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Changes in leaf nutrient traits and photosynthesis of four tree species: effects of elevated [CO_2], N fertilization and canopy positions

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Abstract

Aims

Leaf traits of trees exposed to elevated [CO_2] in association with other environmental factors are poorly understood in tropical and subtropical regions. Our goal was to investigate the impacts of elevated [CO_2] and N fertilization on leaf traits in southern China.

Methods

Four tree species, *Schima superba* Gardn. et Champ. (*S. superba*), *Ormosia pinnata* (Lour.) Merr (*O. pinnata*), *Castanopsis hystrix* AC. DC. (*C. hystrix*) and *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*) were studied in a factorial combination of atmospheric [CO_2] (ambient at ~390 μmol $mol^{-1}$and elevated [CO_2] at ~700 μmol mol^{-1}) and N fertilization (ambient and ambient + 100 kg N ha^{-1} yr^{-1}) in open-top chambers in southern China for 5 years. Leaf mass per unit leaf area (LMA), leaf nutrient concentration and photosynthesis (A_{sat}) were measured.

Important Findings

Results indicated that leaf traits and photosynthesis were affected differently by elevated [CO_2] and N fertilization among species. Elevated [CO_2] decreased LMA in all species, while N fertilization did not affect LMA. Leaf mass-based N concentration (N_M) was significantly greater in *O. pinnata* and *C. hystrix* grown in elevated [CO_2] but was lower in *S. superba*. Leaf mass-based P concentration (P_M) was significantly greater in *C. hystrix* and *A. acuminatissima* exposed to elevated [CO_2] but was lower in *S. superba*. N fertilization significantly increased P_M in *O. pinnata* but decreased P_M in *S. superba*. Photosynthetic stimulation in *O. pinnata*, *C. hystrix* and *A. acuminatissima* was sustained after 5 years of CO_2 fumigation. N fertilization did not modify the effects of elevated [CO_2] on photosynthesis. Leaf traits (N_M, N_A, P_M, P_A) and light-saturated photosynthesis were decreased from the upper to lower canopy. Canopy position did not alter the responses of leaf traits and photosynthesis to elevated [CO_2]. Results suggest that photosynthetic stimulation by elevated [CO_2] in native species in subtropical regions may be sustained in the long term.

Keywords: photosynthesis • elevated CO_2 • nitrogen • phosphorus • subtropical China

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INTRODUCTION

Elevated atmospheric carbon dioxide concentration ([CO_2]) increased short-term photosynthesis in numerous plant studies (Ainsworth and Long 2005; Curtis 1996). In contrast, a reduction in photosynthesis with increased [CO_2], often termed as down-regulation or photosynthetic acclimation, also has been reported (Hyvönen *et al.* 2007; Rey and Jarvis 1998). The different responses to elevated [CO_2] may be dependent on the nutrient availability and light environment affecting the plant net carbon uptake (Curtis and Wang 1998).

Nutrient availability exerts an important control over the response of plants and ecosystems to rising atmospheric CO_2 content (BassiriRad *et al.* 2001; Lloyd and Farquhar 1996). Nitrogen (N) is a major factor affecting plant growth in ecosystems. It was reported that N availability would often affect leaf photosynthesis under elevated [CO_2]. Down-regulation of photosynthesis occurred in plants grown in elevated [CO_2]

with N limitation (Sefcik *et al.* 2007). CO_2 enrichment often leads to decreased leaf N concentration (Stitt and Krapp 1999). High N availability could alleviate the down-regulation of photosynthesis in plants grown in elevated [CO_2] (Reich *et al.* 2006; Sefcik *et al.* 2007). Phosphorus (P) is another factor limiting the response of carbon acquisition of plants and ecosystems to increasing atmospheric CO_2 content. P limitation is common in terrestrial ecosystems (Lambers *et al.* 2006; Nord and Lynch 2009). Elevated [CO_2] requires increasing amounts of P to sustain the increased growth rates (Körner 2006; Lewis *et al.* 2010). It has been observed that photosynthetic responses to elevated [CO_2] generally decrease with decreasing P supply (Campbell and Sage 2006). Continued increases in [CO_2] will induce P limitation of photosynthesis in ecosystems where P is not currently limited (Tissue and Lewis 2010).

Responses of leaves to elevated [CO_2] may be affected by their positions within the canopy due to the direct effect of irradiance or the indirect effects of source–sink relationships, leaf morphology and physiology (Liberloo *et al.* 2007; Tissue *et al.* 2001). Specifically, the vertical profile of a canopy is characterized by a decline of the irradiance from the upper canopy downwards (Liberloo *et al.* 2007). Compared with lower canopy leaves, higher canopy leaves are usually accompanied with higher leaf N (Liberloo *et al.* 2007; Lötscher *et al.* 2003; Yin *et al.* 2003), larger leaf mass per unit leaf area (LMA, Liberloo *et al.* 2007; Rijkers *et al.* 2000), greater number of chloroplasts (Crous and Ellsworth 2004) and higher photosynthetic capacity (Crous and Ellsworth 2004; Del Pozo and Dennett 1999). Those changes may alter impacts of elevated [CO_2] on leaf photosynthetic parameters. It has been hypothesized that greater photosynthetic enhancements by elevated [CO_2] occur in the lower canopy than in the higher canopy (Tissue *et al.* 2001).

In Asia, emission of reactive N increased from 14 Tg N yr^{-1} in 1961 to 68 Tg N yr^{-1} in 2000 and is expected to reach 105 Tg N yr^{-1} in 2030 (Zheng *et al.* 2002). Currently, high atmospheric N wet deposition was found for some forests in southern China (Mo *et al.* 2008). Tropical forests in southern China have often been recognized as N-rich. Meanwhile, tropical forests in this area are generally recognized as P-poor (Reich and Oleskyn 2004; Townsend *et al.* 2007). P limitation to forest primary productivity and other ecosystem processes is widespread in tropical forests (Houlton *et al.* 2008).

Up to date, most studies on the photosynthetic and leaf nutrient responses to elevated [CO_2] have been performed in temperate areas, which are often N limited under natural conditions and with low ambient N deposition. Compared with temperate ecosystems, it is expected that tropical ecosystems will respond differently to increasing N deposition. Nevertheless, it is unclear how plant photosynthesis and leaf nutrients will respond to elevated [CO_2] in the tropical and subtropical regions, where P often is poor but N deposition is increasing. Furthermore, numerous experiments were carried out to study the responses of tree species to elevated [CO_2] and N addition; however, most of these studies focused on just one species or individual plant. Monospecific plant stands may show different responses from mixed counterparts; moreover, elevated [CO_2] has been shown to modify competition among plant species (Kubiske *et al.* 2007; Spinnler *et al.* 2002). In this study, we used open-top chambers to expose four tree species growing with high or low N treatment, to either ambient (~390 μmol mol^{-1}) or elevated (~700 μmol mol^{-1}) [CO_2] for 5 years. The four tree species used were *Castanopsis hystrix* AC. DC. (*C. hystrix*), *Schima superba* Gardn. et Champ. (*S. superba*), *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*) and *Ormosia pinnata* (Lour.) Merr (*O. pinnata*). These species were chosen as they are native and the most widely spread tree species in southern China. *Ormosia pinnata* was the only N_2-fixing species in this experiment. We investigated the combined effects of nitrogen fertilization and long-term elevated [CO_2] on canopy profiles of photosynthetic parameters and related leaf traits. Measurements were taken during the fourth and fifth year of the trees exposed both to elevated [CO_2] and high N addition. This research was carried out to answer the following questions:

- Are leaf traits and photosynthesis affected by elevated [CO_2] and nitrogen fertilization?
- Is photosynthetic stimulation in elevated [CO_2] sustained after 5years?
- Are photosynthetic responses to elevated [CO_2] modified by nitrogen fertilization?
- Whether leaves at the different positions of the crown show different responses to elevated [CO_2]?

MATERIALS AND METHODS

Site and experimental set-up

The experimental site was located in Guangzhou City, Guangdong Province, China (23°20′N and 113°30′E). The area has a monsoon climate characterized by mean annual total solar radiation of 4367.2–4597.3 MJ m^{-2} in the visible waveband and a mean annual temperature of 21.5°C. The annual precipitation ranges from 1600 to 1900 mm, and the mean relative air humidity is 77%. There are two seasons, a wet/rainy season from April to September and a dry season from October to March.

The experiment was carried out in 10 open-top chambers. Each 3-m diameter chamber was 3-m tall (was adjusted to 4.5 m from January 2009) and 0.7-m deep. The above-ground part was wrapped with impermeable and transparent plastic sheets, leaving the top of the chamber totally open. Light intensity in the chamber was 97% of that in open space and no spectral change was detected. Measured rainfall intensity was identical inside and outside of the chambers and the temperature was not significantly different. The below-ground part was delimited by a concrete brick wall that prevented any lateral or vertical water and/or element fluxes with the outside surrounding soil. Three holes at the bottom of the wall were connected to a stainless steel water collection box. Holes were capped by a 2-mm net to prevent losses other than those of leachates. The additional CO_2, supplied from a tank, was distributed in each chamber by a transparent pipe with pinholes. The pipe entwined the inner wall of each chamber in a snake shape and from 2.5 to 0.5 m in height. The distance between two pinholes was 1 cm. To ensure that CO_2 was equally distributed in the entire chamber, the pipe was connected to a fan. CO_2 fumigation was applied with fans daily from 0800 h to 1700 h (except during rain periods). The CO_2 flux from the tank was controlled by a flow meter and CO_2 concentrations on the five planes (2.5, 2, 1.5, 1.0, and 0.5 m in height) in the chambers were periodically monitored using a Licor-6400 (Li-Cor Inc., Lincoln, NE, USA).

In March 2005, soil was collected from a nearby evergreen broadleaved forest. The soil is an ultisol, overlying sandstone and shale bedrocks, with a pH value below 4.5 in all the soil layers. Soil organic C is low, and the primary chemical properties are shown in Table 1 and Liu *et al.* (2008). The soil was collected as three different layers (0–20, 20–40 and 40–70 cm depth), which were then homogenized separately and used to fill the below-ground section of the chambers. One- to two-year old seedlings, grown in a nursery, were transplanted into the chambers with minimal damage to the roots. Each chamber was planted with 48 randomly selected seedlings, with 8 seedlings for each of the following 6 species: *Castanopsis hystrix* AC. DC. (*C. hystrix*), *Schima superba* Gardn. et Champ. (*S. superba*), *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Ormosia pinnata* (Lour.) Merr (*O. pinnata*), *Syzygium hancei* Merr. et Perry (*S. hancei*) and *Pinus massoniana* Lambert (*P. massoniana*). These species are native and the most widely spread tree species in southern China. *Ormosia pinnata* was the only N_2-fixing species in this experiment. As the trees were fast growing, one tree per species was harvested at the end of each year to avoid crowding in the chambers. As most seedlings of *P. massoniana* died in the second year of the experiment, and the seedling leaves of *S. hancei* were too small to be measured accurately with the available instrumentation, we only studied the remaining four species in our experiment.

From April 2005, the chambers were exposed to different treatments. The treatments were as follows: elevated [CO_2] (~700 μmol mol^{-1}) and high N treatment (EN; three chambers), elevated [CO_2] and low N treatment (EU; three chambers), ambient [CO_2] (~390 μmol mol^{-1}) and high N treatment (AN; two chambers) and ambient [CO_2] and low N treatment (AU; two chambers). The high N addition was achieved by spraying NH_4NO_3 solution weekly, with the annual amount of 100 kg N ha^{-1} yr^{-1}. We chose this added N amount because high N deposition was found in our experimental site. In 2006, the wet N deposition was ~56 kg ha^{-1} yr^{-1} in our experimental site (Liu *et al*. 2008). We supposed that N deposition would increase continually in the future in southern China. Except for N, no other fertilizer was used. The seedlings were watered with tap water. About 600 mm extra water was applied in each chamber per year. All other chambers received the same amount of water as the control chambers.

Plant height and basal diameter were measured at the time of planting in early March 2005. They were assessed seven times later in August 2005, November 2005, May 2006, September 2007, January 2008, August 2008 and January 2009, respectively. Plant height was measured from the soil–stem surface to the tip of the apical bud and the diameter was assessed at the soil surface. No significant difference in height and basal diameter of the plant seedlings was found among all treatments at the beginning of the experiment (Table 1S in the online supplementary material). Further information on the experiment design can be found in Liu *et al.* (2008, 2010).

Gas exchange measurements, LMA determination and leaf nutrient assays

Gas exchange measurements of the four tree species were taken in September 2008 and August 2009, 41 and 52 months after treatments were applied. The detailed description of gas exchange and foliar sampling are given as follows. Three trees of each species were randomly chosen within each chamber, and the canopy of each tree was divided into three vertical zones: upper (70–100% of canopy height), middle (30–70% of canopy height) and lower (0–30% of canopy height). In each zone, three new fully expanded leaves were selected at random and gas exchange measurements were carried out between 0900 and 1400 h. The light-saturated rate of photosynthesis (A_{sat}, μmol m^{-2} s^{-1}) was measured at growth [CO_2] and photosynthetic active radiation of 1200 μmol m^{-2} s^{-1} using a portable infrared gas exchange system (Licor-6400; Li-Cor) in each chamber on sunny days. Measurements were conducted within the same week and the trees and canopy zones were measured in random order. Temperature was controlled near ambient (30°C) and kept constant during the measurement of gas exchange. The gas exchange measurements from

Table 1: initial soil chemical properties (mean values ± SD)

Layer (cm)	pH	K (g kg^{-1})	Na (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	P (g kg^{-1})	Organic C (g kg^{-1})	N (g kg^{-1})
0–20	4.15 ± 0.15	6.30 ± 0.73	0.64 ± 0.19	1.03 ± 0.22	1.03 ± 0.13	0.30 ± 0.09	16.33 ± 3.42	0.52 ± 0.15
20–40	4.27 ± 0.15	5.03 ± 1.11	0.63 ± 0.49	0.57 ± 0.27	0.84 ± 0.22	0.18 ± 0.19	7.78 ± 0.91	0.36 ± 0.05
40–60	4.25 ± 0.13	5.49 ± 1.53	1.35 ± 0.63	0.51 ± 0.18	0.83 ± 0.23	0.14 ± 0.07	3.94 ± 1.54	0.29 ± 0.07

the same canopy zone in the same chamber were averaged to give chamber values.

The leaf used for gas exchange measurements was then removed from the plant and the leaf area was measured with a portable leaf area meter (LI-3000A; Li-Cor). Afterward, leaves were placed in paper bags and dried to constant weight in an oven at 70°C and weighed. Leaf mass per unit leaf area (LMA, g m^{-2}) was calculated. The dried leaf samples from the same chamber, species and canopy zone were bulked, finely ground and analyzed for N and P concentration. Foliar N concentrations were determined using the Kjeldahl method as described previously (Xu *et al.* 1993). Foliar P concentrations were determined photometrically after leaf samples were digested with nitric acid (HNO_3). N and P concentrations were expressed on a leaf mass basis (N_M and P_M) (%) and were converted to values expressed on a leaf area basis (N_A and P_A) (g m^{-2}) using LMA values. Photosynthetic nitrogen use efficiency (PNUE, μmol g^{-1} s^{-1}) was calculated as A_{sat} (μmol m^{-2} s^{-1})/leaf N_A (g m^{-2}). Photosynthetic phosphorus use efficiency (PPUE, μmol g^{-1} s^{-1}) was calculated as A_{sat} (μmol m^{-2} s^{-1})/leaf P_A (g m^{-2}). Nitrogen to phosphorus ratio (N:P) was calculated as leaf N_A (g m^{-2})/leaf P_A (g m^{-2}).

Statistical analysis

Data analyses were carried out using the SAS software (SAS Institute Inc., Cary, NC, USA). We chose α equal to 0.05. Data were tested for normality and homogeneity of variance. Analyzed data consisted of light-saturated rate of photosynthesis measured at growth [CO_2], leaf N and P concentrations, LMA, PNUE, PPUE and N:P. Data were analyzed using the following mixed linear model:

$$\text{Dependent variables} = C + N + CP + C \times N + C \times CP + N \times CP + C \times N \times CP,$$

Where C was the effect of the [CO_2] treatments (ambient or elevated CO_2), N was the effect of the N treatments (ambient or high N addition) and CP was the effect of the canopy positions and the interactions between the factors. Repeated measurements for a chamber were stated to follow an unstructured covariance structure. When the effects were significant, they were further analyzed using Tukey multiple comparison test (honestly significant difference). Additionally, simple correlations were performed on the whole set of data using the Pearson correlation coefficients.

RESULTS

Leaf mass per unit leaf area

In general, elevated [CO_2] significantly decreased LMA of all tree species both in 2008 and 2009 (Table 2 and Fig. 1). However, N fertilization treatments had no effect on LMA in all species. Except for *A. acuminatissima*, canopy position significantly affected LMA, with higher LMA found in leaves from upper canopy (Table 2 and Fig. 1). There was no interactive effect of elevated [CO_2] and canopy position on LMA (Table 2 and Fig. 1).

Leaf N concentration (N_M and N_A)

Elevated [CO_2] increased leaf N_M of *O. pinnata* and *C. hystrix* ($P < 0.0001$ and $P < 0.001$, respectively) both in 2008 and 2009 (Fig. 2c, d, e and f); however, it reduced that in *S. superba* ($P < 0.01$) (Fig. 2a and b). Elevated [CO_2] had no effect on N_M in the leaves of *A. acuminatissima* (Fig. 2g and h). N fertilization treatments did not affect N_M for all species. N_M was higher in the upper canopy, except for *S. superba* (Table 2 and Fig. 2). There was no interactive effect of elevated [CO_2] and canopy position on N_M (Table 2 and Fig. 2).

Elevated [CO_2] decreased N_A in the leaves of *S. superba* ($P < 0.0001$), while it had no effect on the other three species. N fertilization increased N_A in the leaves of *O. pinnata* ($P < 0.01$). N_A was higher in the upper canopy of all the tree species (Table 2 and Fig. 3). There was no interactive effect of elevated [CO_2] and canopy position on N_M (Table 2 and Fig. 3).

Leaf P concentration (P_M and P_A)

Elevated [CO_2] significantly reduced P_M of *S. superba* both in 2008 and 2009 ($P < 0.0001$ for both); however, it increased that of *C. hystrix* and *A. acuminatissima* ($P < 0.0001$ for both). Elevated [CO_2] had no effect on P_M in the leaves of *O. pinnata* (Table 2 and Fig. 4). N fertilization significantly increased P_M in the leaves of *O. pinnata* ($P < 0.001$), but significantly decreased P_M in the leaves of *S. superba* ($P < 0.001$). P_M in the leaves of all tree species was significantly affected by the interaction between elevated [CO_2] and N fertilization. P_M was higher in the upper canopy compared to the lower canopy for all the tree species (Table 2 and Fig. 4). No interactive effects of elevated [CO_2] and canopy position on P_M were found in all species (Table 2 and Fig. 4).

Elevated [CO_2] significantly decreased P_A of *S. superba* and *O. pinnata* both in 2008 and 2009 ($P < 0.0001$ and $P < 0.01$, respectively); however, it increased those of *C. hystrix* and *A. acuminatissima* ($P < 0.01$ and $P < 0.001$, respectively). N fertilization significantly increased P_A in the leaves of *O. pinnata* ($P < 0.0001$) only. There was a strong interactive effect of elevated [CO_2] and N fertilization treatments on P_A in the leaves of all tree species. P_A was higher in the upper canopy compared to the lower canopy for all species (Table 2 and Fig. 5). Except for *O. pinnata*, interactive effects of elevated [CO_2] and canopy position on P_A were found in the studied species (Table 2 and Fig. 5).

Light-saturated rate of photosynthesis (A_{sat})

Elevated [CO_2] significantly increased A_{sat} of *O. pinnata* (+51% in 2008 and +42% in 2009; in average), *C. hystrix* (+54% in 2008 and +64% in 2009; in average) and *A. acuminatissima* (+40% in 2008 and +43% in 2009; in average) (except *S. superba*) ($P < 0.0001$ for all three tree species) (Tables 2 and 3). N fertilization did not affect A_{sat} of the tree species, except for *A. acuminatissima* (Tables 2 and 3). For all species, A_{sat}

Table 2: results from statistical analysis for effects of [CO_2] treatments (C), nitrogen treatments (N), canopy positions (CP) and their interactions on LMA, light-saturated net photosynthetic rates (A_{sat}) and nutrient concentrations of four tree species grown in ambient and elevated [CO_2] under low and high N treatments

Parameters	Treatment	*Schima superba*	*Ormosia pinnata*	*Castanopsis hystrix*	*Acmena acuminatissima*
LMA	C	—***	—*	—**	—*
	N	ns	ns	ns	ns
	C* N	ns	ns	—*	ns
	CP	—***	—*	—**	ns
	C*CP	ns	ns	ns	ns
	N*CP	ns	ns	ns	ns
	C* N*CP	ns	ns	ns	ns
Mass-based leaf N concentration (N_M)	C	—**	—***	—**	ns
	N	ns	ns	ns	ns
	C* N	—**	—***	ns	—**
	CP	ns	—***	—***	—**
	C*CP	ns	ns	ns	ns
	N*CP	ns	—***	ns	ns
	C* N*CP	—*	ns	ns	—*
Area-based leaf N concentration (N_A)	C	—***	ns	ns	ns
	N	ns	—**	ns	ns
	C* N	—***	ns	—**	—**
	CP	—***	—***	—***	—**
	C*CP	ns	ns	ns	ns
	N*CP	ns	—*	—**	ns
	C* N*CP	—*	—*	*	ns
Mass-based leaf P concentration (P_M)	C	—***	ns	—***	—***
	N	—***	—***	ns	ns
	C* N	—**	—*	—**	—**
	CP	—***	—***	—***	—***
	C*CP	ns	ns	ns	ns
	N*CP	ns	—*	ns	ns
	C* N*CP	—**	ns	ns	—***
Area-based leaf P concentration (P_A)	C	—***	—**	—**	—***
	N	ns	—***	ns	ns
	C* N	—**	ns	—***	—**
	CP	—***	—***	—***	—***
	C*CP	—***	ns	—*	—***
	N*CP	ns	—*	ns	—*
	C* N*CP	ns	ns	—*	—***
A_{sat}	C	ns	—***	—***	—***
	N	ns	ns	ns	—*
	C* N	ns	ns	ns	ns
	CP	—***	—***	—**	—***
	C*CP	—***	ns	ns	ns
	N*CP	ns	—*	ns	ns
	C* N*CP	ns	ns	ns	ns

The significance of the primary effects and their interactions are shown with the aforementioned abbreviations (*$P \leqslant 0.05$, **$P \leqslant 0.01$ and ***$P \leqslant 0.001$) or as not significant (ns).

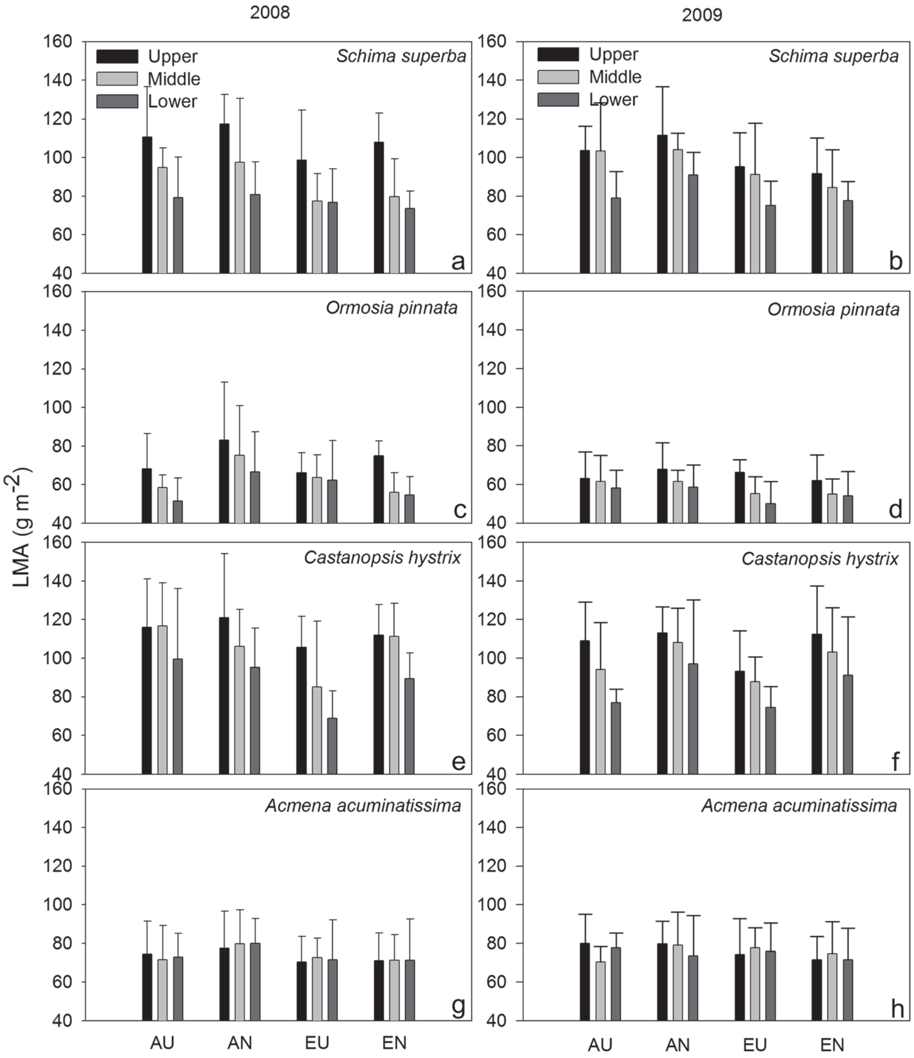

Figure 1: LMA of four species in growth [CO$_2$], N fertilization and different canopy positions in year 2008 and 2009. Growth [CO$_2$] was ambient CO$_2$ (~390 μmol mol^{-1}) or elevated CO$_2$ (~700 μmol mol^{-1}). N fertilization was ambient N or high N (ambient + 100 kg N ha^{-1} yr^{-1}). There were three canopy positions: upper, middle and lower. Growth [CO$_2$] and N fertilization treatments were displayed as follows: AU (ambient CO$_2$ + ambient N), AN (ambient CO$_2$ + high N), EU (elevated CO$_2$ + ambient N) and EN (elevated CO$_2$ + high N). Four species are *Schima superba*, *Ormosia pinnata*, *Castanopsis hystrix* and *Acmena acuminatissima*. Values are means ± standard deviation.

showed significant vertical gradients, decreasing from upper canopy downwards (Tables 2 and 3). Canopy position did not significantly affect of A_{sat} responses to elevated [CO$_2$] except for *S. superba* (Tables 2 and 3).

DISCUSSION

Leaf traits and A_{sat} was affected differently in species by elevated [CO$_2$] and N fertilization

The LMA.

Changes in leaf morphology and anatomy may occur due to the affects of elevated [CO$_2$] on the leaf carboxylation rate per unit leaf area (Luo *et al.*1994; Peterson *et al.* 1999). In our study, the significant lower LMA was found in leaves of tree species grown in elevated [CO$_2$], which is consistent with some studies (Gutiérrez *et al.* 2009; Peterson *et al.* 1999). However, an increase in LMA is common in rising [CO$_2$] (Ghannoum *et al.* 2010; Liberloo *et al.* 2007). It is usually associated with the accumulation of nonstructural carbohydrates (Curtis *et al.* 2000), increases in leaf thickness (Sims *et al.* 1998), changes in mesophyll cell numbers (Vu *et al.* 1989), declines in Rubisco activity and amount, reduction in leaf photosynthesis and decreases in leaf nitrogen content per mass (Gutiérrez *et al.* 2009). In our experiment, decreases in LMA were accompanied with increases in nitrogen content per mass (negative relationship between LMA and N_M; R^2 = 0.438) and the sustained leaf photosynthesis (Fig. 6f; $P <$ 0.001). Furthermore, the reduction in LMA was thought to be beneficial to photosynthesis under elevated [CO$_2$], as decreased LMA increases the nitrogen content on a leaf area

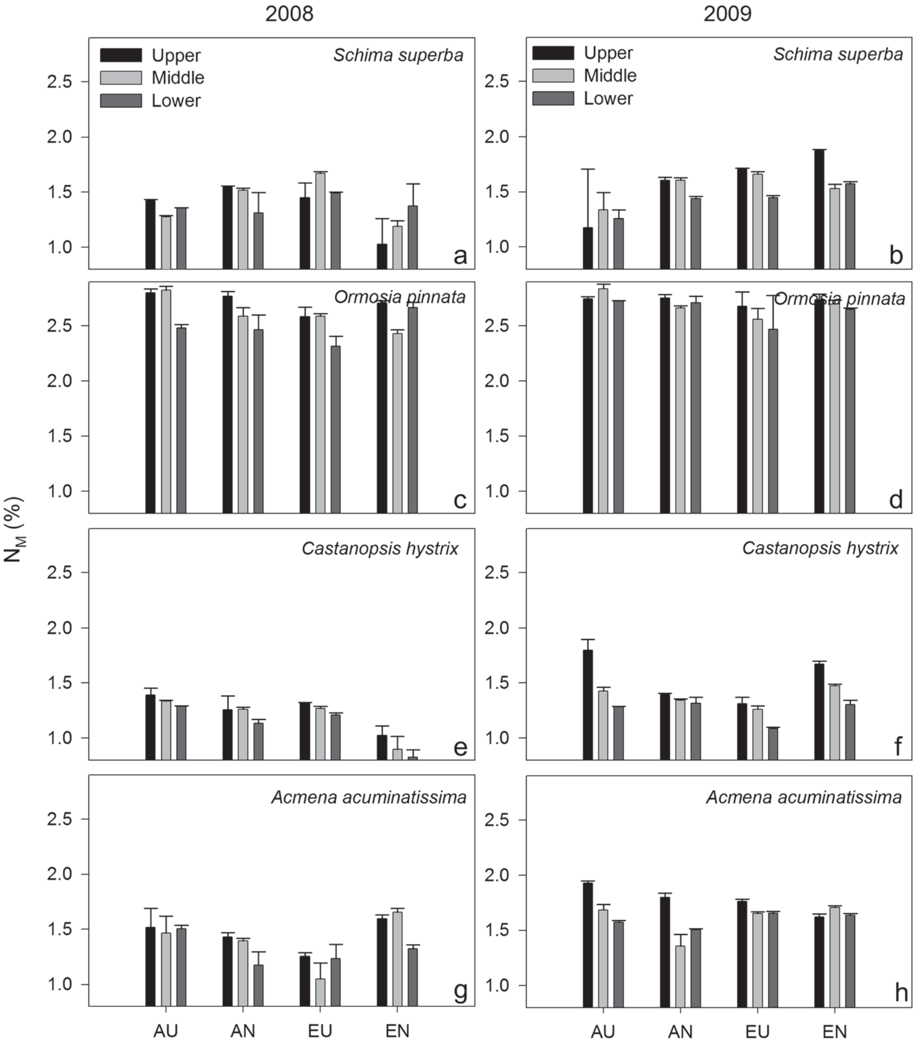

Figure 2: Nitrogen content per mass (N_M) of four species in growth [CO_2], N fertilization and different canopy positions in year 2008 and 2009. Growth [CO_2] was ambient CO_2 (~390 µmol mol^{-1}) or elevated CO_2 (~700 µmol mol^{-1}). N fertilization was ambient N or high N (ambient + 100 kg N ha^{-1} yr^{-1}). There were three canopy positions: upper, middle and lower. Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N), EU (elevated CO_2 + ambient N) and EN (elevated CO_2+ high N). Four species are *Schima superba*, *Ormosia pinnata*, *Castanopsis hystrix* and *Acmena acuminatissima*. Values are means ± standard deviation.

basis, which has the positive relationship with carbon uptake (Luo *et al.*1994).

Leaf N concentration.

It has been established that leaf mass-based N concentration (N_M) is decreased by elevated [CO_2] (Del Pozo *et al.* 2007; Johnson *et al.* 2004). In this study, N_M was significantly greater in the leaves of *O. pinnata* and *C. hystrix* grown in elevated [CO_2] treatments, which is contrary to most previous studies. N_M may be related to many factors. First, N_M would be affected by soil N availability. N deposition is high around Guangzhou City near the experimental site. In 2006, a wet N deposition of ~56 kg ha^{-1} yr^{-1} was measured in the experimental site (Liu et al. 2008). Aber *et al.* (1998) and MacDonald *et al.* (2002) have demonstrated that some northern temperate forests were N saturated when N inputs were above 25–30 kg ha^{-1} yr^{-1}. Since atmospheric N deposition at our research site far exceeds the above-cited critical values, it appears that soil N availability was not a limiting factor for plant growth. From soil total nitrogen analysis, total nitrogen was not affected by elevated [CO_2] and nitrogen addition, which also supports this idea (Liu *et al.* 2010). Although PNUE was greater in the two species under elevated [CO_2] treatments (Table 4), which seems a possible reason for reduced N_M (Stitt and Krapp 1999), it may have little effects on N_M under high N deposition. Second, root uptake of N would also have impacts on N_M. Unpublished data showed that root biomass of *O. pinnata* and *C. hystrix* increased by 22.65% and 17.47% under elevated [CO_2] in year 2009, respectively (Honglang Duan's Master Thesis). Therefore, the increased root growth induced by elevated [CO_2] treatments

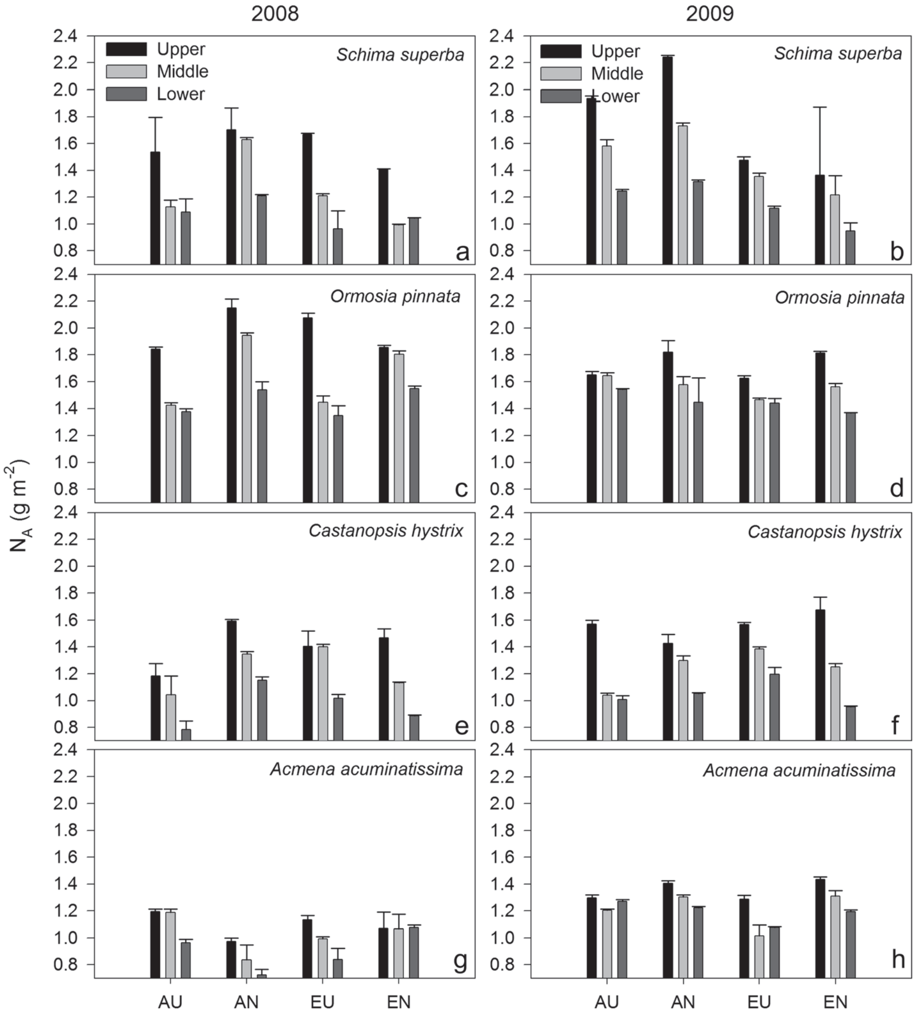

Figure 3: Nitrogen content per unit leaf area (N_A) of four species in growth [CO_2], N fertilization and different canopy positions in year 2008 and 2009. Growth [CO_2] was ambient CO_2 (~390 µmol mol^{-1}) or elevated CO_2 (~700 µmol mol^{-1}). N fertilization was ambient N or high N (ambient + 100 kg N ha^{-1} yr^{-1}). There were three canopy positions: upper, middle and lower. Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N), EU (elevated CO_2 + ambient N) and EN (elevated CO_2 + high N). Four species are *Schima superba*, *Ormosia pinnata*, *Castanopsis hystrix* and *Acmena acuminatissima*. Values are means ± standard deviation.

may have resulted in increased N uptake. In addition, the increased soil moisture in the chambers exposed to elevated [CO_2] accelerated the N uptake by roots (Deng *et al.* 2010). The leaf N_M of *S. superba* in elevated [CO_2] treatments was lower than that in ambient treatments. This may be due to greater PNUE in elevated [CO_2] (Table 4) (Stitt and Krapp 1999) and the limited N uptake for *S. superba* caused by decreased root biomass (decreased by 15.82%; unpublished data).

Leaf P concentration.

Phosphorus (P) is a particularly important limiting nutrient because its supply rate directly controls the photosynthetic response to [CO_2] (Lewis *et al.* 1994; Stocklin *et al.* 1998). It was reported that ecosystems became more P limited with increasing atmospheric [CO_2] concentration above current levels (Vance *et al.* 2003). In our study, however, there was a significant increase of mass-based P concentrations (P_M) in the leaves of *C. hystrix* and *A. acuminatissima* exposed to elevated [CO_2] treatments were found in our study, which supports the previous result found by Norby *et al.* (1986) wherein they studied the effects of CO_2 enrichment on the mineral nutrition of *Quercus alba* seedlings. First, the increased leaf P concentration may be attributed to the greater P uptake by roots. Soil moisture is a key factor of controlling P availability in soils through several processes, such as affecting mineralization processes, influencing P demand for plant growth and impacting microbial activity (Sardans *et al.* 2007). The increased soil moisture in the chambers exposed to elevated [CO_2] (Deng *et al.* 2010) could also lead to the greater P uptake by roots. However, elevated [CO_2] had no effect on P_M in the leaves of *O. pinnata*. Conroy *et al.* (1990) also found little effect of elevated CO_2 on tissue P concentrations in *Pinus radiata* (D. Don). On the one hand, in our study,

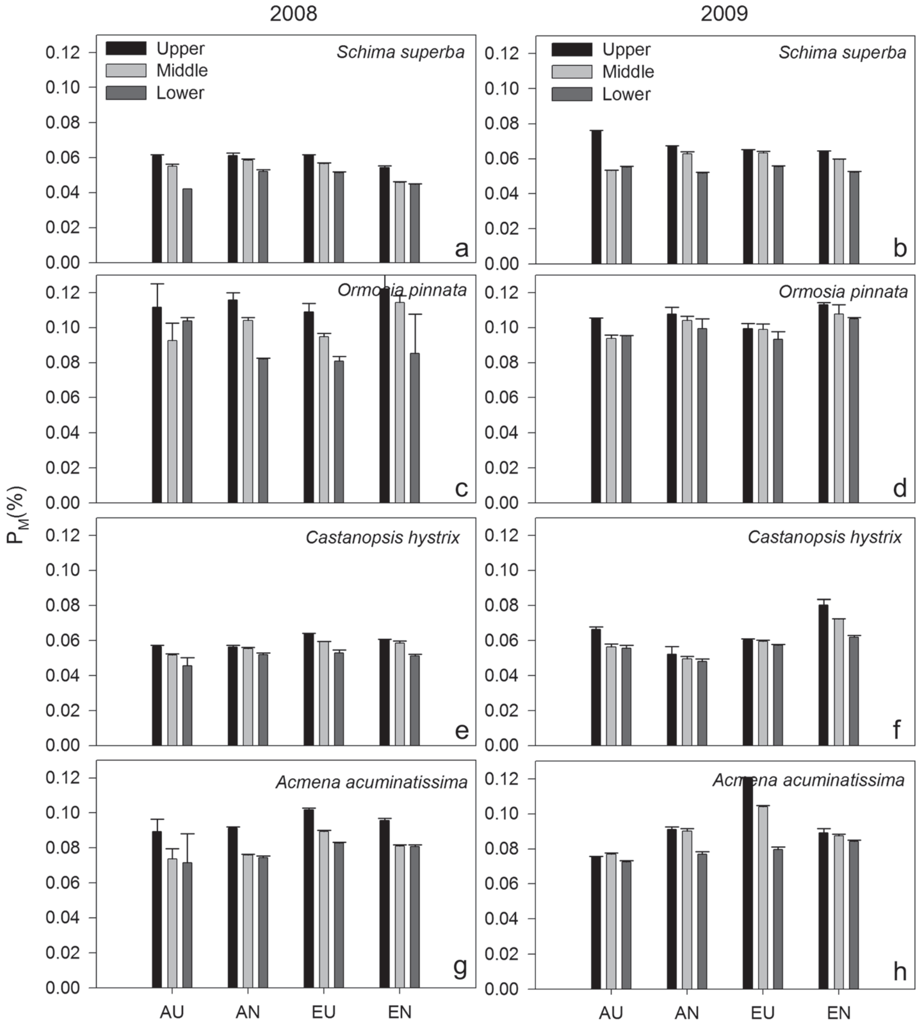

Figure 4: Phosphorus content per mass (P_M) of four species in growth [CO_2], N fertilization and different canopy positions in year 2008 and 2009. Growth [CO_2] was ambient CO_2 (~390 µmol mol^{-1}) or elevated CO_2 (~700 µmol mol^{-1}). N fertilization was ambient N or high N (ambient + 100 kg N ha^{-1} yr^{-1}). There were three canopy positions: upper, middle and lower. Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N) EU (elevated CO_2 + ambient N) and EN (elevated CO_2 + high N). Four species are *Schima superba*, *Ormosia pinnata*, *Castanopsis hystrix* and *Acmena acuminatissima*. Values are means ± standard deviation.

O. pinnata is a N_2-fixing plant. It was reported that the symbiotic N_2-fixing plants had an advantage in P acquisition than non-N-fixing plants (Dyhrman *et al.* 2006). N_2 fixers could rely on extracellular phosphatases, a constitutive N-rich class of enzymes involved in the breakdown of organic P, to enhance local P supplies (Houlton *et al.* 2008). Hence, other stimulation could not change the uptake of P by roots in these N_2 fixers. On the other hand, PPUE of *O. pinnata* was greater in elevated [CO_2], which may decrease the demand for P (Table 4). These two factors may offset each other. P_M in the leaves of *S. superba* grown in elevated [CO_2] treatments was significantly lower than that in ambient [CO_2]. This is consistent with other findings (Johnson *et al.* 1994; Norby *et al.* 1986).

N fertilization significantly increased P_M in the leaves of *O. pinnata* ($P < 0.001$). As a N_2-fixing plant, *O. pinnata* may have the ability to invest N into P acquisition to sustained N_2 fixation in P-limited tropical ecosystems (Houlton *et al.* 2008). The N addition stimulated phosphatase production (Olander and Vitousek 2000; Treseder and Vitousek 2001), thereby increasing P availability.

A_{sat}.

Down-regulation of photosynthesis in elevated [CO_2] has been observed in many previous studies (see Griffin *et al.* 2000; Hyvönen *et al.* 2007; Rogers and Ellsworth 2002; Sefcik *et al.* 2007). This down-regulation in photosynthetic capacity is often associated with a decrease in the amount or activity of Rubisco (the carboxylating enzyme) (Rey and Jarvis 1998; Rogers and Ellsworth 2002), a decrease in leaf carboxylation capacity (V_{cmax}) (Medlyn *et al.* 1999), or a decrease in leaf N concentration (Saxe *et al.* 1998; Stitt and Krapp 1999). However, our data suggested that photosynthetic stimulation

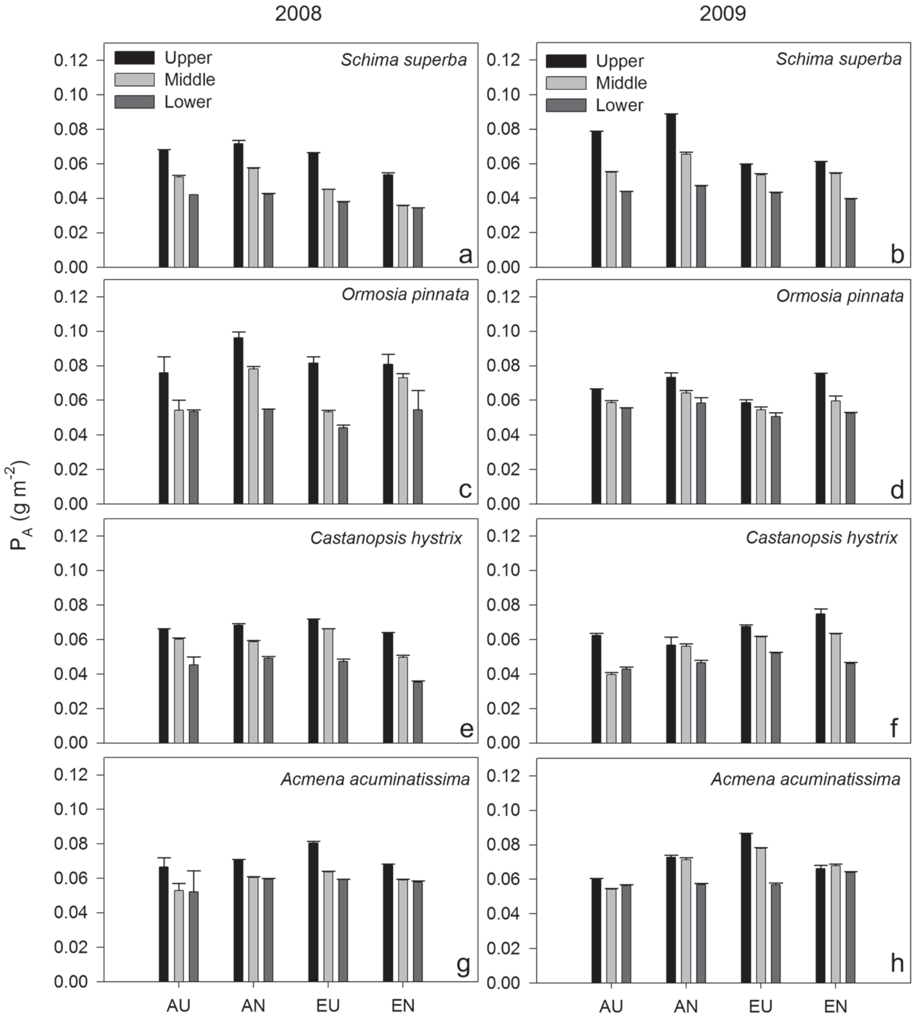

Figure 5: Phosphorus content per unit leaf area (P_A) of four species in growth [CO_2], N fertilization and different canopy positions in year 2008 and 2009. Growth [CO_2] was ambient CO_2 (~390 μmol mol^{-1}) or elevated CO_2 (~700 μmol mol^{-1}). N fertilization was ambient N or high N (ambient + 100 kg N ha^{-1} yr^{-1}). There were three canopy positions: upper, middle and lower. Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N), EU (elevated CO_2 + ambient N) and EN (elevated CO_2 + high N). Four species are *Schima superba*, *Ormosia pinnata*, *Castanopsis hystrix* and *Acmena acuminatissima*. Values are means ± standard deviation.

in *O. pinnata*, *C. hystrix* and *A. acuminatissima* was sustained in elevated [CO_2] after 5 years of fumigation, even under non-fertilized conditions. This is in line with some experiments (Karl-Friedrich Bader et al. 2010; Liberloo *et al.* 2007). Rubisco may be more efficient in elevated [CO_2] due to decreased photorespiration. Thus, less Rubisco would be needed for CO_2 fixation (Liberloo *et al.* 2007). In addition, reduced leaf nutrient concentration (mainly N concentration) is frequently accompanied with down-regulation of photosynthesis. Nevertheless, in our study, elevated [CO_2] did not decrease leaf N and P concentration in *O. pinnata*, *C. hystrix* and *A. acuminatissima* (except *S. superba*), which may also support the sustainability of high net photosynthetic rates in these trees. In addition, the weak relationship between A_{sat} and leaf nutrient concentrations also suggested that photosynthesis could be affected by many factors (Fig. 6).

The light-saturated rates of photosynthesis in all tree species were not affected by the N fertilization. It was reported that plant net photosynthesis was strongly correlated with leaf N concentration (Hikosaka and Osone 2009). However, there was no significant relationship of A_{sat} with leaf N concentration in all species in our study (Fig. 6a and b). Responses of photosynthesis to elevated [CO_2] were not modified by N fertilization, probably due to high ambient N deposition in the experiment site, where N was not a limiting factor (Liu *et al.* 2008; Mo *et al.* 2008).

Leaf traits and A_{sat} response to elevated [CO_2] were not altered by canopy position

In our study, all leaf traits (i.e. LMA, leaf N and P concentration) and A_{sat} were decreased from upper to lower canopy, which is supported by previous studies (Del Pozo and Dennett 1999; Liberloo *et al.* 2007; Lötscher *et al.* 2003; Rijkers *et al.* 2000;

Table 3: mean values (±standard deviation) of rate of photosynthesis (A_{sat}, μmol m^{-2} s^{-1}) measured at their growth CO_2 for upper, middle and lower leaves of four tree species grown in open-top chambers either at ambient (390 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 and at low or high nitrogen supply in September 2008 and 2009

Time	Species	Canopy	AU	AN	EU	EN
2008	*Schima superba*	Upper	9.15 ± 4.58aA	6.45 ± 2.48ab	6.37 ± 3.92ab	4.79 ± 3.83bAB
		Middle	4.66 ± 1.97bB	5.75 ± 2.87ab	8.61 ± 4.36a	6.73 ± 4.67abA
		Lower	3.83 ± 1.78bB	4.47 ± 2.50ab	6.31 ± 3.13a	2.31 ± 0.44bB
	Ormosia pinnata	Upper	7.54 ± 2.86b	8.78 ± 1.80bA	14.74 ± 3.45aA	13.55 ± 2.38aA
		Middle	5.79 ± 2.27b	7.45 ± 2.92bA	11.51 ± 2.53aB	10.54 ± 1.61aB
		Lower	7.39 ± 2.31b	4.78 ± 2.62cB	9.61 ± 0.98abB	10.45 ± 3.91aB
	Castanopsis hystrix	Upper	4.25 ± 2.38A	6.27 ± 1.90A	8.42 ± 5.34A	6.90 ± 5.58
		Middle	3.87 ± 2.38AB	3.87 ± 1.87B	5.48 ± 4.19AB	5.77 ± 2.87
		Lower	2.11 ± 1.42bB	2.30 ± 1.26bB	2.95 ± 2.36bB	5.12 ± 2.73a
	Acmena acuminatissima	Upper	5.20 ± 1.68c	6.35 ± 1.26bcA	8.48 ± 1.60a	7.79 ± 2.11ab
		Middle	5.16 ± 0.87b	5.77 ± 1.93bAB	6.91 ± 2.18ab	8.65 ± 3.14a
		Lower	4.53 ± 2.22bc	4.15 ± 2.37cB	7.78 ± 2.51a	6.27 ± 1.35ab
2009	*Schima superba*	Upper	12.95 ± 2.79A	11.40 ± 3.03A	12.36 ± 5.64aA	10.59 ± 5.18
		Middle	8.46 ± 3.45B	10.12 ± 2.89 A	12.49 ± 5.42A	9.30 ± 4.61
		Lower	5.89 ± 2.76abC	4.83 ± 1.74bB	8.17 ± 3.47aB	8.76 ± 3.79a
	Ormosia pinnata	Upper	8.91 ± 1.35cA	10.94 ± 1.37bcA	12.23 ± 4.14ab	14.25 ± 3.14aA
		Middle	6.85 ± 2.12cB	8.96 ± 1.64bcB	10.95 ± 2.79ab	11.72 ± 2.72aB
		Lower	7.32 ± 1.60cAB	8.29 ± 1.27cB	12.50 ± 1.99a	10.44 ± 1.44bB
	Castanopsis hystrix	Upper	3.04 ± 2.35	2.40 ± 1.54B	4.03 ± 2.23B	2.91 ± 2.38
		Middle	2.49 ± 1.94b	4.35 ± 1.50abA	4.89 ± 1.45abAB	5.31 ± 3.42a
		Lower	2.37 ± 0.81b	2.69 ± 0.54bAB	6.13 ± 1.59aA	4.73 ± 2.51a
	Acmena acuminatissima	Upper	6.82 ± 1.81bA	6.80 ± 1.07b	9.02 ± 2.96abA	9.52 ± 2.34a
		Middle	4.29 ± 1.09cB	6.40 ± 2.09bc	7.76 ± 2.06abAB	8.65 ± 2.77a
		Lower	5.20 ± 1.53bB	5.94 ± 1.68b	6.97 ± 1.76abB	8.73 ± 2.30a

Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N), EU (elevated CO_2 + ambient N) and EN (elevated CO_2 + high N). The mean ± standard deviation within a row followed by different lowercase letters has significant differences among the treatments at $P \leqslant 0.05$. The mean ± standard deviation within each column followed by different uppercase letters has significant differences among the canopy at $P \leqslant 0.05$.

Yin *et al.* 2003). However, it is of greater interest to investigate whether the response of leaf traits and A_{sat} to elevated [CO_2] was altered by canopy position. Our data showed that leaf traits and A_{sat} increased similarly in upper, middle and lower canopy positions under elevated [CO_2] treatments. In other words, we did not find interactive effects of elevated [CO_2] and canopy position on photosynthetic parameters, which is consistent with some studies on coniferous species (Crous and Ellsworth 2004; Griffin *et al.* 2000; Tissue *et al.* 2001). It was predicted that photosynthesis would be enhanced more by elevated [CO_2] in the lower canopy than in the upper canopy due to the increase in apparent quantum yield and the decrease in light compensation point under CO_2 enrichment (Norby *et al.* 1999; Saxe *et al.* 1998; Tissue *et al.* 2001). However, there was no evidence to show this occurred in our experiment.

CONCLUSION

In the study, leaf traits and light-saturated photosynthesis were impacted differently by elevated [CO_2] and N fertilization among species. Specifically, elevated [CO_2] significantly decreased LMA, mass-based N and P concentration (N_M, P_M) and area-based N and P concentration (N_A, P_A) in the leaves of *S. superba*. Mass-based N concentration (N_M) was significantly greater in the leaves of *O. pinnata* and *C. hystrix* grown in elevated [CO_2] ($P < 0.0001$ and $P < 0.001$, respectively) and photosynthetic stimulation of leaves in *O. pinnata*, *C. hystrix* and *A. acuminatissima* was sustained in elevated [CO_2] after 5 years of fumigation, suggesting that they are good species for C fixation in response to the expected rising [CO_2]. However, N fertilization treatments did not affect LMA and N_M in the leaves of all species. N addition did not modify effects of elevated [CO_2] on photosynthesis. Leaf traits (N_M, N_A, P_M, P_A) and light-saturated photosynthesis decreased from the upper to lower canopy. Canopy position did not alter responses of leaf traits and photosynthesis to elevated [CO_2]. The persistent photosynthetic stimulation in most of the native species exposed to elevated [CO_2], plus the sufficient sink capacity and nutrient availability, suggests

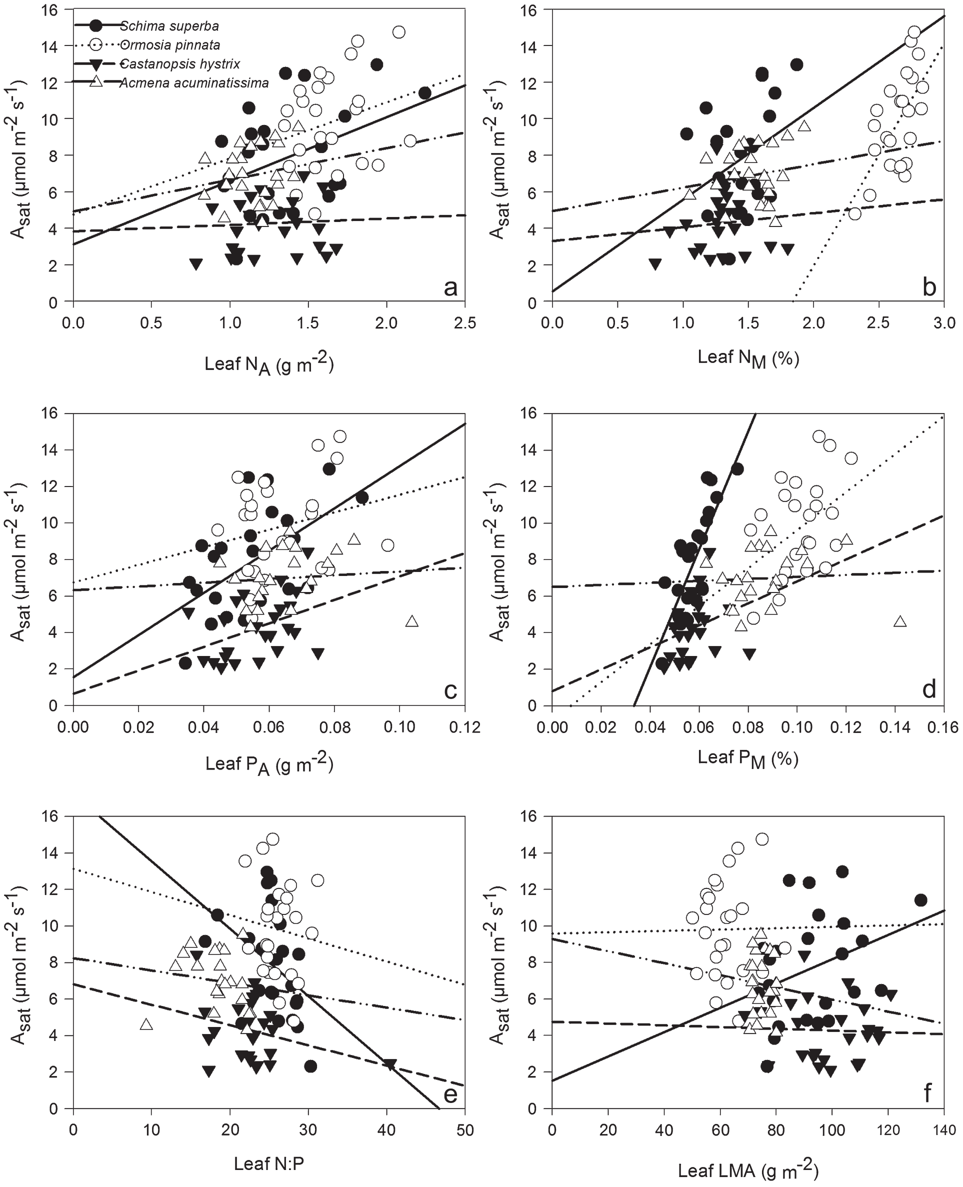

Figure 6: Relationship between (a) A_{sat} and leaf N_A, (b) A_{sat} and leaf N_M, (c) A_{sat} and leaf P_A, (d) A_{sat} and leaf P_M, (e) A_{sat} and leaf N:P and (f) A_{sat} and LMA across growth [CO$_2$] and N fertilization treatments of four species in both year 2008 and 2009. Each data point represents a mean value of each [CO$_2$] and N fertilization treatment in the same canopy position for each species. Lines are linear regression for each species. Four species are *Schima superba, Ormosia pinnata, Castanopsis hystrix* and *Acmena acuminatissima*.

that native tree species could benefit from elevated [CO$_2$] in the future in subtropical China.

SUPPLEMENTARY DATA

Supplementary Table1S is available at *Journal of Plant Ecology* online.

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Conflict of interest statement. None declared.

Table 4: mean values (±standard deviation) of PNUE (μmol g^{-1} s^{-1}), PPUE (μmol g^{-1} s^{-1}) and nitrogen to phosphorus ratio (N:P) at their growth CO_2 for leaves of four tree species grown in open-top chambers either at ambient (390 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 and at low or high nitrogen supply in September 2008 and 2009

Time	Species	Canopy	AU	AN	EU	EN
2008	*Schima superba*	PNUE	6.09 ± 2.77	3.67 ± 0.13	5.83 ± 1.76	4.14 ± 2.37
		PPUE	112.21 ± 32.80	98.65 ± 8.08	151.14 ± 48.76	115.20 ± 65.04
		N:P	19.18 ± 3.33b	26.93 ± 2.80a	25.81 ± 0.81a	28.13 ± 2.08a
	Ormosia pinnata	PNUE	4.51 ± 0.74b	3.67 ± 0.51b	7.39 ± 0.48a	6.75 ± 0.90a
		PPUE	114.68 ± 20.60b	91.25 ± 3.92b	204.76 ± 20.96a	168.01 ± 23.78a
		N:P	25.41 ± 1.06	25.11 ± 2.92	27.71 ± 2.54	25.03 ± 3.26
	Castanopsis hystrix	PNUE	3.33 ± 0.55b	2.93 ± 0.97b	4.75 ± 2.39ab	5.19 ± 0.55a
		PPUE	58.38 ± 10.23b	68.09 ± 22.80ab	87.30 ± 27.79ab	123.09 ± 19.51a
		N:P	17.51 ± 0.40b	23.20 ± 0.26a	19.43 ± 3.21ab	23.65 ± 1.30a
	Acmena acuminatissima	PNUE	4.47 ± 0.21b	6.71 ± 0.28ab	7.90 ± 1.20a	7.08 ± 1.16ab
		PPUE	73.05 ± 27.21	139.44 ± 34.12	123.42 ± 20.37	—
		N:P	16.54 ± 6.68	17.61 ± 3.16	17.50 ± 1.47	—
2009	*Schima superba*	PNUE	5.58 ± 1.00b	4.87 ± 1.10b	8.30 ± 0.96a	8.78 ± 0.99a
		PPUE	151.36 ± 15.20ab	128.66 ± 25.79b	210.07 ± 21.85a	189.24 ± 28.88ab
		N:P	27.34 ± 2.28	26.58 ± 1.33	25.33 ± 0.57	21.63 ± 2.92
	Ormosia pinnata	PNUE	4.74 ± 0.67b	5.81 ± 0.18b	7.64 ± 0.25a	7.67 ± 0.18a
		PPUE	127.66 ± 9.42b	143.79 ± 5.10b	219.01 ± 24.97a	195.19 ± 4.56a
		N:P	27.14 ± 2.04	24.76 ± 0.14	28.60 ± 2.28	25.48 ± 1.12
	Castanopsis hystrix	PNUE	1.94 ± 0.40	2.43 ± 0.69	3.74 ± 1.29	3.64 ± 1.70
		PPUE	55.38 ± 6.91	59.15 ± 17.70	85.41 ± 29.48	74.98 ± 32.69
		N:P	29.67 ± 9.37	24.36 ± 1.53	22.85 ± 0.31	21.16 ± 3.92
	Acmena acuminatissima	PNUE	4.30 ± 0.87b	4.86 ± 0.04b	7.04 ± 0.59a	6.85 ± 0.40a
		PPUE	94.85 ± 17.21b	95.91 ± 7.80b	109.01 ± 11.88ab	135.72 ± 8.18a
		N:P	22.09 ± 0.53a	19.72 ± 1.65ab	15.64 ± 2.99b	19.85 ± 1.58ab

Growth [CO_2] and N fertilization treatments were displayed as follows: AU (ambient CO_2 + ambient N), AN (ambient CO_2 + high N), EU (elevated CO_2 + ambient N) and EN (elevated CO_2 + high N). The mean ± standard deviation within a row followed by different lowercase letters has significant differences among the treatments at $P \leqslant 0.05$. '—' shows missing values.

REFERENCES

Aber J, McDowell W, Nadelhoffer K, *et al.* (1998) Nitrogen saturation in temperate forest ecosystems. BioScience **48:**921–34.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytol* **165**:351–72.

BassiriRad H, Gutschick VP, Lussenhop J (2001) Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. *Oecologia* **126**:305–20.

Campbell CD, Sage RF (2006) Interactions between the effects of atmospheric CO_2 content and P nutrition on photosynthesis in white lupin (*Lupinus albus* L.). *Plant Cell Environ* **29**:844–53.

Conroy JP, Milham PJ, Mazur M, *et al.* (1990) Growth, dry weight partitioning and wood properties of *Pinus radiata* D. Don after 2 years of CO_2 enrichment. *Plant Cell Environ* **13**:329–37.

Crous KY, Ellsworth DS (2004) Canopy position affects photosynthetic adjustments to long-term elevated CO_2 concentration (FACE) in aging needles in a mature *Pinus taeda* forest. *Tree Physiol* **24**:961–70.

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ* **19**:127–37.

Curtis PS, Vogel CS, Wang X, *et al.* (2000) Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO_2 enriched atmosphere. *Ecol Appl* **10**:3–17.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113**:299–313.

Del Pozo A, Dennett MD (1999) Analysis of the distribution of light, leaf nitrogen and photosynthesis within the canopy of *Vicia faba* L. at two contrasting densities. *Aust J Agr Res* **50**:183–9.

Del Pozo A, Pérez P, Gutiérrez D, *et al.* (2007) Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. *Environ Exp Bot* **59**:371–80.

Deng Q, Zhou G, Liu J, *et al.* (2010) Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. *Biogeosciences* **7**:315–28.

Dyhrman ST, Chappell PD, Haley ST, *et al.* (2006) Phosphonate utilization by the globally important marine diazotroph Trichodesmium. *Nature* **439**:68–71.

Ghannoum O, Phillips NG, Conroy JP, *et al.* (2010) Exposure to pre-industrial, current and future atmospheric CO_2 and temperature differentially affects growth and photosynthesis in Eucalyptus. *Global Change Biol* **16**:303–19.

Griffin KL, Tissue DT, Turnbull MH, *et al.* (2000) The onset of photosynthetic acclimation to elevated CO_2 partial pressure in field-grown *Pinus radiata* D. Don. after 4 years. *Plant Cell Environ* **23**:1089–98.

Gutiérrez E, Gutiérrez D, Morcuende R, *et al.* (2009) Changes in leaf morphology and composition with future increases in CO_2 and temperature revisited: wheat in field chambers. *J Plant Growth Regul* **28**:349–57.

Hikosaka K, Osone Y (2009) A paradox of leaf-trait convergence: why is leaf nitrogen concentration higher in species with higher photosynthetic capacity? *J Plant Res* **122**:245–51.

Houlton BZ, Wang YP, Vitousek PM, *et al.* (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature* **454**:327–30.

Johnson DW, Ball JT, Walker RF (1994) Effects of CO_2 and nitrogen on nutrient uptake in ponderosa pine seedlings. *Plant Soil* **168**:144–53.

Johnson DW, Cheng W, Joslin JD, *et al.* (2004) Effects of elevated CO_2 on nutrient cycling in a sweet gum plantation. *Biogeochemistry* **69**:379–403.

Karl-Friedrich Bader M, Siegwolf R, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. *Planta* **232**:1115–25.

Körner C (2006) Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytol* **172**:393–411.

Kubiske ME, Quinn VS, Marquardt PE, *et al.* (2007) Effects of elevated atmospheric CO_2 and/or O_3 on intra- and interspecific competitive ability of aspen. *Plant Biol* **9**:342–55.

Lambers H, Shane MW, Cramer MD, *et al.* (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Ann Bot* **98**:693–713.

Lewis JD, Griffin KL, Thomas RB, *et al.* (1994) Phosphorus supply affects the photosynthetic capacity of loblolly pine grown in elevated carbon dioxide. *Tree Physiol* **14**:1229–44.

Lewis JD, Ward JK, Tissue DT (2010) Phosphorus supply drives nonlinear responses of cottonwood (*Populus deltoides*) to increases in CO_2 concentration from glacial to future concentrations. *New Phytol* **187**:438–48.

Liberloo M, Tulva I, Raïm O, *et al.* (2007) Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed Populus canopy profile (EUROFACE). *New Phytol* **173**:537–49.

Liu JX, Zhang DQ, Zhou GY, *et al.* (2008) CO_2 enrichment increases cation and anion loss in leaching water of model forest ecosystems in southern China. *Biogeoscience* **5**:1783–95.

Liu JX, Zhou GY, Zhang DQ, *et al.* (2010) Carbon dynamics in subtropical forest soil: effects of atmospheric carbon dioxide enrichment and nitrogen addition. *J Soils Sediments* **10**:730–8.

Lloyd J, Farquhar GD (1996) The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. 1. General principles and forest ecosystems. *Funct Ecol* **10**:4–32.

Lötscher M, Stroh K, Schnyder H (2003) Vertical leaf nitrogen distribution in relation to nitrogen status in grassland plants. *Ann Bot* **92**:679–88.

Luo Y, Field CB, Mooney HA (1994) Predicting responses of photosynthesis and root fraction to elevated [CO_2]: interactions among carbon, nitrogen, and growth. *Plant Cell Environ* **17**:1195–204.

MacDonald JA, Dise NB, Matzner E, *et al.* (2002) Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Global Change Biol* **8**:1028–33.

Medlyn BE, Badeck F-W, De Pury DGG, *et al.* (1999) Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant Cell Environ* **22**:1475–95.

Mo JM, Li DJ, Gundersen P (2008) Seedling growth response of two tropical tree species to nitrogen deposition in southern China. *Eur J Forest res* **127**:275–83.

Norby RJ, O'Neill EG, Luxmoore RJ (1986) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. *Plant Physiol* **82**:83–9.

Norby RJ, Wullschleger SD, Gunderson CA, *et al.* (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant Cell Environ* **22**:683–714.

Nord EA, Lynch JP (2009) Plant phenology: a critical controller of soil resource acquisition. *J Exp Bot* **60**:1927–37.

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* **49**:175–90.

Peterson AG, Ball JT, Luo Y, *et al.* (1999) Quantifying the response of photosynthesis to changes in leaf nitrogen content and leaf mass per area in plants grown under atmospheric CO_2 enrichment. *Plant Cell Environ* **22**:1109–19.

Reich PB, Hungate BA, Luo YQ (2006) Carbon-nitrogen interaction in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annu Rev Ecol Evol Syst* **37**:611–36.

Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc Natl Acad Sci USA* **101**:11001–6.

Rey A, Jarvis PG (1998) Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. *Tree Physiol* **18**:441–50.

Rijkers T, Pons TL, Bongers F (2000) The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Funct Ecol* **14**:77–86.

Rogers A, Ellsworth DS (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant Cell Environ* **25**:851–8.

Sardans J, Penuelas J, Estiarte M (2007) Seasonal patterns of root-surface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought. *Biol Fert Soils* **43**: 779–86.

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytol* **139**:395–436.

Sefcik LT, Zak DR, Ellsworth DS (2007) Seedling survival in a northern temperate forest understory is increased by elevated atmospheric carbon dioxide and atmospheric nitrogen deposition. *Global Change Biol* **13**:132–46.

Sims DA, Seemann JR, Luo Y (1998) Elevated CO_2 concentration has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *J Exp Bot* **49**:583–91.

Spinnler D, Egli P, Körner C (2002) Four-year growth dynamics of beech-spruce model ecosystems under CO_2 enrichment on two different forest soils. *Trees* **16**:423–36.

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ* **22**:583–621.

Stocklin J, Schweizer K, Korner C (1998) Effects of elevated CO_2 and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. *Oecologia* **116**:50–6.

Townsend AR, Cleveland CC, Asner GP, *et al.* (2007) Controls over foliar N: p ratios in tropical rain forests. *Ecology* **88**:107–18.

Tissue DT, Griffin KL, Turnbull MH, *et al.* (2001) Canopy position and needle age affect photosynthetic response in field-grown *Pinus radiata* after five years of exposure to elevated carbon dioxide partial pressure. *Tree Physiol* **21**:915–23.

Tissue DT, Lewis JD (2010) Photosynthetic responses of cottonwood seedlings grown in glacial through future atmospheric [CO_2] vary with phosphorus supply. *Tree Physiol* **30**:1361–72.

Treseder KK, Vitousek PM (2001) Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* **82**:946–54.

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol* **157**:423–47.

Vu JCV, Allen LH Jr, Bowes G (1989) Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO_2 enrichment. *Environ Exp Bot* **29**:141–7.

Xu JG, Heeraman DA, Wang Y (1993) Fertilizer and temperature effects on urea hydrolysis in undisturbed soil. *Biol Fert Soils* **16**:63–5.

Yin X, Lantinga E, Schapendonk AHCM, *et al.* (2003) Some quantitative relationships between leaf area index and canopy nitrogen content and distribution. *Ann Bot* **91**:893–903.

Zheng XH, Fu CB, Xu XK, *et al.* (2002) The Asian nitrogen cycle case study. *Ambio* **31**:79–87.