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Differential effects of N and P additions on foliar stoichiometry between species and community levels in a subtropical forest in eastern China

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ABSTRACT

The responses of foliar nitrogen (N) and phosphorus (P) stoichiometry to nutrient additions are important indicators of various ecological processes, especially under climate change. However, how nutrient additions affect foliar N and P stoichiometry from species to community levels remain unclear, especially in P-limited systems. In this study, we conducted a nutrient additions experiment with control, N addition, P addition, and NP addition treatments in a P-limited subtropical forest to examine the effects of nutrient additions on foliar N and P stoichiometry. Our results showed that nutrient additions did not significantly influence foliar N concentration in seven dominant plant species over a three-year experimental period but significantly increased foliar P concentration and decreased foliar N:P ratio in species with lower initial foliar P concentration. The interactive effects of N and P additions on foliar P concentration and N:P ratio were synergistic and antagonistic, respectively. The response ratio of foliar P concentration to nutrient addition had a significantly negative correlation with initial foliar P concentration ($r = -0.644$), but had a positive one with initial foliar N:P ratio ($r = 0.619$). These results suggest that the limiting P dominated the responses of foliar N and P stoichiometry to nutrient additions at species level. At the community level, foliar P concentration and N:P ratio for both community weighted means (CWM) and community non-weighted means (CM) showed weaker positive responses to N and P additions than those at species level and there was no differential response between CWM and CM, suggesting the control of varied species responses on community foliar P concentration and N:P ratio response. Taken together, our results highlight the differential impacts of nutrient additions on foliar N and P stoichiometry between species and community levels, which needs to be considered in models when assessing the responses of ecosystem function to nutrient enrichments.

1. Introduction

Nitrogen (N) and phosphorus (P) are the two major limiting nutrients for plants in most terrestrial ecosystems (Reich et al., 1997; Elser et al., 2007). The foliar N and P stoichiometry (that is, foliar N and P concentrations, or N:P ratio) partially represents the relative availability of N and P in the soil (Güsewell, 2004; Elser et al., 2010). Soil nutrient availability often impacts foliar N and P stoichiometry, which subsequently affects plant photosynthesis, nutrient resorption, plant growth and community composition and ecosystem function (Ostertag, 2010; Marklein and Houlton, 2012; Yuan and Chen, 2015). N and P depositions considered as major contributors to global climate change (IPCC, 2013); depositions have increased three to five-fold since the

industrial and agricultural revolution with increased fossil fuel burning, vast deforestation and fertilizer consumption (Gruber and Galloway, 2008; Zhu et al., 2017), which alters the availability of soil nutrients for plants (Tian et al., 2018). Therefore, the responses of foliar N and P stoichiometry under N and P additions provide a useful insight into the species or ecosystem feedback to N and P depositions because of the close relationships between N and P stoichiometry and ecosystem nutrient availability.

The effects of N and P additions on foliar N and P concentrations have been widely studied in terrestrial ecosystems (Kozovits et al., 2007; Lü et al., 2013; You et al., 2018). However, foliar N and P concentrations showed positive (Lü et al., 2013), neutral (Chen et al., 2015) and negative (Nilsson and Wallander, 2003; Braun et al., 2010)

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responses to nutrient additions in different studies. Besides plant species and climate (Lu et al., 2011), the background soil nutrient availability has been recognized as an important factor controlling the impacts of nutrient additions on foliar N and P concentrations (You et al., 2018). Meta-analysis and site-specific studies showed N addition always increased foliar N concentration and N:P ratio in N-limited systems (Wang et al., 2018; You et al., 2018) through increasing soil N availability. The effects of N addition on foliar P concentration in N-limited systems varied among species, showing negative or neutral result (Wang et al., 2018; You et al., 2018). In P-limited systems, N addition may accelerate P cycling by allocating excess supplied N to phosphatase enzymes (Marklein and Houlton, 2012), which increases foliar P concentration. Although the N and P cycles are coupled (Jiang et al., 2019), the effects of fertilizer type (that is, N or P) and their interactions on foliar N and P concentrations in P-limiting systems remain unclear.

The responses of foliar N and P stoichiometry to nutrient additions varied among species (Wang et al., 2018), due to the differential regulation abilities of species in response to altered soil nutrient status (Yu et al., 2011; Yan et al., 2013). Meanwhile, the community composition (e. g. species abundance or richness) may change after nutrient enrichment (Lu et al., 2010). Therefore, there might be differential responses of foliar N and P stoichiometry to nutrient additions between the species and community levels (Wang et al., 2018). The comparison of nutrient additions impacts on foliar N and P stoichiometry between species and community levels might provide new insights into plant responses to nutrient enrichment and their potential impact on ecosystem function.

At the community level, the effects of nutrient additions on foliar N and P stoichiometry reflected the cumulative results of the species-specific responses as well as community composition change (Volf et al., 2016; Hou et al., 2019). Community weighted means (CWM) and community non-weighted means (CM) are two different ways to quantify the community nutrient traits (e. g. foliar N concentration, P concentration or N:P ratio) (Volf et al., 2016; Wang et al., 2018). The former takes the dominant species and community composition change in consideration, and the latter highlights the dominant and subdominant species (Volf et al., 2016). By comparing CWM and CM would help us to understand the species-specific variation and community composition change in controlling the response of community foliar N and P stoichiometry the N and P additions. Most previously studies comparing the CWM and CM focused on grassland ecosystems or N-limited systems (Volf et al., 2016; Wang et al., 2018). However, what degree the species-specific variation and community composition change driving the response of community function trait varied among different fertilization treatment is not well known in forest ecosystem.

Subtropical forest is the dominant vegetation type in the Yangtze River region of China, where heavy N and P deposition occurs (Zhao et al., 2009; Zhu et al., 2017) and plant growth is limited by P availability (Yan et al., 2010; Wang et al., 2015). However, to our knowledge, no studies have examined how N and P affect and their interaction on foliar nutrients in this region. We initiated a N and P additions experiment in the spring of 2011 in a subtropical broadleaved forest located in Nibong, Zhejiang province (Zheng et al., 2017; Liu et al., 2019). The forest was dominated by *Schima superba* and *Lithocarpus*

glaber. The objectives of this study were to (1) compare the effects of N and P additions and their interactions on foliar N and P stoichiometry at the species and community levels, (2) address the control of varied species-specific responses and community composition change on the response of community foliar N and P stoichiometry to nutrient addition. We hypothesized that: (1) foliar nutrient will be more responsive to P addition than N addition, and foliar P concentration will be more responsive than foliar N to nutrient additions because of the P limitation in this forest; (2) the response of community N and P stoichiometry to nutrient additions would be mainly driven by varied response among different species considering the forest community composition would change little in short-time nutrient additions.

2. Materials and methods

2.1. Study site

The study was conducted in Tiantong Forest Ecosystem Observation and Research Station (29°48′N, 121°47′E, a.s.L. 160 m). This area is characterized by a subtropical monsoon climate, with a mean annual temperature of 16.2 °C and precipitation of 1374 mm. The stand was harvested in the 1960s and has undergone natural re-forestation. The soil type is an Acrisol, with a medium–heavy loam texture and an organic layer about 5 cm thick (Song and Chen, 2007). This forest is P limited, as indicated by the positive response of microbial respiration to P addition (Liu et al., 2019).

2.2. Fertilization treatments

Nutrient addition with four treatments (control: 0 kg N ha^{-1} yr^{-1}; N addition (+N): 100 kg N ha^{-1} yr^{-1}; P addition (+P): 15 kg P ha^{-1} yr^{-1} and NP addition (+NP): 100 kg N ha^{-1} yr^{-1} + 15 kg P ha^{-1} yr^{-1}) was conducted in twelve plots (20 m × 20 m) in the evergreen broadleaved forest (Zheng et al., 2017). Each treatment was replicated in triplicate and randomly assigned. Plots were enclosed with PVC board inserted into the soil to 60 cm depths and separated by at least 10 m from each other. Details of soil properties and plant community characteristics for each treatment before the first fertilization are given in Table 1. From January 2011, fertilizer (NH$_4$NO$_3$ or NaH$_2$PO$_3$ in 20 L of water) was applied monthly over the litter layer. The control plots received 20 L of water to avoid differences in throughfall among the different treatments.

2.3. Leaf and soil sampling and nutrient analysis

Diameter at breast height (DBH) of each stand (DBH > 1 cm) was measured in November 2010 and August 2014. Seven dominant species (*S. superba* and *L. glaber* in the tree layer, *Camellia fraterna*, *Eurya muricata*, *Symplocos sumuntia*, *Eurya rubiginosa* and *Rhododendron ovatum* in the shrub layer) were chosen for leaf sampling, which accounted for about 94% of community biomass and 76% of abundance across the twelve plots. In each plot, young, fully expanded leaves were collected in the summers of 2011, 2012 and 2013. Two individuals of each species with the mean DBH in each plot were chosen. A composite

Table 1
Soil properties and plant community characteristics in control, N addition (+N), P addition (+P) and NP addition (+NP) plots in December 2010 before the first fertilization. Values are means ± SE (n = 3). There was no significant difference among treatments.

Treatments	Soil (0–20 cm)				Plant community	
	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	pH (water)	Tree density (tree ha^{-1})	DBH (cm)
control	33.13 ± 0.08	6.08 ± 0.20	0.45 ± 0.03	4.35 ± 0.03	4125 ± 14	17.47 ± 0.88
+N	32.73 ± 1.57	5.65 ± 0.14	0.40 ± 0.01	4.27 ± 0.03	4883 ± 440	17.05 ± 0.21
+P	32.61 ± 0.83	6.36 ± 0.12	0.40 ± 0.01	4.16 ± 0.02	4117 ± 670	18.65 ± 0.99
+NP	34.04 ± 5.17	5.91 ± 0.18	0.38 ± 0.01	4.23 ± 0.07	3567 ± 816	18.54 ± 1.67

sample of each individual from southern and northern aspects in the upper canopy was obtained. Samples were dried for 48 h at 80 °C before chemical analysis.

Soil samples (0–20 cm depth) were collected in December 2010 before the first fertilization, as well as in the summers of 2011, 2012 and 2013. Five samples per plot were collected using a soil corer (2.5 cm in diameter) and mixed to form a composite sample. Soil samples collected before the first fertilization were analyzed for soil organic carbon content (SOC), pH, total nitrogen concentration (TN) and total phosphorus concentration (TP). Collected fresh soil samples were used to determine available N and P concentrations.

The SOC was determined using the oil bath-K_2CrO_4 titration method (Nelson and Sommers 1996). Soil pH was determined in a soil-to-water ratio of 1:2.5 (v/v) using a pH meter (DM-143-SC, Mettler-Toledo International Inc, Columbus, OH, USA). Foliar N and soil TN were determined by the detection of ammonium. Foliar P and soil TP were determined by the detection of orthophosphateith. Leaf and soil samples were extracted with concentrated sulfuric acid and hydrogen peroxide. Soil ammonium and nitrate concentrations (available N concentration) were determined by extraction with KCL. Soil available P concentration was determined after the detection of phosphate by extraction with sodium bicarbonate. Concentrations in all extracts were determined using an auto chemical analysis meter (SMARTCHEM200, AMS Alliance, Italy).

2.4. Data analysis

Foliar N and P stoichiometry was calculated in two ways: community weighted means (CWM) and community non-weighted means (CM). CWM calculated as Eq. (1) (Yan et al., 2010):

$$Y = \sum_1^i Vi \times Xi / \sum_1^i Vi (i = 7) \tag{1}$$

where Y is foliar N concentration, P concentration or N:P ratio at community level; Xi is the foliar N concentration, P concentration or N:P ratio for species i; and Vi is the importance value (sum of relative abundance, relative dominance and relative frequency) for plant species i (Song, 2017) as the weighted factor. The N or P concentrations for each of the two individuals of each species in one plot were averaged. In Eq. (1), relative abundance is the percentage of the number of trees of a particular species relative to the total number of trees of all species in the community. Relative dominance is the percentage of stand basal area of a particular species relative to the total stand basal area of all species in the community. Relative frequency is the percent of frequency of a particular species relative to the total frequency of all species in the community. During our community investigation, each plot was divided into 16 sub-plots (5 m × 5 m), and the number of times that a particular species appearing in the sub-plots was calculated to obtain the frequency of that species (Song, 2017). For CM, the N or P concentrations and N:P ratio were averaged across all sampled species.

The response ratio (RR) was used to determine the magnitude of the response to nutrient addition, which was calculated as the experimental mean divided by the control mean (Hedges et al., 1999; Zhou et al., 2019). LnRR was calculated as the natural-log-transformed RR to weigh equally the negative and positive responses to nutrient addition (Marklein and Houlton, 2012). A positive value of LnRR indicates an increase of foliar N and P stoichiometry in the fertilized treatment relative to the control, whereas negative value represents a reduction.

Linear-mixed effects models with restricted maximum likelihood (REML) estimation were used to determine the effects of fertilization on foliar N and P stoichiometry of plant species or community. Effects of treatments on foliar N and P stoichiometry, soil nutrient availability, pH and importance value in specific year were analyzed using one-way ANOVA, followed by the Tukey multiple comparison test. Data were natural-log transformed when normality and homogeneity of variance assumptions were not met. Correlation analysis was used to determine

the relationship between the LnRR of foliar N and P stoichiometry and the initial values. Paired T-test was used to compare LnRR of CWM and CM.

Hedge's d was used to calculate the interactive effects of N and P additions on foliar N, P, and N:P ratio at community level with soil available N, P and pH, as described by Gurevitch and Hedges (2001) and Liu et al. (2019). The main effects of N and P additions and their interactions were calculated using Eqs. (2) − (4), respectively.

$$d_A = \frac{(\bar{X}_N + \bar{X}_{N+P}) - (\bar{X}_P + \bar{X}_{CK})}{2s} J(m) \tag{2}$$

$$d_B = \frac{(\bar{X}_N + \bar{X}_{N+P}) - (\bar{X}_N + \bar{X}_{CK})}{2s} J(m) \tag{3}$$

$$d_I = \frac{(\bar{X}_{N+P} - \bar{X}_N) - (\bar{X}_P - \bar{X}_{CK})}{2s} J(m) \tag{4}$$

where $\bar{X}_{CK}$ $\bar{X}_N$ $\bar{X}_P$ and $\bar{X}_{N+P}$ are the means of a variable in control, +N, +P and +NP treatments, respectively; s and m are the pooled standard deviation, and degree of freedom, respectively. The $J(m)$ is the pooled standard deviation and correction term for small sample bias (Hedges and Olkin, 1985). Values of s, m and $J(m)$, were estimated using Eqs. (5)–(7), respectively

$$s = \sqrt{\frac{(n_{CK} - 1)(s_{CK})^2 + (n_N - 1)(s_N)^2 + (n_P - 1)(s_P)^2 + (n_{N+P} - 1)(s_{N+P})^2}{n_{CK} + n_N + n_P + n_{N+P} - 4}} \tag{5}$$

$$m = n_{CK} + n_N + n_P + n_{N+P} - 4 \tag{6}$$

$$J(m) = 1 - \frac{3}{4m - 1} \tag{7}$$

where n_N, n_P, n_{CK}, n_{N+P} are the sample sizes, and s_N, s_P, s_{CK} and s_{N+P} are the standard deviations in the N and P treatments (s_N, s_P), control (s_{CK}), and +NP treatment (s_{N+P}), respectively; The variance of d_I (v_2) of main effects and interactions as well as weighted mean d_I (d_{++}) were estimated using the following equations.

$$v_{2i} = [\frac{1}{n_C} + \frac{1}{n_N} + \frac{1}{n_P} + \frac{1}{n_{N+P}} + \frac{d_I^2}{2(n_C + n_N + n_P + n_{N+P})}]/4 \tag{8}$$

$$d_{++} = \frac{\sum_{i=1}^m \sum_{j=1}^k w_{ij} d_{ij}}{\sum_{i=1}^m \sum_{j=1}^k w_{ij}} \tag{9}$$

where I is the number of groups, k is the number of comparisons in the group and w is weight, which is also the reciprocal of the variance (1/v_2).

When the sample number was > 20, the 95% CI of RR_{++} and d_{++} was calculated as $RR_{++} \pm C_{a/2} \times s(RR_{++})$ and $d_{++} \pm C_{a/2} \times s(d_{++})$, respectively, where $C_{a/2}$ is the two-tailed critical value of the standard normal distribution. The bootstrapping method was conducted to for resampling based on 2500 iterations which was consistent with the calculations of CI of individual and combined effects, when the sample number was > 20. The interaction types were classified as antagonistic, synergistic and additive according to the above calculations (Crain et al., 2008). Specifically, the interactive effect was considered to be additive if the 95% CI overlapped with zero. For factor groups whose individual effects were either both negative and exhibited opposite directions, interactions < 0 were classified as synergistic and > 0 as antagonistic (Crain et al., 2008).

All statistical tests were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and R software (R3.2.1, R Core Team 2015).
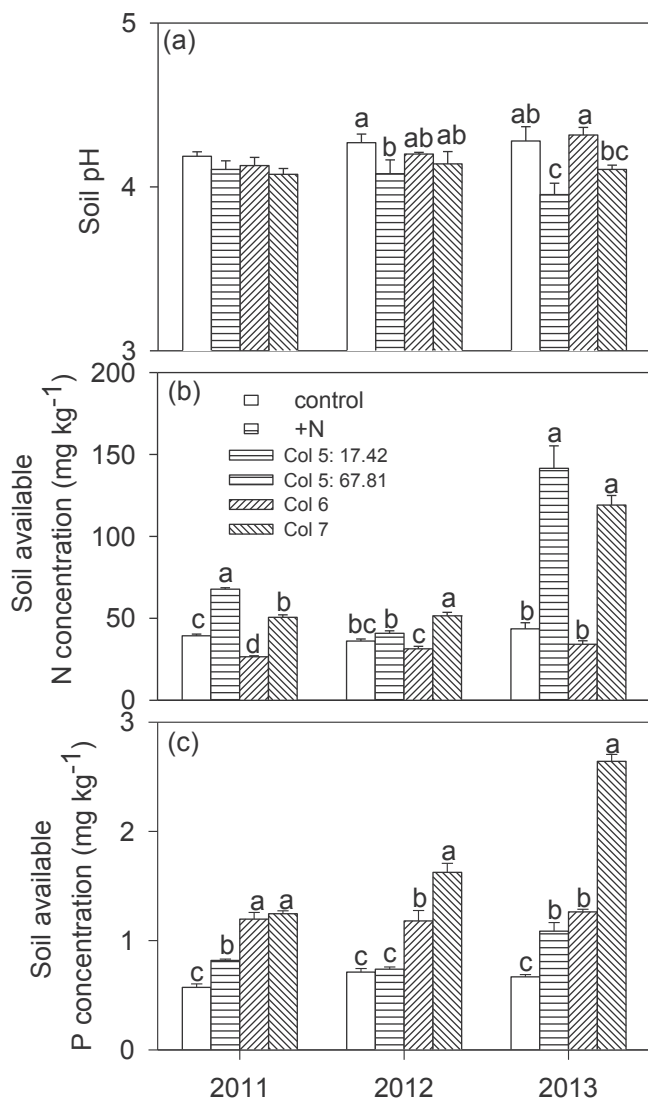
Fig. 1. Soil pH (a), available N concentration (b) and available P concentration (c) in control, +N, +P and +NP treatments in different years. Error bars are ± SE of means for $n = 3$. Different letters indicate significant differences among treatments ($P < 0.05$).

3. Results

3.1. Effects of nutrient additions on soil nutrient availabilities and species importance value

During the whole experiment, soil pH decreased significantly under N and NP additions ($t = -3.328$, $P = 0.005$; $t = -4.08$, $P = 0.001$, respectively), while soil available N concentration increased significantly under N and NP additions ($t = 3.814$, $P = 0.002$; $t = 4.164$; $P < 0.001$, respectively) but decreased under P addition ($t = -5.125$, $P < 0.001$) (Fig. 1, Table S1). Soil available P concentration increased significantly under NP addition ($t = 7.637$, $P < 0.001$). The interactive effects of N and P additions on soil pH and available N concentration were both additive, while a synergistic interaction was observed on soil available P concentration (Fig. 2a–c).

After three-year nutrient additions, the response of community composition was limited (Fig. 3), and only NP addition significantly increased the importance value of *L. glaber*, and P addition significantly increased and decreased importance value of *R. ovatum* and *S. sumuntia*, respectively.

3.2. Effects of nutrient additions on foliar N and P stoichiometry at species level

In the control treatment, the foliar N concentrations ranged from 13.20 ± 0.42 to 16.18 ± 0.86 g kg^{-1} and the foliar P concentrations ranged from 0.75 ± 0.05 to 0.98 ± 0.02 g kg^{-1} across the seven dominant tree species (Table S2). Among these species, *E. rubiginosa* had the highest foliar N:P ratio (21.64 ± 4.32) mainly because of its low foliar P concentration, while *S. sumuntia* had the lowest foliar N:P ratio (15.89 ± 1.15).

Foliar N concentration in most species (except for *R. ovatum* and *S. sumuntia*) showed no significant response to nutrient additions during the experiment (Table 2, Fig. S1). The responses of foliar P concentration and N:P ratio to NP addition were greater than those to N or P addition alone (Table 2, Fig. S2), but these responses were species-specific. For example, N addition significantly reduced foliar P concentration in *C. fraterna* ($t = -2.45$, $P = 0.026$) and *S. sumuntia* ($t = -3.057$, $P = 0.009$) by 14% and 22%, respectively, but induced a significant increase of foliar P concentration by 16% in *E. muricata* ($t = 2.909$, $P = 0.011$). The NP addition significantly increased foliar P concentrations in those species with higher initial foliar N:P ratio (usually lower initial foliar P concentration; that is, *L. glaber* ($t = 4.283$, $P < 0.001$), *E. muricata* ($t = 4.203$, $P < 0.001$), *E. rubiginosa* ($t = 3.386$, $P = 0.004$), and *R. ovatum* ($t = 4.031$, $P < 0.001$)). Consequently, NP addition significantly decreased foliar N:P ratio in these species (Table 2, Fig. S3).

The three-year averaged natural-log-transformed response ratio (LnRR) of foliar P concentration (or N:P ratio) at species level showed the most positive (or negative) values regardless of fertilizer type (Fig. 4). The LnRR values showed that the foliar P concentration (−0.24 to 0.38) and N:P ratio (−0.33 to 0.35) were more responsive than foliar N concentration (−0.06 to 0.07) under nutrient additions. When all species were pooled, LnRR of foliar P concentration were negatively correlated with the initial foliar P concentration ($r = -0.644$, $P < 0.001$), but positively correlated with initial foliar N:P ratio ($r = 0.619$, $P = 0.001$) (Table 3). LnRR of foliar N:P ratio showed the opposite relationships with initial foliar P concentration (or N:P ratio). The significant correlation between LnRR of foliar P concentration and N:P ratio ($r = -0.971$, $P < 0.001$) displayed that changes in foliar N:P ratio were mainly determined by the response of foliar P concentration.

Effects of nutrient additions on foliar N and P stoichiometry at community level The foliar P concentration were both significantly increased by 20% both for CWM and CM under NP addition ($t = 0.012$, $P < 0.001$), while foliar N:P ratio significantly decreased by 13% and 14% for CWM and CM, respectively ($t = 0.005$, $P < 0.001$) (Fig. 5b, e). The interactive effect of N and P additions was additive on foliar N concentration for both CWM and CM, and was synergistic on foliar P concentration, while an antagonistic interaction was observed on foliar N:P ratio (Fig. 2d-f).

The response magnitude of community foliar N and P stoichiometry to N and P additions together was greater than those to N or P addition alone (Fig. 5b, c). The response of foliar stoichiometry for CWM and CM to nutrient additions were both weaker than those at species level. LnRR of foliar P concentration under NP addition was 0.17 for both CWM and CM (Fig. 6b), whereas that at the species level ranged from − 0.09 to 0.35 (Fig. 4f). LnRR of foliar N:P ratio under NP addition for CWM and CM was −0.14 and −0.16 (Fig. 6c), whereas that at the species level ranged from − 0.34 to 0.11 (Fig. 4i). There was no significant difference between Ln RR of CWM and CM (Table 4).
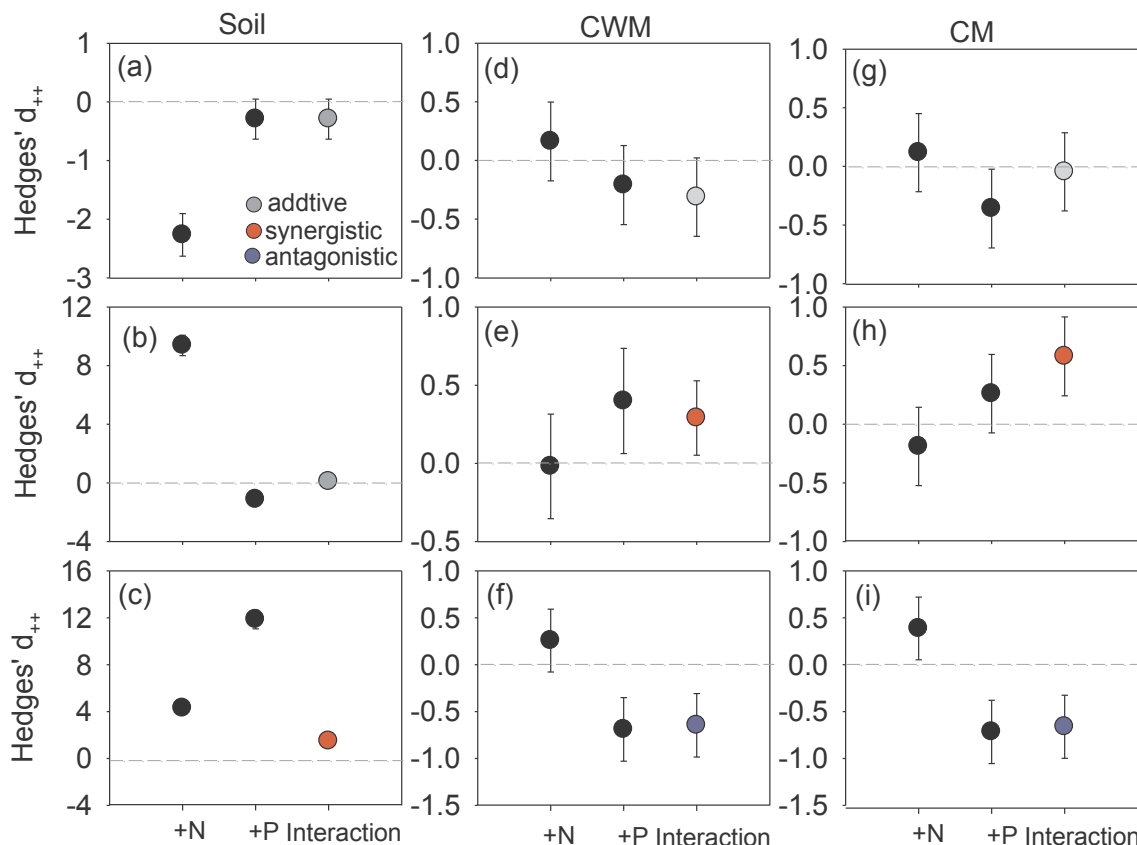
Fig. 2. The main and interactive effects of +N, +P and +NP treatments and their combination on soil pH (a), available N concentration (b) and available P concentration (c), foliar N and P stoichiometry (d-f: N, P concentrations and N:P ratio for CWM; g-i: N, P concentrations and N:P ratio for CM). Values represent means with 95% bootstrap confidence intervals (CIs). If the 95% CIs do not overlap with zero, a response is considered to be significant ($P < 0.05$).

4. Discussion

4.1. Response of foliar N and P stoichiometry to nutrient additions at species level

The response of foliar P concentration to nutrient addition was generally stronger than that of foliar N concentration regardless of fertilizer or species type (Treseder and Vitousek, 2001; Ostertag, 2010). Consistent with former studies and our hypothesis (1), we found that

the foliar P concentration increased under nutrient additions for most species and the responses were much stronger than those of foliar N concentration (Fig. 4). This result might be ascribed to two reasons. First, tree species, especially those growing in P-limited soil, were unable to down-regulate P uptake under high nutrient supplies (Standish et al., 2007), leading to P accumulation in the foliage (Fig. S2). Our results indicated that the response of foliar P concentration to nutrient additions was mediated by the plant capacity for P acquisition. Second, P cycling would be accelerated in response to the extra N supply
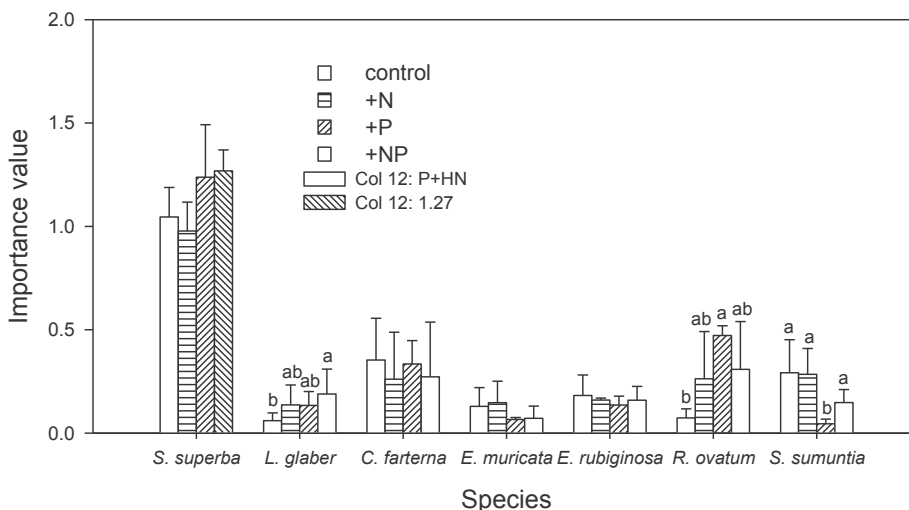

Fig. 3. The effects of N and P additions on importance value of seven dominant species. Error bars are ± SE of means for $n = 3$. Different letters indicate significant differences among treatments ($P < 0.05$).

Table 2
The *t*-values of linear-mixed effects models with REML estimation for effects of N and P additions on foliar N and P stoichiometry of dominant species.

Layer	Species	+N			+P			+NP		
		N	P	N:P ratio	N	P	N:P ratio	N	P	N:P ratio
Tree layer	*S. superba*	0.576	1.146	−1.068	1.171	0.27	0.241	1.093	1.548	−1.741
	L. glaber	−0.401	−1.118	0.948	−0.095	2.133·	−1.667	1.907	4.283***	−2.405*
Shrub layer	*C. fraterna*	0.638	−2.45*	1.912·	−0.507	−0.851	0.689	−0.05	−1.304	1.037
	E. muricata	1.017	2.909*	−1.838·	0.458	1.932·	−1.416	−0.042	4.203***	−3.88**
	E. rubiginosa	1.127	0.439	−0.675	−1.177	1.455	−2.138·	−0.249	3.386**	−3.07**
	R. ovatum	3.187**	0.686	−0.387	0.756	2.827*	−2.104·	1.417	4.031***	−3.948*
	S. sumuntia	2.23*	−3.057**	4.581***	0.202	−1.199	1.465	0.569	−1.064	1.379

·$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(Marklein and Houlton, 2012), because of the positive synergistic interaction of N and P additions on soil P availability (Fig. 2c), which results in the greater response of foliar P than that of foliar N after nutrient additions (Fig. 4).

The initial foliar N and P stoichiometry is often critical in determining the response to nutrient additions (Liu et al., 2013). In this study, the relationship between initial foliar N and response of foliar N was weak, and only the initial foliar P concentration (or N:P ratio) was significantly correlated with the response of foliar P concentration (Table 3). Species with lower foliar P concentration and higher N:P ratio (that is, *E. muricata*, *E. rubiginosa* and *R. ovatum*), indicating stronger P limitation, tended to sequester the supplied N and P, thereby increasing P accumulation and alleviating the inherent P limitation (Olander and Vitousek, 2000; Marklein and Houlton, 2012). Our results indicate that P limitation might could dominate the responses of

species-specific foliar stoichiometry to nutrient additions in this forest.

4.2. Response of foliar N and P stoichiometry to nutrient additions at community level

Meta-analysis showed that N fertility increased foliar N concentration at the global scale (Ostertag and DiManno, 2016; Sardans et al., 2017). However, N addition did not significantly affect the foliar N and P stoichiometry for both CWM and CM in this forest (Fig. 5a, d), which might relate to the nutrient dilution during biomass allocation (Güsewell et al., 2003), because the N-induced increase in aboveground biomass was greater in the + N treatment than that in the control (unpublished data). Although N addition significantly increased soil N availability (Fig. 1b), most available N may be diluted during plant growth (Jiang et al., 2019), leading to the neutral response of foliar N
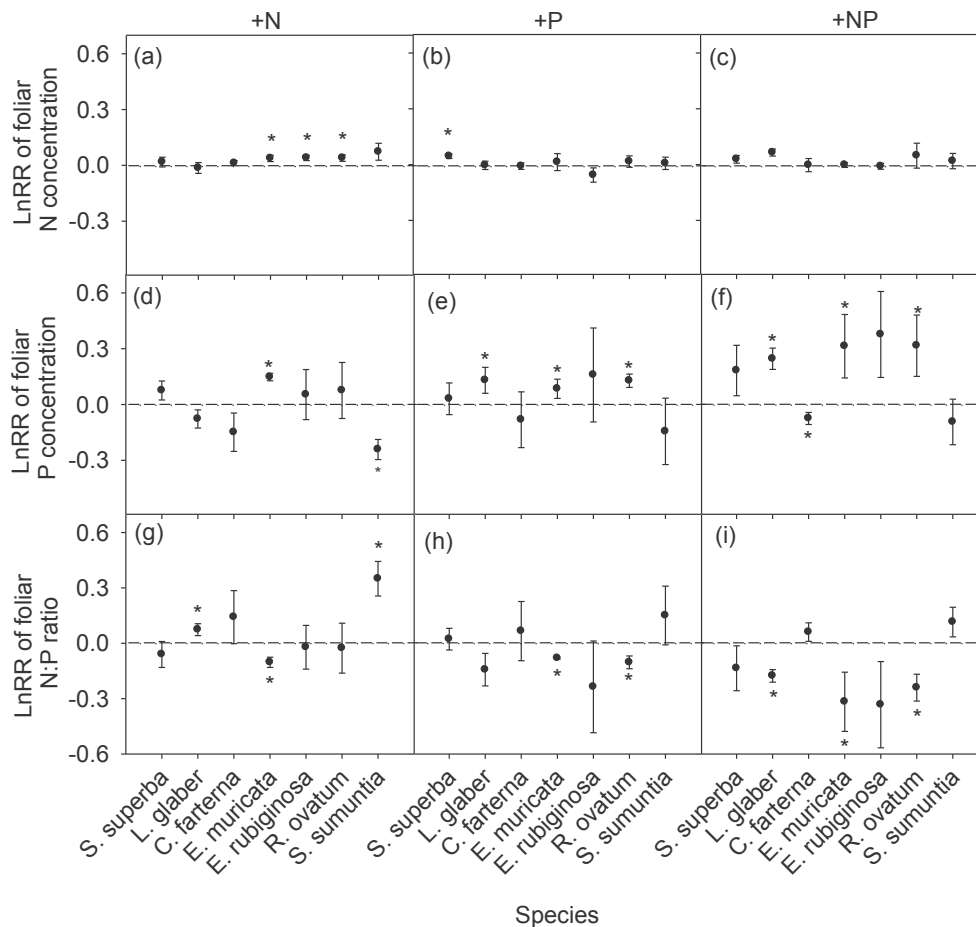

Fig. 4. The natural-log-transformed response ratio (LnRR) of foliar N concentration (a–c), P concentration (d–f) and N:P ratio (g–i) of seven species under +N, +P and +NP treatments. Error bars are ± SE of means for $n = 3$. Asterisks indicate that the LnRR values are significantly different from zero.

Table 3
Pearson correlation coefficients (r) between initial foliar N and P stoichiometry and the natural-log-transformed response ratio (LnRR) of foliar N and P stoichiometry ($n = 21$).

	Initial foliar P	Initial foliar N:P ratio	LnRR of foliar N	LnRR of foliar P	LnRR of foliar N:P ratio
Initial foliar N	0.645***	−0.222	−0.059	−0.174	0.161
Initial foliar P		−0.856***	−0.081	−0.644***	0.637***
Initial foliar N:P ratio			−0.222	0.619**	−0.635***
LnRR of foliar N				−0.059	0.276
LnRR of foliar P					−0.971***

**$P < 0.01$; ***$P < 0.001$.

concentration to N addition. This suggests that N enrichment in P-limited systems did not necessarily exacerbate P limitation of plant growth (Brouwer et al., 2001), rather, it enhanced soil P availability for plants (Fig. 1c), thereby alleviating further P limitation.

The response of foliar P concentration or N:P ratio to N and P addition together for both CWM and CM was stronger than that of N or P addition alone (Fig. 6), due to the interaction of N and P additions. Although P concentration at the community was not significantly affected by N or P addition, the interactive effects of N and P additions on soil available P and foliar P concentration were both synergistic in this system (Fig. 2c, e, h), which was consistent to former studies and highlighted the promoted uptake of P by plants under the N and P addition together (Jiang et al., 2019). Foliar P concentration was increased significantly under NP addition (Fig. 5b, e), which was

consistent to the *meta*-analysis results about tropical forests (Hou et al., 2019; Jiang et al., 2019). The foliar P concentration was determined by the balance between plant growth and P uptake (Güsewell, 2004). In this system, NP addition increased soil available P leading to more nutrient for plant uptake, but did not cause P dilution effect, resulting in the significant increase of foliar P concentration after NP addition (Fig. 5b, e). The significant decrease of foliar N:P ratio under NP treatment because response of N:P ratio was mainly determined by the response of foliar P concentration (Table 3).

The response of community foliar N and P stoichiometry was driven by community change and various species-specific responses (Volf et al., 2016). Through comparing the LnRR of foliar N and P stoichiometry between CWM and CM, we found the response magnitude for CWM and CM was not significantly different (Fig. 6, Table 4). Due to
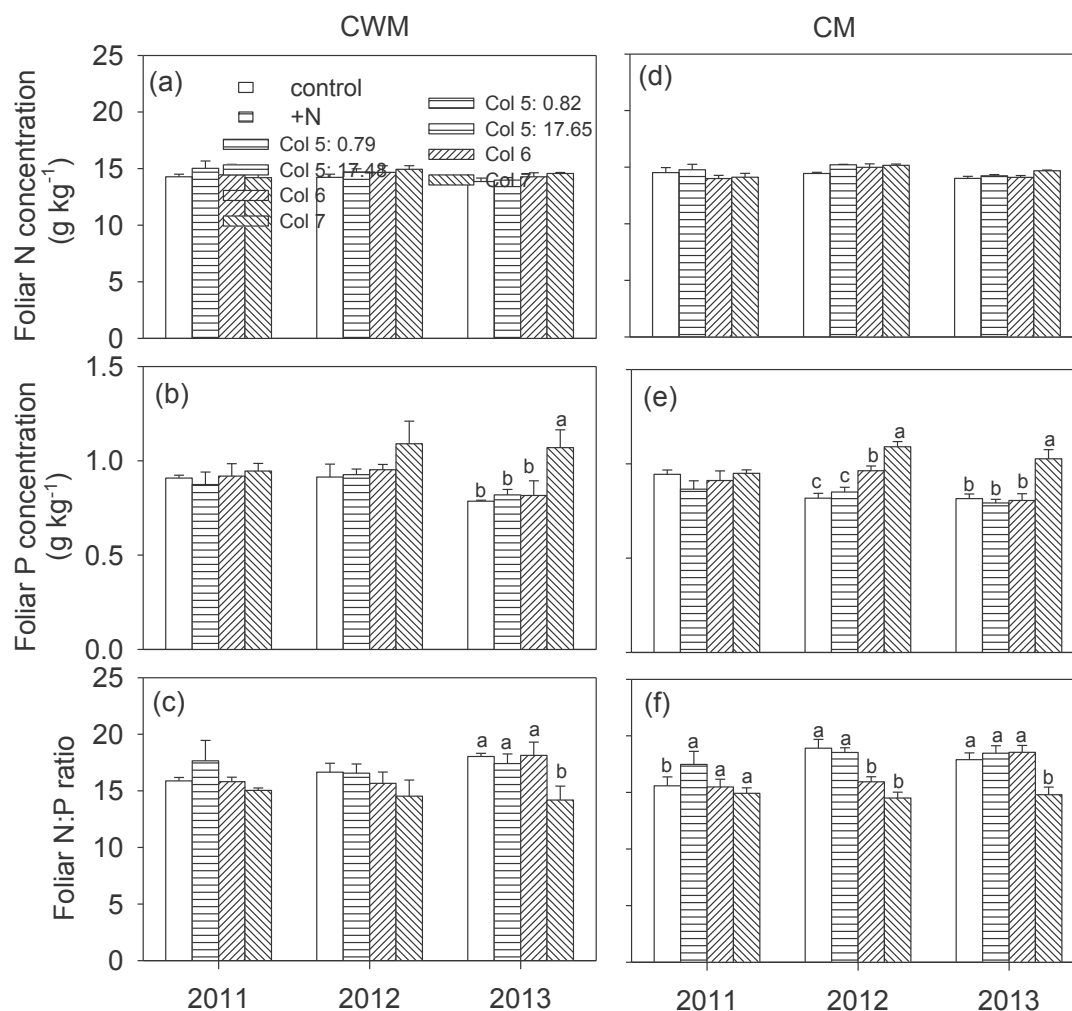

Fig. 5. The foliar N concentration, P concentration and N:P ratio for CWM (a-c) and CM (d-f) under control, +N, +P and +NP treatments. Error bars are ± SE of means for $n = 3$. Different letters indicate significant differences among treatments ($P < 0.05$).
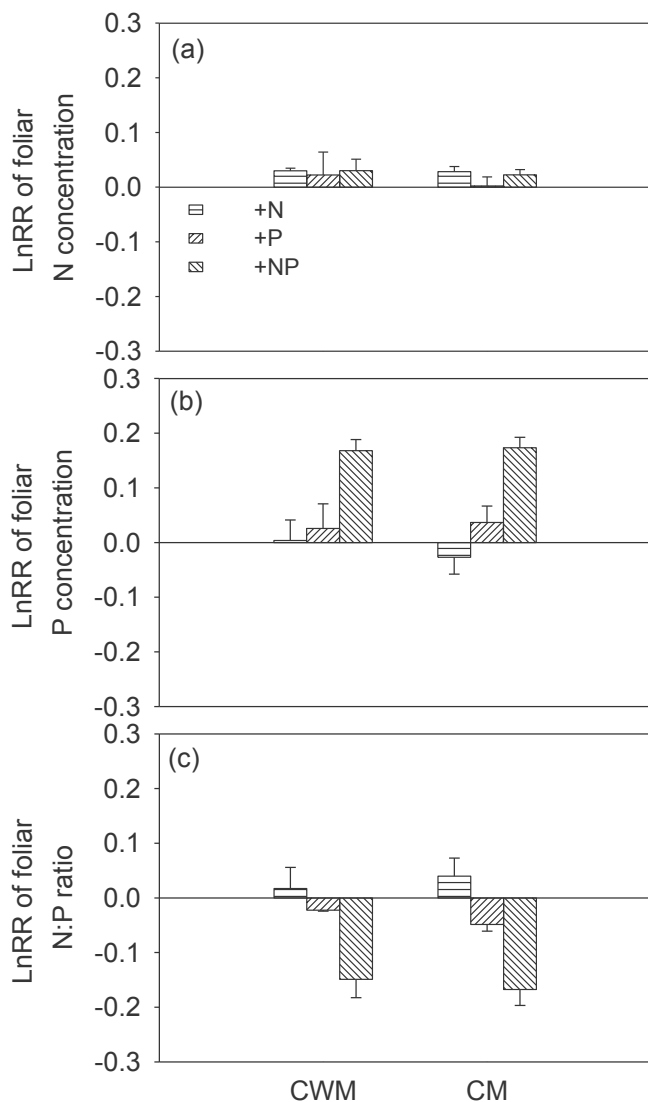
Fig. 6. The natural-log-transformed response ratio (LnRR) of community foliar N concentration (a), P concentration (b) and N:P ratio (c) for CWM and CM under +N, +P and +NP treatments. Error bars are ± SE of means for n = 3.

Table 4
The t-values of paired T-test for the comparison of LnRR of foliar N and P stoichiometry between community weighted means (CWM) and community non-weighted means (CM).

Treatment	LnRR of foliar N	LnRR of foliar P	LnRR of foliar N:P ratio
+N	2.471	1.708	−0.411
+P	1.020	1.634	0.185
+NP	0.832	0.779	−0.493

the complex community composition, the ecosystem function in forest was stable even under long-term nutrient input (Lu et al., 2018). In this forest, the importance value changed little after three-year N and P addition (Fig. 3). These results suggest the significant increase in community foliar P concentration and N:P ratio was mostly driven by the variation of species-specific responses. Thus, the stronger response of foliar P concentration and N:P ratio in several species (i. e. *E. muricata* and *E. rubiginosa*) would be offset by weaker ones in other species, resulting in foliar N and N:P community level was weaker than those at the species level (Fig. 4, Fig. 6).

5. Conclusions

Nutrient additions affected foliar N and P stoichiometry at both species and community levels, with the effect size varying with fertilizer type and the species or site-specific soil nutrient availability. By examining the effects of N and P additions on foliar N and P stoichiometry in seven dominant species in a P-limiting subtropical forest, we found that foliar P concentration in tree species with lower initial foliar P concentration (higher foliar N:P ratio) had a strong positive response to nutrient additions, which was different from the neutral response of foliar N concentration. This indicates that plants with stronger P limitation would use supplied N and P to influence the P dynamics and alleviate the inherent P limitation. The significantly negative correlation between initial foliar P concentration (or N:P ratio) and response ratio of foliar P concentration (or N:P ratio) suggests that limiting P may dominate the response of species foliar nutrient stoichiometry in this system. At the community level, foliar N and P stoichiometry both for CWM and CM showed a weaker positive response to nutrient additions than that at the species level. The same magnitude response of CWM and CM suggests the response of community foliar N and P stoichiometry was mostly driven by varied response among species rather than community composition change. However, the relative importance of intra-specific and inter-specific variation of functional traits on the community N and P stoichiometry response to nutrient additions requires further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106537.

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