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RESEARCH ARTICLE

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Nitrogen fixation and foliar nitrogen do not predict phosphorus acquisition strategies in tropical trees

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Abstract

1. The mechanistic links between nitrogen (N) availability and investment in plant phosphorus (P) acquisition have important implications for plant growth, species distributions, and responses to CO_2 fertilization under global change, especially in P-poor tropical ecosystems. Currently, it is unclear whether investment in strategies that enhance plant P acquisition (arbuscular mycorrhizal, AM; colonization or root phosphatase activity, RPA) are determined primarily by phylogeny, or whether these strategies differ among N_2-fixing legumes and nonfixing plants as a result of differing N availability.
2. We hypothesized that plant N status, which can vary widely independent of N fixation, correlates with investment in P acquisition, because: (a) N and P concentrations scale in plant tissue indicative of coupled demand and (b) plants with more N may have more resources available to allocate to acquisition strategies.
3. We grew seedlings of eight tropical tree species from three families (including three N_2-fixing and one nonfixing legume) under greenhouse conditions in native forest soil for four months. Species represented almost the full range of foliar N observed in tropical trees.
4. Neither foliar N nor P concentrations correlated with investment in P acquisition. Across all species, we found an inverse relationship between investment in AM colonization and RPA, but this trade-off was unrelated to foliar N or P and did not differ between functional types (i.e., N_2 fixers vs. nonfixers).
5. Within legumes (family Fabaceae), two strategies were evident that were unrelated to fixation status. High-fixing *Inga* and nonfixing *Dialium* displayed high foliar N and P concentrations and greater proportional investment in RPA versus AM, while lower fixing *Ormosia* species were characterized by lower foliar nutrient concentrations and proportionally more investment in AM.
6. *Synthesis.* Investment in P acquisition strategies in tropical trees is not dependent on foliar N or functional group, but instead may be controlled in part by resource trade-offs. High diversity in nutrient strategies between related species cautions again the use of simple functional groupings to draw conclusions about nutrient acquisition in tropical trees.

KEYWORDS
arbuscular mycorrhizal fungi, Fabaceae, legume, nitrogen fixation, phosphatase enzymes, phylogeny, plant–soil (below-ground) interactions, stoichiometry

1 | INTRODUCTION

Understanding the interactions between nitrogen (N) and phosphorus (P) acquisition in woody plants is a pressing challenge in tropical ecology. This relationship is central to refining our understanding of nutrient limitation in soil–plant systems and modelling ecosystem function under future global change (Achat, Augusto, Gallet-Budynek, & Loustau, 2016). Phosphorus in particular is thought to limit plant growth in many tropical forests, and to potentially constrain the strength of the tropical forest carbon (C) sink under future CO_2 fertilization (Wieder, Cleveland, Smith, & Todd-Brown, 2015). Currently, feedbacks between C, N, and P are rarely integrated effectively, if at all, into coupled C-climate models. This is largely because doing so requires more experimental data defining the relationship between N availability and P acquisition strategies such as arbuscular mycorrhizal (AM) colonization or root phosphatase enzyme production (Achat et al., 2016).

Current studies point to two hypotheses that may explain differential investment in P acquisition between plant species. The first posits that tropical legumes (family Fabaceae) fix N (paradoxically, given the apparently high N availability in the tropics; Hedin, Brookshire, Menge, & Barron, 2009) in order to invest in P acquisition via the production of N-expensive phosphatase enzymes (Houlton, Wang, Vitousek, & Field, 2008). Houlton et al. (2008) and other studies that have contrasted fixing and nonfixing plants have generally found that phosphatase enzyme production is elevated in the roots and rhizospheres of N_2-fixing legumes (Houlton et al., 2008; Nasto et al., 2014; Png et al., 2017) potentially because they have more N available to invest in enzyme synthesis. Alternatively, there is evidence that phosphatase enzyme production is not dependent on N supply from fixation, but rather may be a phylogenetically conserved trait between groups or species (Png et al., 2017).

Although legumes (which include both N_2-fixing and nonfixing species) typically have high foliar N concentrations, both leguminous and nonleguminous tropical trees exist along a remarkably wide spectrum of foliar N (Townsend, Asner, & Cleveland, 2008; Townsend, Cleveland, & Asner, 2007). This suggests that both groups vary in their ability to acquire N and in the quantity of internal N they may have available to allocate to different functions. In addition, cellular N and P demand are coupled; plants that can obtain sufficient N to maintain higher foliar concentrations are likely to require correspondingly more P to support increased growth or photosynthesis, and indeed there is a clear correlation between N and P in tropical tree foliage (Townsend et al., 2007). If investment in P acquisition is dependent on N availability, then we might expect to see that phosphatase enzyme production or AM colonization vary as a function of foliar N across species, rather than simply grouping in a fixer/nonfixer dichotomy, that is, P acquisition may be a function of internal N availability, rather than functional type. Currently, experiments needed to test this hypothesis, for example, by simultaneously comparing many species along a foliar N gradient under common conditions, are lacking.

Plant investment in P acquisition integrates external supply, internal demand, and the availability of resources that could be allocated to acquisition (Zalamea, Turner, Winter, & Jones, 2016). Phosphorus supply is highly variable among ecosystem types, and is often low in tropical forests growing on highly weathered soils with high sorption capacity (Vitousek, Porder, Houlton, & Chadwick, 2010). Plant availability of soil P varies by chemical species, ranging from soluble phosphate to more complex organic forms that require some breakdown prior to uptake (Dalling, Heineman, Lopez, Wright, & Turner, 2016; Turner, 2008). Phosphorus demand is influenced by growth rate (for allocation to biomolecules such as nucleic acids and ATP; Hidaka & Kitayama, 2011), efficiency of use (Dalling et al., 2016; Kitayama, 2013), and resorption (Dalling et al., 2016). Both N and C (energy) may be invested to increase plant P acquisition in a number of ways. Roots can produce N-rich phosphatase enzymes (*c*. 8%–32% N by weight) that catalyse the release of inorganic P from more complex esters (Dalling et al., 2016; Duff, Sarath, & Plaxton, 1994; Treseder & Vitousek, 2001). Alternatively, plants may allocate C to symbionts like mycorrhizal fungi (Smith & Read, 2008; Treseder & Vitousek, 2001). In the tropics, associations with AM fungi are common and have been shown to increase P acquisition by allowing plants to access inorganic phosphate and expanding effective root area (Smith & Read, 2008). Relatively high rates of C fixation in legumes (supported by greater N availability) may also increase C available to allocate to this symbiosis (Jia, Gray, & Straker, 2004), although a recent global meta-analysis found no relationship between elevated foliar N and photosynthesis in legumes (Adams, Turnbull, Sprent, & Buchmann, 2016). Finally, shifting growth allocation to roots versus shoots may also increase root surface area for P capture (Batterman, Wurzburger, & Hedin, 2013; Olde Venterink, 2011; Treseder & Vitousek, 2001), although this trait may be relatively insensitive to P availability (Zalamea et al., 2016). Each of these mechanisms has interlinked feedbacks. For example, greater photosynthetic rates required to supply C to support mycorrhizal symbioses (to acquire P) may require greater P in the first place. It is also probable that some plants have evolved to invest more heavily in one strategy over another, with demand and resources necessitating trade-offs between them (Nasto et al., 2017; Nasto, Winter, Turner, & Cleveland, in revision).

There is mixed evidence regarding the influence of N supply on investment in P acquisition. If N_2 fixation is driven by a need to allocate N (and/or C) to P acquisition, then we might expect that rates of fixation in legumes correlate with either root phosphatase production or AM colonization. Evidence for this is also mixed; both positive (Nasto et al., 2014) and absent (Batterman et al., 2013; Nasto et al., 2014, 2017 ; Wurzburger & Hedin, 2015) relationships between fixation rates and both of these processes have been observed in tropical trees. When N is supplied externally via the soil, there is evidence that increasing supply increases phosphatase production in subset of both fixing and nonfixing species (Marklein & Houlton, 2012; Olde Venterink, 2011; Treseder & Vitousek, 2001).

Evidence that P acquisition may be constrained primarily by phylogeny is more limited. Png et al. (2017) compared woody legumes and actinorhizal N_2 fixers in a subtropical ecosystem and found that despite similar reliance on N_2 fixation, phosphatase activity was much higher in legumes than in actinorhizal fixers or nonfixers,

leading the authors to conclude that phylogeny was more influential on P acquisition than the functional group. However, the actinorhizal genus studied had much lower foliar N than many legumes (comparable to nonfixers), and thus may differ fundamentally in its N and P requirements, or availability of N to allocate to P acquisition. Olde Venterink (2011) found that nonnodulating temperate legume forbs had consistently higher RPA than nonlegumes at the same level of N supply, also suggesting that high RPA may be a phylogenetically conserved trait. Finally, degree of root colonization by AM fungi has been shown to correlate with root structural traits that are phylogentically conserved across temperate tree families (Valverde-Barrantes, Horning, Smemo, & Blackwood, 2016).

In this study, we hypothesized that N status of individual species, rather than functional group, explains variability in P acquisition. We grew eight tropical tree species (including three N_2-fixing legumes, one nonfixing legume, and four nonlegumes) spanning almost the full range of foliar N concentrations reported for tropical trees (Townsend et al., 2007) and compared metrics of N availability (foliar N, and fixation rate in legumes), seedling growth, foliar P concentrations, and investment in P acquisition (root phosphatase enzyme production and AM colonization). A greenhouse approach using homogenized field soil was selected to control for known strong heterogeneity in local soil nutrient concentrations (especially of N, for example, Osborne et al., 2017) as well as abiotic conditions that would be likely to influence the physiological variables investigated.

2 | MATERIALS AND METHODS

2.1 | Study site and design

We conducted a greenhouse study at the Osa Verde Conservation Campus on the Osa Peninsula of southwestern Costa Rica (8°24′42″ N, 83°19′00″ W) between February and June 2017, in which eight species of native field-collected tree seedlings transplanted from lowland tropical rainforest from were grown in field soil. Mean annual temperature at the site is 26°C, with average rainfall of *c*. 3,500 mm falling predominantly in May–November (Keller, Reed, Townsend, & Cleveland, 2013).

2.2 | Soil substrate

Soils used in the experiment were highly weathered, nutrient-poor Ultisols with an average pH of 5.7 (Osborne et al., 2017) sourced from adjacent primary tropical rainforest. Soils (0–10 cm depth) were collected within a 10 × 10 m area after removing surface litter. Soil was homogenized by hand to break up large aggregates and remove coarse roots, and thoroughly mixed. Plant species included in the study were observed to grow within several hundred metres of the collection site, and so soils were considered a suitable source of mycorrhizal and rhizobial inoculum. After homogenization, subsamples were taken to analyse soil properties. Five samples were dried at 60°C for 3 days and weighed to determine soil moisture. To determine available P concentrations, five air-dried soil samples was shaken in a 1:5 w/v solution of dilute ammonium fluoride solution for 1 min, filtered using glass fibre filters (GF/B), and analysed colorimetrically using a Synergy 2 Microplate Reader (Biotek; Bray & Kurtz, 1945). To determine ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, five samples were extracted immediately by shaking for 1 min every hour for 4 hr in 1:3.75 w/v 2 M KCl, filtered through 0.47-μm syringe filters (Millipore) and immediately frozen. A further five samples were used to determine soil mineralization and nitrification rates at the beginning of the experiment, by incubating at field temperature for 5 days and then extracting as described previously. Concentrations of inorganic N measured at the end of the incubation were used to calculate net N mineralization and nitrification. Soil moisture at time of sampling was relatively low for the system (17%–18%) and measures were made without additional water addition. KCl extracts were analysed for NH_4^+ and NO_3^- concentrations colorimetrically (Miranda, Espey, & Wink, 2001; Weatherburn, 1967) using the plate reader described above. The final five samples were air-dried, homogenized and analysed for total %C and %N using a continuous flow isotope ratio mass spectrometer (Model Delta V Advantage; Thermo-Scientific) at the Cornell University Stable Isotope Laboratory (COIL). Soil variables are summarized in Table S1.

2.3 | Plant material and greenhouse experiment

Field-collected seedlings of eight rainforest tree species were grown in pots under greenhouse conditions for four months. Species consisted of three N_2-fixing legumes: *Inga multijuga* Benth., *Ormosia macrocalyx* Ducke., and *Ormosia coccinea* Aubl. Jacks (all Fabaceae); one nonfixing legume: *Dialium guianense* Aubl. (Fabaceae), and four nonlegumes: *Tapirira guianensis* Aubl., *Spondias mombin* L. (both Anacardiaceae), *Virola koschnyi* Warb., and *Virola sebifera* Aubl. (both Myristicaceae, Figure S1). Phylogenetic relationships between species were generated using PhyloT (phylot.biobyte.de), based on National Centre for Biotechnology Information taxonomy (ncbi.nlm.nih.gov/taxonomy), and visualized with Tree of Life v3.0 (itol.embl.de; Letunic & Bork, 2016, Figure S1).

All species are common in lowland rainforests throughout southern Costa Rica, and were harvested from primary mature rainforest in February 2017. Seedling foliar N concentrations spanned a range from 17–32 mg N g^{-1} (Figure 1). Seedlings were collected from the same cohort (<1 year old) and usually from within the same area (*c*. 50 m × 50 m) where seedlings usually appeared to be offspring of a single parent tree. Roots were washed to remove soil particles. For legumes, abundance of nodules varied by individual. To control for this, existing nodules were gently detached from roots prior to transplanting. The height and number of leaves were recorded prior to planting. An additional 5–10 representative seedlings of each species were measured for height and destructively sampled for biomass (Table S2). Seedlings were divided into root, shoot, and leaf tissue, dried at 70°C for 48 hr and weighed.

Pots (30 cm tall and 10 cm width/depth) were filled with *c*. 2 L of field soil and planted with one seedling each. Fifteen seedlings were

planted per species (n = 15). Seedlings were grown for 4 months in an open-sided greenhouse with 80% sunlight. Plants were watered daily and nothing else was added over the course of the experiment.

2.4 | Harvest and sample analysis

At time of harvest, plants had not exploited the full pot soil volume and none showed evidence of becoming pot bound (roots had exploited <1/2 soil volume). Soil was gently washed from plant roots and tissue samples were gently excised and stored in 50 mM $CaSO_4^-$ at 4°C for phosphatase analysis. Additional root samples were placed in envelopes and air-dried to determine mycorrhizal colonization. Plants were measured for height and number of leaves and were divided into leaf, root, and shoot tissue, and biomass was determined as described above. For legumes, nodules were placed in 50-ml clear acrylic tubes to assess N fixation by the ARA method as per Sullivan et al. (2014). More than one tube was used if necessary, in order to measure fixation for all nodules. After 1 hr of incubation in a 10% acetylene atmosphere, 14 ml of headspace sample was withdrawn and placed in a 10-ml evacuated vial. Nodules were dried to determine biomass. Headspace samples were analysed for ethylene concentration using a Shimadzu GC-2014 equipped with a flame ionization detector (Shimadzu Inc.) at the University of Montana. Blanks were used to control for ethylene produced by nodules, vials or tubes, or photodegraded during transport. To convert between acetylene reduction and N_2 fixation rates, we applied an ethylene:N ratio of 2.8:1 derived from previous experiments at this study site (Sullivan et al., 2014).

Root arbuscular mycorrhizal colonization was analysed by staining with trypan blue (Koske & Gemma, 1989). Roots were cleared in a 10% solution of KOH for 3 days at room temperature, followed by 2 hr at 90°C, rinsed with DI water and acidified in 3% HCl for 12 hr. Roots were soaked for a further 24 hr in trypan blue and de-stained in water for 12 hr. Eight uniformly stained root segments (per seedling) were mounted on slides. Colonization was determined using the magnified intersection method on 50–100 intersections. Results are reported as % root length colonized.

Root phosphomonoesterase (hereafter, phosphatase) activity was determined using a 4-methylumbelliferone (MUB)-linked substrate (Fisher Scientific), adopted from Sinsabaugh et al. (2003). Roots (20–30 mg) were immersed in 1 ml of 50 mM sodium acetate buffer (pH 5), 1 ml of 50 mM sodium acetate buffer (800 μl)/100 mM MUB solution (200 μl), and 1 ml of 50 mM sodium acetate buffer (800 μl)/100 mM MUB (200 μl)/200 mM 4-MUB PO_4^{3-} (200 μl) solution in clear 12-well plates. Plates were shaken at room temperature for 1 hr at 110 rpm and 200 μl subsamples were pipetted from each well into a black 96-well microplate. Each sample included four analytical replicates and negative controls for sample and substrate fluorescence. Microplates were read at 365 nm excitation and 450 nm emission, and enzyme activities were calculated as μmol 4-MUB $P\ g^{-1}$ root hr^{-1}. Assays were conducted within 6 days of root harvesting. Previous analyses have indicated that samples kept at this temperature are stable for at least 1 week (Nasto et al., 2014, 2017).

Dried foliar tissue was analysed for P and N concentrations after grinding finely in a Wiley mill. Foliar N was analysed as described above for soil. Foliar P was analysed by ignition at 500°C for 2 hr and acid digestion in 1 M HCl, followed by phosphate detection using molybdate colourimetry (Kuo, 1996). Relative growth rate (RGR) was calculated according to: $RGR = [(\ln M_f - \ln M_i)/(D_t)]$, where M_f is the final dry mass, M_i is the initial dry mass, and D_t equal to the length of the experiment in days.

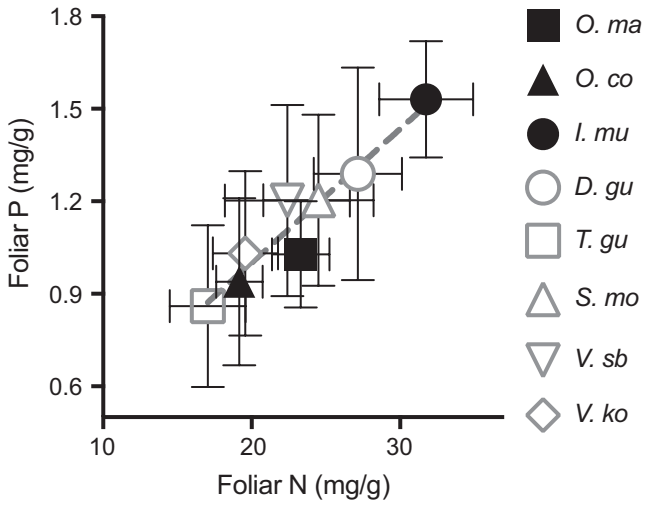

FIGURE 1 Foliar nitrogen to phosphorus ratios for eight tropical tree species analysed in this study. *O. ma = Ormosia macrocalyx*, *O. co = Ormosia coccinea*, *I. mu = Inga multijuga*, *D. gu = Dialium guinense*, *S.mo = Spondias mombin*, *T.gu = Tapirira guianensis*, *V. sb = Virola sebifera and V. ko = Virola koschnyi*. Symbols in black are N_2-fixing species. Dashed line indicates linear regression (R^2 = 0.92, p < 0.001)

2.5 | Statistical analyses

Statistical analyses were performed in JMP Pro 13.1 (SAS Institute). For combinations of continuous variables, best-fit regressions for species means were selected using Akaike's information criteria (AIC) and distribution of residuals was examined to confirm appropriateness of fit. Data were checked for normality using the Shapiro–Wilk test, and if necessary transformed prior to analysis using log or Johnson Si transformations (Moulin et al., 2014). One-way ANOVA followed by Tukey's honest significant difference (HSD) post hoc tests were used to compare traits between plant families and species. Two sample t-tests were used to determine differences between fixing and nonfixing species for each variable. Alpha (α) significance threshold was set at p < 0.05. All data used for analysis are available at https://knb.ecoinformatics.org/#view/doi:10.5063/F1WQ01ZQ (Soper, 2018).

3 | RESULTS

There were no significant relationships between arbuscular mycorrhizal (AM) colonization or root phosphatase activity (RPA) and either

foliar N concentrations (Figure 2a,b), P concentrations (Figure S2a,b) or N:P ratios (data not shown, $p > 0.05$ for all combinations). Across all species, there was an inverse relationship between AM colonization and RPA ($R^2 = 0.49$, Figure 3a, $p < 0.05$). This was also true when fixing and nonfixing species were analysed separately. Overall mean variation across species was greater for AM colonization (which varied fourfold from 15%–60%) than for RPA (which varied approximately twofold from 8.3–15 μmol 4-MUB P g^{-1} root hr^{-1}, Figure 4b,c).

Across all species, RPA declined with relative growth rate (RGR, $R^2 = 0.86$, $p < 0.001$), so that the ratio of AM:RPA showed positive linear correlation with RGR ($R^2 = 0.66$, Figure 3b, $p < 0.001$). As this relationship was heavily influenced by a single very fast growing species (*Spondias mombin*, Figure S3a), the slope of the RGR/AM:RPA and RGR/RPA regressions was not significant when this species was removed from analysis.

The AM colonization and RPA were comparable at the genus, but not family, level. These metrics of P investment did not differ significantly between species within the *Virola* and *Ormosia* genera (Table 1, Figure 4c,d). However, within the families Fabaceae and Anacardiaceae, species that did not occupy the same genus differed significantly in both measures of P acquisition (Table 1, Figure 4c,d).

This contrast was particularly evident within the family Fabaceae. Compared with *Inga multijuga* and *Dialium guianense*, species *Ormosia macrocalyx* and *O. coccinea* were characterized by low foliar N and P concentrations (Figure 4a,b), high C:N ratios, and significantly greater investment in AM colonization compared with RPA (Figure 4c,d and 5a, $p > 0.001$). These differences did not correlate with N_2 fixation however, because *I. multijuga* (characterized by the highest observed N_2 fixation rate) and *D. guianense* (a nonfixer) were most similar to each in these metrics than to *Ormosia* (Figure 5a). N fixation rate in *Inga* exceeded both *Ormosia* species by a factor of at least two when expressed as both per unit nodule biomass (Figure 5b) or per unit total biomass (Figure S4, both $p > 0.001$). Comparing all species together, N_2 fixers did not display elevated investment in either metric of P acquisition compared with nonfixers (Table 1, Figure 4c,d).

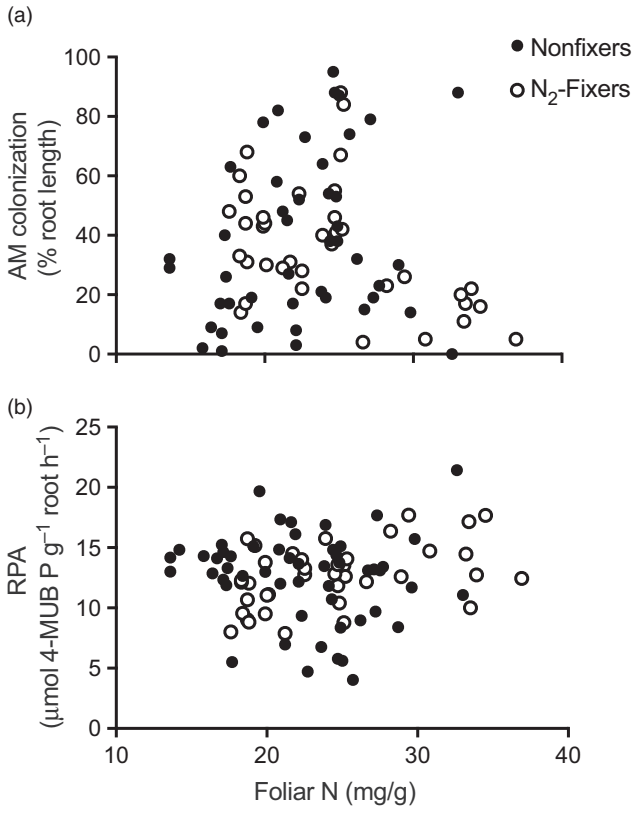

FIGURE 2 Foliar N versus (a) arbuscular mycorrhizal (AM) colonization and (b) root phosphatase activity (RPA) in three N_2-fixing and five nonfixing Costa Rican tropical tree seedlings. No significant relationship exists between either variable ($p > 0.05$)

4 | DISCUSSION

Contrary to our initial hypothesis, we found no evidence that investment in P acquisition by tropical trees is associated with plant N status, nor does it correlate with the ability to fix N_2, or differ substantially between the Fabaceae legume family and other species.

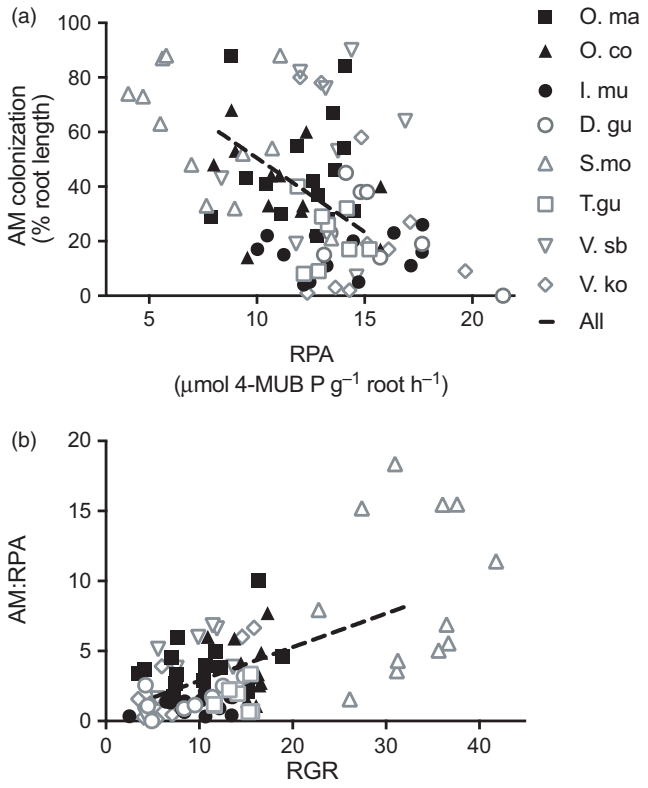

FIGURE 3 Relationship between (a) arbuscular mycorrhizal (AM) colonization and root phosphatase activity (RPA) and (b) relative growth rate (RGR) and AM:RPA ratio in three N_2-fixing and five nonfixing Costa Rican tropical tree seedlings. Symbols in black are N_2-fixing species. *O. ma = Ormosia macrocalyx*, *O. co = Ormosia coccinea*, *I. mu = Inga multijuga*, *D. gu = Dialium guinense*, *S. mo = Spondias mombin*, *T. gu = Tapirira guianensis*, *V. sb = Virola sebifera and V. ko = Virola koschnyi.* Dashed lines indicate linear regressions for species means (data not shown) (a, $R^2 = 0.49$, $p < 0.05$, b, $R^2 = 0.66$, $p < 0.001$)

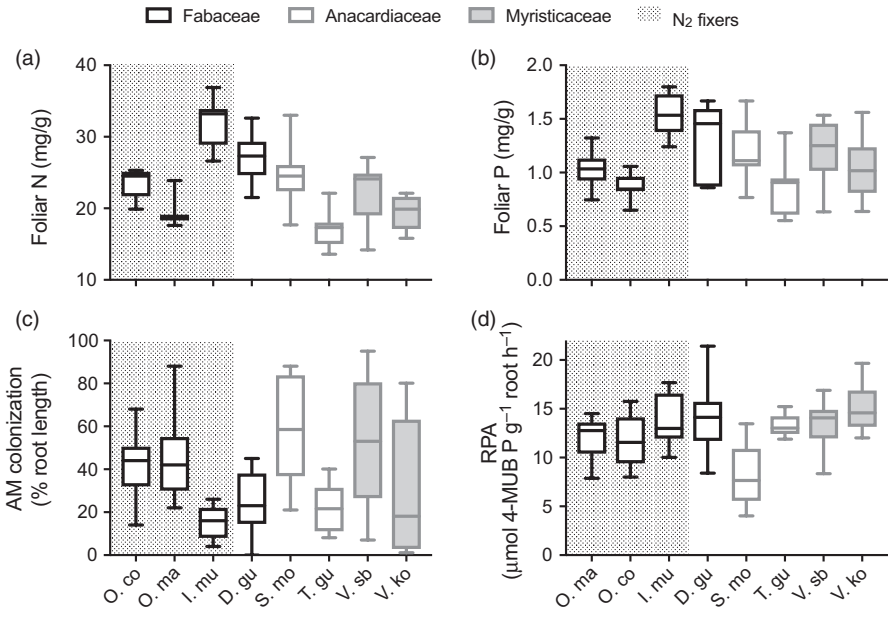

FIGURE 4 Boxplots of (a) foliar nitrogen, (b) foliar phosphorus, (c) arbuscular mycorrhizal (AM) colonization, and (d) root phosphatase activity (RPA) in three N_2-fixing and five nonfixing Costa Rican tropical tree seedlings. *O. ma = Ormosia macrocalyx*, *O. co = Ormosia coccinea*, *I. mu = Inga multijuga*, *D. gu = Dialium guinense* (all Fabaceae), *S. mo = Spondias mombin*, *T. gu = Tapirira guianensis* (both Anacardiaceae), *V. sb = Virola sebifera and V. ko = Virola koschnyi* (both Myristicaceae). There were no significant statistical differences between Fabaceae/non-Fabaceae or N_2 fixers and non-N_2 fixers for any variable ($p > 0.05$)

TABLE 1 Statistical relationships (analysis of variance; ANOVA or *t*-tests) between plant families, species, and functional groups (N_2 fixers/nonfixers) for variables foliar nitrogen and phosphorus concentrations, root phosphatase activity (RPA), or mycorrhizal colonization. NS: not significant, $^{**}p < 0.001$, $^{***}p < 0.0001$. Uppercase letters indicate statistically significant differences between species or families ($p < 0.05$) determined using Tukey's HSD test. Bold letters indicate statistically significant differences within genera. Italics indicate statistically significant differences within families

	Foliar [*N*]		Foliar [P]		RPA		Mycorrhizal colonization	
	p	R^2	***p***	R^2	***p***	R^2	***p***	R^2
Fixer status	NS		NS		NS		NS	
Family	**	0.15	NS		**	0.17	NS	
Fabaceae	A		–		A		–	
Anacardiaceae	B		–		B		–	
Myristicaceae	B		–		A		–	
Species	***	0.7	***	0.42	**	0.37	***	0.39
Fabaceae								
O. macrocalyx	***C***		*BC*		AB		*AB*	
O. coccinea	***DE***		*BC*		B		*AB*	
I. multijuga	*A*		*A*		AB		*C*	
D. guianense	*AB*		*AB*		AB		*BC*	
Anacardiaceae								
T. guianensis	*E*		*C*		*AB*		*BC*	
S. mombin	*BC*		*AB*		*C*		*A*	
Myristicaceae								
V. koschnyi	DE		BC		A		BC	
V. sebifera	CD		ABC		AB		AB	

Instead, we identified a trade-off between investment in arbuscular mycorrhiza versus root phosphomonoesterases. Within the Fabaceae family, we identified two contrasting nutritional strategies (higher foliar N and P with proportionally greater investment in RPA compared with AM, and vice versa) that were not dependent on the presence/absence of fixation. Our data support a growing body of evidence that investment in P acquisition is not dependent on functional type.

With finite resources available, it is not surprising that trade-offs between allocation to different root resource acquisition strategies have been identified in plants (Chen et al., 2016; Lynch & Ho, 2005; Unger, Friede, Hundacker, Volkmar, & Beyschlag, 2016). Both AM and RPA impose a C cost on the plant (Lynch et al., 2005), and across all eight species we observed a negative correlation between percent colonization of AM fungi and RPA indicative of a trade-off between the two (Nasto et al., 2017). Because AM and RPA increase plant access to different soil P pools (inorganic vs. organic), this trade-off may also be related to niche partitioning between co-occurring species (Nasto et al., 2017), and all species in this study commonly co-occur as canopy trees in primary lowland tropical forests. Where a species fell on this trade-off continuum did not

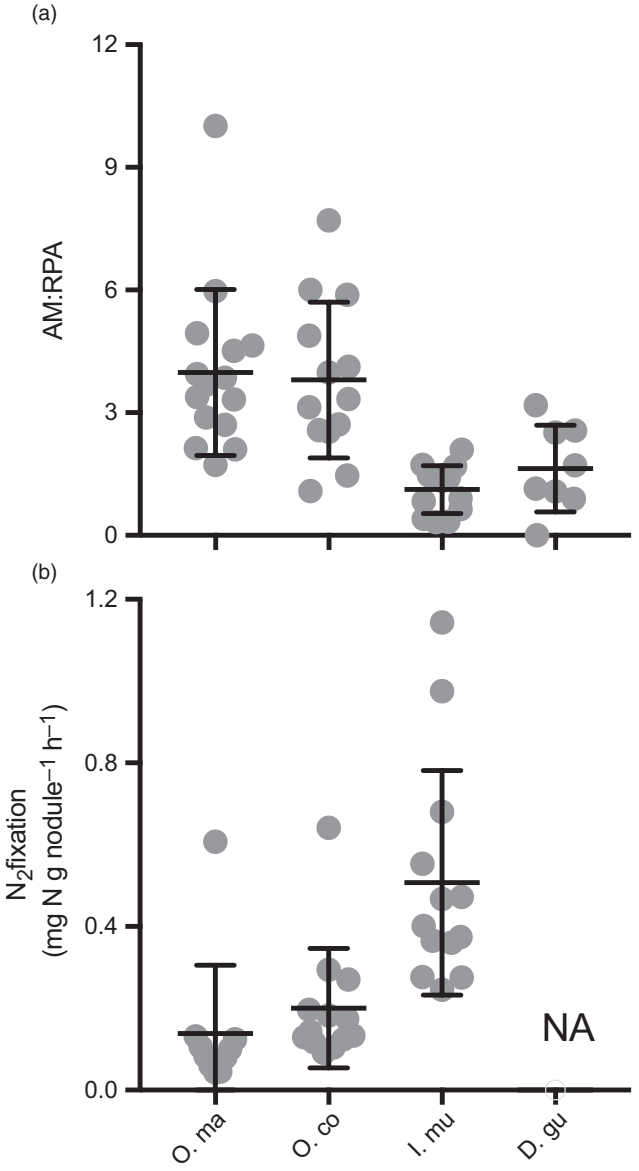

FIGURE 5 (a) Ratio of arbuscular mycorrhizal colonization to root phosphatase (RPA) activity and (b) N_2 fixation (mg N g nodule^{-1} hr^{-1}) for four species in the family Fabaceae. *O. ma = Ormosia macrocalyx*, *O. co = Ormosia coccinea*, *I. mu = Inga multijuga*, *D. gu = Dialium guianense*. *D. guianense* does not fix N_2

appear to be related to its nutrient status, as reflected by foliar N or P concentrations. Instead, the only predictor we identified was relative plant growth rate; however, this relationship was driven disproportionately by a single very fast growing species (*Spondias mombin*) that had relatively low RPA but relatively high AM colonization. In crop species, mycorrhizal colonization has been found to increase photosynthetic rates proportionally more than nutrient uptake (a process of sink stimulation), which may increase C available to allocate to overall plant growth (Kaschuk, Kuyper, Leffelaar, Hungria, & Giller, 2009). When plants support two symbioses (AM and *N*-fixing *Rhizobia*) simultaneously, resource trade-offs between nutrient acquisition strategies could also extend to the C cost of nodulation and maintaining N fixation (Minchin & Witty, 2005), although in some instances these two strategies have also been found to be positively correlated (Ossler, Zielinski, & Heath, 2015). In this study, evidence for a trade-off was mixed, depending on how resource allocation was assessed. Fixation rate (absolute or per unit biomass) was inversely correlated with AM colonization across species, while nodule biomass (total or proportional) was not.

Within Fabaceae, species displayed grouping in P acquisition strategies that was unrelated to the functional trait of N fixation. High N_2-fixer *I. multijuga* (subfamily Caesalpinoideae) and nonfixing legume *Dialium guianense* (Dialoideae) behaved similarly, displaying an apparently contrasting nutrient "lifestyle" compared to lower N_2-fixing species *Ormosia macrocalyx* and *O. coccinea* (Papilionoideae). Compared with *Ormosia*, *I. multijuga* and *D. guianense* were characterized by elevated foliar concentrations of N and P (highest of the eight species examined in this study), by somewhat elevated RPA (+15%) and by lower levels of root AM colonization (-35% to -65%) corresponding to a significant decrease in AM:RPA.

Why have past studies come to conflicting conclusions concerning whether N fixation or the Fabaceae family generally supports greater rates of root P acquisition investment? The high variability within Fabaceae seen here may reflect the fact that this is an especially large (almost 20,000 species) and diverse plant family, with our study species representing three of the currently recognized six subfamilies (Legume Phylogeny Working Group, 2017). Thus, for studies that employ "legume versus non-legume" comparisons with a small number of species, the specific choice of species may disproportionately influence conclusions and explain apparently contrasting results (Nasto et al., 2017). The same is true even in studies that consider a greater number of species, if those selected are generally more closely related (e.g., Olde Venterink, 2011, Png et al., 2017). With regard to fixation, nonfixing legumes are infrequently included in comparisons, which we propose may have led to conflation between fixation and family group. Indeed, when fixing and nonfixing individuals of the same species have been compared, RPA has not been found to vary (Olde Venterink, 2011). The lack of a functional relationship between fixation and investment in P acquisition is further supported by Batterman et al. (2013) and Png et al. (2017), who found no correlation between fixation rate and these variables within or across species at multiple levels of nutrient availability under both glasshouse and field conditions.

Although we found no evidence that N concentrations or enhanced supply of N via fixation enabled greater investment in P acquisition (via AM or RPA), it is nonetheless true that species (in this study and generally) vary substantially in their foliar P concentrations, and that N and P scale closely (Townsend et al., 2007). Thus, some plants must employ alternative mechanisms that foster greater P efficiency or acquisition from a common soil environment. We found no evidence in this study that higher foliar P was related to proportionally greater investment in root biomass (above: below-ground biomass ratios), or varied with growth rate (see Supporting Information). Strategies such as increased resorption rates or leaf longevity also seem unlikely in the case of fast growing seedlings over a relatively short time period (Zalamea et al., 2016). Alternative mechanisms that may

contribute to differences in P acquisition could include production of other classes of phosphatase enzymes such as phosphodiesters (although any P liberated by these enzymes still requires breakdown with the phosphomonosterases measured in this study; Turner, 2008), elevated microbial phosphatase activity in the rhizosphere (Houlton et al., 2008; Keller et al., 2013), generally higher root affinity for P or differences in root architecture that allow exploitation of a larger soil volume (Bucher, 2006; Lynch et al., 2005). Continued characterization of these traits may offer more promising avenues for identifying functional controls over P acquisition.

Overall, we found no evidence for the hypothesis that functional group or N status is a strong control on P acquisition, although whether phylogenetic grouping is an important determinant remains on open question. Although we observed that two pairs of species within the same genera did not differ significantly in P acquisition traits, replication is too low to draw any conclusions from this. However, variability at the family level (especially in a diverse group like Fabaceae), could span almost the full range of variability in this dataset. Given the pressing need to incorporate P dynamics generally into climate and carbon models (Wieder et al., 2015), there is a clear need for more studies that inform how we might go about representing rates and mechanisms of plant P acquisition. Specifically, it may be beneficial to look beyond simple functional groups to a broader suite of conserved traits between related groups, or to explore other physiological traits or trade-offs that may provide greater explanatory value.

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AUTHORS' CONTRIBUTIONS

F.M.S., M.K.N., and C.C.C. conceived of and designed the study; F.M.S., M.K.N., and B.B.O. performed the research; F.M.S. analysed the data and wrote the first draft, and all authors helped revise the manuscript.

DATA ACCESSIBILITY

All data collected for this study are available through the Knowledge Network for Biocomplexity data repository: https://doi.org/10.5063/F1WQ01ZQ (Soper, 2018).

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