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Phosphorus limitation of early growth differs between nitrogen-fixing and non-fixing dry tropical forest tree species

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Summary:

- Tropical forests are often characterized by low soil phosphorus (P) availability, suggesting that P limits plant performance. However, how seedlings from different functional types respond to soil P availability is poorly known but important for understanding and modeling forest dynamics under changing environmental conditions.
- We grew four nitrogen (N)-fixing Fabaceae and seven diverse non-N-fixing tropical dry forest tree species in a shade house under three P fertilization treatments, and evaluated carbon (C) allocation responses, P demand, P-use, investment in P acquisition traits, and correlations among P acquisition traits.
- N-fixers grew larger with increasing P addition in contrast to non-N-fixers, which showed fewer responses in C allocation and P-use. Foliar P increased with P addition for both functional types, while P acquisition strategies did not vary among treatments but differed between functional types, with N-fixers showing higher root phosphatase activity (RPA) than non-fixers.
- Growth responses suggest that N-fixers are limited by P, but non-fixers may be limited by other resources. However, regardless of limitation, P acquisition traits such as mycorrhizal colonization and RPA were non-plastic across a steep P gradient. Differential limitation among plant functional types has implications for forest succession and earth system models.

Keywords: arbuscular mycorrhizas, phosphorus acquisition strategies, photosynthetic phosphorus use efficiency, root phosphatase, seedlings, stoichiometry.

Introduction

Lowland tropical soils have been described as phosphorus (P) limited, which is attributed in part to their advanced weathering state (Walker & Syers, 1976; Vitousek *et al.*, 2010; Cleveland *et al.*, 2011). Once initial soil P stocks are depleted, remaining P is either occluded by secondary minerals or present in organic forms, both of which are difficult for plants to access (Lambers *et al.*, 2008; Turner, 2008).Therefore, plants growing in low P soils invest in either mining (e.g., enzymes that hydrolyze organic compounds) or scavenging traits (symbiosis with mycorrhizal fungi, rapid root growth, or root hair proliferation) to optimize P uptake (Lambers *et al.*, 2008; Zemunik *et al.*, 2015). These P acquisition traits have different resource costs. For instance, root exudates can account for 50% of belowground carbon allocation, while mycorrhizal fungi can demand up to 25% of total carbon assimilated by plants (Lynch & Ho, 2005). Construction of fine roots has a low carbon cost, although their maintenance is higher compared to coarse roots (Laliberté *et al.*, 2015; McCormack *et al.*, 2015). Even though trait costs vary, some traits may be more efficient than others depending on soil P availability (Treseder & Vitousek, 2001; McCormack & Iversen, 2019) and their multifunctionality for acquiring other limiting resources (e.g. water).

Plants with high foliar P tissue concentrations in low P soils can invest more in P acquisition traits (Hayes *et al.*, 2014). They optimize resource use by investing in only a subset of P acquisition traits, and down-regulate these traits when inorganic forms of P are more abundant (i.e., show plasticity) (Ryan *et al.*, 2012; Ushio *et al.*, 2015). However, for most species and functional types it is still unclear which combination of traits (strategy) is the most efficient for acquiring P, in part because most experiments often measure only a handful of root chemical and morphological traits (McCormack *et al.*, 2017), which precludes characterizing an overall plant strategy, especially with regard to symbiotic and physiological traits. Moreover, comparisons across gradients of soil P availability that would elucidate plasticity in investment are limited (Laliberté, 2017). Understanding patterns of plant P use, investment in different P acquisition traits and the coordination among them is important for improving our predictions of plant species abundances and distributions across successional gradients, assessing plant responses to global changes, and defining plant functional types in earth system models (Bardgett *et al.*, 2014; Hanbury-Brown *et al.*, 2022).

In addition to P, plants require multiple other nutrients (Kaspari & Powers, 2016) many of which may also be in short supply and thus affect seedling performance (Santiago *et al.*, 2012). While multiple studies have suggested that tropical forest soils can have an excess of N relative to P (Jenny, 1950), N has still been shown to play a role in nutrient limitation in many cases (e.g., late succession) (Ostertag & DiManno, 2016; Wright, 2019). N and P

concentrations are mechanistically linked by the physiological requirements of organisms (McGroddy *et al.*, 2004), and even though their stoichiometry is constrained, variation in these ratios occurs among species, soil types, and ecosystems (Güsewell, 2004; Townsend *et al.*, 2008).

Plant N and P requirements might be especially linked in leguminous species that acquire N through biological N fixation, common to some Fabaceae subfamilies (Caesalpinioideae and Papilionoideae) and nine other plant families (Soltis *et al.*, 1995; Augusto *et al.*, 2013). N fixation is a P-demanding process, and many empirical and theoretical investigations have suggested that N fixation may thus be coupled with P acquisition (Wang *et al.*, 2007; Marklein & Houlton, 2012). This alternative N acquisition pathway may allow N-fixer species to access more P using this "extra" N, for example by investing it in phosphatase enzyme production (Marklein & Houlton, 2012; Nasto *et al.*, 2014; Png *et al.*, 2019). However, this link may be species-specific and could be phylogenetically conserved (i.e., a characteristic of the Fabaceae family, rather than mechanistically linked to N fixation per se) (Wurzburger & Hedin, 2016; Png *et al.*, 2017; Batterman *et al.*, 2018; Soper *et al.*, 2019), which hinders broad generalizations. To date, most studies exploring how P addition impacts species performance and nutrient acquisition traits have explored few traits and used a small number of species to represent tropical diversity (Huante *et al.*, 1995; Siqueira & Saggin-Júnior, 2001; Batterman *et al.*, 2013; Zalamea *et al.*, 2016), thus limiting the breadth of conclusions that can be drawn.

Nevertheless, broad patterns suggesting the distinctiveness of N-fixing Fabaceae species in terms of growth, development, and physiology are starting to emerge. Tropical forest N-fixers have faster germination rates, higher water use efficiency, and higher seedling growth rates compared to non-fixers (Powers & Tiffin, 2010; Vargas *et al.*, 2015; Adams *et al.*, 2016; Smith-Martin *et al.*, 2017). However, multiple studies conducted in wet forest have suggested that traits like N fixation and root phosphatase activity can differ among N-fixing species (Cernusak *et al.*, 2011; Png *et al.*, 2017; Batterman *et al.*, 2018; Soper *et al.*, 2019). Therefore, the applicability of the N-fixer and non-fixer functional type does not seem appropriate to characterize species functional roles in wet forests (Batterman *et al.*, 2018). By contrast, findings from Waring et al. (2019) suggest that N-fixing and non-fixing trees in tropical dry forests (TDF) respond differently to N and P addition. Model simulations confirm that soil variability influences functional type (fixer vs non-fixer) composition and processes during succession (Medvigy *et al.*, 2019). Therefore, exploring how seedlings from these functional types acquire and use P in a biome that represents 40% of tropical forests

(Murphy & Lugo, 1986), experiences high nutrient limitation due to its intense seasonality (Read & Lawrence, 2006), and has a high abundance of Fabaceae species during early succession (Baribault *et al.*, 2012; Gei *et al.*, 2018) is important for understanding how projected changes in rainfall regimes may affect carbon storage in TDF (Chadwick *et al.*, 2015; Norby *et al.*, 2017; Hanbury-Brown *et al.*, 2022).

To compare the extent to which early growth, performance and trait values of N-fixing and non-fixing TDF seedlings respond to P, we grew plants across a gradient of P availability in a shade house experiment. We measured a range of physiological, morphological, and chemical traits. Specifically, we addressed the following questions: 1) How do N-fixing and non-N-fixing seedlings respond to P availability? 2) How does P addition affect P acquisition traits? 3) How does P addition impact photosynthetic P-use efficiency of N-fixing and non-N-fixing seedlings? And 4) What are the correlations among foliar P concentrations, P use, and acquisition traits? We predicted that: 1) N-fixers show greater growth responses to P addition than non-N-fixers because N-fixers are faster growers. 2) Investment in P acquisition traits varies inversely as a function of soil P availability, and because traits for acquiring P are "expensive", these traits are negatively correlated with each other reflecting trade-offs. 3) Photosynthetic P-use efficiency decreases with P addition for both functional types. 4) Foliar P concentrations are positively correlated with P acquisition traits.

Materials and Methods

Site description

Our study was conducted in a shade house located at Estación Experimental Forestal Horizontes (here after "Horizontes"), part of Área de Conservación Guanacaste (ACG) in northwestern Costa Rica (10.71 N, 85.59 W). Horizontes has a mean annual temperature of 25°C, and a mean annual precipitation of ~1730 mm that mostly falls between late May to early November (Waring *et al.*, 2019; Werden *et al.*, 2020).

Soil collection

Local forest soil from 5-30 cm depth was collected in May 2019 at Horizontes, and then left in the sun for four days covered with plastic to kill any live roots. After that, soils were homogenized and sieved to 5 mm. Soils at the site are Andic and Typic Haplustepts

(Alfaro *et al.*, 2001), with a high clay content (38 ± 1%) and total N:P of 8.3, suggesting P limitation (Waring *et al.*, 2019).

Species selection

We initially selected seeds of 30 species that commonly occur across successional and soil fertility gradients (30 to 1272 total P mg kg^{-1}) in ACG and Área de Conservación Arenal Tempisque (ACAT)- Parque Nacional Palo Verde (10.35 N, 85.35 W) (Powers & Tiffin, 2010; Werden *et al.*, 2018), and had seeds available at the time the experiment was established. Due to logistical constraints (i.e., low germination rates, pests, and high mortality), only 11 species of the original 30 were used in the experiment. We included four N-fixing species from the Papilionoideae subfamily, the most abundant Fabaceae subfamily in the area (Werden *et al.*, 2018) and the one with the highest species richness (Koenen *et al.*, 2021). Papilionoideae species primarily associate with arbuscular mycorrhizal fungi. We caution here that our results should not be extended beyond the Papilionoideae subfamily; however, the fixers and non-fixers used in this study do not differ significantly in distribution characteristics across successional or soil gradients compared to the regional species pool (Methods S1, Table S1, and Fig. S1). Non-fixers included one ectomycorrhizal and six arbuscular mycorrhizal species from seven different families, including one Fabaceae (Table S2 and Fig. S2). We collected most seeds in the ACG and ACAT and purchased seeds of *Tabebuia impegitinosa* (Mart. Ex DC.) Standl. from the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica.

The experiment was conducted during the rainy season from May to October in a 36 m^2 shade house that excluded approximately 55% of PAR (J. Powers, unpublished data). Prior to planting, all seeds were soaked in water overnight. *Hymenaea courbaril* L. seeds were scarified to accelerate germination (Smith-Martin *et al.*, 2017); seeds were placed in 100°C water for 30 s and then in ice water for one minute, this process was repeated three times. Then, between 2-15 seeds (depending on seed size and availability) of each species were planted per pot (11 species x 3 treatments x 7 replicates = 231 pots). Pots were big enough to have a biomass volume ratio < $1gL^{-1}$ (Poorter *et al.*, 2012) (10 x 10 cm and 20 cm deep containing ~2 kg of soil). Pots were randomly arranged within plastic crates to avoid confounding positioning species and treatment effects (8 pots per crate). One week after germination, seedlings were thinned to one per pot. Because we obtained low germination rates for *Lonchocarpus rugosus* Benth. And *H. courbaril,* additional seeds were added. To

minimize differences in soil microbial communities, all seedlings were inoculated with microbial inoculum from three different forest soils collected across the regional P gradient (Smith-Martin *et al.*, 2017; Waring *et al.*, 2019). We prepared the inoculum by combining equal weights of the three soils in 1 L water, shaking them, and adding 10 ml of this solution to each pot 3 weeks after sowing. This treatment was repeated the following week to ensure microbial colonization. When seedlings were ~1 month old they were fertilized every two weeks from July to October with 10 ml of either 0.0016 g ml^{-1} of phosphoric acid (H_3PO_4) (45 kg P ha^{-1} yr^{-1}) (P++) (Waring *et al.*, 2019), 0.00016 g ml^{-1} H_3PO_4 (4.5 kg P ha^{-1} yr^{-1}) (P+), or tap water (P0) to emulate the regional soil P gradient and P application rates used in other fertilization experiments (Siqueira & Saggin-Júnior, 2001; Alvarez-Clare *et al.*, 2013; Waring *et al.*, 2019). Soil moisture was measured in each pot to 5 cm depth three times over the course of the experiment with a SM150 Soil Moisture Sensor (Delta-T Devices, Cambridge, UK). When rainfall was scarce, i.e., lack of rain for > 24 h, seedlings were hand-watered every other day to prevent desiccation. At the conclusion of the experiment, we measured soil pH in distilled water on air-dried soils from all pots using a 1:2.5 soil to solution ratio. Finally, to quantify the distribution of soil P among different P pools, a modified Hedley fractionation was conducted on a subset of soils from the pots at the soil laboratory at CATIE.

Harvesting and biomass

We harvested all seedlings four months after germination over a 10-day period. None of the individuals showed evidence of being pot bound. At the time of harvest, each seedling was separated into root, stem, and leaf fractions. Roots were carefully removed, gently washed in water and weighed. In the case of nodulating species, nodules were removed from the roots (before weighing), counted, and weighed. Leaves were immediately scanned to calculate leaf area using ImageJ software (Schneider *et al.*, 2012). Leaves, stems, roots, and nodules of each seedling were dried at ~60°C for 5 days, and then weighed to calculate total dry biomass (TDB), leaf (LMF), stem (SMF), root (RMF) (Table S3), and nodule (NMF) mass fractions. Root biomass included the tap roots if they were present. Dry weights of leaves were used to calculate specific leaf area (SLA, cm^2 g^{-1}). All statistical analyses were performed using dry weights. SLA did not vary between functional types or treatments (Fig. S3, SLA $p > 0.05$) and will not be further discussed.

Plant performance and nutrient limitation

We measured relative height growth rate (RGR), photosynthetic capacity, and water use efficiency as metrics of plant performance. Stem height was measured from the base of the stem to the apical meristem on each seedling every two weeks after the first true leaves emerged. We used the classic RGR (cmd^{-1}) equation: (ln[final height] – ln[initial height]) / (*final day – initial day)*(Hunt & Cornelissen, 1997; Rees *et al.*, 2010). Photosynthetic capacity was quantified by measuring photosynthetic responses to carbon dioxide concentration (AC_i curves). Gas exchange measurements were performed on one recently matured leaf of three seedlings per species per treatment in situ with a LI-6400XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) at a constant temperature (30°C). Measurements were made at CO_2 concentrations ranging between 0 and 1200 ppm (9 points total), irradiance was 1000 μmol $m^{-2}s^{-1}$ (unlikely to cause photoinhibition of photosynthesis when leaves are measured below or above optimum temperatures) and relative humidity ranged between 60-80%. Measurements were made between 07:00 and 14:00 h and the ambient air temperature generally increased from 25 to 32°C. We then obtained maximum photosynthetic rates (A_{max}) and rubisco maximum carboxylation rate (V_{cmax}) using the 'plantecophys' R package (Duursma *et al.,* 2015).

Finally, we analyzed foliar nutrients, water use efficiency, and photosynthetic phosphorus-use efficiency (PPUE). We bulked all leaves per individual and transported them to the University of Minnesota (UMN) where they were ground and analyzed for total carbon (C), nitrogen (N) and C isotopic ratio ($\delta^{13}C$) on an Isoprime 100 isotope ratio mass spectrometer coupled with an Elementar Pyrocube combustion elemental analyzer (Elementar Americas Inc). Other foliar nutrients (aluminum, boron, calcium, chromium, copper, iron, phosphorus, potassium, magnesium, manganese, sodium, and nickel) were analyzed at the UMN Research Analytical Lab using standard methods in an iCAP™ 7600 ICP-OES (Inductively Coupled Argon Plasma Optical Emission Spectrometer) Analyzer (Thermo Fisher Scientific Inc. Waltham, MA, USA) following digestion in HNO_3. We calculated PPUE from A_{max} and foliar P concentrations (Veneklaas *et al.,* 2012).

Belowground traits

We quantified plant investment in P acquisition using root mass fraction (RMF) and specific root length (SRL)—a morphological response, root phosphatase enzyme activity (RPA)—a physiological response, and % arbuscular mycorrhizal colonization (AMC)—a biotic response representing degree of collaboration with symbionts. SRL was calculated

after scanning all roots with a transparency scanner (Epson Perfection V800, Suwa, Japan; 1-4 images per individual). We placed the roots in a clear polycarbonate tray filled with water to ensure no root overlap and used the Rhizovision software (Seethepalli *et al.*, 2020) to calculate root length of fine root fractions (≤ 2 mm diameter), i.e., excluding any tap root. After scanning, root systems were separated into two subsamples: one sample for RPA, and the other for scoring AMC. RPA was determined using para-nitrophenyl phosphate (*p*NPP) as an analogue substrate for phosphomonoesterase using standard methods (Methods S2; Turner *et al.*, 2001). Finally, we scored arbuscular mycorrhizal colonization (AMC) by staining the roots with alanine blue (Koske & Gemma, 1989) and quantifying colonization microscopically using the magnified intersection method (McGonigle *et al.*, 1990) on ~100 intersections per root fragment. We calculated colonization as the percentage of root length colonized by AM fungi (Methods S3). Ectomycorrhizal colonization was measured using the gridline intersection method (Brundrett *et al.*, 1996). This variable was not included in any of the analyses as only one species was ectomycorrhizal, and these data are not directly comparable to AMC.

While quantifying N fixation activity was not a goal of this experiment, we assessed plant carbon construction investment in fixing structures by quantifying nodule mass and NMF (Methods S4, Table S4 and Fig S4).

Statistical analysis

First, we tested the effects of our experimental treatments on soil pH and soil P fractions using linear models, pooling data by P treatment across all species. Then, to determine how N-fixing and non-fixing seedlings respond to P availability, we analyzed the effects of P addition on TDB, RGR, A_{max}, and $\delta^{13}C$ as a metric of WUE with linear mixed-effects models. In these models, trait values were the response variable. Functional type (N-fixer or non-fixer), treatment (control; P0, low; P+, or high; P++) and their interaction were included as fixed effects. Species identity was included as a random effect to account for variation among species. Seedling initial height was included as a covariable to control for differences in species' sizes. We used a similar model with a beta distribution for SMF and LMF. We checked the distribution of the residuals for all models to ensure that normality and homoscedasticity assumptions were met. When assumptions were not met (TDB, RGR and $\delta^{13}C$), we used a generalized linear mixed-effects model (GLMM) assigning an independent variance structure (varIdent) as a function of species. Finally, we obtained the F-values of a two-way analysis of variance (ANOVA, type III) for the fixed effects and performed multiple

comparisons among treatments and/or functional types using a Tukey post hoc test (Table 1 and Methods S5).

Our second question explored how P addition affects P acquisition traits. The statistical models used to answer this question were similar to the ones used in question 1. Because the residuals did not meet the normality and homoscedasticity assumptions, we used a GLMM with a variance structure (varIdent) as a function of species (RPA, foliar N:P, foliar P, foliar N) or log transformed (SRL) the response variable. AMC and RMF were modeled with a beta distribution (colonization values range from 0 to 1). To answer how PPUE changed with P addition, we ran the same statistical model used in question 1. Then, following Messier *et al.* (2010), we performed a variance partitioning analysis for each trait independently. Importantly, the variance explained for our models corresponds to the group-level variation i.e., functional type, species identity, individual, and treatment. A nested linear model was used for each trait (Messier *et al.*, 2010).

Last, we explored if there were correlations among foliar N and P, PPUE, and nutrient acquisition traits (RPA, AMC, RMF, SRL) conducting a pairwise Pearson correlation analysis with Bonferroni corrections for each functional type separately. We excluded the mycorrhizal colonization data of *Q. oleoides* from the non-fixer correlation because this species had a different mycorrhizal type. Results were considered statistically significant at $p < 0.05$. All statistical analyses were done using R software for statistical computing version 4.0.3 (R Core Team, 2021) and the following packages: nlme (Pinheiro *et al.*, 2019), lme4 (Bates & Maechler, 2011), glmmTMB (Brooks *et al.*, 2017), Hmisc (Harrell, 2019), and car (Fox & Weisberg, 2020).

Results

Soil pH and phosphorus

At the end of the experiment soil pH ranged from 5.56 to 7.07. P addition decreased soil pH by 0.06 (P+) and 0.14 (P++) units compared to the control ($p < 0.01$). However, the differences in pH between the control and low P treatment were not statistically significant ($p > 0.05$). Total P in the Hedley fractions ranged from 548.7 to 1165.9 mg kg^{-1} and confirmed that P fertilization increased soil total P by a factor of 1.4 (P+) and 2.1 (P++) compared to the control ($p < 0.05$). As expected, inorganic fractions experienced the highest increases compared to organic fractions. While P in the Hedley fraction was consistently higher in the high P treatment compared to the other two treatments, the $NaHCO_3$ organic P was the only fraction that did not change among treatments ($p > 0.05$) (Table S5).

Growth and biomass

Early growth of N-fixers was more responsive to P addition than non-fixers (functional type by treatment effect, $p < 0.01$) (Table 1 and Table S6). N-fixers that received P increased their relative growth rates (RGR) by 18% (P+) and 24% (P++) compared to the control ($p < 0.001$), but differences between the two P addition rates were not significant ($p > 0.05$). RGRs for non-fixers were similar among P addition treatments ($p > 0.05$) (Fig.1a and Fig. S5). Similar to RGR, N-fixers had a mean total dry biomass (TDB) three times higher compared to non-fixers across treatments ($p < 0.001$). The differences in TDB depended on the interaction between treatment and functional type ($p < 0.001$). The mean TDB of N-fixers was 39% (P+) and 54% (P++) higher when P was added compared to the control ($p < 0.001$). Mean TDB of non-fixers showed more modest increases of 12% (P+) and 15% (P++) respectively, compared to the control ($p < 0.05$). Mean TDB did not significantly differ between the low and high treatments for either functional type ($p > 0.05$) (Fig. 1b and Fig. S6). Finally, leaf mass fraction did not differ between functional types nor treatments ($p > 0.05$), and allocation to stems differed across P treatments ($p < 0.05$) but not between functional types (Fig. S7).

Photosynthesis and WUE

N-fixers on average had a 36% higher maximum photosynthetic rate (A_{max}) compared to non-fixers ($p < 0.05$). Mean A_{max} increased by 21% for N-fixers when P was added in high quantities (P++) compared to the control ($p < 0.05$, Fig. 1c and Fig. S8). The rubisco carboxylation rate (Vc_{max}) also differed between functional types. N-fixers had a higher mean Vc_{max} compared to non-fixers ($p < 0.05$). However, Vc_{max} values did not vary significantly across P treatments for either functional type ($p > 0.05$). Finally, $\delta^{13}C$ values (interpreted as a proxy for mean water use efficiency; WUE), ranged from -25.9 to -32.42‰ among individuals. The variation in WUE depended upon the interaction between functional types and treatment ($p < 0.05$). N-fixers increased WUE with P addition ($p < 0.05$), while non-fixers had similar WUE across treatments ($p > 0.05$) (Fig. 1d, Fig. S9 and S10).

Foliar P, N, and stoichiometry

N-fixers had lower mean foliar P than non-fixers, and the variation in foliar P depended on the combination of functional type and treatment ($p < 0.01$). P addition increased foliar P of N-fixers by 22% (P+) and 28% (P++) compared to the control ($p < 0.001$), with no significant differences between the two fertilization levels ($p > 0.05$). Foliar P

of non-fixers, increased by 23% (P+) and 44% (P++) respectively compared to the control ($p < 0.001$). High doses of P increased foliar P by 17% compared to the low treatment ($p < 0.001$, Fig. 2a and Fig. S11). In contrast, foliar N was 72% higher for N-fixers compared to non-fixers ($p < 0.001$) and did not vary across treatments for either functional type ($p > 0.05$, Fig. 2b and Fig. S12). Mean foliar N:P of N-fixers was higher across treatments relative to non-fixers, 20.4 for N-fixers compared to 9.8 for non-N-fixers ($p < 0.001$, Fig. 2c and Fig. S13). The variation in N:P depended on the interaction between functional type and treatment ($p < 0.001$). When P was added, N-fixers had lower N:P compared to the control (24.8 P0, 18.6 P+, 16.9 P++) ($p < 0.001$). High rates of P addition had lower N:P compared to the low rates ($p < 0.05$). Non-fixers also showed large changes in N:P with P addition. N:P decreased when P was added (11.4 P0, 8.3 P+, 8.9 P++) ($p < 0.001$). The low and high fertilization treatments had similar N:P ($p > 0.05$). Results for other foliar nutrients appear in the supplement section (Table S7): only boron, chromium, and potassium differed among treatments and functional types.

P-use efficiency

Photosynthetic phosphorus-use efficiency (PPUE) depended upon the interaction between functional types and treatment ($p < 0.05$). N-fixers PPUE was 38% higher than non-fixers PPUE ($p < 0.05$). However, both functional types decreased PPUE with P addition. Fertilized non-fixers decreased PPUE by 27% (P+) and 35% (P++) compared to the control ($p < 0.01$; no difference between P treatments; $p > 0.05$). N-fixers, on the other hand, decreased their PPUE by 19% (P+) compared to the control ($p < 0.05$). However, PPUE for the high P treatment was similar to PPUE of the control and low P ($p > 0.05$) (Fig. 2d).

P acquisition traits

P acquisition traits differed primarily by functional type, rather than in response to P addition (Table 2). Root biomass allocation depended on the combination of functional type and treatment ($p < 0.05$). N-fixers had lower root mass fraction (RMF) compared to non-fixers. RMF of non-fixers only changed between the low and high P additions rates ($p < 0.001$). N-fixers had similar RMF across treatments ($p > 0.05$) (Fig. 3a and Fig. S14). On the other hand, mean root phosphatase activity (RPA) of N-fixers was three times higher compared to non-fixers ($p < 0.01$) and did not decline with P addition for either functional type ($p > 0.05$) (Fig. 3b and Fig. S15). Similar to RPA, the average specific root length (SRL) did not change with P addition for either functional type ($p > 0.05$). However, SRL of non-

fixers was three times higher compared to N-fixers ($p < 0.05$) (Fig. 3c and Fig. S16). Finally, AMC varied substantially across species (from 0-45%, Fig. S17), though the mean (~26%) was similar for both functional groups across treatments ($p > 0.05$, Fig. 3d).

Variance partitioning

Overall, functional type accounted for ~24% of the variance across all traits, species identity ~51%, individual ~25%, and treatment ~0.03%. However, the importance of each source of variation differed among traits. Functional type differences were most important for TDB (45%), RPA (42%), foliar N (76%), and foliar N:P (53%). By contrast, species identity was a dominant source of variation for RGR (78%), $\delta^{13}C$ (76%), AMC (61%), SRL (48%), RMF (75%), foliar P (66%), and PPUE (80%). Individual differences contributed to most of the variation for A_{max} (48%) (Fig. 4).

Correlations among nutrient acquisition traits, PPUE, and foliar nutrients

Non-fixers showed no correlation between foliar P and N ($p > 0.05$). Foliar P was only negatively correlated with PPUE ($r = -0.77$, $p < 0.001$). None of the P acquisition traits were correlated with foliar P ($p > 0.05$). Foliar N was positively correlated with RPA ($r = 0.52$, $p < 0.001$) and SRL ($r = 0.34$, $p < 0.001$). AMC ($r = -0.37$, $p < 0.001$) was negatively correlated with foliar N. Finally, RMF-AMC ($r = 0.53$, $p < 0.001$) and SRL-RPA were positively correlated ($r = 0.49$, $p < 0.01$), while RPA-AMC ($r = -0.22$, $p < 0.001$) was negatively correlated. PPUE was positively correlated with SRL ($r = 0.58$, $p < 0.001$) and negatively correlated with AMC ($r = -0.73$, $p < 0.001$) (Fig. 5a).

N-fixers showed a positive correlation between foliar N and P ($r = 0.59$, $p < 0.001$). In N-fixers, RPA ($r = 0.51$, $p < 0.001$) was the only nutrient acquisition trait positively correlated with foliar P; while PPUE ($r = -0.78$, $p < 0.001$) was negatively correlated with foliar P. The other traits were not correlated with foliar P ($p > 0.05$). Foliar N, on the other hand, was positively correlated with RPA ($r = 0.44$, $p < 0.001$) and negatively correlated with PPUE ($r = -0.54$, $p < 0.001$). SRL-RMF ($r = -0.55$, $p < 0.001$) were the only P acquisition traits negatively correlated. By contrast, RMF-AMC was positively correlated ($r = 0.43$, $p < 0.001$). The other P acquisition traits evaluated were not correlated ($p > 0.05$) (Fig. 5b).

Discussion

Efforts to identify which resource limits productivity in lowland tropical forests have produced mixed results. Some studies have found that P is the only nutrient limiting plant

performance (Vitousek, 1984; Campo & Vázquez-Yanes, 2004; Condit *et al.*, 2013), while other fertilization studies and meta-analyses suggest that at the ecosystem scale there is colimitation by N, P and other macronutrients (Santiago *et al.*, 2012; Wright, 2019). Our study is the first to compare multiple above- and belowground responses to added P for a diversity of dry forest (TDF) species spanning an important functional type distinction (N-fixers vs non-fixers). We found that the growth of N-fixing seedlings (Papilionoideae subfamily) appeared to be P limited, while non-fixers showed little evidence of P limitation suggesting that seedlings and mature trees respond in similar ways to P addition in TDF (Waring *et al.*, 2019). We also found that N-fixers and non-fixers invested differentially in specific sets of P acquisition traits. N-fixers invested more in a key physiological trait (root phosphatase activity), while non-fixers invested more in allocation (greater root mass fraction, RMF) and had distinct morphological traits (higher specific root length, SRL). Finally, contrary to what previous studies conducted in wet tropical forest have suggested (Batterman *et al.*, 2013; Zalamea *et al.*, 2016; Nasto *et al.*, 2019), we found that P acquisition traits were not downregulated even with significant P addition. These differences could be product of seedlings' high nutrient demand in TDF. However, photosynthetic phosphorus-use efficiency was reduced suggesting that plants might be experiencing P luxury consumption (Lawrence, 2003). Although we used the functional type framework to analyze our dataset, the variance partitioning analysis underscored the strong role of individual species identity determining many of the patterns observed (Batterman *et al.*, 2018; Soper *et al.*, 2019), suggesting that broad generalizations about functional type responses regarding some traits should be taken with caution.

Differential P limitation

We found strong evidence that N-fixers responded to P addition, while non-fixers showed little to no response. N-fixers had increased height and biomass gain when P was added, and high doses of P magnified these responses (Fig. 1). Such responses are evidence of P limitation (Vitousek & Howarth, 1991; Batterman *et al.*, 2013). Differential limitation between N-fixers and non-fixers was also reflected in their foliar N:P ratios (> 16 for N-fixers, < 14 for non-fixers) (Fig. 2c); N:P ratios above 16 have been suggested as evidence of P limitation, and values below 14 are indicative of N limitation (Koerselman & Meuleman, 1996). While a previous shade house experiment (Huante *et al.*, 1995) and an ecosystem-scale fertilization study (Waring *et al.*, 2019) have suggested this pattern in both seedlings

and trees in TDF, our experiment is the first to demonstrate it for a larger number of tropical species seedlings growing across a P gradient.

Additionally, RGR and water use efficiency (WUE) did not change for our taxonomically diverse group of seven non-fixing species when P was added, and total dry biomass only slightly compared to the changes N-fixers experienced (Fig. 1). This suggests that non-fixers might be limited by other resources (Santiago *et al.*, 2012; Chou *et al.*, 2018; Waring *et al.*, 2019) Our experimental design does not allow us to determine whether N, some other nutrients, or another resource such as water ultimately limits non-fixers. An obvious follow-up to our experiment would be to test the hypothesis that non-fixing seedlings are limited by N or co-limited by N and P. Overall, our experiment corroborates what Waring *et al.* (2019) suggested at the stand level, i.e., that species-specific responses to nutrient addition depend in part on plant functional type. However, that study lacked the replication to definitively resolve differential P limitation between N-fixers and non-fixers.

Phosphorus-use efficiency

We found that overall N-fixers had a higher PPUE across P treatments compared to non-fixers, suggesting that N-fixers may be more competitive than non-fixers under low resource conditions (Fig. 2d) (Veneklaas *et al.*, 2012). We also observed that PPUE values tended to decrease with P addition, suggesting that seedlings are less efficient at conserving and using P when it is in excess (Lovelock *et al.*, 2004). In fact, the observed increases in foliar P with P addition (Fig. 2a) imply that seedlings might be experiencing P luxury consumption (Cordell *et al.*, 2011). To our surprise, N-fixers in the control and high P treatment had similar PPUE values. This finding could indicate PPUE is a trait with low variability. PPUE seems to be an important functional trait to measure in trees growing on P-poor soils because it shows how species adapt to low-P soils (Hidaka & Kitayama, 2011). Therefore, including traits like PPUE in earth system models may increase our capability to predict future conditions in P-limited tropical forests (Reed *et al.*, 2015; Norby *et al.*, 2016).

Relative investment and down-regulation of P acquisition traits

In theory, once a limiting resource becomes available, plants should down-regulate investment in nutrient acquisition traits and shift biomass allocation patterns to optimize expenditure of other key resources (Bloom *et al.,* 1985). Although we found no evidence of trait downregulation with P addition, seedlings from different functional types invested differentially in P acquisition traits. Non-fixers invested more in less C-costly root traits, such

as exploring more soil volume with high SRL values. Non-fixers had higher biomass allocation to roots overall (Figure 2). By contrast, N-fixers invested in more N-costly traits such as the production of root phosphatases (Yadav & Tarafdar, 2001; Houlton *et al.*, 2008; Guilbeault-Mayers *et al.*, 2020).

Contrary to findings from both tropical and temperate systems, investment in root phosphatase did not decrease with P addition (Marklein & Houlton, 2012; Cabugao *et al.*, 2017, 2021; Lugli *et al.*, 2021). This lack of downregulation response could be related to phylogenetic trait conservation i.e., that certain trait expressions are conserved across lineages, rather than being plastic in response to environmental conditions (Valverde-Barrantes *et al.*, 2015, 2017; Wurzburger & Hedin, 2016; Png *et al.*, 2017). Similarly, percentage of AM colonization did not differ across treatments (Treseder & Allen, 2002). Seedlings from both functional types had equivalent colonization percentages, suggesting that AM colonization could be decoupled from soil P availability as Waring *et al.* (2019) reported in a factorial N and P fertilization experiment at the same site and Powers *et al.* (2005) observed across neotropical rain forests. Even though the major benefit of AM has been considered translocation of inorganic P (Smith & Read, 2010), AM could provide other benefits to tropical dry forest seedlings. For example, AM might help seedlings acquire other nutrients (Siqueira & Saggin-Júnior, 2001; Zangaro *et al.*, 2005; Lambers *et al.*, 2008), facilitate water acquisition or protect them from pathogens (Herre *et al.*, 2007; Laliberté *et al.*, 2015). It is also possible that tree species in TDF lack efficient mechanisms to actively regulate AM colonization (Valverde-Barrantes *et al.*, 2016) or that seedlings growing in pots have to invest more C in mycorrhizal associations because they cannot access existing networks.

The other two P acquisition traits evaluated, SRL and RMF, were higher for non-fixers compared to N-fixers (Fig. 3). RMF was the only trait that increased with P addition for non-fixers, but SRL did not vary (Ostertag, 2001; Wurzburger & Wright, 2015). When plants experience N or P deficiencies, allocation to roots generally increases. However, the effect is stronger for N deficiencies, which is further evidence that non-fixers might be experiencing N limitation (Andrews *et al.,* 1999; Groot *et al.,* 2003). It is also possible that the species used in this experiment were experiencing incomplete downregulation of P acquisition traits when P was added. Tropical N-fixers have shown signs of incomplete downregulation of N-fixation when nutrients were added (Taylor & Menge, 2018, 2021). To our knowledge incomplete downregulation of P acquisition traits has not been reported yet. Finally, the absence of change in P acquisition traits with P addition could also be product of

seedlings' development stage and high nutrient demand, or an indication of the low plasticity of nutrient acquisition traits in TDF tree species (Valverde-Barrantes *et al.*, 2015, 2017).

Evidence for trait integration

We only found a positive correlation between foliar N and P of N-fixers (Townsend *et al.*, 2007; Soper *et al.*, 2019). There was varying evidence for trade-offs (i.e., negative correlations) between pairs of P acquisition traits (McCormack *et al.*, 2015). RMF and AM colonization were positively correlated for both functional types. This suggests that species with higher root biomass could allocate more C to AM fungi and therefore rely more on this symbiotic association for acquiring P (Kong *et al.*, 2016, Bergmann *et al.,* 2020). Similar to what other studies report, AM colonization (specialized on inorganic P uptake) decreased with increases in root phosphatase (a mechanism to access organic P) for non-fixers (Nasto *et al.*, 2017; Soper *et al.*, 2019), indicative of a tradeoff among these P acquisition traits. However, we found that these two traits were not correlated for N-fixers, similar to what has been reported in central Amazon and in a Panamanian montane forest (Steidinger *et al.*, 2015; Lugli *et al.*, 2019). N-fixers' capacity for fast growth may let them prioritize investment in both AM colonization (Ficano *et al.*, 2021) and RPA, traits that allow N-fixers to access both organic and inorganic P (Turner, 2008; Nasto *et al.*, 2014). Finally, RMF decreases with increases in SRL for N-fixers, suggesting that possessing thinner and less C-costly roots may allow N-fixers to allocate less biomass to roots while maintaining a large area for nutrient uptake (Bergmann *et al.*, 2020). Overall, seedlings seem to invest in multiple, complementary traits to acquire different forms of P (Zemunik *et al.*, 2015; Lugli *et al.*, 2019). This investment varies between functional types (Turner *et al.*, 2018) (Fig. 6) and values of these traits are relatively fixed over a large gradient of soil P availability.

Conclusion

Our study provides the first evidence for differences in responses of N-fixing and non-fixing seedlings to P availability in tropical dry forests. These results need to be taken with the caveat that our species only included members of the Papilionaideae subfamily, and it is possible that species from other Fabaceae subfamilies would respond differently. Nevertheless, these results have implications for understanding secondary succession and for defining the behavior of plant functional types in earth system models (Medvigy *et al.*, 2019; Hanbury-Brown *et al.*, 2022). As N-fixing legumes dominate during early succession in tropical dry forests and then give way to non-fixing species, we expect that nutrient limitation

may shift over successional time from P to N limitation. Our analysis also suggests that it is necessary to evaluate multiple nutrient acquisition traits when assessing species responses to nutrient limitation because different functional types might differentially invest in certain nutrient acquisition traits to access a limiting resource. Finally, this multi-species experiment corroborates that the responses of N-fixers and non-fixers to P limitation are consistent at both the ecosystem and seedling level (Waring *et al.,* 2019). These results suggest that nutrient limitation in tropical dry forests is more complex and heterogenous than previously thought (Powers *et al.*, 2015), which requires attention to how this process is implemented in simulation models.

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Author contributions

LT & JSP developed the idea; **LT & JSP** designed the study sampling scheme; **FMS** provided insight into P acquisition traits selection; **LT, Damaris Pereira-Arias, Daniel Perez-Aviles, GVG & OG** performed the sampling. **LT** performed data management and statistical analysis; **JSP, GVG, JG & FMS** provided support on the interpretation of results; **LT** wrote the manuscript with help from **JSP** and edits from all authors.

Data availability

The data that support the findings of this study are available from https://doi.org/10.5061/dryad.9kd51c5n0

References

Adams MA, Turnbull TL, Sprent JI, Buchmann N. 2016. Legumes are different: Leaf nitrogen, photosynthesis, and water use efficiency. *Proceedings of the National Academy of Sciences of the United States of America* 113: 4098–4103.

Alfaro EA, Alvarado A, Chaverri A. 2001. Cambios Edaficos Asociados a Tres Etapas Sucesionales De Bosque Tropical Seco En Guanacaste, Costa Rica. *Agronomia Costarricense* 25: 7–20.

Alvarez-Clare S, Mack MC, Brooks M. 2013. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. *Ecology* 94: 1540–1551.

Andrews M, Sprent JI, Raven JA, Eady PE. 1999. Relationships between shoot to root ratio, growth and leaf soluble protein concentration of *Pisum sativum, Phaseolus vulgaris* and *Triticum aestivum* under different nutrient deficiencies. *Plant, Cell & Environment* 22: 949–958.

Augusto L, Delerue F, Gallet-Budynek A, Achat DL. 2013. Global assessment of limitation to symbiotic nitrogen fixation by phosphorus availability in terrestrial ecosystems using a meta-analysis approach. *Global Biogeochemical Cycles* 27: 804–815.

Bardgett RD, Mommer L, De Vries FT. 2014. Going underground: Root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution* 29: 692–699.

Baribault T, Kobe R, Finley A. 2012. Tropical tree growth is correlated with soil phosphorus, potassium, and calcium, though not for legumes. *Ecological Monographs* 82: 189–203.

Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Dai B, Scheipl F, Grothendieck G. 2011. Package 'lme4'. Linear mixed-effects models using S4 classes. R package version.1(6).

Batterman SA, Hall JS, Turner BL, Hedin LO, LaHaela Walter JK, Sheldon P, van Breugel M. 2018. Phosphatase activity and nitrogen fixation reflect species differences, not nutrient trading or nutrient balance, across tropical rainforest trees. *Ecology Letters* 21: 1486–1495.

Batterman SA, Wurzburger N, Hedin LO. 2013. Nitrogen and phosphorus interact to control tropical symbiotic N2 fixation: A test in inga punctata. *Journal of Ecology* 101: 1400–1408.

Bergmann J, Weigelt A, Van der Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruelheide H, Freschet GT, Iversen CM, Kattge J. 2020. The fungal collaboration gradient dominates the root economics space in plants. *Science Advances 6*: p.eaba3756.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants-an economic analogy. *Annual Review of Ecology and Systematics* 16: 363–392.

Brooks ME, Kristensen K, Benthem KJ, van, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* 9: 378.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in Forestry and Agriculture*. Canberra: Australian Centre for International Agricultural Research

Cabugao KG, Timm CM, Carrell AA, Childs J, Lu T-YS, Pelletier DA, Weston DJ, Norby RJ. 2017. Root and Rhizosphere Bacterial Phosphatase Activity Varies with Tree Species and Soil Phosphorus Availability in Puerto Rico Tropical Forest. *Frontiers in Plant Science* 8: 1–14.

Cabugao KG, Yaffar D, Stenson N, Childs J, Phillips J, Mayes MA, Yang X, Weston DJ, Norby RJ. 2021. Bringing function to structure: Root–soil interactions shaping phosphatase activity throughout a soil profile in Puerto Rico. *Ecology and Evolution* 11: 1150–1164.

Campo J, Vázquez-Yanes C. 2004. Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatán, Mexico. *Ecosystems* 7: 311–319.

Cernusak LA, Winter K, Turner BL. 2011. Transpiration modulates phosphorus acquisition in tropical tree seedlings. 31: 878–885.

Chadwick R, Good P, Martin G, Rowell DP. 2015. Large rainfall changes consistently projected over substantial areas of tropical land. *Nature Climate Change* 6: 177–181.

Chou CB, Hedin LO, Pacala SW. 2018. Functional groups, species and light interact with nutrient limitation during tropical rainforest sapling bottleneck. *Journal of Ecology* 106: 157–167.

Cleveland CC, Townsend AR, Taylor P, Alvarez-Clare S, Bustamante MMC, Chuyong G, Dobrowski SZ, Grierson P, Harms KE, Houlton BZ, *et al.* 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: A pan-tropical analysis. *Ecology Letters* 14: 939–947.

Condit R, Engelbrecht BMJ, Pino D, Péreza R, Turner BL. 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *PNAS* 110: 5064–5068.

Cordell D, Rosemarin A, Schröder JJ, Smit AL. 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84: 747–758.

Duursma RA. 2015. Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data (PC Struik, Ed.). *PLOS ONE* 10: e0143346.

Ficano N, Porder S, McCulloch LA. 2021. Tripartite legume–rhizobia–mycorrhizae relationship is influenced by light and soil nitrogen in Neotropical canopy gaps. *Ecology* 102: 1–11.

Fox J, Weisberg S. 2020. Using car and effects Functions in Other Functions. *Cran* 3: 1–5.

Gei M, Rozendaal DMA, Poorter L, Bongers F, Sprent JI, Garner MD, Aide TM, Andrade JL, Balvanera P, Becknell JM, *et al.* 2018. Legume abundance along successional and rainfall gradients in Neotropical forests. *Nature Ecology and Evolution* 2: 1104–1111.

Groot CCD, Boogaard RVD, Marcelis LFM, Harbinson J, Lambers H. 2003. Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation. *Journal of Experimental Botany* 54: 1957–1967.

Guilbeault-Mayers X, Turner BL, Laliberté E. 2020. Greater root phosphatase activity of tropical trees at low phosphorus despite strong variation among species. *Ecology* 101: 1–9.

Güsewell S. 2004. N:P ratios in terrestrial plants: variation significance. *New Phytologist* 164: 243–266.

Hanbury-Brown AR, Ward RE, Kueppers LM. 2022. Forest regeneration within Earth system models: current process representations and ways forward. *New Phytologist*: 20–40.

Harrell JR, Frank E. 2019. Package 'hmisc'. CRAN2018. 235-6

Hayes P, Turner BL, Lambers H, Laliberté E. 2014. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology* 102: 396–410.

Herre EA, Mejía LC, Kyllo DA, Rojas E, Butler A, Bael S. 2007. Ecological Implications of Anti-Pathogen Effects of Tropical Fungal Endophytes and Mycorrhizae. *Ecology* 88: 550–558.

Hidaka A, Kitayama K. 2011. Allocation of foliar phosphorus fractions and leaf traits of tropical tree species in response to decreased soil phosphorus availability on Mount Kinabalu, Borneo. *Journal of Ecology* 99: 849–857.

Houlton BZ, Wang YP, Vitousek PM, Field CB. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature* 454: 327–330.

Huante P, Rincon E, III FSC. 1995. Responses to Phosphorus of Contrasting Successional Tree-Seedling Species from the Tropical Deciduous Forest of Mexico. *Functional Ecology* 9: 760.

Hunt R, Cornelissen JHC. 1997. Components of relative growth rate and their interrelations in 59 temperate plant species. *New Phytologist* 135: 395–417.

Janos DP, Schroeder MS, Schaffer B, Crane JH. 2001. Inoculation with arbuscular mycorrhizal fungi enhances growth of Litchi chinensis Sonn. trees after propagation by air-layering. *Plant and Soil* 233: 85–94.

Jenny H. 1950. Causes of the high nitrogen and organic matter content of certain tropical forest soils. *Soil Science*, 69: 63-70.

Kaspari M, Powers JS. 2016. Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. *The American Naturalist* 188: S62–S73.

Koenen EJM, Ojeda DI, Steeves R, Migliore J, Bakker FT, Wieringa JJ, Kidner C, Hardy OJ, Pennington RT, Bruneau A, *et al.* 2020. Large-scale genomic sequence data resolve the deepest divergences in the legume phylogeny and support a near-simultaneous evolutionary origin of all six subfamilies. *New Phytologist* 225: 1355–1369.

Koerselman W, Meuleman AFM. 1996. The Vegetation N:P Ratio: A New Tool to Detect the Nature of Nutrient Limitation. *The Journal of Applied Ecology* 33: 1441.

Kong DL, Wang JJ, Kardol P, Wu HF, Zeng H, Deng XB, Deng Y. 2016. Economic strategies of plant absorptive roots vary with root diameter. *Biogeosciences* 13: 415–424.

Koske RE, Gemma JN. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* 92: 486–488.

Laliberté E. 2017. Below-ground frontiers in trait-based plant ecology. *New Phytologist* 213: 1597–1603.

Laliberté E, Lambers H, Burgess TI, Wright SJ. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* 206: 507–521.

Lambers H, Raven JA, Shaver GR, Smith SE. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution* 23: 95–103.

Lawrence D. 2003. The response of tropical tree seedlings to nutrient supply: Meta-analysis for understanding a changing tropical landscape. *Journal of Tropical Ecology* 19: 239–250.

Lovelock CE, Feller IC, Mckee KL, Engelbrecht BMJ, Ball MC. 2004. The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Functional Ecology* 18: 25–33.

Lugli LF, Andersen KM, Aragão LEOC, Cordeiro AL, Cunha HFV, Fuchslueger L, Meir P, Mercado LM, Oblitas E, Quesada CA, *et al.* 2019. Multiple phosphorus acquisition strategies adopted by fine roots in low-fertility soils in Central Amazonia. *Plant and Soil* 450: 49–63.

Lugli LF, Rosa JS, Andersen KM, Ponzio RD, Almeida RV, Pires M, Cordeiro AL, Cunha HFV, Martins NP, Assis RL, *et al.* 2021. Rapid responses of root traits and productivity to phosphorus and cation additions in a tropical lowland forest in Amazonia. New phytologists. 230: 116-128.

Lynch JP, Ho MD. 2005. Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant and Soil* 269: 45–56.

Marklein AR, Houlton BZ. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist* 193: 696–704.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, *et al.* 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* 207: 505–518.

McCormack ML, Guo D, Iversen CM, Chen W, Eissenstat DM, Fernandez CW, Li L, Ma C, Ma Z, Poorter H, *et al.* 2017. Building a better foundation: improving root-trait measurements to understand and model plant and ecosystem processes. *New Phytologist* 215: 27–37.

McCormack ML, Iversen CM. 2019. Physical and Functional Constraints on Viable Belowground Acquisition Strategies. *Frontiers in Plant Science* 10: 1–12.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytologist* 115: 495–501.

McGroddy ME., Daufresne T, Hedin LO. 2004. Scaling of C : N : P Stoichiometry in Forests Worldwide : Implications of Terrestrial Redfield- Type ratios. *Ecology* 85: 2390–2401.

Medvigy D, Wang G, Zhu Q, Riley WJ, Trierweiler AM, Waring BG, Xu X, Powers JS. 2019. Observed variation in soil properties can drive large variation in modelled forest functioning and composition during tropical forest secondary succession. *New Phytologist* 223: 1820–1833.

Messier J, McGill BJ, Lechowicz MJ. 2010. How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters* 13: 838–848.

Murphy PG., Lugo AE. 1986. Ecology of Tropical Dry Forest Author Source : Annual Review of Ecology and Systematics. *Annual Reviews* 17: 67–88.

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC. 2014. Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. *Ecology Letters* 17: 1282–1289.

Nasto MK, Osborne BB, Lekberg Y, Asner GP, Balzotti CS, Porder S, Taylor PG, Townsend AR, Cleveland CC. 2017. Nutrient acquisition, soil phosphorus partitioning and competition among trees in a lowland tropical rain forest. *New Phytologist* 214: 1506–1517.

Nasto MK, Winter K, Turner BL, Cleveland CC. 2019. Nutrient acquisition strategies augment growth in tropical N2-fixing trees in nutrient-poor soil and under elevated CO2. *Ecology* 100: 1–12.

Norby RJ, De Kauwe MG, Domingues TF, Duursma RA, Ellsworth DS, Goll DS, Lapola DM, Luus KA, MacKenzie AR, Medlyn BE, *et al.* 2016. Model–data synthesis for the next generation of forest free-air CO_2 enrichment (FACE) experiments. *New Phytologist* 209: 17–28.

Norby RJ, Gu L, Haworth IC, Jensen AM, Turner BL, Walker AP, Warren JM, Weston DJ, Xu C, Winter K. 2017. Informing models through empirical relationships between foliar phosphorus, nitrogen and photosynthesis across diverse woody species in tropical forests of Panama. *New Phytologist* 215: 1425–1437.

Ostertag R. 2001. Fine-Root Dynamics in Hawaiian Montane Forests. *Ecology* 82: 485–499.

Ostertag R, DiManno NM. 2016. Detecting terrestrial nutrient limitation: A global meta-analysis of foliar nutrient concentrations after fertilization. *Frontiers in Earth Science* 4: 1–14.

Pinheiro J, Bates D, DebRoy S, Sarkar D, Heisterkamp S, Van Willigen B, Maintainer R. 2017. Package 'nlme. Linear and Nonlinear Mixed Effects Models.

Png GK, Lambers H, Kardol P, Turner BL, Wardle DA, Laliberté E. 2019. Biotic and abiotic plant–soil feedback depends on nitrogen-acquisition strategy and shifts during long-term ecosystem development. *Journal of Ecology* 107: 142–153.

Png GK, Turner BL, Albornoz FE, Hayes PE, Lambers H, Laliberté E. 2017. Greater root phosphatase activity in nitrogen-fixing rhizobial but not actinorhizal plants with declining phosphorus availability. *Journal of Ecology* 105: 1246–1255.

Poorter H, Bühler J, Van Dusschoten D, Climent J, Postma JA. 2012. Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology* 39: 839–850.

Powers JS, Becklund KK, Gei MG, Iyengar SB, Meyer R, O'Connell CS, Schilling EM, Smith CM, Waring BG, Werden LK. 2015. Nutrient addition effects on tropical dry forests: a mini-review from microbial to ecosystem scales. *Frontiers in Earth Science* 3: 1–8.

Powers JS, Tiffin P. 2010. Plant functional type classifications in tropical dry forests in Costa Rica: Leaf habit versus taxonomic approaches. *Functional Ecology* 24: 927–936.

R Core Team. 2021. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reed SC, Yang X, Thornton PE. 2015. Incorporating phosphorus cycling into global modeling efforts: A worthwhile, tractable endeavor. *New Phytologist* 208: 324–329.

Rees M, Osborne CP, Woodward FI, Hulme SP, Turnbull LA, Taylor SH. 2010. Partitioning the components of relative growth rate: How important is plant size variation? *American Naturalist* 176: 152–161.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101: 11001–11006.

Ryan MH, Tibbett M, Edmonds-Tibbett T, Suriyagoda LDB, Lambers H, Cawthray GR, Pang J. 2012. Carbon trading for phosphorus gain: The balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell and Environment* 35: 2170–2180.

Santiago LS, Wright SJ, Harms KE, Yavitt JB, Korine C, Garcia MN, Turner BL. 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *Journal of Ecology* 100: 309–316.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.

Seethepalli A, Guo H, Liu X, Griffiths M, Almtarfi H, Li Z, Liu S, Zare A, Fritschi FB, Blancaflor EB, *et al.* 2020. Rhizovision crown: An integrated hardware and software platform for root crown phenotyping. *Plant Phenomics* 2020.

Siqueira JO, Saggin-Júnior OJ. 2001. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza* 11: 245–255.

Smith SE, Read DJ. 2010. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Smith-Martin CM, Gei MG, Bergstrom E, Becklund KK, Becknell JM, Waring BG, Werden LK, Powers JS. 2017. Effects of soil type and light on height growth, biomass partitioning, and nitrogen dynamics on 22 species of tropical dry forest tree seedlings: Comparisons between legumes and nonlegumes. *American Journal of Botany* 104: 399–410.

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG. 1995. Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proceedings of the National Academy of Sciences* 92: 2647–2651.

Soper FM, Nasto MK, Osborne BB, Cleveland CC. 2019. Nitrogen fixation and foliar nitrogen do not predict phosphorus acquisition strategies in tropical trees. *Journal of Ecology* 107: 118–126.

Soper FM, Taylor BN, Winbourne JB, Wong MY, Dynarski KA, Reis CRG, Peoples MB, Cleveland CC, Reed SC, Menge DNL, *et al.* 2021. A roadmap for sampling and scaling biological nitrogen fixation in terrestrial ecosystems. *Methods in Ecology and Evolution* 12: 1122–1137.

Steidinger BS, Turner BL, Corrales A, Dalling JW. 2015. Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. : 121–130.

Taylor BN, Menge DNL. 2018. Light regulates tropical symbiotic nitrogen fixation more strongly than soil nitrogen. *Nature Plants* 4: 655–661.

Taylor BN, Menge DNL. 2021. Light, nitrogen supply, and neighboring plants dictate costs and benefits of nitrogen fixation for seedlings of a tropical nitrogen-fixing tree. *New Phytologist* 231: 1758–1769.

Townsend AR, Asner GP, Cleveland CC. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution* 23: 424–431.

Townsend AR, Cleveland CC, Asner GP, Bustamante MMC. 2007. Controls over foliar N:P ratios in tropical rain forests. *Ecology* 88: 107–118.

Treseder KK, Allen MF. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. *New Phytologist* 155: 507–515.

Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* 82: 946–954.

Turner BL. 2008. Resource partitioning for soil phosphorus: A hypothesis. *Journal of Ecology* 96: 698–702.

Turner BL, Baxter R, Ellwood NTW, Whitton BA. 2001. Characterization of the phosphatase activities of mosses in relation to their environment. *Plant, Cell & Environment* 24: 1165–1176.

Turner BL, Brenes-Arguedas T, Condit R. 2018. Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature*.

Ushio M, Fujiki Y, Hidaka A, Kitayama K. 2015. Linkage of root physiology and morphology as an adaptation to soil phosphorus impoverishment in tropical montane forests. *Functional Ecology* 29: 1235–1245.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB. 2017. A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. *New Phytologist* 215: 1562–1573.

Valverde-Barrantes OJ, Horning AL, Smemo KA, Christopher BB. 2016. Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. *Plant Soil* 57: 613–627.

Valverde-Barrantes OJ, Smemo KA, Blackwood CB. 2015. Fine root morphology is phylogenetically structured, but nitrogen is related to the plant economics spectrum in temperate trees. *Functional Ecology* 29: 796–807.

Vargas GG, Werden LK, Powers JS. 2015. Explaining legume success in tropical dry forests based on seed germination niches: A new hypothesis. *Biotropica* 47: 277–280.

Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible WR, Shane MW, White PJ, *et al.* 2012. Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist* 195: 306–320.

Vitousek PM. 1984. Litterfall, Nutrient Cycling, and Nutrient Limitation in Tropical Forests. *Ecology* 65: 285–298.

Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87–115.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA. 2010. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* 20: 5–15.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15: 1–19.

Wang Y, Houlton BZ, Field CB. 2007. A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production. 21: 1–15.

Waring BG, Pérez-Aviles D, Murray JG, Powers JS. 2019. Plant community responses to stand-level nutrient fertilization in a secondary tropical dry forest. *Ecology* 100: 1–12.

Werden LK, Becknell JM, Powers JS. 2018. Edaphic factors, successional status and functional traits drive habitat associations of trees in naturally regenerating tropical dry forests. *Functional Ecology* 32: 2766–2776.

Werden LK, Calderón-Morales E, Alvarado J. P, Gutiérrez L. M, Nedveck DA, Powers JS. 2020. Using large-scale tropical dry forest restoration to test successional theory. *Ecological Applications* 30.

Wright SJ. 2019. Plant responses to nutrient addition experiments conducted in tropical forests. *Ecological Monographs* 89: 1–18.

Wurzburger N, Hedin LO. 2016. Taxonomic identity determines N2 fixation by canopy trees across lowland tropical forests. *Ecology Letters* 19: 62–70.

Wurzburger N, Wright SJ. 2015. Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. *Ecology* 96: 2137–2146.

Yadav R, Tarafdar J. 2001. Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biology and Fertility of Soils* 34: 140–143.

Zalamea PC, Turner BL, Winter K, Jones FA, Sarmiento C, Dalling JW. 2016. Seedling growth responses to phosphorus reflect adult distribution patterns of tropical trees. *New Phytologist* 212: 400–408.

Zangaro W, Nishidate FR, Regina F, Camargo S, Romagnoli GG, Vandressen J. 2005. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. *Journal of Tropical Ecology* 21: 529–540.

Zemunik G, Turner BL, Lambers H, Laliberté E. 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* 1: 1–4.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Distribution of the most common 82 tree species found in TDFs in Costa Rica across a fertility gradient.

Fig. S2 Distribution of the most common 82 tree species found in TDFs in Costa Rica across a successional gradient.

Fig. S3 Specific leaf area (SLA) of four N-fixing and seven non-N-fixing species of seedlings across phosphorus treatments.

Fig S4. Nodule mass fraction (NMF) across phosphorus treatments of *Dalbergia retusa*, *Gliricidia sepium*, *Lonchocarpus rugosus*, *Lonchocarpus felipei*.

Fig. S5 Relative growth rate (RGR) of species across phosphorus treatments.

Fig. S6 Total dry biomass of species across phosphorus treatments.

Fig. S7 Leaf mass fraction (LMF) and stem mass fraction (SMF) of four N-fixing and seven non-N-fixing species of seedlings across phosphorus treatments.

Fig. S8 Maximum photosynthetic rate (A_{max}) of species across phosphorus treatments.

Fig. S9 Water use efficiency as $\delta^{13}C$ of species across phosphorus treatments.

Fig. S10 Instantaneous water use efficiency (WUE_i) of four N-fixing and seven non-N-fixing species across phosphorus treatments.

Fig. S11 Foliar P of species across phosphorus treatments.

Fig. S12 Foliar N of species across phosphorus treatments.

Fig. S13 Foliar N to P ratios of species across phosphorus treatments.

Fig. S14 Root mass fraction (RMF) of species across phosphorus treatments.

Fig. S15 Root phosphatase activity (RPA) of species across phosphorus treatments.

Fig. S16 Boxplots showing the specific root length (SRL) of species across phosphorus treatments.

Fig. S17 Arbuscular mycorrhizal colonization (AMC) of species across phosphorus treatments.

Methods S1 Focal and regional species distribution.

Methods S2 Root phosphatase activity.

Methods S3 Arbuscular mycorrhizal Colonization.

Methods S4 Data analysis of nitrogen fixation proxies.

Methods S5 Equations used for the data analysis.

Table S1 P-values from t-test comparing the distribution across successional and fertility gradients of the 82 most common tree species found in TDF in Costa Rica.

Table S2 List of species planted with functional trait information.

Table S3 P-Chi-squared values from a one-way analysis of variance of biomass fraction traits and arbuscular mycorrhizal colonization.

Table S4 F-values from a one-way analysis of variance of nodule mass fraction and nodule dry mass.

Table S5 Mean soil pH and P fraction values.

Table S6 -values from a way analysis of variance of plant traits.

Table S7 F-values from way analysis of variance of foliar nutrient.

Fig. 1 Comparison of biomass and growth responses among N-fixing and non-fixing seedling to three levels of P. Reported results come from a one-way analysis of variance for each functional trait. a) Relative growth rate (RGR). b) Total dry biomass. c) Maximum photosynthetic rate (A_{max}). d) Water use efficiency $\delta^{13}C$ (a proxy for water use efficiency) of four N-fixing and seven non-N-fixing species of seedlings across control (P0), low (P+), and high (P++) phosphorus treatments. Green bars represent N-fixing species and gray bars represent non-N-fixing species. Bar heights represent the marginal means extracted from the linear mixed models and error bars represent the standard error. Capital letters represent significant differences between functional types while lower case letters represent differences among treatments within a functional type.

Fig. 2 Comparison of foliar and photosynthetic P-use efficiency responses of N-fixing and non-fixing seedling to three levels of P. Reported results come from a one-way analysis of variance for each functional trait. a) Foliar P. b) Foliar N. c) Foliar N:P, dashed lines indicate P (blue) and N limitation (red) thresholds based on Reich & Oleksyn (2004). d) Photosynthetic P use efficiency (PPUE) of four N-fixing and seven non-N-fixing species of seedlings across control (P0), low (P+), and high (P++) phosphorus treatments. Green bars represent N-fixing species and gray bars non-N-fixing species. Bar heights represent the marginal means extracted from the linear mixed models and error bars represent the standard error. Capital letters represent differences between functional types while lower case letters represent differences within a functional type.

Fig. 3 Comparison of phosphorus acquisition strategies responses of N-fixing and non-fixing seedling to three levels of P. Reported results come from a one-way analysis of variance for each functional trait. a) Root mass fraction (RMF). b) Root phosphatase activity. c) Specific root length (SRL). d) Arbuscular mycorrhizal colonization (AMC) of four N-fixing and seven non-N-fixing species of seedlings across control (P0), low (P+), and high (P++) phosphorus treatments. Green bars represent N-fixing species and gray bare non-N-fixing species. AMC was similar for both functional types. Bar heights represent the marginal means extracted from the linear mixed models for the root phosphatase activity. The raw data means were used for the other three variables since the model outputs were not biological meaningful. Error bars represent the standard error. Capital letters represent differences between functional types while lower case letters represent differences within a functional type.

Fig. 4 Stacked bar plot of variance partitioning for each trait across four nested sources of variation. To determine variance partitioning we used a nested linear model for each trait (Messier et al., 2010, 2017). For this approach we used the same trait sampling scheme as our classification of variation sources: Treatment, Functional type, Species, and Individual. The within factor explains the variation that cannot be explained by the model. Plant functional traits defined as: TDB - total dry biomass, RGR - relative growth rate, A_{max} - maximum photosynthetic rate, $\delta^{13}C$ – proxy for water use efficiency, SRL - specific root length, RMF - root mass fraction, RPA - root phosphatase activity, %AMC - arbuscular mycorrhizal colonization, Foliar P, Foliar N, Foliar N:P, and PPUE – photosynthetic phosphorus use efficiency.

Fig. 5 Pearson correlation coefficients for pairwise relationships among foliar nutrient content (a metric of nutrient demand) and nutrient acquisition traits for a) non-fixing species b) N-fixing species. Significant relationships are denoted with an asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Only significant correlations are represented. Nutrient acquisition traits defined as: RMF - root mass fraction, SRL - specific root length, RPA - root phosphatase activity, AMC – arbuscular mycorrhizal colonization.

Tables

Table 1. F-values from linear mixed models with species as a random effect, and functional type (N-fixer or non-N-fixer) and treatment (control (P0), low (P+), and high (P++) phosphorus) as fixed effects.

Response	Functional type	Treatment	Functional type x Treatment
Relative growth rate	0.0001	**13.87*** **	**6.68*** **
Total dry biomass	**17.11*** **	**6.98***	**13.09*** **
A_{max}	**5.37***	**3.99***	1.24
Vc_{max}	**5.87****	1.27	1.42
$\delta^{13}C$	0.44	**5.38****	**3.35***
Root phosphatase activity	**12.89****	0.58	0.65
Specific root length	**26.93***	0.46	0.16
Foliar P	0.71	**15.28*** **	**5.99****
Foliar N	**55.05*** **	1.19	0.86
Foliar N:P	**34.99*** **	**70.49*** **	**19.87*** **
PPUE	0.56	**3.57***	**3.27***

Statistically significant results are shown in boldface type (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). A_{max} = maximum photosynthetic rate. Vc_{max} = maximum rate of rubisco carboxylase activity. PPUE = Photosynthetic phosphorus use efficiency.

Table 2. Chi-squared values from linear mixed models with a beta distribution with species as a random effect, and functional type (N-fixer or non-N-fixer) and treatment (control (P0), low (P+), and high (P++) phosphorus) as fixed effects.

Response	Functional type	Treatment	Functional type x Treatment
%AMC	3.48	3.30	0.33
Root mass fraction	1.62	3.01	**7.22***
Stem mass fraction	2.98	**6.88***	2.44
Leaf mass fraction	0.18	0.085	3.20

Statistically significant results are shown in boldface type (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). % AMC = arbuscular mycorrhizal colonization.

Figures

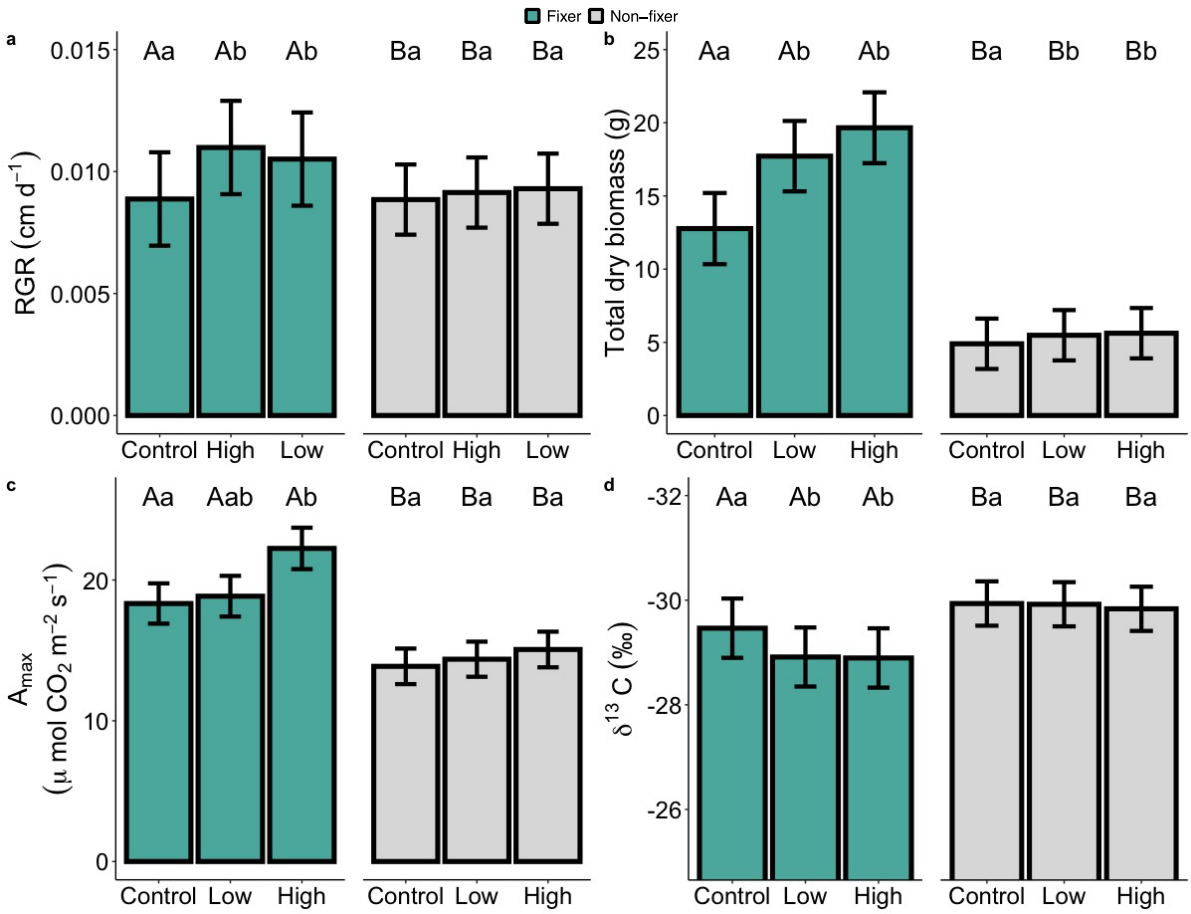

Fig. 1

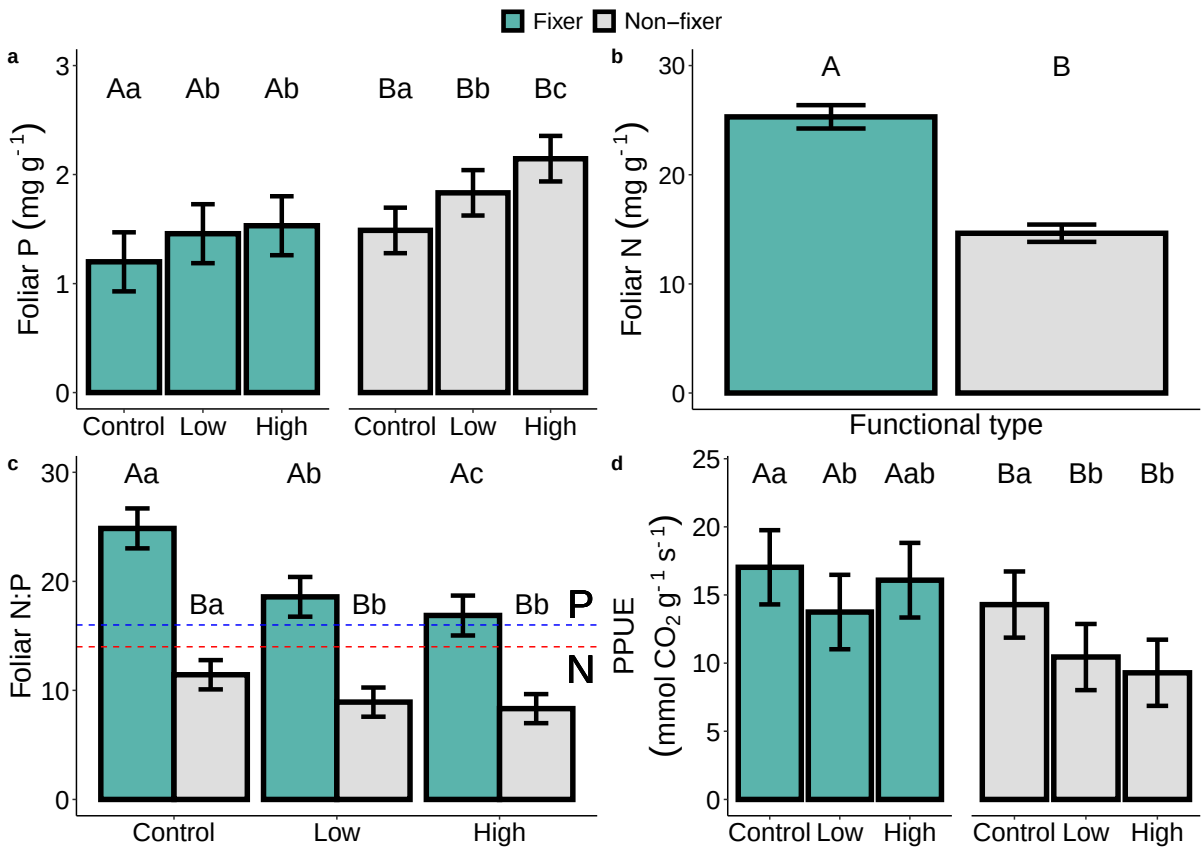

Fig. 2

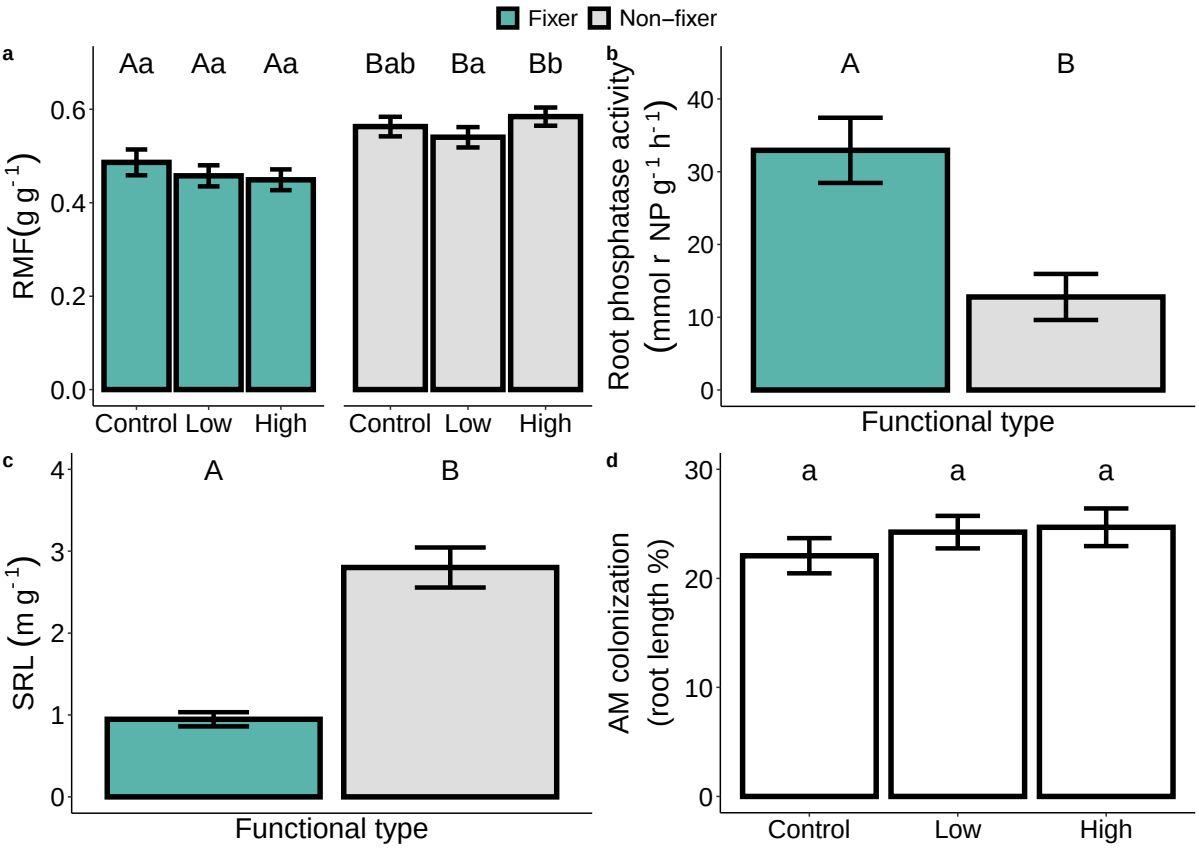

Fig. 3

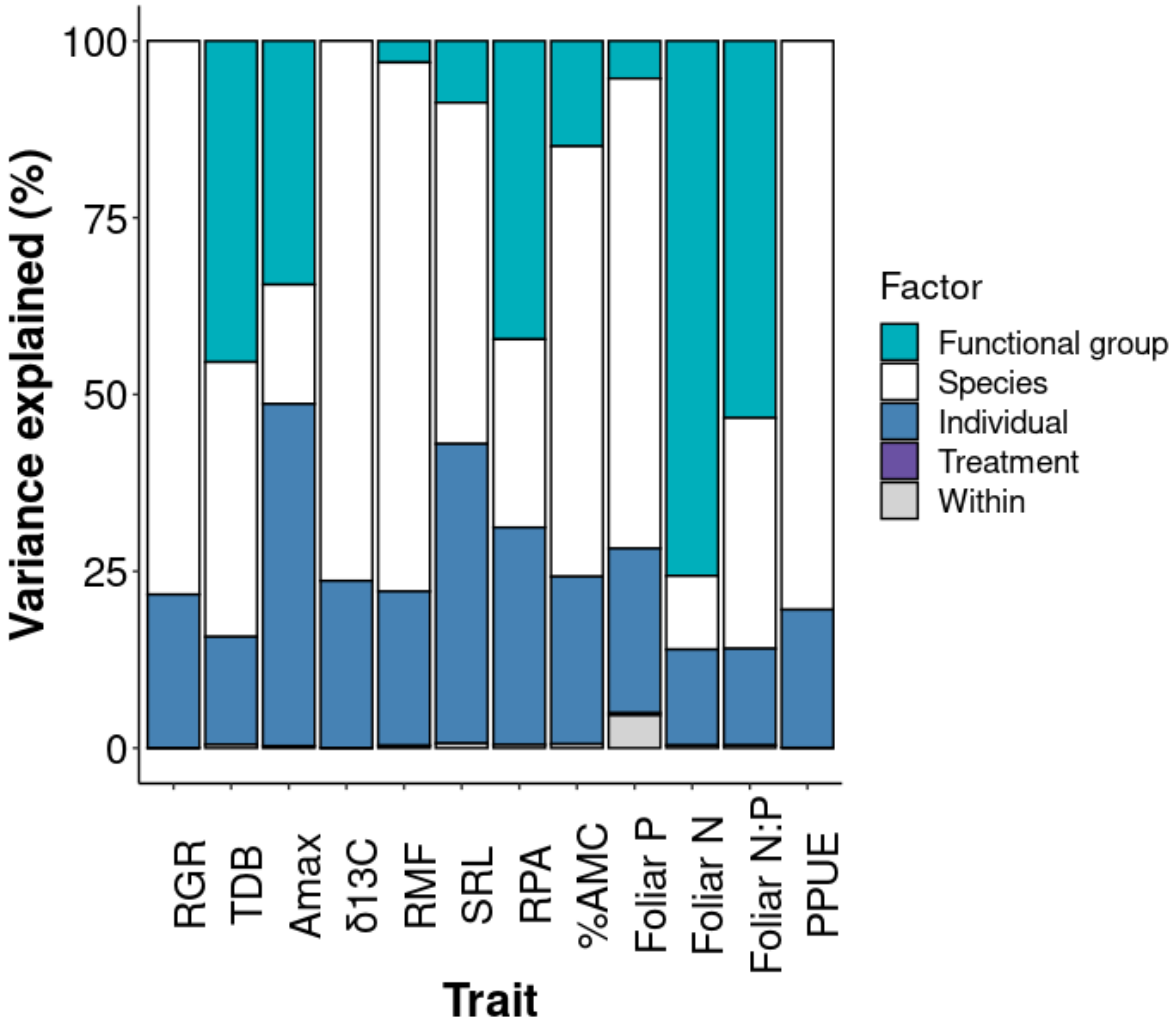

Fig. 4

a. Non-fixing species **b. N-fixing species**

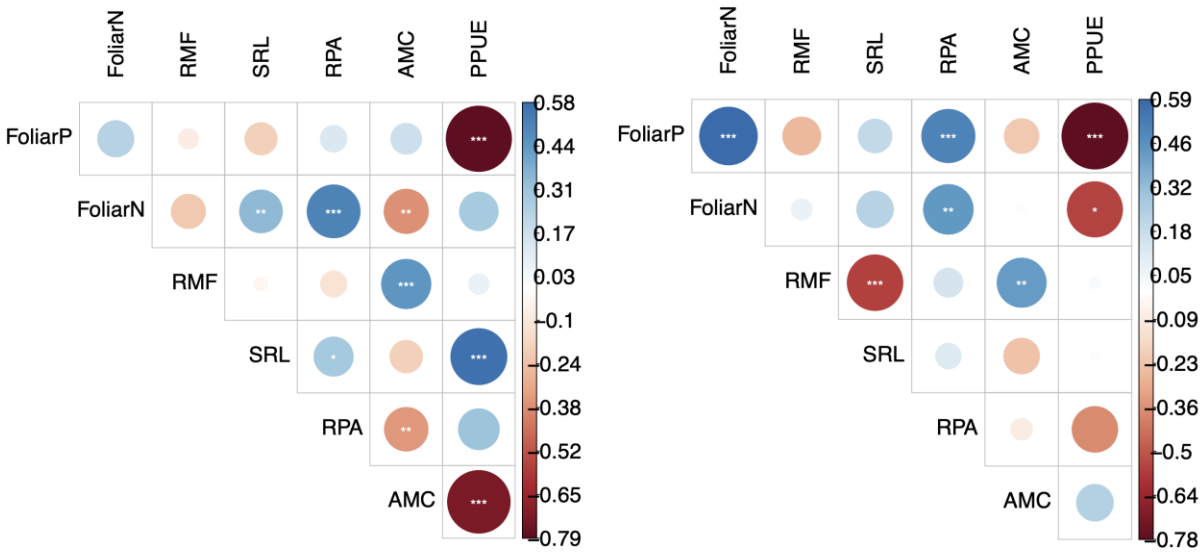

Fig. 5