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The role of nutrient balance in shaping plant root-fungal interactions: facts and speculation

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Microbiota colonizing plant roots and their vicinity were shown not to be just random associations, but compose, at least to some extent, host-selected microbial consortia. The plant physiological status, especially the nutrient status, prompts changes in plant morphology and metabolism, which successively imposes a selective pressure on microbial communities. It is well established that a low phosphate status of the host plant activates the molecular machinery underlying the development of mutualistic associations in the host root with arbuscular mycorrhizal fungi (AMF). We hypothesize that the plant's response to changing nutrient stoichiometry affects processes at the root-mycosphere interface which promote or repress also root interactions with microbial taxa other than AMF. As a consequence, fundamental mechanisms underlying these interactions would be shared in AM host and non-host plants. A detailed understanding of the processes involved in maintenance of plant nutrient homeostasis could contribute to novel strategies in tailoring predominantly parasitic or commensalistic plant–microbe interactions towards beneficial associations.

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Introduction

In terrestrial ecosystems plants are constantly exposed to microbiota undergoing mutually beneficial, commensalistic, and parasitic (even pathogenic) relationships with their photosynthetic host [1,2]. Microbes communicate with plants using a plethora of chemical substances including, for example, volatiles and hormones [3]. Plants perceive microbes via recognition of microbe-associated molecular patterns (MAMPs), molecular signatures which trigger plant innate immunity responses known as MAMP-triggered immunity (MTI) [4]. One would assume that in natural environments the plant innate immunity is always induced due to omnipresent microbes, but as the defense comes with costs like, for example, reduced growth [5], plants constantly exposed to microbes would grow small. It is of note that knowledge of plant innate immunity was mostly established in studies on binary interactions with pathogens, whereas in nature plants interact with a great many microbes, which interact with each other [6]. Not much is known about how plant immunity is imbedded in the response to beneficial microbes, but some MTI-associated receptor kinases are involved not only in pathogenic but also in mutualistic interactions [7,8•,9]. Thus, the concept of plant defense needs to be revisited taking into account the perspective of the holobiont (co-existence of plant host with its microbiota) in spatially complex soil [10].

Plants do activate defense-related or symbiosis-related genes not only in presence of microbes on or in their aboveground or belowground organs, but also in response to imbalances in cellular homeostasis including metabolic perturbations. For instance introducing transgenes encoding heterologous enzymes of primary metabolism into plant cells can affect host sugar metabolism and in turn enhance the resistance to pathogens [11,12]. Homeostatic imbalance often occurs when proliferating roots are exposed to patches of different nutrient concentrations [13]. Soil microbes are integral to the soil carbon, phosphorus and nitrogen cycles [14,15] and as such essential for plant growth. In view of climate change and the forecasted phosphorus crisis [16] sustainable agricultural and soil management in crop production aims at balancing fertilizer input while maintaining high yields and promoting beneficial plant-microbe interactions [17]. Notably, most of the land plants form symbioses with soil fungi in response to nutrient deficiencies [18]. Thus in this review we introduce recent advances in understanding the regulation of plant nutrient homeostasis, its impact on root-associated soil fungi and the involvement of the molecular cross talk between nitrogen and phosphorus in the plant host.

Regulation of bidirectional nutrient transfer in AM symbiosis

Plant colonization of terrestrial ecosystems occurred over 450 Mya [19] comprising the exposure to soil microbiota

such as bacteria and filamentous fungi which emerged much earlier [20]. The appearance and success of land plants were proceeded by the evolution of symbioses of ancient plants with fungi (mycorrhiza formation), as the genes from the symbiotic signaling pathway predated the first land plants and were present in their algal ancestors [21,22••]. Earliest plant lineages like Haplomitriopsida, the basal sister group to all other liverworts, were colonized by fungi belonging to the subphylum Mucoromycotina whereas early divergent liverworts and hornworts hosted Mucoromycotina and Glomeromycotina fungi, sometimes concurrently [23,24].

The process of photosynthesis converts carbon dioxide into sugar and fuels growth of plant and associated microbes. The mutualistic symbiosis between plants and fungi involves reciprocal transfer of essential nutrients, organic carbon, and water. In the arbuscular mycorrhizal symbiosis (AMS) with Glomeromycotina fungi, the predominant type of mycorrhiza formed by approx. 70% of extant land plants [25,26], fungi deliver to the host plant phosphorus (P) and other elements like nitrogen (N) [27] in exchange for organic carbon in the form of fatty acids [28•,29•,30•] and hexoses [31]. It is well established that the host plant controls AMS formation and activates mycorrhiza-specific transport of phosphate ($H_2PO_4^-$ or Pi) predominantly when its Pi status is low [32–35] implying that the mutualistic interaction is regulated by the host's nutrient status. This was further confirmed by the discovery that plant transcription factors from the WRINKLED gene family regulate this symbiosis by binding *cis*-regulatory elements in the promoter sequence of numerous mycorrhiza-regulated genes encoding, for example, mycorrhiza-specific Pi transporter PT4, the H^+-ATPase HA1, and lipid biosynthesis genes like RAM2 [36••,37••] (Figure 1) in a Pi-dependent manner [36••]. In addition, the N status of the plant is also responsible for maintaining a functional AMS [38] as dysfunctional arbuscules developed in the *pt4* mutant only in high nitrate (NO_3^-) conditions whereas at low nitrate or upon activation of the ammonium NH_4^+ transporter AMT2;3, development of functional arbuscules was restored [39,40]. Improving our understanding of the molecular mechanisms underlying the plant's response to nutrient dynamics will facilitate control over host physiology towards optimized interactions with specific fungi and fungal consortia for the benefit of their host.

Interplay between plant nitrogen and phosphorus status

The influence of Pi and nitrate in the establishment of AMS has been pinpointed earlier. In *Medicago truncatula*, for example, combined restriction of Pi and nitrate has an additive and systemic effect favouring increased AMF colonization compared to only Pi limiting conditions. Concomitantly, the plants presented altered expression of defence-related genes and upregulation of genes involved in the biosynthesis of strigolactones [41]. In agreement with these results, Nouri *et al.* show that *Petunia hybrida* growing under nitrate deprivation promoted AMS and counteracted the suppressive effect of high Pi [38]. These results suggest that the plant Pi and nitrate status, respectively, cumulatively influence the interaction with AMF.

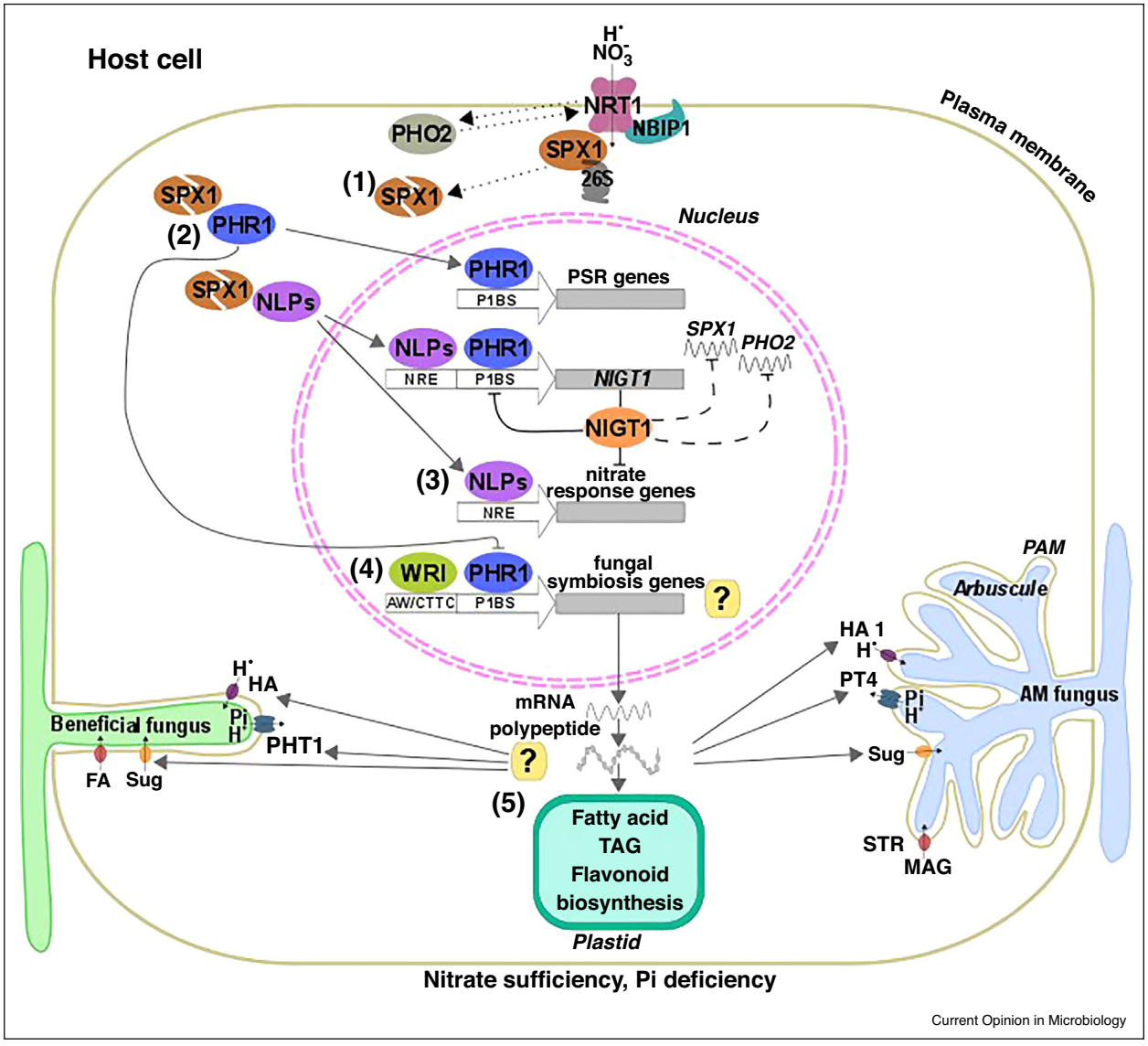
The molecular components regulating the Pi starvation response (PSR) under Pi limiting conditions have been deeply studied in Arabidosis and rice [42]. One of the key transcription factors (TF) in the process is PHR1 which binds to P1BS elements in promoters of Pi starvation-inducible (PSI) genes [43,44]. In Arabidopsis growing at Pi sufficient conditions, SPX1 protein and its orthologs act as Pi-dependent repressors of PHR proteins [45]. PHR1 activity is presumed to play a role in the control of AM-specific Pi transport [46,47].

Recent evidence points towards a regulatory role of nitrate signaling in triggering plant PSR. Genome-wide analysis in Arabidopsis revealed that nitrate influences ~85% of Pi-regulated genes [48], among them are prominent PSR marker genes: IPS1, SPX1 and PHT1;1. Transcript levels were systemically downregulated in the absence of nitrate and dependent on the activity of the nitrate transceptor NRT1.1 [48]. Interestingly, the nitrate effect was impaired in the *pho2* mutant, which positioned this ubiquitin conjugating enzyme E2 at the interface between nitrate and Pi signaling given that the presence of PHO2 also led to enhanced NRT1.1 transcript levels [48]. It is particularly relevant to understand NRT1.1 and PSR interaction because the regulatory mechanism was shown to be conserved in the monocots rice and wheat [48] raising the potential for targeted crop enhancement on soils with low nitrate and P input management.

The report by Hu *et al.* [49••] fills the knowledge gap between dicots and monocots at least in the context of the Pi sensor SPX1. The authors described a regulatory module in rice comprising NRT1.1B, SPX4 (orthologs of Arabidopsis NRT1.1 and SPX1, respectively) and NBIP1. Rice growth under Pi limiting conditions activates the degradation of SPX4, which leads to the coordinated activation of the TFs PHR2 and NLP3 (Arabidopsis PHR1 and NLP7, respectively), thus initiating Pi-uptake and nitrate-uptake responses.

Once the PSR signaling cascade is initiated, PHR1 along with NLP's activate the transcriptional repressors NIGT [50]. The outcome is a dual inhibition of gene expression with opposite effects. On the one hand, the nitrate starvation response is attenuated through direct inhibition of nitrate transporters, TF and genes related to recycling and mobilization of nitrate [51,52]. On the other hand, NIGT directly targets PSR repressors SPX1/2/4 and PHO2 which positively impacts Pi uptake [52], hence resulting in a balanced acquisition of both macronutrients.

Figure 1



Proposed model with host genetic toolbox for nutrient trading with beneficial (mutualistic) fungi.
Nitrate sufficient and Pi deficient concentrations in soil trigger molecular signals regulating plant nutrient homeostasis. Nitrate transceptor NRT1.1 recruits NBIP1 to mediate proteasome degradation of SPX1, which initiates a coordinated release of PHR1 and NLP transcription factors (TFs). Downstream, NLP targets the NRE and along with PHR1 promotes the activity of the transcriptional repressor NIGT1. This in turn allows balanced nitrate and Pi acquisition because NIGT1 inhibits nitrate uptake through direct binding with NRT2 and promotes PSR by repressing *SPX1* and *PHO2*. A further integration node is the feedback loop between PHO2 and NRT1.1, as the presence of PHO2 promotes the protein accumulation of NRT1.1 and vice versa NRT1.1 negatively affects PHO2 abundance. In AM host plants several TFs are activated and bind to the *cis*-regulatory elements present in the promoters of mycorrhiza-inducible genes. Among them there are AP2 TFs WRI5 and CBX1 (WRI1), which regulate expression of genes encoding Pi transporters (e.g. PT4 and HA) and components of lipid biosynthesis. CBX1 was shown to directly target RAM2 involved in fatty acid and cutin biosynthesis [36••,71]. At the cellular level, the root cortical cells of AM host plants are colonized by arbuscular mycorrhizal fungi forming arbuscules, the site of reciprocal transfer of nutrients and organic C in the form of fatty acids and sugars. Much less is known about the regulation of nutrient transfer in non-AM beneficial interactions with root-colonizing soil fungi. We presume that similar to the AM symbiosis fungal colonization leads to activation of transcription factors directing the expression of genes required for bidirectional nutrient exchange. The Pi uptake from beneficial fungi into the host plant likely involves PHT1 family Pi transporters [69,72]. Transcriptional regulation (→); posttranscriptional regulation (▶) and posttranslational control (•••▶); solid grey lines indicate movement of proteins or mRNA between cellular compartments. Unknown regulatory components are indicated with '?'; P1BS, PHR1 binding site; PSR, phosphate starvation response, NRE, nitrate responsive element; WRI, WRINKLED proteins (e.g. CBX1, WRI5); PAM, periarbuscular membrane; MAG, monoacylglycerol; PHT1, Pi transporter from the PHT1 family; HA, proton-ATPase; FA, fatty acid; Sug, sugars. Short summary of the figure: (1) Deregulation of SPX1 upon nitrate uptake; (2) Activation of PHR1; (3) PHR1/NLPs/NIGT1 activation of PSR and nitrate response; (4) Nitrate-dependent PHR1/WRI-mediated regulation of gene modules underlying mutualistic interactions with soil fungi (evidence for Glomeromycotina fungi, matter of speculation in case of other fungi); (5) Biosynthesis of symbiosis-promoting metabolites.

Collectively the model suggests that at low Pi conditions, once nitrate uptake and signaling are attenuated, the plant's resources are directed towards Pi acquisition mainly through the transcriptional and post-translational regulation of SPX1 [52,49••]. Moreover, NIGT regulation indicates that the mechanism is evolutionarily conserved in angiosperms [52,53]. This newly discovered interplay between nitrate and Pi signaling illustrates the complex nuances required to achieve nutrient homeostasis, knowledge which needs to be incorporated in modern strategies aimed at optimizing the use of N and P fertilizers in our crops.

Mutual impact of plant nutrient and mycorrhizal status on the root-associated fungal microbiota

Soil types vary considerably in fertility worldwide, depending on location (climate, geology, vegetation) with corresponding variation in the combination of physico-chemical and biological properties that can facilitate or impede growth of agricultural crops. Fertilizer input to the soil changes the proportion of nutrients available not only to plant, but also to whole microbial communities living in the given area. Moreover, microbes are exposed to the plant-mediated adaptations in the rhizosphere triggered by alterations in the plant nutrient status, for example, changes in root morphology and exudation of metabolites and protons. Several reports show how long-term or short-term P input to the soil changes the root-associated fungal communities in AM non-host plants [54••,55,56•,57,58]. The effect of Pi fertilization triggered moderate changes in the root microbiota structure accounting to 4.5–15% of variance [56•,58], and was associated with higher prevalence of some fungal orders like the Helotiales [54••,56•], among others. It is challenging to uncouple the direct effects of P added to soil on fungi from indirect modifications triggered by plant host responses associated with fertilization, which can be achieved by utilizing split-root experimental systems [32]. In Arabidopsis this was addressed by studying the fungal communities of mutants impaired in the PSR, which showed that PHR1, PHL1, PHF1 and PHO2 are important for establishing the root fungal microbiota under high P [56•,58] as well as low P fertilization regimes in natural soil [58]. The results suggest that the fungi colonizing the root niche are subject to a filtering mechanism determined by the host genotype and molecular cues initiating the PSR [56•,58]. It remains to be shown whether shifts in root fungal communities driven by the plant PSR are consequences of (1) adaptive strategies of the host to beneficial/mutualistic associations with fungi promoting plant growth [54••], (2) niche competition among root colonizers and/or (3) altered plant defense mechanisms [59].

In the case of plants forming an AMS, soil fertilization with Pi introduced minor changes in fungal community structure in roots, manifested mostly by reduced abundance of AM fungi [57,60•]. Interestingly, in low Pi soils, an impaired ability to form a functional AMS in the mycorrhizal host *Lotus japonicus* by mutations in host genes from the common symbiosis signaling pathway or downstream AMS-specific genes, respectively, triggered a significant but minor imbalance in fungal community structure [60•,61]. These studies showed that absence of fully developed and functional arbuscules in mycorrhizal roots was associated with an enrichment of Ascomycota fungi including Helotiales and Nectriaceae species. This implies that the root niche with low abundance of AM fungi can be taken over by particular members of the local soil fungal community. However, these alternative colonizers do not fully complement the wild type phenotype, as, for example, plant biomass and shoot P concentration were still reduced and the expression of PSR and redox-related genes enhanced in mutant plants lacking functional arbuscules and exhibiting reduced colonization by AM fungi [60•]. This highlighted the particular role of AMS in the performance of AM host species.

Fungal members of microbiota beyond the glomeromycotina contribute to plant nutrition and growth

The mutualistic interactions between different species are stabilized when incurred costs by maintaining the cooperation in symbiont and host do not outweigh benefits. It is suggested that the switch from the mutualistic to a non-mutualistic stage can occur during evolution when the mutualism has not become too specialized and the symbiont abundance is scarce [62]. More specifically, the segregation of fungal partners in the AMS may have been stabilized evolutionarily when the host plant developed novel nutrient acquisition strategies or when the symbiont was replaced by a new microbial partner(s) [63].

Accordingly, since the colonization of land, AMS functions/services may as a matter of fact have been compensated in interactions with other fungi which subsequently formed a physiological and genetic basis for a switch from AMS to new mutualistic fungal interactions with plant roots. It is therefore conceptually conceivable that fundamental genetic mechanisms underlying early evolution of AMS [22••] were in part adopted by these newly emerged symbiotic innovations.

While most land plants form an AMS the Brassicaceae plants are an exception because they have lost several genes allowing the development of AMS [64]. Thus the globally used model plant *Arabidopsis thaliana* is considered a non-host for AMF. Although, similar to the situation in mycorrhizal hosts [65], Glomeromycotina fungi are perceived in Arabidopsis roots as was manifested by the upregulation of symbiotic strigolactone biosynthesis

genes after exposure to the fungus, the plant activated costly defense processes in later stages of the interaction accompanied by reduced growth [65]. In contrast, there are several examples of Brassicaceae plants benefiting from associations with Ascomycota and Basidiomycota fungi, suggesting the development of alternative partnerships to compensate for the absence of arbuscular mycorrhiza. These include *Heteroconium chaetospira*, providing nitrogen to*Brassica rapa*, as well as *Serendipita indica*, *Colletotrichum tofieldiae* and Helotiales sp. isolate F229 supplying Pi to their hosts [67–69,54••]. Thus these fungi in part exhibited similar characteristics in Brassicaceae plants like AMF in AM hosts.

In this context it is noteworthy that the beneficial interaction of Arabidopsis with the Ascomycete *Colletotrichum tofieldiae* is controlled by the PSR system and thus by the plant P status [69]. It is thus tempting to speculate that P availability or the plant P status is a primary selective force driving symbiotic trait evolution in the context of the root microbiota. Importantly, Helotiales fungi and *C. tofieldiae* were isolated from plants grown in soils with very low Pi content [54••,69], suggesting low P availability as a driving force in the evolution of novel root symbioses in marginal soils.

In the past two decades many research groups have carefully elucidated fundamental molecular mechanisms underlying AMS development. The models emanating from these studies provide a natural guide to future research on the processes adopted in evolutionary younger symbioses of plant roots with soil-based fungi outside of the Glomeromycotina. The AM mutualism is hypothesized to be evolutionarily stable because the control of the two-way transfer of resources is bidirectional with mechanisms specific for the AM fungi and the mycorrhizal host, respectively [70]. It remains to be tested whether the molecular network which maintains the mutual exchange of C, P and other resources is conserved between AMS and evolutionarily younger beneficial symbioses and how it is mechanistically linked with the primary nitrate response and the PSR, and integrated at the level of the holobiont thriving in its complex three-dimensional environment to maximise nutrient efficiency and fitness of the interacting partners (Figure 1).

Conclusions

Since early emergence on land, plants were capable of forming associations with filamentous eukaryotes and other microbes. Some of these associations evolved into intimate symbioses which to date contribute to improved performance and fitness of the extant symbiotic partners. We speculate that a central regulatory system exists which merges the control of the plant and microbial C:N:P balance, the development of AMS and other beneficial plant-fungus symbioses, and underlying reciprocal transfer of resources. Future mechanistic studies on the impact of this network on plant–microbiota interactions have a great promise to disentangle the complexity of nutrient homeostasis in the plant holobiont – environment context and to optimize root traits that convert soil health into crop yield.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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