



## Negative effects of long-term phosphorus additions on understory plants in a primary tropical forest



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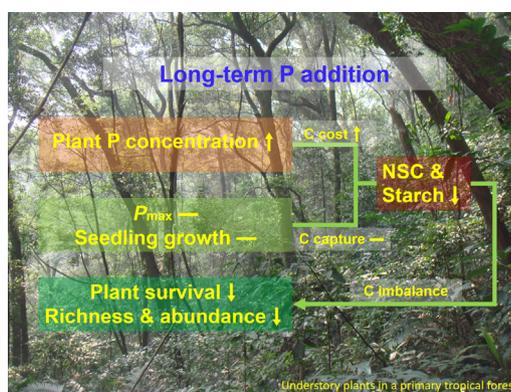
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### HIGHLIGHTS

- Effects of long-term P addition on understory plants were studied in a primary tropical forest.
- P addition increased plant P concentration, but not photosynthetic capability and growth.
- P addition decreased the survival rate of young seedlings.
- P addition reduced the species richness and density of understory plants.
- Negative effects due to C imbalance from increased cost to maintain higher P concentration

### GRAPHICAL ABSTRACT



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### ABSTRACT

Human activities have disturbed global phosphorus (P) cycling by introducing substantial amounts of P to natural ecosystems. Although natural P gradients and fertilization studies have found that plant community traits are closely related to P availability, it remains unclear how increased P supply affects plant growth and diversity in P-deficient tropical forests. We used a decadal P-addition experiment (2007–2017) to study the effects of increased P input on plant growth and diversity in understory layer in tropical forests. We monitored the dynamics of seedling growth, survival rate, and diversity of understory plants throughout the fertilization period under control and P addition at  $15 \text{ g P m}^{-2} \text{ yr}^{-1}$ . To identify the drivers of responses, P concentration, photosynthesis rate and nonstructural carbon were analyzed. Results showed that long-term P addition significantly increased P concentrations both in soil pools and plant tissues. However, P addition did not increase the light-saturated photosynthesis rate or growth rate of the understory plants. Furthermore, P addition significantly decreased the survival rate of seedlings and reduced the species richness and density of understory plants. The negative effects of P addition may be attributed to an increased carbon cost due to the tissue maintenance of plants with

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higher P concentrations. These findings indicate that increased P supply alone is not necessary to benefit the growth of plants in ecosystems with low P availability, and P inputs can inhibit understorey plants and may alter community composition. Therefore, we appeal to a need for caution when inputting P to tropical forests ecosystems.

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## 1. Introduction

Phosphorus (P) is an essential nutrient for plant metabolism and growth (Vitousek et al., 2010). However, P deficiency is widespread, as approximately 43% of the global natural land area is P-limited (Du et al., 2020). Currently, human activities are changing P cycling by introducing external P to natural and semi-natural ecosystems. Anthropogenic modification has doubled the global P influx since the industrial revolution (Peñuelas et al., 2013). Elevated atmospheric P deposition has occurred due to increasing agricultural pollution and fossil fuel combustion-related sources in many regions such as Europe and Asia (Pan et al., 2021). In the future, the role of P will become increasingly important because the areas affected by external P inputs are growing. Increased P availability may alter the structure and function of terrestrial ecosystems (Steffen et al., 2015), such as their productivity and species diversity (Ceulemans et al., 2014; Zemunik et al., 2016; Wassen et al., 2021). Therefore, understanding the long-term effects of P inputs on plant growth and diversity is critical for predicting changes in ecosystem dynamics in the context of global change.

Humid tropical forests are generally P-depleted because soil parent materials are highly weathered, with the majority of P being leached out (Walker and Syers, 1976). The rest of the P in soils mostly occludes in secondary minerals and is not available to plants (Vitousek et al., 2010; Yang et al., 2013). This limitation of P is an important basis for biogeochemical niche partitioning (Turner, 2008), which shapes the pattern of species composition in tropical forests (Zemunik et al., 2016). Moreover, P limitation is becoming popular with increasing nitrogen (N) deposition in the tropics (Marklein and Houlton, 2012). Accordingly, tropical forests may be very sensitive to external P input. There is increasing concern regarding the inputs of P in the tropics. First, the application of P fertilizers is widespread in the tropics, especially when native tropical forests are converted to plantations such as coffee and rubber plantations or soya bean fields (Perfecto et al., 1996; Rodrigues et al., 2016). Second, P addition has been proposed as a practical measure to alleviate the negative effects of N deposition (Chen et al., 2016). For example, the addition of P was found to decrease nitrate (NO<sub>3</sub><sup>-</sup>) leaching and nitrous oxide (N<sub>2</sub>O) emissions (Chen et al., 2016) and mitigate the inhibition of N deposition on methane (CH<sub>4</sub>) uptake in tropical forests (Zhang et al., 2011; Yu et al., 2017). However, after P addition, increased soil P availability would persist for a long time because of the strong adsorption of P by iron and aluminum oxides (Bouwman et al., 2009; Lambers et al., 2013). Considering that tropical forests support the highest species diversity and production in terrestrial ecosystems (Raven et al., 2020), knowledge about the responses of P-depleted tropical forests to P inputs has particular significance.

Increased P supply is expected to alleviate P limitation (Siddique et al., 2010; Hou et al., 2020) and benefit photosynthetic C assimilation because P-containing metabolites play key roles in the Calvin cycle (Ågren et al., 2012; Soons et al., 2017). However, the effects of P addition may vary among different species and functional groups because of a variety of functional traits related to P acquisition and use within a community (Alvarez-Clare et al., 2013; Lambers et al., 2015; Phoenix et al., 2020). In light of the species-specific responses to P addition (Mayor et al., 2014), a decrease in plant diversity is expected due to the intensification of interspecific competition (Soons et al., 2017; Stiles et al., 2017). Some experimental evidence also supports this view (Hautier et al., 2009; Soons et al., 2017). It has also been reported that species richness decreases with elevated P availability along several regional

gradients (Condit et al., 2013; Ceulemans et al., 2014; Wassen et al., 2021). Considering that most of the experimental fertilization studies were conducted in N-limited but P-rich temperate ecosystems (Soons et al., 2017), it is interesting to know whether the interspecific competition induced by P application can be echoed in N-rich but P-poor tropical ecosystems (Vitousek et al., 2010; Lu et al., 2021).

Recent studies in tropical forests have challenged the view that increased P supply would benefit plant growth in P-depleted environments. First, whether tropical forests are P-limited is still controversial because some plant species can grow fast despite the extremely low P environment (Alvarez-Clare et al., 2013; Turner et al., 2018). Thus, the fertilization effects may not be fully applicable to tropical forests because most species in this region may adapt to low P habitats. Second, experimental studies failed to observe a consistent response to P addition in tropical forests. For example, the effect of P addition on the photosynthetic capacity and growth of tropical plants varied in different studies, with no response to P addition for a large proportion of plants (Burslem et al., 1995; Mo et al., 2019). The effects of P addition also depend on the specific functional properties of plants including shade tolerance (Lawrence, 2003; Alvarez-Clare et al., 2013). It has been reported that the growth of shade-tolerant species is less susceptible to P addition (Lawrence, 2003), while increased tissue P may increase the metabolic costs and influence the survival rate. These phenomena or uncertainties indicate that the consequences of P inputs on plants in P-deficient tropical forests are still not clear and require further study.

In this study, we explored the impacts of enhanced P input in tropical forests on the growth and diversity of understorey plants using a long-term P addition experiment (2007 to 2017) that was conducted in a primary tropical forest in Southern China. Understorey layer plants were selected because they are critically important in forest regeneration and species turnover (Zalamea et al., 2016) and are relatively sensitive to the dynamics of resources compared to canopy trees (Gilliam, 2007; Lu et al., 2010; Dirnboeck et al., 2014). Previous studies on soil microbial biomass, phosphatase activities, and foliar P absorption suggest that the bioavailability of P is low in this forest (Wang et al., 2018; Zhou et al., 2018). Thus, we hypothesized that long-term P addition would increase plant tissue P concentration, photosynthetic capability, and growth, thus decreasing species abundance in the understorey layer through interspecific competition.

## 2. Methods

### 2.1. Study site

Our study was performed at the Dinghushan Biosphere Reserve in the central Guangdong province in Southern China (23° 10' 12" N, 112° 32' 42" E). The reserve occupies an area of 1150 ha, and over 1300 native vascular plant species have been recorded (Zhou, 1982). The mean annual temperature of the area is 21 °C, with the coldest month in January (mean of 12.6 °C) and the warmest month in July (mean of 28.0 °C). The mean annual precipitation is 1927 mm, with 75% of the rainfall falling from March to August (Huang and Fan, 1983). The soil is lateritic red earth (oxisols, the U.S. soil taxonomy system), which forms from a sandstone regolith and extends >60-cm deep (Mo et al., 2003). Fine roots mostly grow in the top 0–10 cm soil.

The experimental plots were established in the evergreen broadleaf forest (250–300 m elevation), which has been well protected from human disturbance for more than 400 years (Zhou et al., 2006). The

forest is a regional climax and occupies 20% of the reserve (Mo et al., 2003). The stratified aboveground community of the forest comprises canopy, sub-canopy, and understory plants. Dominant species in the canopy and subcanopy layers are native evergreen tree species, including *Castanopsis chinensis* Hance, *Machilus chinensis* Hemsl, *Schima superba* Gardn. et Champ, *Cryptocarya chinensis* Hemsl, and *Syzygium rehderianum* Merr. et Perry. Seedlings, shrubs, and herbaceous plants constitute the understory layer, accounting for a large proportion of plant diversity (Tables S1 and S2). Canopy openness is generally less than 5%, without any large forest gaps (Lu et al., 2010). The forest floor has a typical thin litter layer (1–2 cm) due to the rapid decomposition.

## 2.2. Experimental treatments

The P addition experiment was established in 2007 (Liu et al., 2012), including two treatments of blank control and P addition at  $15 \text{ g P m}^{-2} \text{ year}^{-1}$ . The dose of P addition was designed to saturate chemical and microbial P immobilization processes under high precipitation (1927 mm), and thereby meet plant nutrient requirements because most of the added P would be immobilized in soil minerals by chemical sorption (Olander and Vitousek, 2004). The level of P addition followed the fertilization experiment in Costa Rica by Cleveland and Townsend (2006). Each treatment consisted of five replicates of  $5 \times 5\text{-m}$  plots. All the plots were laid out randomly on the same side of the mountain, and each plot was surrounded by an approximately 10-m buffer strip (Supplementary material, Fig. S1). The initial conditions of these plots did not differ in terms of understory plant abundance and soil properties (Liu et al., 2012). In the P-addition plots, P in the form of monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ ) was dissolved in 5 L of deionized water and sprayed on the forest floor using a backpack sprayer every other month (equivalent to 0.5 mm rainfall each time) from February 2007 and continued through December 2017, while the control plots received the equivalent amount of water. The side effects of sodium (Na) introduced by the P fertilizer were negligible because soil exchangeable Na was found to be very low in all plots ( $<1.6 \text{ mmol kg}^{-1}$ ) due to the high annual rainfall (Mao et al., 2017).

## 2.3. Field survey of understory plants

In each plot, a  $1\text{-m}^2$  subplot was established in 2007 to monitor the understory plants. Investigations of the understory plants were conducted annually in the subplots since 2008. All individuals, including the tree seedlings, woody shrubs, lianas, and herbaceous plants, were identified and measured to calculate species richness (total number of species), abundance (stem density), and coverage (percentage) for all vascular plants with height  $< 1.5 \text{ m}$  on the subplot scale. The coverage of individuals was estimated using the square-grid method. Additionally, the total number of seedlings (for the present year) was recorded to analysis the survival rate, which is calculated from the number of germinating seedlings alive in the next year/total germinating seedlings. The growth of the tree seedlings (height  $> 25 \text{ cm}$ ) in the subplots was tracked to compare the treatment effects on the growth rates. Growth rates were calculated by averaging the increases in height during the positive growth period, as perishing of the upper part is a common phenomenon in the seedling stage. We also estimated the aboveground biomass of all the investigated plants in each plot using a previously constructed regression model (Supplementary Material Table S3). To evaluate the effect of P addition on community composition at the whole plot scale ( $25 \text{ m}^2$ ), we further determined the species richness, abundance, and coverage and calculated the Shannon-Wiener index ( $H'$ ) and Pielou's evenness index ( $E$ ) for all plants in each plot in September 2016 using Eqs. (1)–(3):

$$P_i = \frac{N_i}{N} \quad (1)$$

$$\text{Shannon–Wiener index } (H') = -\sum P_i \ln P_i \quad (2)$$

$$\text{Pielou's evenness index } : E = \frac{H'}{\ln S} \quad (3)$$

where  $P_i$  is the proportion of individuals of species  $i$  to the total individual number,  $N_i$  and  $N$  indicates individuals of species  $i$  and total individuals, respectively, in each plot, and  $S$  is the total number of species in each plot.

## 2.4. Sampling and analyses

The soil properties were monitored since the establishment of the study (Mao et al., 2017). In July 2017, after 10 years of P addition, 0–10-cm mineral soils were sampled. In each plot, six cores with a 5-cm diameter of soil were drilled and combined into a composite sample. Soil samples were sieved using a 2-mm mesh and air-dried. Living fine roots were hand-sorted during soil sieving, and then washed carefully with deionized water. Soil pH was measured in a 1: 2.5 soil: water suspension. The available soil P was extracted using Bray-1 solution, composed of 0.03 M ammonium fluoride ( $\text{NH}_4\text{F}$ ) and 0.025 M hydrogen chloride (HCl) (Liu et al., 1996). Total P was analyzed after digestion with sulfuric acid and perchloric acid (Liu et al., 1996). Extractable P and total P were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 2000 DV, Perkin Elmer, Shelton, CT, USA). Soil available N was extracted using 2 M potassium chloride (KCl) solution, and then determined using TOC Analyzers (TOC-VCSH, Shimadzu, Tokyo, Japan). Soil organic carbon (SOC) and total N were determined using an elemental analyzer (IsoPrime100, Isoprime, Cheadle Hulme, UK).

We assessed the photosynthetic capacity of seven species (*Ardisia lindleyana* D. Dietrich, *Carallia brachiata* Merr, *Calamus rhabdocladus* Burret, *Cryptocarya chinensis* Hemsl, *Cryptocarya concinna* Hance, *Aidia canthioides* Masam, and *Gnetum montanum* Markgr) co-occurring in all plots in both the dry season (January) and wet season (July) of 2015. In each plot, two to four medium individuals of each species were selected, and 5–10 fully mature leaves were measured from an individual plant. Light-saturated photosynthetic rates ( $P_{\text{max}}$ ) were measured in situ between 9:00 and 11:30 A.M. using a Li-6400 portable photosynthesis system (LI-COR, Inc. Lincoln, NE, USA). The light intensity was set at  $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (to reach light saturation), and all measurements were performed under ambient carbon dioxide ( $\text{CO}_2$ ) concentrations (approximately  $400 \mu\text{mol mol}^{-1}$ ). Leaves and connected branches (including only four tree species, *A. canthioides*, *C. brachiata*, *C. chinensis*, and *C. concinna*) from the same individuals were sampled for chemical analysis. All plant samples, including leaves, branches, and fine roots, were oven-dried at  $65^\circ \text{C}$  for 48 h and ground to a fine and homogeneous powder. Plant tissue P concentration was determined by ICP-OES after digestion with nitric acid and perchloric acid ( $\text{HClO}_4$ ) (Liu et al., 1996). Non-structural carbohydrate (NSC) concentrations in leaves and fine roots were determined by the Anthrone colorimetric method (Li et al., 2016). In brief, ethanol-soluble fractions were analyzed for soluble sugar, whereas solid residues were hydrolyzed and extracted by 9.2 M  $\text{HClO}_4$  and analyzed for starch. Total NSC was reported as soluble sugar plus starch content in glucose equivalents based on a standard curve for glucose.

## 2.5. Statistical analysis

The effects of P addition on soil properties, seedling growth, survival rates, aboveground biomass, and plant diversity indices (within a  $25\text{-m}^2$  plot) were tested using independent sample  $t$ -tests. The differences in plant P concentration and  $P_{\text{max}}$  between treatments (control and P addition) were tested using general linear mixed effect models, with species as the random factor. For any species, an independent sample  $t$ -test was used to examine the differences in plant P concentration and  $P_{\text{max}}$ . The

significance of P addition effects on plant diversity indices (richness, abundance, and coverage) in subplots was determined using repeated-measures analysis of variance (ANOVA). Greenhouse-Geisser epsilon values were used to correct for violations of the compound symmetry assumption of repeated-measures ANOVA. The pattern of species composition at the whole plot level was characterized via rank/abundance curves after combining individuals in all five replicates of each treatment. All descriptive statistical analyses were performed using the SPSS software (version 19.0, SPSS, Chicago, IL, USA). Significant statistical differences were set at a threshold of  $p < 0.05$ .

### 3. Results

#### 3.1. Soil chemistry

In the control plots, available P was generally  $< 4 \text{ mg kg}^{-1}$  soil, and total P was averaged at  $0.2 \text{ g kg}^{-1}$  soil in the top 0–10 cm of the mineral soil (Table 1). The decadal P addition significantly increased the extractable soil P and total P to  $38.6 \text{ mg kg}^{-1}$  and  $0.56 \text{ g kg}^{-1}$ , respectively. However, long-term P addition had no significant effect on the other general soil properties such as SOC, total N, and soil pH (Table 1).

#### 3.2. Plant traits

Accompanied by the increased extractable soil P, plant P concentration increased significantly for almost all species under P addition (Fig. 1; Fig. S2), with an average increase of 54%, 162%, and 396% for leaves, branches, and fine roots, respectively. However, P addition did not affect the photosynthetic capacity ( $P_{max}$ ) of all understory species in either the dry or wet seasons (Fig. 2a; Fig. S3). We tracked the growth of 15 tree seedlings (all  $> 25 \text{ cm}$ ) in ten subplots since the start of this study. These seedlings grew very slowly (average increase of  $4 \text{ cm year}^{-1}$  in height), and P addition did not significantly affect their growth rates and coverage (Fig. 2b). In addition, we recorded 275 germinating seedlings during the study period and found no significant difference between the control and the P-addition plots (Fig. 2c). However, the survival rates were remarkably lower in the P-addition plots than in the control plots (6% vs. 21%,  $p < 0.001$ ; Fig. 2d). Phosphorus addition decreased NSC ( $p < 0.05$ ) and starch ( $p < 0.01$ ) in the fine roots (Fig. 3), but it had no significant effect on the leaves (Fig. S4).

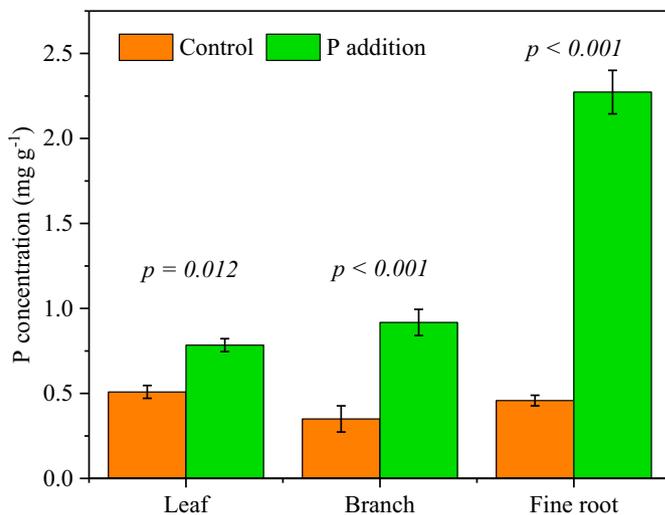
#### 3.3. Plant diversity

Within the  $1\text{-m}^2$  subplots and the  $25\text{-m}^2$  plots, we recorded 17 and 45 taxa, respectively (Supplementary Material, Tables S1 and S2). Across all plots, the most abundant species were *A. lindleyana*, *G. montanum*, *C. concinna*, and *C. chinensis*, accounting for approximately 50% of the total plant density. In the subplots, P addition significantly decreased the species richness ( $p = 0.04$ ) and abundance ( $p = 0.02$ ), but not the coverage ( $p = 0.10$ ) (Fig. 4). Across all plots, P addition significantly decreased the species richness ( $p < 0.01$ ) and abundance ( $p = 0.03$ ) but had no effect on the total species coverage ( $p = 0.39$ ;

**Table 1**

Comparison of selected soil properties between the control and P treatment in the studied forest. Sampling was conducted in July 2017, after 10 years of continuous P addition. Values are means with standard error (SE) in parentheses ( $n = 5$ ). Significant difference between the control and P treatment is marked by  $p$ -values. *ns* denotes no significant difference among treatments.

Soil characteristics	Control	P addition	Treatment effect
SOC ( $\text{g kg}^{-1}$ )	33.5 (2.6)	31.2 (2.7)	<i>ns</i>
Available P ( $\text{mg kg}^{-1}$ )	3.8 (0.3)	38.6 (4.1)	$p < 0.001$
Total P ( $\text{g kg}^{-1}$ )	0.20 (0.01)	0.56 (0.05)	$p < 0.001$
Available N ( $\text{mg kg}^{-1}$ )	22.8 (1.2)	19.9 (2.7)	<i>ns</i>
Total N ( $\text{g kg}^{-1}$ )	1.54 (0.08)	1.39 (0.12)	<i>ns</i>
Soil pH ( $\text{H}_2\text{O}$ )	3.68 (0.04)	3.72 (0.04)	<i>ns</i>



**Fig. 1.** Phosphorus concentrations of understory plant leaves, branches, and sand level fine roots in the control and P-addition plots. Values are means, and error bars are standard error (SE,  $n = 5$ ), according to linear mixed effect models (leaf and branch) and independent-samples  $t$ -test (fine root). Significant difference between the control and P treatment is marked by  $p$ -values.

Fig. 5). The  $H'$  and  $E$  were significantly lower in the P addition plots than in the control plots ( $p = 0.03$ ,  $p = 0.03$ , Fig. 5). For seedling demography, the rank/abundance curve of the species showed a decline in abundance for most species in the sequence under P addition (Fig. S5). In particular, the dominant species showed the largest decrease in the number of individuals (Fig. S5).

### 4. Discussion

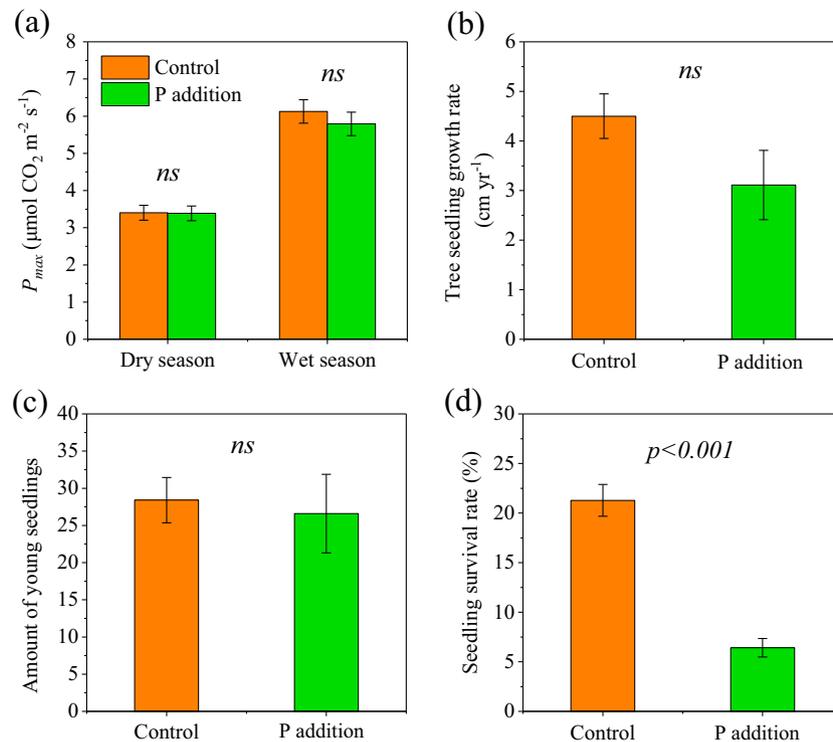
#### 4.1. Responses of soil P and plant P to long-term P addition

Soil total P concentration in the control plots measured in the present study ranged from  $0.17$  to  $0.24 \text{ g kg}^{-1}$ , which is within the range of the lowest levels measured worldwide (Yang et al., 2013). Similarly, foliar P concentration of understory plants in this forest ranged from  $0.3$  to  $1.0 \text{ mg g}^{-1}$ , falling at the lowest level across terrestrial ecosystems (Güsewell, 2004). Our results are comparable with those measured in other tropical forests (Townsend et al., 2007; Chen et al., 2018); thus, they are in line with the view that humid tropical regions are P-deficient, given that soils have been highly weathered and have lost most of their parent material through leaching during pedogenesis (Vitousek et al., 2010).

As expected, long-term P addition significantly increased soil extractable P concentration (from  $3.8$  to  $38.6 \text{ mg kg}^{-1}$ ) and total P concentration (from  $0.2$  to  $0.56 \text{ g kg}^{-1}$ ). The total P level in P-addition plots was still below the average level of the soils in China ( $5.9 \text{ g P kg}^{-1}$ ) (Zhang et al., 2005). It confirms that a high load of P addition is a reliable method for elevating soil P availability in humid tropical forests. In addition, P addition greatly increased plant P concentrations, especially in the fine roots (Fig. 1). These results are consistent with many other studies in tropical forests (Burslem et al., 1995; Schreeg et al., 2014; Alvarez-Clare et al., 2013), suggesting that P addition satisfies the plants P demand.

#### 4.2. Responses of photosynthesis and seedling growth to long-term P addition

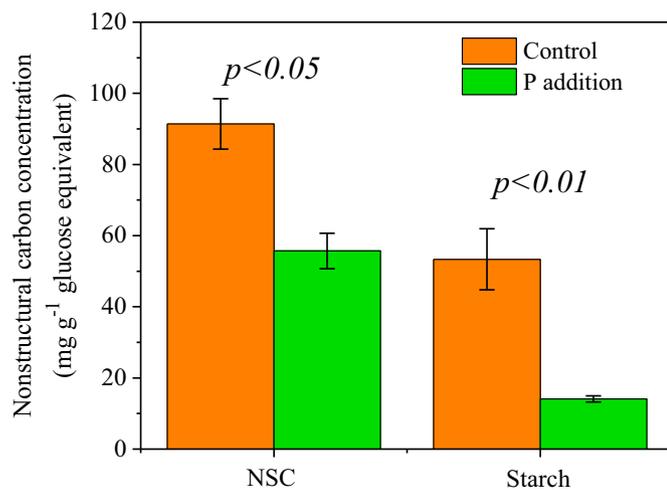
We found that P addition had no significant effect on the photosynthetic capability and tree seedling growth of the understory plants, although P concentrations of plant tissues was elevated following the decadal P addition. Studying a lowland tropical forest in Panama,



**Fig. 2.** Effect of P addition on maximum photosynthesis rate ( $P_{max}$ ) of seven understory plants in different seasons in 2015 (a); tree seedling growth rate during tracking survey in  $1 \text{ m}^2$  subplots ( $n = 8$  in control and  $n = 7$  in P-addition plots) (b); total amount of germinating seedlings during the study period (2008–2017) (c); and the survival rate of germination seedlings in the next year investigation (d). Values are means, and error bars are standard error (SE,  $n = 5$ ), according to linear mixed effect models (a) and independent-samples  $t$ -test (b, c, d);  $ns$  denotes no significant difference among treatments.

Santiago et al. (2012) also found that P addition increased the tissue P concentration in young tree seedlings by 16%, but it had no effect on their growth. These results contrast with our expectation regarding the positive response of plant growth to P addition, which is widely accepted in P-depleted ecosystems (Ceccon et al., 2003; Burslem et al., 1995; Herbert and Fownes, 1995). Based on Liebig's law of the minimum that plant growth is controlled by the resource in the lowest supply, we suggest two plausible reasons for the lack of positive responses of photosynthesis and growth.

First, a high canopy closure may limit plant growth to a greater extent than soil P deficiency. Studies have shown that the response of plants to nutrient addition depends on irradiation when the canopy is

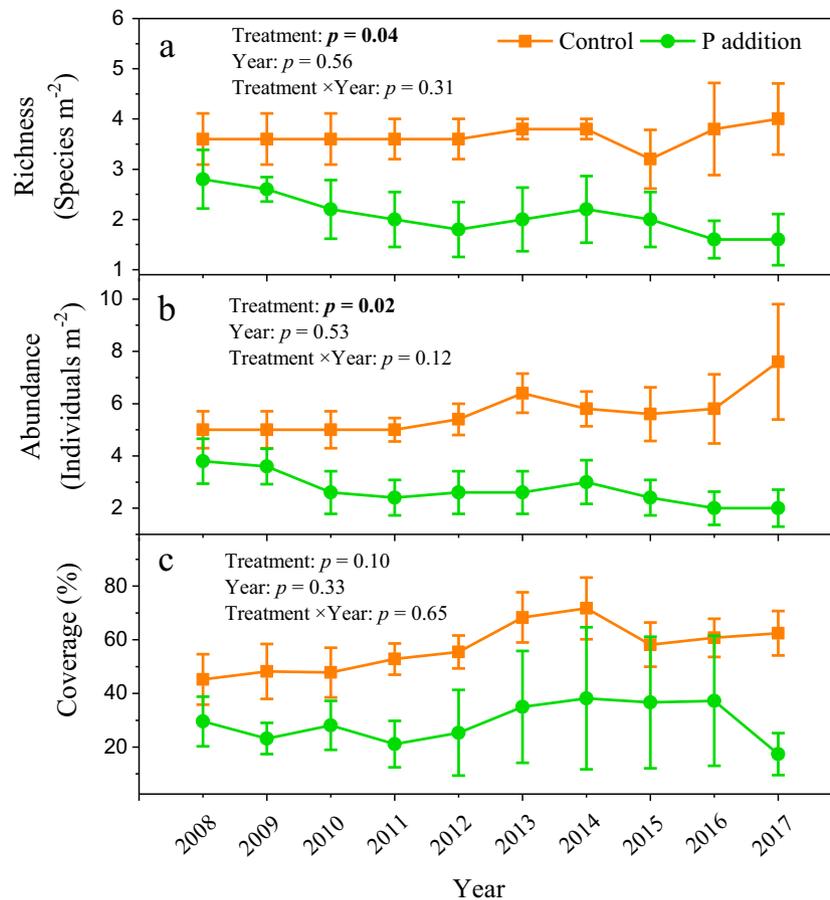


**Fig. 3.** Fine root nonstructural carbon (NSC) and starch content in the control and P treatment. Error bar is standard error (SE,  $n = 5$ ). Significant differences between the two treatments are marked by  $p$ -values according to independent-samples  $t$ -test.

closed, and nutrient addition effects occur only when light is sufficient (Veendaaal et al., 1996; Hautier et al., 2009). In the present study, the understory plants persisted well under the deep shade, with both a high leaf area index ( $>6.5$ ) and canopy closure ( $>95\%$ ) (Ren and Peng, 1997; Lu et al., 2010). The growth rates of tree seedlings were very low in both the control and P-addition plots (approximately  $4.5 \text{ cm yr}^{-1}$ ; Fig. 2b) but rapid in the forest gap caused by a typhoon ( $>10 \text{ cm yr}^{-1}$ , unpublished data), suggesting a strong inhibition effect of shading on plant growth. Second, the combined limitation of other nutrients (e.g., potassium [K], calcium [Ca], magnesium [Mg]) may also contribute to the lack of response of photosynthetic capability and growth rate (Burslem et al., 1995), considering that old acidic soils (oxisol and ultisol) are usually exhausted in such cations. One convincing case is that tree growth rates were stimulated by K addition or K + P addition, but not P addition alone across all five species of the understory layer in a tropical forest of Panama (Santiago et al., 2012), demonstrating a stronger limitation of K over P.

#### 4.3. Responses of plant diversity to long-term P addition

In this study, P addition significantly decreased the survival rate of young seedlings by two-thirds of that of the controls over the study period (Fig. 2d). Comparably, Newbery et al. (2002) also found that two years of P fertilization hindered the survival of young seedlings in a central African rainforest. In contrast, our results differ from a 2.7-year study in Costa Rica, which showed that P addition increased the percentage of seedling survival from 59% to 78% (Alvarez-Clare et al., 2013). Other studies have also reported no response of survival rates of young seedlings to P addition (Cárate-Tandalla et al., 2015). However, the negative effects of P addition on young seedling survival can lead to a reduction in recruitment and changes in species turnover in the long run. This is confirmed by the decrease in understory plant diversity (e.g., species richness, stem density, and diversity indices) in response to long-term P additions in this study (Figs. 4 and 5).



**Fig. 4.** Temporal patterns of responses of plant diversity (richness, abundance, and cover) to P addition treatment in understory layer for years 2008–2017. Values and error bars are means ( $\pm$  SE) among plots ( $n = 5$ ). Significant difference between the two treatments is marked by  $p$ -values according to repeated measures analysis of variance.

In general, enhanced nutrient supply can stimulate plant growth and alter competitive interactions due to heterogeneous nutrient limitation among different species or functional groups (Gilliam, 2006; Hautier et al., 2009; Soons et al., 2017). Altered plant diversity under P addition has been observed in many studies in various ecosystems (Soons et al., 2017) such as tundra (Theodose and Bowman, 1997), grassland (Stiles et al., 2017), savanna (Bustamante et al., 2012), and secondary forests (Siddique et al., 2010). However, the response of plant diversity to P inputs has rarely been evaluated in humid tropical forests, where P is deficient, and species are highly diverse. Our results were not in line with the competitive exclusion principle because P addition had no positive effect on the increase in tree seedling growth (Fig. 2), plant coverage (Fig. 4), and understory plant biomass (Fig. 5). There was no superior performance of the individual species under the P addition.

We suggest that an increase in metabolic cost for P storage may account for the negative effects of P addition on seedling survival and diversity, considering that P addition did not stimulate C capture in the deep shade understory layer. The remarkable increase in P concentration in the leaves, branches, and fine roots indicates a “luxury consumption” of P (Fig. 1). Large amounts of P stored in tissues require additional energy and are expected to increase metabolic costs involving processes of P transportation and synthesis of P-containing metabolites (Chapin, 1990; Raven et al., 2018). As a result, increased C cost without enhanced aboveground C assimilation potentially exhausts plant C storage, as we found decreases in total NSC and starch content of fine roots where most of the P accumulated (Fig. 5). The amount of NSC stored is an indicator of survival potential (Poorter and Kitajima, 2007). Plants die when they cannot mobilize sufficient resources to heal injuries or to maintain the growth of tissues (Waring, 1987; Wiley et al., 2017). In our study, P addition-induced decreases in C storage were consistent with a lower

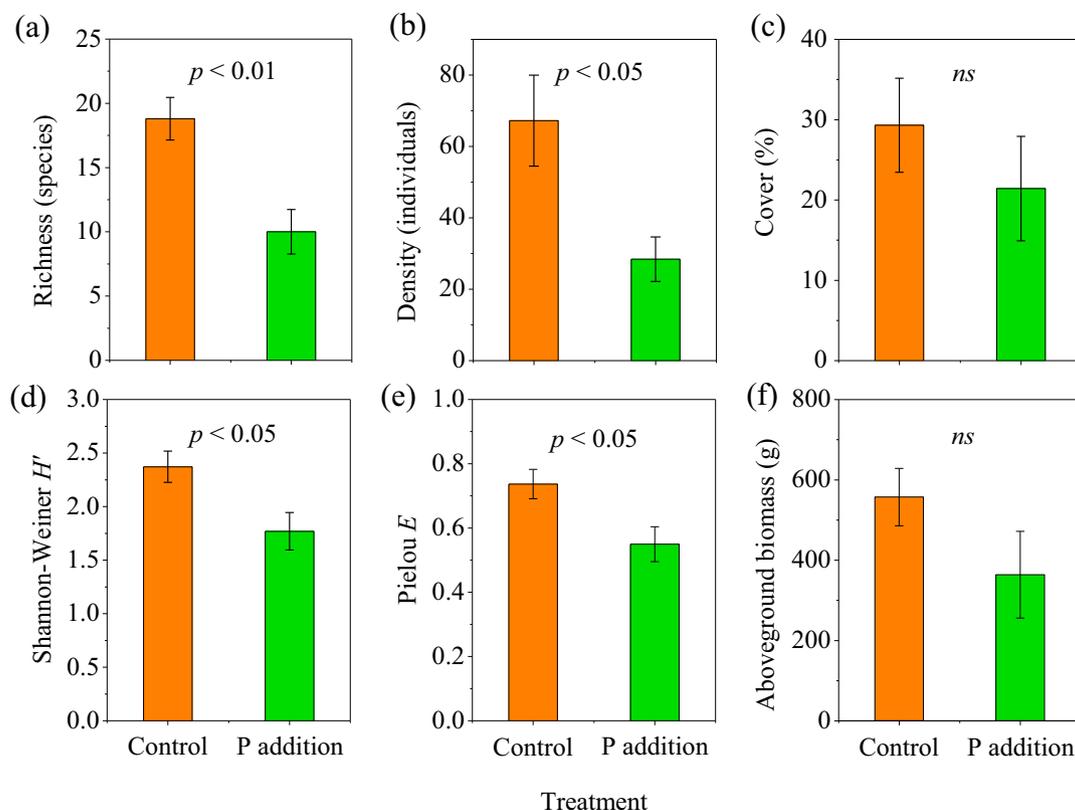
survival potential, indicating that understory plants may be impacted by a C imbalance.

## 5. Conclusions

Using results from our long-term P addition experiment, we showed that chronic P inputs greatly increased plant P concentration in this N-rich but P-poor forest, but these failed to stimulate foliar net photosynthesis and seedling growth. High canopy closure is the main reason for the lack of a positive response under P supply. We further found that long-term P addition can decrease the survival rate of young seedlings and understory species diversity. We propose that the negative effect of P addition on understory plants is likely the result of increased C costs rather than competition exclusion mechanisms among species. Further studies are required to clarify the underlying mechanisms, especially the magnitude of elevated costs and responses of mycorrhizal symbiont to P addition. Our findings suggest that enhanced P inputs may have a profound negative effect on turnover and community composition of understory plant species in tropical forests, and such effects need to be fully considered when trying P amendments are used as an approach to maintain biodiversity in P-limited tropical forests.

## CRediT authorship contribution statement

Q.M., X.L. and J.M. conceived of the study, Q.M., X.L., C.W. and F.Z. performed the field experiment and laboratory analysis, Q.M. and X.L. analyzed the data and wrote the draft of the manuscript. F.S.G. J.M., P.G., D.E., G.G., Q.Y. and H.C. contributed to the revision of the manuscript.



**Fig. 5.** Characteristics of understory plant diversity in the control and P treatment at the  $5 \times 5$  m plot scale (a, richness; b, density; c, total cover of understory layer; d, Shannon-Wiener index  $H'$ ; e, Pielou's evenness index  $E$ ; f, total aboveground biomass). Values are means, and error bars are standard error (SE,  $n = 5$ ) according to independent-samples  $t$ -test; ns denotes no significant difference among treatments.

## Declaration of competing interest

All authors have no conflict of interest to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.149306>.

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