

# Seedling growth response of two tropical tree species to nitrogen deposition in southern China

Jiangming Mo · Dejun Li · Per Gundersen

Received: 7 March 2007 / Revised: 15 December 2007 / Accepted: 21 January 2008  
© Springer-Verlag 2008

**Abstract** Seedling growth response of two tropical tree species (*Schima superba* and *Cryptocarya concinna*) to simulated N deposition was studied during a period of 11 months. One-year-old seedlings were grown in forest soil treated with N as  $\text{NH}_4\text{NO}_3$  at Control–no N addition, N5–5, N10–10, N15–15, and N30–30  $\text{g N m}^{-2} \text{ year}^{-1}$ . The objective was to examine the effects of N addition on seedling growth and compare this effect between the two tropical tree species of different species-N-requirement. Results showed that both species responded significantly to N addition and exhibited positive effect to lower rate of N addition and negative effect to higher rate of N addition on growth parameters (height and stem base diameter, biomass production, and net photosynthetic rate). The highest values were observed in the N10 plots for *S. superba* and in the N15 plots for *C. concinna*, but the lowest values were observed in the N30 plots for both species. However, the reduction in the N30 plots was more pronounced for *S. superba* than for *C. concinna* relative to the control plots. Our

findings suggest that response of seedling growth of tropical tree species to atmospheric N deposition may vary depending on rate of N deposition and species-N-requirement.

**Keywords** Nitrogen deposition · Species-N-requirement · Forest dynamic · Global change · Tropics

## Introduction

Elevated nitrogen (N) deposition and its ecological effects on forest ecosystems are of increasing concern worldwide (Matson et al. 1999, 2002; Galloway et al. 2003; Magill et al. 2004). High N input to coniferous stands in Europe has been linked to nutritional disorders in plants, reduced growth and reduced tolerance to frost, insects and pathogens (Nihlgård 1985; Boxman et al. 1998). Results from N addition experiments at the Harvard Forest showed that long-term N addition decreased net primary production and increased tree mortality (Magill et al. 2004). McNulty et al. (1996) concluded that if N additions continue, high elevation spruce-fir forests could eventually be converted into stands of birch, maple, or both. In a seedling experiment on two temperate conifer tree species, Nakaji et al. (2001, 2002) found that net photosynthetic rate and biomass production of *Cryptomeria japonica* seedlings were increased by N addition, while those of *Pinus densiflora* seedlings were significantly reduced by the highest N addition, and thus concluded that *P. densiflora*, characteristic from nutrient-poor habitats, is more sensitive to high N deposition than *C. japonica*.

However, most studies on the consequences of enhanced N deposition have been performed on temperate trees. To our knowledge, information regarding response of subtropical and tropical trees to increased N deposition is non-existent.

---

Communicated by A. Merino.

J. Mo (✉) · D. Li  
South China Botanical Garden,  
The Chinese Academy of Sciences, Dinghu,  
Zhaoqing, Guangdong 526070, China  
e-mail: mojm@scib.ac.cn

D. Li  
State Key Laboratory of Organic Geochemistry,  
Guangzhou Institute of Geochemistry,  
The Chinese Academy of Sciences,  
Guangzhou 510640, China

P. Gundersen  
Danish Centre for Forest, Landscape and Planning,  
University of Copenhagen, Hoersholm Kongevej 11,  
2970 Hoersholm, Denmark

With the increase in population and in per-capita energy and food consumption in the less-industrialized areas, many of which locate in tropics and subtropics, N deposition there is expected to increase dramatically. For example, over 40% of all N fertilizers are now used in the tropics and subtropics and over 60% will be used there by 2020 (Galloway et al. 2003). At the same time, fossil fuel use is expected to increase by several times in many areas of the tropics over the coming decades (Hall and Matson 1999). As a consequence, large areas there will be experiencing more than doubled N deposition fluxes in 2050 relative to those in 1990s (Galloway et al. 2004).

In Asia, the use and emission of reactive N increased from 14 Tg N year<sup>-1</sup> in 1961 to 68 Tg N year<sup>-1</sup> in 2000 and is expected to reach 105 Tg N year<sup>-1</sup> in 2030 (Zheng et al. 2002). Currently, this leads to high atmospheric N deposition (30–73 kg N ha<sup>-1</sup> year<sup>-1</sup>) in some forests of southern China (Huang et al. 1994; Ren et al. 2000; Xu et al. 2001; Zhou and Yan 2001). For example, the annual flux of N deposition from rainfall to the tropical forests of Dinghushan Biosphere Reserve increased from 36 to 38 kg N ha<sup>-1</sup> year<sup>-1</sup> during the last decade (Huang et al. 1994; Zhou and Yan 2001). In temperate regions of Europe and North America, forests receiving inorganic N deposition below 8–10 kg N ha<sup>-1</sup> year<sup>-1</sup> retain all N input. However, above this level of deposition, forests often leach nitrate and the ecosystem will move toward N saturation with significant biological and ecological response (Aber et al. 1998; MacDonald et al. 2002). At inputs above 25–30 kg N ha<sup>-1</sup> year<sup>-1</sup>, all the forests studied in Europe were N saturated (as defined in Aber et al. 1998; MacDonald et al. 2002).

With atmospheric N deposition in some Chinese forests currently exceeding the above-cited critical values, it is possible that excessive N inputs could saturate the retention capacity of these Chinese forest ecosystems. This interpretation is partially supported by the recent studies in the Dinghushan Biosphere Reserve, where a mature forest ecosystem with long-term high N deposition exhibited no significant positive and even some negative response to N additions in litter decomposition (Mo et al. 2006), and this negative response became stronger with experimental time (Fang et al. 2007), suggesting that the forest has been N saturated (Mo et al. 2006; Fang et al. 2007). In addition, other studies in Dinghushan Biosphere Reserve also indicated that the mature forests had been experiencing a decline in tree growth. For example, the biomass of woody plants declined by 15% from 1994 to 1999 (Zhang et al. 2002), and there has been a decreasing trend of litter production during the last two decades (Guan et al. 2004). These changes in productivity could be an effect of the high atmospheric N deposition in this region. However, little information is available about the response of Chinese forest trees to excess soil N, especially the tropical forests in

southern China, where industry and agriculture has recently been increasing rapidly (Mo et al. 2006).

*Schima superba* Chardn. & Champ. and *Cryptocarya concinna* Hance are the most representative broadleaf tree species in the tropical forests of southern China. In general, *S. superba* grows in relatively nutrient-poor soils, whereas *C. concinna* grows in relatively nutrient-rich soils (Wang et al. 1982; Kong et al. 1997). In view of an increasing concern about the effects of N deposition on tropical forest trees in southern China, we conducted an N addition experiment on seedlings of *S. superba* and *C. concinna*. The objective was to examine the effects of N addition on seedling growth and compare this effect between the two tropical tree species of different species-N-requirement. We hypothesize that: (1) seedling growth of these two tropical tree species responds positively to lower rate of N addition and negatively to higher rate of N addition; and (2) *S. superba*, characteristic from nutrient-poor habitats, is less sensitive to lower rate of N addition and more sensitive to higher rate of N addition than *C. concinna*.

## Materials and methods

### Site description

This study was conducted in the UNESCO/MAB Dinghushan Biosphere Reserve (DHSBR), which lies in the middle part of Guangdong Province in southern China (112°10'E, 23°10'N). The reserve has a monsoon climate and is located in a subtropical/tropical moist forest life zone (sensu Holdridge 1967). The mean annual rainfall of 1,927 mm has a distinct seasonal pattern, with 75% of it falling from March to August and only 6% from December to February (Huang and Fan 1982). Annual average relative humidity is 80%. Mean annual temperature is 21.0°C, with an average temperature of the coldest (January) and hottest (July) month of 12.6 and 28.0°C, respectively.

The 1,200 ha reserve is mainly covered by three forest types: pine forest (early stage), pine-broadleaf mixed forest (mid stage) and monsoon evergreen broadleaf forest (climax stage) in different successive stages (Mo et al. 2003). These three forest types vary in degree of human impact, stages of succession, site conditions and species assemblages as well (Mo et al. 2003). The mixed forest and the broadleaf forest cover more than half of the total forest area in subtropical and tropical China (Wang et al. 1982; Mo et al. 1995, 2003; Kong et al. 1997). For our experiment we chose two tree species, *S. superba* and *C. concinna*. The former is a dominant species in both mixed forest and broadleaf forest, while the latter occurs only in the broadleaf forest. In addition, *Castanopsis Chinensis*–*Schima Superba*–*Cryptocarya Concinna* community is typical in

the broadleaf forest type in the study region (Wang et al. 1982; Kong et al. 1997; Mo et al. 2003).

Our research site was established at a small flat open land of pine-broadleaf mixed forest, which is at the bottom of a small hill. Nitrogen deposition in precipitation was  $36 \text{ kg N ha}^{-1} \text{ year}^{-1}$  in 1990 and  $38 \text{ kg N ha}^{-1} \text{ year}^{-1}$  in 1999 (Huang et al. 1994; Zhou and Yan 2001). The soil is lateritic red earth formed from sandstone (oxisols) with a depth of about 30 cm and relatively well drained. Soil properties in the top 10 cm were measured by using the samples collected in January 2003 before the first application of N treatment. Soil pH, total C, total N, C/N ratio and bulk density were  $5.03 \pm 0.03$ ,  $13 \pm 1 \text{ mg g}^{-1}$ ,  $1.0 \pm 0.1 \text{ mg g}^{-1}$ ,  $13 \pm 1$  and  $1.26 \pm 0.05 \text{ g cm}^{-3}$ , respectively ( $n = 3$ , mean  $\pm$  SE).

### Experimental design

The experiment was arranged with a completely randomized design with five N treatments. There were three replicates for each treatment (see below). Soil (0–30 cm in depth) in the research site was mixed to obtain a uniform mixture before the start of experiment in October 2002 and all weeds were removed. This research site was covered by weeds before homogenizing of the soil and removal of weeds. Fifteen plots of dimensions  $4 \text{ m} \times 5 \text{ m}$  were established and each plot was surrounded by a 1-m wide buffer strip. On October 25, 2002, 1-year-old seedlings of two tree species were obtained from an experimental field of South China Agricultural University and were transplanted by hand. Forty seedlings of each species were separately planted in each plot with a spacing of  $0.5 \times 0.5 \text{ m}$ . Throughout the experiment weeds were removed regularly by hand. Five N addition treatments (in three replicates) were established: Control (without N added), N5 ( $5 \text{ g N m}^{-2} \text{ year}^{-1}$ ), N10 ( $10 \text{ g N m}^{-2} \text{ year}^{-1}$ ), N15 ( $15 \text{ g N m}^{-2} \text{ year}^{-1}$ ) and N30 ( $30 \text{ g N m}^{-2} \text{ year}^{-1}$ ). All plots and treatments were laid out randomly. Nitrogen fertilizer as  $\text{NH}_4\text{NO}_3$  was applied twice a month (middle and end of each month) in equal amounts every time from January 15, 2003. The fertilizers were dissolved in 10 L of water and sprayed on the plots using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of fertilizer. The Control plots were only sprayed with 10 L water without N added.

### Soil pH values, and soil available N and P

Mineral soils of the upper 10 cm were sampled on November 10, 2003. In each plot, three soil cores (2.5 cm in diameter) were collected randomly, and combined to one composite sample in the field. In the laboratory, soils were sieved (2 mm) and mixed thoroughly by hand. One 10-g

sub-sample from each composite sample was shaken for 1 h in 50 ml 2 mol/L KCl, and filtered through pre-leached Whatman no.1 filters; the filtrate was frozen immediately for later analysis. Another 10-g sub-sample was dried at  $105^\circ\text{C}$  to constant weight (at least 24 h) to determine gravimetric moisture content, and all samples are reported on  $105^\circ\text{C}$  basis. Extractable  $\text{NH}_4^+\text{-N}$  was determined colorimetrically by the indophenol blue method and  $\text{NO}_3^-\text{N}$  was analyzed by copper–cadmium reduction method (Liu 1996).

Mineral soils collected in November 2003 were also analyzed for soil pH and available P. Soil pH was measured in distilled water suspension using glass electrode, after shaking for 1 h at a ratio of 25 ml water to 10 g mineral soil. Available P was analyzed colorimetrically after extraction by a combination of ammonium chloride and hydrofluoric acid (Liu 1996).

### Measurements of plant growth, net photosynthetic rate and plant tissue nutrient status

Height and stem base diameters of 10 randomly selected seedlings from each plot and each species were measured on January 10, April 10, July 10 and November 10, 2003. In November 2003, all seedlings in the whole research site were investigated to determine the survival rate of seedlings.

Net photosynthetic rate ( $P_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured with a CI-301 portable photosynthesis system (CID, Inc., USA) on November 15, 2003. Every 2 h, from 7:00 to 17:00, five to six leaves (from 1 or 2 seedlings per plot) per species from each treatment were chosen for measurement. On November 25, 2003, three randomly selected seedlings from each plot and each species were harvested to determine the dry mass and element concentrations of the plant organs. Each seedling was cut at the root collar, and the root was extracted from the soil and hand washed (Wang et al. 1998). Harvested seedlings were separated into leaves, shoot (stem + branches) and roots. All the plant organs were dried at  $60^\circ\text{C}$  for 1 week and weighted to determine weight of whole plant and each organ (Wang et al. 1998).

All plant materials were ground to pass a 0.15-mm-mesh sieve. Sub-samples were dried at  $105^\circ\text{C}$  to constant weight to determine gravimetric moisture content, and all samples are reported on  $105^\circ\text{C}$  basis. Nitrogen concentrations in plant tissues were determined with semimicro-Kjeldahl digestion (Bremner and Mulvaney 1982) followed by detection of ammonium with a Wescan ammonia analyzer. Nutrient analyses for other elements in plant tissues (P, K, Ca, and Mn) used the methods given in Anderson and Ingram (1989): samples were first digested with concentrated sulfuric acid and hydrogen peroxide (with appropriate catalysts), and total phosphorus was determined by the

colorimetric method and the available cations by the atomic absorption method.

### Statistical analyses

One-way analysis of variance (ANOVA) was used to test the effects of N treatment on *S. superba* and *C. concinna* seedlings. For seedling height and basal diameter, with measurement carried out continuously throughout the study period, a repeated-measure ANOVA was also used to test difference among treatments. For seedling survival rate, angular transformation of percentage data was carried out prior to statistical analysis to meet the assumptions of variance homogeneity. A multiple comparison with Duncan's test was made after ANOVA for the differences in seedling growth parameters (height and stem base diameter, biomass production, and net photosynthetic rate), and in survival rate and nutrients. Relationship between soil pH values and soil  $\text{NO}_3\text{-N}$  concentration was examined with linear regression analysis. Vector analysis was also used to compare dry matter-weight, concentration and content of P and K in seedling leaves for *S. superba* and *C. concinna* (Haase and Rose 1995). All analyses were conducted using SPSS 10.0 for windows. Statistical significant differences were set with  $p$  values  $< 0.05$  unless otherwise stated.

Leaf weight ratio (LWR) means the ratio of the leaf dry weight to the whole dry weight of a seedling, and the same for shoot weight ratio (SWR) and root weight ratio (RWR). Root-aboveground part ratio (RAR) was calculated by dividing the aboveground part dry weight by the root dry weight of a seedling.

## Results

### Soil pH values, and soil available N and P

After 11 months of N treatment, soil extractable  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations increased with increasing level of N addition and these increases were more pronounced in  $\text{NO}_3\text{-N}$  than in  $\text{NH}_4\text{-N}$  (Table 1). However, N addition did not influence soil available P. Soil pH values decreased

with increasing level of N addition and exhibited negative linear relationship with soil  $\text{NO}_3\text{-N}$  concentration ( $p = 0.033$ ) (Table 1).

### Growth and biomass allocation

The response of biomass production (total dry matter) to N addition generally showed a positive effect of lower rate of N addition and no significant effect of higher rate of N addition for both species (Table 2). The highest values were observed in the N10 plots for *S. superba* and in the N15 plots for *C. concinna*. This increase was less pronounced for *S. superba* (increased by 68% in N10 plots relative to the control) than for *C. concinna* (increased by 110% in N15 plots relative to the control). The lowest total biomass was found in the N30 for both species; however, the reduction was higher for *S. superba* (decreased by 40% relative to the control) than for *C. concinna* (decreased by 15% relative to the control). Similar trend was also found for the response of root biomass production to N addition (Table 2).

Nitrogen addition significantly decreased RAR relative to the control for both species (Table 2). This decrease was higher for *S. superba* (decreased by 27% relative to the control on average) than for *C. concinna* (decreased by 23% relative to the control on average). Nitrogen addition had negative effect on SWR while positive effect on RWR. There was no significant N effect on LWR in all treatments for both species except for *S. superba* in N30 plots (Table 2).

Values of both seedling height and stem base diameter for both species generally followed the similar pattern than biomass production (Fig. 1). The pattern above became more pronounced with time, indicating the cumulative effect of N addition. Repeated-measure ANOVA showed significant statistical significant difference in both seedling height and stem base diameter for both species over the entire study period ( $p < 0.05$ , Fig. 2).

### Net photosynthetic rate and survival rate of seedlings

Similar to the response of biomass production, net photosynthetic rate (Pn) was increased by lower rate of N addition and decreased by higher rate of N addition for both

**Table 1** Response of mineral soil properties (0–10 cm depth) to N deposition (Means with S.E. in parentheses,  $n = 3$  for all samples; available N and P in  $\text{mg kg}^{-1}$ )

	Control	N5	N10	N15	N30
$\text{NH}_4^+\text{-N}$	1.6(0.2)c	2.4(0.1)c	2.5(0.2)c	3.5(0.4)b	4.8(0.4)a
$\text{NO}_3^-\text{N}$	4.1(0.9)c	8.2(1.9)bc	15.7(1.2)b	17.2(4.0)ab	19.1(3.0)a
Available P	14.2(1.6)	15.9(2.2)	15.8(5.2)	14.6(2.4)	15.8(1.3)
pH	5.16(0.03)a	4.88(0.14)ab	4.88(0.09)ab	4.75(0.14)b	4.66(0.15)b

Note Different letter within a single row indicate significant difference ( $p < 0.05$ )

\* Data were measured after 11 months of nitrogen addition

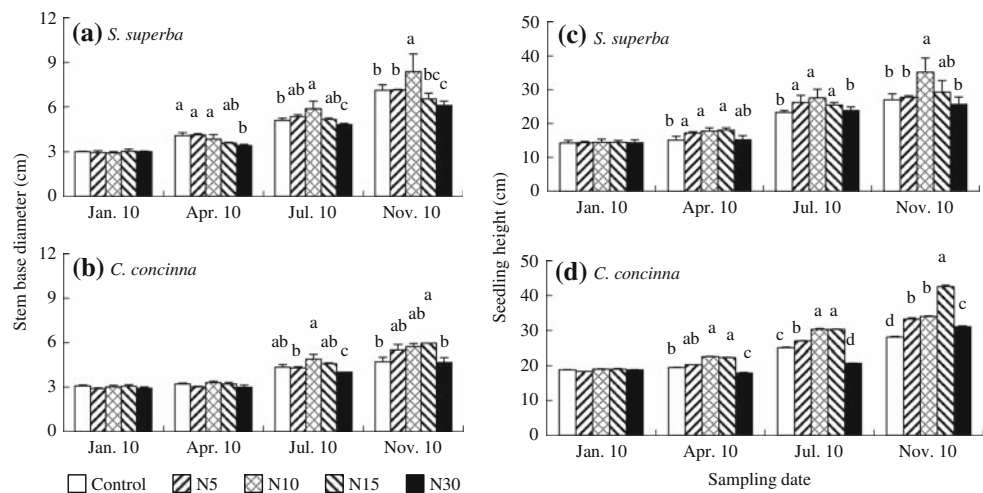
**Table 2** Biomass and its allocation among leaves, shoots and roots after 11 months of N addition (Means with S.E. in parentheses,  $n = 3$  for all samples)

Plots	Total (g)	Root-weight (g)	Leaf-weight ratio	Shoot-weight ratio	Root-weight ratio	RAG* part ratio
<i>S. superba</i>						
Control	7.07(0.31)b	2.38(0.28)b	0.30(0.02)a	0.37(0.01)b	0.34(0.02)a	0.53(0.06)a
N5	5.01(0.60)b	1.38(0.22)b	0.24(0.04)ab	0.49(0.05)ab	0.27(0.01)b	0.40(0.02)b
N10	11.85(1.39)a	3.47(0.30)a	0.29(0.03)a	0.41(0.01)ab	0.30(0.02)ab	0.42(0.04)ab
N15	6.66(1.24)b	1.55(0.31)b	0.31(0.02)a	0.46(0.03)ab	0.23(0.01)b	0.31(0.02)b
N30	4.25(0.27)b	1.18(0.03)b	0.21(0.01)b	0.51(0.02)a	0.28(0.02)ab	0.41(0.04)ab
<i>C. concinna</i>						
Control	4.59(0.73)b	1.35(0.09)ab	0.31(0.04)	0.39(0.02)b	0.30(0.03)a	0.44(0.05)a
N5	6.51(1.22)ab	1.76(0.20)ab	0.36(0.03)	0.36(0.02)b	0.28(0.02)ab	0.38(0.05)a
N10	6.30(1.05)ab	1.50(0.24)ab	0.34(0.01)	0.42(0.01)ab	0.24(0.01)bc	0.32(0.02)b
N15	9.62(2.04)a	2.07(0.54)a	0.36(0.01)	0.43(0.02)ab	0.21(0.02)c	0.29(0.02)b
N30	3.92(1.09)b	1.00(0.22)b	0.30(0.01)	0.44(0.01)a	0.26(0.02)abc	0.36(0.02)ab

Note: Means followed by different letter among treatments differed significantly ( $p < 0.05$ )

\* RAG, Root-aboveground

**Fig. 1** Effects of nitrogen addition on seedling stem base diameter (a, b) and height (c, d) of two tropical tree species in southern China. Different letter above the columns indicates significant difference among treatments ( $p < 0.05$ ).  $\text{NH}_4\text{NO}_3$  solution was sprayed twice a month and began on 15 January 2003



species (Fig. 3a), with the highest values observed in the N10 plots for *S. superba* (increased by 40% relative to the control) and in the N15 plots for *C. concinna* (increased by 52% relative to the control). The lowest Pn was also found in N30 for both species, with the relative reduction of 28 and 9% for *S. superba* and for *C. concinna*, respectively (Fig. 3a).

No significant difference was found on survival rate of seedlings for both species between control and all levels of N treatments except for N30 plots, in which the reduction in seedling survival rate was higher for *S. superba* (reduced by 45% relative to the control) than for *C. concinna* (reduced by 22% relative to the control) (Fig. 3b).

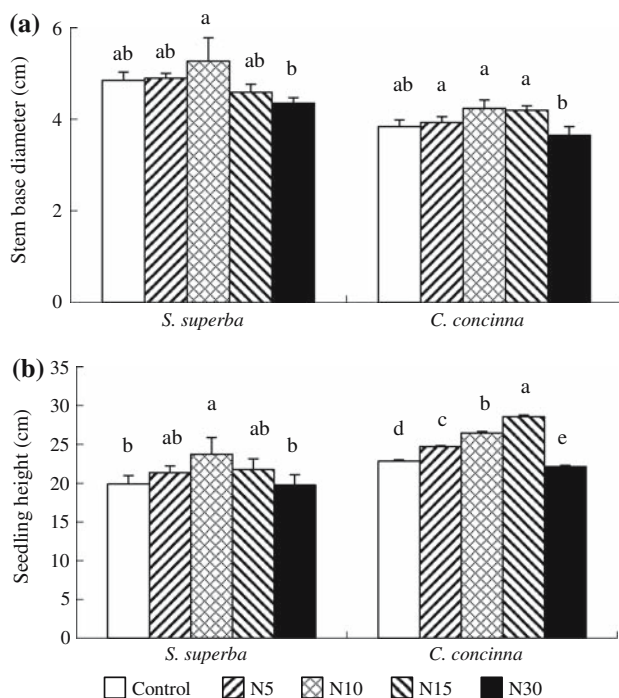
### Nutrition

Leaf N concentration generally increased with N addition with some variations between species (Table 3). In

comparison with those in control plots, leaf N concentration for *C. concinna* significantly increased in all N treatments except for N5, whereas for *S. superba*, such significant increase was observed in treatment N30 only (Table 3). The concentration of P and K were reduced by N addition, resulted in significant increase in ratios of N/P and N/K for both species. However, there was no significant effect of N addition on concentrations of the remaining elements (Table 3).

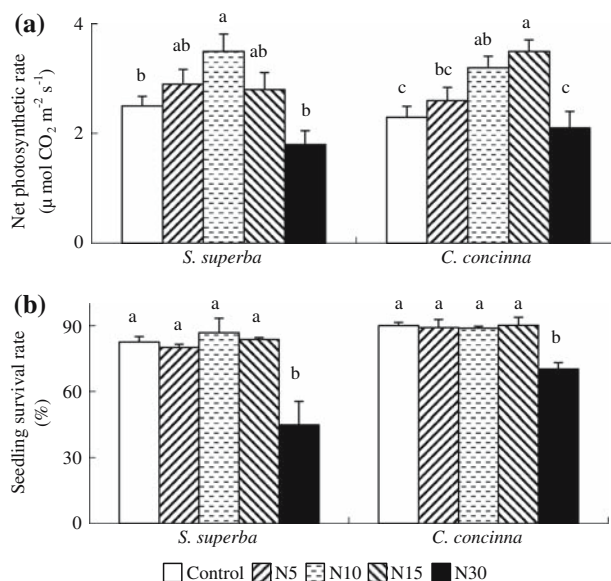
### Discussion

Biomass production, height, stem base diameter, and net photosynthetic rate showed a positive response to the lower rates of N addition but responded negatively to the higher rates of N addition with the most negative response seen at the N30 rate. The positive response mentioned above suggests that N is likely a limiting factor for seedling growth in



**Fig. 2** Overall effect of nitrogen addition on seedling stem base diameter (a) and height (b) of two tropical tree species in southern China. Each column represents the mean value across all sampling dates in a given treatment within the same species. Mean values sharing the same letter are not significantly different among treatments within the same species ( $p < 0.05$ , repeated-measure ANOVA)

the study soil, and is similar to the results found in a seedling experiment in temperate region (Brown et al. 1996). Our findings are consistent with the results found for a temperate conifer tree species (*C. japonica*) in a seedling experiment, in which the biomass production and net photosynthetic rate for seedlings increased with increasing rate of N addition from control to N5.7 ( $5.7 \text{ g N m}^{-2} \text{ year}^{-1}$ )



**Fig. 3** Effects of nitrogen addition on net photosynthetic rate (a) and survival rates (b) of seedling of two tropical tree species in southern China. Different letter above the columns indicates significant difference among treatments ( $p < 0.05$ ). Data were measured after 11 months of nitrogen addition

and then decreased with increasing level of N addition from N5.7 to N34 ( $34 \text{ g N m}^{-2} \text{ year}^{-1}$ ) (Nakaji et al. 2001, 2002). Similar results were also reported for other temperate tree species by several authors (McNulty et al. 1996; Boxman et al. 1998; Sikström et al. 1998). Thus, in effect of N deposition on seedling growth threshold may also be existent for tropical tree species.

The occurrence of threshold may be related to the soil N availability. It has been reported that when the amount of available N in forest soil does not exceed the requirement

**Table 3** Nutrient concentrations and their ratios in seedling leaves after 11 months of N addition (Means with S.E. in parentheses,  $n = 3$  for all samples)

	N ( $\text{mg g}^{-1}$ )	P ( $\text{mg g}^{-1}$ )	K ( $\text{mg g}^{-1}$ )	Ca ( $\text{mg g}^{-1}$ )	Mn ( $\text{mg g}^{-1}$ )	N/P ratio	N/K ratio
<i>S. superba</i>							
Control	8.8(0.7)b	1.0(0.1)a	3.8(0.2)a	7.2(0.4)	0.5(0.1)	8.4(0.3)b	2.3(0.2)c
N5	10.2(1.8)ab	0.7(0.2)b	3.4(0.3)ab	7.0(0.1)	0.4(0.1)	14.5(3.6)a	3.0(0.5)bc
N10	9.8(1.9)ab	0.8(0.1)ab	2.9(0.1)bc	6.1(1.0)	0.3(0.1)	12.0(2.0)a	3.5(0.8)bc
N15	11.2(1.6)ab	0.9(0.1)ab	2.5(0.1)c	7.3(1.0)	0.4(0.0)	12.4(1.2)a	4.5(0.6)ab
N30	13.9(1.4)a	0.9(0.1)ab	2.3(0.3)c	7.1(0.6)	0.5(0.1)	15.4(1.3)a	6.0(0.3)a
<i>C. concinna</i>							
Control	11.3(1.6)b	2.9(0.9)a	4.7(0.6)a	7.5(0.5)	0.3(0.1)	3.9(0.8)c	2.4(0.2)b
N5	9.7(0.5)b	2.0(0.4)ab	3.7(0.6)ab	7.7(0.7)	0.2(0.0)	5.4(1.4)c	2.8(0.4)b
N10	15.2(1.0)a	1.7(0.1)ab	3.1(0.6)ab	6.2(0.6)	0.2(0.0)	8.8(0.2)b	5.3(1.1)a
N15	15.3(0.9)a	1.1(0.1)b	2.7(0.3)b	5.9(0.4)	0.2(0.0)	13.9(2.0)a	5.7(0.4)a
N30	15.5(0.8)a	1.4(0.1)b	2.9(0.6)b	6.2(0.6)	0.2(0.0)	11.1(1.3)ab	4.0(0.4)ab

Note: Means followed by different letter among treatments differed significantly ( $p < 0.05$ )

for tree growth, an increase in the atmospheric N input usually stimulates tree growth; however, the current input of atmospheric N to forests is in excess of plant requirements and is adversely affecting tree growth (Nihlgård 1985; Aber et al. 1989, 1998). In the present study, under high rate of N addition soil might have been in excess of N. Nitrogen in excess of plant demand may increase leaching of nutrients and soil acidification (Emmett et al. 1998) and cause nutritional imbalances (Boxman et al. 1998). Thus, the negative effect of higher N addition on seedling growth in our study may be related to the following: First, the low soil pH value. Due to the large amount of N addition, both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the higher N treatment plots would likely exceed the demand of plants and microbes. It is well known that  $\text{NO}_3^-$  leaching out of the system would leave the protons in the soil, and the greater affinity of protons for cation exchange sites in the soil will displace a base cation, which will balance the charge of the leaching  $\text{NO}_3^-$ . The amount of  $\text{NO}_3^-$  leaching was significantly correlated with decreasing soil pH value (Macdonald et al. 2002; Chen et al. 2004). In the present study, soil  $\text{NO}_3^-$  concentration increased with increasing level of N addition and exhibited negative linear relationship with soil pH values ( $p = 0.033$ ) (Table 1). If the  $\text{NH}_4^+$  ion is taken up, one proton is released. If the added  $\text{NH}_4^+$  ion is nitrified, two protons are released, and leaching of resulting  $\text{NO}_3^-$  also gives rise to the increase of soil protons and base cation loss. So both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  addition would lead to soil acidification and base cation loss (Matson et al. 1999). The low pH values in the higher N-treated plots would be a cause leading to the negative effect of N addition on seedling growth, as it was reported that significant reductions in dry matter production of seedlings can be induced by low soil pH value (Nakaji et al. 2001, 2002).

Second, a change in root biomass or nutrient imbalance. Increases in soil N availability can influence the availability and uptake of other nutrients for plant growth by reducing the root biomass (Schulze 1989), diluting other nutrient concentrations in the tree tissues (Aber et al. 1995; Ericsson 1995; Brække 1996), and lower availability of soil base cation as discussed above. These are supported by our findings. The lowest root dry matter (biomass) was found in N30 treatment plots for both species (Table 2). Nitrogen addition decreased RAR for both species (Table 2). These results suggest that seedlings in the N treatments invested relatively less biomass to roots when compared with the control plots. The same trends were also found by several authors (Aerts et al. 1991; Bertson et al. 1995; Wang et al. 1998). Nitrogen addition increased leaf N concentrations but decreased the concentrations of P and K (Table 3). This was confirmed by the results of vector analysis (figure not showed). As a result, ratios of N/P and N/K in N treated plots, especially higher N treated plots were significantly

increased for both species ( $p < 0.05$ ). For example, foliar N/P ratio was 80% higher in N30 plots than in the control plots (Table 3). Changes in root biomass and foliar chemistry were significantly associated with the reduction in seedling/tree growth (Aber et al. 1995; McNulty et al. 1996; Schaberg et al. 1997; Nakaji et al. 2001, 2002; Krupa 2003). The threshold effect of N deposition has also been observed in the study on soil fauna in the same research site (Xu et al. 2007).

An interesting finding was that the highest values for the seedling growth parameters (biomass production, height and stem base diameter, and net photosynthetic rate) were observed in N10 ( $10 \text{ g N m}^{-2} \text{ year}^{-1}$ ) plots for *S. superba* and in N15 ( $15 \text{ g N m}^{-2} \text{ year}^{-1}$ ) plots for *C. concinna*. This positive effect of lower rate of N addition was less pronounced for *S. superba* than for *C. concinna*, suggesting that *S. superba* is less sensitive to lower rate of N deposition than *C. concinna*. For the negative effect of higher rate of N addition, however, *S. superba* was more pronounced than *C. concinna*. For example, the reduction in net photosynthetic rate in N30 plots was 28% for *S. superba* and 9% for *C. concinna* relative to the control plots (Fig. 3a).

The different response to N addition between these two species could be related to the species-N-requirement. Assuming that the N treatment, which resulted in maximum biomass production, is characterized by a well-balanced nutritional status, optimal ratios were calculated for each species (Berger and Glatzel 2001). In this experiment, the optimal N/Ca/K/P/Mn ratio in seedling leaves was 34/21/10/3/1 for *S. superba* and 96/37/17/7/1 for *C. concinna*. These ratios suggest that *S. superba* demands less N than *C. concinna* for seedling growth. This interpretation is supported by the fact that leaf N concentration is generally lower for *S. superba* than for *C. concinna* even in the same habitat (Wang et al. 1982; Kong et al. 1997). For example, leaf N concentration was  $16 \text{ mg g}^{-1}$  for *S. superba* and  $23 \text{ mg g}^{-1}$  for *C. concinna* in trees for an adjacent tropical forest (Mo et al. 2000), and was  $9 \text{ mg g}^{-1}$  for *S. superba* and  $11 \text{ mg g}^{-1}$  for *C. concinna* in seedlings for the present study (Table 3).

The interpretation above is supported by the results found in a seedling experiment for two temperate tree species (Nakaji et al. 2001, 2002). In that experiment, Nakaji et al. (2001, 2002) found that net photosynthetic rate and biomass production of *C. japonica* seedlings were increased by the N addition, while those of *P. densiflora* seedlings were significantly reduced by the highest N addition. They attributed this different response to the species-N-requirement, because *C. japonica*, characteristic of nutrient-rich habitats, had higher species-N-requirement compared with *P. densiflora* (Nakaji et al. 2001, 2002).

Our finding has a fundamental implication for the forest dynamics and forest management in the study region.

*S. superba* grows in relatively nutrient-poor soils, whereas *C. concinna* grows in relatively nutrient-rich soils and the former is a dominant tree species in both pine-broadleaf mixed and mature forests, while the latter occurs only in the mature forest. With the increasing N input to forests in this region, the relatively nutrient-poor mixed forests may be suitable for the growth of *C. concinna* and the population of *S. superba* in the nutrient-rich mature forest may be reduced.

**Acknowledgments** We would like to thank the constructive comments, from two anonymous reviewers and the editor, which have greatly improved the quality of the paper. This study was founded by National Natural Science Foundation of China (No. 40730102, 30670392) and Key Project of Chinese Academy of Sciences Knowledge Innovation Program (KZCX2-YW-432).

## References

- Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in Northern forest ecosystems. *Bioscience*. 39:378–386
- Aber JD, Magill A, McNulty SG, Boone RD, Nadelhoffer KJ, Downs M, Hallet R (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air Soil Poll* 85:1665–1670
- Aber JD, McDowell W, Nadelhoffer KJ, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in Northern forest ecosystems, hypotheses revisited. *BioScience* 48:921–934
- Aerts R, Boot RGA, vander Aart PJM (1991) The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87:551–559
- Anderson JM, Ingram JSI (1989) Tropical soil biology and fertility: a handbook of methods. CAB International, Wallingford
- Berger TW, Glatzel G (2001) Response of *Quercus petraea* seedlings to nitrogen fertilization. *Forest Ecol Manage* 149:1–14
- Berntson GM, Farnsworth EJ, Bazzaz FA (1995) Allocation, within and between organs, and the dynamics of root length changes in two birch species. *Oecologia* 101:139–447
- Boxman AW, Blanck K, Brandrud TE, Emmett BA, Gundersen P, Hogervorst RF, Kjønaas OJ, Persson H, Timmermann V (1998) Vegetation and soil biota response to experimentally-changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. *Forest Ecol Manage* 101:65–80
- Brække FH (1996) Needle analyses and graphic vector analyses of Norway spruce and Scots pine stands. *Trees* 11:23–33
- Bremner JM, Mulvaney CS (1982) Nitrogen-total. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, Part 2: chemical and microbial properties*, Agronomy Monograph 9. Agronomy Society of America, Madison, pp 595–624
- Brown KR, Thompson WA, Camm EL (1996) Effects of N addition rates on the productivity of *Picea Sitchensis*, *Thuja plicata*, and *Tsuga heterophylla* seedlings. II. Photosynthesis,  $^{13}\text{C}$  discrimination and N partitioning in foliage. *Trees* 10:198–205
- Chen XY, Mulder J, Wang YH, Zhao DW, Xiang RJ (2004) Atmospheric deposition, mineralization and leaching of nitrogen in subtropical forested catchments, South China. *Environ Geochem Hlth* 26:179–186
- Emmett BA, Reynolds B, Silgram M, Sparks TH, Woods C (1998) The consequences of chronic nitrogen addition on N cycling and soil-water chemistry in a Sitka spruce stand North Wales. *For Ecol Manage* 101:165–175
- Ericsson T (1995) Growth and shoot/root ratio of seedlings in relation to nutrient availability. *Plant Soil* 168:205–214
- Fang H, Mo JM, Peng SL, Li ZA, Wang H (2007) Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. *Plant Soil* 297:233–242
- Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The nitrogen cascade. *BioScience* 53:341–356
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vorosmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226
- Guan LL, Zhou GY, Zhang DQ, Liu JX, Zhang QM (2004) Twenty years of litterfall dynamics in subtropical evergreen broad-leaved forests at the Dinghushan forest ecosystem research station. *Acta Phytoecol Sin* 28:449–456 (in Chinese with English abstract)
- Hall SJ, Matson PA (1999) Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature* 400:152–155
- Haase DL, Rose R (1995) Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *Forest Sci* 41:54–66
- Holdridge LR (1967) Life zone ecology. Tropical Science Center, San Jose
- Huang ZF, Fan ZG (1982) The climate of Ding Hu Shan. *Trop Subtrop Forest Ecosyst* 1:11–23 (in Chinese with English abstract)
- Huang ZL, Ding MM, Zhang ZP, Yi WM (1994) The hydrological processes and nitrogen dynamics in a monsoon evergreen broad-leaved forest of Dinghu shan. *Acta Phytoecol Sin* 18:194–199 (in Chinese with English abstract)
- Kong GH, Huang ZL, Zhang QM, Liu SZ, Mo JM, He DQ (1997) Type, structure, dynamics and management of the lower subtropical evergreen broad-leaved forest in the Dinghushan Biosphere Reserve of China. *Tropics* 6:335–350
- Krupa SV (2003) Effects of atmospheric ammonia ( $\text{NH}_3$ ) on terrestrial vegetation: a review. *Environ Pollut* 124:179–221
- Liu GS, Jiang NH, Zhang LD, Liu ZL (1996) Soil physical and chemical analysis and description of soil profiles. Standards Press of China, Beijing, pp 121–265 (in Chinese)
- Macdonald JA, Dise NB, Matzner E, Armbruster M, Gundersen P, Forstner M (2002) Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Glob Change Biol* 8:1028–1033
- Magill AH, Aber JD, Currie WS, Nadelhoffer KJ, Martin ME, McDowell WH, Melillo JM, Steudler PA (2004) Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecol Manage* 196:7–28
- Matson PA, McDowell WH, Townsen AR, Vitousek PM (1999) The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* 46:67–83
- Matson PA, Lohse KA, Hall SJ (2002) The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio* 31:113–119
- McNulty SG, Aber JD, Newman SD (1996) Nitrogen saturation in a high elevation New England spruce-fir stand. *Forest Ecol Manage* 84:109–121
- Mo JM, Brown S, Lenart M, Kong G (1995) Nutrient dynamics of a human-impacted pine forest in a MAB reserve of subtropical China. *Biotropica* 27:290–304
- Mo JM, Zhang DQ, Huang ZL, Yu QF, Kong GH (2000) Distribution pattern of nutrient elements in plants of Dinghushan lower subtropical evergreen broad-leaved forest. *J Trop Subtrop Bot* 8:198–206 (in Chinese with English abstract)
- Mo JM, Brown S, Peng SL, Kong GH (2003) Nitrogen availability in Disturbed, Rehabilitated and Mature Forests of Tropical China. *Forest Ecol Manage* 175:573–583



- Mo JM, Brown S, Xue JH, Fang YT, Li ZA (2006) Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant Soil* 282:135–151
- Nakaji T, Fukami M, Dokiya Y, Izuta T (2001) Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. *Trees* 15:453–461
- Nakaji T, Takenaga S, Kuroha M, Izuta T (2002) Photosynthetic response of *Pinus densiflora* seedlings to high nitrogen load. *Environ Sci* 9:269–282
- Nihlgård B (1985) The ammonium hypothesis. An additional explanation to the forest die back in Europe. *Ambio* 14:2–8
- Ren R, Mi F, Bai N (2000) A chemometrics analysis on the data of precipitation chemistry of China. *J Beijing Polytechnic Univ* 26:90–95 (in Chinese with English abstract)
- Schaberg PG, Perkins TD, McNulty SG (1997) Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. *Can J For Res* 27:1622–1629
- Schulze ED (1989) Air pollution and forest decline in a spruce (*Picea abies*) forest. *Science* 244:776–783
- Sikström U, Nohrstedt H, Pettersson F (1998) Stem-growth response of *Pinus sylvestris* and *Picea abies* to nitrogen fertilization as related to needle nitrogen concentration. *Trees* 12:208–214
- Wang Z, He D, Song S, Chen S, Chen D, Tu M (1982) The vegetation of Dinghushan Biosphere Reserve. *Trop Subtrop Forest Ecosyst* 1:77–141 (in Chinese with English abstract)
- Wang JR, Hawkins CDB, Letchford T (1998) Relative growth rate and biomass allocation of paper birch (*Betula papyrifera*) populations under different soil moisture and nutrient regimes. *Can J For Res* 28:44–55
- Xu YG., Zhou GY, Lou TS, Wu ZM, He ZC (2001) Soil solution chemistry and element budget in the forest ecosystem in Guangzhou. *Acta Ecol Sin* 21:1760–1861 (in Chinese with English abstract)
- Xu GL, Mo JM, Fu SL, Gundersen P, Zhou GY, Xue JH (2007) The response of soil fauna to simulated nitrogen deposition—a nursery experiment in subtropical China. *J Environ Sci* 19:603–609
- Zhang YM, Zhou GY, Wen DZ, Zhang DQ, Zhang QM (2002) Biomass dynamics of the *Castanopsis chinensis*–*Schima superba*–*Cryptocarya concinna* community of monsoon evergreen broad-leaved forest in Dinghushan Reserve. *Trop Subtrop Forest Ecosyst* 9:10–17 (in Chinese with English abstract)
- Zheng X, Fu C, Xu X, Xiaodong Y, Huang Y, Chen G, Han S, Hu F (2002) The Asian nitrogen cycle case study. *Ambio* 31:79–87
- Zhou GY, Yan JH (2001) The influence of region atmospheric precipitation characteristics and its element inputs on the existence and development of Dinghushan forest ecosystems. *Acta Ecol Sin* 21:2002–2012 (in Chinese with English abstract)