



Effects of elevated carbon dioxide and nitrogen addition on foliar stoichiometry of nitrogen and phosphorus of five tree species in subtropical model forest ecosystems

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ABSTRACT

The effects of elevated carbon dioxide (CO₂) and nitrogen (N) addition on foliar N and phosphorus (P) stoichiometry were investigated in five native tree species (four non-N₂ fixers and one N₂ fixer) in open-top chambers in southern China from 2005 to 2009. The high foliar N:P ratios induced by high foliar N and low foliar P indicate that plants may be more limited by P than by N. The changes in foliar N:P ratios were largely determined by P dynamics rather than N under both elevated CO₂ and N addition. Foliar N:P ratios in the non-N₂ fixers showed some negative responses to elevated CO₂, while N addition reduced foliar N:P ratios in the N₂ fixer. The results suggest that N addition would facilitate the N₂ fixer rather than the non-N₂ fixers to regulate the stoichiometric balance under elevated CO₂.

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1. Introduction

Nitrogen (N) and phosphorus (P) are fundamental nutrients for plant growth. They are commonly limited for primary production and other ecosystem processes (Vitousek and Howarth, 1991; Vitousek et al., 2010). Foliar N and P concentrations can reflect plant and soil nutrient status (Vitousek et al., 1995; Townsend et al., 2007), and be related to the functioning of plants and vegetation composition (Koerselman and Meuleman, 1996; Güsewell, 2004). Foliar N:P ratios are determined by the uptake and losses of N and P. The variations in foliar N:P ratios vary with species and depend on nutrient conditions to which plants are exposed (Güsewell, 2004). Foliar N:P ratios have been proved to be useful to assess N versus P limitation to primary production in terrestrial ecosystems with a growing focus on ecological stoichiometry (Finzi et al., 2004; Reich and Oleksyn, 2004; Han et al., 2005; Townsend et al., 2007).

Atmospheric carbon dioxide (CO₂) concentration has increased globally by approximately 35% since the industrial revolution, and is predicted to reach 700 ppm by the end of this century (Houghton et al., 2001). A number of studies have revealed that elevated CO₂

can greatly affect plant nutrient concentrations and cause an elemental imbalance in plants (Loladze, 2002; Finzi et al., 2004; Johnson et al., 2004; Nguyen et al., 2006). Elevated CO₂ does not promote nutrient uptake at the same rate as dry matter accumulation, and consequently, causes the nutrient dilution in tissues (Cotrufo et al., 1998; Loladze, 2002). A frequently observed effect of elevated CO₂ is a decrease in plant N concentration, despite the diversity of the experimental systems and plant species studied (Cotrufo et al., 1998; Larsen et al., 2011). Due to growth dilution, there were several observations on reductions in P in tissues due to elevated CO₂ where P was apparently not a limiting factor (Norby et al., 1986; Johnson et al., 1997). The effect of elevated CO₂ on P nutrition is, however, challenged by the evidence that fixed C in belowground under elevated CO₂ may lead to increases in P release and uptake from the soils (Lagomarsino et al., 2008; Lukac et al., 2010). These findings led to the speculation that P concentration in tissues could not be significantly influenced by elevated CO₂ (Johnson et al., 2004). The changes in foliar N and P by elevated CO₂ may lead to a shift in foliar N:P ratios. On the contrary, the stoichiometric flexibility in foliar N:P ratios may affect ecosystem production and decomposition processes, and then influence N and P cycles in natural ecosystems (Wang et al., 2010). Therefore, our understanding of foliar N and P stoichiometry in response to high

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CO₂ can help us to understand N and P cycling under the global change.

The atmospheric deposition of N-containing compounds has grown over the past years due to human activities (Matson et al., 2002). N enrichment has been considered to greatly affect ecosystems in response to elevated CO₂ due to the strong coupling between C and N cycles (Norby et al., 1999; Luo et al., 2004; Liu et al., 2011a). Progressive N limitation develops if elevated CO₂ causes C and N to sequester into long-lived plant biomass and soil organic matter pools, while N limitation might be alleviated when the sequestered N is compensated by additional N supply (Luo et al., 2004). If N limitation is removed, other nutrients, particularly P, will become limited to plant growth under the condition of elevated CO₂ (Aerts and Chapin, 2000). Thus, foliar N and P stoichiometry would be better used as an index to reflect the shift between N and P limitation to ecosystems under elevated CO₂ together with N addition.

Until recently, most studies of foliar N and P stoichiometry in response to elevated CO₂ have been conducted in temperate forests, which are often limited by N. Compared with the temperate ecosystems, tropical forests that are generally recognized as N rich but P-poor, will respond differently to increasing N deposition (Matson et al., 1999). It is unclear that with the increasing N deposition, how foliar N and P stoichiometry responds to elevated CO₂ in the tropics and subtropics. In this study, we used open-top chambers to study the effects of elevated CO₂ alone and together with N addition on foliar N and P stoichiometry in five native and widely spread tree species in subtropical ecosystems over 5 years after the treatments began. Our objectives were to examine: (1) how foliar N and P stoichiometry in the tree species would respond to elevated CO₂; (2) how the responses of foliar N and P stoichiometry in tree species to elevated CO₂ would be altered by N addition; and (3) the differences between N₂ fixers and non-N₂ fixers in response to elevated CO₂ and N addition.

2. Material and methods

2.1. Study site and experimental setup

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 south subtropical monsoon climate with an average annual relative humidity of 77%. The annual precipitation ranging from 1600 mm to 1900 mm has a distinct seasonal pattern, with about 80% of it falling in the wet season (from April to September) and 20% occurring in the dry season (from October to March). The mean annual temperature is 21.5 °C, and the mean annual total solar radiation reaches 4.37–4.60 GJ m⁻² in the visible waveband.

Ten open-top chambers were built for this experiment, which were located in an open area to receive full light and rain. The open-top chamber and the experiment design have been described in detail elsewhere (Liu et al., 2008). From April 2005, the chambers were exposed to different treatments. Briefly, three chambers received an elevated CO₂ (ca. 700 μmol mol⁻¹) and high N treatment (NH₄NO₃ applied at 100 kg N ha⁻¹ yr⁻¹) (CN), three chambers received an elevated CO₂ treatment (without any N fertilizer application) (CC), two chambers received high N treatment (with ambient CO₂) (NN), and finally two chambers acted as a control (with ambient CO₂ and no N fertilizer application) (CK). Soils in all chambers were collected from a nearby evergreen broadleaved forest. One- to two-year-old seedlings were transplanted in the chambers without damaging the roots. Each chamber was planted with eight seedlings for each of the following six species: *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Castanopsis hystrix* J. D. Hooker et Thomson ex A. De Candolle. (*C. hystrix*), *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*), *Pinus massoniana* Lamb. (*P. massoniana*), *Schima superba* Gardn. et Champ. (*S. superba*) and *Syzygium hancei* Merr. et Perry (*S. hancei*). The total 48 seedlings were randomly located in each chamber. These species are native, and the most widely spread tree species in southern China. *O. pinnata* is a N₂ fixer and the other tree species is non-N₂ fixers. As trees were growing fast, one tree per species was harvested at the end of each year to avoid excess crowd in each chamber.

2.2. Sample collection and measurement

As *P. massoniana* died in the second year of this experiment, we only studied the other five species in our experiment, which are *A. acuminatissima*, *C. hystrix*, *O. pinnata*, *S. hancei* and *S. superba*. From 2005 to 2009, one tree for each species in

each chamber was randomly chosen to be harvested for sampling at the end of each year. All the leaves on a harvested tree were pooled together for one sample.

Leaf samples were dried for 72 h at 70 °C in an oven and then finely ground prior to N and P analyses. Foliar N concentration was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Foliar P concentration was measured photometrically after leaves were digested with nitric acid (HNO₃). Mass foliar N:P ratio was used in our study.

2.3. Data analysis

Data analyses were carried out using the SPSS software. Analyzed data consisted of foliar N, foliar P and foliar N:P ratio. Variable normality and residual homoscedasticity were checked. We chose α equal to 0.05. Repeated-measures ANOVA was used to examine the effects of elevated CO₂ and N addition on foliar N, foliar P and foliar N:P ratios over the time. Subsequently, the effects were further analyzed using ANOVA, followed by Tukey multiple comparison test when they were significant for each sampling time. Additionally, in order to examine the major drivers of shifts in foliar N:P ratios, we used the response ratio (RR) to calculate the relationships of the RR of foliar N:P ratio with the RR of N or P under elevated CO₂ or N addition. RR is the ratio of the variable in the experimental group to that in the control group (Hedges et al., 1999).

3. Results

3.1. Foliar N

The effect of sampling time on foliar N in the tree species was significant, except for *O. pinnata*, on which the effect of sampling time was close to a statistically significant level ($P = 0.077$) (Table 1). The average concentration of foliar N across all species was 15.0 mg g⁻¹, ranging from 8.0 mg g⁻¹ to 30.8 mg g⁻¹. For the CK treatment, foliar N ranged from 9.0 mg g⁻¹ to 26.9 mg g⁻¹ with an average of 14.9 mg g⁻¹. Among the species, *O. pinnata* showed the significantly highest foliar N as compared with the other species.

Elevated CO₂ had a significantly negative effect on foliar N in *A. acuminatissima* and *S. hancei* in the first year of the experiment, which showed the lowest values in the CC treatments (Fig. 1). However, elevated CO₂ did not significantly affect foliar N in the other non-N₂ fixers over the time (Table 1). Foliar N in *O. pinnata* showed a positive response to elevated CO₂, but the differences did not arrive at a significant level. N addition significantly increased foliar N in all the species, except for *S. superba*, in which foliar N showed little response to N addition (Table 1).

The interaction of elevated CO₂ and N addition only significantly affected foliar N in *S. hancei*, with the highest value in the CN treatment but the lowest one in the CC treatment.

Table 1

Repeated-measures ANOVA for effects of carbon dioxide (C) and nitrogen (N) treatments, sampling time and their interactions on foliar N, foliar phosphorus (P) and foliar N:P ratio of five tree species grown under various CO₂ and N treatments. Asterisks indicate that the effects of primary factors or their interactions are significant (* $P < 0.05$, ** $P < 0.01$). No significance is shown with ns.

	Species	C	N	C*N	Time	Time*C	Time*N	Time*C*N
Foliar N	<i>A. acuminatissima</i>	ns	*	ns	**	**	ns	ns
	<i>C. hystrix</i>	ns	*	ns	**	ns	ns	ns
	<i>O. pinnata</i>	ns	*	ns	ns	ns	ns	ns
	<i>S. hancei</i>	*	*	**	**	*	ns	ns
	<i>S. superba</i>	ns	ns	ns	*	ns	ns	ns
Foliar P	<i>A. acuminatissima</i>	ns	ns	ns	**	**	**	**
	<i>C. hystrix</i>	ns	ns	ns	**	ns	ns	ns
	<i>O. pinnata</i>	ns	**	ns	**	ns	ns	ns
	<i>S. hancei</i>	**	*	ns	ns	ns	ns	ns
	<i>S. superba</i>	ns	ns	ns	*	ns	ns	ns
Foliar N:P ratio	<i>A. acuminatissima</i>	ns	ns	ns	**	ns	ns	**
	<i>C. hystrix</i>	ns	ns	ns	*	ns	ns	ns
	<i>O. pinnata</i>	ns	ns	ns	*	ns	ns	ns
	<i>S. hancei</i>	**	ns	ns	ns	ns	ns	ns
	<i>S. superba</i>	*	ns	*	**	*	*	*

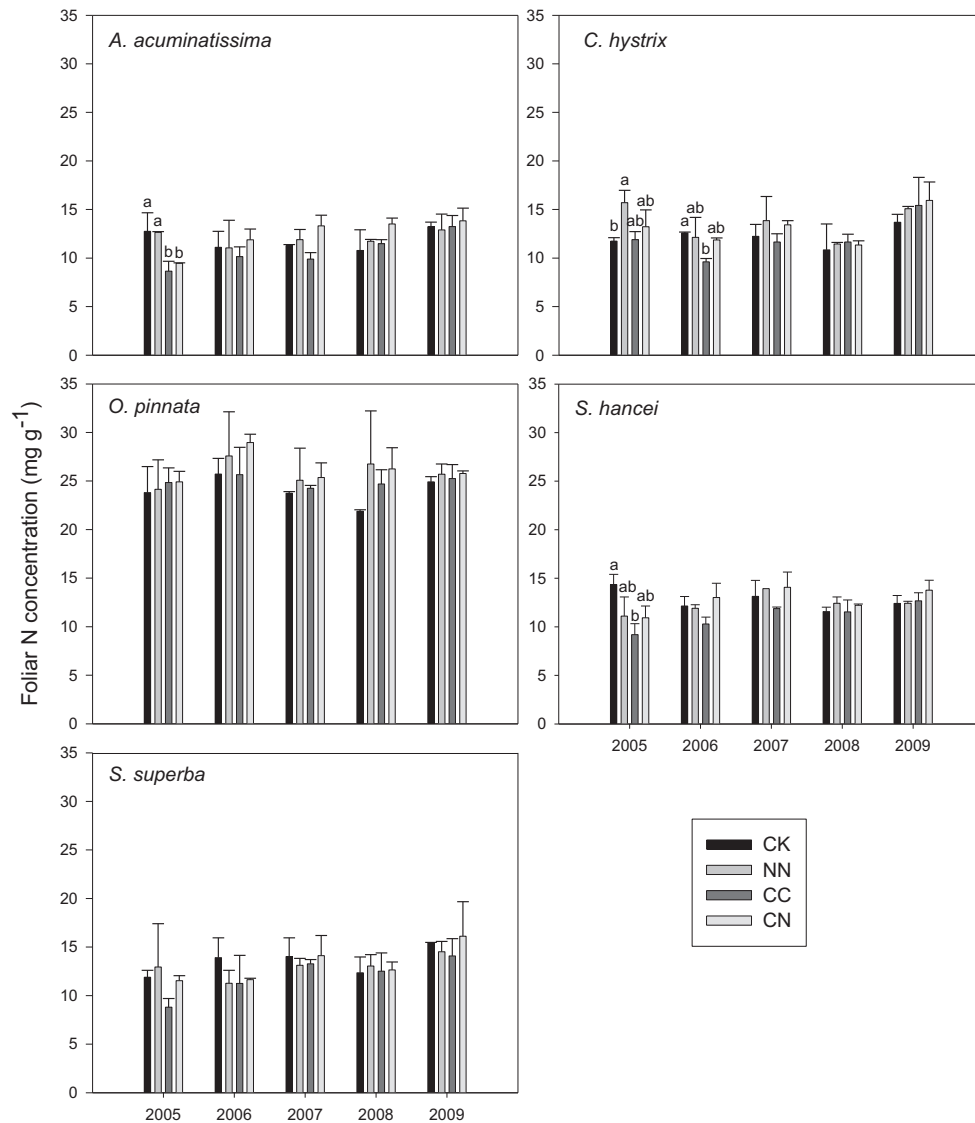


Fig. 1. Foliar nitrogen (N) concentrations (mg g^{-1}) of five tree species exposed to the various CO_2 and N treatments for five years. Error bars represent standard deviation. Different lowercase letters denote significant differences between treatments in each year for each species. $n = 3$ for the CC and CN treatments, and $n = 2$ for the CK and NN treatments. CK, Ambient CO_2 + Ambient N; NN, Ambient CO_2 + High N; CC, Elevated CO_2 + Ambient N; and CN, Elevated CO_2 + High N.

3.2. Foliar P

There was a significant effect of sampling time on foliar P in all tree species, except for *S. hancei* (Table 1). Foliar P across all species was in the range of 0.30 mg g^{-1} to 1.49 mg g^{-1} , with an average of 0.65 mg g^{-1} . The average foliar P in the CK treatment was 0.57 mg g^{-1} . *O. pinnata* showed the significantly greatest foliar P among the five tree species (Fig. 2).

Foliar P in all tree species generally showed an increasing trend in response to elevated CO_2 , while this positive effect was only significant in *S. hancei*. For *S. hancei*, the differences between the treatments arrived at a significant level in 2009, with significantly higher foliar P in the CC or CN treatment than in the CK treatment (Fig. 2). For *A. acuminatissima*, the effect of elevated CO_2 differed with the sampling time. Elevated CO_2 significantly reduced foliar P in *A. acuminatissima* in 2005, but significantly increased it in 2008 (Fig. 2). We found that N addition had a significantly positive effect on foliar P in *O. pinnata* or *S. hancei* (Table 1), with the greatest values in the CN treatment. As for *A. acuminatissima*, a positive effect of N addition on foliar P occurred in 2008 (Fig. 2), with the

significantly higher value in the CN treatment than in the other treatments.

The significantly interactive effects of elevated CO_2 and N addition on foliar P were only found in *A. acuminatissima* in 2006 and 2008.

3.3. Foliar N:P ratios

Sampling time significantly affected foliar N:P ratios in all tree species, except for *S. hancei*. Across all the species, foliar N:P ratios varied from 9.4 to 48.1, with an average of 23.5. The mean value of foliar N:P ratios in the CK treatment was 26.2. The values of foliar N:P ratios also significantly varied with the tree species, with the highest one in *O. pinnata*.

Elevated CO_2 significantly decreased foliar N:P ratios in *S. hancei* over the time. Foliar N:P ratios in *A. acuminatissima* and *C. hystrix* were showed a decreasing trend when exposed to elevated CO_2 , with the lowest values in the CC treatment. However, the same trend did not apply to that in *O. pinnata*. As for *S. superba*, a significantly negative effect of elevated CO_2 on foliar N:P ratios was

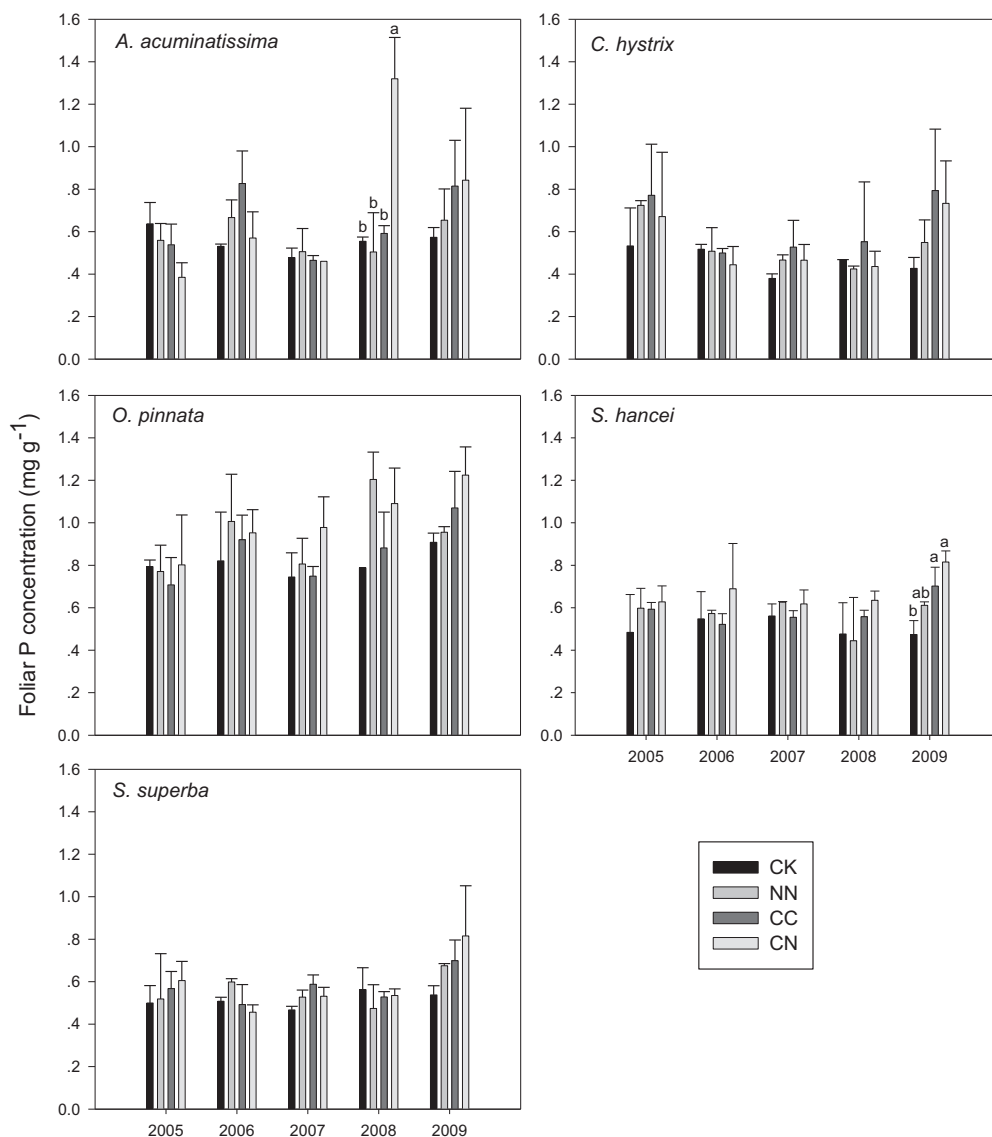


Fig. 2. Foliar phosphorus (P) concentrations (mg g^{-1}) of five tree species exposed to the various CO_2 and N treatments for five years. Error bars represent the standard deviation. Different lowercase letters denote significant differences between treatments in each year for each species. $n = 3$ for the CC and CN treatments, and $n = 2$ for the CK and NN treatments. CK, Ambient CO_2 + Ambient N; NN, Ambient CO_2 + High N; CC, Elevated CO_2 + Ambient N; and CN, Elevated CO_2 + High N.

observed in 2005 and 2009, with the lowest values in the CC treatment (Fig. 3). N addition exerted little effects on foliar N:P ratios in the non- N_2 fixers over the time (Table 1). As for the N_2 fixer, foliar N:P ratio showed no response to N addition at the beginning of this experiment. However, when considering the time from 2007 to 2009, foliar N:P ratios in *O. pinnata* were significantly reduced by the N addition.

The interactive effect of elevated CO_2 and N addition on foliar N:P ratios occurred in some tree species at different sampling times, such as *A. acuminatissima* in 2006, or *S. superba* in 2006 and 2009.

3.4. Major drivers of shifts in foliar N:P ratios

For all species pooled together, the responsive ratio (RR) of foliar N:P ratio was significantly related to the RR of foliar P rather than the RR of foliar N under elevated CO_2 or N addition (Fig. 4). The shifts of foliar N:P ratio in each species in response to elevated CO_2 or N addition were largely driven by foliar P. The RR of foliar N:P ratio was weakly associated with the RR of foliar N under elevated

CO_2 or N addition in each species (Fig. 4a, b). However, the RR of foliar N:P ratio was significantly negatively correlated with the RR of foliar P under elevated CO_2 or N addition in each species, except for *S. hancei*, in which the negative relationship between the RRs of foliar N:P ratio and foliar P under elevated CO_2 was close to a significant level ($P = 0.085$) (Fig. 4c, d).

4. Discussion

4.1. General pattern of foliar N and P stoichiometry

Foliar N, foliar P and foliar N:P ratios were generally significantly affected by the sampling time. One sampling campaign therefore was rather limited to draw any reliable conclusion on foliar N and P and their stoichiometry. During the five years of the experiment, averaged foliar N across all species in the CK treatment (14.9 mg g^{-1}) was higher when compared with the leaves in worldwide evergreen plants reported by Aerts (1996) (13.7 mg g^{-1} for foliar N, 1.02 mg g^{-1} for foliar P), while foliar P in the CK treatment (0.57 mg g^{-1}) was quite lower. These differences were

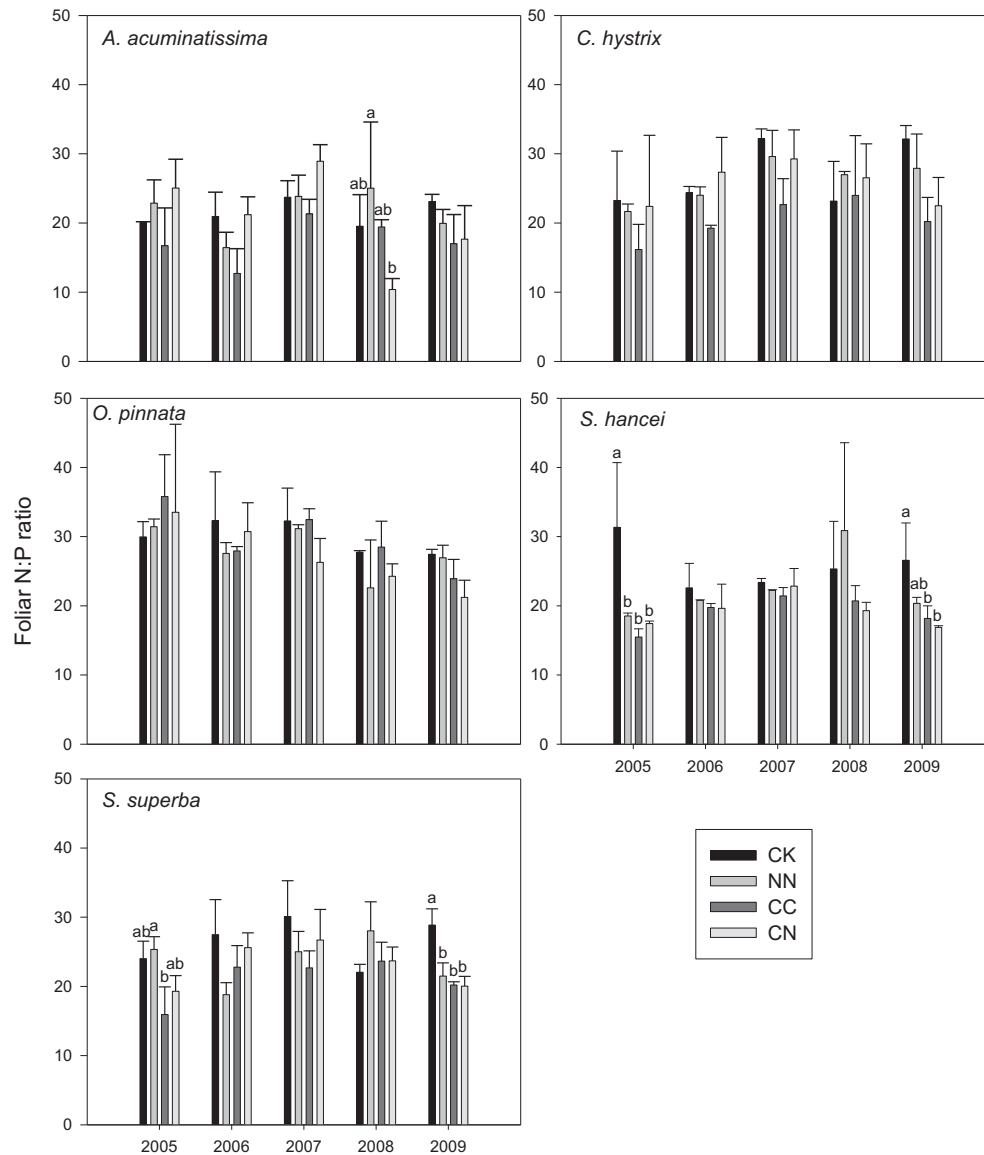


Fig. 3. The ratio of foliar nitrogen and phosphorus (foliar N:P ratio) of five tree species exposed to the various CO₂ and N treatments for five years. Error bars represent standard deviation. Different lowercase letters denote significant differences between treatments in each year for each species. $n = 3$ for the CC and CN treatments, and $n = 2$ for the CK and NN treatments. CK, Ambient CO₂ + Ambient N; NN, Ambient CO₂ + High N; CC, Elevated CO₂ + Ambient N; and CN, Elevated CO₂ + High N.

probably attributed to considerable atmospheric N deposition ($56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for the wet N deposition), which led to high N availability, and low soil available P (2.13 mg kg^{-1} for 0–20 cm) in our region (Liu et al., 2008). Foliar N and foliar P were both lower than those of broadleaves of evergreen woody plants in China reported by Han et al. (2011) (16.7 mg g^{-1} for foliar N and 0.99 mg g^{-1} for foliar P). This is probably due to the different sampling seasons. Wet season, which has higher soil temperature and moisture than dry season, can facilitate the release of N and P through high decomposition (Deng et al., 2010) and generate relatively high foliar N and P. However, foliar N:P ratios in this area were quite high. This might reflect that plant growth at our site is much more limited by P than by N as foliar N:P ratios are often suggested to infer potential N- or P- limitation of terrestrial net primary productivity (Koerselman and Meuleman, 1996; Güsewell, 2004; Reich and Oleksyn, 2004; Townsend et al., 2007).

Foliar N, foliar P and foliar N:P ratios varied significantly with the tree species, indicating different requirements of N and P, and

different abilities of competing for N or P among the species (Güsewell and Koerselman, 2002). Our results showed that *O. pinnata*, as a N₂ fixer, had the highest foliar N, foliar P and foliar N:P ratios. The high foliar N in *O. pinnata* was consistent with the results from other surveys and experiments (Townsend et al., 2007; Nardoto et al., 2008; Alvarez-Clare and Mack, 2011; Inagaki et al., 2011; Liu et al., 2011b), suggesting that legumes generally have an “N-demanding lifestyle” (McKey, 1994). N₂ fixers can invest N into P acquisition (Houlton et al., 2008), which results in relatively high foliar P. It has been reported that N₂ fixers often have higher N:P ratios than co-occurring non-N₂ fixers (Güsewell et al., 2003).

4.2. Effects of elevated CO₂ on foliar N and P stoichiometry

Foliar N or P is generally reduced by elevated CO₂ because of nutrient dilution through accumulation of non-structural carbohydrates in elevated CO₂ (Poorter et al., 1997; Cotrufo et al., 1998;

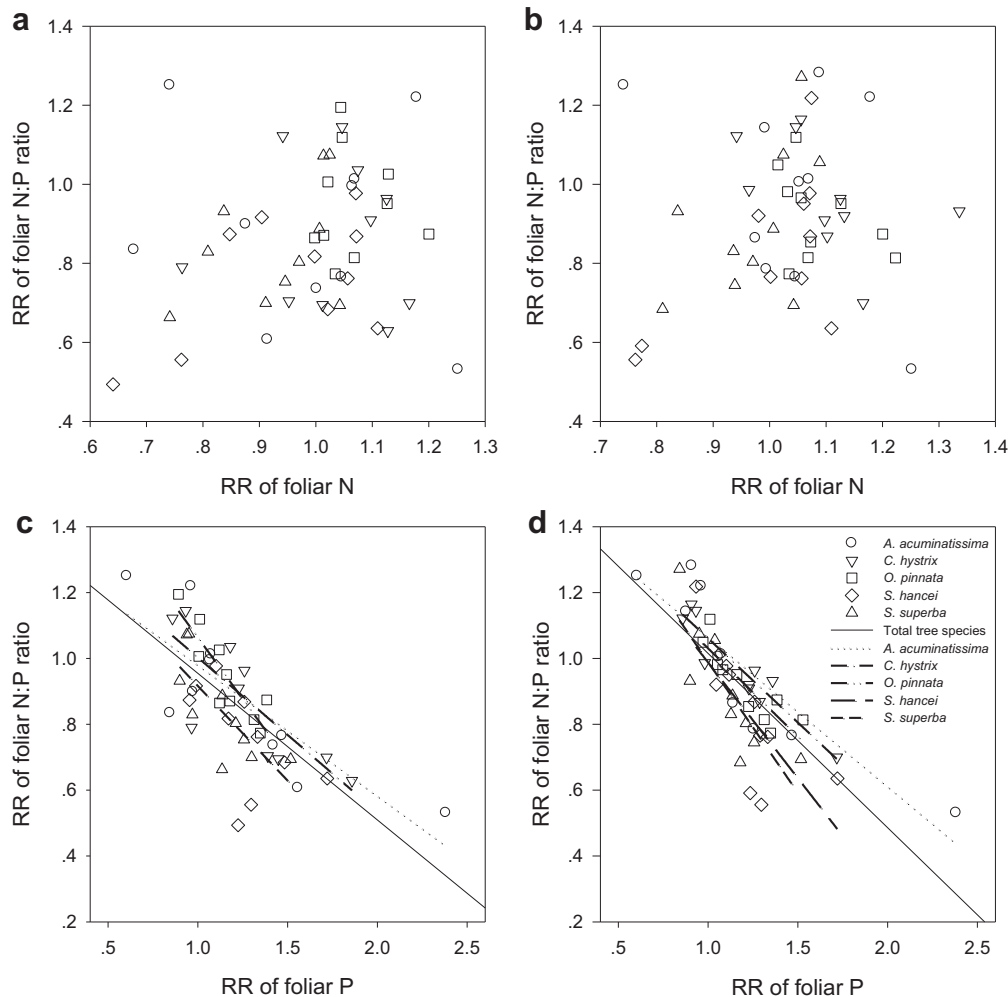


Fig. 4. Relationships between responsive ratio (RR) of foliar nitrogen (N) or foliar phosphorus (P) and RR of foliar N:P ratio under elevated CO_2 or N addition: (a) the relationship between RR of foliar N and RR of foliar N:P ratio under elevated CO_2 ; (b) the relationship between RR of foliar N and RR of foliar N:P ratio under N addition; (c) the relationship between RR of foliar P and RR of foliar N:P ratio under elevated CO_2 ; (d) the relationship between RR of foliar P and RR of foliar N:P ratio under N addition. Lines were plotted for significant relationships with $P < 0.05$.

Güsewell, 2004; Norby and Iversen, 2006; Teng et al., 2006). However, this phenomenon did not apply to our study. Our results showed that foliar N was little affected by elevated CO_2 , while foliar P exhibited a positive response to elevated CO_2 . N or P dilution due to elevated CO_2 only occurred in *A. acuminatissima* or *S. hancei* in the first year of the experiment. Previous studies in our experiment found that elevated CO_2 treatment increased soil moisture remarkably (Deng et al., 2010), which could stimulate soil microbial processes and result in increases in rates of litter decomposition and nutrient mineralization (Niklaus et al., 1998).

Our results showed that foliar N:P ratios in the non- N_2 fixers were generally reduced by elevated CO_2 although the differences were not significant in some species. This is due to the stimulation of P uptake by plants under elevated CO_2 , which drives lowered foliar N:P ratios. There is clear evidence that the response ratio (RR) of foliar N:P ratio was much more related to that of foliar P than to that of foliar N for all the non- N_2 species under elevated CO_2 (Fig. 4a, c). On the contrary, as for *O. pinnata*, its foliar N:P ratio was little influenced by elevated CO_2 due to its up-regulation of both foliar N and P under elevated CO_2 . Therefore, the non- N_2 fixers could be favored by elevated CO_2 to regulate the balance of foliar N and P in the P-poor soils to some extent.

4.3. Effects of N addition on foliar N and P stoichiometry

N addition almost had a significantly positive effect on foliar N in plant species except for *S. superba*, which was a sensitive species to acid deposition (Liu et al., 2007). The positive effect was consistent with other studies (McNulty et al., 2005; Knops et al., 2007; Rowe et al., 2008; Cui et al., 2010). The increased foliar N with N addition indicates that plants can use or absorb excess N to serve as a sink for N in the dry season.

No or a positive response of foliar P to N addition in the tree species, is somewhat in contradiction to the results of a decline in foliar P by N addition (Flückiger and Braun, 1998; Nilsson and Wallander, 2003; Braun et al., 2010), which was due to an inhibition of mycorrhiza by the N addition or a fixation of P as a result of N load in acidified soils. N addition was found to exert a positive effect on phosphatase activity (Johnson et al., 1999; Pilkington et al., 2005), which is important for P acquisition (Duff et al., 1994; Huang et al., 2011). The high phosphatase activity could increase P acquisition to compensate the dilution of foliar P and prevent future P deficiency. Consequently, this results in no decline or even an increase in foliar P concentration in the tree species with the N addition, especially under elevated CO_2 .

The responses of foliar N:P ratios to the N addition in the non-N₂ fixers were not obvious during the experiment. It is not supported by the assumptions that high N deposition was related to high N:P ratios and the effects would be strengthened by P limitation (Brouwer et al., 2001; Güsewell, 2004). Our results showed that the shifts in foliar N:P ratios in response to the N addition were strongly determined by foliar P rather than foliar N dynamics (Fig. 4b, d). Although N addition increased foliar N, N addition did not necessarily induced an increase in foliar N/P ratio as high N availability could also increase P uptake by plants through increasing P mineralization. Moreover, the significantly decreased foliar N:P ratio by the N addition was found in *O. pinnata* from 2007 to 2009. There were probably two mechanisms for the results. Firstly, the N₂ fixer is adept at acquiring P by investing added N to produce phosphatase to mineralize P in P-poor soils, which is supported by the theory of a trade-off between N and P developed by Houlton et al. (2008). The second mechanism is that faster growing rates lead to decreased foliar N:P ratios. The previous study in our experiment showed that the growths of *O. pinnata* in the CN and NN treatments were higher than in the other treatments (Zhao et al., 2011). As an increase in allocation to P-rich ribosomal RNA is required to support the faster growth rates (Elser et al., 2000; Sterner and Elser, 2002), there is often a negative relationship between the N:P ratio and growth rate. *O. pinnata* thus displayed lowered foliar N:P ratios in the CN and NN treatments. In this case, our results indicate that N addition could be conducive to the N₂ fixer in competition for P with the non-N₂ fixers under elevated CO₂.

4.4. The interactive effects among CO₂ treatment, N treatment and sampling time on foliar N and P stoichiometry

The interactive effects of CO₂ and N treatments on foliar N, foliar P and foliar N:P ratio varied with the tree species and sampling time. Elevated CO₂ and N addition interact with each other in stimulating plant growth (Hungate et al., 2003), which could lead to complex interactions on foliar nutrient concentrations. The reason for higher foliar N of *S. hancei* in the CN treatment than in the CC treatment could be explained by increases in N uptake by the N addition to offset the growth dilution of N under elevated CO₂. The interactive effects of elevated CO₂ and N addition on foliar P and foliar N:P ratios in *A. acuminatissima* or foliar N:P ratios in *S. superba* were variable at the different sampling times (Table 1). Thus, it should be cautious to draw any conclusion on the responses of foliar N, foliar P and foliar N:P ratios to the interaction of elevated CO₂ and N addition with only one sampling campaign. In contrast with the non-N₂ fixers, there was no interactive effect of CO₂ and N treatments on foliar N, foliar P or foliar N:P ratios in *O. pinnata*. This was because foliar N, foliar P and foliar N:P ratios showed great responses to the N addition rather than elevated CO₂.

5. Conclusions

Our results showed that high foliar N but low foliar P resulted in high foliar N:P ratios, indicating that plant growth may be more limited by P than by N in our region. Foliar N and P and foliar N:P ratios in the N₂ fixer (*O. pinnata*) were all higher than in the non-N₂ fixers (*A. acuminatissima*, *C. hystrix*, *S. hancei* and *S. superba*). Elevated CO₂ exerted little effect on foliar N due to high soil N availability in our area, while it increased foliar P and caused low foliar N:P ratio irrespective of the statistical insignificance in some species. Foliar N or foliar P in some tree species was increased by the N addition. The relationships of the RR of foliar N:P ratio to that of foliar N or foliar P suggest that changes in foliar N:P ratio were much more driven by foliar P than by foliar N under both elevated

CO₂ and N addition. Foliar N:P ratios in the non-N₂ fixers were not affected by the N addition, while those in the N₂ fixer were decreased by the N addition from 2007 to 2009. Our results imply that compared with the non-N₂ fixers, the N₂ fixer could have a competitive advantage for P by N addition under elevated CO₂.

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