Functional Ecology

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Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability

Running Head: Response of foliar traits to N and P addition

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Summary

1. Nitrogen (N) and phosphorus (P) are essential nutrients for plant metabolism and their availability often limits primary productivity. Whereas the effects of N-availability on photosynthetic capacity are well established, we still know relatively little about the effects of P availability at a foliar level, especially in P-limited tropical forests.

2. We examined photosynthetic capacity, leaf mass per area (LMA), and foliar P fractions in five woody plant species after six years of N and P fertilization in a lowland tropical forest.

3. Foliar N:P ratios indicated P limitation of the unfertilized plants; accordingly photosynthetic P-use efficiency (PPUE) and LMA decreased with P addition, and foliar N and P concentrations increased, whereas N addition had little effect on measured foliar traits. However, P addition enhanced photosynthetic capacity only in one species, and not in other four species. We then assessed plant acclimation to low P availability by quantifying four fractions of foliar P representing different functional pools: structural P, metabolic P (including inorganic P), nucleic acid P, and residual P. We found that P addition enhanced the concentrations of metabolic, structural, and nucleic acid P fractions in all species, but the magnitude of the effect was species-specific.

4. Our findings indicate that tropical species acclimate to low P availability by altering allocation of foliar P to meet the demand of P for photosynthesis. Importantly, species typical of lowland tropical forests in East Asia maintained their photosynthetic rate under low P availability. We conclude that P limitation of leaf photosynthetic capacity may not be as common as previously assumed due to plant acclimation mechanisms in low-P tropical forests. Species-specific strategies to allocate P to different foliar fractions represent a potentially important adaptive mechanism for plants in P-limited systems.

Introduction

Nitrogen (N) and phosphorus (P) are essential nutrients for photosynthetic carbon assimilation, and the most common nutrients limiting net primary productivity in terrestrial ecosystems. Nitrogen availability constrains plant productivity in many temperate and boreal forests by limiting leaf initiation and expansion (Vos & Biemond 1992) and the synthesis of Rubisco and other photosynthetic proteins (Evans 1989). However, plant productivity in tropical forests is generally regarded as P limited, rather than N limited, because soil P availability generally declines with bedrock weathering and soil age (Walker & Syers 1976). Quaternary glaciation exposed fresh bedrock over a large area of temperate and boreal regions, but not in the tropics (Vitousek 1984). In accordance, biogeochemical theory indicates that old, strongly weathered lowland tropical forests should be P limited (Vitousek 1984; Vitousek & Howarth 1991; Vitousek *et al.* 2010). However, tropical forests maintain the greatest plant biomass and the fastest rates of many biological processes (i.e. decomposition, N transformation) on Earth (Tanner *et al.* 1990). Therefore, identifying the strategies that tropical plants have evolved to use P efficiently under low soil P availability is an important topic in plant ecology (Hidaka & Kitayama 2009; Reed *et al.* 2011; Hidaka & Kitayama 2013; Lambers *et al.* 2015).

Shifting P allocation in leaves is an important mechanism for plants to acclimate to low soil P availability (Hidaka & Kitayama 2011). Foliar P is functionally divided into four major fractions: 1) metabolic P, including low-molecular-weight phosphate esters (e.g., ADP, ATP, and sugar phosphates), and inorganic phosphate (P_i); 2) nucleic acid P, most of which is contained in ribosomal RNA; 3) structural P in membrane phospholipids; and 4) residual P in phosphorylated proteins and unidentified residues (Kedrowski 1983; Hidaka & Kitayama 2011; Lambers *et al.* 2011). Of these, metabolic P is of particular interest for studying P limitation, because P-containing metabolites have key roles in the Calvin-Benson cycle, and insufficient metabolic P could limit maximum photosynthetic rates (Ågren, Wetterstedt & Billberger 2012). Nucleic acid P generally represents 40–60% of the organic P pool in leaves (Veneklaas *et al.* 2012). Over 85% of nucleic acid P is contained in RNA, especially ribosomal RNA (rRNA), and a high P allocation to

rRNA sustains rapid protein synthesis, which is required for growth and photosynthesis (Hidaka & Kitayama 2011). Therefore, there is generally a positive correlation between rRNA content and protein content, as well as growth rates, over a range of taxa (Elser *et al.* 2000). Structural P accounts for 10-20% of all foliar P (Veneklaas *et al.* 2012), and is contained mainly in phospholipids, which are an essential component of plasmalemma and organelle membranes. Finally, residual P can represent 20% of total foliar P in tropical trees (Hidaka & Kitayama 2011) and probably mostly comprises phosphorylated proteins. Generally, the concentration of the residual P fraction is relatively constant, because phosphorylated proteins are essential for many metabolic processes. However, under extremely P-limiting conditions, phosphatases may also dephosphorylate phosphorylated proteins (Schlüter *et al.* 2013), leading to a reduction in residual P concentrations.

Under P deficiency, photosynthesis is generally reduced (Turnbull, Warren & Adams 2007) due to feedback inhibition resulting from reduced leaf growth (Dissanayaka *et al.* 2018) or because orthophosphate (Pi) in the cytosol becomes limiting (Warren 2011). Plants also tend to increase their leaf mass per unit area (LMA; Chiera, Thomas and Rufty (2002), and increase their photosynthetic P-use efficiency (Hidaka & Kitayama 2009) when P supply is limited. Ellsworth *et al.* (2015) suggest that P-limitation of photosynthetic capacity is likely common in the field. However, since photosynthesis involves a series of chemical and physical processes that are dependent on nutrient supply, some degree of acclimation to P availability can be expected for plants growing on low-P soils. There is evidence that tree species growing on P-poor soils in tropical montane forests reduce their overall need for foliar P by decreasing the metabolic P fraction (Hidaka & Kitayama 2011). However, it is unclear whether such a reduction in metabolic P affects leaf photosynthetic capacity, because plant vacuoles serve as a reservoir of P_i (Mimura 1995), which buffers direct P_i restriction of photosynthesis. Although generally a short-term acclimation to low P availability, this buffering effect has been observed in many species (Rao & Terry 1995; Schachtman, Reid & Ayling 1998; Warren 2011). Another important P-saving mechanism under low P availability involves the replacement of phospholipids (structural P) in

membranes by sulfolipids and galactolipids (Rouached, Arpat & Poirier 2010; Lambers *et al.* 2012; Veneklaas *et al.* 2012), which can maintain foliar P metabolite concentrations at very low P availability. However, this mechanism appears to be an evolutionary adaptation to low P availability, and has so far only been reported in Proteaceae from severely P-impoverished soils in Australia (Lambers *et al.* 2012; Prodhan, Finnegan & Lambers 2019).

Lowland tropical forest trees have experienced long-term low P status; adaptations to P limitation thus likely evolved in these species, but field-based evidence for this is lacking. A recent study in a Panamanian tropical forest showed that P limitation of plant growth is species-specific, but does not translate into a community-wide response, because some species are adapted to low P availability and able to grow rapidly, despite low soil P availability (Turner, Brenes-Arguedas & Condit 2018). The finding redefined our understanding of P limitation in species-rich tropical forests; however, we do not fully understand the mechanisms by which tropical species are able to acclimate to low-P soils and maintain rapid growth.

Given the importance of photosynthetic capacity for plant performance, a better understanding of species-specific foliar P-allocation patterns and their relationship with photosynthetic capacity in low-P tropical forests is needed. We investigated this within an existing fertilization experiment in lowland tropical forest in China, using five woody plant species with distinct distributions: three species are limited to the lowland tropics (stenotopic), one species occurs in both tropical and subtropical forests of East Asia, and is also found in temperate regions (eurytopic; Fig. S1). To understand how plants acclimate to low-P soils through shifts in foliar traits and P-allocation pattern, we measured leaf photosynthesis, photosynthetic P-use efficiency (PPUE), LMA, leaf N and P concentrations, and leaf P fractions after six years of N- and P fertilization. We hypothesized that:

1) Fertilisation with P would have a much stronger effect on foliar traits (i.e. photosynthetic capacity, LMA, N and P concentrations) than fertilisation with N.

2) The response of photosynthetic capacity to P fertilization would be species-specific, whereby the maximum photosynthetic rate of stenotopic tropical species would be unaffected by P addition, as they are adapted to the low soil P availability.

3) Species-specific changes in the allocation of P to different leaf fractions (structural P, metabolic P, nucleic acid P, and residual P) explain the maintenance of photosynthetic capacity under low P availability.

Material and methods

Site description

The study was carried out at Xiaoliang Research Station for Tropical Coastal Ecosystems of the Chinese Academy of Sciences (21°27′N, 110°54′E), located in the southwest of Guangdong Province, China. This region is characterized by a tropical monsoon climate with a mean annual temperature of 23°C. Annual rainfall ranges from 1400 to 1700 mm, with dry season from November to March. The soil is classified as a latosol developed from granite (Wang *et al.* 2014). Annual wet N deposition in the region was *c.* 40 kg N ha^{-1} in 2011 and 2012 (Mo *et al.* 2015; Chen *et al.* 2016).

The study site was located in secondary broad-leaf mixed forest on coastal land (*c.* 5 km from the coast) with a very small slope. The forest was restored from *Eucalyptus exserta* plantation by introducing 312 plant species between 1964 and 1975. Thereafter, natural colonization during succession displaced almost all of the planted species, resulting in a typical secondary evergreen tropical forest with the biodiversity and structural complexity of natural forest (Mo *et al.* 2015; Chen *et al.* 2016).

Experimental design

An N- and P-fertilization experiment was established in a randomized block design within the secondary tropical forest in September 2009 (Zhao *et al.* 2014; Chen *et al.* 2016). Four 10-m × 10-m plots were established within each of five replicate blocks; adjacent blocks were separated by 50 m. Four treatments, N-addition (+N), P-addition (+P), N- and P-addition (+NP), and a control treatment (CT, no addition of mineral nutrients) were assigned randomly to the four plots within each block. The edges of each plot were trenched to a depth of 20-cm and surrounded by a 2-m wide buffer. Since most fine roots are distributed

in surface soils, the trenches largely inhibited the transfer of nutrients among treatments, as evidenced by clear differences between fertilized and unfertilized treatments in extractable soil P after six years of fertilization in 2015 (Table 1). Fertilizers were applied every two months from 2009 - 2015 to give total amounts of N and P equivalent to 100 kg ha^{-1} yr^{-1}. Briefly, for every fertilizer application, 476.6 g NH$_4$NO$_3$ (equal to 166.6 g N) and/or 808 g Na$_2$HPO$_4$ (equal to 166.6 g P) were dissolved in 30 L groundwater and then applied to the corresponding plots uniformly using a backpack sprayer near the soil surface; 30 L of groundwater were also applied to control plots (Wang *et al.* 2014; Li *et al.* 2015). The amount of added water in each plot was equivalent to 0.08% and 0.35% of rainfall inputs in the wet and dry seasons, respectively (Mo *et al.* 2015).

Measurement of foliar traits

We used previous vegetation surveys at the study site to identify the 10 most common understory species, and selected five common species that occurred in sufficient numbers in all experimental treatments. *Clerodendrum cyrtophyllum* Turcz.is a eurytopic shrub species, which is distributed widely from temperate forest to tropical forest in East Asia (Fig. S1). *Schefflera octophylla* (Lour.) Harms is a small tree that can grow to 15 m height and can be found in subtropical and tropical forests (He & Zeng 1978). *Syzygium bullockii* Hance. and *Psychotria rubra* (Lour.) Poir. are shrubs or small trees (Chen & Pie 1982; Chen 1984; Chen 1999), typical of lowland tropical forests (i.e. stenotopic species). *Uvaria microcarpa* Champ. Ex Benth is a shrub only found in tropical forests (i.e. a stenotopic species, Jiang and Li (1979). We selected individuals with a height of *c*. 2 m for consistency of *in situ* foliar level measurements. We measured foliar photosynthetic rates, leaf mass per area (LMA) and foliar N and P concentrations on mature leaves of all five species after six years of fertilization with N and/or P in 2015. Leaf photosynthetic rates were measured using a portable open-system infrared gas analyzer (LI-6400, LI-COR Biosciences, Lincoln NE, USA). Measurements were carried out during seven days in August 2015 between 8:00 AM and 12:00 PM on fully expanded healthy sun-exposed mature leaves. Three to seven individuals per species were measured in each treatment. Rates of CO$_2$ exchange were measured *in situ* under controlled cuvette conditions set to 1000 μmol m^{-2} s^{-1} PAR, 400 μmol CO$_2$ mol^{-1}, a chamber temperature of 25 ±1ºC and a vapor pressure deficit of 0.5-1.0 kPa. The photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} was the same as previous studies in tropical forests (Hidaka & Kitayama 2009; Hidaka & Kitayama 2013), and is generally a saturating light value for photosynthesis in these understory species. Leaves measured for CO$_2$-assimilation rates were subsequently collected to determine LMA using a portable leaf area meter (LI-3000A, LI-COR Biosciences) and a second leaf sample was collected from the same branch for chemical analyses. All leaf samples were stored on ice, returned to the lab

and washed within 6 h. After determination of leaf area, the first leaf samples were dried to constant weight at 50ºC for 72 h and weighed to determine dry mass and LMA (g m^{-2}). The second leaf sample was freeze-dried for analysis of foliar N and P concentrations (mg g^{-1} dry weight) and P fractions. Leaf samples were ground after removing petioles and main veins, and foliar P concentrations were measured spectrophotometrically after digestion with sulfuric acid (H_2SO_4); foliar N concentrations were determined using the Kjeldahl method (Wang *et al.* 2013). Photosynthetic nutrient use efficiency for N (PNUE) and P (PPUE) was defined as the rate of net photosynthesis per unit N or P expressed on a leaf dry mass basis, and photosynthetic capacity is given on dry mass basis (A_{mass}; nmol CO_2g^{-1}s^{-1}) and leaf area basis (A_{area}; umol CO_2m^{-2}s^{-1}).

Measurement of P fractions

Foliar P was partitioned into four fractions: structural P, metabolic P (including P_i), nucleic acid P and residual P, using sequential extraction (Kedrowski (1983) with modifications (Hidaka and Kitayama (2011). First, *c.* 0.5 g of freeze-dried leaf sample was weighed into a 50-ml centrifuge tube (tube 1); it was then extracted twice with 7.5 ml 12:6:1 CMF (chloroform, methanol, formic acid, v/v/v). The liquid extract was transferred into a clean tube (tube 2). The residue from the initial extraction was then extracted twice with 9.5 ml 1:2:0.8 CMW (chloroform, methanol, water, v/v/v), and the liquid solvent was also added to tube 2. The remaining residue was then mixed with 9.5 ml water-washed chloroform and the supernatant was also transferred to tube 2, and mixed thoroughly. The upper phase of the extract in tube 2 was transferred to a clean tube (tube 3) and the lower lipid-rich phase was used to determine structural P.

All liquid extracts in tube 3 were mixed with those remaining in tube 1, and 5 ml methanol (85% v/v) was added; the tube was then placed in a vacuum dryer for 48 h to remove residual chloroform and methanol. The extract was refrigerated (4ºC) for 1 h. First, 1 ml TCA (trichloroacetic acid, 100%) and then 10 ml TCA was added and the extract was shaken for 1 h before being centrifuged at 3000 g. The supernatant was analyzed for metabolic P.

Finally, the residue was mixed with 35 ml TCA (2.5%, w/v), extracted for 1 h in a hot water bath (95ºC), and centrifuged at 3000 g after cooling to room temperature. The liquid layer was measured for nucleic acid P and the residue from this final extraction was analyzed for residual P. All foliar P fractions were measured spectrophotometrically after digestion with sulfuric acid (H_2SO_4) and are expressed on a dry mass basis (mg g^{-1}).

Data analyses

All data analyses were conducted in R version 3.1.0 (R Core Team 2017), using the nlme package (Pinheiro *et al.* 2016) for linear mixed effects models and the FactoMineR package (Le, Josse & Husson 2008) for multivariate analyses. As our experiment used a randomized block design, we first used linear models (Analysis of Variance) to assess the block effect on soil nutrients before fertilization (Table 1), and found no significant block effect on soil extractable N or P. We then used linear mixed effects models (*lme* function) to examine the effects of N-addition, P-addition and species identity on leaf parameters. In the initial model, N-addition, P-addition, species and their interaction were considered fixed effects, and plots within blocks as the random effect. We compared nested models using likelihood ratio tests and AICs to check for model improvement (Pinheiro & Bates 2000). There was generally a better model fit (lower AIC values) with interaction terms; we thus selected the full factor model and assessed the significance of each fixed effect using the *anova* function. As there were significant interactions between treatments and species (Table 2), we also investigated species-specific responses to N- and P addition using separate linear models with block as an error term (Table S1). Where models for individual species were significant, post-hoc tests (Tukey's HSD) were conducted for multiple comparisons among treatments. Results are reported as significant at p<0.05.

Results

All measured foliar traits varied strongly among the five study species and the responses of foliar traits to nutrient additions were often species-specific (Table 2), indicating different nutrient-use or allocation strategies in our study species.

Photosynthesis and leaf mass per area (LMA)

Although there was no overall effect of N- and/or P addition on photosynthetic rate, significant interactions indicated species-specific responses to N and P addition (Table 2). Both of A_{eara} and A_{mass} showed the similar patterns among species and fertilization treatments (Fig.1 & Table 2). Analyses for each species individually showed that A_{mass} was affected by the interaction of N and P in *C. cyrtophyllum* and *S. octophylla* (Table S1)*. A_{mass} in *C. cyrtophyllum* (i.e. the eurytopic species) increased only when P was added alone, resulting in significantly higher A_{mass} in the +P treatment than in the +NP, +N or control treatments (Fig. 1). By contrast, A_{area} in *S. octophylla* was significantly lower in the +P treatment than in the control, but the negative effect of P addition was ameliorated by the addition of N (Fig. 1).

Leaf mass per area (LMA) in the control ranged from 40 g m^{-2} to 71 g m^{-2} (Fig. 1; Table 2), and decreased significantly with P addition (Table 2), with the strongest reduction in *C. cyrtophyllum* and *S. octophylla* (*p=0.006* and *p=0.02*, respectively; Fig 1, Table S1). There was no effect of N addition on LMA for any species (Fig. 1; Table S1).

Foliar N and P concentrations and N:P ratios

The response of foliar N concentrations (hereafter 'foliar [N]') to fertilizations was strongly influenced by species identity and treatment, but overall foliar [N] was slightly but significantly higher in treatments with added N (Table 2). Foliar P concentrations (hereafter

foliar [P]) increased markedly with P addition in all species (Fig. 2; Table 2). Foliar [P] increased by 33% - 107% in +P treatments compared with controls, and by 27% - 121% in +NP treatments. Foliar N:P ratios in control plots indicated P limitation at the study site: *C. cyrtophyllum* had the highest foliar N:P ratio of 33; *P. rubra* and *S. octophylla* had similar intermediate N:P ratios (25 and 24, respectively), whereas the foliar N:P ratios in *S. bullockii* and *U. microcarpa* were somewhat lower (20 and 21, respectively). A decline in foliar N:P ratios was observed in all species as a result of foliar P accumulation in the +P and +NP treatments (Fig. 2). Nitrogen addition did not affect foliar N:P ratios in any species, except *S. bullockii* (Fig. 2, Table S1), which had a higher foliar N:P ratio with N addition as a result of increased foliar [N].

Photosynthetic phosphorus- and nitrogen-use efficiency

PNUE was not affected by N addition, whereas PPUE decreased with P addition (Table 2; Fig. S2). PPUE was 9% to 56% lower in the +P treatment and 18% to 51% lower in the +NP treatment compared to the controls. Species identity modified the response of PNUE and PPUE to N and P additions (Table 2), but individual analyses only showed significantly higher PNUE in *C. cyrtophyllum* in the +P treatment, and lower PPUE in *C. cyrtophyllum* and *S. octophylla* in the +NP and +P treatments, respectively (Fig. S2).

Foliar phosphorus fractions

Overall, only residual P increased with N addition (Table 2) and this result was largely due to an increase in residual P in *C. cyrtophyllum* in the +NP treatment (Table 3). There was a strong increase in all P fractions with P-addition (Tables 2 and 3), and species identity

significantly influenced the response of metabolic P, nucleic acid P and residual P, and marginally influenced the response of structural P (P × species interactions; Table 2). Metabolic P (which includes P_i) increased the most in response to P-addition, followed by structural P and nucleic acid P (Table 3 & Table S2). The pattern for residual P was less clear: there was a significant increase of residual P in *C. cyrtophyllum* and *P. rubra* in the +P and +NP treatments, but not in any other species (Table 3).

Discussion

It is thought that the productivity of lowland tropical forests is limited by P, rather than N (Thomas, Montagu & Conroy 2006; Pasquini & Santiago 2012). However, experimental manipulations have failed to detect a consistent response to P addition in species-rich lowland tropical forests (Cleveland *et al.* 2011; Wright *et al.* 2018). A recent study indicated that P limitation is widespread at the level of individual species, but not at the community level, because some species grow relatively rapidly on infertile soils, despite extremely low P availability (Turner, Brenes-Arguedas & Condit 2018). Our study showing altered allocation to foliar P fractions after P addition provides a potential mechanism to explain how tropical species can maintain photosynthetic capacity under low P availablity.

Fertilization effects on foliar traits and P fractions

Increased LMA has been widely reported as a sign of N or P deficiency in crop species (Chiera, Thomas & Rufty 2002; Assuero, Mollier & Pellerin 2004) and although we observed increased foliar [N] in response to N-addition in two species (*C. cyrtophyllum* and *S. bullockii*), we measured no corresponding change in LMA. By contrast, LMA declined markedly with P addition in all five species, especially in *C. cyrtophyllum* and *S. octophylla* (Fig. 1), suggesting that P addition alleviated P deficiency in these species. This supports our first hypothesis that foliar traits would show a greater response to P than N addition. Species-specific responses of foliar nutrients and LMA to fertilization (Table 2, Fig. 1) may reflect differences in their life history strategies and resource requirements (Sayer & Banin 2016). Overall, these results provide evidence for P limitation of tree growth in the studied forest.

Despite several lines of evidence suggesting P limitation in tropical forests (Vitousek *et al.* 2010; Turner, Brenes-Arguedas & Condit 2018; Wright *et al.* 2018), photosynthetic capacity was only increased by P addition in the eurytopic species *C. cyrtophyllum* which is consistent with our second hypothesis and demonstrates that species growing on low-P soils can maintain photosynthetic capacity. Similarly, there was no response of photosynthetic rate to N and P fertilization in understory species of a tropical plantation (Zhu, Lu & Mo 2014) or in response to P addition in lowland tropical forest understory seedlings after 10 years of P fertilization (Pasquini & Santiago 2012). The results, combined with LMA results, also agree with studies of several crop species which demonstrated that photosynthesis is far less sensitive to fertilization than leaf growth (Chiera, Thomas & Rufty 2002; Assuero, Mollier & Pellerin 2004; Dissanayaka *et al.* 2018).

Various adaptive strategies allow plants to acclimate to low P availability. Generally, plants on P-impoverished soils have evolved physiological mechanisms for maximizing soil P acquisition, such as increasing root surface area, carboxylate exudation, and phosphatase release (Reed *et al.* 2011; Zemunik *et al.* 2015). Our results show that the low P availability in tropical forests does not necessarily affect rates of photosynthesis. PPUE decreased with P addition in our study, showing that the unfertilized plants have efficient P-use strategies and maintain functions despite low availability of soil P. Increased PPUE has been observed in response to decreasing P availability along a natural soil fertility gradient, where plants were able to maintain rates of photosynthesis despite a remarkable decline in foliar [P] (Hidaka & Kitayama 2009). Changes in foliar P allocation may help explain how plants can maintain stable rates of photosynthesis when P availability is low.

Vacuoles in plant cells serve as a reservoir of P_i (Mimura 1995), which is required for export of triose phosphates from chloroplasts and for photophosphorylation. Accordingly, we observed large increases in metabolic [P] (most of which is likely to be [P_i]; (Veneklaas *et al.* 2012), with P addition in all species. The ability of plants to take up and store additional P_i or a relatively low capacity to down-regulate P_i uptake (Ostertag 2010), may explain the observed large changes in metabolic [P]. However, since photosynthetic capacity was unaffected by P addition in the stenotopic species, we expect these plants were able to maintain sufficient metabolic [P], despite low soil P status at the study site.

The mechanism by which plants maintain sufficient metabolic P concentrations to support stable rates of photosynthesis has been reported in crop plants (Schlüter *et al.* 2013) and Proteaceae from severely P-impoverished soils in Australia (Lambers *et al.* 2015), but has never been reported for tropical lowland forests. In this study, the metabolic P fraction includes P_i, and when P availability decreases, excess P_i stored in vacuoles (Veneklaas *et al.*

2012) is released to maintain the $[P_i]$ in the cytosol, followed by release of P from membrane phospholipids (Mimura 1995; Schachtman, Reid & Ayling 1998; Lambers *et al.* 2012). As plant growth rates decrease, nucleic acid P will eventually also be used to maintain the cytosolic $[P_i]$ in cells. Rates of photosynthesis will therefore only be affected when the cytosolic $[P_i]$ can no longer be maintained (Schachtman, Reid & Ayling 1998). Hence, under low soil P availability, a certain proportion of foliar P is allocated to metabolic [P] (including P_i) to maintain photosynthesis which could draw P from structural P (Hidaka & Kitayama 2013), although we found little evidence for this link in our study, possibly because the plants constitutively function at low phospholipid concentrations, as in Proteaceae (Lambers *et al.* 2012; Prodhan, Finnegan & Lambers 2019). By contrast, we observed the expected increases in all foliar P fractions with P-addition, with the largest increase in $[P_i]$ due to luxury uptake and storage of P_i (Ostertag 2010).

Our current knowledge of the residual P fraction is insufficient to explain why P-addition resulted in a negative relationship between photosynthetic rates and residual [P] in this study (Fig. S3); further work is needed to clarify the functional role of the residual P fraction.

Insights into species-specific acclimation to P availability through foliar P allocation

The distinct responses of foliar traits among the five study species revealed different P requirements and allocation. It is noteworthy that *C. cyrtophyllum*, a eurytopic species with a wide distribution from temperate to tropical zones, not only had the highest foliar [P] of all species, regardless of treatment (Fig. 2), but was also the only species showing increased photosynthetic capacity with P addition. The substantial increase in nucleic acid [P], combined with the decline in LMA, suggests that leaf growth increased with P addition in this species, but we did not measure this directly. These results, combined with the increased PNUE with P addition, suggest that *C. cyrtophyllum* had the highest P requirement of all the studied species, and its photosynthetic capacity was limited by low P availability in this tropical forest. The high plasticity of *C. cyrtophyllum* for re-allocating P in response to P availability may also help to explain its wide distribution in temperate and tropical forests (Fig. S1).

Schefflera octophylla, which is distributed in subtropical and tropical forest, also showed lower LMA with P addition as well as increased concentrations of foliar P and all P fractions, except residual P, but its photosynthetic capacity actually declined in the +P treatment. The distinct responses of foliar traits in *S. octophylla* to individual nutrients compared with the +NP treatment suggests that leaf area in this species is potentially P-limited, but that maintaining foliar N:P stoichiometry may be more important for photosynthesis than

increases in either nutrient alone.

Syzygium bullockii, which only occurs in tropical forest, showed the greatest increase in structural [P] in response to P addition, whereas LMA and photosynthetic capacity were not affected by fertilization (Fig. 2). This suggests that *S. bullockii* utilizes more P from phospholipids to maintain photosynthetic rates and leaf area when P availability is low. Increased nucleic acid and structural P fractions with P fertilization in this species also suggest that foliar growth is P limited. Finally, the lack of changes in photosynthetic capacity or LMA in *P. rubra* and *U. microcarpa* following fertilization suggest that they are well adapted to low-P soils. Both species only occur in tropical forests and *P. rubra* in particular showed no evidence of P limitation, whereas small increases in metabolic and nucleic acid [P] in *U. microcarpa* (Table S2), suggest that some foliar processes may be P-limited.

Conclusions

In tropical forests on P-poor soils, plants exhibit morphological, physiological, molecular, and biochemical adaptions to low P availability. Although we found some evidence for P limitation in this tropical forest, four species in this study maintained their photosynthetic capacity, despite low P availability. Our measurements of foliar P fractions demonstrate that the study species acclimated to low P availability by reducing P allocation to non-metabolic foliar P fractions to meet their demand for metabolic P to maintain photosynthetic capacity. We conclude that P limitation of leaf photosynthetic capacity may not be as common as previously assumed for lowland tropical forests, and some species have a high capacity to maintain their photosynthetic rate in low-P soils. The species-specific strategies to allocate P to different foliar fractions represents a potentially important adaptation mechanism for plants in P-limited systems.

Author contributions:

F.W. and Z.L. planned and designed the research. Q.M., B.Z., Y.L. and S.Y. conducted field work. F.W., Q.M. and E.S. analyzed data. F.W., Q.M., E.S., H.L., J.T., M.H., Z.L. and X.Z. wrote the manuscript.

Data accessibility:

Conflicts of interests:

The authors declare that they have no conflict of interests.

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Second Language Abstract: Chinese

氮和磷是植物代谢过程中的重要养分也是限制生态系统初级生产力的主要因子。尽管我们对氮如何影响植物光合能力有了比较全面的认识，但在低磷的热带森林中，我们对土壤磷如何影响植物光合功能还缺乏系统的了解。本研究通过在热带森林的野外氮磷添加实验中测定不同植物的叶片光合能力和叶片功能性状以及叶片磷组分，系统的了解了这些植物在叶片尺度上的低磷环境的适应机制。叶片氮磷比的结果表明该森林是磷限制的生态系统，施磷降低了叶片磷利用效率和比叶重但增加了叶片磷和氮的浓度，而施氮对叶片性状无显著影响。施磷仅增加了一个广布种的叶片光合能力而对其他四个狭布种无显著影响。我们进一步了解了叶片磷组分的变化，发现施磷处理增加了所有物种的代谢磷、结构磷和核酸磷组分，但其增加的尺度因种而异。这些结果表明热带森林植物通过改变叶片中磷的分布来满足光合作用对磷的需求，并且东亚地区的热带森林的典型植物能够在低磷的土壤环境中维持相对稳定的光合速率。通过该项研究，我们认为由于热带植物进化的适应机制，低磷对热带森林植物的光合能力的限制比预想的要小的多。不同植物的叶片磷组分的分配策略是其重要的对低磷环境的适应机制。

Tables:

Table 1 General soil chemical properties (0-10 cm) in fertilization treatments in a secondary coastal forest in China before the start of fertilizer application in September 2009 and after six years of fertilization treatments in May 2015; where CT is control, and +N, +P or +NP denote fertilization with nitrogen (N), phosphorus (P) or both nutrients, respectively; means ±SE are shown for n=5. Different superscript letters indicate significant differences among treatments at $p<0.05$. TP: total P concentration, TN: total N concentration, AP: available phosphorus; data for 2009 are reported in Li et al. (2015).

Date	Variables	CT	+N	+P	+NP
Sep. 2009	pH (H_2O)	4.0±0.06	4.0±0.05	4.0±0.05	4.0±0.09
	AP(mg kg^{-1})	4.10±0.56	3.79±0.42	4.06±0.37	3.70±0.60
	NO_3^--N (mg	2.88±0.35	2.72±0.11	2.68±0.31	2.35±0.33
	NH_4-N(mg kg^{-1})	2.12±0.12	1.85±0.13	1.81±0.11	2.03±0.17
	TN (g kg^{-1})	2.71±0.15	2.34±0.21	2.66±0.10	2.68±0.19
	TP (g kg^{-1})	0.40±0.03	0.38±0.02	0.42±0.02	0.43±0.03
May. 2015	pH (H_2O)	3.8±0.02	3.8±0.06	3.9±0.06	3.9±0.06
	AP (mg kg^{-1})	4.60^c±0.57	5.60^c±0.69	71.4^a±2.8	53.2^b±9.15
	NO_3^--N (mg	6.02±0.75	7.42±1.58	3.11±0.46	6.04±1.85
	NH_4-N(mg kg^{-1})	3.55±0.36	4.11±0.46	4.19±0.73	4.16±0.50
	TN (g kg^{-1})	1.31±0.05	1.36±0.04	1.25±0.09	1.50±0.12
	TP (g kg^{-1})	0.35^b±0.02	0.39^b±0.01	0.65^a±0.02	0.58^a±0.05

Table 2 Significance (p-values) of terms from linear mixed model analysis for foliar traits, nutrient concentrations and phosphorus fractions of five woody species in a tropical forest following six years of fertilization with nitrogen (N) and phosphorus (P), where S is species; N is N-addition; P is P-addition; A_{area} is photosynthetic rates per unit area; A_{mass} is photosynthetic rates per unit mass; LMA is leaf mass per unit area; PNUE is photosynthetic N-use efficiency and PPUE is photosynthetic P-use efficiency.

Fixed effect	A_{area}	A_{mass}	LMA	N concentration	P concentration	N:P ratios	PNUE	PPUE	Metabolic P	Nucleic P	Structural P	Residual P
S	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
N	*ns*	*ns*	*ns*	*<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.009*
P	*ns*	*ns*	*0.001*	*0.001*	*<0.001*	*<0.001*	*ns*	*0.005*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
N×S	*0.020*	*ns*	*ns*	*0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.001*
P×S	*ns*	*ns*	*ns*	*0.001*	*0.001*	*0.005*	*ns*	*0.038*	*0.006*	*0.016*	*ns*	*<0.001*
N×P	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.004*
N×P×S	*<0.001*	*0.005*	*ns*	*0.038*	*ns*	*ns*	*0.002*	*0.040*	*ns*	*ns*	*ns*	*0.027*

Table 3 Concentrations of foliar phosphorus (P) fractions (mg g^{-1} dry weight) of five woody plant species in a tropical forest following six years of fertilization with nitrogen (+N), phosphorus (+P) or both nitrogen and phosphorus (+NP) compared with controls (CT); means ±S.E. are given. Different superscript letters indicate significant differences among treatments at p<0.05.

Species	Treatment	Metabolic P	Nucleic acid P	Structural P	Residual P
Clerodendrum cyrtophyllum	CT	0.49±0.14	0.24^{b}±0.06	0.32±0.03	0.12^{b}±0.03
	+N	0.49±0.05	0.33^{ab}±0.01	0.35±0.05	0.14^{b}±0.01
	+P	1.36±0.25	0.44^{ab}±0.06	0.54±0.08	0.27^{b}±0.09
	+NP	1.23±0.26	0.47^{a}±0.04	0.42±0.06	0.49^{a}±0.27
Syzygium bullockii	CT	0.15^{a}±0.02	0.24^{ab}±0.02	0.09±0.05	0.24±0.02
	+N	0.13^{a}±0.01	0.18^{b}±0.01	0.10±0.04	0.19±0.02
	+P	0.43^{a}±0.20	0.30^{a}±0.03	0.28±0.08	0.24±0.01
	+NP	0.23^{ab}±0.05	0.26^{ab}±0.03	0.21±0.02	0.23±0.01
Psychotria rubra	CT	0.12±0.02	0.18^{a}±0.01	0.13±0.03	0.42^{b}±0.01
	+N	0.20±0.07	0.19^{a}±0.03	0.16±0.02	0.46^{ab}±0.02
	+P	0.30±0.07	0.24^{a}±0.02	0.16±0.02	0.51^{a}±0.03
	+NP	0.19±0.03	0.21^{a}±0.02	0.15±0.03	0.53^{a}±0.02
Schefflera octophylla	CT	0.21^{b}±0.02	0.17^{b}±0.01	0.35±0.03	0.10±0.01
	+N	0.24^{b}±0.01	0.16^{b}±0.01	0.36±0.03	0.10±0.00
	+P	0.47^{a}±0.02	0.26^{a}±0.01	0.47±0.03	0.10±0.01
	+NP	0.90^{a}±0.28	0.33^{a}±0.05	0.52±0.18	0.11±0.01
Uvaria microcarpa	CT	0.27±0.06	0.27±0.04	0.21±0.04	0.25±0.03
	+N	0.27±0.02	0.27±0.00	0.25±0.03	0.22±0.01
	+P	0.66±0.15	0.33±0.03	0.31±0.03	0.23±0.02
	+NP	0.60±0.15	0.27±0.02	0.31±0.04	0.21±0.00

Figures Caption

Fig. 1 Box-plots of area-based and mass-based photosynthetic rates (A_{area} and A_{mass}) and leaf mass per unit area (LMA) for five woody species in a tropical forest following six years of fertilization with nitrogen (+N), phosphorus (+P) or both nutrients (+NP) compared to controls (CT). Different letters within panels indicate post-hoc significant differences among treatments at $p<0.05$.

Fig. 2 Box-plots of foliar nitrogen (N) and phosphorus (P) concentrations and N to P ratio for five species in a tropical forest following six years of fertilization with N (+N), P (+P) or both nutrients (+NP) compared to controls (CT). Different letters within panels indicate significant post-hoc differences among treatments at $p<0.05$.