and Rhodanobacter, significantly decreased, while the overall number of bacterial species was higher, in both a SL biosynthetic gene *DWARF 17* (d17)

and receptor *DWARF 14* (d14) mutant in rice [14•]. Additionally, changes in bacterial and fungal composition were observed in sorghum cultivars differing in their exudate SL composition [15]. Altogether, these findings suggest that SLs play an important role in shaping the rhizosphere microbiome community.

Flavonoids are another class of signaling molecules involved in the interaction between plants and microbes.

Under low nitrogen, plants exude flavonoids that act as chemo-attractants and nodulation gene (Nod) inducers for symbiotic Rhizobia. Amongst the Nod-inducing flavonoids, apigenin and luteolin were both strong chemo-attractants while naringenin produced a very low response in *Rhizobium leguminosarum* biovar phaseoli [16], suggesting selective roles of different flavonoids. Intriguingly, flavonoids are also produced by other families of plants that don't establish nodulation with Rhizobia [17]. New research in maize showed that root-exuded flavonoids play a role in the recruitment of Oxalobacteraceae that induce lateral root formation, thereby facilitating nitrogen uptake [18••].

In addition to these well-studied symbiotic signaling molecules, the evidence for the existence of other microbial signaling molecules is gradually increasing. For instance, benzoxazinoids (BXs) are signaling molecules considered as chemo-attractants for several beneficial microbes [19,20••]. The presence of the BX, 2,4-dihydroxy-7-methoxy-*2H*-1,4-benzoxazin-3(*4H*)-one (DIMBOA), in the root exudate of maize resulted in the recruitment of a beneficial rhizobacterial strain *Pseudomonas putida* KT2440 [19]. Interestingly, Cotton *et al.* [20••] revealed BXs not only act as signaling molecules for beneficial microbes but also as endogenous regulators controlling root metabolism, which may be the actual cause for the changes in the host-associated microbiome [21]. The BXs also play a role in plant defense against insect herbivory and in iron uptake, and their production is affected by environmental conditions and abiotic stresses such as photoperiod, water deficiency, light intensity, and jasmonic acid [22,23]. Whether there is a relationship between certain abiotic stress responses, changes in the BXs and changes in the microbial community is, however, still elusive. Further research, using metabolomics and microbiome analyses, should shed more light on this.

Also, the presence of coumarins in root exudates has been reported to reshape the root microbial composition by improving or inhibiting the growth of specific microbes. Stringlis *et al.* [24••] showed that the antimicrobial coumarin, scopoletin, selectively suppresses the growth of pathogenic *Verticillium dahlia* and *Fusarium oxysporum*, while having no growth-inhibiting effect on beneficial rhizobacteria *Pseudomonas simiae* and *Pseudomonas capeferrum*. In addition to their effect on the microbiome, coumarins improve iron acquisition by reducing Fe^{3+} into Fe^{2+}, which can be taken up through IRON-REGULATED TRANSPORTER 1 (IRT1) [25], and/or chelating insoluble Fe^{3+} and facilitating its transport into the roots [26]. This is particularly pivotal for plants growing in alkaline soils, in which iron availability is restricted. Under Fe deficiency, transcription factors of the bHLH family were shown to be involved in the regulation of transcription factor FER-LIKE IRON DEFICIENCY (FIT) and subsequent upregulation of *MYB72* and genes involved in coumarin biosynthesis [27]. The expression of *MYB72* is also induced upon inoculation with root colonizing *P. simiae* WCS417 [28], which triggers induced systemic resistance (ISR) in Arabidopsis. Intriguingly, iron deficiency and ISR have separate signaling pathways that both trigger *MYB72*, and ISR-inducing rhizobacteria enhance the production of coumarin even under sufficient iron [28]. β-Glucosidase, *BGLU42*, has a dominant role in the formation of the coumarin scopoletin, by hydrolysis of scopolin, required to allow excretion from the root (Figure 1a) [24••,26,29], through ATP-binding cassette (ABC) transporter, ABCG37 [30]. Considering the antibiotic effect of coumarins, it seems that secretion of coumarins not only facilitates Fe uptake, but also aids beneficial microbes to outcompete other microbes in the same root niche [26,28].

Role of microbial metabolites in the plant–microbiome interaction

In addition to plant signaling molecules, microbe–microbe metabolic interactions, through quorum sensing (QS), play an important role in shaping microbial communities. *N*-acyl homoserine lactone (AHL), a QS molecule produced by Gram-negative bacteria, has been extensively studied and was shown to not only act as a signaling molecule within and between bacterial species, but also between bacteria and higher organisms [31,32]. Recently, other QS molecules were identified, that is, *cis*-2-unsaturated fatty acids, pyrones, alkyl quinolones and dialkyl resorcinols, but little is known on the species integrating these signals [2]. Intriguingly, plants also release QS-mimicking compounds such as AHL-mimicking molecules [8], rosmarinic acid [7] and the flavanone naringenin [9] that interfere with root microbiome assembly. Plant produced gamma-aminobutyric acid and proline modulate the concentration of QS molecule of *Agrobacterium* spp. [33]. This mechanism, termed 'Quorum Quenching'(QQ) or 'Quorum Interfering' (QI), benefits the plant by inhibiting or inducing the bacterial QS system. Upon uncovering such a mechanism, several attempts were made to generate genetically engineered plants able to modify QS in bacteria. The introduction of an AHL synthase from *Yersinia enterocolitica* into *Nicotiana tabacum* resulted in AHL production by the latter, altering quorum sensing in root bacteria [34]. Recently, transgenic AHL-producing *N. tabacum* were also shown to be resistant to *Pseudomonas syringae* [35]. Apart from QS molecules, microbial-produced volatile organic compounds (mVOCs) — such as terpenes, indoles, alkenes, and short-chain fatty acids — have also been well studied for their effect on plants and their role in the interaction with other microorganisms and have been extensively reviewed [4,36]. Here we give just a number of examples on the importance of these mVOCs. For instance, 2,3-butanediol and acetoin were two of the first volatiles discovered in Bacillus strains that induced plant growth promotion in Arabidopsis [4]. In addition,

dimethylhexadecylamine (DMHDA) and indole from PGPRs, increase root length and numbers, and root hair density in Arabidopsis by interfering with auxin signaling [37]. Many species of *Pseudomonas* and *Bacillus* have been reported to produce mVOCs that inhibit plant pathogens directly by altering the transcriptional expression levels of several genes involved in motility and pathogenicity or indirectly through enhancing induced systemic resistance in plants [36]. Moreover, mVOCs produced by *Collimonas pratensis* and *Serratia plymuthica* can promote the growth and induce chemotactic motility of the beneficial *Pseudomonas fluorescens* [38].

In vitro studies of microbial signaling molecules are limited by the fact that just a small fraction of the microbes present in soil is culturable [39]. Yet, recent advances in bioinformatics and biotechnology have helped to circumvent this limitation. For example, transposon-insertion sequencing (TIS) methods, which integrate random mutations in the genome with high-throughput sequencing, have provided a powerful way to investigate microbial gene functions [40,41]. In the past few years, this method has also successfully been applied to studies on plant-associated bacteria (reviewed by Ref. [40]). Furthermore, recent advances in multi-omics data integration coupled with precise gene editing are offering new perspectives to dig deeper into the mechanisms [5]. From the microbe side, genetic engineering can be utilized to remove functions from specific bacterial strains from soil communities as demonstrated recently by Finkel *et al.* [42]. In their study, inter-specific conjugative transfer of suicide vectors from *Escherichia coli* to *Variovorax* spp. was used to knock out the auxin-degrading operon in *Variovorax* spp., which suppressed its ability to release root growth inhibition [42].

The presence of a plethora of different microbial species highlights an important feature of the rhizosphere microbiome, the cumulative and synergistic effects of individual microbial species [10,43]. Recently, synthetic communities (SynComs) of microorganisms have emerged as a promising tool to investigate microbial community dynamics and their metabolic interactions [42–44]. For instance, a combination of *Chitinophaga* sp. and *Flavobacterium* sp. showed to suppress the fungal root pathogen *Rhizoctonia solani* more efficiently than when they were used individually [44]. Similarly, a synthetic community consisting of seven bacterial species, mainly belonging to Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria suppressed maize seedling blight (*Fusarium verticillioides*) colonization and growth [43].

Perception of signaling molecules in the rhizosphere

Signaling molecules are defined as a chemical language understood by the organisms able to read it. For only a fraction of signaling compounds, the mechanism underlying this perception has been unraveled. In bacteria, the perception of AHLs and AHL-mimics has been investigated over the two past decades and extensively reviewed [45,46]. Also, for some plant-produced signaling molecules, microbial perception has been studied. Apigenin and luteolin, for example, are low-nitrogen induced flavonoid chemoattractants for Rhizobia. Competition experiments suggest that apigenin and luteolin are recognized by a common receptor, but that there is also a separate receptor for luteolin alone [16]. The same two flavonoids also interact with the transcriptional regulator Nodulation D (NodD). It is unclear whether NodD perception is also responsible for the chemoattraction, likely it is not, as the afore mentioned flavonoid naringenin only induces a very low response for chemoattraction in Rhizobia but strongly activates the NodD protein. Activation of NodD promotes transcription of *nod* genes involved in rhizobial lipo-chitooligosaccharide (LCO) production, so called nodulation factor or Nod factors, NFs [47,48]. The NF signaling molecules released by rhizobia are then perceived by the plant Lysin motif (LysM) receptors, which triggers a signaling cascade allowing rhizobia to reach the root cortex [49]. Similarly, chemical communication of AM fungi with plants is mediated by Myc-LCOs (Myc factors), which have a similar structure as the Nod factors, with tetrameric and pentameric chitin backbones [49]. Moreover, it was found in rice that Myc factors have a high affinity to a LysM-type receptor (OsMYR1) [50]. Because of the relatively similar structure and common signaling components, it was proposed that signaling and perception of Nod and Myc factors have the same evolutionary origin [2,49].

The perception and subsequent downstream signaling of many other signaling molecules are still a conundrum. For instance, the perception mechanisms of strigolactones in AM fungi and, possibly, bacteria are largely unknown [51] although they have been largely elucidated in plants [52]. A recent study using pathogenic fungi showed that genes implicated in reactive oxygen species homeostasis were differentially expressed in the presence of the SL analog GR24 [53]. The authors suggested that the perception of SLs in AM fungi first evolved as a defense mechanism against foreign molecules and was repurposed during co-evolution with land plants to mediate host perception. Another potential receptor for SLs, the SL-INDUCED PUTATIVE SECRETED PROTEIN 1 (SIS1), has been identified in AM fungi *Rhizophagus irregularis* [54] (Figure 1d). Further investigation of the complete molecular mechanism behind SL perception in micro-organisms is needed. Likewise, the perception mechanism of many specialized metabolites produced by microbes, such as mVOCs and QS molecules, by plants, is still largely unknown [36].

In addition to specialized metabolites, several studies have demonstrated a role for amino acids in mediating plant–microbe interactions [55]. In bacteria, amino acids bind to specific types of receptors known as MCPs (methyl-accepting chemotaxis proteins) [56]. Indeed, several MCPs have been identified in *Bacillus subtillis*, but only McpB displayed binding affinity towards amino acids [57,58]. A recent study reported that McpB is involved in the perception by *B. subtillis* of amino acids exuded by the roots of *Arabidopsis thaliana* [58]. Recognition of amino acids by these chemoreceptors modulates the activity of HISTIDINE KINASE (CHEMOTAXIS PROTEIN A; CheA), which phosphorylates the CHEMOTAXIS PROTEIN Y (CheY) response regulator (Figure 1c). The phosphorylated CheY then interacts with the flagellar motor and subsequently influences bacterial behavior by changing the rotation and/or speed of the flagella [59,60]. A better insight into the receptors perceiving amino acids as well as homologous proteins of CheA and CheY in beneficial bacteria may shed more light on the perception of amino acids, and possibly also other plant exuded metabolites, in the rhizosphere.

Conclusions and future perspectives

The mechanisms underlying metabolic interactions between plants and microbes have been studied using advanced genetic approaches. Assessing the function of specific genes can be achieved by gain and loss of function mutations using a wide range of techniques and by functional analysis of proteins and genetic elements. So far, only a small fraction of the metabolites exuded by plants from their roots into the rhizosphere have been investigated for their role in beneficial microbe recruitment and we are therefore only just starting to understand these metabolic recruitment mechanisms. Future work should focus on discovering new signaling molecules, uncovering their role in the rhizosphere, as well as understanding the perception and signal transduction processes in the signal receiving organisms. This will require rapid development of multiple disciplines such as multi-omics data integration, protein modeling, and advanced engineering tools in both plants and microbes. Owing to the high complexity of interactions influencing soil microbial communities, assessing the role played by specific species or genera in the plant stress response remains challenging. However, as our understanding of the interaction between microbial species at the ecological level increases and new gene-editing tools and delivery systems become available, targeted microbial engineering in the field is coming within reach. One could foresee knocking out quorum sensing functions or receptors of specific strains of bacteria using, for instance, site-directed endonucleases to target specific microbial strains or their functions, allowing for eliminating one or more specific micro-organisms. Ultimately, advances in the discovery of mechanisms underlying beneficial microbe recruitment will result in the development of new biotechnological approaches to engineer the plant–microbiome interaction, which will eventually result in a more sustainable agriculture.

Author contributions

DA, SJ and LD outlined the paper. DA and SJ reviewed the scientific literature; DA, SJ and LD wrote the draft. HB and LD revised the draft; all authors read and agreed upon the final manuscript.

Conflict of interest statement

Nothing declared.

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