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Seedling growth responses to phosphorus reflect adult distribution patterns of tropical trees

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Summary

- Soils influence tropical forest composition at regional scales. In Panama, data on tree communities and underlying soils indicate that species frequently show distributional associations to soil phosphorus. To understand how these associations arise, we combined a pot experiment to measure seedling responses of 15 pioneer species to phosphorus addition with an analysis of the phylogenetic structure of phosphorus associations of the entire tree community.
- Growth responses of pioneers to phosphorus addition revealed a clear tradeoff: species from high-phosphorus sites grew fastest in the phosphorus-addition treatment, while species from low-phosphorus sites grew fastest in the low-phosphorus treatment. Traits associated with growth performance remain unclear: biomass allocation, phosphatase activity and phosphorus-use efficiency did not correlate with phosphorus associations; however, phosphatase activity was most strongly down-regulated in response to phosphorus addition in species from high-phosphorus sites.
- Phylogenetic analysis indicated that pioneers occur more frequently in clades where phosphorus associations are overdispersed as compared with the overall tree community, suggesting that selection on phosphorus acquisition and use may be strongest for pioneer species with high phosphorus demand.
- Our results show that phosphorus-dependent growth rates provide an additional explanation for the regional distribution of tree species in Panama, and possibly elsewhere.

Introduction

Improvements in our ability to characterize the availability and spatial distribution of potentially limiting soil resources (ter Steege *et al.*, 2006; Engelbrecht *et al.*, 2007; Baldeck *et al.*, 2013b; Chang *et al.*, 2013; Condit *et al.*, 2013) have renewed interest in the role of edaphic niche differentiation in the assembly of tropical tree communities. Variation in nutrient availability correlates with local tree species distributions (John *et al.*, 2007), and with compositional turnover (i.e. β-diversity) within communities (Paoli *et al.*, 2006; Baldeck *et al.*, 2013a) and across regions (Potts *et al.*, 2002; Tuomisto *et al.*, 2003). These studies provide compelling evidence that tree distributions are linked to variation in soil resources, yet it remains unclear whether species respond to individual limiting soil nutrients, or to a range of correlated soil variables.

The extent to which tree species distribution patterns are influenced by environmental factors in lowland tropical forests was explored recently for 550 species across the Isthmus of Panama (Condit *et al.*, 2013). The region is characterized by strong gradients of dry-season moisture deficit and nutrient availability, the latter resulting from marked variation in geology and associated soils. Using species presence and absence for 550 tree species at 72 sites across the 65-km-wide Isthmus, in combination with data on soil nutrients and climate, Condit *et al.* (2013) used Gaussian logistic regressions to determine the strength of species associations with these variables. They showed that dry season intensity and readily exchangeable soil phosphorus (P) were the two strongest predictors of species distributions. In particular, more than half of the tree species studied had pronounced associations with either high- or low-P soils. Among-species comparisons of the strength of associations between tree species distributions and soil nutrients can be made using P effect sizes (Condit *et al.*, 2013), which reflect the estimated change in the probability of occurrence of a species across the gradient in P availability when other resources are held constant. Positive effect sizes indicate species that occur predominantly on high-P soils, while negative effect sizes indicate species that occur predominantly on low-P soils. The magnitude of an effect size therefore represents the extent to which species show positive, negative or no significant association with soil P across the landscape. At present, the underlying physiological mechanisms that determine these P associations, and therefore species distributions, remain unknown.

Pot experiments have been widely used to study the adaptations and growth response of tropical trees to different amounts of resource availability (e.g. Kitajima, 1994; Huante *et al.*, 1995a, b; Dalling *et al.*, 2004; Dent & Burslem, 2009; Garrish *et al.*, 2010). To understand how P associations have arisen and are maintained, we combined an analysis of the phylogenetic structure of P effect sizes of tree species included in the Condit *et al.* (2013) study, with a pot experiment to identify the traits associated with species affinities for soils with either high or low-P concentrations. We grew seedlings of 15 fast-growing tree species in P-poor soil (0.13 mg P kg^{-1}), to determine whether species P effect sizes reflect relative growth performance, biomass allocation, or root phosphatase production. To isolate the effect of P, seedlings were fertilized with a full nutrient solution with (+P) or without (−P) P.

Assuming that trade-offs in the ability to grow in high vs low-P soils drive observed species distributions, we predicted a negative correlation between species growth rates in low- and high-P soil. Furthermore, we predicted that when P is limiting, species that occur on low-P soils (i.e. that have negative effect sizes) would invest more in P acquisition and have higher P-use efficiency (PUE) and phosphatase enzyme activity than species that occur on soils with high concentrations of P (i.e. positive effect sizes). By contrast, we predicted that species that naturally occur on high-P soils would depend to a greater extent on inorganic P sources (i.e. lower investment in phosphatase enzymes) and have less conservative P use.

Our predicted tradeoff in species growth rates across soils differing in P availability contrasts with previous pot experiments which have shown positive correlations between growth in low and high light (Kitajima, 1994; Dalling *et al.*, 2004), soil fertility (Dent & Burslem, 2009) and soil NPK treatments (Huante *et al.*, 1995a). Positive correlations, however, may be a consequence of the inclusion of light-demanding and shade-tolerant species with contrasting physiology and resource allocation patterns. In this study, we restricted our pot experiment to pioneer species with similar life histories. To explore whether selection on P-use traits of pioneers, which have high growth rates and therefore high P demand, differs from those of the large community of untested shade-tolerant species, we also use a community phylogenetic approach to compare whether P effect sizes are more dissimilar at the genus and family level among clades that include pioneer species than among clades composed of slower-growing, shade-tolerant species. Finally, we used growth data for a subset of 84 tree species present in the Barro Colorado Island (BCI) 50 ha forest dynamics plot to test the prediction that variance in growth rates of species from clades with more dissimilar (overdispersed) P effect sizes is greater than those with more similar (underdispersed) effect sizes.

Materials and Methods

Species and study site

Seeds of 15 species were collected on Barro Colorado Nature Monument in central Panama (9°05′N, 79°45′W; Table 1). All the species used in this study are considered pioneers, with small seeds, a strong tendency to recruit into gaps, and high rates of growth, mortality and dispersal (Condit *et al.*, 1996; Dalling *et al.*, 1998). We focused on pioneer species because seedlings of larger-seeded, shade-tolerant trees can utilize P reserves from seeds for prolonged periods (Kitajima, 2002; Slot *et al.*, 2013). We consider *Trema micrantha* (*sensu lato*) to represent two species (Yesson *et al.*, 2004): *T. micrantha* 'brown' is restricted to landslides and road embankments, while *T. micrantha* 'black' occurs mostly in treefall gaps (Silvera *et al.*, 2003; Yesson *et al.*, 2004; Pizano *et al.*, 2011).

To ensure that seedlings were of sufficient size to survive transplantation and that seedlings used in the experiment were in a similar developmental stage, we raised seedlings under 30% full sun and high red : far-red irradiance (*c.* 1.4). Seedlings with fully expanded cotyledons were transplanted in 2.65 l tree pots (Steuewe & Sons Inc., Corvallis, OR, USA) containing a 50 : 50 mixture of sieved soil and silica sand. We collected unsterilized forest soil from the Santa Rita ridge that has among the lowest resin-extractable P (0.13 mg P kg^{-1}) in the regional plot network used by Condit *et al.* (2013) (range 0.1–22.8 mg P kg^{-1}) and a total exchangeable base concentration of 1.4 $cmol_c$ kg^{-1}. This soil is developed on pre-Tertiary basalt and preliminary analyses indicate it is an Ultisol in the Soil Taxonomy system (Soil Survey Staff, 1999).

The tree pots were placed underneath a rain shelter with a glass roof on tables *c.* 0.8 m above a concrete surface at the Santa Cruz Field Facility of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07′N, 79°42′W). The shelter had no sidewalls, and therefore air temperature, wind speed and relative humidity were similar to ambient conditions. Initial dry mass and leaf area of plants were determined at the time of transplant from at least four representative seedlings. Seedlings of four species were grown during the 2012 wet season, and the remaining 11 species were grown at the same location during the 2013 wet season (Table 1).

Nutrient treatments

We grew six seedlings of each species in each of two nutrient treatments. In one treatment the soil–sand mix was fertilized with a full nutrient solution except P, while in the other, the soil–sand mix was fertilized with the same nutrient solution plus P. Seedlings received 150 ml of the nutrient solution once a week. Each solution contained the following chemical concentrations of nutrients: 4 mM KNO_3, 1.5 mM $MgSO_4$ and 4 mM $CaCl_2$. In addition, 1.3 mM P was added as NaH_2PO_4. Both solutions included micronutrients and Fe as ethylenediaminetetraacetic acid iron (III)-sodium salt. Plants were hand-watered at least once a day except when the seedlings were fertilized.

Harvest measurements

To avoid a pot size effect on seedling performance (Poorter *et al.*, 2012), we harvested the seedlings of each species when the dry

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Table 1 Species list, growth period, harvest date and phosphorus (P) effect sizes determined from species distributions across the Isthmus of Panama (Condit *et al.*, 2013), species relative growth rate (RGR) and root phosphatase activity

Species	Growth period (d)	Harvest date	P effect size	RGR ($mg\ g^{-1}\ d^{-1}$)		Phosphatase activity ($\mu mol\ pNP\ g^{-1}\ h^{-1}$)	
				+P	−P	+P	−P
Luehea seemannii Triana & Planch.	62	December 2013	1.2	96.4	53.4	273.1	355.7
Cecropia longipes Pittier	25	August 2012	1.2	154.4	29.7	883.2	1298.7
Cecropia peltata L.	29	September 2012	1.0	165.0	46.1	759.1	1113.0
Guazuma ulmifolia Lam.	62	December 2013	0.7	90.0	48.9	247.2	402.4
Ficus insipida Willd.	90	January 2014	0.5	62.8	40.4	116.5	142.5
Trema micrantha 'black' (L.) Blume	56	December 2013	0.4	124.5	76.3	902.3	1253.2
Trema micrantha 'brown' (L.) Blume	54	December 2013	0.4	112.5	69.8	673.7	601.8
Cecropia obtusifolia Bertol.	31	September 2012	0.3	152.8	62.7	724.5	725.0
Ochroma pyramidale (Cav. ex Lam.) Urb.	48	December 2013	−0.2	106.4	76.7	541.6	511.2
Ficus tonduzzii Standl.	69	September 2013	−0.2	84.2	44.9	82.2	93.9
Luehea speciosa Willd.	56	September 2013	−0.4	111.4	84.9	633.6	555.5
Apeiba tibourbou Aubl.	54	December 2013	−0.7	92.1	67.6	275.4	256.1
Cecropia insignis Liebm.	46	September 2012	−0.9	110.2	67.8	1086.8	1262.1
Trichospermum galeottii (Turcz.) Kosterm.	63	October 2013	−1.1	103.7	94.9	330.2	322.7
Apeiba membranacea Spruce ex Benth.	83	October 2013	−1.1	52.4	54.6	270.2	281.6

*p*NP, *para*-nitrophenol.

plant biomass to pot volume ratios were, on average, $< 1\ g\ l^{-1}$ in the +P treatment. Therefore, seedlings of different species grew for different time periods, but were harvested with similar biomass. At harvest time, seedlings were separated into root, leaf and stem fractions. Roots were first gently washed with tap water and *c.* 500 mg of fresh fine root mass per seedling was collected and washed twice with reverse osmosis water and stored overnight at 4°C for phosphatase assays (see below). Leaf area was measured with an automated leaf area meter (LI-3000A; Li-Cor, Lincoln, NE, USA) and final biomass was measured after drying for at least 72 h at 60°C.

Relative growth rate (RGR) was calculated according to the following equation: $RGR = [(\ln W_f - \ln W_i)/(\Delta t)]$, where W_f is the final dry mass, W_i is the initial dry mass, and Δt is the duration of the experiment. Net assimilation rate (NAR; biomass increment per unit leaf area) and root mass fraction (RMF, root mass per unit whole-plant dry mass) were calculated from harvest data.NAR was calculated according to the equation used by Williams (1946): $NAR = [(W_f - W_i) \times (\ln A_f - \ln A_i)]/[(A_f - A_i) \times (\Delta t)]$, where W_f and W_i are the final and initial dry masses, respectively, A_f and A_i are the final and initial leaf areas, respectively, and Δt is the duration of the experiment.

Phosphatase activity was determined using *para*-nitrophenyl phosphate (*p*NPP) as an analogue substrate for phosphomonoesterase (Turner *et al.*, 2001). About 500 mg of fresh fine roots per seedling were placed in a glass vial with 9 ml of sodium acetate-acetic acid buffer (pH 5.0) and placed in a shaking water bath at 26°C (mean soil temperature in Panamanian lowland forests). The assay was initiated by adding 1.0 ml substrate (50 mM *p*NPP) and incubated for 30 min. The reaction was terminated by removing 0.5 ml of buffer solution and adding it to 4.5 ml of terminator solution (0.11 M NaOH) in a glass test tube. After vortexing, the absorbance was measured at 405 nm against *para*-nitrophenol (*p*NP) standards. Enzyme activity was expressed in $\mu mol\ pNP\ g^{-1}$ dry mass h^{-1}.

Leaf material from each harvested seedling was ground and analyzed for total nitrogen (N) and P. Foliar N concentration was measured by automated combustion and thermal conductivity detection on a Thermo Flash EA1112 analyzer (CE Elantech, Lakewood, NJ, USA). Foliar P was determined by ignition (550°C, 1 h) and dissolution of the ash in 1 M HCl, with phosphate detection by automated molybdovanadate colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Finally, foliar PUE was calculated as foliar plant productivity (i.e. foliar RGR) per unit mass of foliar P.

Data analysis

Using means of six seedlings per plant species grown in each P treatment, we calculated the treatment percentage difference in RGR, root phosphatase activity, NAR, RMF, foliar P concentration, foliar N : P ratio and foliar PUE. The treatment percentage difference was calculated as the difference between the +P and −P treatments for each trait value divided by the average of the trait values and multiplied by 100.

To determine whether growth responses to P addition influence species distributions, relationships between the treatment means, as well as the percentage difference in RGR, root phosphatase activity, NAR, RMF, foliar P concentration, foliar N : P ratio, foliar PUE and P effect sizes were explored using linear regression models. For all the species included in this study, the P effect sizes – defined as the first-order parameter of the logistic model for the species distribution relative to P – were obtained from Condit *et al.* (2013) (https://repository.si.edu/handle/10088/19529). The effect sizes represent the strength of the relationship between soil P and the probability of species occurrence,

where P was measured using anion exchange resins, the most biologically available form of P. To account for the nonindependence of traits within related plant lineages, phylogenetic independent contrasts (PICs) (Felsenstein, 1985) were calculated using the PIC function in the package APE (Paradis *et al.*, 2004) for R v.2.15.3 (R Core Team, 2013), and using a phylogeny constructed with Phylomatic (Webb & Donoghue, 2005).

We used linear mixed-effects models to test the prediction that P treatments, P effect sizes and their interaction affect RGR, root phosphatase activity, NAR, RMF, foliar P concentration, foliar N : P ratio and foliar PUE. All dependent variables were log-transformed before analysis and results were considered to be statistically significant at $P < 0.05$. We coded each species as random and P treatments and P effect sizes as fixed effects. Statistical analyses were carried out in R using the NLME package (Pinheiro *et al.*, 2013). Results of these models are summarized in Supporting Information Table S1.

Phylogenetic structure of P effect sizes across Panamanian trees

We examined the extent to which particular clades of tropical trees showed higher, lower, or random dispersion of P effect sizes across a community phylogeny created for all 550 tree species included in Condit *et al.* (2013). The community phylogeny was constructed using the placement of each species within the taxonomic hierarchy (family, genus, species) using the software PHYLOMATIC (Webb & Donoghue, 2005). Average pairwise distances between effect sizes were computed across all subtrees for the community phylogeny. Observed pairwise distances were compared with the distribution of 999 null values for each tree, which were computed by randomly shuffling the tip names across the phylogeny, creating all possible subtrees, and calculating mean pairwise trait distances for each novel subtree. Nodes with greater or less than expected effect sizes were determined by the rank of the observed value for the observed subtree compared with a subtree with randomly placed trait values. The percentage of species belonging to overdispersed, underdispersed and nonsignificant clades was determined by counting the number of tips included in each category. To compare the strength of the effect of soil P relative to other soil nutrients quantified by Condit *et al.* (2013), we also examined the extent to which particular tree clades showed higher, lower or random dispersion of inorganic N and calcium (Ca) effect sizes. As some clades will appear to be over- or underdispersed by chance, we quantified the frequency of these species associations with inorganic N as a 'null model' (none of the species analyzed by Condit *et al.* (2013) showed significant distributional associations with N). Soil Ca was used because this was the third strongest predictor of species distributions, after dry-season moisture and P.

We used data from sapling growth on the BCI 50 ha plot, (Feeley *et al.*, 2007) to test whether dispersion of P effect sizes measured for tree species across the region predicts variance in RGR. The Feeley *et al.* (2007) dataset reports RGR based on the growth of individuals of 1–5 cm diameter at breast height between 2000 and 2005 in the 50 ha forest dynamics plot, and includes 44% and 48% of the species included in overdispersed and underdispersed clades, respectively. Because RGR values are not normally distributed within each group, we normalized the data using the natural logarithm and then performed an F test using the VAR.TEST function on R, to ask if the overdispersed clades have greater variance in RGR than the underdispersed clades.

Results

To confirm that we had imposed P limitation in our pot experiment, we measured foliar N and P concentrations in each of the nutrient addition treatments. For all species, higher foliar N : P ratios were found in the −P treatment ($F_{1,116} = 61.75$, $P < 0.001$; Fig. S1a, Table S2). We also found significantly higher foliar P in the +P treatment ($F_{1,116} = 63.94$, $P < 0.001$; Fig. S2a).

Consistent with our prediction, there was a clear tradeoff manifested across species in the ability to grow in low- vs high-P soils. Species that occur naturally on soils with a high concentration of readily exchangeable (i.e. bioavailable) P grew slower in the −P treatment, but faster in the +P treatment, compared with species that occur on low-P soils (P effect size × P treatment interaction, $F_{1,125} = 164.56$, $P < 0.001$; Fig. 1a). This shift in relative growth performance was also reflected in a positive relationship between the percentage difference in species RGR in response to P addition, and species P effect sizes ($r^2 = 0.60$, $n = 15$, $P < 0.001$; Fig. 1b). This relationship was also maintained after accounting

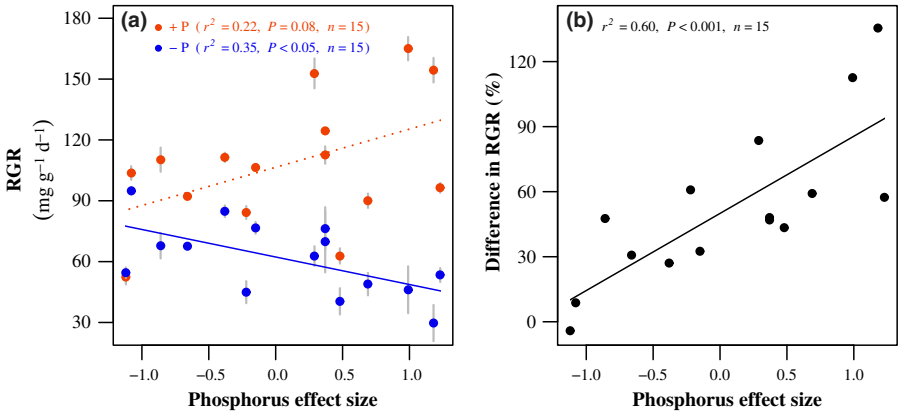

Fig. 1 Growth response to phosphorus (P) addition. (a) Relationship between the relative growth rate (RGR) for each P treatment and the species P effect sizes. Vertical error bars correspond to ± SE. +P, with P; –P, without P. (b) Relationship between the percentage difference in species RGR in response to P addition, and species P effect sizes. Solid lines represent significant relationships and the dotted line represents a marginally significant relationship.

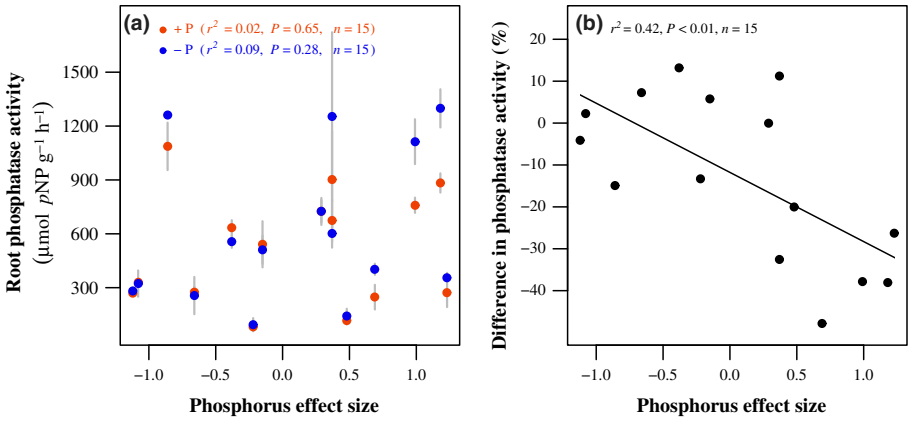

Fig. 2 Phosphatase activity (expressed by root dry mass) response to phosphorus (P) addition. (a) Relationship between the root phosphatase activity for each P treatment and the species P effect sizes. Vertical error bars correspond to ± SE. +P, with P; −P, without P; *p*NP, *para*-nitrophenol. (b) Relationship between the percentage difference in root phosphatase activity in response to P addition and species P effect sizes. The line represents a negative and significant relationship.

for phylogenetic nonindependence of these traits within related plant lineages using PICs ($r^2 = 0.70$, $n = 14$, $P < 0.001$). A similar pattern was also observed for net assimilation rate (NAR; P effect size × P treatment interaction, $F_{1,125} = 12.93$, $P < 0.001$; $r^2 = 0.60$, $n = 15$, $P < 0.001$; after controlling for phylogenetic relatedness, $r^2 = 0.76$, $n = 14$, $P < 0.001$; Table S2, Fig. S3a,b).

Growth responses, however, did not entirely reflect predicted changes in P acquisition traits. Root phosphatase activity was only marginally different between P treatments (P treatment effect, $F_{1,110} = 3.74$, $P = 0.056$; Fig. 2a), although the decline in phosphatase activity with P addition was greater for species with more positive P effect sizes (P effect size × P treatment interaction, $F_{1,110} = 6.86$, $P < 0.01$; Fig. 2a). Furthermore, we found a significant negative relationship between the percentage difference in phosphatase activity in response to P addition and species P effect sizes ($r^2 = 0.42$, $n = 15$, $P < 0.01$; Fig. 2b). This relationship was also maintained after controlling for phylogeny ($r^2 = 0.43$, $n = 14$, $P < 0.01$). We also found a significant negative relationship between the percentage difference of RGR and the percentage difference in phosphatase activity, in response to P addition ($r^2 = 0.36$, $n = 15$, $P < 0.01$; Fig. 3), indicating that species that showed the greatest growth response to the +P treatment were also species in which phosphatase activity was reduced the most in the +P treatment.

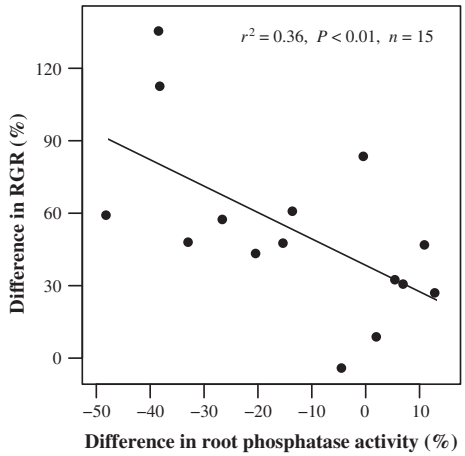

Fig. 3 Relationship between the percentage difference in relative growth rate (RGR) and the percentage difference in root phosphatase activity in response to phosphorus addition. The line represents a negative and significant relationship. This negative and significant relationship was also maintained after controlling for phylogeny (phylogenetic independent contrasts, $r^2 = 0.32$, $n = 14$, $P < 0.05$).

In common with root phosphatase activity, other traits related to P acquisition (i.e. root mass fraction) and use efficiency (i.e. foliar P and PUE) did not correlate with P effect sizes when P treatments were evaluated separately (Figs S2a, S4a, S5a). However, we did find a significant positive relationship between the percentage difference in root mass fraction in response to P addition and species effect sizes before ($r^2 = 0.37$, $n = 15$, $P < 0.05$; Fig. S4b) and after controlling for phylogeny ($r^2 = 0.33$, $n = 14$, $P < 0.05$). No relationship was found between foliar P concentrations or foliar PUE in response to P addition and species effect sizes (foliar P, $r^2 = 0.01$, $n = 15$, $P = 0.75$; PUE, $r^2 = 0.23$, $n = 15$, $P = 0.07$; Figs S2b, S5b).

Analysis of P effect sizes for the entire tree meta-community showed that 16% of the 550 species analyzed by Condit *et al.* (2013) belong to overdispersed clades in which effect sizes are more dissimilar than expected by chance ($P < 0.05$; Fig. 4). An additional 17% of species are in underdispersed clades, indicating potential niche conservatism ($P < 0.05$; Fig. 4). Although only a small fraction of clades were overdispersed, about half of the species included in our pot experiment, which represent many of the most common pioneer tree species in Neotropical forests, are members of overdispersed clades (Fig. 4). Overdispersed clades include the orders Malvales, Brassicales and Sapindales (all within the Malvidae in the Rosids clade), while taxa that belong to underdispersed clades are broadly distributed across the Rosids clade, and are mainly represented by shade-tolerant, old-growth species (e.g. Asterids, Fabales). When the phylogenetic analysis was repeated using N, (our null model where no effect sizes were significantly different from zero), we found that 7% of species belonged to overdispersed clades, and 19% to underdispersed clades ($P < 0.05$). The Ca results showed that 24% of species analyzed belong to overdispersed clades and 28% of species to underdispersed clades ($P < 0.05$). However, none of species in our pot experiment were in overdispersed clades for Ca. Finally, we also found that the clades that were overdispersed for P had significantly greater variance in RGR than underdispersed clades for saplings of species that occur on the BCI 50 ha forest dynamics plot ($F_{37,45} = 1.81$, $P = 0.03$).

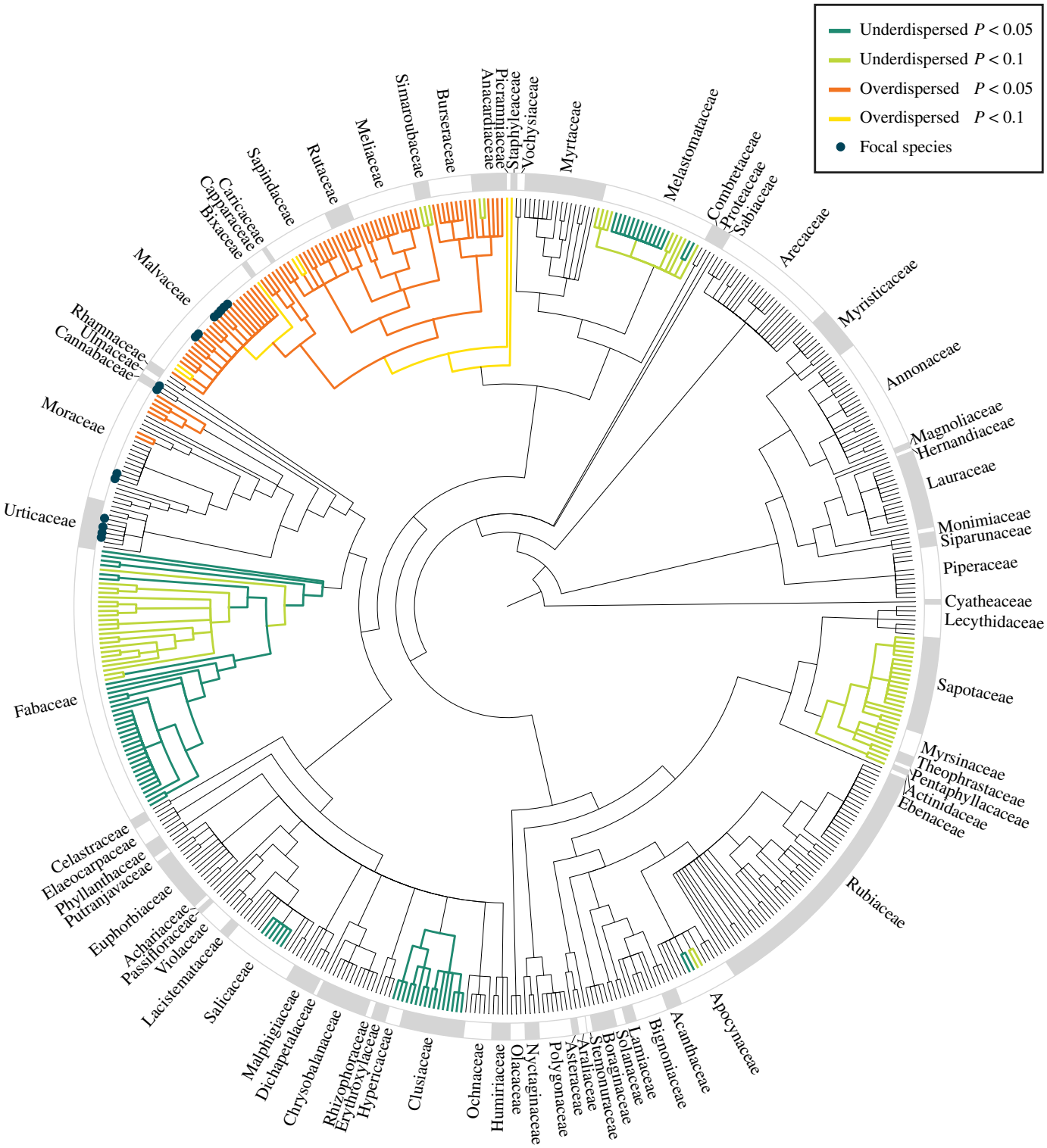

Fig. 4 Phylogenetic structure of phosphorus (P) associations. The community phylogeny was created for all the 550 species included in Condit *et al.* (2013) with clades showing the phylogenetic signal for the P effect sizes. Clades are colored according to the phylogenetic signal: orange and yellow clades, phylogenetic overdispersion; dark and light green clades, phylogenetic underdispersion; black clades, clades that did not differ from the random expectation.

Discussion

Growth responses of tropical tree seedlings to P addition in our pot experiment were remarkably consistent with the distributional affinities for P of saplings and adult trees described by the P effect sizes of Condit *et al.* (2013). Furthermore, our results suggest that the regional distribution patterns of trees in Panama may arise in part as a direct consequence of tradeoffs in growth performance among species in response to soil P. These differences in growth at the seedling stage seem unrelated to root

biomass allocation or PUE. Instead, differences in allocation to other P acquisition traits are likely to underpin species responses to P availability.

We found for the first time that in addition to differences in drought sensitivity among tree species (Engelbrecht *et al.*, 2007), tradeoffs in growth performance potentially underlie regional tree species distributions across the Isthmus of Panama. Our finding that species with high RGR also had higher plasticity in growth rate than species with lower RGR is consistent with a previous study (Huante *et al.*, 1995a). However, our finding of a tradeoff in growth rates across P treatments was not observed in earlier pot (Huante *et al.*, 1995a; Dent & Burslem, 2009) and field transplant experiments (Fine *et al.*, 2004; Palmiotto *et al.*, 2004). In the most similar study, Huante *et al.* (1995a) compared growth rates of 34 woody species in a pot experiment and found a positive correlation in species growth rates across treatments altering N, P, and potassium availability. The contrasting results of these studies may reflect several differences in the Huante *et al.* (1995a) experiment: the use of diverse plant functional groups with large intrinsic differences in allocation to growth, potentially confounding effects of seed nutrient reserves when including large-seeded species in growth experiments (Kitajima, 2002), or the potential absence of species that are adapted to sites with contrasting soil nutrient availability.

Plant roots synthesize a variety of phosphatase enzymes that hydrolyze organic P-containing compounds, releasing inorganic P for absorption by roots (Richardson *et al.*, 2005; Turner & Engelbrecht, 2011). Here we assayed acid phosphomonoesterase, which catalyzes the hydrolysis of a wide range of organic and condensed inorganic phosphates that are abundant in tropical forest soils (Turner & Engelbrecht, 2011). Contrary to our expectation, we did not find support for the hypothesis that investment in phosphomonoesterase explains species distributional affinities for P-poor soils. Paradoxically, six out of the nine species for which the phosphatase activity was greater on the −P treatment have positive P effect sizes. This result suggests that phosphomonoesterase enzymes may be more important for P acquisition in species from relatively P-rich soils. The ability to down-regulate phosphatase synthesis may also provide a competitive advantage for these species on high-P soils. Our results also indicate that the species that were unable to finely regulate phosphatase synthesis between the P treatments grew the least in response to P addition.

While root mass fraction was quite variable among species, this variation was not associated with species P effect sizes for either of the P treatments. In addition, we found a weak positive relationship between the percentage difference in RMF in response to P addition and the species P effect sizes, driven by higher plant biomass and associated RMF in the +P treatment. In general, biomass allocation may be relatively insensitive to P availability (but see the case of *Heliocarpus pallidus* in Huante *et al.*, 1995b). In a pot experiment that independently varied soil N and P availability to seedlings of the tropical pioneer tree *Ficus insipida*, biomass allocation responded strongly to variation in N availability, but did not respond to variation in P availability (Garrish *et al.*, 2010). Similarly, a fertilization experiment in the field in Panama found that seedling root-to-shoot biomass ratio did not respond to P addition when compared with unfertilized seedlings on the control plots (Santiago *et al.*, 2012).

In contrast to biomass allocation, foliar P responds strongly to P availability. Nutrient addition experiments along a Hawaiian soil chronosequence, and in lowland forest in Panama, showed that foliar P responded more strongly to P addition than did foliar N to N addition (Ostertag, 2010; Santiago *et al.*, 2012; Mayor *et al.*, 2014). While foliar P concentration also responded strongly to P addition in the present study, neither foliar P concentration nor the percentage difference of its change in response to P addition correlated with P effect sizes. This result suggests that differences in PUE are unlikely to contribute to the observed species responses in seedlings. However, foliar P concentrations from shade leaves of 137 adult trees collected on BCI correlated positively with their species P effect sizes (Dalling *et al.*, 2016), suggesting that differences in PUE may be more important in shade-tolerant species or in adult trees.

In the absence of response to P limitation in terms of phosphomonoesterase production, or in the allocation of biomass or P use among species, it is likely that other factors affecting P uptake play roles that were not identified in our pot experiment. One possible explanation is that these species invest in other classes of phosphatase enzymes (e.g. phosphodiesterase or phytase; Turner & Engelbrecht, 2011) that were not assayed in this experiment. Alternatively, species differences in the costs and benefits of inorganic P uptake via arbuscular mycorrhizal (AM) fungi may be important (Smith & Read, 2008; Plassard & Dell, 2010). All the species used in our pot experiment associate with AM fungi, as do pioneer species more generally (Kiers *et al.*, 2000; Mangan *et al.*, 2010; Pizano *et al.*, 2011). Other physiological features associated with tolerance of low-P conditions, including remobilization of internal P (Raghothama, 1999) and increased leaf longevity (Veneklaas *et al.*, 2012), are unlikely to be important to fast-growing seedlings in our short-term experiment.

As yet it is unclear whether the growth responses to P observed for pioneer species in our pot experiment extend to the much larger guild of slower-growing shade-tolerant trees. In a glasshouse experiment, where seedlings of a tropical forest pioneer and a shade-tolerant species were grown on different amounts of P supply, Raaimakers & Lambers (1996) found that both species took up similar amounts of P over time, but the pioneer species used it to increase growth rate, while the shade-tolerant species maintained the same growth rate and stored the extra P. To begin to address whether the responses of pioneer species observed here might differ from shade-tolerant trees, we compared the distribution of effect sizes for pioneers with that of the entire tree community, and tested whether the variation in effect sizes among species was reflected in variation in growth rates among saplings growing on the 50 ha forest dynamics plot on BCI.

For P, we found that 16% of the 550 species included in Condit *et al.* (2013) belong to clades in which effect sizes are overdispersed. Overdispersion, where effect sizes of closely related species are less similar than expected at random, is consistent with interspecific competition driving trait differentiation. By contrast, only 7% of the species included in Condit *et al.* (2013)

belong to overdispersed clades for N, consistent with the absence of significant effect sizes for this nutrient, while 24% of species belong to overdispersed clades for Ca. These results suggest that across all taxa, P does not have an inordinate effect on trait distribution across the phylogeny relative to other soil resources. However, a much larger fraction of the pioneers in our pot experiment (47%) were associated with overdispersed clades for P than the tree community in general, suggesting that the high tissue turnover rates and P concentrations necessary to sustain the high photosynthetic rates of pioneers (Reich *et al.*, 2009) may be important for trait differentiation and potentially for lineage diversification. By contrast, none of the pioneers in the study were in clades overdispersed for Ca. Nonetheless, P associations still have a measurable impact on shade-tolerant trees on BCI, reflected in the correlation between foliar P concentration and effect sizes (Dalling *et al.*, 2016), and in the observation that saplings of species from overdispersed clades growing in the BCI 50 ha forest dynamics plot have significantly higher variance in growth rates than those of underdispersed clades.

In conclusion, differences among species in their ability to grow under distinctive soil P conditions can act as a proximate mechanism determining tree species distributions across the Isthmus of Panama. Furthermore, our phylogenetic analysis suggests an evolutionary hypothesis for the diversification of species with high P demand across environments with contrasting P availability. Thus, the high local heterogeneity in soil P environments in Panama, in combination with climate variation (Engelbrecht *et al.*, 2007), may allow tree species to coexist at the regional scale, and potentially in other lowland tropical forests world-wide.

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Author contributions

P-C.Z., J.W.D., B.L.T. and K.W. designed the study. P-C.Z., J.W.D., C.S., F.A.J., B.L.T. and K.W. collected data and performed the statistical analyses. P-C.Z, J.W.D. and B.L.T. wrote the manuscript and all authors contributed to revisions.

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Supporting Information

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

Fig. S1 Foliar N : P ratio response to phosphorus addition.

Fig. S2 Foliar phosphorus concentration response to phosphorus addition.

Fig. S3 Net assimilation rate response to phosphorus addition.

Fig. S4 Root mass fraction response to phosphorus addition.

Fig. S5 Foliar phosphorus-use efficiency in response to phosphorus addition.

Table S1 Results of generalized linear mixed effect models

Table S2 Species list and traits information

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