

Authors' response to editor' comments on the manuscript bg-2016-416 “*Contrasting growth responses among plant growth forms to nitrogen fertilization in a subtropical forest in China*” by Di Tian et al.

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**To the editor:**

Dear Dr. Zaehle,

Enclosed please find our revised version of the manuscript (bg-2016-416) “*Contrasting growth responses among plant growth forms to nitrogen fertilization in a subtropical forest in China*” for possible publication in *Biogeosciences*.

Thank you very much for your helpful comments and suggestions which allowed us to improve the manuscript. We have carefully studied the comments and rephrased the introduction, results and discussion in the updated version. The point-by-point responses and the revised manuscript with changes marked are attached with this letter at the bottom.

We hope that our manuscript is now acceptable for *Biogeosciences* and look forward to hearing from you soon. Thank you!

On behalf of all the authors,

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**[Comment]** 1. you added fish-eye observations, but they need to be mentioned as a separate section in the results (including a figure or a table), and not buried in the discussion.

**[Reply]** Many thanks for your helpful and insightful comment. We added a separate table (i.e. Table 2) in the Result and described the effect of nitrogen fertilization on the canopy cover (Lines 238 to 239 at Page 8) as follows: The indicator of forest canopy (i.e. [I-Fmv]) showed no significant differences between unfertilized and fertilized plots with 3.4 years of N fertilization (Table 2).

**Table 2** The indicators of canopy cover (i.e. [I-Fmv]) of the three treatments in our experiments. *n* indicates the number of replicates; SE indicates the standard error.

Treatment	<i>n</i>	Canopy cover	
		mean	SE
CK	3	0.77	0.01
N50	3	0.76	0.04
N100	3	0.72	0.01

**[Comment]** 2. your text reads as if there had been largely no or a negative response, but this does not reflect Figure 2 panel c which suggests that the growth rate of large trees has almost doubled. Maybe this is not statistically significant, because of the large variation, but this is too large a difference to be ignored. This is one of the points where the limitations of the study design need to be more carefully addressed, as the small plot size and low replication reduce the statistical power of the experiment. It is important to mention the limitation of the design not only in the Methods section, but also in case it does affect the interpretation of the Results.

**[Reply]** Thank you very much for your insightful comment and suggestion. Indeed, our results showed the plot-averaged absolute and relative growth rates of basal area and aboveground biomass of trees were not affected by N fertilization. However, across the individuals of *C. eyrei*, the small trees 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 has not significantly changed with the N fertilization. Moreover, 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, indicating contrast responses of plant growth forms to N fertilization.

As you pointed out, the Figure 2 panel c did suggest that the growth rate of large trees has almost doubled and the significance of N fertilization on the large trees might be hidden by the large variation

or low replication. We rephrased the interpretation of Figure 2 panel c in Result 3.2 at page 9 as follows: “As opposed to the negative responses of small trees to N fertilization, the basal area increment and RGR of median (DBH of 10-30 cm) and large *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-3c and 3e-3f,  $p>0.05$  in all cases)” [Lines 261-266].

In addition, we added the discussion on the influence of low replication and high spatial heterogeneity on plant growth, especially of the large *C. eyrei* individuals in Discussion 4.1 as follows: “Furthermore, our observation of large trees with DBH >30 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” [Lines 329-340].

**[Comment] 3.** Figure S1 provides good information in addition on what is going on at the sites, and should not be buried in the SI. Please include this, and a paragraph outlining the implications of this data into the main manuscript.

**[Reply]** Thanks for your suggestions. Yes, following your suggestion, we moved Figure SI to the main context as Figure I. We also added Figure I panel c to show the changes of soil N:P ratio with the effect of N fertilization. Corresponding to these changes, we added the descriptions on detail method and statistical analysis used in Figure I in the Methods section. Moreover, we added a new paragraph in Results to state the changes of soil N and P contents and pH as follows: “3.4 years of N fertilization significantly increased the N content of 0-10 cm soil ( $p=0.03$ ), especially in NI00 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 fertilization also decreased mildly soil pH and aggravated soil acidification (Fig. 1d,  $p=0.05$ )” [Lines 239-243].

**[Comment] 4.** As reviewer#1 already commented, please remove the reference to the

unpublished material of the P fertilisation experiment (e.g. L289-298), because without proper explanation of the experiment design, results and their uncertainty, these data appear unsubstantiated.

**[Reply]** Thank you. We have removed the references and related P fertilization experiment in the revised manuscript.

**[Comment]** 5. As reviewer #1 already commented, the environmental relevance of the magnitude of the fertilisation is questionable. Please down-tone any reference to N deposition in the introduction (e.g. first paragraph), as your experiment is not designed to address the likely response of N deposition on these ecosystems.

**[Reply]** Thank you for the helpful suggestions. We have carefully checked the whole text and rephrased the interpretation of N deposition to avoid misleading wording. Especially, we rephrased several sentences in Introduction section as well in Abstract, to down-tone the effects of N deposition.

# 1 **Contrasting growth responses among plant growth forms to nitrogen** 2 **fertilization in a subtropical forest in China**

3  
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## 19 20 **Abstract**

21 Reactive nitrogen (N) increase in the biosphere ~~Atmospheric nitrogen (N) deposition~~ has  
22 been a noteworthy aspect of global change-, producing considerable ecological effects on the  
23 functioning and dynamics of the terrestrial ecosystems. A number of observational studies  
24 have explored responses of plants to experimentally simulated N deposition-enrichment in  
25 boreal and temperate forests. Here we asked how the dominant trees and different plant  
26 growth forms respond to experimental N ~~deposition-enrichment~~ in a subtropical forest in  
27 China. We conducted a 3.4-year N fertilization experiment in an old-aged subtropical  
28 evergreen broad-leaved forest in eastern China with three treatment levels applied to nine 20\_  
29 m×20 m plots and replicated in three blocks. We divided the plants into trees, saplings, shrubs  
30 (including tree seedlings), and ground-cover plants (ferns) according to the growth forms, and  
31 then measured the absolute and relative basal area increments of trees and saplings and the  
32 aboveground biomass of understory shrubs and ferns. We further grouped individuals of the  
33 dominant tree species *Castanopsis eyrei* into three size classes to investigate their respective  
34 growth responses to the N fertilization. Our results showed that the plot-averaged absolute

35 and relative growth rates of basal area and aboveground biomass of trees were not affected by  
36 N fertilization. Across the individuals of *C. eyrei*, the small trees with a DBH (diameter at  
37 breast height) of 5-10 cm has declined by 66.4% and 59.5%, respectively, in N50 (50 kg N  
38 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  
39 trees with a DBH of >10 cm has not significantly changed with the N fertilization. The  
40 growth rate of small trees, saplings and the aboveground biomass of understory shrubs and  
41 ground-cover ferns decreased significantly in the N fertilized plots. Our findings suggested  
42 that N might not be a limiting nutrient in this mature subtropical forest, and the limitation of  
43 other nutrients in the forest ecosystem might be aggravated by the enhanced N  
44 ~~deposition~~availability, potentially resulting in an adverse effect on the development of natural  
45 subtropical forest.

46

47 **Key-words:** *Castanopsis eyrei*, N fertilization, plant growth, shrub layer, subtropical forest,  
48 tree layer, ground-cover fern

## 49 1 Introduction

50

51 Reactive nitrogen (N) increase in the biosphere, especially atmospheric N deposition,  
52 ~~Atmospheric nitrogen (N) deposition~~ is a globally prevalent phenomenon (Galloway *et al.*  
53 2004). It has become a serious environmental issue in China, especially in the southeastern  
54 regions, with ~~the~~ drastic increase of ~~nitrogen-N oxides emissions~~ entering terrestrial  
55 ecosystems, ~~—~~ which producing considerable ecological effects on the functioning and  
56 dynamics of the terrestrial ecosystems (Liu *et al.* 2013; Gu *et al.* 2015). ~~On the one hand,~~  
57 ~~most forest ecosystems show increased productivity and stand biomass with N deposition~~  
58 ~~(Magnani *et al.* 2007). A recent study employing a model simulation suggests that N~~  
59 ~~deposition has contributed to a 4.8% increase in the total carbon (C) storage of China's~~  
60 ~~forests between 1981 and 2010 (Gu *et al.* 2015). On the other hand, N deposition has reduced~~  
61 ~~species richness in terrestrial ecosystems (Lu *et al.* 2010; Dirnböck *et al.* 2014) and, in~~  
62 ~~extreme cases, can cause N saturation with negative effects on ecosystem functioning in~~  
63 ~~forest ecosystems (Aber *et al.* 1998).~~

64

65 Since the 1990s, ~~N deposition has been~~ the simulated ~~with~~ N-fertilization experiments have  
66 been conducted in various forest ecosystems to explore the responses of plants and other  
67 organisms to the potential high nitrogen deposition N enrichment and changes of soil N  
68 availability (e.g., Wright & Tietema 1995; Bobbink *et al.* 2010; Fowler *et al.* 2015). Although  
69 a number of studies have reported a general positive effect of N enrichment on plants in  
70 N-limited forests and a negative effect of excess N have been shown in a number of studies  
71 (e.g., Aber *et al.* 1998<sup>89</sup>; Högberg *et al.* 2006; Gilliam 2006; Thomas *et al.* 2010), specific  
72 responses of plants appeared to be highly growth form-dependent and ecosystem-dependent  
73 (LeBauer & Treseder 2008; Bedison & McNeil 2009; Dirnböck *et al.* 2014).—

74

75 Due to the widespread ~~high amount of~~ heavy N deposition in Europe and America, numerous  
76 studies that focused on the growth responses of plants to N enrichment have been carried out  
77 in boreal and temperate forests during the past several decades (Magill 2000; Högberg *et al.*  
78 2006). These studies showed that most trees have a positive growth response to N  
79 fertilization and therefore have higher potential carbon sequestration because the status of N  
80 limitation was largely alleviated by the increasing N inputs (e.g., Thomas *et al.* 2010;  
81 BassiriRad *et al.* 2015). However, the understory plants in these forest ecosystems  
82 inconsistently showed general negative responses to N enrichment with declined biomass or

83 shifted community structure (Rainey *et al.* 1999; Du *et al.* 2014; Dirnböck *et al.* 2014). In  
84 addition to the opposite responses of trees and understory plants to N enrichment, differences  
85 remained in the effects of N enrichment on single plant growth form in these forests.  
86 Generally, the limited light availability in these ecosystems with high tree canopy cover was  
87 ascribed to the negative effects of N fertilization (Strengbom & Nordin 2008).

88  
89 Recently, the effects of N ~~deposition~~ enrichment on tropical forests raised researchers'  
90 concern. Fertilization experiments in tropical forests showed different growth responses of  
91 trees to nutrient addition among individual size levels, understory shrubs and tree seedlings  
92 (Wright *et al.* 2011; Pasquini & Santiago 2012; Santiago *et al.* 2012) which contrasted with  
93 the ones found for trees in the previously described experiments. For example, phosphorus (P)  
94 fertilization enhanced the growths of small trees and seedlings but had no effect on median  
95 and large trees, while N addition did not show any significant effect on plant growth in a  
96 lowland tropical forest (Alvarez-Clare *et al.* 2013). In addition to the ubiquitous concept that  
97 P was a critical element driving plant growth in tropical forests (Vitousek *et al.* 1991),  
98 heterogeneous nutrient limitation that the growths of plants were co-limited by multiple  
99 nutrients was further proposed to explain why diverse plants respond differently to ~~nutrient-N~~  
100 ~~addition~~ enrichment (Wright *et al.* 2011; Alvarez-Clare *et al.* 2013; Wurzbürger & Wright  
101 2015). Nevertheless, the patterns of specific nutrient limitation and responses of plants to  
102 ~~added-nutrient~~ N enrichments among diverse forest ecosystems need further exploration.

103  
104 As most of the nutrient fertilization experiments have focused on boreal forests, temperate  
105 forests and lowland tropical forests, few studies have investigated the effects of N ~~deposition~~  
106 enrichment on subtropical forests despite their broad distribution throughout the world and  
107 great contribution to global ~~C~~ carbon sink (Zhou *et al.* 2013; Yu *et al.* 2014; Huang *et al.*  
108 2015). With ~~the~~ increasing N ~~deposition~~ deposited in ~~the~~ subtropical ~~region~~ ecosystems,  
109 ~~especially~~ in ~~central and southeastern~~ eastern China (Du *et al.* 2014), it ~~i~~'s important to  
110 diagnose the nutrient limitation and evaluate the responses of different plant growth forms to  
111 N ~~deposition~~ enrichment in subtropical forests for the assessment of carbon sequestration and  
112 community dynamics.

113  
114 To better predict the responses of subtropical forests and different plant growth forms to N  
115 ~~deposition~~ enrichment, we carried out a 3.4-year N fertilization experiment with three  
116 treatment levels applied to nine 20 m × 20 m plots and replicated in three blocks in a



117 subtropical forest in south-eastern China. We attempt to explore whether N is a limiting  
118 element in the old-aged evergreen broad-leaved subtropical forest. We hypothesize a positive  
119 response of trees to N fertilization, but a negative response of understory growth forms to N  
120 fertilization due to the expansion of canopy crown and consequent reduction of light  
121 availability.

122

## 123 **2 Materials and methods**

124

### 125 **2.1 Study site and experimental design**

126 The N fertilization experiment site was located at 30°01'47" N latitude and 117°21'23" E  
127 longitude at an altitude of 375 metres in the natural conservation zone of Guniujiang in Anhui  
128 Province, eastern China. As a commendable representative of the typical subtropical  
129 broadleaved evergreen forest, the Guniujiang experimental site is an important part of the  
130 NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du *et al.*  
131 2013), because of its representativeness in both species composition and landscape structure  
132 in the subtropical evergreen forest region. The study area has a humid climate with strong  
133 summer monsoons with an annual average precipitation of 1,700 mm and an average annual  
134 temperature of 14.9 °C. The soil in this area has been classified as yellow brown earth  
135 (Chinese Soil Taxonomic Classification), and the pH<sub>H2O</sub> value at 0-10 cm soil depth was  
136 4.58±0.05 (mean±SE). The total **nitrogen****N**, **phosphorus****P**, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub>-N content in the  
137 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>,  
138 respectively (Li *et al.* 2015).

139

140 The study was conducted in a well-protected, mature subtropical evergreen forest (>300 year  
141 age) with a three-layered vertical structure: the canopy tree layer (DBH>5 cm and height>5  
142 m); the understory layer of saplings, shrubs and seedlings (DBH<5 cm and height<5 m); and  
143 the ground-cover layer (ferns and herbs). The average density and basal area of trees were  
144 1,219 trees ha<sup>-1</sup> and 36.35 m<sup>2</sup> ha<sup>-1</sup>, respectively; *Castanopsis eyrei* was the dominant species  
145 (which was also an important species at some other sites in subtropical forests) and accounted  
146 for 87% of the total aboveground biomass of trees. The understory saplings and shrubs  
147 contained several species, including *Cleyera japonica*, *Camellia cuspidata*, *Rhododendron*  
148 *ovatum*, *Eurya muricata*, *Cinnamomum japonicum*, *Cinnamomum subavenium*, *Sarcandra*  
149 *glabra*, and *C. eyrei*, and other native subtropical evergreen species (Table 1). Two fern  
150 species (*Woodwardia japonica* and *Dryopteris hwangshanensis*) and an orchid (*Cymbidium*

151 *tortisepalum* var. *longibracteatum*) appeared on the floor layer, while *W. japonica* exclusively  
152 dominated the floor layer with a coverage of 10%-20%.

153

154 We began N fertilization in March 2011. A randomized block design was used to avoid spatial  
155 heterogeneity. We chose three blocks with similar stand growth, species composition and site  
156 condition to establish three N treatments in each block: CK (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N50 (50 kg N  
157 ha<sup>-1</sup> yr<sup>-1</sup>), and N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>). ~~As the amount of wet N deposition in this region  
158 was 5.9-7.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>, we applied N fertilization at these two levels to simulate the  
159 extreme N deposition cases.~~ In total, nine 20 m × 20 m plots were established with a 5-10 m  
160 buffer zone between each plot. The total NH<sub>4</sub>NO<sub>3</sub> was divided into 12 dosages and applied to  
161 the forest in each month at regular intervals. NH<sub>4</sub>NO<sub>3</sub> in dosages of 0.48 kg/plot and 0.95  
162 kg/plot were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly in  
163 N50 and N100 plots using a back-hatch sprayer. The unfertilized plots (controls) were  
164 similarly treated with 15 L of fresh water without NH<sub>4</sub>NO<sub>3</sub>.

165

## 166 **2.2 Sampling and measurement**

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

173

174

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

175 where DBH<sub>1</sub> represents the initial DBH (cm) of trees measured in March 2011, and X<sub>2</sub> and X<sub>1</sub>  
176 (mm) represent the widths of gaps on the tapes measured in July 2014 and at the beginning of  
177 the experiment, respectively.

178

179 The basal area is a common indicator for weighing the biomass of trees. Therefore, tree basal  
180 area increments were calculated to indicate the responses of tree biomass to the N fertilization.  
181 First, to test community-level responses of tree layer to N fertilization, we calculated the sum  
182 of total basal area increase (m<sup>2</sup> ha<sup>-2</sup> year<sup>-1</sup>) of all trees in a plot after 3.4 years of N  
183 fertilization and divided this value by the period of N fertilization (3.4 years) to obtain the  
184 annual basal area increase rate of the trees (dead trees were not included). Second, relative

185 annual basal area growth rate (RGR,  $\text{m}^2 \text{m}^{-2} \text{year}^{-1}$ ) was used to eliminate the conceivable  
186 interferential effects resulting from the differences in the number and size of original  
187 individuals among plots according to the following equation, similar to Alvarez-Clare et al.'s  
188 method (2013):

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

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

193  
194 Because *C. eyrei* was the only dominant species in the tree layer, we separated it from other  
195 tree species and grouped its individuals into three classes based on their DBH values (i.e.,  
196 5-10 cm, 10-30 cm and >30 cm) to investigate the effects of N fertilization on the growth of  
197 trees after removing the plant species and original size factors. During the monitoring of tree  
198 growth, dead trees were recorded. Then, we calculated the aboveground biomass increments  
199 of trees and the proportion of dead biomass using allometric equations (see Table S1).

200  
201 We examined the effects of N fertilization on understory tree saplings distributed in the plots  
202 according to their sizes and characteristics. For small trees with  $\text{DBH} < 5$  cm and  $\text{height} > 2$  m  
203 (defined as “saplings”), DBH was measured at the beginning of N fertilization and in July  
204 2014. Then, annual basal area growth rate and RGR of saplings were calculated based on  
205 DBH changes. For very small trees or shrubs with  $\text{DBH} < 5$  cm and  $\text{height} < 2$  m (defined as  
206 “shrubs/seedlings”), we set two  $5 \text{ m} \times 5 \text{ m}$  subplots in each plot along a diagonal direction  
207 and investigated the abundance, dominance, basal diameter (diameter at 10 cm above the  
208 ground), height and crown diameters of all shrubs/seedlings inside the subplots at two  
209 specific times. The first time was at the beginning of N fertilization (March 2011), and the  
210 second was in July 2014. The length, width and number of fern leaves were measured  
211 carefully in the above-mentioned subplots, and the allometric equations for seven dominant  
212 species were then obtained (Table S1). Because the average aboveground biomass of  
213 shrubs/seedlings and ferns showed no significant differences across three N treatments before  
214 N fertilization in March 2011, we regarded the distribution of these understory  
215 shrubs/seedlings and ferns to be homogeneous among the three treatments. Then we  
216 identified the effects of N fertilization by comparing the aboveground biomass of  
217 shrubs/seedlings and ferns in 2014 among the different treatments. Meanwhile, to investigate  
218 the canopy cover and understory light availability, we used a digital camera (Canon, Japan)

219 with a fisheye lens (Sigma circular fisheye) to take photographs of canopy. In each subplot,  
220 we put the camera at 1m above ground and took 5 photos upwards from understory.

221

222 In addition, to further explore the influences of N fertilization on plants' growth from  
223 biogeochemical aspect ~~at the Discussion part~~, we measured soil N, P content and pH (~~for~~  
224 ~~details, see “Methods of soil sampling and nutrient detection” in the Supplementary~~  
225 ~~Materials~~). Specifically, we set three subplots randomly within each plot and collected three  
226 subsamples of 0-10 cm soil for each subplot using a hand-held steel soil borer (3 cm in  
227 diameter), during investigation of the understory plants investigation. Then, the three  
228 subsamples were mixed together to form one sample per plot and transported to a laboratory  
229 and air dried naturally. After air-dried, soil samples were ground with a ball mill (NM200,  
230 Retsch, Haan, Germany) and screened through a 100 mesh sieve. The N concentration of soil  
231 was measured using an elemental analyser (2400 Series2 CHNS/O Elemental Analyzer,  
232 Perkin-Elmer, USA). After acid digestion of the samples, soil P concentrations were  
233 measured using a flow injection analysis instrument (AutoAnalyzer3, Bran+Lubbe City,  
234 Germany). ~~In this study, mass total N and mass total P were used. Soil pH was measured by~~  
235 dry soil in water suspension with a water:soil ratio of 1:2.5.

236

### 237 **2.3 Statistical analysis**

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

251

### 252 **3 Results**

253

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

The indicator of forest canopy (i.e., [1-Fmv]) showed no significant differences between unfertilized and fertilized plots with 3.4 years of N fertilization (Table 2). 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 fertilization also decreased mildly 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. 1a2a~2c). 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. 1d2d).

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. 2a3a-32ff). 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. 2a3a,  $p_{\text{treat}}=0.025$ ). Specifically, the N50 and N100 fertilization decreased the absolute basal area increments of small individual trees at rates of  $2.2 \text{ cm}^2 \text{ tree}^{-1} \text{ year}^{-1}$  and  $1.98 \text{ cm}^2 \text{ tree}^{-1} \text{ year}^{-1}$ , 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. 2d3d,  $p_{\text{treat}}=0.19>0.05$ ). ~~Inconsistent with~~ As opposed to the negative responses of small trees to N fertilization, the basal area increment and RGR of median *C. eyrei* individuals ~~with~~ (DBH of 10-30 cm) and large *C. eyrei* individuals ~~with~~ (DBH of >30cm) showed no significant responses to N fertilization, although but the averaged growth rate of large *C. eyrei* individuals with DBH of >30cm in N50 plots almost doubled the value of the corresponding large individuals in unfertilized plots (Fig. 2b3b-32c and 32ee-32f,  $p_{\text{treat}}>0.05$  in all cases).

### 3.2-3 Growth responses of understory saplings, shrubs/seedlings, and ferns to N fertilization

288 Responses of understory saplings to N fertilization were similar to those of small dominant  
289 trees. Although the annual absolute increments of basal area increments of saplings showed  
290 no significant response to N fertilization (Fig. [3a4a](#),  $p=0.72>0.05$ ), the RGR of sapling  
291 growing in N50 and N100 plots showed a substantial decrease at rates of  $0.021 \text{ m}^2 \text{ m}^{-2} \text{ yr}^{-1}$   
292 and  $0.019 \text{ m}^2 \text{ m}^{-2} \text{ yr}^{-1}$ , respectively, compared to sapling growing in unfertilized plots (Fig.  
293 [3b4b](#),  $p_{\text{treat}} < -0.001$ ). In addition, a general negative effect of N fertilization also occurred on  
294 understory shrubs and ground-cover ferns. The aboveground biomass of seven predominant  
295 shrubs/seedlings was drastically decreased by 69.4% and 79.1% in N50 and N100 fertilized  
296 plots, respectively, compared with those in the unfertilized plots (Fig. [4a5a](#),  $p < 0.01$ ).  
297 Remarkably, the aboveground biomass of ground-cover ferns significantly declined by 92.4%  
298 and 93.4% in N50 and N100 fertilized plots (Fig. [4b5b](#),  $p < 0.05$ ).

299

## 300 4 Discussion

301

### 302 4.1 Growth responses of trees to N fertilization

303 Nutrient limitation was generally determined through evaluating ecosystem feedbacks to  
304 nutrient addition (Vitousek 1991; Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013). When the  
305 forest ecosystems showed a positive response to added nutrient, e.g., plant growth or rates of  
306 physiological processes were promoted, the added nutrient then could be interpreted as  
307 limiting to the ecosystem, otherwise, as not limiting to the ecosystem (Santiago 2015). We  
308 initially expected positive growth responses of trees exposed to N fertilization in this  
309 subtropical forest because N availability in the soil would be enhanced by N fertilization and  
310 the potential N limitation of plants in the forest ecosystem could be alleviated. However,  
311 contrary to our expectation, we did not observe strong positive growth responses of trees to N  
312 fertilization (Figs. [1-2](#) and [2-3](#)). Across individual trees of different sizes and plant growth  
313 forms, we only observed substantial negative responses of small trees (5-10 cm DBH; Fig. [2a](#)  
314 [3a](#) and [2d3d](#))—and saplings (Fig. [3a4a](#)–[3b](#) and [4b](#)) and weak responses of median and large  
315 trees (>10 cm DBH) to N fertilization (Fig. [2b3b](#)–[2e-3c](#) and [2f3ef](#)–[2e3fe](#)), which further  
316 demonstrated that the growth of trees in this old-aged subtropical forest was not essentially  
317 limited by N as hypothesized.

318

319 Contrasted with previous positive responses of trees to N fertilization in boreal and temperate  
320 forests which were considered as N limited ecosystems (Högberg *et al.* 2006; Thomas *et al.*  
321 2010; BassiriRad *et al.* 2015), our finding of the unchanged responses of trees to N

322 fertilization was partly consistent with observations of trees from tropical forests (e.g.,  
323 Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013). Studies from mature tropical forests have  
324 revealed that P availability was a critical element shaping tree species distribution and  
325 productivity (Santiago 2016; Dalling *et al.* 2016). Given the similar high-weathered soil  
326 properties, humid climatic conditions and dominant evergreen broadleaf trees in mature  
327 subtropical forest as those in wet tropical forest, we speculated that P limitation, rather than N  
328 limitation, might have played a key role in influencing growth of plants in subtropical forest.

329  
330 The N and P stoichiometry of soil might have objectively provided indicators of P limitation  
331 with the effects of N fertilization in this subtropical forest, because soil N contents and N:P  
332 ratio in N fertilized plots were remarkably higher than those in unfertilized plots (Fig. 1).

333 Additionally, limitation of other nutrients, such as K (potassium) which was highlighted in  
334 tropical forests, and their combination as well as heterogeneous nutrient limitation of specific  
335 species, plant growth forms and individuals in different sizes may warrant further  
336 consideration in subtropical forests (Wright *et al.* 2011; Santiago *et al.* 2012; Alvarez-Clare *et*  
337 *al.* 2013). \_\_\_

338 ~~As a supplement, we used a P fertilization experiment conducted in another subtropical forest~~  
339 ~~with similar community structure nearby our experiment site to check if P limits plant growth.~~  
340 ~~We applied 50 kg ha<sup>-1</sup> yr<sup>-1</sup> P (P<sub>2</sub>O<sub>5</sub>) to the forest and measured the growth of the dominant~~  
341 ~~tree species (*C. sclerophylla*) following the same steps presented in the ‘Materials and~~  
342 ~~methods’ section in this paper. After two years’ P fertilization, we found that the annual~~  
343 ~~absolute basal area increments and relative basal area in P fertilized plots were 56.0% and~~  
344 ~~101.5% higher, respectively, than in unfertilized plots ( $p=0.02$  and  $p=0.03$ , respectively,~~  
345 ~~unpublished data). Our results from N fertilization and the supplementary P fertilization~~  
346 ~~experiments indicate that plant growth in subtropical forest ecosystems might be highly~~  
347 ~~limited by P, although it is in great need for further verification in the next studies. Similarly,~~  
348

349 Moreover, the high spatial heterogeneity in old-aged subtropical forest, similar to tropical  
350 forests, could be a possible explanation for the lack of significant responses of plot-averaged  
351 basal area growth, RGR, aboveground biomass of trees with a DBH of >5cm and the  
352 proportion of dead trees to N fertilization. In eastern China, the distributions of subtropical  
353 forest stands are quite topographically fragmented, while relative flat stands are required to  
354 avoid N losses and minimize spatial heterogeneity among experimental treatments. The  
355 actual distribution and topography of the subtropical forests limited the number of

356 replications in the N fertilization experiment. This limitation might reduce the statistic power  
357 of N treatment on plot-averaged plant growth rate which has been pointed out in previous  
358 studies ([Wright \*et al.\* 2011](#); [Alvarez-Clare \*et al.\* 2013](#)). Furthermore, our observation of large  
359 trees with DBH >30 cm showed that the averaged growth rate of large *C. eyrei* individuals in  
360 N50 plots almost doubled the value of the corresponding large individuals in unfertilized  
361 plots. Nevertheless, the results of ANOVA showed that the effect was not significant. As the  
362 number of large trees in the experiment was relatively less than the small trees, the low  
363 replication and high spatial site heterogeneity might have reduced the statistical power of N  
364 fertilization on the large trees. Thus, fertilization experiments with more homogeneous plots  
365 and more replicates are warranted to further strengthen these findings. Overall, given the  
366 negative and potential positive effects of N fertilization on small and large trees, it is of  
367 urgent necessity to conduct long-term monitoring of the trees which would provide  
368 alternatives for accurately evaluating the forest dynamics with N fertilization under the  
369 enhanced global N deposition.

370

#### 371 **4.2 Growth responses of small trees, understory saplings, shrubs/seedlings and ferns to** 372 **N fertilization**

373 Although the positive responses of small or juvenile trees to nutrient fertilization has been  
374 reported in boreal, temperate and tropical forest (e.g., [Högberg \*et al.\* 2006](#); [Bedison &](#)  
375 [McNeil 2009](#); [Alvarez-Clare \*et al.\* 2013](#)), our results showed a remarkable negative effect of  
376 N fertilization on small-sized plants including trees, understory saplings, shrubs/seedlings and  
377 ferns. During our field investigation, we also found that the average proportion of dead trees  
378 (Fig. [4d](#)) tended to increase in N fertilized plots although the result was not statistically  
379 significant ( $p_{\text{treat}}=0.50$ ). Additionally, the ground-cover ferns in N100 plots almost  
380 disappeared after 3.4-year N fertilization (personal observation). Given the high stand density  
381 in this mature subtropical forest, we suggest that N fertilization might potentially lead to  
382 increased self- and alien-thinning of individuals through decreasing understory light  
383 availability.

384

385 The pivotal role of light availability in the eco-physiological processes of understory growth  
386 forms has been widely recognized ([Santiago 2015](#)). Due to the limited light availability,  
387 understory plants may not be able to incorporate the added nutrient and promote their  
388 photosynthetic rates ([Alvarez-Clare \*et al.\* 2013](#)). ~~Nevertheless~~ However, a study conducted in  
389 tropical forest with thick canopy showed that photosynthetic process could be enhanced by



390 nutrient addition even under low light availability (Pasquini & Santiago 2012). In a sharp  
391 contrast, the study conducted in an Australian rainforest revealed that understory seedlings  
392 increased growth when the light availability was high, but showed no significant response to  
393 nutrient fertilization in low lights (Thompson *et al.* 1988). These studies, together with our  
394 field observations, suggest that the growth of understory plants is largely co-limited by  
395 nutrient and light availability in the local environment. Further, our results of forest canopy  
396 cover estimated by photographic fisheye showed no significant differences between  
397 unfertilized ( $0.77 \pm 0.01$ ) and N fertilized plots ( $0.76 \pm 0.04$  and  $0.72 \pm 0.01$  in N50 and N100  
398 plots, respectively), which was consistent with the findings of Lu *et al.* (2010). Although the  
399 understory light irradiance fluctuated largely during a day and was very hard to detect  
400 precisely, our measurements of forest canopy cover provided a rough evaluation for light  
401 availability. The results might indicate that other factors in addition to the low light  
402 availability in this old-aged forest had also played a crucial role in influencing understory  
403 plants during 3.4 years' N fertilization.

404

### 405 **4.3 Potential N saturation and plant growth**

406

407 The striking biomass reduction of the understory plants, especially ferns, in response to N  
408 fertilization in our study well corroborated the similar findings in an old-aged tropical forest  
409 at Mt. Dinghushan in China (Lu *et al.*, 2010). Also, consistent with previous studies obtained  
410 from boreal, temperate and tropical forests (Rainey *et al.* 1999; Alvarez-Clare *et al.* 2013;  
411 Dirnböck *et al.* 2014), our experiment revealed that understory small-sized plants responded  
412 sensitively to nutrient fertilization, which might indicate a possibility of N saturation in the  
413 subtropical forest. According to the definition of N saturation addressed by Aber *et al.*  
414 (1998) (i.e., N availability in the forest ecosystem exceeded the demand of plants and  
415 microbes), the drastic decrease of understory ferns, shifted composition of understory plant  
416 community, and cation imbalances of understory species after 7 years' chronic N fertilization  
417 at Harvard Forest, USA, could be interpreted as useful indicators of N saturation (Rainey *et*  
418 *al.* 1999). Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Mt.  
419 Dinghushan also showed signs of N saturation, such as significant increases in nitrate (NO<sub>3</sub>-)  
420 leaching, inorganic N concentration and N<sub>2</sub>O emissions of soils, and soil acidification (Lu *et*  
421 *al.* 2014; Chen *et al.* 2015). In our experiment, ~~we observed mild~~ the soil acidification and  
422 increased soil N concentration in high N fertilized plots (Fig. S1). ~~C-~~ Combined with the  
423 negative responses of understory plants, ~~we~~ suggest that the 3.4-year N fertilization in this

424 mature subtropical forest site has potentially caused N saturation. ~~Nevertheless, but~~ further  
425 observations are still required to explore the mechanisms underlying the changes of different  
426 growth forms with the effects of N enhancement in the subtropical forests.

427

## 428 **5. Conclusion**

429

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

442

443

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452 | **References**

- 453 | Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty,  
454 | S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest  
455 | ecosystems: hypotheses revisited, *BioScience*, 48, 921-934, 1998.
- 456 | Alvarez-Clare, S., Mack, M.C., and Brooks, M.: A direct test of nitrogen and phosphorus  
457 | limitation to net primary productivity in a lowland tropical wet forest, *Ecology*, 94,  
458 | 1540-1551, 2013.
- 459 | BassiriRad, H., Lussenhop, J.F., Sehtiya, H.L., and Borden, K.K.: Nitrogen deposition  
460 | potentially contributes to oak regeneration failure in the Midwestern temperate forests of  
461 | the USA, *Oecologia*, 177, 1-11, 2015.
- 462 | Bedison, J.E., and McNeil, B.E.: Is the growth of temperate forest trees enhanced along an  
463 | ambient nitrogen deposition gradient?, *Ecology*, 90, 1736-1742, 2009.
- 464 | Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante,  
465 | M., Cinderby, S., Davidson, E., and Dentener, F.: Global assessment of nitrogen  
466 | deposition effects on terrestrial plant diversity: a synthesis, *Ecol. Appl.*, 20, 30-59, 2010.
- 467 | Chen, H., Gurnesa, G.A., Zhang, W., Zhu, X.M., Zheng, M.H., Mao, Q.G., Zhang, T., and  
468 | Mo, J.M.: Nitrogen saturation in humid tropical forests after 6 years of nitrogen and  
469 | phosphorus addition: Hypothesis testing, *Functional Ecol.*, 30(2), 305-313, 2015.
- 470 | Dalling, J. W., Heineman, K. –Lopez, O. R., Wright, S. J., and Turner, B. L. in : *The*  
471 | *Paradigm of Phosphorus Limitation, Tropical tree physiology*, eds. Goldstein, G., and  
472 | Santiago, L.S., Springer International Publishing, Switzerland, pp.261-274, 2016.
- 473 | Dirnböck, T., Grandin, U., Bernhardt - Römermann, M., Beudert, B., Canullo, R., Forsius,  
474 | M., Grabner, M.T., Holmberg, M., Kleemola, S., and Lundin, L.: Forest floor vegetation  
475 | response to nitrogen deposition in Europe, *Global Change Biol.*, 20, 429-440, 2014.
- 476 | Du, E.Z., Zhou, Z., Li, P., Hu, X.Y., Ma, Y.C., Wang, W., Zheng, C.Y., Zhu, J.X., He, J.S., and  
477 | Fang, J.Y.: NEECF: a project of nutrient enrichment experiments in China's forests, *J.*  
478 | *Plant Ecol.*, 6, 428-435, 2013.
- 479 | Du, E.Z., Liu, X.Y., and Fang, J.Y.: Effects of nitrogen additions on biomass, stoichiometry  
480 | and nutrient pools of moss *Rhytidium rugosum* in a boreal forest in Northeast China,  
481 | *Environ. Poll.*, 188, 166-171, 2014.
- 482 | Fowler, Z. K., Adams, M. B., and Peterjohn, W. T.: Will more nitrogen enhance carbon  
483 | storage in young forest stands in central Appalachia?, *For. Ecol. Manage.*, 337, 144-152,  
484 | 2015.

485 | Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P.,  
486 | Asner, G.P., Cleveland, C., Green, P., and Holland, E.: Nitrogen cycles: past, present,  
487 | and future, *Biogeochemistry*, 70, 153-226, 2004.

488 | [Gilliam, F.S.: Response of the herbaceous layer of forest ecosystems to excess nitrogen](#)  
489 | [deposition, \*J. Eco.\*, 94, 1176-1191, 2006.](#)

490 | Gu, F.X., Zhang, Y.D., Huang, M., Tao, B., Yan, H.M., Guo, R., and Li, J.: Nitrogen  
491 | deposition and its effect on carbon storage in Chinese forests during 1981–2010, *Atmos.*  
492 | *Environ.*, 123, 171-179, 2015.

493 | Huang, Y.M., Kang, R., Mulder, J., Zhang, T., and Duan, L.: Nitrogen saturation, soil  
494 | acidification, and ecological effects in a subtropical pine forest on acid soil in southwest  
495 | China, *J. Geophys. Res.*, 120, 2457-2472, 2015.

496 | Högberg, P., Fan, H.B., Quist, M., Binkley, D., and Tamm, C. O.: Tree growth and soil  
497 | acidification in response to 30 years of experimental nitrogen loading on boreal forest,  
498 | *Global Change Biol.*, 12, 489-499, 2006.

499 | [LeBauer, D.S., and Treseder, K.K.: Nitrogen limitation of net primary productivity in](#)  
500 | [terrestrial ecosystems is globally distributed, \*Ecology\*, 89\(2\), 371-379, 2008.](#)

501 | Li, P., Han, W.X., Zhang, C., Tian, D., Xu, X.X., and Fang, J.Y.: Nutrient resorption of  
502 | *Castanopsis eyrei* varies at the defoliation peaks in spring and autumn in a subtropical  
503 | forest, Anhui, China, *Ecol. Res.*, 30, 111-118, 2015.

504 | Liu, X.J., Zhang, Y., Han, W.X., Tang, A.H., Shen, J.L., Cui, Z.L., Vitousek, P., Erisman, J.  
505 | W., Goulding, K., and Christie, P.: Enhanced nitrogen deposition over China, *Nature*,  
506 | 494, 459-462, 2013.

507 | Lu, X.K., Mao, Q.G., Gilliam, F. S., Luo, Y.Q., and Mo, J.M.: Nitrogen deposition  
508 | contributes to soil acidification in tropical ecosystems, *Global Change Biol.*, 20,  
509 | 3790-3801, 2014.

510 | Lu, X.K., Mo, J.M., Gilliam, F.S., Zhou, G.Y., and Fang, Y.T.: Effects of experimental  
511 | nitrogen additions on plant diversity in an old - growth tropical forest, *Global Change*  
512 | *Biol.*, 16, 2688-2700, 2010.

513 | Magill, A. H., Aber, J. D., Berntson, G. M., McDowell, W. H., Nadelhoffer, K. J., Melillo, J.  
514 | M., and Steudler, P.: Long-term nitrogen additions and nitrogen saturation in two  
515 | temperate forests, *Ecosystems*, 3, 238-253, 2000.

516 | ~~Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle,~~  
517 | ~~A., Hari, P., Jarvis, P. G., and Kolari, P.: The human footprint in the carbon cycle of~~

518 [temperate and boreal forests, Nature, 447, 849-851, 2007.](#)

519 Pasquini, S., and Santiago, L.: Nutrients limit photosynthesis in seedlings of a lowland  
520 tropical forest tree species, *Oecologia*, 168, 311-319, 2012.

521 [R Development Core Team. R: A Language and Environment for Statistical Computing, R  
522 Foundation for Statistic Computing, Vienna, 2010.](#)

523 Rainey, S. M., Nadelhoffer, K. J., Silver, W. L., and Downs, M. R.: Effects of chronic  
524 nitrogen additions on understory species in a red pine plantation, *Ecol. Appl.*, 9, 949-957,  
525 1999.

526 Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N., and Turner,  
527 B.L.: Tropical tree seedling growth responses to nitrogen, phosphorus and potassium  
528 addition, *J. Ecol.*, 100, 309-316, 2012.

529 Santiago, L. S.: Nutrient limitation of eco-physiological processes in tropical trees, *Trees*, 29,  
530 1291-1300, 2015.

531 Santiago, L. S., and Goldstein, G.: Is Photosynthesis Nutrient Limited in Tropical Trees?  
532 Tropical tree physiology, Springer International Publishing, 299-318, 2016.

533 Strengbom, J., and Nordin, A.: Commercial forest fertilization causes long-term residual  
534 effects in ground vegetation of boreal forests, *For. Ecol. Manage.*, 256, 2175-2181,  
535 2008.

536 [Thimonier, A., Sedivy, I., Schleppei, P.: Estimating leaf area index in different types of mature  
537 forest stands in Switzerland: a comparison of methods, Eur J Forest Res., 129, 543-562,  
538 2010.](#)

539 Thompson, W., Stocker, G.C., and Kriedemann, P.E.: Growth and photosynthetic response to  
540 light and nutrients of *flindersia brayleyana* F. Muell., a rainforest tree with broad  
541 tolerance to sun and shade, *Funct Plant Boil.*, 15, 299-315, 1988.

542 Thomas, R.Q., Canham, C.D., Weathers, K.C., and Goodale, C.L.: Increased tree carbon  
543 storage in response to nitrogen deposition in the US, *Nature Geosci.*, 3, 13-17, 2010.

544 Vitousek, P. M., and Howarth, R. W.: Nitrogen limitation on land and in the sea: how can it  
545 occur? *Biogeochemistry*, 13, 87-115, 1991.

546 Wright, R. F., and Tietema, A.: Ecosystem response to 9 years of nitrogen addition at Sogndal,  
547 Norway, *For. Ecol. Manag.*, 71, 133-142, 1995.

548 Wright, S.J., Yavitt, J.B., Wurzburger, N., Turner, B.L., Tanner, E.V., Sayer, E.J., Santiago,  
549 L.S., Kaspari, M., Hedin, L.O., and Harms, K.E.: Potassium, phosphorus, or nitrogen  
550 limit root allocation, tree growth, or litter production in a lowland tropical forest,  
551 *Ecology*, 92,1616-1625, 2011.

552 | Wurzburger, N., and Wright, S. J.: Fine - root responses to fertilization reveal multiple  
553 | nutrient limitation in a lowland tropical forest, *Ecology*, 96, 2137-2146. 2015.

554 | Yu, G.R., Chen, Z., Piao, S.L., Peng, C.H., Ciais, P., Wang, Q.F., Li, X.R., and Zhu, X. J.:  
555 | High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon  
556 | region, *P. Natl. Acad. Sci. USA*, 111, 4910-4915, 2014.

557 | Zhou, G.Y., Peng, C.H., Li, Y.L., Liu, S.Z., Zhang, Q.M., Tang, X.L., Liu, J.X., Yan, J.H.,  
558 | Zhang, D.Q., Chu, and G.W.: A climate change-induced threat to the ecological  
559 | resilience of a subtropical monsoon evergreen broad-leaved forest in Southern China,  
560 | *Global Change Biol.*, 19, 1197-1210, 2013.

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

Growth forms	Species	Growth variable		
		TBA (m <sup>2</sup> ha <sup>-1</sup> )	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 & 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 m <sup>-2</sup> )		
Ferns	<i>Woodwardia japonica</i>	1.19 $\pm$ 0.23		

565



566  
567

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

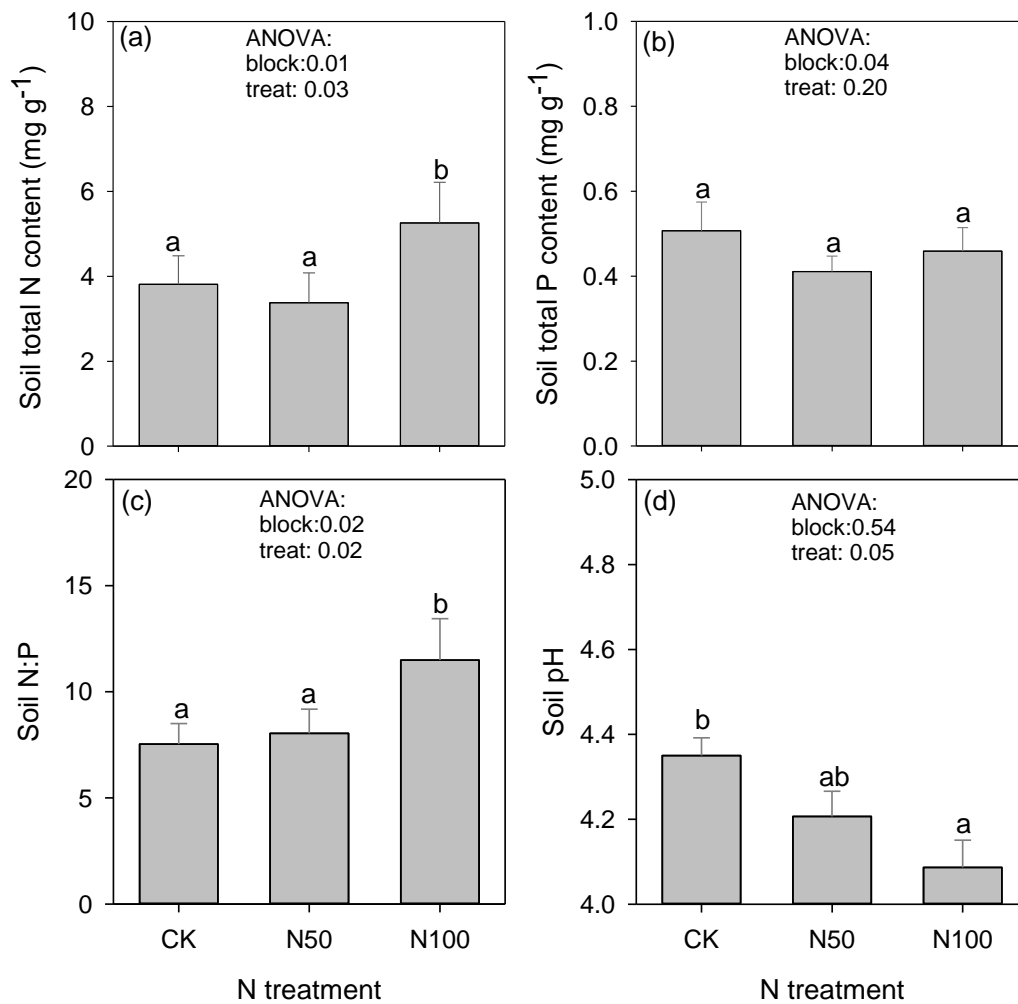
<u>Treatment</u>	<u><i>n</i></u>	<u>Canopy cover</u>	
		<u>mean</u>	<u>SE</u>
<u>CK</u>	<u>3</u>	<u>0.77</u>	<u>0.01</u>
<u>N50</u>	<u>3</u>	<u>0.76</u>	<u>0.04</u>
<u>N100</u>	<u>3</u>	<u>0.72</u>	<u>0.01</u>

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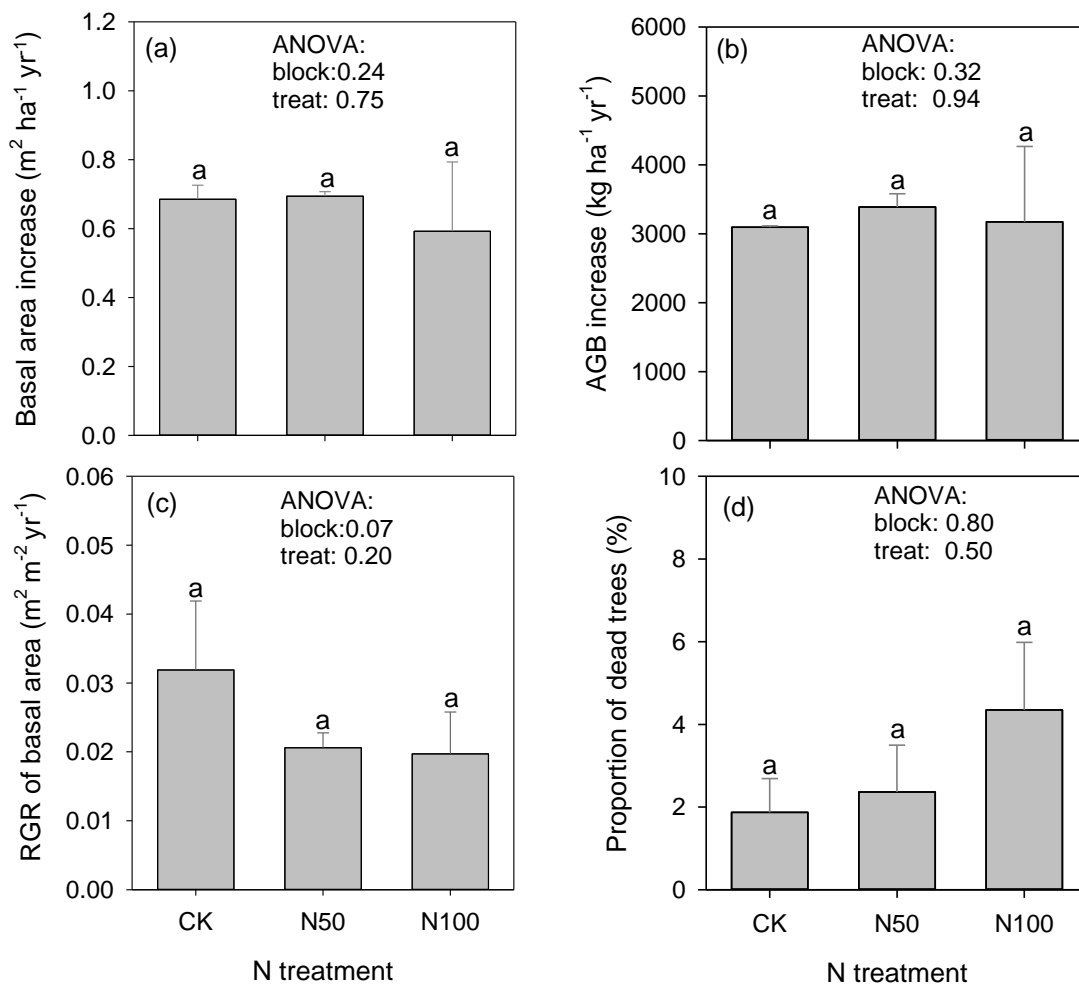
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**Figure 1** Effects of N fertilization on soil nutrient content, N:P ratio and pH (mean  $\pm$  se, n=3) at the soil depth of 0-10 cm. (a) Total N content per gram soil; (b) total P content per gram soil; (c) N:P ratio and (d) soil pH. Numbers in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



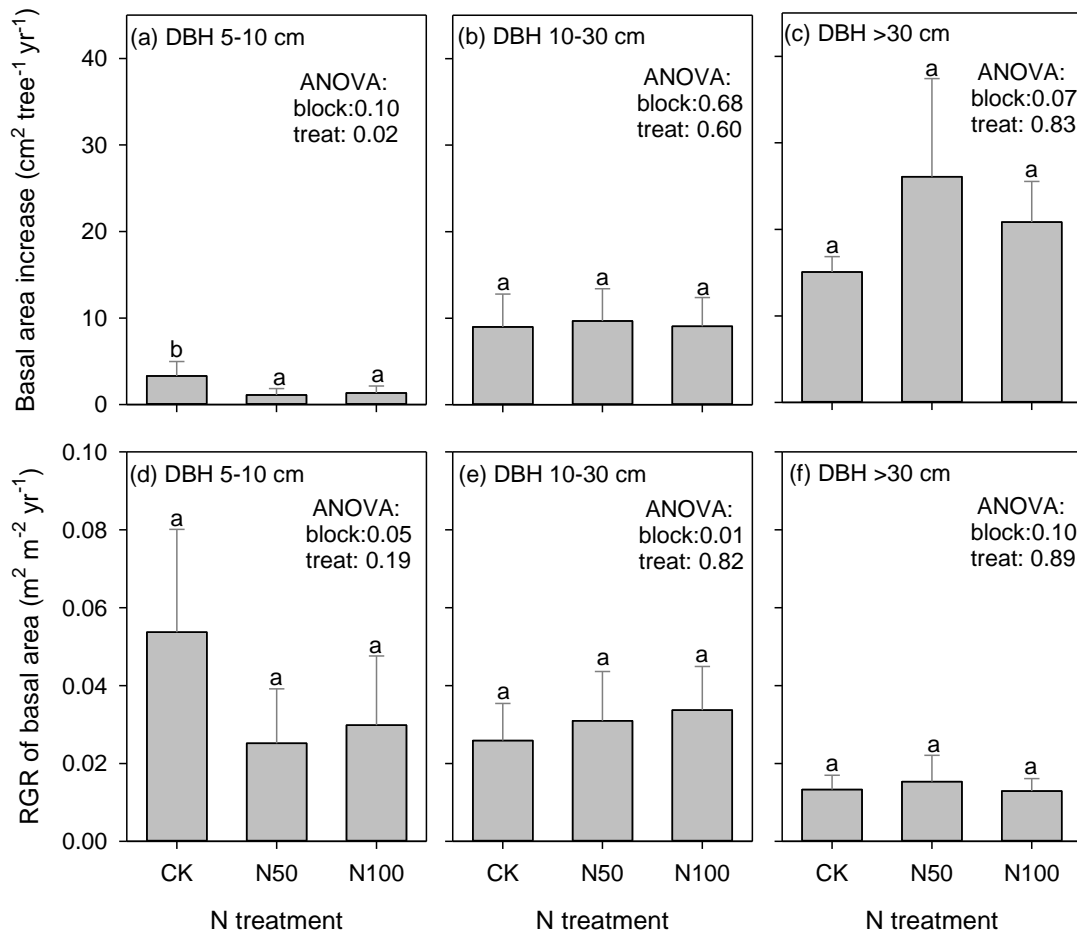
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573 **Figure 1-2** Effects of N fertilization on the growth and mortality of all trees (mean  $\pm$  se). (a)  
 574 Absolute basal area increase of all trees; (b) aboveground biomass increase of all trees; (c)  
 575 relative growth rate of total tree basal area; and (d) the proportion of all dead trees. The  
 576 proportion of dead trees was calculated using the aboveground biomass of all dead trees  
 577 during the experiment divided by the total aboveground biomass of all trees in 2014.  
 578 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis  
 579 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  
 580 N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).



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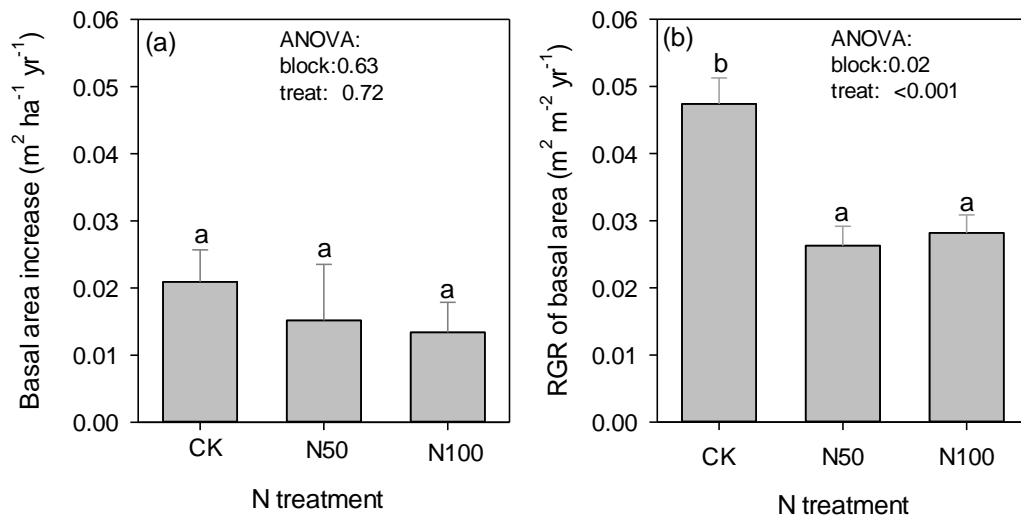
582 **Figure 2-3** Effects of N fertilization on the growth (mean  $\pm$  se) of *C. eyrei* by DBH classes  
 583 (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth  
 584 increase rate of basal area. Numbers in these figures indicate the results of ANOVA. The N  
 585 treatment on x-axis represents three levels of N fertilization: CK (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N50 (50  
 586 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).



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588 | **Figure 3-4** Effects of N fertilization on the growth of saplings (mean  $\pm$  se). (a) Absolute basal  
589 | area increase and (b) the relative growth rate of basal area. Numbers in these figures indicate  
590 | the results of ANOVA. The N treatment on x-axis represents three levels of N fertilization:  
591 | 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>).

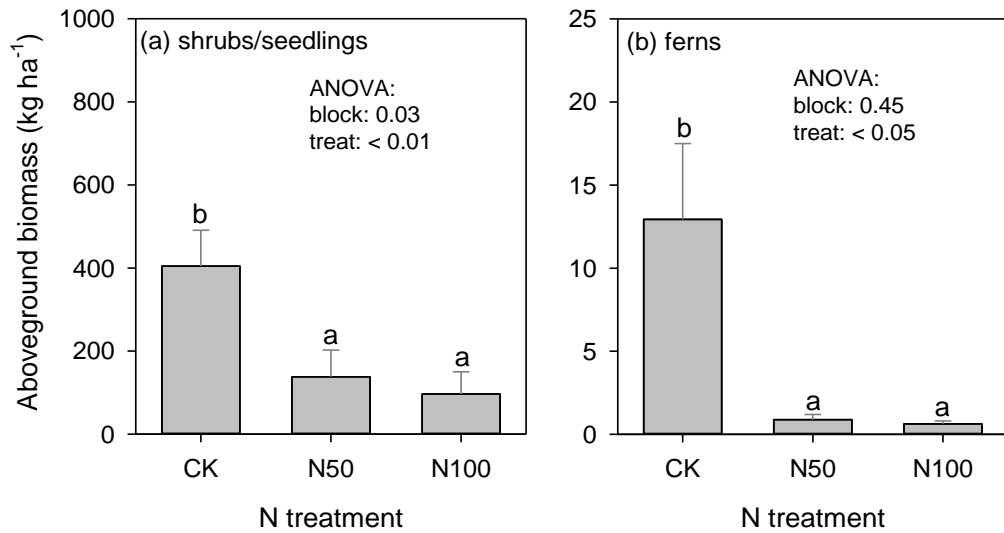
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594 | **Figure 4-5** Effects of N fertilization on the aboveground biomass of shrubs, seedlings and  
595 ferns. Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean  $\pm$  se).  
596 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis  
597 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  
598 N100 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

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