

Phosphorus disequilibrium in the tripartite plant-ectomycorrhiza-plant growth promoting rhizobacterial association

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Abstract

Plant roots and rhizospheres are colonized by an extensive and diverse microbial community. These microbes may form mutualistic, commensal, and/or pathogenic relationships and influence agricultural and forest productivity. Symbiotic ectomycorrhizal (EcM) fungi colonize the roots of many tree species, and the literature on these associations extensively describes their influence on plant nutrient relations and response to environmental stress. Similarly, soil bacteria ubiquitously colonize roots and rhizospheres and many of these bacteria may also play roles in influencing tree productivity. In particular, plant growth promoting rhizobacteria (PGPR) positively affect plant growth by altering nutrient availability in soils and inducing changes in plant hormone balance, plant stress resistance, and immunity pathways. In nature, EcM fungi and soil PGPR co-exist and the interaction and composition of this multi-tiered rhizosphere community aids in the acquisition of nutrient resources from soils as well as host plant response to environmental stress. The assembly of EcM communities is influenced by tree species and environmental conditions, and the tree and EcM species further influence PGPR community structure. Functionally, these symbiotic associations exhibit unique expression profiles and ecophysiological activities within the tripartite association. EcM and PGPR mediate production of complex arrays of exudates, including organic acids, siderophores, enzymes, and other organic compounds, which alter nutrient equilibria in soils, leading to increased access to phosphorus (P) and other macro- and micronutrients. As a metaorganism, the tripartite ectomycorrhizas increase the ecological breadth of host trees and influence the structure and function of forested ecosystems.

Keywords: Ectomycorrhizal fungi, mineral weathering, mycorrhizal helper bacteria, nutrient scavenging, phosphate

1. Introduction

Microbes in the rhizosphere play a significant role in the relationship of trees to soils and environmental stresses. Soil microbes are numerous and diverse, and play roles that are pathogenic, commensal, and

symbiotic in nature (Barton and Northup, 2011). They increase the ecological breadth of trees, *i.e.*, broaden the conditions under which many tree species can function (Adriaensen *et al.*, 2005;

Augé, 2004; Seguel *et al.*, 2013). Together with the terrestrial vegetative community, the root-associated microbial community influences and mediates the biogeochemical cycling of nutrients, such as NP and sulfur (S), and thus is critical in forest productivity and the provision of ecosystem services (Barton and Northup, 2011; Berg and Smalla, 2009; Chapman *et al.*, 2006).

The roots of the vast majority of forest tree species form mycorrhizal associations (Smith and Read, 2008) with species in boreal, temperate, and tropical forests forming relationships with ectomycorrhizal (EcM) fungi. In exchange for C in the form of sugars, ECM fungi integrate the host tree into soil nutrient cycles and provide physical, physiological, and biochemical access to nutrients in the soil that the host tree would otherwise be unable to access (Buée *et al.*, 2007; Phillips and Fahey, 2006; Zhao and Running, 2010). For example, both N and P are limited in forests due to high biological demand in soils and/or recalcitrance of mineral and organic forms of these critical nutrients that lead to their inherent limitation to forest tree roots. EcM fungi possess metabolic pathways that allow access to these nutrients and, thus, provide access to their hosts as well (Buée *et al.*, 2007; Chalot and Brun, 1998; Phillips and Fahey, 2006; Plassard and Dell, 2010; Zhao and Running, 2010). This ecological access to nutrients extends beyond the influence of the rhizosphere itself into the region of influence of mycorrhizas in the soil—the mycorrhizosphere.

In addition to accessing nutrients from recalcitrant soil pools, EcM fungi and the mycorrhizosphere provide a unique niche in which soil microbes, including a vast community of bacteria, reside and these may also contribute metabolic and ecological enhancement of nutrient acquisition (Frey-Klett *et al.*, 2007). Here, these plant growth promoting rhizobacteria (PGPR), or mycorrhizal helper bacteria (MHB), receive benefits from the host mycorrhiza,

including C and a physical niche, and may provide numerous direct and indirect benefits to host trees and influence their associated EcM fungi. Benefits of PGPR to the host include stimulation of root growth, nutrient acquisition, and modification of rhizosphere microbial communities (Cassán *et al.*, 2014; Frey-Klett *et al.*, 2007; Hryniewicz *et al.*, 2010; Persello-Cartieaux *et al.*, 2003; Rogers *et al.*, 2012), all of which improve tree performance and resistance to environmental stress.

For the purpose of this review, we are focusing on the tripartite relationships of the roots of tree species with symbiotic EcM fungi and plant growth promoting rhizobacteria. In addition, we are focusing further on the role of the tripartite association in the acquisition of limiting resources, notably P, from the soil ecosphere.

2. Building Community—the Formation of Mycorrhizosphere Associations

Plant-mycorrhizal associations have existed since the colonization of land by autotrophic plant ancestors (Graham and Miller, 2005). Since these first arbuscular-like mycorrhizal relationships, selection and specialization have led to numerous and diverse mycorrhizal associations, some more narrow and some more cosmopolitan in nature. These include the arbuscular mycorrhizas, ericoid mycorrhizas, arbutoid mycorrhizas, monotropoid mycorrhizas, orchid mycorrhizas, and ectomycorrhizas, the focus of this review.

Ectomycorrhizas typically form between roots of woody plants and fungi belonging to the Basidiomycota, Ascomycota, and Zygomycota (Tedersoo *et al.*, 2010). The ecological and physiological benefits of the EcM fungal association are well studied (Smith and Read, 2008), and include improvements in host nutrient and water acquisition as well as enhanced stress resistance

(Cairney, 2012; Chalot and Brun, 1998; Courty *et al.*, 2010; Plassard and Dell, 2010; van Hees *et al.*, 2006). These associations vary in host-fungus specificity: the associations may be narrow, perhaps reflecting specialization to specific ecological conditions or strategies to limit competition (den Bakker *et al.*, 2004; Molina *et al.*, 1992) or may be broad, reflecting greater flexibility of fungi to occupy broad soil ecological niches (Rosling *et al.*, 2003).

Also present in the rhizospheres of trees are a wide variety of bacteria (Brown *et al.*, 2012; Calvaruso *et al.*, 2007; Frey-Klett *et al.*, 2007), which, when providing growth benefit to the host, are termed plant growth promoting rhizobacteria (PGPR) (Compant *et al.*, 2010; Khan *et al.*, 2009; Persello-Cartieaux *et al.*, 2003; Rodríguez and Fraga, 1999). As with mycorrhizal symbiosis, the plant-PGPR relationship is probably ancient and based on the availability of carbon (C) from the autotrophic host root that may be exchanged for benefit to the host plant. These benefits include access to nutrients, biological control in the rhizosphere, and alteration of phytohormone levels, which increase host vigor, stress resistance, and broaden the ecological niche of the host (Babalola, 2010; Barriuso *et al.*, 2008; Bent *et al.*, 2001; Compant *et al.*, 2010; Persello-Cartieaux *et al.*, 2003).

2.1. EcM Community Selection

Generalist ECM fungi, such as *Amanita muscaria*, may be found in association with many host genera across widely different environments. Such generalization would promote extensive EcM species radiation and may reflect adaptability of the EcM fungus (Cairney, 2000; Courty *et al.*, 2010; Taylor *et al.*, 2006). In contrast, specialization may reflect historical bottlenecks, such as in glacial refugia, or ecological specialization that constrained the number of host-symbiont combinations (Brundrett, 2009;

Courty *et al.*, 2010; den Bakker *et al.*, 2004). As host-symbiont specialization is retained, there must exist unique host-fungal combinations that function to provide optimum benefit within the ecological range of the symbiont pair.

Although the controls over host-EcM fungal specificity are not yet described, patterns of specificity suggest that the plant host influences, to some extent, the ecology of the mycorrhizosphere. Culture studies have noted distinct host-EcM specificities. Massicotte *et al.* (1994) noted strong mycorrhization preferences among six tree species and 15 EcM fungal species, with none of the fungi having broad host ranges. However, a study of host-EcM fungal specificity among five tree species grown in soil indicated that specificity is significantly less strict, but still present (Massicotte *et al.*, 1999). Further, host-EcM specificity was low among five tropical tree species and 39 EcM fungi collected from field soils in New Guinea (Diedhiou *et al.*, 2010). Interestingly, in this study, specificity varied by host-species life stage, with seedlings tending to support a more diverse EcM community, a pattern supported for *Populus balsamifera* along a glacial chronosequence (Helm *et al.*, 1996), but not for *Pinus thunbergii* in a forest with low EcM fungal species diversity to begin with (Obase *et al.*, 2009). Thus, it is difficult to make generalizations regarding the development of EcM fungal communities. Community structure varies with host species as well as the age or stage of the host, depends on the surrounding edaphic conditions, and may be restricted by the diversity of propagules from the surrounding ecosystem (Elliott *et al.*, 2007).

2.2. PGPR Community Selection

The structuring of bacterial communities by plant hosts may also favor plant performance under specific environmental conditions. Evidence indicates that specific plant host-bacterial combinations may

be encouraged, perhaps as a result of the “biased rhizosphere effect” (Hartmann, *et al.* 2009). According to this hypothesis, host exudation profiles enrich the root zone in specific bacterial groups due to the presence of both stimulatory and inhibitory factors (Babalola, 2010; Hartmann *et al.*, 2009). This selection may, in turn, alter root function/exudation and further bias the maintenance of a limited number of bacterial groups in the plant rhizosphere (Hartmann *et al.*, 2009). In the field, the majority of forest tree roots will be colonized by symbiotic EcM. As this is the norm, an investigation of host tree-bacterial community selection will be nearly impossible to separate from selection mediated by the resident EcM fungal community. Several reports indicate that specific EcM fungal species influence the composition of the microbial community formed in the mycorrhizosphere. Roots of *Betula pubescens*, for example, form mycorrhizas with numerous EcM species and these species differentially influence bacterial and

ascomycete communities (Izumi and Finlay, 2011). Across the EcM associates, both cosmopolitan and EcM-specific bacterial profiles were found (Figure 1), with additional selection existing for ascomycetes (Izumi and Finlay, 2011). Similar bacterial community selection has been reported between *Lactarius vellereus* and *Lactarius subdulcis* mycorrhizas of *Fagus sylvatica* (Mogge *et al.*, 2000) and noted for *Rhizopogon* sp. (Kretzer *et al.*, 2009).

Interestingly, endophytic bacterial communities of mature *Populus deltoides* trees from upland and bottomland soils in Tennessee, USA were dramatically different from rhizosphere samples, suggesting that a further level of selection and potential host specificity leads to a narrow community of bacterial living within the root proper (Gottel *et al.*, 2011). Elucidation of the functional attributes of endophytic communities vis-à-vis rhizospheric communities remains a potentially fruitful area of research.

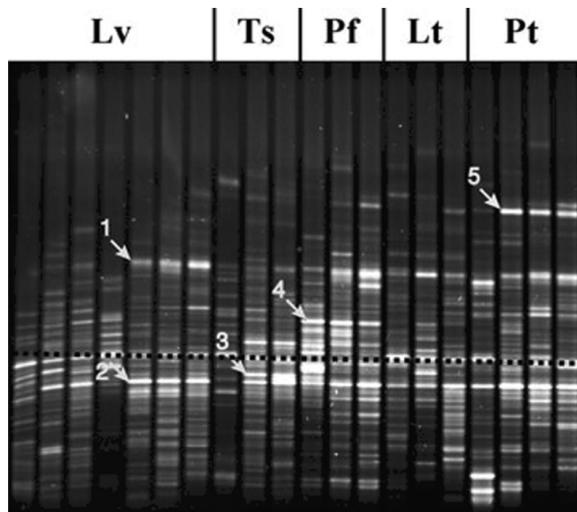


Figure 1. DGGE profile of the bacterial communities associated with the mycorrhizas of *Betula pubescens*. EcM mycorrhizas: Lv = *Leccinum variicolor*; Ts = *Tomentellopsis submollis*; Pf = *Piloderma fallax*; Lt = *Lactarius torminosus*; Pt = *Pseudotomentella tristis*. Bacterial associate sequence homology: 1 = *Acidobacteriaceae*; 2 = *Rhodopseudomonas palustris*; 3 = *Rhizobium leguminosarum*; 4 = *Mesorhizobium* sp.; 5 = *Mucilaginibacter* sp. Note the cosmopolitan associates (bands 1, 2) and EcM-specific associates (bands 3, 5). Reproduced from Izumi and Finlay (2011) with permission.

2.3. PGPR-EcM Community Interactions

That the EcM fungi and PGPR communally occupy the same physical space raises questions regarding co-selection and mutualistic or antagonistic relationships between the two groups and subsequent functional implications for the host tree. Indeed, several reports suggest that there may be complex interactions that ultimately influence structural and functional aspects of the mycorrhizospheric community, and thus will influence the ecological breadth of the plant-EcM-bacterial tripartite association.

In addition to the influence of EcM fungal species on PGPR communities noted above, mycorrhizospheric bacterial isolates have been found to alter relationships between EcM and host tree roots. *Streptococcus* sp. stimulated both mycelial growth *in vitro* and colonization of roots of *Picea abies* by *A. muscaria* (Riedlinger *et al.*, 2006). Garbaye and Bowen (1989; 1987) found that bacterial isolates from the mycorrhizospheres of *Pinus radiata* differentially influenced mycorrhization by *Hebeloma crustuliniforme*, *Paxillus involutus*, and *Rhizopogon luteolus*. Similar influences were noted for *Pseudomonas fluorescens* and the *Acacia holosericea*-*Pisolithus* sp. symbiosis (Founoune *et al.*, 2002) and for *Sphingomonas paucimobilis* and *Ralstonia pickettii* in symbiosis with *Salix viminalis* (Hryniewicz *et al.*, 2010). In an uncontrolled soil experiment, inoculation of *Pinus pinea* seedlings with *Arthrobacter* sp., but not *Staphylococcus* spp., stimulated EcM root tip production and, further, each PGPR fostered a different EcM fungus to dominate the root system (Barriuso *et al.*, 2008). These community structural controls over EcM populations will have functional implications for the tree host.

2.4. Communication in the Tripartite Community

Accessing soil resources is the central role of the complex root-symbiont community. An effective balance between supply and demand of nutrients and the deployment of C into the rhizosphere will require communication between partners and the integrated expression of their genomes. These interactions between mycorrhizosphere members may result from the production of diffusible compounds that may trigger metabolic and growth responses in symbiosis. Actinomycetes produce compounds that regulate the growth of soil-borne fungi and these exudates may function to structure the microbial communities of tree roots (Riedlinger, *et al.* 2006, Keller, *et al.* 2006). The PGPR *Streptomyces* strain AcH 505, for example, produces several compounds that regulate fungal activity (Keller, *et al.* 2006). One such exudate, auxofuran, is a compound with structural similarity to auxin and it stimulates the growth of *A. muscaria in vitro* (Riedlinger, *et al.* 2006, Keller, *et al.* 2006) and the formation of mycorrhizas *in symbio* (Schrey *et al.*, 2005). Auxofuran was also found to induce changes in gene expression in *A. muscaria*, with up-regulation of a number of metabolism-related genes such as acetoacetyl-CoA synthetase to support ergosterol production (Schrey *et al.*, 2005). Genes related to cell-cell interactions, stress response, and metabolism were differentially expressed in *Laccaria bicolor* when challenged with beneficial, neutral, or antagonistic bacteria (Deveau *et al.*, 2014), with the PGPR *P. fluorescens* BBc6R8 inducing greater expression of transcription factors and transcripts related to chromatin structure and stress response. A large number of *P. fluorescens* isolates altered gene expression in *L. bicolor* and increased root colonization of three *Populus* species (Labbe *et al.*, 2014), again supporting the complex relationships

underlying microbial community structure in the mycorrhizosphere.

While little information on EcM fungi-PGPR crosstalk is available for tree species, there is evidence that coordinated messages are exchanged that reflect nutrient demands of the plant hosts. Root exudates from nutrient stressed maize plants altered gene expression in *Bacillus amyloliquefaciens* *in vitro*, with the greatest changes under N limitation (Carvalhais *et al.* 2013). These conditions induced stress response genes in *B. amyloliquefaciens*, which may reflect a selection pressure on the PGPR community by the host (Carvalhais *et al.*, 2013). Exudates from P, iron (Fe), and potassium (K) deficient plants had much lesser effects on gene expression in *B. amyloliquefaciens* in exponential growth phase and, by and large, induced up-regulation of chemotaxis-, motility-, and transport-related genes, again suggesting that, under these nutrient conditions, the host may provide signals that select PGPR community assemblages. In support of this, Zyško *et al.* (2012) noted that gene expression in *Pseudomonas aeruginosa* growing the rhizosphere of *Lolium perenne* was broadly altered by P limitation, which may have reflected alterations in root exudation in response to P starvation. Conversely, colonization of *Arabidopsis thaliana* by *Bacillus subtilis* activated the Fe acquisition machinery to increase Fe uptake in through elevated transcription factors that stimulated ferric reductase and an iron transporter (Zhang *et al.*, 2009).

Clearly, complex signals and responses between mycorrhizosphere microbes and perhaps the plant host function to establish the structure and function of the community of the tripartite symbiosis. As the acquisition of nutrient resources is perhaps the keystone attribute of root symbiotic associations, the role of the tripartite symbiosis in the biological cycling of recalcitrant and limiting nutrients such as P is critical to forest ecosystem productivity.

3. Changes in Phosphorus Disequilibrium by Structured Mycorrhizosphere Communities

Soil nutrient limitation, especially N and P, limits the productivity of many natural and planted forests (Hou *et al.*, 2012; St. Clair *et al.*, 2008; Wardle *et al.*, 2004). To overcome P limitation, trees rely on integrated physiological acclimation systems that increase phosphate (P_i) availability in the rhizosphere and P_i uptake (Plaxton and Tran, 2011). Additionally, trees depend on the activity of symbiotic mycorrhizal fungi and PGPR to meet their nutritional demands (Calvaruso *et al.*, 2006; Plassard and Dell, 2010; Yang *et al.*, 2009). Symbiont stimulation of nutrient acquisition by forest trees is fostered by changes in root surface area and P_i acquisition affinity, increased soil exploration by EcM hyphae, and changes in exudation profiles of enzymes and small molecular weight compounds produced by both EcM fungi and PGPR.

3.1. Modulation of P_i Transporter Affinity by EcM Fungi

Stimulation of P uptake by EcM fungi will lead to increased dissolution of P_i complexes by altering equilibria concentrations in the soil solution. Increases in the affinity of P_i uptake will be more effective than increasing P_i uptake capacity (Machado and Furlani, 2004). Increased P_i acquisition by roots of *Populus tremuloides* colonized by *L. bicolor* resulted from higher affinity for P_i uptake by mycorrhizal roots ($k_m = 6.5 \mu\text{M}$) than NM roots ($k_m = 36.9 \mu\text{M}$) (Desai *et al.*, 2014). Similarly, the EcM fungi *P. involutus*, *Thelephora terrestris*, and *Suillus bovinus* increased P_i uptake due to the higher affinity of EcM roots in *Pinus sylvestris* (Van Tichelen and Colpaert, 2000). These changes in P_i uptake affinity may reflect changes in P_i transporter expression in the host plant

resulting from symbiosis and/or the operation of EcM transporters with innately greater affinity for P_i in the mycorrhizosphere (Becquer *et al.*, 2014; Loth-Pereda *et al.*, 2011; Plassard and Dell, 2010). In *Populus trichocarpa*, for example, the expression of several P_i -transporter genes was differentially regulated by EcM (as well as AM symbionts) (Loth-Pereda *et al.*, 2011), reflecting the role of mycorrhizal community diversity in influencing P_i acquisition potential of the host under P limitation. Such increases in the affinity for P_i uptake induced by EcM fungal colonization will alter chemical equilibria in the mycorrhizosphere and maintain the flow of P_i to the host plant.

3.2. Changes in Exudation Mediated by EcM Fungi

Working in concert with changes in uptake kinetics, the association of EcM with the short roots of trees has been shown to alter chemical equilibria of P_i -containing minerals in the soil. Such changes in mineral solubility may result from changes in the deposition of a variety of C-containing compounds capable of chelating metals such as aluminum (Al), Fe, and calcium (Ca), which often control P_i solubility. Mineral weathering may reflect enhanced production by the host root and/or EcM fungus of common exudates, such as low molecular weight organic acids (LMWOAs), or the production of novel compounds by EcM, including siderophores such as ferricrocin, that aid in dissolution reactions in the mycorrhizosphere (Baldwin, 2005; Becquer *et al.*, 2014; Hryniewicz *et al.*, 2010; Johannsson *et al.*, 2009; Plassard and Dell, 2010).

Several studies reporting exudation rates for LMWOAs suggest that colonization of tree roots by EcM fungi either reduces or does not affect the production of organic acids, including malate, citrate, and oxalate, which would alter P_i -containing compound dissolution in the mycorrhizosphere. In

P. tremuloides, colonization of roots by *L. bicolor* reduced the exudation of LMWOAs at any specific P_i concentration in the rhizosphere, an effect due to the change in response thresholds resulting from enhanced P_i uptake (Desai *et al.*, 2014). Oxalate production by *Pinus pinaster* mycorrhizas were symbiont dependent, with those formed with *Rhizopogon roseolus*, but not *Hebeloma cylindrosporum*, excreting oxalate (Casarin *et al.*, 2003). Similarly, Johannsson *et al.* (2008, 2009) noted limited differences in LMWOA exudation in *P. sylvestris* colonized by several EcM symbionts. However, roots of *P. sylvestris* colonized by six EcM species produced 6.7- to 13.6-fold greater dissolved organic C (DOC) in comparison with nonmycorrhizal roots (Johannsson *et al.*, 2008), suggesting that novel C-containing compounds produced by roots colonized by EcM fungi may play important roles in mycorrhizospheric processes. In contrast, exudation by roots of *P. sylvestris* colonized by *H. longicaudum*, *P. involutus*, or *Piloderma croceum* exhibited generally similar overall exudation, although the profile of LMWOAs varied between EcM and nonmycorrhizal roots (Van Schöll *et al.*, 2006). Colonization of roots of *P. tremuloides* by *L. bicolor* similarly altered organic acid profiles in the mycorrhizosphere, but, in contrast to Johannsson *et al.* (2008), reduced the flux of DOC to the rhizosphere, especially when P_i in the environment was limiting (Desai *et al.*, 2014). Such differences may reflect differences between hosts and/or symbionts in physiological processing of C.

Several novel compounds produced by EcM fungi may alter chemical equilibria in the rhizosphere. Hydroxymate siderophores, for example, play roles in the Fe nutrition of plants through their capacity to chelate and solubilize sparingly soluble Fe-containing minerals (Marschner and Marschner, 2012). At the same time, the production of such metal-chelating molecules may also alter P_i availability. Isolates of *P. involutus* differed in their production of siderophores,

with an isolate with greater production stimulating the growth of *S. viminalis* to a greater extent (Hryniewicz *et al.*, 2010). In *P. sylvestris* mycorrhizas, for example, *H. crustuliniforme* hyphae produced ferricrocin (Van Hees *et al.*, 2006). Exudates from *Pisolithus tinctorius* with high affinity for AL were collected by metal ion affinity chromatography and compounds ranging between 400 and 2000 Da were identified as compounds active in metal binding (Baldwin, 2005). That these masses are large compared to LMWOAs (citrate = 191, for example), there appears to be a collection of large exudate molecules yet to be described with the potential to mediate nutrient dissolution reactions.

3.3. Changes in P_o Scavenging Enzymes Mediated by EcM Fungi

In addition to stimulating the production of metal-chelating compounds by host roots or producing novel EcM fungal-derived weathering agents, the EcM symbiosis may alter the cycling of P in the mycorrhizosphere through the production of organic P (P_o)-scavenging enzymes, such as acid phosphatases (APases) and phytases (Courty *et al.*, 2010). These enzymes have the capacity to split phosphate ester bonds in organic complexes, freeing up P_i for subsequent uptake. As the forest soil P pool may be dominated by P_o (Ali *et al.*, 2009; Criquet *et al.*, 2004) that is otherwise not available to plants, access via EcM fungi associated with tree roots would greatly enhance the P nutrition of the host.

EcM fungi exhibit a wide range of capacities to access P_o . Numerous *in vitro* studies suggest that EcM may access a broad range of P-monoesters, such as sugar phosphates, nucleotide phosphates, and polyphosphates, as well as phytic acid (Louche *et al.*, 2010; Nygren and Rosling, 2009). In soils, Ali *et al.* (2009) found that mycorrhizas of *P. pinaster* exhibited a range of APase activities and were

responsive to environmental P limitation, increasing as soil extractable P_i declined. Similarly, APase rates varied widely between *Nothofagus oblique* mycorrhizal with *P. tinctorius*, *P. involutus*, *Cenococcum geophilum*, and *Descolea antarctica* (Alvarez *et al.*, 2012). Interestingly, Courty *et al.* (2011) found that phosphatase rates of 40 genotypes of *Populus* varied extensively, and, while rates were greatly increased by the association of *L. bicolor* with roots, patterns of variation among host genotypes did not change, which suggests that APase activity is under control of the host tree, yet stimulated by the EcM symbiont.

3.4. Modulation of P_i Transporter Affinity by PGPR

The association of PGPR with the roots of many plant species often stimulates growth and nutrient acquisition due to changes in a variety of both environmental and plant factors (Bhattacharyya and Jha, 2012; Compant *et al.*, 2010; Persello-Cartieaux *et al.*, 2003; Yang *et al.*, 2009). Among these may be changes in host root ion transporter behavior that would favorably benefit nutrient acquisition. As P_i is available sparingly in most soils, especially those not managed by fertilization, changes in P_i transport affinity or capacity would benefit plant P_i acquisition from the rhizosphere. Unlike EcM fungi, however, PGPR do not form a fungal mantle that serves as an interface between the soil solution and root cortical cells. It is this fungal sheath that may mediate changes in ion transport noted above—indeed it may be difficult, at the flux level, to disentangle EcM from host uptake systems in the mycorrhiza. For PGPR, however, changes in root ion uptake would have to result from changes in host physiology modulated by a signal from the bacteria.

PGPR provide significant benefits to their hosts in P-limiting environments. In *Populus tremuloides*, for

example, inoculation with *P. fluorescens* significantly improves plant performance at low P_i *in vitro* (Figure 2). Such benefits may result from alterations in P_i transporter expression in the host root, such as those noted for mycorrhizal fungi, above. Given the growth and nutrient-stimulating effects of PGPR on many plant species, it is surprising that data on ion uptake/flux is limited (Mantelin and Touraine, 2004). At the same time, linkages between developmental growth changes induced by PGPR and nutrient demand confound assessment of potential direct mediation of plant transport system activity by PGPR (Mantelin and Touraine, 2004). In one

of few studies in (Mantelin and Touraine, 2004). In one of few studies in this area, Bertrand *et al.* (2000) noted that inoculation of roots of *Brassica napus* by *Achromobacter* stimulated NO_3^- and K^+ uptake rates while increasing H^+ extrusion. These findings suggest that there was an increase in energization of cortical cells by the PGPR that may have stimulated the driving forces for ion transport (Bashan, 1990). Given the extensive data showing PGPR stimulation of plant growth and nutrient efficiency, there seems to be ample room for research on the nutrient transport mechanisms underlying these benefits.

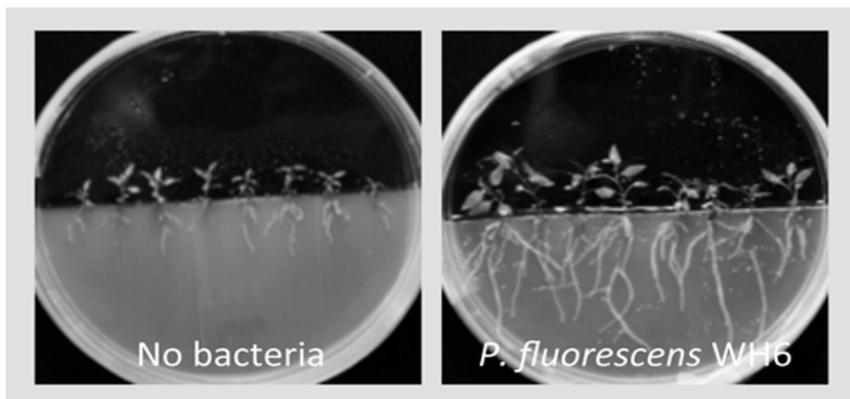


Figure 2. Growth of *Populus tremuloides* on nutrient agar containing 25 μM P_i . Plants were not inoculated (left) or inoculated with *Pseudomonas fluorescens* strain WH6 (right) (Desai and Collart, unpublished).

3.5. Changes in Exudation Mediated by PGPR

PGPR play a significant role in producing a suite of compounds that weather minerals in the rhizosphere (Bhattacharyya and Jha, 2012; Cassán *et al.*, 2014; Compant *et al.*, 2010; Persello-Cartieaux *et al.*, 2003; Yang *et al.*, 2009), which establishes their functional role as “phosphate solubilizing bacteria” (Khan *et al.*, 2009; Rodriguez and Fraga, 1999). Additionally, PGPR may stimulate exudation of compounds

by their host plants capable of altering nutrient disequilibria, which could also play a role in altering nutrient solubility equilibria in the rhizosphere. However, research in this area is scarce. In one of the few reports assessing the influence of PGPR on host root exudation patterns, Liu *et al.* (2013) found that *Fraxinus americana*, inoculated with *B. subtilis*, exhibited significant increases in the root exudation of sugars, amino acids, and organic acids. These changes suggest that the PGPR may influence nutrient/energy

resource flow from the plant to the rhizosphere, which could also alter P-containing mineral solubility.

3.6. Changes in P_o Scavenging Enzymes Mediated by PGPR

Many of the benefits of PGPR are derived from increased cycling of P in the plant rhizosphere, and most data related to the P benefit of PGPR has focused on the dissolution of sparingly soluble inorganic P complexes. However, access to soil P_o reserves would also benefit host nutrition, especially in forest soils where P_o is prevalent. A variety of PGPR exhibit both APase and phytase activities (Franco-Correa *et al.*, 2010; Idriss *et al.*, 2002; Turan *et al.*, 2012) and, in some cases, this translates to improved plant performance. In relation to forest tree species, Li *et al.* (2013) isolated 17 phytate-degrading bacterial strains from *Populus euramericana* and *Pinus massoniana* plantation soils. As with EcM fungi, phytic acid degradation activity varied extensively among isolates.

4. Phosphorus Disequilibrium in Complex Mycorrhizosphere Communities

Microbe-mediated changes in root system/mycorrhiza P_i uptake affinities and rates as well as in C flux to the mycorrhizosphere will alter mineral weathering in the soil (Finlay *et al.*, 2009; Finlay, 2008). Reactions driven by acidification and ligand binding will foster P_i (and organic P-containing compound) release from minerals and complexes in the mycorrhizosphere. Release of phosphatases and phytases would foster the cycling of organic-P molecules. Both processes would increase P_i availability to the tripartite association and the plant host.

4.1. Increasing Mineral P_i Dissolution

EcM fungi colonize the vast proportion of root absorptive surfaces of forest trees and fungi integrate their host plants into biogeochemical cycles by facilitating the weathering of soil minerals (Blum *et al.*, 2002; Finlay *et al.*, 2009). Although the role of each EcM species in a soil may vary, many increase H^+ extrusion, organic acid and DOC exudation, and weathering of soil minerals.

At a scale focusing on biogeochemical evidence, isotopic signatures indicated that apatite was a significant source of Ca (and hence P) in forests dominated by spruce and fir, but not by sugar maple (Blum *et al.*, 2002). Similarly, soil analyses from the mycorrhizospheres of *Abies lasiocarpa* indicated that *Piloderma* sp. facilitated significant changes in cation availability (Arocena and Glowa, 2000) and microscopic evaluation of soil minerals associated with EcM hyphae indicted biofilms, structural modifications, and dissolution patterns indicative of biological weathering (Augusto *et al.*, 2000; Saccone *et al.*, 2012; van Breemen *et al.*, 2000). In a root exclusion study, rock phosphate dissolution in a hyphal compartment in a field soil was correlated with soil acidification mediated by fungal hyphae of *Pinus radiata* (Liu *et al.*, 2005).

Experiments at the plant level, where environmental conditions are more controlled, support field observations that the capacity of EcM fungi in mineral weathering varies by species. For example, Wallander (2000) noted significant weathering of apatite mediated by *Suillus variegatus* on *P. sylvestris*, and this dissolution was related to oxalate concentrations in the root zone. Nonmycorrhizal seedlings also facilitated apatite dissolution, but to a lesser extent. Similarly, *R. roseolus*, which facilitated oxalate exudation in *P. pinaster*, accessed hydroxyapatite in soils (Casarin *et al.*, 2004). The dissolution of

berlinite by *Pinus rigida* colonized by *P. tinctorius* was mediated by unidentified exudates, however nonmycorrhizal plants were unable to breakdown this compound (Cumming and Weinstein, 1990). Dissolution of iron ore varied among *P. tinctorius*, *P. involutus*, *L. bicolor*, and *Suillus tomentosus* on *Pinus patula*, which produced fungal-specific exudation profiles (Adeleke *et al.*, 2012).

One of the best-noted roles of PGPR in promoting plant growth is their ability to solubilize P from a variety of P-containing minerals (Gyaneshwar *et al.*, 2002; Khan *et al.*, 2009; Rodriguez *et al.*, 2006). There is a vast literature supporting siderophore-mediated mineral dissolution (Leong, 1986; Loper and Henkels, 1999; Rodríguez and Fraga, 1999). In soils, certain *Burkholderia glathei* isolates facilitated the dissolution of biotite in the rhizosphere of *P. sylvestris*, and increased acquisition of K⁺ and Mg²⁺ for the plant (Calvaruso *et al.*, 2006). Such benefits may be direct, *i.e.*, bacteria produce new compounds that are functionally more active in the weathering process, or indirect, *i.e.*, the bacteria stimulate plant root growth and increase their capacity to weather minerals (Calvaruso *et al.*, 2006). Similarly, PGPR were capable of promoting the growth of several cactus species through the production of organic acids that acidify the substrate and increase the dissolution of rhyodacite other substrates (Lopez *et al.*, 2012; Puente *et al.*, 2004).

To support chemical weathering of substrates, several *in vitro* studies have investigated the production of H⁺ and organic acids by PGPR and their impacts on mineral dissolution. Chen *et al.* (2006) assayed 36 environmental strains selected for P-solubilizing capacity and noted that dissolution of tricalcium phosphate was associated with culture acidification. Further, isolates produced unique profiles of citric acid, gluconic acid, lactic acid, succinic acid, and propionic acid (as well as three unknowns), although

there was no clear pattern suggesting any particular acid was more effective than others (Chen *et al.*, 2006). In 45 *Collimonas* strains, tricalcium orthophosphate and biotite solubilization was associated with acidification of the media, although these strains also produced gluconic acid that may serve as a weathering agent (Uroz *et al.*, 2009). Interestingly, of two effective phosphate-solubilizing bacteria, only *Enterobacter aerogenes* conferred a growth benefit to host *Phaseolus vulgaris* plants grown with tricalcium phosphate, suggesting that *in vitro* screening may not reflect performance *in symbio* or that host-PGPR compatibility also needs to be considered when deploying PGPR in a management strategy.

Few studies have assessed combinations of EcM and PGPR in affecting nutrient acquisition, interactions between symbionts, and plant performance. In *P. sylvestris*, dual colonization with *Agrobacterium* and *Laccaria laccata* increased weathering of phlogopite, accompanied by losses of K⁺, Mg²⁺, and Fe³⁺ (Leyval and Berthelin, 1991). Interestingly, in this study, inoculation with *Agrobacterium* increased the production of citrate, malate, fumarate, and lactate by roots, whereas roots inoculated with *L. laccata* or both symbionts exhibited a strong suppression of organic acid exudation. Furthermore, *Agrobacterium* increased root colonization by *L. laccata*, pointing to the importance of mycorrhizospheric community ecology when addressing plant-soil interactions. Koele *et al.* (2009), similarly working with *P. sylvestris*, *L. bicolor*, and *Scleroderma citrinum* as well as *B. glathei* and *Collimonas* sp., noted that symbionts differentially altered biotite weathering, especially aiding in Mg²⁺ access and uptake. Importantly, PGPR strain population persistence was elevated in mycorrhizas versus bulk or rhizosphere soils and was also EcM species dependent, again pointing to important ecological (and related functional) attributes of the tripartite association (Koele *et al.*, 2009).

In the field, Calvaruso *et al.* (2007) assessed microbial functional diversity in a *Quercus petraea* forest by isolating 264 bacterial strains from soils, rhizospheres, and *S. citrinum* mycorrhizas. Assessment of nutrient mobilizing capacity of these isolate indicated that EcM have a strong

influence on the functional ecology of microbial populations, with increased selection for strains that exhibit elevated Fe and P solubilization activity from the bulk soil to the fungal sheath (Figure 3).

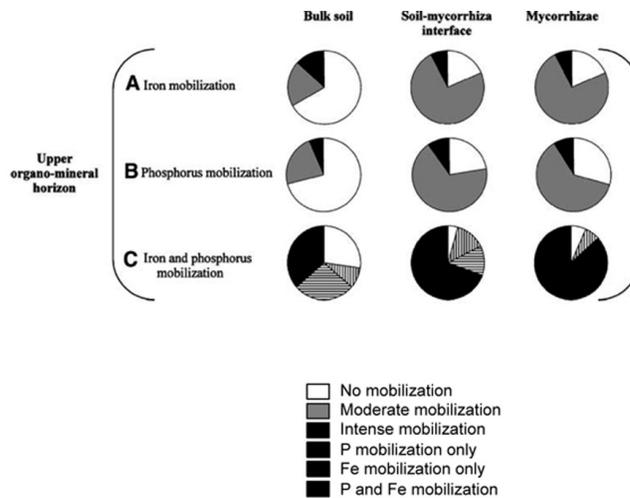


Figure 3. Iron and phosphorus mobilization capacities of microbial communities increase from the bulk soil to the soil-mycorrhiza interface to the mycorrhizae of *Quercus petraea*-*Scleroderma citrinum* mycorrhizas. Changes in mobilization capacity depicted in pie charts and legend reflect EcM structuring of microbial communities in these microenvironments. Reproduced from Calaruso *et al.* (2007) with permission.

4. 2. Increasing Organic-P Cycling

Accessing soil P_0 reserves and transferring P to the plant host would be a significant benefit of microbial symbionts in forest soils, and several *in symbio* studies indicate that EcM fungi provide this capacity to forest trees. Mycorrhizas of *N. oblique* formed with *P. tinctorius*, *P. involutus*, *C. geophilum*, and *D. antartica* exhibited a wide range of APase activities, and P concentration of host shoots was positively correlated with mycorrhiza APase activity across

all symbionts (Alvarez *et al.*, 2012). In addition to correlations of APase activity and plant performance all symbionts (Alvarez *et al.*, 2012). In addition to correlations of APase activity and plant performance in soils, several authors have noted that EcM fungal APase activity is associated with depletion of soil P_0 pools, providing functional support to the reports on APase activity. Native EcM hyphae from roots of *P. radiata* exhibited elevated APase activity that was associated with depletion of P_0 fractions in a forest soil (Liu *et al.*, 2005), and *P. involutus* hyphae

extracted P from leaf litter and transferred P to host *P. sylvestris* (Perez-Moreno and Read, 2000). Given the wide range in APase and phytase capacities of EcM species reported, it is clear that selection by the host or edaphic environment of the EcM community has the potential to increase P acquisition by forest trees (Buée *et al.*, 2007; Courty *et al.*, 2006; Taniguchi *et al.*, 2008).

Little information is available on Po access by PGPR and P transfer to host plants. Two *P. fluorescens* and

one *Rhanelia aquatilis* strains with high phytase activity provided significant growth enhancement to *P. euramericana* (368% increase in height growth) and *P. massoniana* (69% increase in height growth) seedlings in a low P forest soil (Li *et al.*, 2013). More work in this area, especially aligning *in vitro* enzyme activities with performance *in symbio*, would further elucidate the roles of PGPR in accessing P in the rhizosphere (see also Becquer *et al.* 2014).

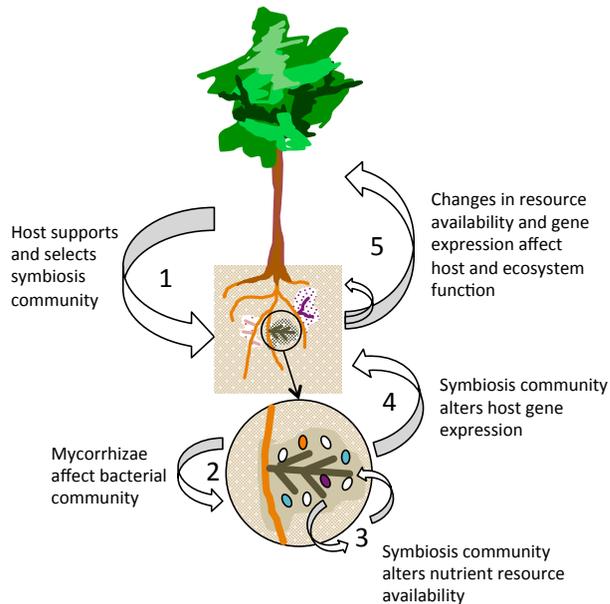


Figure 4. Interactions affecting the acquisition of soil nutrient resources in trees. Selection of the microbial community by host and edaphic factors creates a specialized tree-mycorrhiza-rhizobacterial metaorganism that deploys the genome resources of all symbionts to explore soils, alter nutrient availability, increase acquisition, and alter metabolic pathways to acclimate to nutrient limitation.

5. Conclusions

Within soils, biogeochemistry and demand both function to limit Pi availability at the tree root-soil interface. Phosphorus limitation leads to a variety of metabolic stresses and adjustments that serve as acclimation

mechanisms to nutrient limitation. The association of symbiotic EcM fungi and PGPR with tree roots greatly influences these stress and acclimation spones by altering P availability, acquisition, and metabolism in the mycorrhizosphere.

While, there is limited process-based understanding of these interactions, it is clear that there are functional ecological processes involved, including host plant selection of EcM communities and subsequent selection of PGPR associates, which creates a metaorganism interacting with the soil environment (Figure 4). Interactions among the partners lead to changes in metagenome expression and deployment of combined physical, physiological, and metabolic systems to explore soils, alter nutrient availability, increase nutrient acquisition, and alter metabolic pathways. Interactions among the partners lead to changes in metagenome expression and deployment of combined physical, physiological, and metabolic systems to explore soils, alter nutrient availability, increase nutrient acquisition, and alter metabolic pathways functioning to acclimate to nutrient limitation (Figure 4). The complexity of these relationships and responses influence the functional ecology of the mycorrhizosphere and the larger scale productivity of forested ecosystems.

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