

# Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests

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## Abstract

Increased atmospheric carbon dioxide (CO<sub>2</sub>) concentrations and nitrogen (N) deposition induced by human activities have greatly influenced the stoichiometry of N and phosphorus (P). We used model forest ecosystems in open-top chambers to study the effects of elevated CO<sub>2</sub> (ca. 700 μmol mol<sup>-1</sup>) alone and together with N addition (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) on N to P (N : P) ratios in leaves, stems and roots of five tree species, including four non-N<sub>2</sub> fixers and one N<sub>2</sub> fixer, in subtropical China from 2006 to 2009. Elevated CO<sub>2</sub> decreased or had no effects on N : P ratios in plant tissues of tree species. N addition, especially under elevated CO<sub>2</sub>, lowered N : P ratios in the N<sub>2</sub> fixer, and this effect was significant in the stems and the roots. However, only one species of the non-N<sub>2</sub> fixers showed significantly lower N : P ratios under N addition in 2009, and the others were not affected by N addition. The reductions of N : P ratios in response to elevated CO<sub>2</sub> and N addition were mainly associated with the increases in P concentrations. Our results imply that elevated CO<sub>2</sub> and N addition could facilitate tree species to mitigate P limitation by more strongly influencing P dynamics than N in the subtropical forests.

**Keywords:** carbon dioxide, N addition, N : P ratios, N<sub>2</sub> fixer, subtropical forests

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## Introduction

Environmental changes induced by anthropogenic activities, such as increasing carbon dioxide (CO<sub>2</sub>) and enhanced nitrogen (N) deposition, have profoundly altered N and phosphorus (P) cycles in natural ecosystems (Vitousek *et al.*, 1997; Perring *et al.*, 2008; Sardans *et al.*, 2012). It has been observed that elevated CO<sub>2</sub> decreased N and P concentrations in plants because of growth dilution (Johnson *et al.*, 1997; Cotrufo *et al.*, 1998). Plant could also respond to enhanced N deposition with increases in N and/or P concentrations in tissues (Fujita *et al.*, 2010; Huang *et al.*, 2012; Marklein & Houlton, 2012; Sardans *et al.*, 2012). The alterations in N and/or P concentrations in plants contribute to a shift in N to P (N : P) ratios, which, in turn, influences plant production and decomposition processes (Serner & Elser, 2002; Wang *et al.*, 2010; Huang *et al.*, 2011b).

N : P ratios in plants can provide insight into nutrient limitation, vegetation composition and functioning in ecosystems under future environmental change

(Güsewell, 2004; Elser *et al.*, 2010). The studies of N : P ratios in plants, which were exposed to elevated CO<sub>2</sub> and N addition, have mainly focused on leaves. However, the responses of other plant tissues, such as stems and roots, to elevated CO<sub>2</sub> and N addition are different from leaves (Gifford *et al.*, 2000; Xia & Wan, 2008). Garrish *et al.* (2010) found that the homeostatic control over N : P ratios was stronger in leaves than in stems or roots. This is because the allocation or storage of excess N or P uptake from soils could preferably occur in stems and roots rather than in leaves when one of these nutrients limits plant growth but the other does not (Dyckmans & Flessa, 2001; Cernusak *et al.*, 2010). These suggested that N : P ratios in leaves would be less responsive to elevated CO<sub>2</sub> or N addition than those in stems and roots when soil N or P cycling was altered by the environmental changes. Moreover, leaves are metabolically more active than stems and roots. The nutrient in leaves can be acquired through the nutrient movement within plants as well as nutrient supply in soils (Lukac *et al.*, 2010), which directly reflects the nutrient need for short-term physiological activities in plants. However, nutrients in stems and roots, functioning differently and with longer lifespans compared with leaves, are expected to more represent the results of long-term impacts of environmental changes (Xu

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*et al.*, 2009). Based on these, it is necessary to investigate the responses of N : P ratios in different plant tissues (rather than leaf centric) to elevated CO<sub>2</sub> or N addition.

High N deposition with the range 30–73 kg N ha<sup>-1</sup> yr<sup>-1</sup> was observed in some forests of Southern China (Mo *et al.*, 2006). As N availability increases, P could become increasingly limiting to plant growth in response to elevated CO<sub>2</sub> (Gress *et al.*, 2007). Previous studies have demonstrated that foliar N : P ratios in subtropical mature forests were high and exceeded the break point that often suggests P limitation (N/P > 20) at a vegetation level (Güsewell, 2004; Huang *et al.*, 2011a). Therefore, unraveling the responses of organ-specific N : P ratios to elevated CO<sub>2</sub> with the increased N deposition in tropical and subtropical forests would improve our understanding of how plant species adjust their stoichiometry of N and P to cope with environmental changes in P limitation conditions.

The N<sub>2</sub> fixer has the ability of direct access to N from the atmosphere. It appears that the N<sub>2</sub> fixer would suffer less from progressive N limitation than the non-N<sub>2</sub> fixer under elevated CO<sub>2</sub> (Luo *et al.*, 2004; Johnson, 2006). It has been suggested that the N<sub>2</sub> fixer is adept at investing N into phosphatase production to reduce P limitation compared with the non-N<sub>2</sub> fixer in P-limited conditions (Houlton *et al.*, 2008). Previous studies also showed that the rate of P cycling was greater in the stands of the N<sub>2</sub> fixer than those of the non-N<sub>2</sub> fixer, although the influences of the N<sub>2</sub> fixer on soil P availability were variable (Giardina *et al.*, 1995; Compton & Cole, 1998; Binkey *et al.*, 2000). Therefore, the responses of the N<sub>2</sub> fixer to elevated CO<sub>2</sub> would differ from those of the non-N<sub>2</sub> fixer when P limitation is induced by the increasing N.

This study was designed to investigate N : P ratios in different plant tissues, including leaves, stems and roots, of five tree species (four non-N<sub>2</sub> fixers and one N<sub>2</sub> fixer) in response to elevated CO<sub>2</sub> and N addition in subtropical model forest ecosystems established in open-top chambers. N and P concentrations in the three plant tissues of the five tree species were measured at the end of each year from 2006 to 2009. The objectives of this study were (1) to estimate how N : P ratios in different plant tissues of the tree species would respond to elevated CO<sub>2</sub>, (2) to evaluate how N addition would influence their N : P ratios under high soil N availability, and (3) to investigate whether N addition would change their N : P ratios in response to elevated CO<sub>2</sub>.

## Materials and methods

### Study site

The study was conducted at South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China (23°20' N

and 113°30' E). The area has a typical monsoon and humid climate with the mean relative air humidity at 77%. The annual precipitation ranges from 1600 to 1900 mm with 80% occurring from April to September. The mean annual temperature is 21.5 °C with the mean annual total solar radiation of 4367.2–4597.3 MJ m<sup>-2</sup> in the visible waveband.

### Experimental design

We used 10 open-top chambers exposed to full light and rain in an open space to model subtropical forest ecosystems. Each cylindrical chamber had a 3 m diameter, with a 3 m high above-ground section (adjusted to 4.5 m later) and a 0.7 m deep below-ground section. The above-ground section was wrapped with transparent and impermeable plastic sheets. The below-ground section was surrounded by concrete brick wall to prevent the lateral or vertical movement of water and/or element from the surrounding soils. There were three holes at the bottom of the wall. The holes, which were capped by a 2 mm net to prevent losses other than those of leachates, were connected to a stainless steel water collection box (Liu *et al.*, 2011).

In March 2005, soils classified as ultisol overlying sandstone and shale bedrocks were collected from a nearby evergreen broad-leaved forest after harvesting. Three different layers of soils (0–20, 20–40, and 40–70 cm) were homogenized separately and then placed into the below-ground part of chamber correspondingly. The initial soil chemical properties were presented in Table 1.

Six tree species, which are native and the most widely spread in Southern China, were selected. They included *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Castanopsis hystrix* Hook. f. et Thomson ex A. DC. (*C. hystrix*), *Syzygium hancei* Merr. et Perry (*S. hancei*), *Pinus massoniana* Lamb. (*P. massoniana*), *Schima superba* Gardn. et Champ. (*S. superba*) and *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*). Each chamber was planted with eight 1- to 2-year-old seedlings for each tree species. There were total of 48 seedlings randomly located in each chamber. *P. massoniana* was a typical pioneer species and the other tree species were representative species of the climax community in subtropical forests. When they grew together, *P. massoniana* died in the second year of our experiment due to the competitive exclusion by the other species. Thus, we only studied the other five species in this experiment.

From April 2005, the chambers were randomly exposed to four treatments with two levels of CO<sub>2</sub> concentrations (elevated CO<sub>2</sub> and ambient CO<sub>2</sub>) and two levels of N additions (with and without N fertilizer). Three chambers received an elevated CO<sub>2</sub> (ca. 700 μmol mol<sup>-1</sup>) with N fertilizer (NH<sub>4</sub>NO<sub>3</sub> applied at 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) (EN), three chambers received an elevated CO<sub>2</sub> without N fertilizer (EU), two chambers received ambient CO<sub>2</sub> (ca. 390 μmol mol<sup>-1</sup>) with N fertilizer (AN) and finally, two chambers acted as controls (ambient CO<sub>2</sub> and without N fertilizer) (AU). The elevated CO<sub>2</sub> treatments were achieved by supplying additional CO<sub>2</sub> from a tank until the CO<sub>2</sub> concentration of the chamber was ca. 700 μmol mol<sup>-1</sup>. The additional CO<sub>2</sub> was distributed by a transparent pipe entwined the inner wall of the chamber in a

**Table 1** Initial soil chemical properties

Depth (cm)	pH	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Total P (g kg <sup>-1</sup> )	NH <sub>4</sub> -N (mg kg <sup>-1</sup> )	NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )
0–20	4.15	16.33	0.52	0.30	9.30	17.40	2.13
	(0.15)	(3.42)	(0.15)	(0.09)	(1.73)	(8.34)	(0.93)
20–40	4.27	7.78	0.36	0.18	9.74	4.19	0.42
	(0.15)	(0.91)	(0.05)	(0.19)	(1.46)	(2.57)	(0.21)
40–60	4.25	3.94	0.29	0.14	8.68	4.94	0.33
	(0.13)	(1.54)	(0.07)	(0.07)	(2.31)	(2.70)	(0.12)

The values in brackets are standard deviations. C, carbon; N, nitrogen; P, phosphorus. Data in the table, except for NH<sub>4</sub>-N and NO<sub>3</sub>-N, were cited from Liu *et al.* (2008). Soil available P was extracted by acid-ammonium fluoride solution (0.03 mol L<sup>-1</sup> NH<sub>4</sub>F–0.025 mol L<sup>-1</sup> HCl) (Bray & Kurtz, 1945) and determined by molybdate colorimetry.

snake shape in the height of 0.5–2.5 m. The pipe contained pinholes, and the distance between pinholes was 1 cm. A fan was connected to the pipe to make CO<sub>2</sub> equally distributed in the whole chamber. The CO<sub>2</sub> flux was controlled by a flowmeter. The CO<sub>2</sub> concentrations on the five planes (0.5, 1.0, 1.5, 2, and 2.5 m in height) in the chamber were periodically monitored with a Licor-6400 (LI-COR Inc., Lincoln, NE, USA). The N addition was achieved by spraying seedlings once a week with a total amount of NH<sub>4</sub>NO<sub>3</sub> at 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>. No other fertilizer was used. The seedlings were watered with tap water as the walls of the chambers in the below-ground sections blocked lateral and vertical water fluxes. All other chambers received the same amount of water as the AU chambers.

#### Sample collection and measurement

Samples were collected from the five tree species, consisting of *A. acuminatissima*, *C. hystrix*, *O. pinnata*, *S. hancei* and *S. superba*. One tree per species in each chamber was randomly harvested at the end of each year from 2006 to 2009. The leaf, stem and root samples were collected from the harvested trees for N and P analysis.

Samples were dried for 72 h at 70 °C and then finely ground. N concentration was measured using the Kjeldahl method (Bremner & Mulvaney, 1982). P concentration was measured photometrically after samples were digested with nitric acid (HNO<sub>3</sub>). Mass N : P ratios were used in our study.

#### Data analysis

Data were confirmed with the Kolmogorov–Smirnov test for normality and with the Levene's test for homogeneity of variance prior to statistical analysis. Data were log transformed when normality and homogeneity of variance assumptions were not satisfied. Repeated-measures ANOVA was used to investigate the effects of elevated CO<sub>2</sub> and N addition on N : P ratios in each plant tissue of tree species over the experimental time. Significant differences among treatments in each sampling time were analyzed using ANOVA, followed by Tukey multiple comparison test. As there were differences in N and P stoichiometry between plant tissues (leaves, stems and roots) of the five tree species, an effect-size index, relative effect (RE)

(Duval *et al.*, 2011), was used to estimate the responses of N : P ratios to elevated CO<sub>2</sub> and N addition in different plant tissues of the five tree species. RE is quantified by the ratio of the variable in the experimental group to the control group minus one. To investigate the drivers of changes in N : P ratios, the correlations of the RE of N : P ratios with the RE of N or P concentrations under elevated CO<sub>2</sub> and N addition were further performed, respectively.

## Results

### Overall pattern of N : P ratios

N : P ratios in plant tissues of the five tree species generally differed among years (Table 2). In the AU treatments, N : P ratios in the stems and roots of the five tree species were relatively lower in 2006 and 2008 than in 2007 and 2009 (Fig. 1), which were mainly contributed to the changes in P concentrations rather than N (Table S1 and S2). Such variations among years were associated with the change in precipitation, with relatively higher values in 2006 and 2008 than in 2007 and 2009 (Figure S1). *O. pinnata* had higher N : P ratios in the AU treatments than the other tree species, with an average value of 29.9 in leaves, 40.4 in stems and 44.1 in roots, respectively. The high N : P ratios in *O. pinnata* were mainly attributed to its high N concentration (Table S1). The averaged N : P ratios in the non-N<sub>2</sub> fixers in the AU treatments were 25.3 for leaves, 20.1 for stems and 23.0 for roots.

### Effects of elevated CO<sub>2</sub> on N : P ratios

Elevated CO<sub>2</sub> tended to lower N : P ratios in some plant tissues of tree species (RE < 0) (Fig. 2). In leaves, elevated CO<sub>2</sub> significantly decreased N : P ratios in *S. hancei* in 2009 and *S. superba* in 2009, whereas it lowered the values in *A. acuminatissima* in 2008 only under N addition. The significantly lower N : P ratios in the stems under elevated CO<sub>2</sub> were detected in *A. acuminatissima*

**Table 2** Results from statistical analysis for effects of elevated CO<sub>2</sub> (C) and nitrogen addition (N), year and their interactions on N : P ratios in plant tissues of five tree species

Species	Tissues	C	N	Year	C × N	C × year	N × year	C × N × year
A	Leaf	ns	ns	**	ns	*	ns	**
	Stem	**	ns	**	ns	ns	ns	**
	Root	ns	ns	**	ns	ns	ns	*
B	Leaf	ns	ns	ns	ns	ns	ns	ns
	Stem	ns	ns	**	ns	ns	ns	ns
	Root	ns	ns	**	ns	**	ns	ns
C	Leaf	*	ns	**	ns	**	**	**
	Stem	ns	ns	**	ns	ns	ns	ns
	Root	ns	ns	**	*	ns	ns	ns
D	Leaf	ns	ns	ns	ns	ns	*	*
	Stem	ns	ns	**	ns	**	ns	**
	Root	**	ns	**	*	**	ns	ns
E	Leaf	ns	ns	**	ns	ns	ns	ns
	Stem	ns	*	ns	ns	*	ns	ns
	Root	ns	*	**	ns	**	ns	ns

A, *Acmena acuminatissima*; B, *Castanopsis hystrix*; C, *Syzygium hancei*; D, *Schima superba*; E, *Ormosia pinnata*. Asterisks indicate the effects of primary factors or their interactions are significant (\* $P < 0.05$ , \*\* $P < 0.01$ ). No significance is shown with ns.

over the experimental time, *S. superba* in 2009 and *O. pinnata* in 2009 (Table 2; Figs 1 and 2). Elevated CO<sub>2</sub> also decreased N : P ratios in the roots of *C. hystrix*, *S. superba* and *O. pinnata* in 2009 (Figs 1 and 2). We found that the reductions in N : P ratios were mainly related to the enhanced P concentrations under elevated CO<sub>2</sub> (Table S2).

#### Effects of N addition on N : P ratios

N addition had no significant effects on N : P ratios in the plant tissues of *A. acuminatissima*, *C. hystrix* and *S. hancei*, although there were some positive effects on N concentrations in some species (Table 2 and Table S1). In 2009, N : P ratios in the three plant tissues of *S. superba* were significantly lower in the AN and EN treatments than in the AU treatments (Fig. 1). However, N addition, especially under elevated CO<sub>2</sub>, led to significantly lowered N : P ratios in the stems and roots of *O. pinnata* (RE < 0) (Table 2; Fig. 3). There was also a trend toward decreasing N : P ratios in its leaves under N addition ( $P = 0.051$ ). The declines in N : P ratios were all due to the increases in P concentrations under N addition (Table S2).

#### The interaction of elevated CO<sub>2</sub> and N addition on N : P ratios

The interactive effects of elevated CO<sub>2</sub> and N addition on N : P ratios varied with tree species and plant tissues. N : P ratios in the three plant tissues of *C. hystrix* and *O. pinnata* were not significantly affected by the interaction of elevated CO<sub>2</sub> and N addition (Table 2). The EU and AN treatments tended to low N : P ratios

in the three plant tissues of *A. acuminatissima* in 2006, the three plant tissues of *S. superba* in 2009, and the roots of *S. hancei* in 2009, whereas the combination of elevated CO<sub>2</sub> and N addition (EN treatments) did not strengthen this effect (Fig. 1). In 2008, N : P ratios in the leaves of *A. acuminatissima* and the stems of *S. superba* were increased by the AN treatments and not affected by the EU treatments, whereas they were decreased by the EN treatments (Fig. 1).

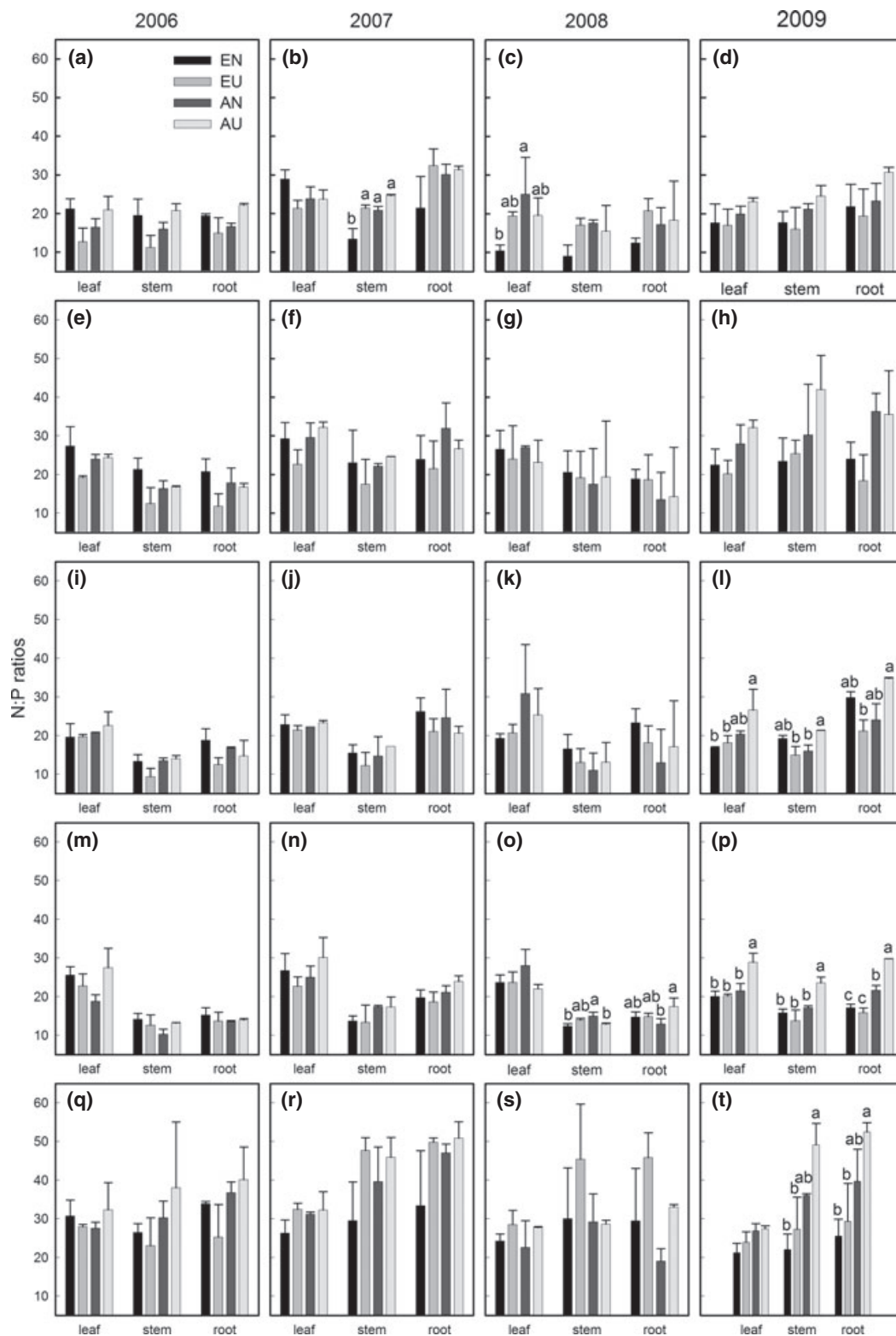
#### Drivers of the shifts in N : P ratios in response to elevated CO<sub>2</sub> and N addition

Data from all the plant tissues and the tree species were pooled together to analyze the drivers of the shifts in N : P ratios under elevated CO<sub>2</sub> and N addition. We found that the RE of N : P ratios under elevated CO<sub>2</sub> exhibited a significantly positive association with the RE of N concentrations ( $r^2 = 0.055$ ,  $P < 0.001$ ) (Fig. 4a), but a significantly negative relationship to the RE of P concentrations ( $r^2 = 0.555$ ,  $P < 0.001$ ) (Fig. 4b). On the other hand, the RE of N : P ratios under N addition showed a weak association with the RE of N concentrations ( $r^2 = 0.012$ ,  $P > 0.05$ ) (Fig. 4c), but it was significantly and negatively related to the RE of P concentrations ( $r^2 = 0.569$ ,  $P < 0.01$ ) (Fig. 4d).

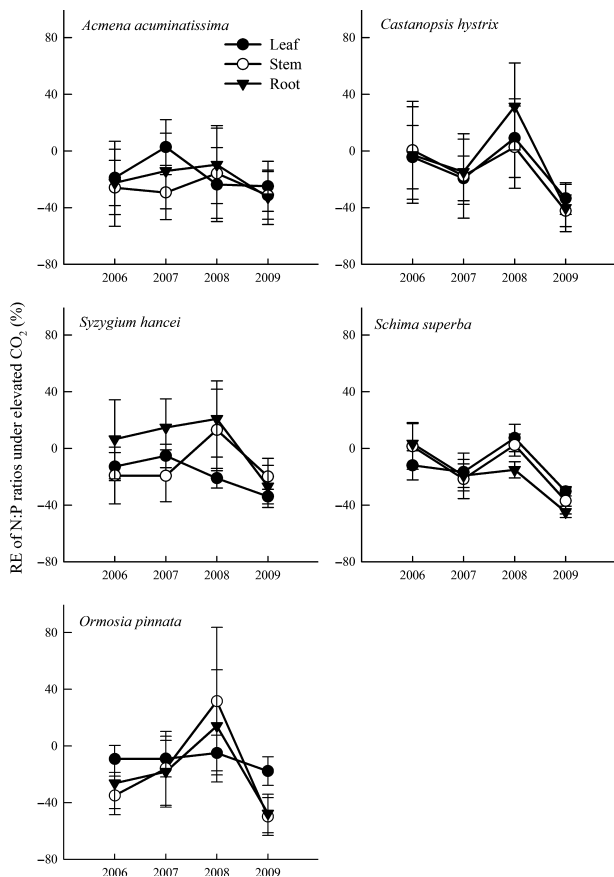
## Discussion

### Nutrient limitation of tree species

N : P ratios in the stems and roots of the five tree species presented relatively higher values in the dry years

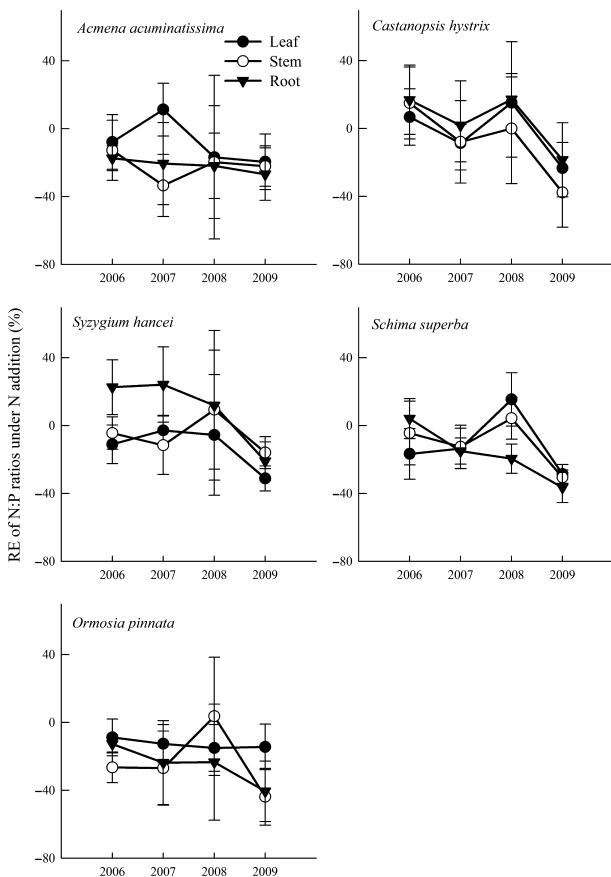


**Fig. 1** Nitrogen to phosphorus (N : P) ratios in plant tissues of five tree species under different CO<sub>2</sub> and N treatments. Error bars are standard deviations. Different lowercase letters indicate significant differences between the treatments in the same plant tissue of tree species in each year. (a–d), *Acmena acuminatissima*; (e–h), *Castanopsis hystrix*; (i–l), *Syzygium hancei*; (m–p), *Schima superba*; (q–t), *Ormosia pinnata*. EN, elevated CO<sub>2</sub> + N fertilized; EU, elevated CO<sub>2</sub> + N unfertilized; AN, ambient CO<sub>2</sub> + N fertilized; AU, ambient CO<sub>2</sub> + N unfertilized.



**Fig. 2** Relative effect (RE) of nitrogen to phosphorus (N : P) ratios in five tree species under elevated  $\text{CO}_2$ . Error bars are standard deviations.

(2007 and 2009) than in the wet years (2006 and 2008). The pattern, however, was not observed in the leaves, which supported the idea that the weaker homeostatic control over N : P ratios in stems and roots than in leaves (Garrish *et al.*, 2010). The lower N : P ratios in the wet years depended mainly on increases in P concentrations (Table S1 and S2). This is consistent with the results from the previous studies, showing the strong control of water availability on P dynamics (Cernusak *et al.*, 2010; Renteria & Jaramillo, 2011). Enhanced P uptake was probably due to the greater release of P through increased litter decomposition by great water availability (Deng *et al.*, 2010). Conversely, N concentrations did not exhibit any increases in the wet year. It adds the evidence to the suggestion that N is not a limiting factor for plant growth because of great soil N availability caused by high atmospheric N deposition ( $56 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$  for the wet N deposition) in our region (Liu *et al.*, 2008). Although there were differences in N : P ratios between the  $\text{N}_2$  fixer and the non- $\text{N}_2$  fixers due to plant functional and physiological variability (McKey, 1994), the mean N : P ratios in the

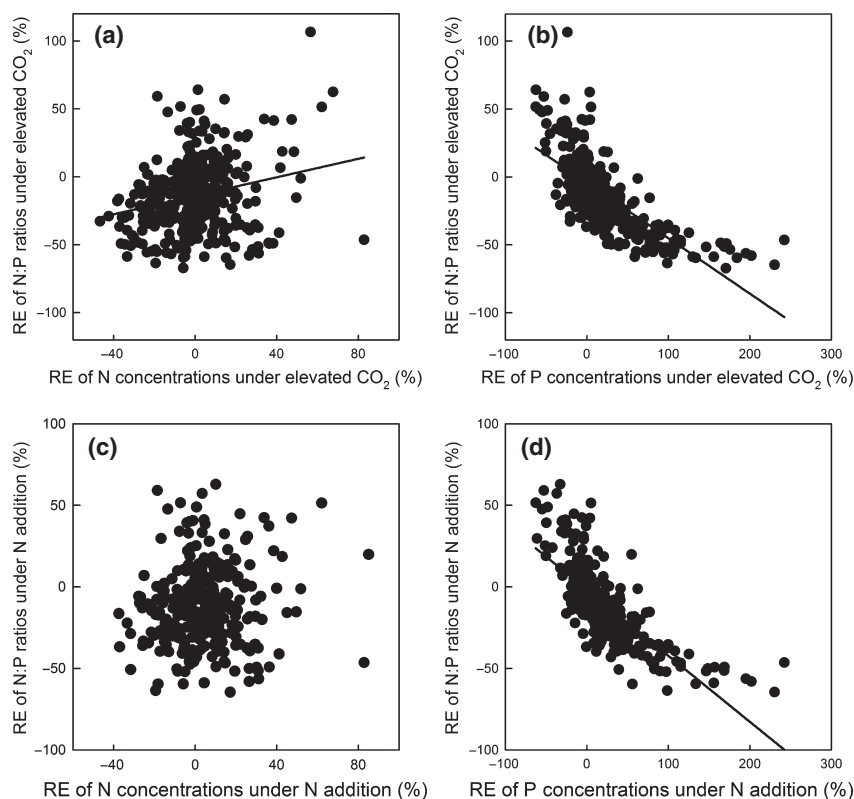


**Fig. 3** Relative effect (RE) of nitrogen to phosphorus (N : P) ratios in five tree species under N addition. Error bars are standard deviations.

plant tissues of the five tree species were greater than 20. If we accepted the proposal that N : P ratios below 10 and above 20 often indicate N- and P-limited biomass production, respectively (Güsewell, 2004), plant growth would be suffering more from P than N limitation in the region.

#### *Lowered N : P ratios under elevated $\text{CO}_2$ in some plant tissues of tree species*

The response of N : P ratios in the five tree species to elevated  $\text{CO}_2$  varied with plant tissues. When different plant tissues were considered, it was recognized that the survey of N : P ratios in leaves of tree species may not provide powerful insight into the tree species in response to elevated  $\text{CO}_2$ . The significant effects of elevated  $\text{CO}_2$  on N : P ratios were detected mostly after 2008, 3 years after the experiment began. These further confirmed that the response of plants to elevated  $\text{CO}_2$  in a relatively long-term period was quite different from that observed over a shorter term study (Gifford *et al.*, 2000). Our results showed that N : P



**Fig. 4** Correlations of the RE of nitrogen to phosphorus (N : P) ratios with the RE of N or P concentrations under elevated CO<sub>2</sub> (a, b) and N addition (c, d). Lines were plotted for significant relationships at the confidence level of  $P < 0.05$ .

ratios in some plant tissues of the five tree species were lowered by elevated CO<sub>2</sub> and that the declines in N : P ratios were primarily involved in P increases. The previous study in our experiment has demonstrated that elevated CO<sub>2</sub> led to a remarkable increase in soil moisture (Deng *et al.*, 2010), which could stimulate soil microbial processes and facilitate litter decomposition and nutrient mineralization (Niklaus *et al.*, 1998). Moreover, elevated CO<sub>2</sub> tends to increase plant root biomass (Luo *et al.*, 2006), which was also observed in our previous study (Zhao *et al.*, 2011). The expansion of root system under elevated CO<sub>2</sub> may improve P acquisition. Therefore, the great P uptake under elevated CO<sub>2</sub> resulted in lower N : P ratios. As the N : P ratio in plant tissues is linked to nutrient limitation (Güsewell, 2004), the repressed N : P ratio due to elevated CO<sub>2</sub> suggests elevated CO<sub>2</sub> could facilitate tree species to relieve the stress of P limitation in subtropical forests.

#### *Greater influences of N addition on N : P ratios of the N<sub>2</sub> fixer than those of the non-N<sub>2</sub> fixers*

N addition, especially under elevated CO<sub>2</sub>, significantly decreased N : P ratios in *O. pinnata*. The effects were stronger in the stems and roots than in

the leaves. This further demonstrated that the stems and roots would be more sensitive to environmental changes than the leaves (Garrish *et al.*, 2010). The reduction in N : P ratios in *O. pinnata* was contributed to the greater P concentrations under N addition. *O. pinnata*, as a N<sub>2</sub> fixer, is characterized by N-demanding lifestyle and luxurious N use (McKey, 1994; Inagaki *et al.*, 2011). The N<sub>2</sub> fixer is adept at investing N to P acquisition via producing phosphatases, a constitutively N-rich class of enzymes involved in P mineralization, to increase local P supplies (Treseder & Vitousek, 2001; Houlton *et al.*, 2008; Marklein & Houlton, 2012). In this case, N addition could facilitate *O. pinnata* in holding a substantial competitive advantage in P acquisition, especially under elevated CO<sub>2</sub>. N addition did not increase N : P ratios in the non-N<sub>2</sub> fixers, and it even lowered N : P ratios in *S. superba* in 2009. This is inconsistent with the suggestion that high N deposition often led to great N : P ratios and this effect would be strengthened by low P availability (Brouwer *et al.*, 2001; Güsewell, 2004). Our results showed that P concentrations were increased to promote the nutrient equilibration although the N concentrations in some plant tissues were increased by N addition. This is supported by the suggestion that terrestrial plants

can allocate excess N to increase P cycling rates, thus delaying the onset of P limitation to plant growth (Marklein & Houlton, 2012). N : P ratios in the non-N<sub>2</sub> fixers, however, were not so remarkably decreased by N addition as those in *O. pinnata*. Thus, N addition would favor the N<sub>2</sub> fixer to balance N and P under elevated CO<sub>2</sub>, which will alter species composition of ecosystems.

#### *The role of N addition in the effects of elevated CO<sub>2</sub> on N : P ratios in plant tissues of tree species*

Previous studies have suggested that N is likely to affect the responses of terrestrial ecosystem to elevated CO<sub>2</sub> (Hungate *et al.*, 2003; Luo *et al.*, 2004; Reich *et al.*, 2006). However, the N limitation on the effects of elevated CO<sub>2</sub> may be weak or absent if soil N is sufficient (Reich *et al.*, 2006). There was clear evidence that N addition did not change the responses of *C. hystrix* to elevated CO<sub>2</sub>. Elevated CO<sub>2</sub> produced lower N : P ratios without N addition in most of the plant tissues in the other non-N<sub>2</sub> fixers if there were interactive effects of elevated CO<sub>2</sub> and N addition. This indicates that N availability in the region is not a limiting factor for plants to adjust N and P stoichiometry under elevated CO<sub>2</sub> in P-poor soils. Moreover, when both CO<sub>2</sub> and N were added, high N availability led to lower N : P ratios in *O. pinnata*. These suggest that the current soil N availability in the region is not enough for *O. pinnata* to hold an advantage in P acquisition under elevated CO<sub>2</sub>.

#### *P driving the shifts of N : P ratios in response to global change*

The changes in N : P ratios showed stronger relationships with the P than N concentrations under both elevated CO<sub>2</sub> and N addition (Fig. 4). This indicates that the N : P ratio in response to the global change is largely dependent on P changes in the region. Elevated CO<sub>2</sub> often has a diluting effect on N due to the greater accumulation of C in tissues (Cotrufo *et al.*, 1998). Our results showed that N concentrations were reduced by elevated CO<sub>2</sub> at the beginning and then unchanged (Table S1), whereas P concentrations were increased by elevated CO<sub>2</sub>. This implies that N is not limiting to plant growth and can be readily acquired to match increased C assimilation. When N was enough for plant growth in response to elevated CO<sub>2</sub>, the allocation to P-rich ribosomal RNA was increased to support faster growth rates (Sterner & Elser, 2002; Elser *et al.*, 2010). In addition, N addition had greater influences on P than N concentrations. This confirms the idea that increasing N availability can alter P

cycling (Marklein & Houlton, 2012). Therefore, our results suggest that elevated CO<sub>2</sub> and N addition may more strongly influence P cycling than N cycling in the N-rich but P-poor environment.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Nitrogen concentrations (mg g<sup>-1</sup>) in plant tissues of five tree species under different CO<sub>2</sub> and N treatments.

**Table S2.** Phosphorus concentrations (mg g<sup>-1</sup>) in plant tissues of five tree species under different CO<sub>2</sub> and N treatments.

**Figure S1.** Total annual precipitation and mean annual temperature in Guangzhou City, China, during the experimental period from 2006 to 2009.

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