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Research

Nutrient acquisition, soil phosphorus partitioning and competition among trees in a lowland tropical rain forest

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Summary

- We hypothesized that dinitrogen (N_2)- and non-N_2-fixing tropical trees would have distinct phosphorus (P) acquisition strategies allowing them to exploit different P sources, reducing competition.
- We measured root phosphatase activity and arbuscular mycorrhizal (AM) colonization among two N_2- and two non-N_2-fixing seedlings, and grew them alone and in competition with different inorganic and organic P forms to assess potential P partitioning.
- We found an inverse relationship between root phosphatase activity and AM colonization in field-collected seedlings, indicative of a trade-off in P acquisition strategies. This correlated with the predominantly exploited P sources in the seedling experiment: the N_2 fixer with high N_2 fixation and root phosphatase activity grew best on organic P, whereas the poor N_2 fixer and the two non-N_2 fixers with high AM colonization grew best on inorganic P. When grown in competition, however, AM colonization, root phosphatase activity and N_2 fixation increased in the N_2 fixers, allowing them to outcompete the non-N_2 fixers regardless of P source.
- Our results indicate that some tropical trees have the capacity to partition soil P, but this does not eliminate interspecific competition. Rather, enhanced P and N acquisition strategies may increase the competitive ability of N_2 fixers relative to non-N_2 fixers.

Introduction

Soil nutrient availability strongly influences patterns of plant productivity, species distribution and community composition in tropical forests (Gentry, 1988; Clinebell *et al.*, 1995; Palmiotto *et al.*, 2004; John *et al.*, 2007; Condit *et al.*, 2013). Although nitrogen (N) is presumed to cycle in excess of demand in many tropical forests (e.g. Hedin *et al.*, 2009), phosphorus (P) is often thought to limit many tropical ecosystem processes (Vitousek, 1984; Cleveland *et al.*, 2011; Condit *et al.*, 2013). Yet, somewhat paradoxically, lowland tropical rain forests contain a disproportionate number of trees (Fabaceae) capable of symbiotic dinitrogen (N_2) fixation (Ter Steege *et al.*, 2006) – a plant–microbial mutualism that enables plants to convert atmospheric N_2 into biologically available forms – compared with temperate and high-latitude forests (Rundel, 1989; Menge *et al.*, 2014; Menge & Crews, 2016). However, N_2 fixation is an energetically expensive process and requires significant carbon (C) and P investments from host plants to support their microbial symbionts (Vitousek & Howarth, 1991). Thus, from a resource economics perspective, N_2-fixing trees should be at a competitive disadvantage and less abundant in the N-rich, P-poor rain forests common throughout the lowland tropics compared with the N-poor, P-rich temperate and high-latitude forests. This disparity between theory and observation highlights our incomplete understanding of how soil nutrients influence plant performance across the tropics.

Previous attempts to identify mechanisms that may promote relatively high abundances of N_2-fixing trees in lowland tropical rain forests have pointed to sizeable and rapid N losses via leaching and/or denitrification (Hedin *et al.*, 2009), the facultative nature of the N_2 fixation strategy (Menge *et al.*, 2009, 2014), evolutionary history (Sheffer *et al.*, 2015), higher survival rates of N_2-fixing trees relative to other trees (Menge & Chazdon, 2016) or the advantage of N_2-fixing trees over non-N_2-fixing trees in the acquisition of soil P (Houlton *et al.*, 2008). For example, N_2-fixing trees often show high rates of phosphatase activity, either on or around their roots. Thus, it is possible that N_2-fixing trees can more readily invest in these N-rich enzymes and mineralize more P from ester-bound forms to release phosphate (PO_4^{3-}) ions before plant uptake (McGill & Cole, 1981; Olander & Vitousek, 2000; Treseder & Vitousek, 2001; Allison, 2005; Houlton *et al.*, 2008; Marklein & Houlton, 2011). Alternatively, actively N_2-fixing trees may up-regulate photosynthesis (Harris *et al.*, 1985;

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Jia *et al.*, 2004), which could allow for relatively greater investment in arbuscular mycorrhizal (AM) fungi than in non-N_2-fixing trees to scavenge distant pools of labile PO_4^{3-} ions, or access other forms of P (e.g. crystalline-bound P via the production of chelating compounds; Marschner, 1995; Comerford, 1998; Smith & Read, 2008). If N_2-fixing trees have higher rates of photosynthesis, this could provide them with an additional advantage over non-N_2-fixing trees in the acquisition of soil P.

In a previous study that simultaneously measured and compared AM colonization and root phosphatase activity between nodulated and non-nodulated tree roots in a lowland tropical rain forest, Nasto *et al.* (2014) showed that, although both P acquisition strategies were indeed higher on nodulated than non-nodulated roots, the difference in AM colonization between the two root types was much greater than the more subtle differences in root phosphatase activity. For example, AM colonization on nodulated roots was 124% greater than that on non-nodulated roots, whereas root phosphatase activity was only 28% greater for nodulated than for non-nodulated roots (Nasto *et al.*, 2014). Furthermore, previous research has suggested that relatively small increases in AM colonization can yield relatively large increases in P uptake capacity, whereas relatively large increases in phosphatase enzyme activity are needed to yield similar rates of P uptake (Treseder & Vitousek, 2001). If these observations apply broadly to tropical N_2-fixing and non-N_2-fixing trees, it would imply that N_2-fixing trees may disproportionately rely on AM fungi to acquire inorganic P, whereas non-N_2-fixing trees may rely more strongly on organic P mineralized via enzymes. Thus, instead of strongly competing for soil P, N_2-fixing and non-N_2-fixing trees may effectively partition soil P by predominantly exploiting different chemical P compounds (Turner, 2008). Such soil P partitioning could facilitate the coexistence of N_2-fixing and non-N_2-fixing trees via the formation of complementary soil niches – a phenomenon that has been documented for N in a variety of temperate ecosystems (McKane *et al.*, 2002; Weigelt *et al.*, 2005; Kahmen *et al.*, 2006; Oelmann *et al.*, 2007) and recently for P in a montane tropical ecosystem (Steidinger *et al.*, 2014).

Here, we used an observational study in a lowland tropical rain forest in Costa Rica to examine whether soil P acquisition strategies (i.e. root phosphatase activity and AM colonization) vary among seedlings of two N_2-fixing and two non-N_2-fixing tree species. In addition, we performed a potted seedling experiment to assess whether N_2-fixing trees and non-N_2-fixing trees may avoid strong competition by exploiting different chemical P compounds. To do this, we grew the same N_2-fixing and non-N_2-fixing species used in the observational study alone and in competition (i.e. one N_2-fixing and one non-N_2-fixing seedling together) and provided them with different forms of soil P. Next, we determined whether seedling growth (i.e. relative growth rate (RGR) and change in leaf area) and foliar [P] among seedlings responded differently to the chemical P compounds when grown alone. Finally, we assessed whether the response ratios (i.e. seedlings in competition relative to alone) of RGR (competitive response), foliar [P], P acquisition strategies and N_2 fixation among seedlings responded differently to the chemical P compounds when grown in competition. Given the difference in AM colonization relative to the difference in root phosphatase activity between nodulated and non-nodulated roots observed previously (Nasto *et al.*, 2014), we hypothesized that N_2-fixing and non-N_2-fixing trees may have somewhat unique and dominant P acquisition strategies, allowing them to exploit, and perform better on, different chemical P compounds. Specifically, we hypothesized that the N_2-fixing seedlings would predominantly exploit inorganic P via AM fungi, whereas the non-N_2-fixing seedlings would predominantly exploit organic P via phosphatase enzymes. If so, P partitioning could reduce competition for P.

Materials and Methods

Study site

We conducted this study in a mature lowland tropical rain forest near the Piro Biological Station on the Osa Peninsula, southwestern Costa Rica (8°24′42″N, 83°19′00″W). The mean annual temperature is *c.* 26°C and the mean annual precipitation is *c.* 3500 mm (Keller *et al.*, 2013). The region experiences a dry season from December through April with heavy rains common throughout the rest of the year. The forest has high biomass with a species-rich assemblage of large-statured canopy trees (mean canopy height of 45 m; Taylor *et al.*, 2015). Putative N_2-fixing tree species are common in the forest and represent 5–13% of the total tree abundance (Sullivan *et al.*, 2014). The soils are classified as highly weathered, nutrient-poor Ultisols (Berrange & Thorpe, 1988) derived from Pliocene greywacke-type marine and continental conglomerates, sandstones and siltstones (Buchs *et al.*, 2009; Gardner *et al.*, 2013). Surface soil (0–10 cm) has a pH in water of 5.7 (Osborne *et al.*, 2017), an inorganic N concentration (ammonium + nitrate) of 5.5 mg kg^{-1} and an extractable P concentration of 1.2 mg kg^{-1} (Nasto *et al.*, 2014).

Observational study

We used two N_2-fixing (*Inga marginata* Willd. and *Inga thibaudiana* DC.) and two non-N_2-fixing (*Brosimum utile* Kunth Oken and *Tapirira guianensis* Aubl) tree species common in Neotropical lowland rain forests for both the observational and experimental studies (Supporting Information Table S1). In May 2015, 10 seedlings of each species were harvested from the same cohort (< 1 yr old). After harvesting, roots were excised and stored at 5°C in 20-ml scintillation vials containing 50 mM calcium sulfate ($CaSO_4$) solution to maintain their structural integrity and reduce hypo-osmotic conditions for future root phosphatase activity and AM colonization analyses. In addition, two to three nodules were excised from each N_2-fixing seedling and placed in acrylic tubes for the acetylene reduction assay (ARA) to approximate the N_2 fixation rates.

We measured root phosphatase activity using a 4-methylumbelliferone (4-MUB)-linked substrate adopted from Sinsabaugh *et al.* (2003). Briefly, roots were gently washed with 50 mM $CaSO_4$ to remove any attached soil particles.

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Subsequently, 20–30 mg of roots 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 4-MUB solution (200 μl), and 1 ml of 50 mM sodium acetate buffer (800 μl)/100 mM 4-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 h at 110 rpm and 200-μl subsamples from each well were pipetted 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 root phosphatase activities were calculated as μmol 4-MUB-P g^{-1} root h^{-1}. Assays were performed within 1 wk of sampling.

We measured AM colonization by staining roots with trypan blue (Koske & Gemma, 1989). Roots were cleared in a 10% potassium hydroxide solution for 3 d, rinsed with tap water and placed in a 3% hydrogen chloride (HCl) solution for 12 h for acidification. The HCl solution was replaced with trypan blue for 24 h and the roots were de-stained in water for 12 h. Ten cleared and uniformly stained root pieces per seedling were mounted on slides and root colonization was quantified using the magnified intersection method (McGonigle *et al.*, 1990) on *c.* 50 intersections. Colonization is reported as the percentage root length colonized by AM fungi.

We measured N_2 fixation on the N_2-fixing seedlings using the ARA method (Hardy *et al.*, 1968; Sullivan *et al.*, 2014) by the excision of nodules from N_2 fixer roots and incubation in a 50-ml clear acrylic tube for 1 h with a 10% acetylene atmosphere. After incubation, 14-ml headspace samples were removed from the tubes with a syringe, injected into 10-ml glass vials and returned to the laboratory for analysis by gas chromatography using a Shimadzu GC-2014 equipped with a flame ionization detector (Shimadzu Inc., Kyoto, Japan). Ethylene produced from the nodules without acetylene exposure, ethylene produced from tubes and glass vials, ethylene within our acetylene and ethylene lost as a result of photodegradation during transport were accounted for. To convert acetylene reduction rates into N_2 fixation rates, an ethylene : N conversion ratio of 2.8 : 1 that was previously generated from this site was applied (Sullivan *et al.*, 2014).

Experimental study

Concurrent with the observational study, we harvested an additional 100 seedlings of each species from the same cohort and gently washed them with water to remove any attached soil particles. We transplanted 50 seedlings of each species alone into 1-l pots. Given the large number of chemical P compounds included in our study, a full-factorial experiment in which all species competed against each other was not possible. However, we randomly chose to also transplant 50 seedlings of *I. marginata* alongside *T. guianensis* and *I. thibaudiana* alongside *B. utile* into 1-l pots so that each contained one competing pair. None of the seedlings of the N_2-fixing species had nodules at the time of transplant. All pots were filled with acid-washed sand and native soil in a sand : soil ratio of 85 : 15. The individual seedlings and competing pairs were assigned to one of five treatments ($n = 10$). The treatments differed in the form of P, and included: (1) PO_4^{3-} (inorganic orthophosphate); (2) phosphorite (crystalline phosphate); (3) glucose PO_4^{3-} (a simple phosphomonester); (4) phytate (sodium salt of *myo*-inositol hexakisphosphate); and (5) a P-free control (Table S2). These treatments represent a continuum of P acquisition costs (Turner, 2008; Steidinger *et al.*, 2014) and a hypothetical divide between the forms of P that are commonly acquired by AM fungi (inorganic: PO_4^{3-} and phosphorite) versus P mineralizing enzymes (organic: glucose PO_4^{3-} and phytate). All individual seedlings and competing pairs were grown in an open-air glasshouse under full natural light conditions to reduce potential C limitation to their symbiotic partners (i.e. N_2-fixing bacteria and AM fungi). Initial stem height and number of leaves for each seedling were recorded for use as potential covariates (Steidinger *et al.*, 2014). Five of the 10 seedlings of each species from the observational study were used to estimate initial seedling mass, leaf area and foliar [P] (Table 1).

All pots were fertilized every other day with 30 ml of a solution containing the appropriate chemical P compound (with the exception of the no-P control and the phosphorite treatment, which was added to the appropriate pots in a 5-g batch at the beginning of the experiment) and all other essential nutrients (Table S2). By the time of harvest, the PO_4^{3-}-, glucose PO_4^{3-}- and phytate-treated pots had each received a total of 40 mg of P in addition to the 0.18 mg of available P in the sand : soil matrix. Every 3 d, all pots were watered with 30 ml of rainwater collected adjacent to the glasshouse.

All of the seedlings were harvested once signs of P deficiency were qualitatively detected (*c.* 3 months). After harvesting, a suite of seedling growth and nutrient acquisition metrics was measured. First, leaf area was measured by taking photographs and importing them into ImageJ (Schneider *et al.*, 2012). The change in leaf area was calculated as the difference between the initial and final leaf area. Second, nodules from the N_2-fixing seedlings were excised for immediate N_2 fixation analysis using ARA. Third, roots were excised and stored for root phosphatase activity and AM colonization analyses, which were performed within 1 wk of sampling. Fourth, plants were dried at 70°C for 48 h and weighed to determine total (aboveground and belowground) dry biomass. Lastly, foliar [P] was measured by ignition (500°C, 2 h) and acid digestion (1 M HCl), with phosphate detection by

Table 1 Mean values (standard errors) of all variables from the initial cohort of the four tropical tree species ($n = 5$)

Species	Functional group	Mass (g)	Leaf area (cm^2)	Foliar [P] ($mg\ g^{-1}$)
Inga marginata	N_2 fixer	0.15 (0.01) a	5.65 (0.23) a	0.41 (0.05)
I. thibaudiana	N_2 fixer	0.20 (0.03) a	6.75 (0.28) a	0.49 (0.03)
Brosimum utile	Non-N_2 fixer	1.04 (0.08) b	30.87 (1.75) b	0.39 (0.01)
Tapirira guianensis	Non-N_2 fixer	0.54 (0.04) c	15.09 (0.41) c	0.51 (0.17)

Different letters indicate significant differences among species ($P < 0.05$).

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molybdate colorimetry using a Synergy 2 Microplate Reader (Biotek, Winooski, VT, USA).

Plant dry biomass was used to calculate RGR,

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

where M_f is the final mass, M_i is the initial mass and dt is the duration of the experiment in days. Then, RGR was used to calculate the competitive response,

$$\text{Competitive response} = \frac{\mathrm{RGR_c}}{\mathrm{RGR_a}}$$

where $\mathrm{RGR_c}$ is RGR in competition and $\mathrm{RGR_a}$ is RGR alone. Response ratios of foliar [P], root phosphatase activity, AM colonization and N_2 fixation were calculated in a similar manner. The $\log_{10}$ of all responses was taken: positive values represent an increase in competitive response, foliar [P] and nutrient acquisition strategy when seedlings were grown in competition relative to alone, whereas negative values represent a decrease when seedlings were grown in competition relative to alone.

Statistical analyses

Before statistical analyses, data were tested for normality using the Shapiro–Wilk test and homoscedasticity using the Levene test, and all data that did not fit the assumptions were log transformed. For the observational study, nested ANOVA with Fisher's least significant difference *post-hoc* comparisons was used to determine differences in root phosphatase activity and AM colonization between functional groups and among species. In addition, a two-sample *t*-test was used to assess differences in N_2 fixation between the N_2-fixing species. Also, simple linear regression and a calculated Pearson correlation coefficient were used to determine a relationship between root phosphatase activity and AM colonization. Lastly, nested ANOVA with Fisher's least significant difference *post-hoc* comparisons was used to determine differences in initial mass, leaf area and foliar [P] between functional groups and among species before the start of the seedling experiment.

When seedlings were grown alone, nested ANCOVA with functional group/species and treatment as model variables, and initial height and number of leaves as covariates, was used to determine differences in RGR, change in leaf area and foliar [P] between functional groups and among species. For each species × dependent variable combination, linear models were used to assess the significance of treatment responses relative to the no-P control. Lastly, simple linear regressions and Pearson correlation coefficients were used to determine relationships between variables. When seedlings were grown in competition, nested ANCOVA with functional group/species and treatment as model variables, and initial height and number of leaves as covariates, was used to determine differences in competitive response and the response ratios of foliar [P], P acquisition strategies and N_2 fixation between functional groups and among species. In addition, single-sample *t*-tests were used to determine whether competitive responses and response ratios were different from zero. All statistical analyses were performed using the open-source R software v.2.15.3 (R Development Core Team, 2015). For all data, significance was determined when $P < 0.05$.

Results

Observational study

Overall, there were differences in root phosphatase activity and AM colonization between functional groups ($P < 0.001$ and $P < 0.001$, respectively) and among species ($P = 0.001$ and $P = 0.10$, respectively; Fig. 1). By functional group, the N_2-fixing seedlings had higher root phosphatase activity (Fig. 1a) and lower AM colonization than the non-N_2-fixing seedlings (Fig. 1c). Among species, *I. marginata* had the highest root phosphatase activity (Fig. 1b) and the lowest AM colonization (Fig. 1d), whereas *B. utile* had the lowest root phosphatase activity (Fig. 1b) and the highest AM colonization (Fig. 1d). The N_2-fixing *I. thibaudiana* and non-N_2-fixing *T. guianensis* had similar and intermediate root phosphatase activities (Fig. 1b) and AM colonization (Fig. 1d). In addition, root phosphatase activity and AM colonization were negatively correlated among seedlings across the four species ($P = 0.03$, $r = 0.35$; Fig. 2). Lastly, and between the two N_2-fixing species, *I. marginata* had higher N_2 fixation rates than *I. thibaudiana* ($P = 0.05$; Fig. 1e).

Experimental studies

At the time of transplant, there were differences in initial mass and leaf area between functional groups ($P < 0.001$ and $P < 0.001$, respectively) and among species ($P < 0.001$ and $P < 0.001$, respectively; Table 1). By functional group, the non-N_2-fixing seedlings had greater mass and leaf area than the N_2-fixing seedlings. Among species, *B. utile* had greater mass and leaf area than *T. guianensis*, whereas the two N_2-fixing species had the lowest and were not different from each other. Foliar [P] did not vary between functional groups ($P = 0.97$) or among species ($P = 0.55$).

When grown alone, seedling growth and foliar [P] responded to the chemical P compounds in a variety of ways (Table S3), but of most importance were the species × treatment interactions (Fig. 3). First, the N_2-fixing *I. marginata* had higher RGR, greater change in leaf area and higher foliar [P] with additions of glucose PO_4^{3-} (Fig. 3). Second, the N_2-fixing *I. thibaudiana* had higher RGR and greater change in leaf area with additions of inorganic PO_4^{3-}, but foliar [P] did not differ with the addition of different chemical P compounds (Fig. 3). Third, the non-N_2-fixing *B. utile* had higher RGR and foliar [P] with additions of inorganic PO_4^{3-} and phosphorite, and greater change in leaf area with additions of inorganic PO_4^{3-} only (Fig. 3). Lastly, the non-N_2-fixing *T. guianensis* had higher foliar [P] with additions of phosphorite, but RGR and change in leaf area did not differ with the addition of different chemical P compounds (Fig. 3).

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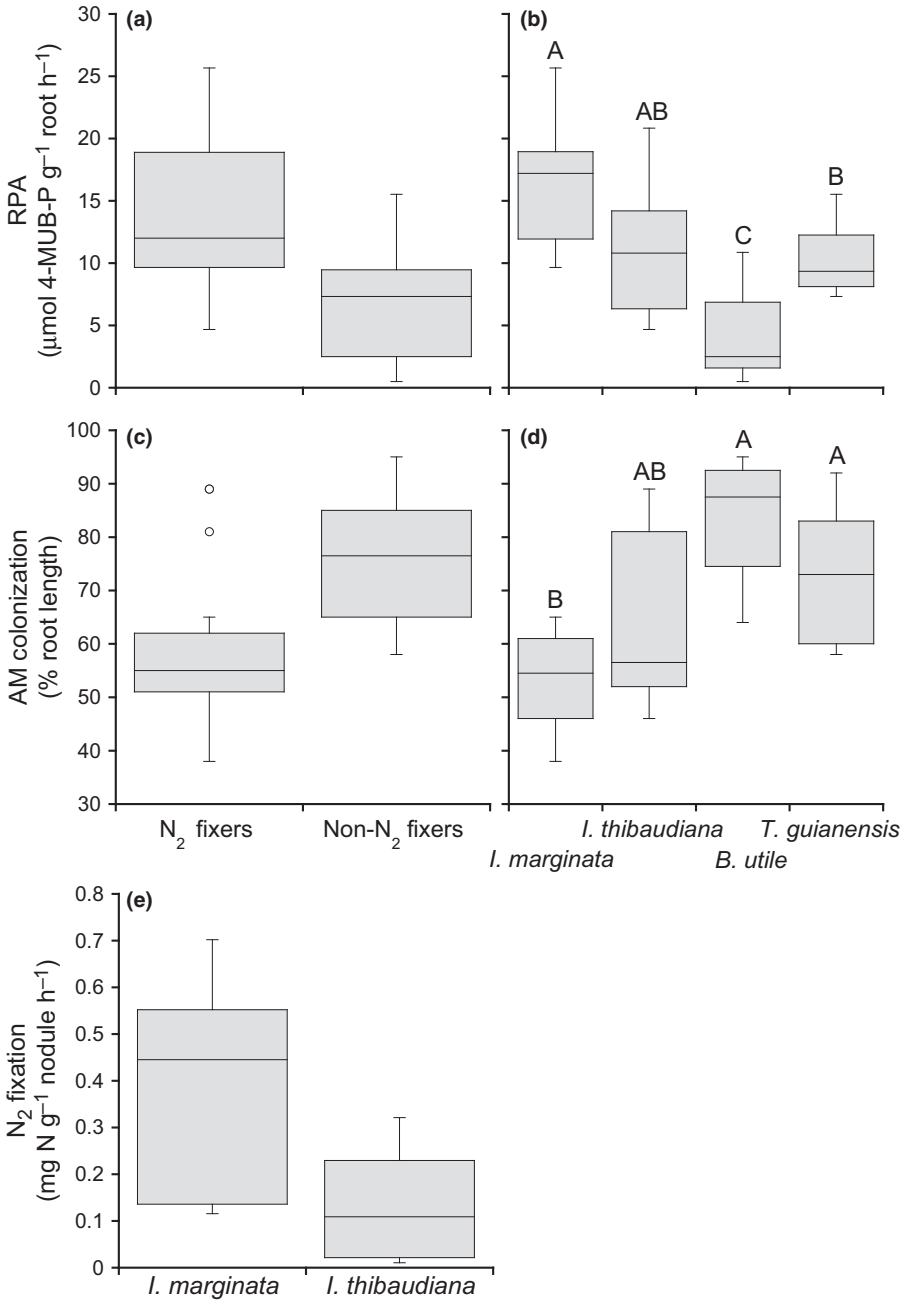

Fig. 1 Boxplots of root phosphatase activity (RPA) expressed as µmoles of 4-MUB-P per gram root mass and hour between functional groups (a) and among species (b), arbuscular mycorrhizal (AM) colonization expressed as the percentage root length between functional groups (c) and among species (d), and N_2 fixation expressed as milligrams of N per gram nodule and hour between the two N_2-fixing species (e) from the observational study. The two N_2-fixing tree species are *Inga marginata* and *I. thibaudiana*, and the two non-N_2-fixing tree species are *Brosimum utile* and *Tapirira guianensis*. Boxplot lines represent medians, boxes represent quartile values and points represent outliers. Different letters in (b) and (d) indicate differences among species ($P < 0.05$). 4-MUB, 4-methylumbelliferone.

Root phosphatase activity, AM colonization and N_2 fixation also responded to the chemical P compounds in a variety of ways when the seedlings were grown alone (Table S3). However, of most importance was how the P acquisition strategies related to RGR among seedlings across species (Fig. 4), as well as N_2 fixation rates among seedlings across the N_2-fixing species (Fig. 5). The ratio of AM colonization to root phosphatase activity was negatively correlated with the RGR of the N_2-fixing *I. marginata* ($P < 0.001$, $r = 0.63$; Fig. 4a), whereas the ratio was positively correlated with the RGR of the non-N_2-fixing *B. utile* ($P < 0.001$, $r = 0.78$; Fig. 4c) and *T. guianensis* ($P = 0.05$, $r = 0.46$; Fig. 4d). The ratio of AM colonization to root phosphatase activity was not correlated with the RGR of the N_2-fixing *I. thibaudiana* ($P = 0.36$). Lastly, and across the two N_2-fixing species, N_2 fixation was positively correlated with root phosphatase activity ($P < 0.001$; Fig. 5a), but not with AM colonization ($P = 0.54$; Fig. 5b).

When grown in competition (*I. marginata* with *T. guianensis*, and *I. thibaudiana* with *B. utile*), the competitive response and the response ratio of foliar [P] varied between functional groups ($P < 0.001$ and $P < 0.001$, respectively) and among species ($P < 0.01$ and $P = 0.05$, respectively), but not among treatments ($P = 0.49$ and $P = 0.19$, respectively; Table S4). By functional group, the N_2-fixing seedlings showed a greater competitive response and foliar [P] response ratio than the non-N_2-fixing seedlings (Fig. 6a,b). However, only the competitive response of

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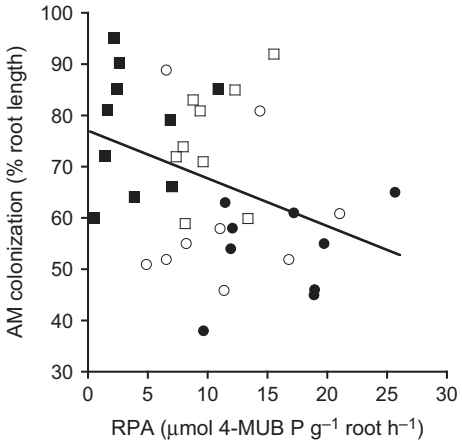

Fig. 2 Relationship between root phosphatase activity (RPA), expressed as μmoles of 4-MUB-P per gram root mass and hour, and arbuscular mycorrhizal (AM) colonization, expressed as percentage root length across species, from the observational study ($P = 0.03$, $r = 0.35$). The two N_2-fixing tree species are *Inga marginata* (closed circles) and *I. thibaudiana* (open circles), and the two non-N_2-fixing tree species are *Brosimum utile* (closed squares) and *Tapirira guianensis* (open squares). 4-MUB, 4-methylumbelliferone.

N_2-fixing *I. thibaudiana* was greater than zero ($P < 0.001$), whereas the competitive response of non-N_2-fixing *T. guianensis* was less than zero ($P < 0.01$). By contrast, the foliar [P] response ratios of both N_2-fixing *I. marginata* and *I. thibaudiana* were greater than zero ($P < 0.01$ and $P < 0.001$, respectively), and the foliar [P] response ratios of *B. utile* and *T. guianensis* were less than zero ($P < 0.001$ and $P < 0.01$, respectively). In addition, the root phosphatase activity response ratio varied between functional groups ($P = 0.05$), but not among species ($P = 0.38$) or treatments ($P = 0.17$; Table S4). By functional group, the N_2-fixing seedlings showed a greater root phosphatase activity response ratio than the non-N_2-fixing seedlings, and the response ratios of both N_2-fixing *I. marginata* and *I. thibaudiana* were greater than zero ($P < 0.001$ and $P < 0.001$, respectively; Fig. 6c). By contrast, the AM colonization response ratio did not vary between functional groups ($P = 0.09$), among species ($P = 0.68$) or treatments ($P = 0.71$; Table S4). However, the response ratios of both N_2-fixing *I. marginata* and *I. thibaudiana* were greater than zero ($P < 0.01$ and $P < 0.01$, respectively; Fig. 6d). Lastly, the response ratio of N_2 fixation did not vary among species ($P = 0.06$) or treatments ($P = 0.16$; Table S4), and both *I. marginata* and *I. thibaudiana* had N_2 fixation response ratios greater than zero ($P = 0.01$ and $P = 0.05$, respectively; Fig. 6e).

Discussion

Our study demonstrates the potential for soil P partitioning among some tropical forest trees; yet, the ability to exploit different chemical P compounds did not predict competitive outcomes. We hypothesized that N_2-fixing and non-N_2-fixing tree species would have somewhat distinct P acquisition strategies allowing them to exploit different chemical P compounds. Our results indicate that the N_2-fixing and non-N_2-fixing species studied here do indeed partition soil P, and the predominant

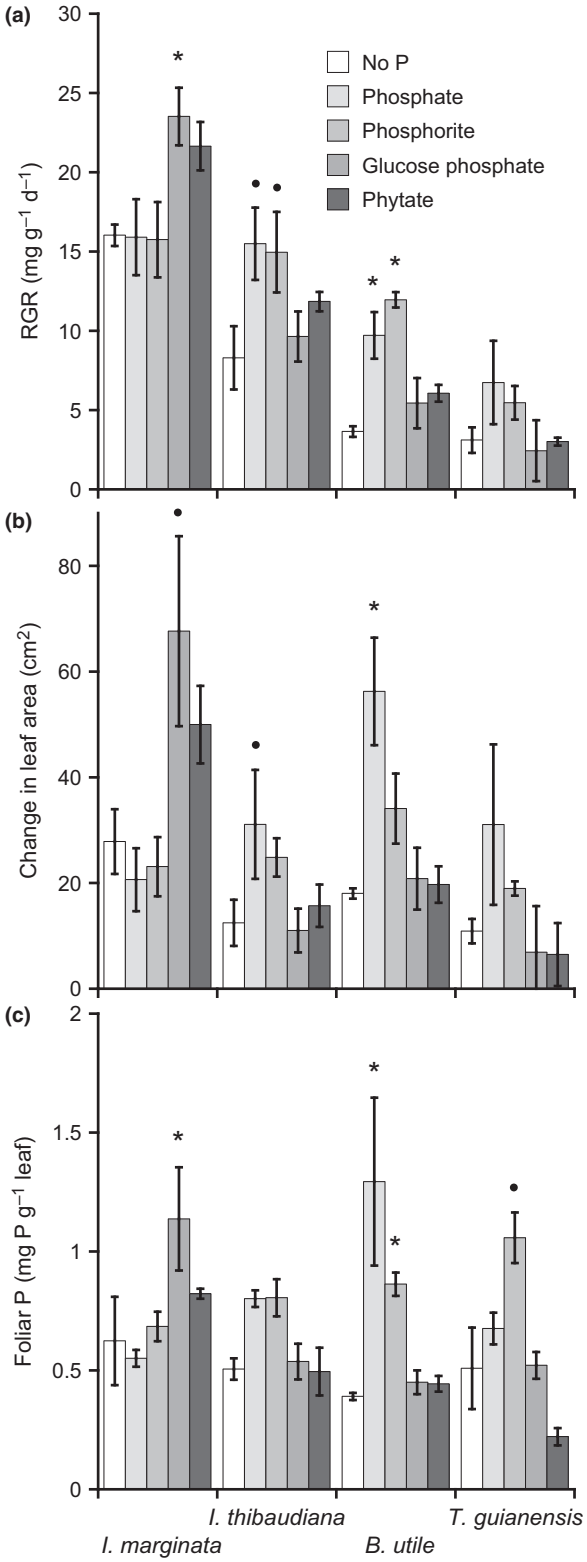

Fig. 3 Mean values ± SE for relative growth rate (RGR) expressed as milligrams of new mass per gram total mass and day (a), change in leaf area expressed as cm^2 (b), and foliar [P] expressed as milligrams of P per gram leaf (c) for the two N_2-fixing species, *Inga marginata* and *I. thibaudiana*, and the two non-N_2-fixing species, *Brosimum utile* and *Tapirira guianensis*, grown alone in the experimental study. Within each species, treatments with differences from the no-P control are indicated: •, $P < 0.1$; *, $P < 0.05$.

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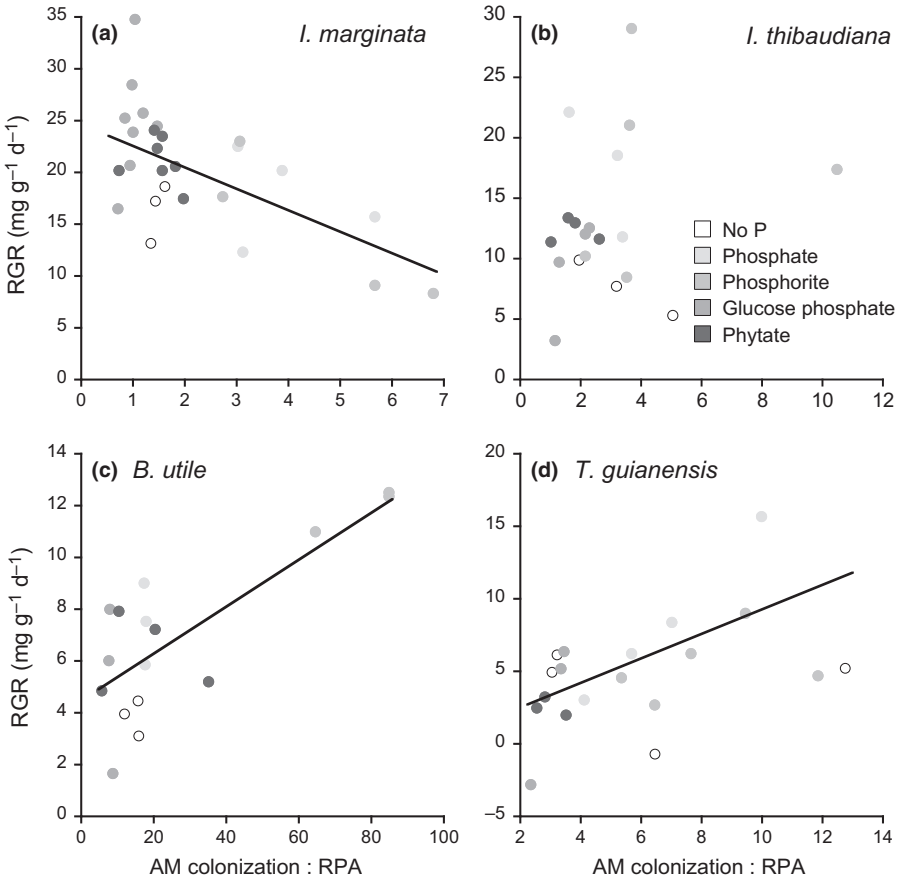

Fig. 4 Relationship between the arbuscular mycorrhzal (AM) colonization to root phosphatase activity (RPA) ratio and relative growth rate (RGR), expressed as milligrams of new mass per gram total mass and day, for the two N_2-fixing species, *Inga marginata* (a; $P < 0.001$, $r = 0.63$) and *I. thibaudiana* (b; $P = 0.36$), and the two non-N_2-fixing species, *Brosimum utile* (c; $P < 0.001$, $r = 0.78$) and *Tapirira guianensis* (d; $P = 0.05$, $r = 0.46$), grown alone in the experimental study. A large ratio indicates a predominant reliance on AM fungi, whereas a small ratio indicates a predominant reliance on RPA.

form of P that they exploit may be related to trade-offs in their investment in root phosphatases versus AM fungi. For example, the N_2-fixing species with relatively high rates of phosphatase activity and N_2 fixation predominantly exploited organic P, whereas the N_2-fixing species with relatively low rates of N_2 fixation and the non-N_2-fixing species with relatively high AM colonization predominantly exploited inorganic P. Interestingly, this distinction in P partitioning did not necessarily follow functional groups. Instead, partitioning was species specific and perhaps related to N_2 fixation rates. Nonetheless, despite soil P partitioning at the species level, the N_2-fixing species in our study competed more effectively for P than did the non-N_2-fixing species. Although it is possible that the N_2-fixing species also competed more effectively for N (via N_2 fixation), we did not explicitly test the role of N in influencing competitive outcomes. Nonetheless, our results show both the potential for soil P partitioning at the species level, and also that the N_2-fixing tree species studied here may be competitively superior in the acquisition of nutrients that often limit growth in lowland tropical rain forests (e.g. Wright *et al.*, 2011).

P partitioning is related to a predominant P acquisition strategy

In the observational study, *I. marginata* had the highest rates of root phosphatase activity among the four species (Fig. 1). When grown alone, the N_2-fixing *I. marginata* showed greater seedling growth (i.e. higher RGR and greater change in leaf area) and higher foliar [P] with organic P as the sole P source (Fig. 3), and the AM colonization to root phosphatase activity ratio was negatively correlated with RGR (Fig. 4a). Together, these data suggest that *I. marginata* may exploit organic P via a predominant reliance on root phosphatases. By contrast, when grown alone, the N_2-fixing *I. thibaudiana* showed greater seedling growth (i.e. higher RGR and greater change in leaf area) with inorganic P as the sole P source (Fig. 3). Although it is possible that *I. thibaudiana* predominantly exploited inorganic P via AM fungi, there was no correlation between its ratio of P acquisition strategies and RGR (Fig. 4b), nor was its level of AM colonization in the observational study different from that of the other species (Fig. 1). Together, these data indicate a lack of reliance on one P acquisition strategy over another and are in stark opposition to *I. marginata*. These differences within the N_2-fixing functional group, however, may be related to their species-specific differences in N_2 fixation rates.

In both the observational and experimental studies, *I. marginata* had higher N_2 fixation rates than *I. thibaudiana* (Fig 1 and Fig. 5a, respectively). If N_2 fixation is indeed a mechanism that allows N_2-fixing trees to invest fixed N_2 into root phosphatases (Houlton *et al.*, 2008), it would help to explain why, when grown alone in the experimental study, *I. marginata* showed positive growth responses with organic P as the sole P source. In other words, the *rate* of N_2 fixation, rather than the general capacity to fix N_2, may have influenced its ability to

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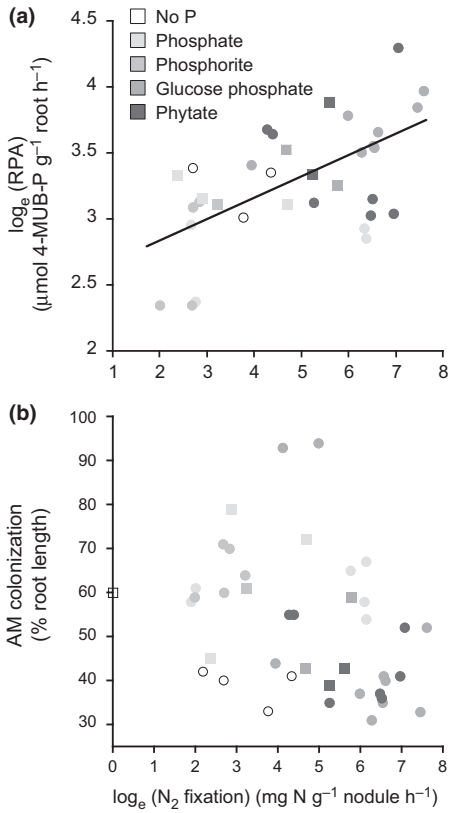

Fig. 5 Relationship between the log of N_2 fixation, expressed as milligrams of N per gram nodule and hour, and the log root phosphatase activity (RPA), expressed as μmol of 4-MUB-P per gram root mass and hour (a; $P < 0.001$; $r = 0.56$), and arbuscular mycorrhizal (AM) colonization, expressed as percentage root length (b; $P = 0.54$), for the two N_2-fixing tree species, *Inga marginata* (circles) and *I. thibaudiana* (squares), grown alone in the experimental study. 4-MUB, 4-methylumbelliferone.

acquire organic P. In addition, when the seedlings were grown alone in the experimental study, the N_2 fixation rates of both N_2-fixing species were highest when they were provided with organic P as the sole P source, and N_2 fixation rates were positively correlated with root phosphatase activity (Fig. 5a). Although the true directionality of this N and P relationship remains unclear (i.e. fixing N_2 to acquire P or acquiring P to fix N_2), it provides further evidence of a link between N_2 fixation and organic P acquisition.

In the observational study, the non-N_2-fixing species *B. utile* and *T. guianensis* had the greatest levels of AM colonization among the four species (Fig. 1). When grown alone, both non-N_2-fixing species generally showed greater seedling growth (i.e. higher RGR and greater change in leaf area) and higher foliar [P] with inorganic P as the sole P source (Fig. 3), and their AM colonization to root phosphatase activity ratios were positively correlated with their RGRs (Fig. 4c,d). Together, these data suggest that *B. utile* and *T. guianensis* may exploit inorganic P via a predominant reliance on AM fungi. However, it is surprising that, although the seedlings in the experimental study were grown under conditions of P limitation, only the two non-N_2-fixing species seemed to respond positively to inorganic P, especially PO_4^{3-}, as the sole P source.

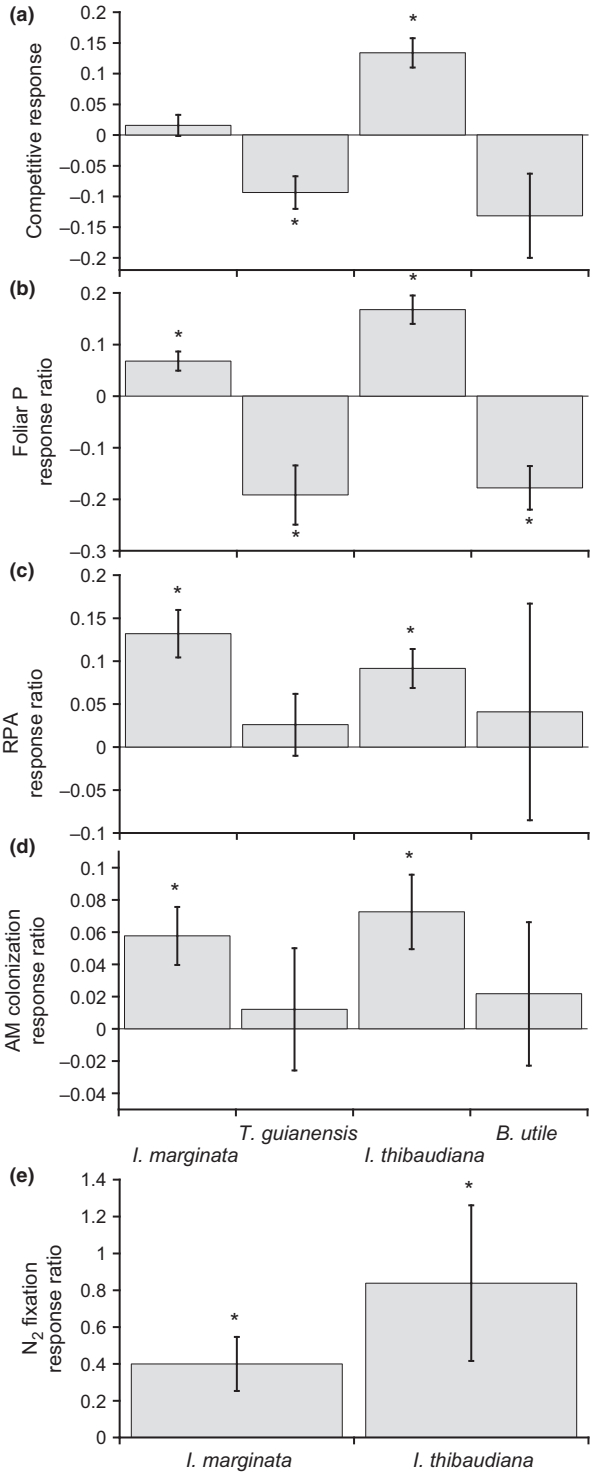

Fig. 6 Mean values ± SE for competitive response (a), and the response ratios of foliar [P] (b), root phosphatase activity (RPA; c), arbuscular mycorrhizal (AM) colonization (d) and N_2 fixation (e) for the N_2-fixing *Inga marginata* and the non-N_2-fixing *Tapirira guianensis* grown in competition, and the N_2-fixing *I. thibaudiana* and the non-N_2-fixing *Brosimum utile* grown in competition in the experimental study. A positive response indicates that competition stimulated growth or nutrient acquisition strategies, whereas a negative response indicates that competition suppressed growth or nutrient acquisition strategies. Asterisks indicate cases in which the competitive responses and response ratios are different from zero ($P < 0.05$).

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We offer two possible reasons why the greater levels of AM colonization may have benefited the two non-N_2-fixing species in the acquisition of inorganic P. First, although labile inorganic P (i.e. PO_4^{3-}) is often assumed to be the most readily available form of plant-available P (e.g. Walker & Syers, 1976), AM fungi are most advantageous in acquiring this form of P when host trees have minimal root development (Smith & Read, 2008). Indeed, other studies from Neotropical rain forests have shown that the root length (on both a per unit plant and root biomass basis) is lower for *B. utile* and *T. guianensis* than for *I. marginata* (Pavlis & Jeník, 2000; Brenes-Arguedas *et al.*, 2013). Second, despite bulk additions of PO_4^{3-} to the experimental pots, some of this added P may have become unavailable via rapid mineral surface adsorption onto the iron and aluminum sesquioxide clay-dominated soils added to the sand : soil medium (Uehara & Gillman, 1981; Olander & Vitousek, 2004). Although it is widely assumed that sorbed forms of inorganic P are largely inaccessible to plants over short time scales, previous studies have suggested that, like an extensive root network, mycorrhizal hyphae may be capable of releasing iron- and aluminum-bound P by altering the mycosphere pH and/or producing chelating compounds (Marschner, 1995; Comerford, 1998; Smith & Read, 2008). Thus, the high levels of AM colonization on *B. utile* and *T. guianensis* roots found in the observational study (Fig. 1) may have allowed them to exploit a greater proportion of the added inorganic P (both PO_4^{3-} and phosphorite), promoting positive growth responses for the seedlings exposed to inorganic P additions.

Overall, our results suggest that some tropical forest tree species may exhibit a trade-off in P acquisition strategies that enable them to exploit different chemical P compounds despite P limitation. This highlights the importance of simultaneously measuring AM colonization with other P acquisition strategies (e.g. phosphatases). However, it is important to note that differences in AM colonization across the species studied here could have been driven by other factors. For example, levels of AM colonization can vary depending on the initial size and growth rate of a tree (although we control for the differences in initial size of the host plant), such that tree species with a high RGR may have low levels of AM colonization as a result of a dilution effect (e.g. *I. marginata*). Also, tropical tree species can harbor different AM fungal taxa (Husband *et al.*, 2002) that differ in their allocation to intra- versus extraradical structures (Hart & Reader, 2002). Thus, the quantification of intraradical AM colonization alone may not be representative of the host tree's total investment in AM fungi. These two species-specific drivers of AM colonization could contribute to the differences we found here, as well as the contrasting results found in non-species-specific studies (e.g. Nasto *et al.*, 2014).

Although soil P partitioning may be just one of many possible factors explaining our results, if it does occur in forests, it could contribute to the coexistence of lowland tropical trees via interspecific differences in P acquisition (i.e. niche complementarity) (*sensu* Loreau & Hector, 2001; Turner, 2008; Steidinger *et al.*, 2014). Our study is not the first to show evidence of niche complementarity via soil nutrient acquisition (e.g. McKane *et al.*, 2002; Weigelt *et al.*, 2005; Kahmen *et al.*, 2006; Oelmann *et al.*, 2007; Ashton *et al.*, 2010), but very few have demonstrated niche complementarity with respect to soil P. Of those that have (Ahmad-Ramli *et al.*, 2013; Steidinger *et al.*, 2014), niche complementarity via soil P partitioning was demonstrated across broad plant functional groups (e.g. AM, ectomycorrhizal, ericoid mycorrhizal and/or non-mycorrhizal plants) that are known to have distinctly different abilities to acquire different chemical P compounds (Smith & Read, 2008). Our study, however, may be the first to demonstrate that soil P partitioning can occur in some tropical tree species *within* a mycorrhizal type (i.e. AM). This is a surprising result given that, despite the hyperdiversity of lowland tropical rain forest, nearly all lowland tropical trees in this system are AM, and AM fungi have a putatively narrowly defined role in P acquisition (Smith & Read, 2008). If our results apply broadly, it would imply that the nature of P acquisition of any tropical AM tree may not be defined by the ability of a tree to host AM fungi, but rather the relative contribution to P acquisition by AM fungi versus other P acquisition strategies.

P partitioning does not influence competitive outcomes

Despite compelling evidence from our experimental study that some lowland tropical rain forest tree species have the potential to partition soil P, our results showed that the two N_2-fixing species were competitively superior to the two non-N_2-fixing species regardless of the P source added (Fig. 6a). For example, both root phosphatase activity and AM colonization response ratios were positive and greater in the N_2-fixing species than in the non-N_2-fixing species (Fig. 6c,d), indicating an enhanced capacity to acquire inorganic and organic P when grown in competition. Although we did not measure P uptake directly, the roles of phosphatases and AM fungi in the acquisition of soil P have been well established (Lambers *et al.*, 2008; Smith & Read, 2008). Thus, it is likely that the enhanced P acquisition strategies led to greater uptake, which may be reflected in the positive foliar [P] response ratios (Fig. 6b). In addition, the N_2-fixing species had positive N_2 fixation response ratios, indicating an enhanced capacity to acquire N when grown in competition. Lastly, the N_2-fixing species had positive and greater competitive responses than the non-N_2-fixing species (Fig. 6a), indicating enhanced and greater RGRs than the non-N_2-fixing species when grown in competition. It is important to note, however, that although previous studies have shown that larger plants tend to have smaller RGRs than smaller plants (e.g. Paine *et al.*, 2012), we argue that this negative size-dependent effect of plant size in RGR is not applicable to our study, as we used seedlings rather than mature trees, and accounted for the initial differences in seedling size (Table 1) in our statistical approach (see earlier).

Our results are consistent with those from other pot studies using species from other ecosystems (Baker *et al.*, 1994; Bi & Turvey, 1994; Little *et al.*, 2002), in which N_2-fixing plants

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up-regulated N_2 fixation and increased their growth when competing against non-N_2-fixing plants. The N_2-fixing species studied here appeared to similarly up-regulate N_2 fixation, as well as their P acquisition strategies, to stimulate growth. This could provide a mechanistic explanation for the enhanced competitive response of the N_2-fixing species versus the non-N_2-fixing species.

Conclusion

Phosphorus is thought to limit ecosystem processes in some lowland tropical rain forests (Vitousek, 1984; Cleveland *et al.*, 2011; Condit *et al.*, 2013). Yet, soil nutrient availability is highly heterogeneous (Townsend *et al.*, 2008), and recent fertilization studies have shown nutrient colimitation to tree growth (Wright *et al.*, 2011; Alvarez-Clare *et al.*, 2013). Although it remains unclear how, or if, the results from our seedling experiment may translate into an understanding of nutrient acquisition by a broader set of mature canopy trees in natural forest, we argue that the ability to fix high rates of N_2, as well as the relative contribution of root phosphatases versus AM fungi to P acquisition, could drive soil P partitioning. If so, this could represent a critical and underappreciated process with the potential to facilitate coexistence, even among tree species belonging to a single mycorrhizal functional group. However, P partitioning alone may not create a strong enough effect to minimize interspecific competition. In our study, growth responses in the absence of competition did not predict competitive outcomes, highlighting the importance of studying plant performance within the context of plant–plant interactions. Rather, the ability to fix N_2 – and any associated enhanced capacity to acquire soil P – may simply represent one of many factors (e.g. light, herbivory, pathogens and parasites) that influence competition, and, subsequently, productivity, species distribution and community composition in diverse lowland tropical rain forest.

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Author contributions

M.K.N., Y.L. and C.C.C. conceived of or designed the study. M.K.N., B.B.O., C.S.B. and P.G.T. performed the research. M.K.N. analyzed the data. M.K.N. wrote the manuscript. B.B.O., Y.L., G.P.A., C.S.B., S.P., P.G.T., A.R.T. and C.C.C. contributed to revisions.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 The four focal tree species used in the observational and experimental studies organized by family, functional group

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and geographic distribution (accessed through GBIF Data Portal, http://data.gbif.org)

Table S2 The concentration and chemical form of all macronutrients and micronutrients in the hydroponic feed solution (*sensu* Steidinger *et al.*, 2014)

Table S3 *F*-table for nested ANCOVA of relative growth rate, change in leaf area, foliar [P], root phosphatase activity, AM colonization and N_2 fixation, with initial number of leaves and height entered as covariates, when the seedlings were grown alone in the experimental study

Table S4 *F*-table for nested ANCOVA of competitive response and the response ratios of foliar [P], root phosphatase activity, AM colonization and N_2 fixation, with initial number of leaves and height entered as covariates, when the seedlings were grown in competition in the experimental study

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