

Photosynthesis acclimation, leaf nitrogen concentration, and growth of four tree species over 3 years in response to elevated carbon dioxide and nitrogen treatment in subtropical China

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Abstract

Purpose Up to date, most studies about the plant photosynthetic acclimation responses to elevated carbon dioxide (CO₂) concentration have been performed in temperate areas, which are often N limited under natural conditions and with low ambient N deposition. It is unclear whether photosynthetic downregulation is alleviated with increased N availability, for example, from increased N deposition due to fossil fuel combustion in the tropics and subtropics. Awareness of plant photosynthetic responses to elevated CO₂ concentration will contribute to the better understanding and prediction of future forest productivity under global change.

Materials and methods Four tree species, *Schima superba* Gardn. et Champ., *Ormosia pinnata* (Lour.) Merr, *Castanopsis hystrix* AC. DC., and *Acmena acuminatissima* (Blume) Merr. et Perry were exposed to a factorial combination of atmospheric CO₂ concentration (ambient and elevated CO₂ concentration at ca. 700 μmol CO₂ mol⁻¹) and N deposition (ambient and ambient + 100 kg N ha⁻¹ year⁻¹) in open-top chambers in southern China for 3 years since March 2005.

Light-saturated net photosynthetic rate, leaf N concentration, and tree growth of all species were measured.

Results and discussion The CO₂ treatments did not affect light-saturated net photosynthetic rate of all species grown with the high N treatment. However, *S. superba* grown with the low N treatment (ambient) had 23% and 47% greater net photosynthesis in the ambient CO₂ concentration than those in the elevated CO₂ concentration for December 2006 and November 2007 (20 and 31 months after the treatments were applied), respectively, and *A. acuminatissima* grown with the low N treatment had 173%, 26%, and 121% greater net photosynthesis in trees grown in the ambient CO₂ concentration than those in the elevated CO₂ concentration for July 2006 (16 months after the treatments), December 2006 (20 months), and November 2007 (31 months), respectively, whereas, photosynthetic acclimation was not found for *C. hystrix* and *O. pinnata*. With the photosynthetic acclimation of *S. superba* and *A. acuminatissima*, we also found that the elevated CO₂ concentration decreased significantly leaf N concentration in trees of *S. superba* and *A. acuminatissima* grown with the low N treatment, respectively.

Conclusions *C. hystrix* seems to be a good species for C fixation under global climate change, particularly with the rising CO₂ concentration. We demonstrated that the relative responses to elevated CO₂ concentration and N treatment differ among tree species and functional types in the tropical and subtropical areas.

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1 Introduction

Elevated atmospheric CO₂ concentration has increased short-term photosynthesis in numerous plant studies (Curtis 1996; Xu and Chen 2006). In contrast, a reduction in photosynthesis with increased CO₂ concentration, often termed downregulation or photosynthetic acclimation, also has been reported (Rey and Jarvis 1998; Hyvönen et al. 2007). Photosynthesis reduction appears to be brought about by end product inhibition, resulting from an imbalance in the supply and demand of carbohydrates (Jones et al. 1996; Cheng et al. 1998). Substantial production of carbohydrates induced by higher photosynthetic rates at elevated CO₂ partial pressure initiates a feedback mechanism that ultimately reduces photosynthetic capacity. The lacks of sufficient “sink capacity” for incoming photosynthate lead to a negative feedback effect and downward acclimation of photosynthetic capacity.

In the long term, photosynthetic rates and tree growth responses to elevated CO₂ concentration are dependent on environmental and genetic factors affecting the plant's ability to develop new sinks for C (Hyvönen et al. 2007; Cai et al. 2010). Environmental and genetic factors can affect photosynthetic capacity and thus the nature of acclimation to CO₂ concentration (Wolfe et al. 1998; Robredo et al. 2010). Among all the factors which influence photosynthetic capacity, genotypic variation and N availability are the most pronounced. Acclimation is more profound for C₃ plants compared to species with the C₄ or CAM photosynthetic pathways (Wolfe et al. 1998; Ainsworth and Long 2005). Within the C₃ species, genotypic variation in sink capacity for photosynthates is likely a very important factor in determining the magnitude and nature of acclimation responses to CO₂ concentration (Wolfe et al. 1998; Ainsworth and Long 2005). In a comparison of the growth responses of 156 species to elevated CO₂ concentration, it has been concluded that inherently fast-growing C₃ species exhibit stronger CO₂ responses than slow-growing species (Poorter 1993; Hovenden 2003). Nitrogen availability is important for leaf photosynthesis because leaf chlorophyll concentration, Rubisco (ribulose-1-5-biphosphate carboxylase), and plant growth all increase with the greater N availability (Kutik et al. 1995; Xu et al. 2002; Bondada and Syvertsen 2003; Huang et al. 2008). Downregulation of photosynthesis would occur with elevated CO₂ concentration exposure due to the N limitation effect (Sefcik et al. 2006, 2007; Hyvönen et al. 2007). Plants grown with high N availability would increase light-saturated photosynthetic rate, alleviating the downregulation responses for plants grown under elevated CO₂ concentration (Saxe et al. 1998; Medlyn et al. 1999; Reich et al. 2006; Sefcik et al. 2007).

In Asia, emission of reactive N increased from 14 Tg N year⁻¹ in 1961 to 68 Tg N year⁻¹ in 2000 and is expected to

reach 105 Tg N year⁻¹ in 2030 (Zheng et al. 2002). Currently, this leads to high atmospheric N deposition (NH₄⁺-N, NO₃⁻-N) in precipitation for some forests of southern China (30–73 kg N ha⁻¹ year⁻¹, Ren et al. 2000; Mo et al. 2006). The N deposition in Guangzhou City of southern China increased from 46 kg N ha⁻¹ year⁻¹ in 1988 to 73 kg N ha⁻¹ year⁻¹ in 1990 (Ren et al. 2000). High N deposition in this area leads to higher N availability in most forest ecosystems. Up to date, most studies about the downregulation responses to elevated CO₂ concentration have been performed in temperate areas, which are often N limited under natural conditions and with low ambient N deposition. It is unclear whether photosynthetic downregulation is alleviated with increased N availability, for example, from increased N deposition due to fossil fuel combustion in the tropics and subtropics. It is also unclear that with the increasing N deposition how plant photosynthesis and growth respond to elevated CO₂ concentration in the tropics and subtropics.

Numerous experiments were carried out to study the responses of trees species to the elevated CO₂ concentration and N addition, however most of these studies just focused on one or individual species. Relative responses to elevated CO₂ concentration differ among tree species and functional types within a forest ecosystem (Curtis and Wang 1998). In this study, we used open-top chambers to expose four tree species, growing with high or low N treatment, to either ambient (about 390 ppm) or elevated (about 700 ppm) CO₂ concentration for 3 years. These four tree species are: *Castanopsis hystrix* Hook.f. & Thomson ex A. DC., *Schima superba* Gardn. and Champ., *Acmena acuminatissima* (Blume) Merr. et Perry, and *Ormosia pinnata* (Lour.) Merr. All these native species are distributed widely in subtropical areas. *C. hystrix* and *S. superba* are pioneer tree species and grow very fast. *O. pinnata* is an N-fixing species. *A. acuminatissima* is a native understory species, growing relatively slowly. Light-saturated photosynthetic rate, leaf N concentration, and tree growth were measured periodically for about 3 years in our study. Variations in the above parameters among the four tree species were examined. We hypothesized: (1) long-term growth in elevated CO₂ concentration would lead to the downward photosynthetic acclimation in mature leaves and (2) the magnitude of downward acclimation would be different among the different species grown in the elevated CO₂ concentration.

2 Materials and methods

2.1 Open-top chamber design

The experiment was carried out in 10 open-top chambers. Each cylindrical chamber had a diameter of 3 m, 3 m high above-ground section (was adjusted to 4.5 m later), and

0.7 m below-ground section. The above-ground section was wrapped with impermeable and transparent plastic sheets, leaving the top totally open. Sunlight intensity in the chamber was 97% of that in open space with no spectral change detected. Rainfall intensity and air temperature were also identical inside and outside the chambers. In the treatments with elevated CO₂ concentration, CO₂ was distributed in each chamber by a transparent pipe with pinholes. A fan was connected to the pipe to ensure equal distribution of CO₂ in the entire chamber. The CO₂ flux from the tank was controlled by a flowmeter, and the CO₂ concentrations in the chambers were periodically examined using a Licor-6400 (LI-COR Inc., Lincoln, NE, USA). The open chambers used in the experiment were located in an open space where they all were exposed to full light and rainfall. The distance between elevated CO₂ chambers and ambient CO₂ chambers is about 150 m. We considered the prevailing wind direction. The elevated atmospheric CO₂ concentration of the elevated CO₂ chambers will not affect the CO₂ concentration of the ambient CO₂ chambers.

2.2 Experimental design

The study was carried out 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 4,367.2–4,597.3 MJ m⁻² in the visible waveband and a mean annual temperature of 21.5°C. The annual precipitation ranges from 1,600 to 1,900 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.

In March 2005, we collected the soil from a nearby evergreen broadleaved forest. The soil was a lateritic soil and was collected as three different layers (0–20, 20–40, and 40–70 cm depth) that were homogenized separately and used to fill the below-ground section of the chambers. One to 2-year-old seedlings grown in a nursery were transplanted in the chambers with minimal damage to the roots. In order to determine responses of different tree species to elevated CO₂ concentration and N over a short time frame, seedlings were planted at a high density. Each chamber was planted with 48 randomly located seedlings, eight for each of six species: *C. hystrix*, *S. superba*, *A. acuminatissima*, *O. pinnata*, *Syzygium hancei* Merr. et Perry, and *Pinus massoniana* Lambert. These species were selected because they are native and the most widely spread tree species in southern China. As trees were growing fast, one tree per species was harvested at the end of each year to avoid excess crowd in each chambers. As most seedlings of *P. massoniana* died in the second year of our experiment, and the seedling leaves of *S. hancei* are too small which could not be measured for the photosynthesis using our instrument, we only studied the other four species in our experiment.

From April 2005, the chambers were exposed to different treatments. The three chambers received an elevated CO₂ concentration and high N treatment (NH₄NO₃ applied at 100 kg N ha⁻¹ year⁻¹), three chambers received the high CO₂ concentration treatment (with the low N treatment: ambient without any N fertilizer application), two chambers the high N treatment (but with ambient CO₂ concentration), and finally two chambers were used as a control (with the ambient CO₂ concentration and the low N treatment) and did not receive the high CO₂ or high N treatment. The high CO₂ concentration treatments were achieved by supplying additional CO₂ from a tank until a concentration of ca. 700 ppm CO₂ was reached in the chambers. The high N addition treatments were achieved by spraying seedlings once a week for a total amount of NH₄NO₃ at 100 kg N ha⁻¹ year⁻¹. 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. More detailed information about the experimental design has been reported previously (Liu et al. 2008, 2010).

2.3 Photosynthesis measurements

We examined the influence of CO₂ exposure, N treatment, measurement month, and their interactions on leaf light-saturated net photosynthesis for the four tree species. All measurements were made between 0900 and 1200 hours and 1400 and 1600 hours on sunny days in July 2005, October 2005, July 2006, December 2006, and November 2007 (3, 6, 16, 21, and 31 months after the treatments were applied), respectively. At least 2 days were chosen during each of the above measurement months. Four fully expanded leaves (the current year leaves) per tree in the middle canopy were randomly selected for measurements. Light-saturated rate of photosynthesis was measured at ambient CO₂ concentration and photosynthetic active radiation of 1,200 μmol m⁻² s⁻¹ using a portable infrared gas exchange system (Licor 6400, Lincoln, NE, USA) in each chamber. Preliminary measurements showed that this light intensity was above the light-saturation point for all leaves. At each measurement, temperature and air humidity were controlled according to the environmental conditions.

2.4 Measurements of plant growth and leaf N concentration

Plant growth was measured as the increment in plant dimensions over the 3-year study period to determine how CO₂ and N treatments and their interactions affected growth for each tree species. Only the growth of the trees alive until the end of the experiment was considered. Tree height and basal diameter were measured at the time of planting in

early March 2005. Tree height and basal diameter were assessed for five times later: in August 2005, November 2005, May 2006, September 2007, and January 2008 (4, 7, 13, 29, and 33 months after the treatments were applied), respectively. Tree height was measured from the soil–stem surface to the tip of the apical bud, and the diameter was assessed at the soil surface.

Leaf N concentration was measured in January 2006, January 2007, and July 2007 (9, 21, and 27 months after the treatments were applied), respectively. In January 2006 and January 2007, we chose one tree per species in each chamber and harvested all the leaves on this tree and pooled for the N measurements. In July 2007, depending on the leaf sizes, about 8 to 20 mature leaves were collected per species in each chamber. Leaf samples were dried for 3 days at 70°C in an oven and then ground prior to N analysis. Foliar N concentrations were determined using the Kjeldahl method as described previously (Xu et al. 1993).

2.5 Data analysis

Data analyses were carried out using the SAS software (SAS Institute Inc., Cary, NC, USA). We chose α equal to 0.05. Variables normality and residual homocedasticity were checked. Analyzed data consisted of light-saturated rate of photosynthesis measured at common CO₂ concentration, leaf N concentration, and tree basal diameter and height. Data were analyzed using the following mixed linear model:

$$\text{Dependent variables} = C + N + \text{species} + \text{time} + C \times N + C \times \text{species} + C \times \text{time} + N \times \text{species} + N \times \text{time} + \text{species} \times \text{time} + \text{species} \times C \times \text{time} + \text{species} \times N \times \text{time} + C \times N \times \text{species} + \text{time} \times C \times N.$$

Where C was the effect of the CO₂ concentration treatments (ambient or ca.700 ppm), N the effect of the N treatments (ambient or high N addition), time the effect of the measurement months, and the interactions between the factors. Repeated measurements for a chamber were stated to follow an unstructured covariance structure. The interaction of species \times $C \times N \times$ time never significantly affected any parameters, and it was subsequently removed from the model and was not displayed in Section 3.

When the effects were significant, they were further analyzed using Tukey multiple comparison test (HSD). Additionally, simple correlations were performed on the whole set of data using the Pearson correlation coefficients.

3 Results

3.1 Leaf N concentration

Leaf N concentrations primarily were affected by the species, measurement month, and CO₂ and N treatments (Table 1). *O. pinnata* had the highest N concentration

compared to the other three species (Fig. 1). For the same species, leaf N concentrations change with month. Except for *O. pinnata*, the other three species' N concentrations were significantly affected by the CO₂ treatments. For *S. superba*, elevated CO₂ concentration decreased significantly leaf N concentration in trees grown with the low N treatment in January and July 2007 (21 and 27 months after the treatments were initially imposed, $p < 0.001$) and in trees grown with the high N treatment in July 2007 ($p < 0.001$). For *A. acuminatissima*, decreased N concentrations were found in the leaves of trees grown with the low N treatments and exposed to the elevated CO₂ concentration in January 2006 and July 2007 (9 and 27 months after the treatments, $p < 0.0001$ and $p < 0.001$, respectively). For *C. hystrix*, elevated CO₂ concentration only decreased significantly leaf N concentration in trees grown with the low N treatment in January 2007. The high N treatment only increased significantly leaf N concentration in the trees of *O. pinnata* and *C. hystrix* over the 3-year period.

3.2 Light-saturated rate of photosynthesis

In our experiment, light-saturated rate of photosynthesis measured at common CO₂ concentration was dependent on the tree species, CO₂ exposure, N treatment, measurement month, and their interactions (see Table 1). Among all the factors, the species and measurement month affected the photosynthesis the most (F values are 269.75 and 218.57, respectively). Elevated CO₂ concentration did not affect light-saturated net photosynthetic rate of *S. superba* grown under the high N treatment. However, when *S. superba* was grown under the low N treatment, there were 23% and 47% greater net photosynthesis in the ambient CO₂ concentration than those in the elevated CO₂ concentration in December 2006 and November 2007 (20 and 31 months after the treatments), respectively (Fig. 2), suggesting that photosynthetic acclimation happened. With *O. pinnata* grown under the high N treatment, elevated CO₂ concentration treatment increased net photosynthesis by 30% and 140% in December 2006 and November 2007, respectively. With *O. pinnata* grown under the low N treatment, CO₂ treatment significantly increased light-saturated net photosynthesis by 70%, 109%, and 142% in July 2005, October 2005, and December 2006, respectively. No photosynthetic acclimation was found for *O. pinnata* during the study period. For *C. hystrix*, only when they were grown in the low N treatment in October 2005, elevated CO₂ concentration increased significantly net photosynthesis ($p < 0.0001$). There was also no photosynthetic acclimation for this species. For *A. acuminatissima* grown in the high N treatment, there was 177% greater net photosynthesis in trees grown in the elevated CO₂ concentration than those in the ambient CO₂ concentration in November 2007. Where-

Table 1 Analysis of net light-saturated rate of photosynthesis (A_{sat}) measured at common CO_2 concentration, basal diameter, height, and leaf N concentration for four tree species (species) grown under ambient and elevated CO_2 concentration (C) and high and low N treatments (N) during a number of measurement months (time) over the 3-year study period

Source	A_{sat}		Basal diameter		Height		Leaf N concentration	
	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F
Species	269.75	<0.0001	228.48	<0.0001	215.77	<0.0001	662.57	<0.0001
C	12.20	0.0005	5.78	0.02	24.53	<0.0001	9.62	0.003
Species×C	7.27	<0.0001	9.43	<0.0001	9.65	<0.0001	7.55	0.004
N	14.45	0.0002	21.19	<0.0001	32.83	<0.0001	13.02	0.0005
Species×N	2.62	0.0495	12.75	<0.0001	7.34	<0.0001	2.25	ns
C×N	2.08	ns	0.21	ns	2.30	ns	5.21	0.02
Species×C×N	9.73	<0.0001	0.73	ns	1.82	ns	0.27	ns
Time	218.57	<0.0001	1,047.54	<0.0001	969.73	<0.0001	44.72	<0.0001
Species×time	30.02	<0.0001	24.42	<0.0001	28.35	<0.0001	9.83	<0.0001
C×time	10.08	<0.0001	2.28	0.03	1.66	ns	1.91	ns
Species×C×time	1.86	ns	1.36	ns	1.72	0.01	1.48	ns
N×time	5.51	0.001	2.74	0.008	3.95	0.0003	1.09	ns
Species×N×time	3.01	0.0065	1.83	0.005	0.79	ns	1.99	ns
C×N×time	5.32	0.0013	0.13	ns	0.47	ns	2.55	ns
R ²	0.71		0.84		0.83		0.95	

as, for *A. acuminatissima* grown in the low N treatment, there were 173%, 26%, and 121% greater net photosynthesis in trees grown in the ambient CO_2 concentration than

those in the elevated CO_2 concentration in July 2006, December 2006, and November 2007, respectively (see Fig. 2). Overall, the low N treatment led to photosynthetic

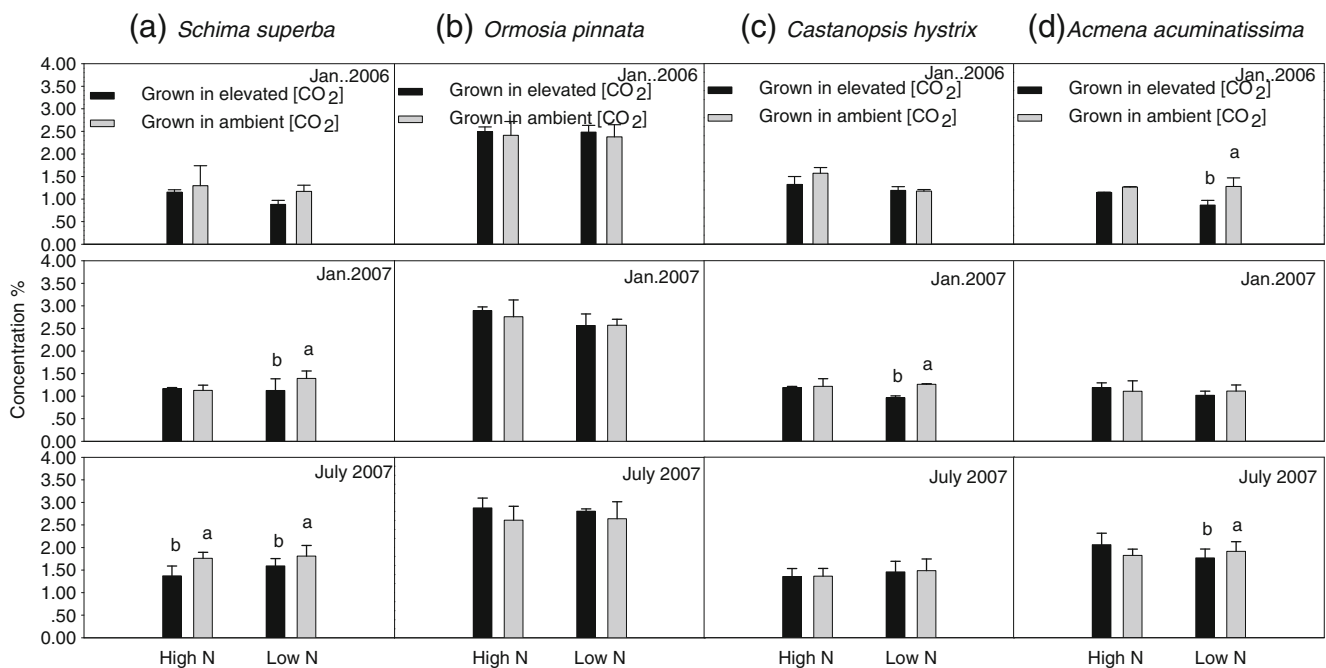


Fig. 1 Leaf N concentrations of four tree species: **a** *S. superba*, **b** *O. pinnata*, **c** *C. hystrix*, and **d** *A. acuminatissima* grown in ambient and elevated CO_2 concentration under high and low N treatments for three measurement months of January 2006, January 2007, and July 2007

(9, 21, and 27 months after the treatments were applied), respectively. Different letters indicate significant differences at the confidence level of $p < 0.05$ between elevated CO_2 concentration and ambient CO_2 concentration treatments but at the same N treatment

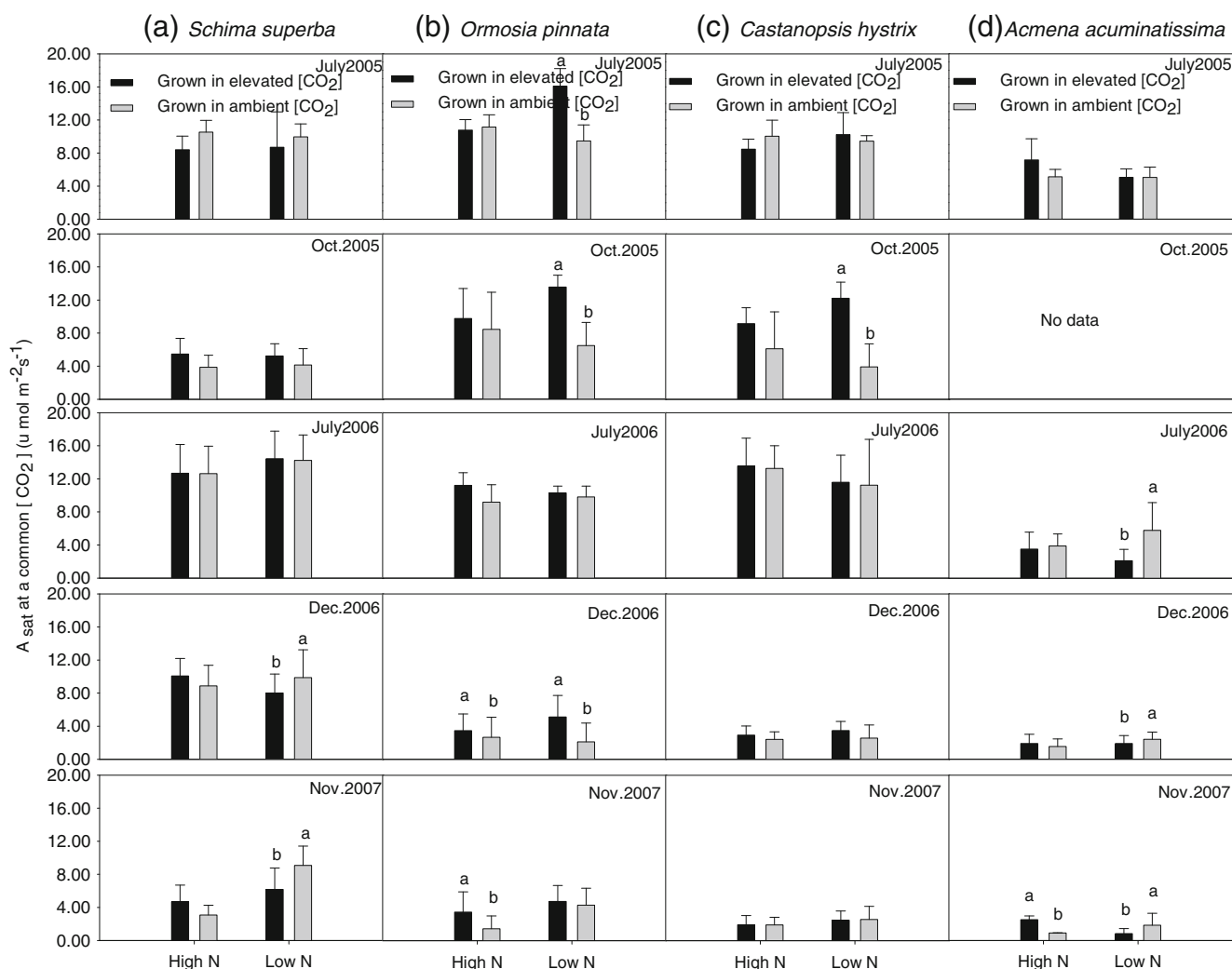


Fig. 2 Net light-saturated rate of photosynthesis (A_{sat}) measured at common CO₂ concentration of four tree species: **a** *S. superba*, **b** *O. pinnata*, **c** *C. hystrix*, and **d** *A. acuminatissima* grown in ambient and elevated CO₂ concentration under high and low N treatments for five measurement months of July 2005, October 2005, July 2006,

December 2006, and November 2007 (3, 6, 16, 21, and 31 months after the treatments were applied), respectively. Different letters indicate significant differences at the confidence level of $p < 0.05$ between elevated CO₂ concentration and ambient CO₂ concentration treatments but at the same N treatment

acclimation for species of *S. superba* and *A. acuminatissima*. Photosynthetic acclimation responded to be earlier for *A. acuminatissima* than for *S. superba*.

3.3 Plant growth

Tree growth shown as basal diameter and height in our experiment differed with the species (see Table 1, $p < 0.0001$) and generally increased with the elevated CO₂ and N treatments ($p < 0.0001$, see Table 1). There was a significant CO₂ concentration treatment by species interaction for both basal diameter and height growth ($p < 0.0001$, see Table 1). For *S. superba*, the high N treatment significantly increased the basal diameter and height growth (Figs. 3 and 4). The basal diameter and height growth of *O. pinnata* were not affected significantly by both the CO₂ and N treatments.

Elevated CO₂ concentration significantly increased the growth of *C. hystrix* ($p = 0.02$ and $p = 0.01$, respectively, Figs. 3 and 4), which translated into greater basal diameter and height for *C. hystrix* grown in the elevated CO₂ concentration over time (see Figs. 3 and 4). At the early growth stage, the high CO₂ concentration treatment increased the height growth of *A. acuminatissima* grown with the high N treatment, but this early greater height growth disappeared later. This was more obvious in the trees grown with the low N treatment (see Fig. 4).

4 Discussion

Many studies have shown that elevated CO₂ concentration can reduce foliar nutrient concentrations (Johnson et al.

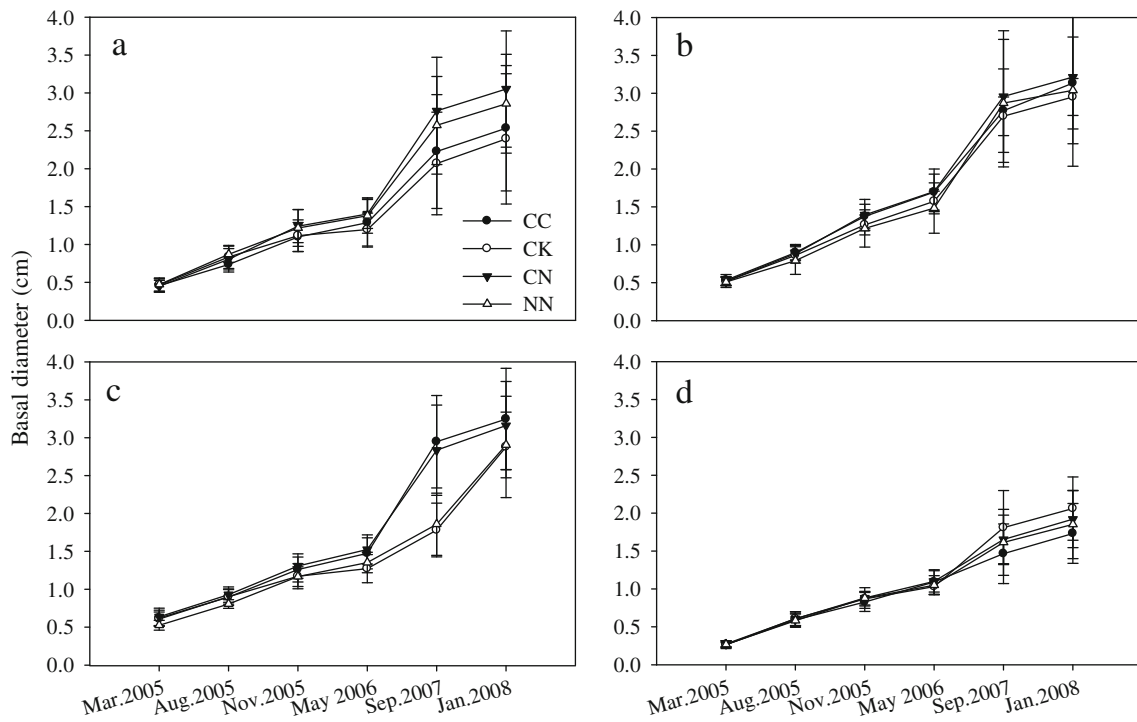


Fig. 3 Basal diameter growth of four tree species: **a** *S. superba*, **b** *O. pinnata*, **c** *C. hystrix*, and **d** *A. acuminatissima* grown in ambient and elevated CO₂ concentration under high and low N treatments for six

measurement months of March 2005, August 2005, November 2005, May 2006, September 2007, and January 2008, respectively. Treatments are: CK control, NN high N, CC high CO₂, CN high CO₂ + high N

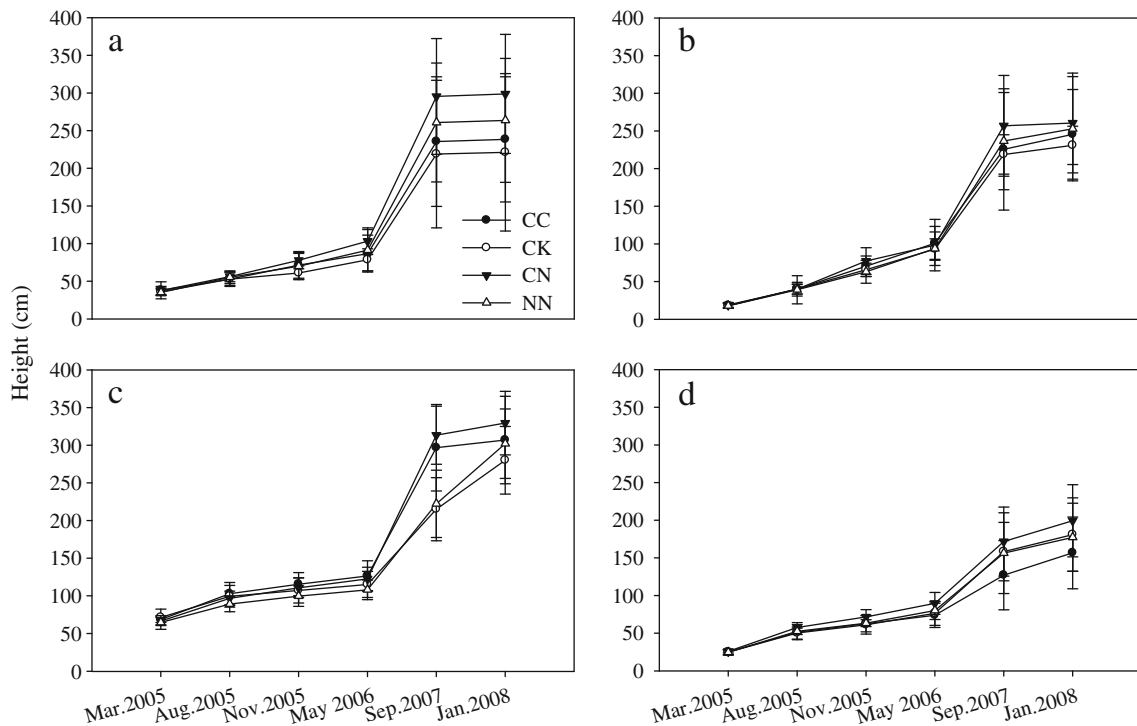


Fig. 4 Tree height growth of four species: **a** *S. superba*, **b** *O. pinnata*, **c** *C. hystrix*, and **d** *A. acuminatissima* grown in ambient and elevated CO₂ concentration under high and low N treatments for six measurement

months of March 2005, August 2005, November 2005, May 2006, September 2007, and January 2008, respectively. Treatments are: CK control, NN high N, CC high CO₂, CN high CO₂ + high N

2003, 2004; Finzi et al. 2002; Hyvönen et al. 2007). Above-ground tissue N concentrations consistently decreased with the CO₂ enrichment were reported (Blank and Derner 2004; Johnson et al. 2004). The same results were obtained for some species in our experiment, especially when the species were grown with the low N treatment. The highest N concentration was found in leaves of *O. pinnata* as it is an N₂-fixing species.

Downregulation of photosynthesis would occur with elevated CO₂ concentration exposure due to the N limitation effect (Sefcik et al. 2006, 2007). Effects of elevated CO₂ concentration on leaf C assimilation occurred largely through changes in leaf N level (Ellsworth et al. 2004; Hyvönen et al. 2007). In our experiment, downregulation of photosynthesis only happened in the leaves of *S. superba* and *A. acuminatissima* grown in the elevated CO₂ concentration with the low N treatment. Compared to the other treatments, these two species grown in the elevated CO₂ concentration with the low N treatment had lower leaf N concentrations, which supports the finding of the downregulation of photosynthesis in the subtropical environment. Sheu and Lin (1999) also reported that decreased photosynthetic rate with elevated CO₂ concentration was observed in the leaves of *S. superba* when measured in the ambient CO₂ concentration over a long-term exposure of 6 months. *O. pinnata* is an N fixation species. It is understandable that no photosynthetic acclimation was found for this species since greater N concentrations were shown in the leaves of this species. It has also been reported that the N-fixing ability of legumes generally enhances their responses to elevated CO₂ concentration (Hebeisen et al. 1997; Lüscher et al. 1998, 2000). In the Soy FACE experiment, non-nodulating soybeans showed downregulation of photosynthesis in elevated CO₂ concentration, while nodulating ones maintained the same photosynthetic capacity under both ambient and elevated CO₂ concentration (Ainsworth et al. 2004). Lüscher et al. (2000) also found that under elevated CO₂ concentration, effectively nodulating *Medicago sativa* increased harvestable biomass and N yield, while ineffectively nodulating plants were negatively affected by elevated CO₂ concentration. *C. hystrix* is not an N fixation species, however no photosynthetic acclimation was shown for this species over time, because *C. hystrix* is a fast-growing species which probably has the large sink. Hovenden (2003) reported in his experiment that downregulation of photosynthesis was only in the slow-growing poplar clones. Hence, *C. hystrix* is a very good species for C fixation under elevated CO₂ concentration.

The N treatment affected the growth of *S. superba* which was proved by the low N concentration found in the leaves of *S. superba* grown with the low N treatment. Relatively low photosynthesis rate in the leaves of *S. superba* grown with the low N treatment and elevated CO₂ concentration in

the later measurement period also supported the finding. During our measurement, *O. pinnata* growth was not affected by the CO₂ concentration treatments, which is contrary with most of previous studies where tree growth was positively affected by elevated CO₂ concentration (Xiao et al. 2005; Ceulemans et al. 1996; Sefcik et al. 2007). However, we hypothesized that the greater growth would soon be found for the *O. pinnata* grown with the elevated CO₂ concentration as downregulation of photosynthesis was not found for this species. The N treatments did not affect *O. pinnata* growth, which attributed that this species was an N fixation species. The elevated CO₂ concentration treatment increased significantly the growth of *C. hystrix*. Consistent high photosynthetic rate in the seedlings of *C. hystrix* grown with the elevated CO₂ concentration led to consistently greater basal area and height growth of *C. hystrix* exposed to the high CO₂ concentration. Elevated CO₂ concentration had no significant effect on the growth of *A. acuminatissima* as photosynthetic acclimation was found in the trees grown with the elevated CO₂ concentration during the earlier measurement month.

The responses of different plant species to elevated CO₂ concentration varied greatly (Ceulemans et al. 1996; Wullschleger 1997; Curtis and Wang 1998; Ainsworth and Long 2005; Luo et al. 2005). It is important to incorporate as many of these sources of variation as possible into estimates of the CO₂ concentration enhancement of photosynthesis because predictions of future forest productivity rely on these values. Our results showed that there were variations of leaf photosynthesis and tree growth for the four tree species in response to elevated CO₂ concentration. The magnitude of photosynthetic acclimation differed between C₃ functional groups in our experiment. Net light-saturated rate of photosynthesis tended to be reduced to a greater extent in the slow-growing species (*A. acuminatissima*) than in the fast-growing species (*C. hystrix*) or legume (*O. pinnata*). This result is consistent with the other reports (Hovenden 2003; Ainsworth and Long 2005).

Elevated CO₂ concentration increased the growth of *C. hystrix* significantly, while the high N treatment increased the growth of *S. superba*. Our first hypothesis was partly supported as the long-term growth in the elevated CO₂ concentration only led to the downward photosynthetic acclimation in two species. Our second hypothesis was largely supported as the magnitude of downward acclimation differed among the species grown in the elevated CO₂ concentration.

Few experiments about the downregulation responses to elevated CO₂ concentration have been performed in subtropical China with a high ambient N deposition. In the experiment, we found that photosynthetic downregula-

tion still happened in two species grown with the elevated CO₂ concentration under the high ambient N deposition for the 3-year study period. At the same time, the consistently high net photosynthesis was also obtained for one species grown with the elevated CO₂ concentration under the high ambient N deposition. As we only studied four tree species in our experiment and no other such experiments were carried out in subtropical China, more research should be done to improve the understanding of how different tree species would respond to climate change and N deposition in terms of biogeochemical cycles and forest ecosystem productivity in the subtropical and tropical environments, particularly over longer periods (>5 years).

5 Conclusions

Four tree species, *S. superba* Gardn. et Champ., *O. pinnata* (Lour.) Merr., *C. hystrix* AC. DC., and *A. acuminatissima* (Blume) Merr. et Perry were exposed to the elevated CO₂ concentration and ambient N deposition in open-top chambers for about 3 years; photosynthetic acclimation was found for *S. superba* and *A. acuminatissima*, which indicates that the long-term tree growth in the elevated CO₂ concentration would lead to the downward photosynthetic acclimation in mature leaves of these species in subtropical China, even with the high ambient N deposition. However, photosynthetic acclimation was not found for the fast-growing species (*C. hystrix*) and the N fixation species (*O. pinnata*). The photosynthetic acclimation of *S. superba* and *A. acuminatissima* was attributed to the relative low leaf N concentration for these two species. The fast growth of *C. hystrix* exposed to the elevated CO₂ concentration highlights that *C. hystrix* should be a good species for C fixation in response to the expected rising CO₂ concentration in the future.

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