



# Growth responses of trees and understory plants to nitrogen fertilization in a subtropical forest in China

Di Tian<sup>1</sup>, Peng Li<sup>1</sup>, Wenjing Fang<sup>1</sup>, Jun Xu<sup>2</sup>, Yongkai Luo<sup>3</sup>, Zhengbing Yan<sup>1</sup>, Biao Zhu<sup>1</sup>, Jingjing Wang<sup>2</sup>, Xiaoniu Xu<sup>2</sup>, and Jingyun Fang<sup>1</sup>

<sup>1</sup>Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing, 100871, China

<sup>2</sup>Department of Forestry, Anhui Agricultural University, 230036, Hefei, Anhui, China

<sup>3</sup>State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China

Correspondence to: Jingyun Fang (jyfang@urban.pku.edu.cn)

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**Abstract.** Reactive nitrogen (N) increase in the biosphere has been a noteworthy aspect of global change, producing considerable ecological effects on the functioning and dynamics of the terrestrial ecosystems. A number of observational studies have explored responses of plants to experimentally simulated N enrichment in boreal and temperate forests. Here we investigate how the dominant trees and different understory plants respond to experimental N enrichment in a subtropical forest in China. We conducted a 3.4-year N fertilization experiment in an old-aged subtropical evergreen broad-leaved forest in eastern China with three treatment levels applied to nine 20 m × 20 m plots and replicated in three blocks. We divided the plants into trees, saplings, shrubs (including tree seedlings), and ground-cover plants (ferns) according to the growth forms, and then measured the absolute and relative basal area increments of trees and saplings and the aboveground biomass of understory shrubs and ferns. We further grouped individuals of the dominant tree species, *Castanopsis eyrei*, into three size classes to investigate their respective growth responses to the N fertilization. Our results showed that the plot-averaged absolute and relative growth rates of basal area and aboveground biomass of trees were not affected by N fertilization. Across the individuals of *C. eyrei*, the small trees with a DBH (diameter at breast height) of 5–10 cm declined by 66.4 and 59.5 %, respectively, in N50 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and N100 fertilized plots (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>), while the growth of median and large trees with a DBH of > 10 cm did not significantly

change with the N fertilization. The growth rate of small trees, saplings, and the aboveground biomass of understory shrubs and ground-cover ferns decreased significantly in the N-fertilized plots. Our findings suggested that N might not be a limiting nutrient in this mature subtropical forest, and that the limitation of other nutrients in the forest ecosystem might be aggravated by the enhanced N availability, potentially resulting in an adverse effect on the development of natural subtropical forest.

## 1 Introduction

Reactive nitrogen (N) increase in the biosphere, especially atmospheric N deposition, is a globally prevalent phenomenon (Galloway et al., 2004). It has become a serious environmental issue in China, especially in the southeastern regions, with drastic increase of N entering terrestrial ecosystems which produces considerable ecological effects on the functioning and dynamics of the terrestrial ecosystems (Liu et al., 2013; Gu et al., 2015). Since the 1990s, the simulated N-fertilization experiments have been conducted in various forest ecosystems to explore the responses of plants and other organisms to the potential high N enrichment and changes of soil N availability (e.g. Wright and Tietema, 1995; Bobbink et al., 2010; Fowler et al., 2015). Although a number of studies have reported a general positive effect of N enrichment on plants in N-limited forests and a negative effect of excess N

(e.g. Aber et al., 1998; Högberg et al., 2006; Gilliam, 2006; Thomas et al., 2010), specific responses of plants appeared to be highly growth form-dependent and ecosystem-dependent (LeBauer and Treseder, 2008; Bedison and McNeil, 2009; Dirnböck et al., 2014).

Due to the widespread heavy N deposition in Europe and America, numerous studies that focused on the growth responses of plants to N enrichment have been carried out in boreal and temperate forests during the past several decades (Magill, 2000; Högberg et al., 2006). These studies showed that most trees have a positive growth response to N fertilization and therefore have higher potential carbon sequestration because the status of N limitation was largely alleviated by the increasing N inputs (e.g. Thomas et al., 2010; BassiriRad et al., 2015). However, the understory plants in these forest ecosystems inconsistently showed general negative responses to N enrichment with declined biomass or shifted community structure (Rainey et al., 1999; Du et al., 2014; Dirnböck et al., 2014). In addition to the opposite responses of trees and understory plants to N enrichment, differences remained in the effects of N enrichment on single plant growth form in these forests. Generally, the limited light availability in these ecosystems with high tree canopy cover was ascribed to the negative effects of N fertilization (Strengbom and Nordin, 2008).

Recently, the effects of N enrichment on tropical forests have raised researchers' concern. Fertilization experiments in tropical forests showed different growth responses of trees to nutrient addition among individual size levels, understory shrubs, and tree seedlings (Wright et al., 2011; Pasquini and Santiago, 2012; Santiago et al., 2012) which contrasted with the ones found for trees in the previously described experiments. For example, phosphorus (P) fertilization enhanced the growths of small trees and seedlings but had no effect on median and large trees, while N addition did not show any significant effect on plant growth in a lowland tropical forest (Alvarez-Clare et al., 2013). In addition to the ubiquitous concept that P was a critical element driving plant growth in tropical forests (Vitousek et al., 1991), the heterogeneous nutrient limitation that the growths of plants were co-limited by multiple nutrients was further proposed to explain why diverse plants respond differently to N enrichment (Wright et al., 2011; Alvarez-Clare et al., 2013; Wurzbarger and Wright, 2015). Nevertheless, the patterns of specific nutrient limitation and responses of plants to N enrichments among diverse forest ecosystems need further exploration.

As most of the nutrient fertilization experiments have focused on boreal forests, temperate forests, and lowland tropical forests, few studies have investigated the effects of N enrichment on subtropical forests despite their broad distribution throughout the world and great contribution to the global carbon sink (Zhou et al., 2013; Yu et al., 2014; Huang et al., 2015). With increasing N deposited in the subtropical ecosystems in southeastern China (Du et al., 2014), it is important to diagnose the nutrient limitation and evaluate the responses

of different plant growth forms to N enrichment in subtropical forests for the assessment of carbon sequestration and community dynamics.

To better predict the responses of subtropical forests and different plant growth forms to N enrichment, we carried out a 3.4-year N fertilization experiment with three treatment levels applied to nine 20 m × 20 m plots and replicated in three blocks in a subtropical forest in southeastern China. We attempt to explore whether N is a limiting element in the old-aged evergreen broad-leaved subtropical forest. We hypothesize a positive response of trees to N fertilization, but a negative response of understory growth forms to N fertilization due to the expansion of canopy crown and consequent reduction of light availability.

## 2 Materials and methods

### 2.1 Study site and experimental design

The N fertilization experiment site was located at 30°01'47" N latitude and 117°21'23" E longitude at an altitude of 375 m in the natural conservation zone of Guniujiang in Anhui Province, eastern China. As a commendable representative of the typical subtropical broad-leaved evergreen forest, the Guniujiang experimental site is an important part of the NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du et al., 2013), because of its representativeness in both species composition and landscape structure in the subtropical evergreen forest region. The study area has a humid climate with strong summer monsoons with an annual average precipitation of 1700 mm and an average annual temperature of 14.9 °C. The amount of wet N deposition in this region was 5.9–7.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The soil in this area has been classified as yellow-brown earth (Chinese Soil Taxonomic Classification), and the pH<sub>H<sub>2</sub>O</sub> value at 0–10 cm soil depth was 4.58 ± 0.05 (mean ± SE). The total N, P, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N content in the soil at 0–10 cm depth were 3.23 (0.37), 0.32 (0.02), 0.012 (0.001), and 0.002 (0.0006) mg g<sup>-1</sup>, respectively (Li et al., 2015).

The study was conducted in a well-protected, mature subtropical evergreen forest (> 300 year age) with a three-layered vertical structure: the canopy tree layer (DBH > 5 cm and height > 5 m); the understory layer of saplings, shrubs, and seedlings (DBH < 5 cm and height < 5 m); and the ground-cover layer (ferns and herbs). The average density and basal area of trees were 1219 trees ha<sup>-1</sup> and 36.35 m<sup>2</sup> ha<sup>-1</sup>, respectively; *Castanopsis eyrei* was the dominant species (which was also an important species at some other sites in subtropical forests) and accounted for 87 % of the total aboveground biomass of trees. The understory saplings and shrubs contained several species, including *Cleyera japonica*, *Camellia cuspidata*, *Rhododendron ovatum*, *Eurya muricata*, *Cinnamomum japonicum*, *Cinnamo-*

**Table 1.** Growth measurements for four plant growth forms in this study before N fertilization. Numbers in the tables represent means (or mean  $\pm$  standard error,  $n = 9$ ) of plants across all plots. TBA: total basal area of trees; DBH: diameter at breast height (1.3 m); Basal diameter: diameter at 10 cm above the ground.

Growth forms	Species	Growth variable		
		TBA ( $\text{m}^2 \text{ha}^{-1}$ )	DBH (cm)	Height (m)
Trees	<i>Castanopsis eyrei</i>	$32.5 \pm 2.7$	$15.7 \pm 3.6$	$11.8 \pm 2.1$
Saplings	<i>C. eyrei</i>	$0.61 \pm 0.10$	$3.81 \pm 0.04$	$2.59 \pm 0.06$
		Coverage (%)	Basal diameter (mm)	Height (cm)
Shrubs and seedlings	<i>Cleyera japonica</i>	2.89	$9.24 \pm 5.13$	$79.8 \pm 40.82$
	<i>Camellia cuspidata</i>	8.60	$7.01 \pm 0.62$	$60.1 \pm 4.37$
	<i>Rhododendron ovatum</i>	5.97	$16.81 \pm 8.91$	$167.5 \pm 65.02$
	<i>Eurya muricata</i>	3.04	$7.00 \pm 1.57$	$111.0 \pm 38.16$
	<i>Cinnamomum japonicum</i>	2.85	$4.44 \pm 1.46$	$51.1 \pm 26.59$
	<i>Cinnamomum subavenium</i>	5.03	$2.77 \pm 0.64$	$29.9 \pm 7.54$
	<i>Sarcandra glabra</i>	2.92	$3.60 \pm 0.11$	$35.7 \pm 3.69$
		Density (shoots $\text{m}^{-2}$ )		
Ferns	<i>Woodwardia japonica</i>	$1.19 \pm 0.23$		

*mum subavenium*, *Sarcandra glabra*, and *C. eyrei*, and other native subtropical evergreen species (Table 1). Two fern species (*Woodwardia japonica* and *Dryopteris hwangshanensis*) and an orchid (*Cymbidium tortisepalum* var. *longibracteatum*) appeared on the floor layer, while *W. japonica* exclusively dominated the floor layer with a coverage of 10–20 %.

We began N fertilization in March 2011. A randomized block design was used to avoid spatial heterogeneity. We chose three blocks with similar stand growth, species composition, and site condition to establish three N treatments in each block: CK ( $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), N50 ( $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), and N100 ( $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). In total, nine  $20 \text{ m} \times 20 \text{ m}$  plots were established with a 5–10 m buffer zone between each plot. The total  $\text{NH}_4\text{NO}_3$  was divided into 12 dosages and applied to the forest in each month at regular intervals.  $\text{NH}_4\text{NO}_3$  in dosages of 0.48 and  $0.95 \text{ kg plot}^{-1}$  were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly in N50 and N100 plots using a back-hatch sprayer. The unfertilized plots (controls) were similarly treated with 15 L of fresh water without  $\text{NH}_4\text{NO}_3$ .

## 2.2 Sampling and measurement

In March 2011, the species of all trees higher than 2 m in each plot were labelled and their initial DBH (1.3 m) was measured. Then, autonomous band dendrometers made of aluminium tape and springs were installed on trees with a DBH greater than 5 cm. After 1 month to allow the tapes and springs on the trees to become stable, we began to measure the changes in the gaps on the tapes using vernier callipers (measured in July 2014) and then calculated tree DBH ac-

cording to the following equation:

$$\text{DBH} = \text{DBH}_1 + \frac{X_2 - X_1}{3.14 \times 10},$$

where  $\text{DBH}_1$  represents the initial DBH (cm) of trees measured in March 2011, and  $X_2$  and  $X_1$  (mm) represent the widths of gaps on the tapes measured in July 2014 and at the beginning of the experiment, respectively.

The basal area is a common indicator for weighing the biomass of trees. Therefore, tree basal area increments were calculated to indicate the responses of tree biomass to the N fertilization. First, to test community-level responses of tree layer to N fertilization, we calculated the sum of total basal area increase ( $\text{m}^2 \text{ha}^{-2} \text{yr}^{-1}$ ) of all trees in a plot after 3.4 years of N fertilization and divided this value by the period of N fertilization (3.4 years) to obtain the annual basal area increase rate of the trees (dead trees were not included). Second, relative annual basal area growth rate (RGR,  $\text{m}^2 \text{m}^{-2} \text{yr}^{-1}$ ) was used to eliminate the conceivable interferential effects resulting from the differences in the number and size of original individuals among plots according to the following equation, similar to the method of Alvarez-Clare et al. (2013):

$$\text{RGR} = \frac{\ln(2014 \text{ BA}) - \ln(2011 \text{ BA})}{3.4},$$

where RGR represents the relative annual basal area growth rate ( $\text{m}^2 \text{m}^{-2} \text{yr}^{-1}$ ), BA indicates the sum of basal area of all trees in each plot, and 3.4 (years) is the N fertilization period.

Because *C. eyrei* was the only dominant species in the tree layer, we separated it from other tree species and grouped its individuals into three classes based on their DBH values

(i.e. 5–10, 10–30 and > 30 cm) to investigate the effects of N fertilization on the growth of trees after removing the plant species and original size factors. During the monitoring of tree growth, dead trees were recorded. Then, we calculated the aboveground biomass increments of trees and the proportion of dead biomass using allometric equations (see Table S1 in the Supplement).

We examined the effects of N fertilization on understory tree saplings distributed in the plots according to their sizes and characteristics. For small trees with DBH < 5 cm and height > 2 m (defined as “saplings”), DBH was measured at the beginning of N fertilization and in July 2014. Then, annual basal area growth rate and RGR of saplings were calculated based on DBH changes. For very small trees or shrubs with DBH < 5 cm and height < 2 m (defined as “shrubs/seedlings”), we set two 5 m × 5 m subplots in each plot along a diagonal direction and investigated the abundance, dominance, basal diameter (diameter at 10 cm above the ground), height, and crown diameters of all shrubs/seedlings inside the subplots at two specific times. The first time was at the beginning of N fertilization (March 2011), and the second was in July 2014. The length, width, and number of fern leaves were measured carefully in the above-mentioned subplots, and the allometric equations for seven dominant species were then obtained (Table S1). Because the average aboveground biomass of shrubs/seedlings and ferns showed no significant differences across three N treatments before N fertilization in March 2011, we regarded the distribution of these understory shrubs/seedlings and ferns to be homogeneous among the three treatments. Then we identified the effects of N fertilization by comparing the aboveground biomass of shrubs/seedlings and ferns in 2014 among the different treatments. Meanwhile, to investigate the canopy cover and understory light availability, we used a digital camera (Canon, Japan) with a fisheye lens (Sigma circular fisheye) to take photographs of canopy. In each subplot, we put the camera at 1 m above ground and took five photos upwards from understory.

In addition, to further explore the influences of N fertilization on plants' growth from the biogeochemical aspect, we measured soil N, P content and pH. Specifically, we set three subplots randomly within each plot and collected three subsamples of 0–10 cm soil for each subplot using a handheld steel soil borer (3 cm in diameter), during investigation of the understory plants. Then, the three subsamples were mixed together to form one sample per plot and transported to a laboratory and air-dried naturally. After being air-dried, soil samples were ground with a ball mill (NM200, Retsch, Haan, Germany) and screened through a 100-mesh sieve. The N concentration of soil was measured using an elemental analyser (2400 Series2 CHNS/O elemental analyser, Perkin-Elmer, USA). After acid digestion of the samples, soil P concentrations were measured using a flow injection analysis instrument (AutoAnalyser3, Bran + Luebbe, Germany). Soil

**Table 2.** The indicator of canopy cover (i.e.  $[1 - F_{mv}]$ ) of the three treatments in our experiments.  $n$  indicates the number of replicates. SE indicates the standard error.

Treatment	$n$	Canopy cover	
		mean	SE
CK	3	0.77	0.01
N50	3	0.76	0.04
N100	3	0.72	0.01

pH was measured by dry soil in water suspension with a water : soil ratio of 1 : 2.5.

### 2.3 Statistical analysis

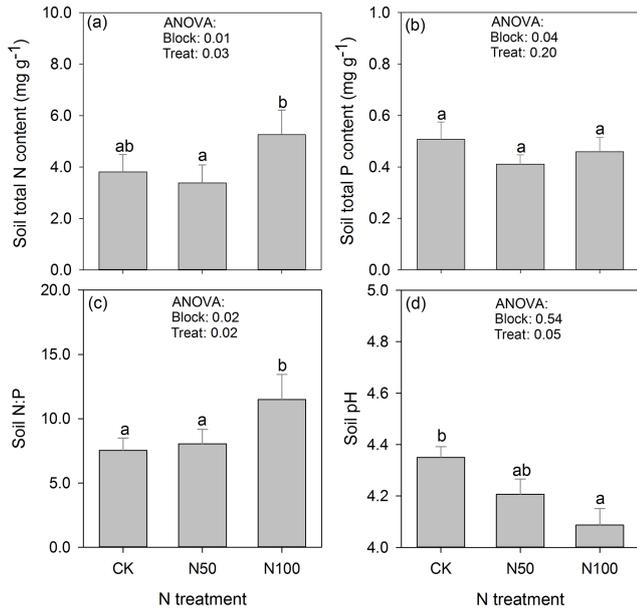
We used an analysis of variance (ANOVA) to evaluate the effects of N fertilization on soil N and P content, soil pH, tree basal area increments, RGR, aboveground biomass increments, proportion of dead trees, and aboveground biomass of shrubs/seedlings and ferns. Block and N treatment were both regarded as fixed factors in the statistical model. We excluded the interactions between block and N treatment from the model because they do not have ecological meaning. Tukey's honest significant difference (HSD) tests were used to conduct the multi-comparisons among the three N treatments. For the estimation of canopy cover, we followed the detailed procedures of weighted ellipsoidal method using the software of Hemisfer (version 2.16.6) to obtain values of vertical total gap fraction ( $F_{mv}$ ) which indicate the proportion of projected light spots to the total projected area (Thimonier et al., 2010). Then we obtained the values of  $[1 - F_{mv}]$  to indicate canopy cover. All statistical analyses were performed in R.3.2 (R Development Core Team, 2010), and all figures were drawn in SigmaPlot 12 (Systat, 2010).

## 3 Results

### 3.1 Effects of N fertilization on canopy cover, soil N and P contents, and pH

The indicator of forest canopy (i.e.  $[1 - F_{mv}]$ ) showed no significant differences between unfertilized and fertilized plots with 3.4 years of N fertilization (Table 2). Although the fish eye measurements did not provide evidence for the changes in total forest cover with the effects of N fertilization, there still may be a shift between the contribution of overstory and understory trees to the total forest cover.

The 3.4 years of N fertilization significantly increased the N content of 0–10 cm soil ( $p = 0.03$ ), especially in N100 plots (Fig. 1a), but showed no significant effect on soil P content (Fig. 1b,  $p > 0.05$ ), thus leading to a significant increase in soil N : P ratio (Fig. 1c,  $p = 0.02$ ). Additionally, the N fer-



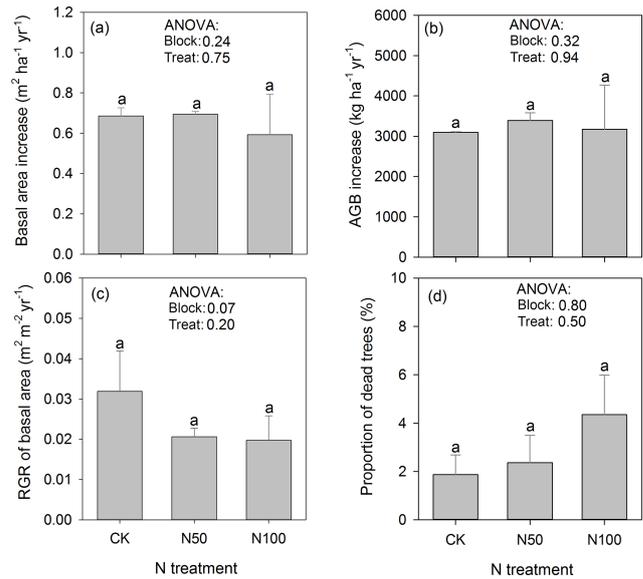
**Figure 1.** Effects of N fertilization on soil nutrient content, N : P ratio, and pH (mean  $\pm$  SE,  $n = 3$ ) at the soil depth of 0–10 cm. (a) Total N content per gram soil, (b) total P content per gram soil, (c) N : P ratio and (d) soil pH. Numbers in these figures indicate the results of ANOVA.

tilization also decreased soil pH and aggravated soil acidification (Fig. 1d,  $p = 0.05$ ).

### 3.2 Growth responses of trees to N fertilization

The increments of absolute basal area, aboveground biomass, and RGR of all trees at plot level showed no significant response to N fertilization during 3.4-year N fertilization (Fig. 2a–c). Compared with the unfertilized plots, N50 and N100 fertilized plots showed a tendency toward higher averaged proportions of dead trees' aboveground biomass despite no significant difference between them (Fig. 2d).

Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent responses of absolute basal area increments and RGR to N fertilization (Fig. 3a–f). The small trees with a DBH of 5–10 cm growing under unfertilized plots showed greater basal area increments than those growing under N-fertilized plots (Fig. 3a,  $p = 0.02$ ). Specifically, the N50 and N100 fertilization decreased the absolute basal area increments of small individual trees at rates of 2.2 and 1.98 cm<sup>2</sup> tree<sup>-1</sup> yr<sup>-1</sup>, respectively, which indicated that the decreasing degrees of the absolute basal area of small trees reached 66.4 and 59.5 % in N50 and N100 plots. The small individual trees also showed a tendency toward lower averaged RGR in N-fertilized plots although no significant difference was detected between them (Fig. 3d,  $p > 0.05$ ). As opposed to the negative responses of small trees to N fertilization, the basal area increment and RGR of median *C. eyrei* individuals (DBH of 10–30 cm) and large

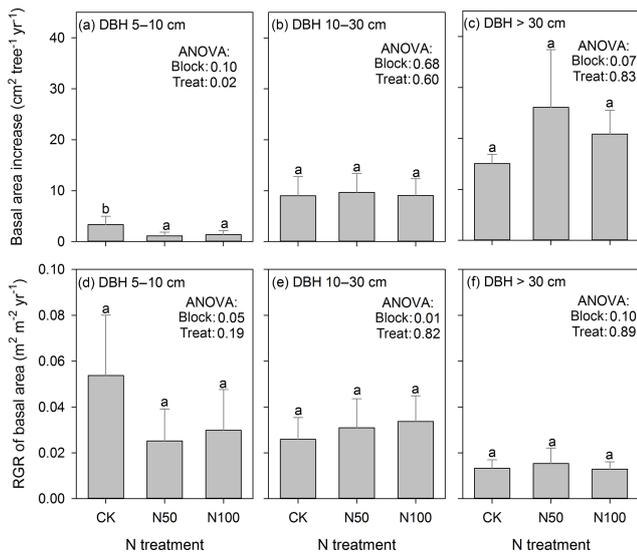


**Figure 2.** Effects of N fertilization on the growth and mortality of all trees (mean  $\pm$  SE). (a) Absolute basal area increase of all trees, (b) aboveground biomass increase of all trees, (c) relative growth rate of total tree basal area, and (d) the proportion of all dead trees. The proportion of dead trees was calculated using the aboveground biomass of all dead trees during the experiment divided by the total aboveground biomass of all trees in 2014. Numbers in these figures indicate the results of ANOVA. The N treatment on the x axis represents three levels of N fertilization: CK (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N50 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

*C. eyrei* individuals (DBH of > 30 cm) showed no significant response to N fertilization, but the averaged growth rate of large *C. eyrei* individuals in N50 plots almost doubled the value of the corresponding large individuals in unfertilized plots (Fig. 3b, c, e and f,  $p > 0.05$  in all cases).

### 3.3 Growth responses of understory saplings, shrubs/seedlings, and ferns to N fertilization

Responses of understory saplings to N fertilization were similar to those of small dominant trees. Although the annual absolute increments of basal area increments of saplings showed no significant response to N fertilization (Fig. 4a,  $p > 0.05$ ), the RGR of sapling growing in N50 and N100 plots showed a substantial decrease at rates of 0.021 and 0.019 m<sup>2</sup> m<sup>-2</sup> yr<sup>-1</sup>, respectively, compared to sapling growing in unfertilized plots (Fig. 4b,  $p < 0.001$ ). In addition, a general negative effect of N fertilization also occurred on understory shrubs and ground-cover ferns. The aboveground biomass of seven predominant shrubs/seedlings was drastically decreased by 69.4 and 79.1 % in N50 and N100 fertilized plots, respectively, compared with those in the unfertilized plots (Fig. 5a,  $p < 0.01$ ). Remarkably, the aboveground biomass of ground-cover ferns significantly declined



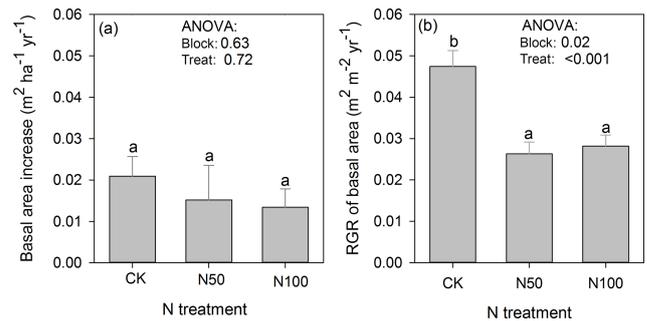
**Figure 3.** Effects of N fertilization on the growth (mean  $\pm$  SE) of *C. eyrei* by DBH classes (5–10, 10–30 and > 30 cm). (a–c) Absolute basal area increase and (d–f) relative growth increase rate of basal area. Numbers in these figures indicate the results of ANOVA. The N treatment on the x axis represents three levels of N fertilization: CK (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N50 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

by 92.4 and 93.4 % in N50 and N100 fertilized plots (Fig. 5b,  $p < 0.05$ ).

## 4 Discussion

### 4.1 Growth responses of trees to N fertilization

Nutrient limitation was generally determined through evaluating ecosystem feedbacks to nutrient addition (Vitousek, 1991; Santiago et al., 2012; Alvarez-Clare et al., 2013). When the forest ecosystems showed a positive response to added nutrient, e.g. plant growth or rates of physiological processes were promoted, the added nutrient then could be interpreted as limiting to the ecosystem – and otherwise, as not limiting to the ecosystem (Santiago, 2015). We initially expected positive growth responses of trees exposed to N fertilization in this subtropical forest because N availability in the soil would be enhanced by N fertilization and the potential N limitation of plants in the forest ecosystem could be alleviated. However, contrary to our expectation, we did not observe strong positive growth responses of trees to N fertilization (Figs. 2 and 3). Across individual trees of different sizes and plant growth forms, we only observed substantial negative responses of small trees (5–10 cm DBH; Fig. 3a and d) and saplings (Fig. 4a and b) and weak responses of median and large trees (> 10 cm DBH) to N fertilization (Fig. 3b, c, e, f), which further demonstrated that the growth



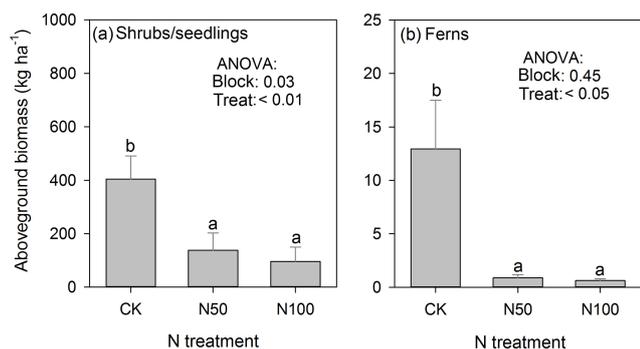
**Figure 4.** Effects of N fertilization on the growth of saplings (mean  $\pm$  SE). (a) Absolute basal area increase and (b) the relative growth rate of basal area. Numbers in these figures indicate the results of ANOVA. The N treatment on the x axis represents three levels of N fertilization: CK (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N50 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

of trees in this old-aged subtropical forest was not essentially limited by N as hypothesized.

Contrasted with previous positive responses of trees to N fertilization in boreal and temperate forests which were considered as N limited ecosystems (Högberg et al., 2006; Thomas et al., 2010; BassiriRad et al., 2015), our finding of the unchanged responses of trees to N fertilization was partly consistent with observations of trees from tropical forests (e.g. Santiago et al., 2012; Alvarez-Clare et al., 2013). Studies from mature tropical forests have revealed that P availability was a critical element shaping tree species distribution and productivity (Santiago and Goldstein, 2016; Dalling et al., 2016). Given the similar high-weathered soil properties, humid climatic conditions, and dominant evergreen broadleaf trees in mature subtropical forest as those in wet tropical forest, we speculated that P limitation, rather than N limitation, might have played a key role in influencing growth of plants in subtropical forest.

The N and P stoichiometry of soil might have objectively provided indicators of P limitation with the effects of N fertilization in this subtropical forest, because soil N contents and N : P ratio in N-fertilized plots were remarkably higher than those in unfertilized plots (Fig. 1). Additionally, limitation of other nutrients, such as K (potassium) which was highlighted in tropical forests, and their combination as well as heterogeneous nutrient limitation of specific species, plant growth forms, and individuals in different sizes may warrant further consideration in subtropical forests (Wright et al., 2011; Santiago et al., 2012; Alvarez-Clare et al., 2013).

Moreover, the high spatial heterogeneity in old-aged subtropical forest, similar to tropical forests, could be a possible explanation for the lack of significant responses of plot-averaged basal area growth, RGR, aboveground biomass of trees with a DBH of > 5 cm, and the proportion of dead trees to N fertilization. In eastern China, the distributions of subtropical forest stands are quite topographically frag-



**Figure 5.** Effects of N fertilization on the aboveground biomass of shrubs, seedlings and ferns. Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean  $\pm$  SE). Numbers in these figures indicate the results of ANOVA. The N treatment on the x axis represents three levels of N fertilization: CK ( $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), N50 ( $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and N100 ( $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ).

mented, while relatively flat stands are required to avoid N losses and minimize spatial heterogeneity among experimental treatments. The actual distribution and topography of the subtropical forests limited the number of replications in the N fertilization experiment. This limitation might reduce the statistic power of N treatment on plot-averaged plant growth rate, as has been pointed out in previous studies (Wright et al., 2011; Alvarez-Clare et al., 2013). Furthermore, our observation of large trees with DBH  $> 30 \text{ cm}$  showed that the averaged growth rate of large *C. eyrei* individuals in N50 plots almost doubled the value of the corresponding large individuals in unfertilized plots. Nevertheless, the results of ANOVA showed that the effect was not significant. As the number of large trees in the experiment was relatively less than the small trees, the low replication and high spatial site heterogeneity might have reduced the statistical power of N fertilization on the large trees. Thus, fertilization experiments with more homogeneous plots and more replicates are warranted to further strengthen these findings. Overall, given the negative and potential positive effects of N fertilization on small and large trees, it is of urgent necessity to conduct long-term monitoring of the trees which would provide alternatives for accurately evaluating the forest dynamics under the enhanced global N deposition.

#### 4.2 Growth responses of small trees, understory saplings, shrubs/seedlings, and ferns to N fertilization

Although the positive response of small or juvenile trees to nutrient fertilization has been reported in boreal, temperate, and tropical forest (e.g. Högberg et al., 2006; Bedison and McNeil, 2009; Alvarez-Clare et al., 2013), our results showed a remarkable negative effect of N fertilization on small-sized plants including trees, understory saplings,

shrubs/seedlings, and ferns. During our field investigation, we also found that the average proportion of dead trees (Fig. 2d) tended to increase in N-fertilized plots although the result was not statistically significant ( $p = 0.50$ ). Additionally, the ground-cover ferns in N100 plots almost disappeared after 3.4-year N fertilization (personal observation). Given the high stand density in this mature subtropical forest, we suggest that N fertilization might potentially lead to increased self- and alien thinning of individuals through decreasing understory light availability.

The pivotal role of light availability in the eco-physiological processes of understory growth forms has been widely recognized (Santiago, 2015). Due to the limited light availability, understory plants may not be able to incorporate the added nutrient and promote their photosynthetic rates (Alvarez-Clare et al., 2013). However, a study conducted in tropical forest with thick canopy showed that photosynthetic process could be enhanced by nutrient addition even under low light availability (Pasquini and Santiago, 2012). In sharp contrast, the study conducted in an Australian rainforest revealed that understory seedlings increased growth when the light availability was high, but showed no significant response to nutrient fertilization in low lights (Thompson et al., 1988). These studies, together with our field observations, suggest that the growth of understory plants is largely co-limited by nutrient and light availability in the local environment. Further, our results of forest canopy cover estimated by photographic fisheye showed no significant differences between unfertilized and N-fertilized plots, which was consistent with the findings of Lu et al. (2010). Although the understory light irradiance fluctuated widely during a day and was very hard to detect precisely, our measurements of forest canopy cover provided a rough evaluation for light availability and a potential shift between the contribution of overstory and understory trees to the total forest cover which could partly explain the differences in the responses of trees with different sizes (i.e. different DBH classes). The results might indicate that other factors in addition to the low light availability in this old-aged forest had also played a crucial role in influencing understory plants during 3.4 years of N fertilization.

#### 4.3 Potential N saturation and plant growth

The striking biomass reduction of the understory plants, especially ferns, in response to N fertilization in our study well corroborated the similar findings in an old-aged tropical forest at Mt Dinghushan in China (Lu et al., 2010). Also, consistent with previous studies obtained from boreal, temperate, and tropical forests (Rainey et al., 1999; Alvarez-Clare et al., 2013; Dirnböck et al., 2014), our experiment revealed that understory small-sized plants responded sensitively to nutrient fertilization, which might indicate a possibility of N saturation in the subtropical forest. According to the definition of N saturation addressed by Aber et al. (1998) (i.e.

N availability in the forest ecosystem exceeded the demand of plants and microbes), the drastic decrease of understory ferns, shifted composition of understory plant community, and cation imbalances of understory species after 7 years of chronic N fertilization at Harvard Forest, USA, could be interpreted as useful indicators of N saturation (Rainey et al., 1999). Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Mt Dinghushan also showed signs of N saturation, such as significant increases in nitrate ( $\text{NO}_3^-$ ) leaching, inorganic N concentration, and  $\text{N}_2\text{O}$  emissions of soils, and soil acidification (Lu et al., 2014; Chen et al., 2015). In our experiment, the soil acidification and increased soil N concentration in high-N-fertilized plots (Fig. 1) combined with the negative responses of understory plants suggest that the 3.4-year N fertilization in this mature subtropical forest site has potentially caused N saturation. Nevertheless, further observations are still required to explore the mechanisms underlying the changes of different growth forms with the effects of N enhancement in the subtropical forests.

## 5 Conclusion

Contrasting growth responses among plant growth forms to N fertilization were present in the mature subtropical evergreen forest in this study. Overall growth of trees at the plot level showed no significant response to the N fertilization; however, if the dominant tree species, *C. eyrei*, was grouped into three DBH classes, the basal area increment of small trees with a DBH of 5–10 cm declined 66.4 and 59.5% in N50 and N100 fertilized plots, respectively, while the growth of median and large trees with a DBH of > 10 cm showed weak responses to N fertilization. The growths of understory saplings, shrubs/seedlings, and ground-cover ferns showed a negative response to N fertilization. Our results indicated that N might not be a limited nutrient in this subtropical forest and that other nutrient and light availability may potentially co-limit growth of plants with different growth forms. Our data also suggested that even short-term N fertilization might have caused N saturation in this mature subtropical forest and the limitation of other nutrients might be amplified with increasing N addition.

*Data availability.* All of the original data used in this study can be found in the Supplement.

**The Supplement related to this article is available online at <https://doi.org/10.5194/bg-14-3461-2017-supplement>.**

*Author contributions.* JF and XX designed the experiment. DT and PL conducted the N fertilization experiment. DT, PL, WF, JX, and JW collected data during the experiment. YL contributed the allometric equations for shrubs. DT, ZY, and JF wrote the paper. All authors shared discussion and revisions of the paper.

*Competing interests.* The authors declare that they have no conflict of interest.

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