

# Effects of nitrogen and phosphorus addition on leaf nutrient characteristics in a subtropical forest

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## Abstract

**Key message** P deposition can alleviate P limitation in subtropical forests and P deposited in conjunction with high quantities of N does not enhance P limitation.

**Abstract** Increasing nitrogen (N) and phosphorus (P) deposition could influence plant growth and survival to varying extents. N and P deposition is essential for subtropical forests where plant growth is limited by lower soil P availability in highly weathered soils. However, whether N and P deposition can increase leaf P concentration and alleviate P limitation is unclear. We investigated changes in N and P concentrations, N:P ratios, and resorption for six dominant species (two tree species and four understory species), following 2 years of N and P additions in a subtropical forest in southern China. P addition either alone or together with the addition of N increased green leaf P concentrations (except in *Schima superba*) and senesced leaf P and decreased N:P ratios (except in *Pinus massoniana*), but had no influence on P resorption. N addition had no apparent influence on leaf N concentrations, N:P ratios, or N resorption in all species. The considerable influence of P addition can be explained by rising soil P availability. Our results suggest that subtropical forests are P limited and that increasing P deposition

can moderately alleviate the limitation of P. Furthermore, P deposited in conjunction with high quantities of N does not enhance P limitation, as it is insensitive to elevated N input.

**Keywords** Leaf N concentration · Leaf P concentration · N:P · Nutrient resorption · Nutrient limitation · Mixed forest

## Introduction

Nitrogen (N) and phosphorus (P) are common elements that are necessary for plant growth in terrestrial ecosystems. N is needed for the synthesis of amino acids, proteins, and the enzymes that catalyze photosynthesis. P is the primary constituent of ATP and is an important element in nucleic acids, phospholipids, and other cellular metabolites (Elser et al. 1996). The supply of soil N and P determines plant productivity to a large extent. To explore which of these vital elements limit primary productivity, many experiments studying N and P fertilization have been conducted over the last few decades (Elser et al. 2007; Li et al. 2016), where the findings of such studies have helped elucidate the influence of increasing N and P deposition on the structure and function of terrestrial ecosystems (Bennett et al. 2001; Elser and Bennett 2011; Galloway et al. 2004).

The most important index of N or P limitation for plant growth is leaf N or P concentration. Generally, the positive relationships between leaf N or P and plant productivity have been widely observed in most N- and P-limited ecosystems (Gusewell 2004; Li et al. 2016). Furthermore, with the exception of external soil conditions (available N and P), leaf N and P concentration can be influenced by the internal resorption of N and P by plants (Aerts 1996; Killingbeck 1996). A common strategy for plants is to resorb N and P

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from senescing leaves prior to abscission, where it is estimated that approximately 50% of leaf N and P are recycled via this method of resorption (Yuan and Chen 2009a, b), thereby increasing the leaf N and P use efficiency. Therefore, exploring the influence of N and P deposition on leaf N and P concentrations and leaf resorption can improve our understanding of N and P limitation in terrestrial ecosystems.

Subtropical forests can be used as a model to study the effect of P limitation. With the high temperatures and quantities of precipitation common to subtropical forests, soils are depleted in P as a result of substantial weathering and high fractions of recalcitrant P (Walker and Syers 1976). Coincidentally, this is also the region with the highest rate of N deposition (20–30 kg N hm<sup>-2</sup> year<sup>-1</sup>) (Zhu et al. 2015). Furthermore, we have previously observed that P deposition occurs at a very low rate (0.34 kg hm<sup>-2</sup> year<sup>-1</sup>) in subtropical forest ecosystems (Zhu et al. 2016). This imbalance in atmospheric N and P deposition could alter nutrient availability and further increase P limitation in these regions (Li et al. 2016; Peñuelas et al. 2013).

Given the aforementioned scenarios of P limitation in subtropical forests, we hypothesize that elevated P deposition will directly enhance soil P availability and subsequently increase leaf P concentration. To date, while the potential effect of increasing P deposition has been tested in other P-limited ecosystems, such as tropical forests (Lawrence 2001; Mayor et al. 2014; Tanner et al. 1990) and N-saturated grasslands (Huang et al. 2016; Long et al. 2016), it has not been tested in subtropical forests to the best of our knowledge. Furthermore, while it is possible that N deposition indirectly influences plant P concentration via enhanced P uptake by stimulating phosphatase activity and subsequently organic P mineralization (Marklein and Houlton 2012; Rodríguez and Fraga 1999), N deposition could also decrease P uptake by roots by increasing the toxicity of soil cations such as Fe<sup>2+</sup>, Mn<sup>2+</sup>, Al<sup>3+</sup> after soil acidification (Tian and Niu 2015). Thus, given the higher soil N availability and lower labile P substrates in subtropical forests, we hypothesized that the influence of N deposition on plant P concentration would be minimal.

Leaf N and P concentrations are influenced by leaf N and P resorption to a certain degree. Species with low P concentrations (<0.5 mg g<sup>-1</sup>) in senesced leaves were regarded to have a high P resorption efficiency (Killingbeck 1996), while leaf P concentrations in many species from subtropical forests are approximately 0.5 mg g<sup>-1</sup>, suggesting high P resorption efficiencies. We hypothesize that leaf P resorption is relatively stable under conditions of N and P addition owing to the constrained flexibility of leaf P resorption in long-term low P soils (Harrington et al. 2001; Treseder and Vitousek 2001). Leaf N resorption should have no significant response to N and P addition, as subtropical forests typically have optimal green leaf N concentrations above

7.0 mg g<sup>-1</sup> (Killingbeck 1996). However, the aforementioned hypotheses have not been well tested in subtropical forests (Chen et al. 2015).

Here, we conducted an experiment with N and P addition in a secondary subtropical forest in southern China. N and P, individually and in combination, were applied on a monthly basis. In practice, we sampled six dominant plant species—including dominant trees and understory shrubs and herbs—and investigated the effects of N and P addition on N and P concentrations, N:P ratios, and resorption efficiencies in a field-based experiment. The main objectives were to (1) investigate the response of leaf N and P concentration and resorption in subtropical forests, and (2) to test the aforementioned hypotheses.

## Materials and methods

### Study site

The experiment was carried in a subtropical mixed pine and broadleaf forest (23°9'41"N, 112°32'36"E, 50 m elevation) in the Dinghu Mountain region of southern China. This area is characterized by a typical subtropical monsoon and humid climate. The mean annual temperature is approximately 21.5 °C, where the mean monthly temperature ranges from 12.6 °C in January to 28.0 °C in July (Liu et al. 2013). The mean annual precipitation is approximately 1927 mm, approximately 75% of which falls during March–August (Liu et al. 2012). Zonal vegetation was evergreen broad-leaved forests, but it was heavily destroyed due to land conversion for agricultural production. The vegetation was restored during 1930–1950 by planting *Pinus massoniana* (PM). The forests were naturally invaded and colonized by broadleaf species, *Schima superba* (SS). Hence, it is a transitional forest from pine to monsoon evergreen broadleaf forest.

The soil is lateritic red earth developed from sandstone, with a depth of 30–60 cm. The mean bulk density of the top soil (0–10 cm) is 1.4 g cm<sup>-3</sup>, while the average soil pH is 4.1. The averages of soil total carbon, total N, and total P concentrations for the top 10 cm of soil are 13.6, 0.79, and 0.14 g kg<sup>-1</sup>, respectively. Precipitation-based inorganic N deposition in 2013 was 22.8 kg N ha<sup>-1</sup> year<sup>-1</sup>, with an NH<sub>4</sub><sup>+</sup>-N to NO<sub>3</sub><sup>-</sup>-N ratio of 1.4:1, while P deposition was 0.34 kg N ha<sup>-1</sup> year<sup>-1</sup> (Zhu et al. 2016).

### Experimental design and sampling

Our research site was established at the bottom of a hill with a slope around 30°. Stand density was ca. 150 stems ha<sup>-1</sup> for PM and ca. 960 stems ha<sup>-1</sup> for SS. The average of volume was about 0.18 m<sup>3</sup> tree<sup>-1</sup> for PM (DBH,

22.7 cm; height, 11.0 m) and 0.06 m<sup>3</sup> tree<sup>-1</sup> for SS (DBH, 11.2 cm; height, 9.5 m). Topographic position and slope were considered to ensure uniformity among plots. The experimental design included four treatments with four replicates (total of 16 plots): control (CK, no N and P addition), N addition (N, 100 kg N ha<sup>-1</sup> year<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>), P addition (P, 100 kg P ha<sup>-1</sup> year<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>), and N plus P addition (NP, 100 kg N ha<sup>-1</sup> year<sup>-1</sup> and 100 kg P ha<sup>-1</sup> year<sup>-1</sup>). Each plot was 20 m × 20 m, and was separated from neighboring plots by a 10-m buffer. All fertilizers were dissolved in 50 L of water and sprayed using a backpack sprayer, and were applied monthly from June 2013 to June 2015. In lieu of a fertilizer solution, control groups were sprayed with water (equivalent to 0.5 mm of rainfall).

Young green leaves from six species were collected in June 2015 for green leaf nutrient measurement. The present study included two tree species (SS and PM), and four dominant understory species [two shrubs: *Psychotria rubra* (PR) and *Ixora chinensis* (IC), and two herbs: *Dicranopteris dichotoma* (DD) and *Lophatherum gracile* (LG)]. Three healthy mature trees were randomly selected in each plot in June 2015. One twig from sun-exposed branch within the upper two-third of the crown was collected using a tall tree trimmer with a bamboo pole. The current-year fully expanded leaves were detached from each twig and were bulked to yield one sample. For four understory species, mature leaves from 3 to 5 healthy individuals per plot were collected by hand and mixed to one sample. Two 1 × 1 m nylon mesh traps (about 0.5 m high from the ground) were used to collect senesced tree leaves in each plot. Senesced tree leaves fallen in December 2015 with no signs of decay were pooled per plot and evenly mixed to yield one sample for senesced leaf nutrient measurement. Senesced leaves with no signs of decay from PR were collected and measured the nutrient content of senesced leaves, while the quantities of senesced leaves from the other three species were not sufficient to be collected and analyzed. All samples were dried at 65 °C for 48 h and ground to homogeneity with a ball mill for chemical analyses. Five soil cores (5 cm diameter) were collected from each plot at 0–10 cm depth and bulked into one sample. Soil samples for ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) measurement were obtained every 2 months from August 2013 to June 2015. Soil P content was measured only in June 2015. Soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were determined by an extraction using a solution of 2 M KCl, followed by a colorimetric analysis on an AutoAnalyzer (SEAL AA3, Norderstedt, Germany). Soil inorganic N was calculated as the sum of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations. Soil available P was extracted using an NH<sub>4</sub>F–HCl solution as described by Bray (1945).

## Chemical analyses

Leaf N concentrations were measured using the micro-Kjeldahl method (Sparks 1996), while leaf P concentrations were analyzed by the ammonium molybdate method after persulfate oxidation (Sparks 1996). Leaf N resorption efficiency (NRE) and leaf P resorption efficiency (PRE) were calculated as the proportion of N and P recovered from a senesced leaf (Killingbeck 1996; Yuan and Chen 2009b):

$$\text{NRE} = \frac{N_{\text{green}} - N_{\text{senesced}}}{N_{\text{green}}}, \quad (1)$$

$$\text{PRE} = \frac{P_{\text{green}} - P_{\text{senesced}}}{P_{\text{green}}}, \quad (2)$$

where N<sub>green</sub> and P<sub>green</sub> were the N and P concentrations of green leaves, and N<sub>senesced</sub> and P<sub>senesced</sub> were the N and P concentration of senesced leaves.

## Statistical analyses

A two-way ANOVA was used to test the impacts of N, P, and NP addition on green leaf and senesced leaf N and P concentrations, green leaf N:P ratio, and leaf N and P resorption efficiencies. Dunnett's test was conducted to determine the significant differences between different experimental treatments. Pearson correlations were used to investigate the relationship between green leaf N and P concentrations and soil available N and P concentrations. All statistical analyses were performed using the SPSS 18.0 software package (SPSS, Chicago, IL, USA). The significance level was set at P = 0.05 for all calculations.

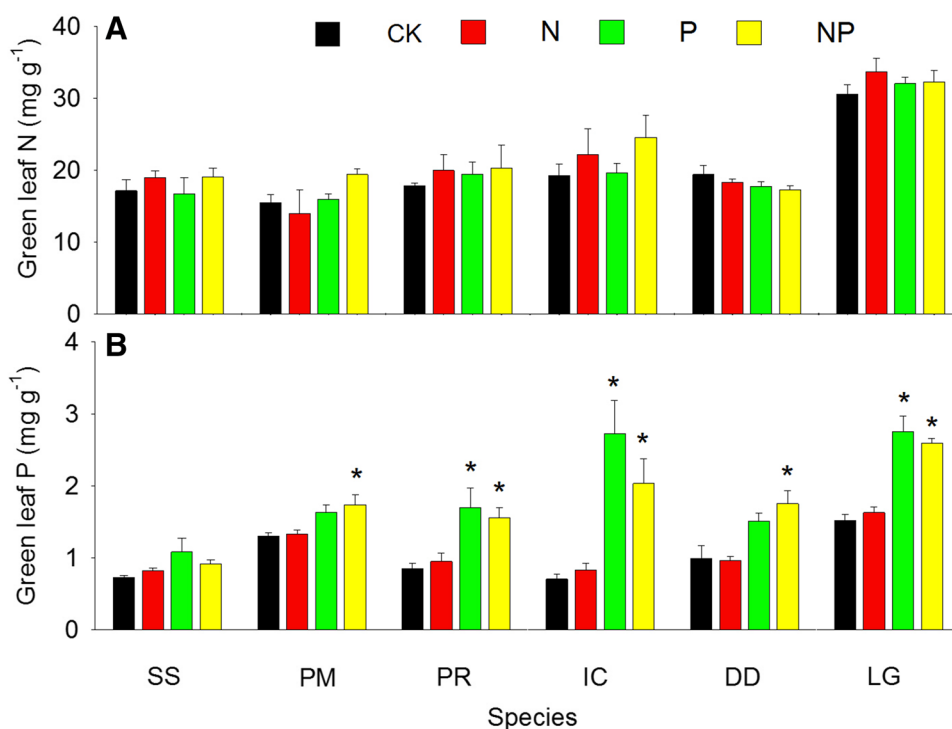
## Results

### Changes in leaf N, P, and related resorption efficiencies

Neither N or P addition alone, nor the addition of NP combined, changed green leaf N concentrations in any species in the present study (Fig. 1a). P addition increased green leaf P concentration by an average of 41% (Fig. 1b). No interactions of N and P addition were detected. More specifically, P addition increased green leaf P concentrations in *P. rubra*, *I. chinensis*, and *L. gracile*. NP addition enhanced green leaf P concentrations in all species with the exception of *S. superba*. The increase in green leaf P concentration by P addition was observed to be higher in understory species than in tree species.

Similarly, senesced leaf N concentrations in *S. superba*, *P. massoniana*, and *P. rubra* exhibited no changes in N in

**Fig. 1** Effects of nitrogen (N) and phosphorus (P) addition (CK: control, N: N addition, P: P addition, NP: both N and P addition) on green leaf N and P concentrations in a subtropical forest. Data represent mean and standard error ( $n=4$ ). Asterisks indicate significant differences from the control treatment ( $P<0.05$ ). SS, *Schima superba*; PM, *Pinus massoniana*; PR, *Psychotria rubra*; IC, *Ixora chinensis*; DD, *Dicranopteris dichotoma*; LG, *Lophatherum gracile*



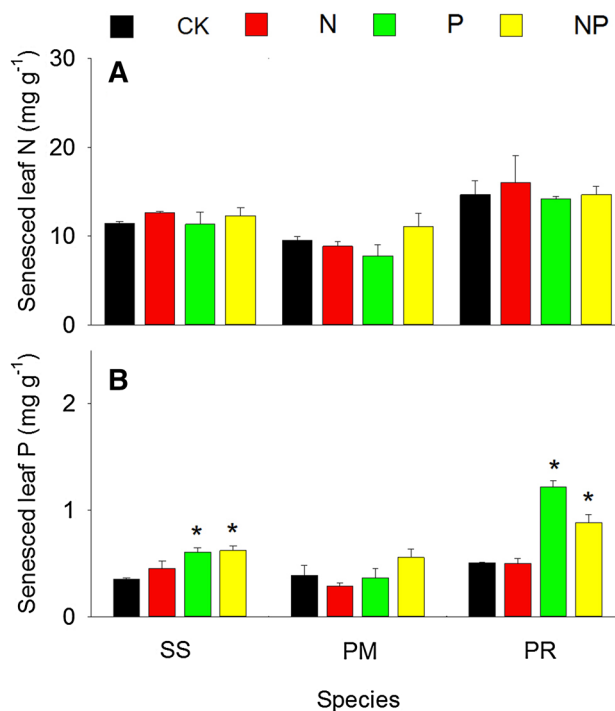
response to N or P addition, either individually or in combination (Fig. 2a). P and NP addition increased senesced green leaf P concentrations in both *S. superba* and *P. rubra* (Fig. 2b). The positive effect of P addition was stronger in *P. rubra* than in *S. superba*.

N addition exerted no effect on green leaf N:P ratio in any of the species tested (Fig. 3). *S. superba*, *P. rubra*, *I. chinensis*, *D. dichotoma*, and *L. gracile* exhibited significantly lower N:P ratios in the P treatments, and *I. chinensis*, *D. dichotoma* and *L. gracile* exhibited significantly lower N:P ratios in the NP treatments in comparison to CK. The two understory herbs—*D. dichotoma* and *L. gracile*—displayed the lowest N:P ratios of all six species. In the tree species, the N:P ratio was lower in *P. massoniana* than in *S. superba*.

Leaf NRE and PRE in *S. superba*, *P. massoniana*, and *P. rubra* did not change with both when added individually and in combination (Fig. 4). On average, NRE and PRE in the three plant species tested were 32.3% (Fig. 4a) and 50.3% (Fig. 4b), respectively, although PRE in *P. massoniana* was higher than that of *S. superba*.

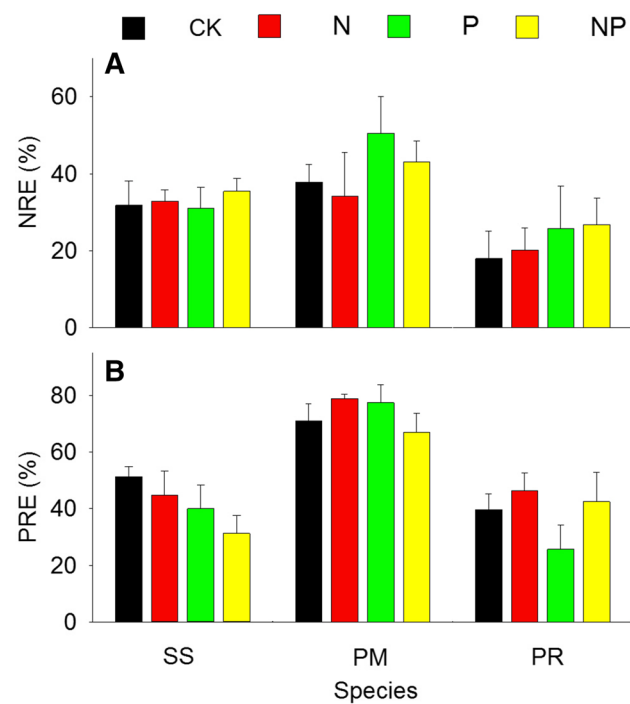
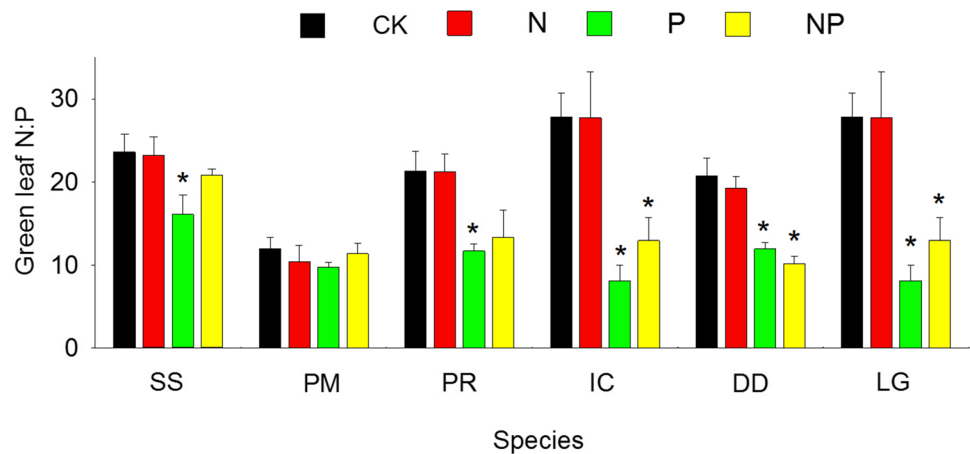
### Soil available N and P concentrations

N addition significantly increased soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (Table 1). Soil available P concentration was eight times higher in P and NP treatments (24.26 and 27.97  $\text{mg kg}^{-1}$ , respectively on average) than in the CK group (2.73  $\text{mg kg}^{-1}$ ), while the addition of N (2.82  $\text{mg kg}^{-1}$ ) did not change it. There were no



**Fig. 2** Effects of nitrogen (N) and phosphorus (P) addition (CK: control, N: N addition, P: P addition, NP: both N and P addition) on senesced leaf N and P concentrations in a subtropical forest. Data represent mean and standard error ( $n=4$ ). Asterisks indicate significant differences from the control treatment ( $P<0.05$ ). SS, *Schima superba*; PM, *Pinus massoniana*; PR, *Psychotria rubra*

**Fig. 3** Effects of nitrogen (N) and phosphorus (P) addition (CK: control, N: N addition, P: P addition, NP: both N and P addition) on green leaf N:P ratios in a subtropical forest. Data represent mean and standard error ( $n=4$ ). Asterisks indicate significant differences from the control treatment ( $P<0.05$ ). SS, *Schima superba*; PM, *Pinus massoniana*; PR, *Psychotria rubra*; IC, *Ixora chinensis*; DD, *Dicranopteris dichotoma*; LG, *Lophatherum gracile*



**Fig. 4** Effects of nitrogen (N) and phosphorus (P) addition (CK: control, N: N addition, P: P addition, NP: both N and P addition) on leaf N resorption efficiency (NRE) and leaf P resorption efficiency (PRE) in the subtropical forest. Data represent mean and standard error ( $n=4$ ). Asterisks indicate significant differences from the control treatment ( $P<0.05$ ). SS, *Schima superba*; PM, *Pinus massoniana*; PR, *Psychotria rubra*

interactions of N and P addition on soil properties. The average pH value in the control soil was 3.96, while N addition significantly decreased soil pH to 3.90, and P addition increased pH to 4.04.

**Table 1** Results ( $F$  values) of two-way ANOVA of nitrogen (N) addition, phosphorus (P) addition, and the interaction of N and P addition on soil available N and P and pH in a subtropical forest

	NH <sub>4</sub> -N	NO <sub>3</sub> -N	In-N	Available P	pH
N	8.02**	18.32**	19.26**	0.48	10.65**
P	0.01	1.14	0.25	71.93**	4.70*
N*P	0.47	0.52	0.80	0.43	0.83

\* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$

**Relationships of N and P concentrations between plants and soil**

Leaf N:P stoichiometry was sensitive to soil available P rather than soil available N. More specifically, leaf P concentrations in all species were significantly and positively correlated with soil available P, while leaf N concentrations had no apparent relationship to soil available N and P, except in *P. massoniana* (Table 2). As a result, leaf N:P ratios were negatively correlated with soil available P in nearly all species, and exhibited no relationship with soil available N. In addition, plant N and P were not correlated with soil pH values.

**Discussion**

In a subtropical coniferous and broadleaved mixed forest in southern China, P addition generally increased leaf P concentrations and decreased N:P ratios, while N addition did not alter leaf N or P concentrations. Therefore, our results confirm the hypothesis that the subtropical forest is indeed P-limited and not N-limited. Our findings also support the hypothesis that N and P addition exerted no effects on leaf N and P resorption in the subtropical forest ecosystem in southern China.

**Table 2** Pearson's product-moment correlation (*r* values) between green leaf nutrients [nitrogen (N) concentrations, phosphorus (P) concentrations, and N:P ratios] and soil nutrients (available N and P)

Species	Green leaf N		Green leaf P		Green leaf N:P	
	Inorganic N	Available P	Inorganic N	Available P	Inorganic N	Available P
<i>Schima superba</i>	0.198	0.013	− 0.115	0.530*	0.212	− 0.510*
<i>Pinus massoniana</i>	0.202	0.614*	0.034	0.620*	0.106	0.019
<i>Psychotria rubra</i>	0.269	0.074	− 0.173	0.912***	0.188	− 0.893***
<i>Ixora chinensis</i>	0.345	0.225	− 0.301	0.733**	0.243	− 0.707**
<i>Dicranopteris dichotoma</i>	− 0.037	− 0.418	0.029	0.657**	− 0.027	− 0.773***
<i>Lophatherum gracile</i>	0.261	0.126	− 0.144	0.928***	0.169	− 0.873***
All species	0.105	0.102	− 0.121	0.562***	0.13	− 0.575**

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

### P-acquiring ability, rather than P resorption, influenced leaf P concentration

The 41% increase in green leaf P after P addition observed in the present study was consistent with the average range (30–60%) of previously reported increases in green leaf P in tropical forest ecosystems under conditions of P addition (Li et al. 2016; Yuan and Chen 2015). Our findings demonstrate that while P addition increased leaf P concentrations, it did not influence leaf P resorption (Figs. 1, 4). This suggests that leaf P was regulated directly by an external supply and not by the internal remobilization of P. These findings are consistent with the meta-analysis of Yuan and Chen (2015), who found that P addition increased both green and senesced leaf P concentrations in conifers and evergreen angiosperms, while having no influence on PRE. The underlying reasons for the directly proportional increases in P concentration in both green and senesced leaves under conditions of P addition are poorly understood. Generally, it has been shown that nutrient use efficiency would decrease with increasing nutrient availability, both in the field (Yuan and Chen 2009a; Yuan et al. 2011) and in fertilization experiments (Yuan and Chen 2015). Whether the effects of P addition on PRE vary with the length of the fertilization period requires further investigation. With regard to specific types of forest ecosystems, previously published information regarding the effect of P addition on PRE in subtropical forests is lacking (Yuan and Chen 2015).

Furthermore, the ability to uptake soil available P by leaf P accumulation is an important survival strategy for many plant species. Previous studies have reported that some plant species growing in P-poor environments may not downregulate P uptake under conditions where a higher supply of P is available (Lambers and Shane 2007; Standish et al. 2007). However, in such cases, the addition of P is not a common condition that plants are likely to experience in the wild. It is possible that high leaf P concentration under conditions of P addition may not

necessarily be correlated with high physiological demand, or that leaf P concentrations reach a level of saturation. In addition, to paint a more complete picture of the effects of P addition on leaf P concentration in subtropical forest, and ultimately on plant productivity, longer-term studies will be necessary.

The influence of P addition on leaf P concentration was independent of soil N availability, as no significant differences between P and NP treatments were observed in the present study (Fig. 1). Our findings were consistent with the results of previous studies performed on different growth types of plants or in P limited tropical forests (Li et al. 2016; Yuan and Chen 2015). For instance, Mayor et al. (2014) reported that there was no interaction between N and P fertilization in four common tree species in a lowland tropical forest in Panama following 13 years of N, P, and NP addition. Additionally, based on a meta-analysis, Yuan and Chen (2015) observed no significant differences in the P effect on leaf P concentrations in evergreen angiosperms. The most plausible reason for these observations is that there was no effect of N addition on soil P supply (Table 1). In soils, the supply of P originates from two processes: from organic P mineralization or from P addition. Soil pH is another important factor that influences organic P mineralization, as lower soil pH will decrease soil phosphatase activity. Based on our observations, soils under conditions of P addition exhibited significantly higher pH values (mean =  $4.04 \pm 0.15$  SD during 2013–2015) than soils under conditions of NP addition (mean =  $3.93 \pm 0.14$  SD during 2013–2015). This was likely because N addition intensifies soil acidification to some extent. These findings suggest that soil P mineralization rates should be lower under conditions of NP addition. However, the amount of  $100 \text{ kg P ha}^{-1} \text{ year}^{-1}$  from externally available P addition was much higher than the quantity of mineralized P owing to the lower amounts P substrates of available in subtropical soils. Hence, the differences in soil P mineralization under conditions of varying levels of acidity can be ignored. These findings also highlight the fact that

high ratios of atmospheric N and P deposition in the future could have the potential to increase leaf P concentrations.

Plant responses to P addition were species specific, signifying that shifts in community composition could occur in response to P availability in subtropical forests. Forests in southern China are typically broad-leaved evergreen forests. The forest investigated in the present study was a mixed coniferous and broad-leaved secondary forest developing from a *P. massoniana* plantation. *P. massoniana*, which is being replaced by *S. superba*, exhibited a stronger response to P addition in comparison to the nutrient-conserving *S. superba*. In comparison to *S. superba*, the higher P resorption efficiency of *P. massoniana* and lower green leaf N:P indicate a higher P use efficiency. These characteristics of *P. massoniana* suggest that P addition would be more favorable for *P. massoniana* than it would be for *S. superba*. Therefore, P addition could have potential to delay the succession of subtropical forests in this region, but this effect should be less because the amount of ambient P deposition was low ( $0.34 \text{ kg hm}^{-2} \text{ year}^{-1}$ ) in this region (Zhu et al. 2016) although P was one of the key factors contributing to forest succession. In addition, the lower P concentration in *S. superba* contributed to the higher leaf N:P in *S. superba* than in *P. massoniana*, which were consistent with the result of these two species in Han et al. (2005). However, when scaling up to the level of functional group, we should notice that there was no significant difference between the N:P values of broadleaf and coniferous species in China, even only for tropical and subtropical regions (Han et al. 2005).

### Responses of plant P limitation to external N and P inputs

Plant productivity in most tropical and subtropical forests is limited by P availability, which have been confirmed by previous fertilization experiments (Cleveland et al. 2002; Mayor et al. 2014; Townsend et al. 2007) and meta-analyses (Elser et al. 2007; Li et al. 2016). This is also true in the present study, as indicated by the response of leaf P concentration to P treatments. Meanwhile, the significant positive effect of P addition on the growth rate of small tree (< 15 cm DBH) showed in Li et al. (2017) based on this experiment also supported P addition can alleviate P limitation in subtropical forests. Furthermore, Olde Venterink (2016) highlighted “Chemical facilitation” which was named as a non-added nutrient can be enhanced by one added nutrient through changing soil chemical conditions (e.g., soil acidity). Our study provides new evidence of no chemical facilitation via N and P addition.

Liebig’s Law of the minimum, a theory that applies to the effects of nutrient limitation on plant growth, developed from the idea that growth is governed not by the

availability of all resources, but by the availability of the most limiting resource (single-resource limitation). Expanding on this idea, Harpole et al. (2011) reported four types of nutrient limitations on plant growth: single limitation, simultaneous co-limitation, independent co-limitation, and serial limitation; while Olde Venterink (2016) argued that chemical facilitation between nutrients is common. For example, N addition has been shown to decrease soil pH (Tian and Niu 2015), and subsequently increase P availability for plants through P desorption (Jin et al. 2014; Wang et al. 2011). In the present study, soil acidity induced by the addition of  $\text{NH}_4\text{NO}_3$  exerted no influence on soil P availability (Table 1; Fig. 1), as P is adsorbed to Fe or Al oxides and hydroxides in acidic soils (Hinsinger 2001). The rise in soil pH under conditions of P addition (Table 1) likely exerted a positive effect on soil P availability; however, this increase of soil available P caused by high soil pH was much less in comparison to the amount of P addition. Our findings demonstrate that the subtropical coniferous and broadleaved mixed forest is solely limited by P availability, and that the possibility of chemical facilitation between nutrients related to soil acidity is unlikely.

Unexpectedly, our findings are not consistent with the perspective that atmospheric deposition with a high N:P ratio, owing to high N deposition and low P deposition, would exacerbate P limitation in subtropical forests (Li et al. 2016; Zhu et al. 2016). The general view of enhanced P limitation under conditions of N addition, based on stimulated plant production by N addition, and then plant P demand based on N:P homeostasis, mostly occurs in N and P co-limited temperate forests (Braun et al. 2010; Gress et al. 2007). In the present case, this is not true because leaf N and P are insensitive to N addition. Hence, at the very least, the influence of high atmospheric N:P deposition would be overestimated for this type of forest.

Under normal conditions, plants are generally able to downregulate nutrient uptake under varied natural soil conditions. However, when the soil environment shifts from conditions of high P limitation to conditions of no P limitation in a short period of time, an excessive P uptake by plants may occur, even to saturated or toxic levels (Ostertag 2010). In this type of situation, leaf P concentration or N:P ratio may not reflect the actual demands of plant growth under conditions of P addition. Thus, the findings of this 2-year study could be further elucidated and verified by the long-term applications of potentially limiting nutrients to subtropical forest ecosystems.

**Author contribution statement** HY conceived the idea, conducted the analysis and wrote the manuscript.

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### Compliance with ethical standards

**Conflict of interest** The author declare that she has no conflict of interest.

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