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Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species

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Summary

1. We hypothesized that tropical plant species with different mycorrhizal associations reduce competition for soil phosphorus (P) by specializing to exploit different soil organic P compounds.
2. We assayed the activity of root/mycorrhizal phosphatase enzymes of four tree species with contrasting root symbiotic relationships--arbuscular mycorrhizal (angiosperm and conifer), ectomycorrhizal and non-mycorrhizal--collected from one of three soil sites within a montane tropical forest. We also measured growth and foliar P of these seedlings in an experiment with P provided exclusively as inorganic orthophosphate, a simple phosphomonoester (glucose phosphate), a phosphodiester (RNA), phytate (the sodium salt of *myo*-inositol hexakisphosphate), or a no-P control.
3. The ectomycorrhizal tree species expressed twice the phosphomonoesterase activity as the arbuscular mycorrhizal tree species, but had similar phosphodiesterase activity. The non-mycorrhizal Proteaceae tree had markedly greater activity of both enzymes than the mycorrhizal tree species, with root clusters expressing greater phosphomonoesterase activity than fine roots.
4. Both the mycorrhizal and non-mycorrhizal tree species contained significantly greater foliar P than in no-P controls when limited to inorganic phosphate, glucose phosphate, and RNA. The ectomycorrhizal species did not perform better than the arbuscular mycorrhizal tree species when limited to organic P in any form. In contrast, the non-mycorrhizal Proteaceae tree was the only species capable of exploiting phytate, with nearly three times the leaf area and more than twice the foliar P of the no-P control.
5. Our results suggest that arbuscular and ectomycorrhizal tree species exploit similar forms of P, despite differences in phosphomonoesterase activity. In contrast, the mycorrhizal tree species and non-mycorrhizal Proteaceae appear to differ in their ability to exploit phytate. We conclude that resource partitioning of soil P plays a coarse but potentially ecologically important role in fostering the coexistence of tree species in tropical montane forests.

Introduction

Plant species and their symbiotic associates encounter soil nutrients in chemical forms that present different barriers to acquisition, providing an opportunity for specialization to exploit different fractions of the total nutrient pool. This phenomenon is well documented with respect to soil nitrogen (N), with evidence for preferential uptake of different chemical forms leading to species coexistence within a site via the formation of complementary soil niches and/or between sites via habitat-specialization on soils dominated by different chemical forms (McKane et al. 2002; Weigelt, Bol, & Bardgett 2003; Oelmann et al. 2007; although see Andersen & Turner 2013). A similar phenomenon may occur for soil phosphorus (P), which exists in diverse organic forms that are susceptible to different classes of enzymatic attack before they can be exploited by plants (Turner 2008; Ahmad-Ramli, Thomas, & Johnson 2013). Here we compare the capacity of tropical tree species from four distinct functional groups to exploit P from different organic sources in order to gauge the potential for partitioning of the total soil P pool.

Phosphorus is an essential plant nutrient with virtually no atmospheric presence (Marschner 1995). Physiological and bio-chemical studies suggest that plants respond to changes in soil P availability with a diversity of acquisition strategies (*reviewed in* Vance et al. 2003). Organic P constitutes a major proportion of the total P in most soils (Harrison 1987) and is present in compounds that must be mineralized by different classes of phosphatase enzyme before the phosphate can be taken up by plants. Mineralization of organic P represents the combined effects of plants, free living soil microbes, and root-associated mutualists, particularly mycorrhizal fungi, which account for as much as 80% of the total P uptake by plants (Douds *et al.* 2000). The different forms of soil P can be placed along a gradient of increasing investment required for mineralization, depending on their solubility and the type and number of enzymes that must be produced in order to liberate phosphate (Turner 2008) (Figure 1). Thus, simple phosphomonoesters (e.g. glucose-phosphate, mononucleotides) require hydrolysis by the enzyme phosphomonoesterase, while phosphodiesters (e.g. DNA, RNA) require both phosphomono- and phosphodiesterase to liberate phosphate. Phytate (salts of *myo*-inositol hexakisphosphate) is hydrolyzed

by a special class of phosphomonoesterases called phytases (Figure 1). Of these organic forms, phytate is the most resistant to hydrolysis, due in part to its strong stabilization on mineral soil surfaces. It must therefore be solubilized prior to enzymatic hydrolysis, which presumably represents the most metabolically expensive cost of acquisition among the organic P compounds (Turner 2008).

We compared the phosphatase enzyme activity and performance of tropical tree species from the following functional groups—arbuscular mycorrhizal angiosperms, arbuscular mycorrhizal conifers, ectomycorrhizal angiosperms, and non-mycorrhizal proteoid plants. Most plant species form associations with arbuscular mycorrhizal (AM) fungi, which produce a network of hyphae in the soil and leaf litter that are thought to predominately increase the acquisition of inorganic P (Dodd et al. 1987; Tarafdar & Marschner 1994; Joner, van Aarle, & Vosatka 2000). However, tropical conifers in the family Podocarpaceae, which form arbuscular mycorrhizas inside specialized root nodules (Dickie & Holdaway 2011), are associated with P-deficient soils enriched in phosphodiesters and phytic acid (Richardson et al. 2004; Turner et al. 2007). This suggests that either podocarps or their fungal partners might have the ability to utilize recalcitrant organic P (Turner 2008), although this has not been tested (Dickie & Holdaway 2011). A third group of tree species associate with ectomycorrhizal (EM) fungi, which have evolved from saprotrophs and are known to exploit organic P via the exudation of phosphatase enzymes (Phillips & Fahey 2006; Smith & Read 2008). Finally, tropical non-mycorrhizal trees from the family Proteaceae are restricted to soils with very low P availability (Lambers et al. 2012). Proteaceae trees form cluster roots, or densely packed rootlets produced from the main root axis, that exude abundant phosphatase enzymes capable of mineralizing organic P (Adams & Pate 1992; Watt & Evans 1999; Lambers *et al.* 2006; Lambers *et al.* 2008).

Plant species capable of exploiting organic P should have high root/mycorrhizal phosphatase activity. In addition, plant species capable of exploiting recalcitrant organic P should elicit growth, allocational, and nutritional reactions when limited to phosphodiesters and phytate. These include (relative to a no-P control) (i) increased foliar P concentration; (ii) increased growth; (iii) increased leaf area (Shipley & Meziane 2002); and (iv) increased specific leaf area (SLA), a functional leaf trait

describing the ratio of leaf area to leaf mass that correlates positively with plant nutrition (Meziane & Shipley 2001).

We hypothesized that differences in mycorrhizal associations among tree species would result in a gradient in the ability to exploit organic P, with taxa arrayed in the following order (from lowest to highest ability): AM angiosperm trees, AM podocarp conifers, EM trees, and NM trees (Proteaceae). We tested this hypothesis by using root phosphatase assays of tree species representative of different P-acquisition strategies from a tropical montane forest, and by conducting a growth experiment in which seedlings of the same tree species were supplied with one of a series of compounds as the sole P source.

Methods

We conducted two separate experiments. In the first experiment, plant species were collected from the field, potted in field soil, transferred to the lab and exposed to artificial enzyme substrates in order to assay phosphomono- and diesterase activity. In the second experiment, a separate group of field-collected seedlings from the same plant species were grown in a shade-house in the field in a sand growth medium with P provided in one of 4 different chemical forms (with a no-P control).

Study site

The study was conducted in the Fortuna Forest Reserve along the Cordillera Central of western Panama. The reserve supports lower montane forest between 1000 and 1500 m a.s.l., with mean annual rainfall of 4900–7200 mm and mean annual temperature of 19–22°C (Cavelier, Solis, & Jaramillo1996; Andersen, Turner, & Dalling 2010). Photosynthetically active radiation under (PAR) cloudy conditions that prevail in the montane forest at Fortuna are 290 μmol photons m^2 s^{-1}. Seedlings were collected close to permanent 1-hectare forest census plots at Chorro, Honda B, and Hornito (see Andersen et al. 2012, for site descriptions). Soils at Chorro and Honda B are formed on rhyolitic tuff; the surface soil (0-10 cm) has pH in water of <4.0 and low concentrations of inorganic N (0.63 and 3.40 μg cm^{-3} extracted in KCl, respectively) and extractable P (2.74 and 4.10 μg cm^{-3} by Mehlich-3 extraction, respectively) compared to

other nearby sites (Andersen et al. 2012). In contrast, Hornito has more fertile soils developed on porphyritic dacite, with higher pH (5.8) and greater concentrations of inorganic N and extractable P (4.52 and 10.92 μg cm^{-3}, respectively). The Chorro plot is dominated primarily by canopy palms (*Colpothrinax aphanopetela*), but also contains the AM focal species *Podocarpus oleifolius* (~8% of basal area). The Honda B plot is dominated by the EM emergent canopy tree *Oreomunnea mexicana* (Juglandaceae), which accounts for 42% of the basal area of the plot. The Hornito plot supports a species rich assemblage that includes AM, EM, and NM tree species, including *Mollinedia* (0.3% of basal area) and *Roupala* (2% of basal area), but lacks *Podocarpus* and contains a relatively low abundance of *Oreomunnea* relative to Honda B (Anderson *et al.* 2010; J. Dalling *unpublished data*).

Plant species collection

Four focal tree species representative of different root morphologies and/or mycorrhizal association were chosen for both of our experiments (Table 1). Sixty seedlings from the same cohort (<1 year old) of each species were selected to minimize differences in initial size in February of 2010, and were transplanted to the greenhouse. Seedlings were harvested from live soils where they reached abundances sufficient to allow for replication in our two experiments. We did not include a cohort grown in sterile soil as a control as we were not attempting to quantify the relative contribution of plant, mycorrhizal fungi, and other root associated microbes to plant nutrition and phosphatase activity. While this approach lacks precision in terms of the influence of specific host-microbial interactions, it does sample a composite of ecologically relevant interactions that potentially influence growth and resource acquisition among our four tree species under natural conditions (e.g., mycorrhizal fungi, symbiotic and free-living bacteria).

Mycorrhizal Colonization Assays

At the end of the two experiments, we harvested five 10 cm fine root segments from each plant. Roots were put in cassettes and then cleared in 10% KOH at 90 ˚C for several hours and leached in H_2O_2 for up to 30 min or until completely transparent. The roots were then acidified in 2% HCl for 30 min and stained

in trypan blue for 1 hour. Finally the roots were preserved in acidified glycerol. Percent infection of EM and AM fungi was quantified for each root using the protocol outlined in McGonigle et al. (1990). Using 200x magnification, presence of infection was scored at 1 mm intervals for the entire length of the root section. We scored vesicles, arbuscules, and AM hyphae as positive AM encounters (Brundrett 2009; Treseder 2013) while the EM Hartig net and external mantel were scored as positive EM encounters (Brundrett 2004). Separate quantification of AM arbuscules was not possible due to difficulty detecting these structures in our stained root samples. The percentage of infection was calculated as the ratio of 1 mm intervals that contained AM or EM fungal structures to the total number of intervals observed.

For the root phosphatase assays we were interested only in the infection of the root segments (2-3 cm) that were exposed to enzyme substrates. For the AM species, we considered root segments colonized by AM fungi if they contained vesicles, arbuscules, or AM hyphae. Dried roots were rehydrated in water overnight prior to clearing and staining (Hetrick, Wilson, & Hartnett 1989). For the EM species (*Oreomunnea*), we assayed only infected root segments, although we selected these at random and without respect to EM morphology or exploration type.

Experiment 1: Phosphatase Assays

To assay phosphomonoesterase and phosphodiesterase 10–14 individuals of each species were potted for 10 months in field soil from the sites from which they were collected before roots were harvested for analysis. The assay was based on a protocol modified from Antibus, Sinsabaugh, & Linkins (1992) and focuses on the activity of surface-bound and extra-cellular acid phosphatases associated directly with plant roots and the fungal mantle (in EM plants). Plant roots were rinsed gently in deionized water and adhering soil particles removed with forceps. For each plant, five terminal fine root segments (5 cm) were placed in 4.9 mL of 20 mM acetate buffer adjusted to pH 5.0 and incubated at 26°C in a shaking water bath. Assays were initiated by the addition of 0.1 mL of 25 mM substrate (final concentration 0.5 mM) of either *para*-nitrophenyl phosphate (phosphomonoesterase substrate; Fisher Scientific, Pittsburgh, PA) or

bis-*para*-nitrophenyl phosphate (phosphodiesterase substrate; Sigma-Aldrich, St Louis, MI), and incubated for 45 minutes. A blank (roots but no substrate) and a control (substrate but no roots) were included with each assay to correct for color not originating from phosphatase activity. To terminate the reaction, 0.5 mL of the assay solution was added to 4.5 mL of 0.125 M NaOH. Absorbance was determined at 405 nm against *para*-nitrophenol standards between 0 and 10 μM. Roots were dried at 105°C for 24 h to determine dry mass. Enzyme activity was expressed as μmol product mg^{-1} dry root mass h^{-1}.

Experiment 2: Seedling Growth and Allocation Experiment

To determine how plant performance varied when grown with different P sources, eight seedlings of each species were assigned to each of five treatments. Source seedlings were grown in the pots containing soil from their source of origin and were transplanted 1-2 weeks after collection into the treatments. At transplant roots were gently washed with water and the seedlings transferred into 1 L pots (8.3 cm diameter x 40 cm height containers) filled with acid washed sand along with ~1 mL of the original live soil. Seedlings were assigned to treatments to minimize differences in mean stem height and leaf number among treatments. The experiment was conducted during the wet season in lower montane forest with a high frequency to cloud immersion and >4000mm rainfall per year. All plants were grown at the Fortuna Forest Reserve in a 4 x 8 meter greenhouse under shade cloth, which reduced PAR to ~18% of that found under open sky, approximating light conditions found under small gaps in the forest canopy. Maximal temperature variation in the greenhouse was between 17°C at night and up to 36°C during midday sunny periods. Treatments differed in the supply of P, provided as: (1) inorganic orthophosphate (2) a simple phosphomonoester (glucose 6-phosphate), (3) sodium phytate (sodium salt of *myo*-inositol hexakisphosphate), (4) a phosphodiester (RNA), and (5) a P-free control (No-P) (Table 2). Initial stem height and leaf number were recorded for each replicate for use as potential covariates. Six additional seedlings of each species were harvested before the experiment began to estimate initial seedling mass and allocational variables (Table 3). Plants of the same species and treatment were grouped together in

individual racks containing eight replicated each, which were distributed randomly on two benches and rotated every five days so that each rack occupied each position in the greenhouse for an equal amount of time.

Experimental and control plants were fertilized on alternate days with 30 mL of a solution containing all essential nutrients. First, a no-P nutrient stock solution was prepared using Scott's Dark Weather Feed® fertilizer (Scott's MiracleGro, Maryville, Ohio), supplemented with $MgSO_4$. For the P treatment solutions, a solution of the appropriate chemical form of P was added to the nutrient stock before fertilization (Table 2). Additionally, every three days each plant received 30 mL of rainwater collected adjacent to the greenhouse. After ~3.5 months, plants were harvested and leaf area measured using a flatbed scanner. Plants were dried at 70°C for 24 h and weighed. Foliar P was determined by ignition (550°C, 1 h) and acid digestion (1 M H_2SO_4), with phosphate detection by automated molybdate colorimetry.

Plant dry mass was used to calculate relative growth rate (RGR),

$$RGR = \frac{\ln(M_f - M_i)}{dt}$$

where M_f is final mass, M_i is initial mass estimated as the average dry mass of the pre-experimental harvest, and dt is the duration of the experiment in days. Leaf area was calculated as the difference between initial and final leaf area, where initial leaf area was estimated as the mean leaf area of the pre-experimental cohort for each species separately (Table 3). Specific leaf area was calculated as the ratio of total leaf area over dry mass.

Statistical Analysis

For experiment 1, differences in phosphomonoesterase and phosphodiesterase activity among species and root forms were analyzed using ANOVA with post-hoc comparisons made using Fisher's least significant difference (LSD). The same analysis was conducted on differences in dry mass, total and specific leaf area, and foliar P concentration among species from the pre-experimental harvest from experiment 2. For

species exposed to different chemical forms of P in experiment 2, differences in relative growth rate, total and specific leaf area, and foliar P were analyzed in ANOVA with species and treatment used as model variables and initial height and number of leaves used as covariates. In addition, for each species x dependent variable combination, linear models were used to assess the significance of treatment responses relative the no-P control (intercept). All analyses were performed using R version 2.15.3 (R Core Team, 2013).

Results

Mycorrhizal Colonization Assays

No mycorrhizal fungi were observed in roots of *Roupala*, confirming its non-mycorrhizal status. There were significant differences among the mycorrhizal tree species in percentage root length colonized by mycorrhizal fungi: *Oreomunnea* (EM) 63%, *Podocarpus* (AM) 37%, and *Mollinedia* (AM) 18%. Root length colonized within individual P treatment groups from experiment 1 was highly variable (supplementary figure 1). We performed an ANOVA on the colonization percentages with plant species and P treatment as fixed factors. Plant species differed significantly in their extent of colonization, whether we compared all three mycorrhizal tree species ($F_{2,10}$=13.7, $p<0.001$), or just the two AM tree species ($F_{1,6}$=8.7, $p<0.01$), while there were no significant treatment or species x treatment interaction effects in either model.

We recorded similar levels of colonization in the enzyme assay roots as those observed in the growth-experimental plants (20% infected roots in *Mollinedia* and 33% in *Podocarpus*, and no infection in *Roupala*). We found no significant differences in the frequency of AM structures among treatments or between AM species (Chi-square test, $p>0.05$).

Initial Measures

Oreomunnea (EM) seedlings were larger than the three other species, with significantly greater initial mass and total leaf area. Leaves of *Mollinedia* had significantly greater SLA than those of *Podocarpus*,

while the other species did not differ significantly from one another. *Roupala* had markedly greater foliar P than the other species, with approximately three times the P concentration of *Oremmunea* (EM) and twice the foliar P of *Mollinedia* and *Podocarpus* (both AM) (table 3).

Experiment 1: Phosphatase Assays

Overall, there were significant differences among species in phosphatase activity. *Roupala* (NM) produced significantly greater phosphomono- and phosphodiesterase activity than the other focal species (Figure 2a-b). *Roupala* root clusters produced significantly greater phosphomonoesterase than fine roots excised from the same seedlings (Figure 2a), while values did not differ for phosphodiesterase (Figure 2b). The AM species *Podocarpus* and *Mollinedia* did not differ in either phosphomono- or phosphodiesterase activity, while *Oreomunnea* (EM) expressed twice the phosphomonoesterase activity of *Podocarpus* and *Mollinedia*, these differences were marginally significant ($p=0.10$ and 0.06, respectively; Figure 2b). There were no significant differences between infected and uninfected root segments of the arbuscular mycorrhizal tree species for either phosphatase assay.

Experiment 2: Seedling Growth and Allocation Experiment

Seedlings of our study species showed a diversity of responses to experimentally applied P sources (Table 4). However, of particular interest for this study was whether species differed in their responses to different P sources, manifested as a species × treatment interaction. We found highly significant species × treatment interaction effects ($p<0.001$) for all dependent variables (Figure 3a-d).

The growth rate of the AM species was significantly enhanced with the addition of inorganic P and marginally inhibited by RNA. *Mollinedia* (AM) differed from *Podocarpus* in exhibiting greater growth in the glucose phosphate treatment than the No-P control ($p=0.06$) (Figure 3a). A similar pattern was observed for leaf area, although inhibition in the RNA treatment was insignificant for both AM species (Figure 3b). In contrast, *Oreomunnea* (EM) growth and leaf area was largely insensitive to differences in P form, with the exception of significant inhibition by RNA causing negative growth (Figure 3a) and

change in leaf area (Figure 3b), indicating a net loss in plant biomass over the experimental period. The growth rate of *Roupala* (NM) was highest in the phytate treatment, but this difference was not significant due to the high growth rate maintained by plants in the no-P control (Figure 3a). Six of eight *Roupala* seedlings in the No-P controls developed root clusters, while this occurred in none of the seedlings in the other treatments. *Roupala* seedlings in the phytate treatment developed nearly three times the leaf area (Figure 3b) and twice the specific leaf area relative to plants in the no-P control (Figure 3c).

Foliar P patterns for all of the mycorrhizal tree species were significantly greater than the control in the RNA, glucose phosphate, and inorganic phosphate treatments (Figure 3d). *Roupala* (NM) was unique among the tree species in exhibiting significantly greater foliar P in the phytate treatment, with greater than twice the foliar P as the no-P control (Figure 3d). Although *Podocarpus* (AM) had marginally greater foliar P in the phytate treatment relative its no-P control ($p=0.06$), it exhibited low but variable growth when limited to phytate (Figure 3a).

Discussion

Our study demonstrates that non-mycorrhizal *Roupala* exhibited both a markedly greater ability to mineralize mono- and diester-P than coexisting mycorrhizal tree seedlings and a unique ability to exploit phytate, the most metabolically expensive form of organic P presented in our experiment (Turner 2008) (Figure 1). In contrast, we failed to find evidence for differences in the ability to exploit organic P between ecto- and arbuscular mycorrhizal tree seedlings. Although the fungal roots of ectomycorrhizal tree seedlings expressed twice the phosphomonoesterase activity as the fine roots of an arbuscular mycorrhizal angiosperm and conifer, this difference did not translate into growth or nutritional benefits when seedlings with comparable mycorrhizal infection were limited to organic P. An analysis of foliar P levels suggests that the lack of growth benefit in ectomycorrhizal seedlings was not related to P limitation, as both the arbuscular and ectomycorrhizal species had significantly greater P levels in monoester, diester, and inorganic P treatments relative to their no-P controls. Similarly, differences within the arbuscular mycorrhizal group suggest that both species exhibited similar competencies for exploiting

organic P, with *Mollinedia* somewhat better able to augment growth and leaf development when limited to monoester P. Overall, our results present strong evidence for the potential of non-mycorrhizal and mycorrhizal tree species to exploit different fractions of the soil P pool, while suggesting that the mycorrhizal tree species exploit similar forms of P despite exhibiting a diversity of growth responses.

Studies from arctic tundra found that competitively dominant plant species specialize to acquire chemical forms of soil N that constitute the largest fraction of the total N pool (McKane et al. 2002; Ashton et al. 2010). Podocarps are known to dominate very low P soils with high proportions of organic P in phosphodiester and phytate forms (Turner *et al.,* 2007; Turner, 2008). Thus, we hypothesized that podocarps would have a greater potential for exploiting recalcitrant organic P relative to other AM tree species. However we found no evidence to support this hypothesis—the two AM tree species studied here had nearly identical phosphatase activity, while *Mollinedia* (AM) was better able to grow and develop leaves when limited to monoester P. Several studies in tropical systems have failed to find a relationship between plant species distribution and preferred forms of soil N (e.g., Andersen & Turner 2013; Russeo et al. 2013), suggesting that plants' preferences for chemical fractions of a total nutrient pool cannot always be predicted by edaphic specialization alone.

Podocarps house AM fungi in specialized root nodules, an adaptation that increases the volume of root cortex available for mycorrhizal fungal colonization while minimizing the cost of producing new cell wall and membrane material (Dickie & Holdaway 2011). This unique root morphology may explain in part why *Podocarpus* had nearly twice the AM fungal infection of *Mollinedia*. Although differences in AM fungal colonization are widely associated with significant increases in host performance, the proportion of variation in growth and P acquisition explained by differences in root length colonized is generally low (e.g., around 10% in a meta-analysis by Treseder 2013). Thus, it is not particularly unusual that the greater root infection rate in *Podocarpus* did not appear to influence seedling performance in our first experiment. This may simply reflect the low ability of AM fungal hyphae relative to plant roots to mineralize organic P (Dodd et al. 1987; Tarafdar & Marschner 1994; Joner, van Aarle, & Vosatka 2000).

Alternatively, our sampling of seedlings root systems may have failed to detect the functional components of mycorrhizal fungi associated with P acquisition. For example, our measurements of root length colonized did not include the external absorptive structures (extraradical mycorrhizal hypha), nor differentiate between parts of mycorrhizal fungi associated with resource storage (vesicles), transfer (internal hypha), and exchange (arbuscules)—a relatively common (e.g., Treseder 2014; Brundrett 2004), if unfortunate limitation. Further, our sampling design relied on intensive sampling of a small number of large (~10 cm) contiguous root segments, rather than on a larger number of smaller root segments. This approach has the potential to over-estimate root length colonized in some samples due to repeated observations of the same continuous AM symbiont, which may have contributed to the lack of difference in root length colonized observed among P treatments in our first experiment.

Our finding that ectomycorrhizal roots have greater phophomonoesterase activity than arbuscular mycorrhizal roots is consistent with several comparative studies (e.g., Antibus, Bower, & Dighton 1997; Phillips & Fahey 2006). Surprisingly, while the phosphomonoesterase assay suggests that EM tree species have a greater ability than AM tree species to exploit organic P, the rest of our results imply otherwise. Ectomycorrhizal root tips of *Oreomunnea* had, on average, slightly less phosphodiesterase activity than root tips from the AM tree species. Further, in our P addition experiment *Oreomunnea* differed from the AM tree species primarily in exhibiting a markedly more negative reaction to RNA (e.g., a net loss of leaf area over the course of the experiment).

The growth responses of *Oreomunnea* (EM) seedlings were largely insensitive to P nutrition. This is consistent with a finding by Moyersoen, Alexander, & Fitter (1998), who found that EM fungal colonization increased P uptake but had no impact on the growth of tropical trees. Although EM trees have been broadly characterized as more capable of exploiting nutrients in organic forms than AM trees (Phillips, Brzotek, & Midgely 2013), our findings challenge both the generality of this phenomenon and its relevance to ecological niche partitioning. Previous studies in mixed AM and EM forests have failed to

find evidence for niche-differentiation in the spatial distribution of infected roots (Moyersoen, Fitter, & Alexander 1998) or in the distribution of tree species in different soil types (Moyersoen, Becker, & Alexander 2001). While our results suggest that while *Oreomunnea* (EM) may be better able to mineralize some organic P substrates, we can suggest no conditions of soil P that would enhance *Oreomunnea* seedling performance relative to those of the AM or NM trees we investigated.

Our finding that *Roupala* (NM) has a considerable capacity for mineralizing organic P is consistent with several studies conducted on the Proteaceae (Adams & Pate, 1992: Watt & Evans, 1999; Lambers et al. 2011). Our result is unique in comparing the organic P use of Proteaceae to coexisting tree species representing different functional groups of mycorrhiza. However, interpreting *Roupala*'s potential to exploit different P forms is complicated by the high growth rate it maintained in the no-P control, which was likely supported by initial P-reserves. *Roupala* had markedly greater foliar-P concentrations potentially available for remobilization to growing tissue relative to the other species at the beginning of the experiment. While this also should have impacted *Roupala* in the phytate treatment, responses to the control and phytate groups were qualitatively different. For example, *Roupala* maintained three times the leaf area in the phytate treatment relative to the control, although overall biomass differences between these treatments remained small because leaves in the phytate treatment had twice the SLA. Further, *Roupala* in the phytate treatment had twice the foliar P of the controls, while the no-P controls were the only treatment to develop root clusters, which is a physiological response to extreme P limitation in Proteaceae (Watt & Evans 1999). Together, these results suggest *Roupala* reaped several qualitative benefits from phytate, while seedlings in the no-P control made efficient use of initial reserves but began responding to P starvation by the end of the experiment.

Although Proteaceae trees are rare in the Fortuna Forest Reserve (0.4 % of individuals >10 cm DBH; J. Dalling, unpublished data), P partitioning between mycorrhizal and non-mycorrhizal trees likely plays an important role in other forest systems. For example, Proteaceae are common in forests in central and southern Chile, where they are specialized on newly formed volcanic soils and glacial moraines that are high in P bound in forms with low bio-availability (i.e., sorbed to Al and Fe oxides) (Borie & Rubio

2003). Lambers et al. (2012) suggested that Proteaceae on these soils act as ecosystem engineers, mobilizing large pools of recalcitrant phosphate and converting and reintroducing it into the soil via leaf litter composed of relatively more labile P. This process facilitates the coexistence of Proteaceae and mycorrhizal tree species that are specialized to acquire P at a low cost of acquisition. Our results demonstrate that *Roupala*, but none of the mycorrhizal tree species, was able to exploit phytate-P, suggesting yet another large pool of soil P exists for mobilization.

The depressed growth and leaf area amongst mycorrhizal tree species when limited to phosphodiesters (RNA) as a P source is not caused by P-deficiency. All of the species produced significant phosphodiesterase activity and had significantly greater foliar P concentration in the phosphodiester treatment than in the no-P control. It appears that either phosphodiesters posed a formidable metabolic cost for plants to acquire or possibly produced a toxic effect inhibiting plant growth. It is remarkable to note that although the four plant species investigated here mineralized diester-P at around one tenth the rate of monoester-P, they exhibited greater foliar P concentrations in the RNA relative to the glucose phosphate treatments [~60 and 70% greater in *Oreomunnea* (EM) and *Roupala* (NM), respectively]. Although it is possible that the negative response to RNA is an experimental artifact of unknown origin, we note that growth depression (% growth and leaf area reduction relative to controls) in the RNA treatment was least in *Roupala* (NM) and greatest in *Oreomunnea* (EM), the species with the highest and lowest phosphodiesterase activity, respectively. If root mineralization mitigates the growth inhibition of RNA, this would explain why *Roupala* (NM) was able to maintain similar growth and leaf development in the RNA treatment relative to the glucose and inorganic phosphate treatments. This is of potential ecological importance, as plants may encounter large pools of nucleic acid P, particularly in strongly weathered soils where it forms a large proportion of the total organic P (Turner *et al.,* 2007; Turner & Engelbrecht 2011).

We found evidence for differences in the forms of soil P that can be exploited by plant species belonging to two coarsely divided functional groups—mycorrhizal and Proteaceae trees. Similarly, Ahmad-Ramli et al. (2013) found differences in the capacity of an arbuscular and ericoid mycorrhizal

plant species to acquire P from simple monoester or phytate-P. These examples of marked differences in preference for different forms of soil P between functional groups contrasts with studies that have failed to find evidence of P partitioning and niche complementarity among plant and fungal species within the AM functional group (Reynolds et al. 2006; Vogelsang, Reynolds, & Bever 2006). The evidence thus far suggests that P partitioning applies primarily at a coarse level among functional groups of plants.

Our assays of phosphatase activity, although consistent with previous comparative work (e.g., Antibus, Bower, & Dighton 1997; Tederesoo *et al.* 2012), involved the removal of terminal root segments and were likely disruptive of fungal hypha. In the case of AM plants, roots often express markedly greater phosphatase activity than hypha (Joner, van Aarle, & Vosatka 2000), such that our assays represent the bulk of the plant-fungal activity. In contrast, our assays may underestimate the potential of EM fungi to mineralize organic P, as both the fungal mantle and extra-radical hypha can express high phosphatase activity (Finlay 2008; *but see* Tedersoo et al. 2012).

By conducting our study in a montane forest, we were able to study several different types of mycorrhizal association that are hypothesized to differ in their capacity for organic P exploitation. However, our experimental design involved isolating seedlings from one another, and thus excluded the influences of competition (e.g., Ahmad-Ramli et al. 2013) and facilitation among multiple hosts (e.g., Li, Zhang, & Tang 2004) and the formation of common hyphal networks (e.g., Simard & Durall 2004), all of which may influence organic P acquisition under field conditions. Further, while our selection of focal tree species represents one of only two non-mycorrhizal trees in the Proteaceae, and the only abundant ecto-mycorrhizal tree at Fortuna, we compared only two AM tree species for which we had strong *a priori* expectations for different responses to organic P. More extensive comparative studies within the AM functional group may yet reveal a stronger potential for soil P partitioning, while studies on how potential to exploit P from different chemical forms influences species performance under field conditions (e.g., P^{32} tracer studies with multiple P sources under field conditions) are necessary to elucidate the ecological relevance of P partitioning. With these caveats, we suggest that Proteaceae trees can exploit

forms of organic P that are unavailable to mycorrhizal tree species, a process with the potential to facilitate the coexistence of these functional groups.

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Data Accessibility

All data associated with this manuscript have been archived in the Dyrad data repository (doi:10.5061/dryad.3gc55).

Table 1. Focal species for enzyme assays and growth experiment organized by family, mycorrhizal status, geographic distribution (Accessed through GBIF Data Portal, data.gbif.org, 2012-04-09), and collection site at the Fortuna Forest Reserve.

Species	Family	Mycorrhizal status	Distribution	Collection site
Mollinedia darienensis	Monimiaceae	Arbuscular mycorrhizal	Costa Rica to southern Colombia	Hornito
Podocarpus oleifolius	Podocarpaceae	Arbuscular mycorrhizal within nodulated roots	Southern Mexico, throughout Central America, and along western South	Chorro

			America into Bolivia	
Oreomunnea mexicana	Juglandaceae	Ectomycorrhizal	Southern Mexico to central Panama	Honda B
Roupala montana	Proteaceae	Nonmycorrhizal—forms cluster roots	Southern Mexico, throughout Central America, into southern Brazil	Hornito

Table 2. The concentration (mM) and chemical form of all macro and micronutrients in the hydroponic feed solution. Note that experimental treatments received one of the four possible forms of P, while the different forms of N were provided simultaneously (percentages indicate the relative mass of each form).

Nutrient	**Chemical Form**	**Concentration in feed solution (mM)**
Phosphorus (P)	1. Sodium phosphate monobasic (Sigma S0761) 2. D-glucose 6-phosphate disodium salt hydride (Sigma G7250) 3. *myo*-Inositol hexakisphosphate (Sigma P0109) 4. RNA from torula yeast (Sigma R6625)	0.833
Nitrogen (N)	82.6 % nitrate 13.9% urea 3.5% ammonia	24.9
Potassium (K)	Potassium nitrate	8.95
Magnesium (Mg)	Magnesium sulfate	2.00

Sulfur (S)	Magnesium sulfate	16.4
Calcium (Ca)	Calcium nitrate	6.40
Iron (Fe)	Fe-EDTA	0.021
Manganese (Mn)	Mn-EDTA	0.011
Copper (Cu)	Cu-EDTA	0.001
Boron (B)	Boric acid	0.015
Zinc (Zn)	Zn-EDTA	0.001
Molybdenum (Mo)	Ammonium molybdate	.0218

Table 3. Mean values (and standard errors) for all variables from the initial, pre-experimental harvest (n=8). Different letters indicate significant differences among species according to Fisher's LSD (p<0.05).

Species	**Mass (g)**	**Leaf area (cm^2)**	**SLA ($cm^2\ mg^{-1}$)**	**Foliar P (ppm)**
Mollinedia (AM)	0.20 (0.02) b	20.87 (2.54) b	0.26 (0.01) a	1.04 (0.06) a
Podocarpus (AM)	0.13 (0.03) b	11.58 (2.58) b	0.21 (0.02) b	0.92 (0.19) a
Oreomunnea (EM)	1.51 (0.32) a	131.30 (21.33) a	0.24 (0.01) ab	0.49 (0.04) a
Roupala (NM)	0.21 (0.07) b	20.30 (4.20) b	0.24 (0.01) ab	3.08 (0.58) b

Table 4. F-table for ANOVA of all dependent variables, with initial number of leaves and height entered as covariates

	Model Statement				
Dependent Variable	*Species (df=3)*	*Treatment (df=4)*	*Species x Treatment (df=12)*	*Initial Leaf # (df=1)*	*Initial Height (df=1)*
Relative growth rate	31.3***	12.9***	2.3*	4.5*	3.6 •
Leaf area	14.0***	10.5***	6.3***	1.8	0.1
Specific leaf area	2.4 □	3.1*	2.6**	0.1	1.5
Foliar P	19.7***	38.9***	6.6****	0.7	0.3

□ p<0.10, * p<0.05, ** p<0.01, *** p<0.001

Figure 1. A conceptual figure adapted from Turner (2008) displaying examples representative of common functional groups of soil P along a gradient of increasing cost of acquisition, determined by the metabolic cost of synthesizing and exuding phosphatase enzymes and organic acids capable of mineralizing and solubilizing organic P, respectively.

Figure 2a-b. Boxplots of root/mycorrhizal a) phosphomonoesterase and b) phosphodiesterase activity expressed as the release of *para*-nitrophenol (*p*NP) per hour and root dry mass for *Mollinedia* (AM), *Podocarpus* (AM), *Oreomunnea* (EM), *Roupala* fine roots (NM) and *Roupala* root clusters (NM), with different letters indicating significant differences according to Fisher's LSD ($p<0.05$).

Figure 3a-d. Mean values +/- standard error for (a) relative growth rate, (b) leaf area, (c) specific leaf area, and (d) foliar P. Within each species, treatments with significant or marginally significant differences from the no-P control are indicated with either * ($p<0.05$) or □ ($p<0.10$), respectively, according to analysis of variance using linear models.

Supplemental Figure 1. Mean root length colonized +/- standard error for the three mycorrhizal tree species, with different letters indicating significant differences among species according to Fisher's LSD ($p<0.05$).

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