

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

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19
20 **Abstract**

21 Atmospheric nitrogen (N) deposition has been a noteworthy aspect of global change. A
22 number of observational studies have explored responses of plants to N deposition in boreal
23 and temperate forests. Here we asked how the dominant trees and different plant growth
24 forms respond to experimental N deposition in a subtropical forest in China. We conducted a
25 3.4-year N fertilization experiment in an old-aged subtropical evergreen broad-leaved forest
26 in eastern China with three treatment levels applied to nine 20×20 m plots and replicated in
27 three blocks. We divided the plants into trees, saplings, shrubs (including tree seedlings), and
28 ground-cover plants (ferns) according to the growth forms, and then measured the absolute
29 and relative basal area increments of trees and saplings and the aboveground biomass of
30 understory shrubs and ferns. We further grouped individuals of the dominant tree species
31 *Castanopsis eyrei* into three size classes to investigate their respective growth responses to
32 the N fertilization. Our results showed that the plot-averaged absolute and relative growth
33 rates of basal area and aboveground biomass of trees were not affected by N fertilization.
34 Across the individuals of *C.eyrei*, the small trees with a DBH (diameter at breast height) of

35 5-10 cm has declined by 66.4% and 59.5%, respectively, in N50 (50 kg N ha⁻¹ yr⁻¹) and N100
36 fertilized plots (100 kg N ha⁻¹ yr⁻¹), while the growth of median and large trees with a DBH
37 of >10 cm has not significantly changed with the N fertilization. The growth rate of small
38 trees, saplings and the aboveground biomass of understory shrubs and ground-cover ferns
39 decreased significantly in the N fertilized plots. Our findings suggested that N might not be a
40 limiting nutrient in this mature subtropical forest, and the limitation of other nutrients in the
41 forest ecosystem might be aggravated by the enhanced N deposition, potentially resulting in
42 an adverse effect on the development of natural subtropical forest.

43

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

46 **1 Introduction**

47

48 Atmospheric nitrogen (N) deposition is a globally prevalent phenomenon (Galloway *et al.*
49 2004). It has become a serious issue in China with the drastic increase of nitrogen oxides
50 emissions, producing considerable effects on terrestrial ecosystems (Liu *et al.* 2013). On the
51 one hand, most forest ecosystems show increased productivity and stand biomass with N
52 deposition (Magnani *et al.* 2007). A recent study employing a model simulation suggests that
53 N deposition has contributed to a 4.8% increase in the total carbon (C) storage of China's
54 forests between 1981 and 2010 (Gu *et al.* 2015). On the other hand, N deposition has reduced
55 species richness in terrestrial ecosystems (Lu *et al.* 2010; Dirnböck *et al.* 2014) and, in
56 extreme cases, can cause N saturation with negative effects on ecosystem functioning in
57 forest ecosystems (Aber *et al.* 1998).

58

59 Since the 1990s, N deposition has been simulated with N-fertilization experiments in forest
60 ecosystems to explore the responses of plants and other organisms to nitrogen deposition (e.g.,
61 Wright & Tietema 1995; Bobbink *et al.* 2010; Fowler *et al.* 2015). Due to the widespread
62 high amount of N deposition in Europe and America, numerous studies that focused on the
63 growth responses of plants have been carried out in boreal and temperate forests during the
64 past several decades (Magill 2000; Högberg *et al.* 2006). These studies showed that most
65 trees have a positive growth response to N fertilization and therefore have higher potential
66 carbon sequestration because the status of N limitation was largely alleviated by the
67 increasing N inputs (e.g., Thomas *et al.* 2010; BassiriRad *et al.* 2015). However, the
68 understory plants in these forest ecosystems inconsistently showed general negative
69 responses to N enrichment with declined biomass or shifted community structure (Rainey *et al.*
70 1999; Du *et al.* 2014; Dirnböck *et al.* 2014). In addition to the opposite responses of trees
71 and understory plants to N enrichment, differences remained in the effects of N enrichment
72 on single plant growth form in these forests. Generally, the limited light availability in these
73 ecosystems with high tree canopy cover was ascribed to the negative effects of N fertilization
74 (Strengbom & Nordin 2008).

75

76 Recently, the effects of N deposition on tropical forests raised researchers' concern.
77 Fertilization experiments in tropical forests showed different growth responses of trees to
78 nutrient addition among individual size levels, understory shrubs and tree seedlings (Wright
79 *et al.* 2011; Pasquini & Santiago 2012; Santiago *et al.* 2012) which contrasted with the ones

80 found for trees in the previously described experiments. For example, phosphorus (P)
81 fertilization enhanced the growths of small trees and seedlings but had no effect on median
82 and large trees, while N addition did not show any significant effect on plant growth in a
83 lowland tropical forest (Alvarez-Clare *et al.* 2013). In addition to the ubiquitous concept that
84 P was a critical element driving plant growth in tropical forests (Vitousek *et al.* 1991),
85 heterogeneous nutrient limitation that the growths of plants were co-limited by multiple
86 nutrients was further proposed to explain why diverse plants respond differently to nutrient
87 addition (Wright *et al.* 2011; Alvarez-Clare *et al.* 2013; Wurzburger & Wright 2015).
88 Nevertheless, the patterns of specific nutrient limitation and responses of plants to added
89 nutrient among diverse forest ecosystems need further exploration.

90

91 As most of the nutrient fertilization experiments have focused on boreal forests, temperate
92 forests and lowland tropical forests, few studies have investigated the effects of N deposition
93 on subtropical forests despite their broad distribution throughout the world and great
94 contribution to global C sink (Zhou *et al.* 2013; Yu *et al.* 2014; Huang *et al.* 2015). With the
95 increasing N deposition in subtropical region, especially in central and eastern China (Du *et al.*
96 2014), it's important to diagnose the nutrient limitation and evaluate the responses of
97 different plant growth forms to N deposition in subtropical forests for the assessment of
98 carbon sequestration and community dynamics.

99

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

107

108 **2 Materials and methods**

109

110 **2.1 Study site and experimental design**

111 The N fertilization experiment site was located at 30°01'47" N latitude and 117°21'23" E
112 longitude at an altitude of 375 metres in the natural conservation zone of Guniujiang in Anhui
113 Province, eastern China. As a commendable representative of the typical subtropical

114 broadleaved evergreen forest, the Guniujiang experimental site is an important part of the
115 NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du *et al.*
116 2013), because of its representativeness in both species composition and landscape structure
117 in the subtropical evergreen forest region. The study area has a humid climate with strong
118 summer monsoons with an annual average precipitation of 1,700 mm and an average annual
119 temperature of 14.9 °C. The soil in this area has been classified as yellow brown earth
120 (Chinese Soil Taxonomic Classification), and the $\text{pH}_{\text{H}_2\text{O}}$ value at 0-10 cm soil depth was
121 4.58 ± 0.05 (mean \pm SE). The total nitrogen, phosphorus, NH_4^+ -N and NO_3 -N content in the soil
122 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^{-1} ,
123 respectively (Li *et al.* 2015).

124

125 The study was conducted in a well-protected, mature subtropical evergreen forest (>300 year
126 age) with a three-layered vertical structure: the canopy tree layer (DBH>5 cm and height>5
127 m); the understory layer of saplings, shrubs and seedlings (DBH<5 cm and height<5 m); and
128 the ground-cover layer (ferns and herbs). The average density and basal area of trees were
129 $1,219 \text{ trees ha}^{-1}$ and $36.35 \text{ m}^2 \text{ ha}^{-1}$, respectively; *Castanopsis eyrei* was the dominant species
130 (which was also an important species at some other sites in subtropical forests) and accounted
131 for 87% of the total aboveground biomass of trees. The understory saplings and shrubs
132 contained several species, including *Cleyera japonica*, *Camellia cuspidata*, *Rhododendron*
133 *ovatum*, *Eurya muricata*, *Cinnamomum japonicum*, *Cinnamomum subavenium*, *Sarcandra*
134 *glabra*, and *C. eyrei*, and other native subtropical evergreen species (Table 1). Two fern
135 species (*Woodwardia japonica* and *Dryopteris hwangshanensis*) and an orchid (*Cymbidium*
136 *tortisepalum* var. *longibracteatum*) appeared on the floor layer, while *W. japonica* exclusively
137 dominated the floor layer with a coverage of 10%-20%.

138

139 We began N fertilization in March 2011. A randomized block design was used to avoid spatial
140 heterogeneity. We chose three blocks with similar stand growth, species composition and site
141 condition to establish three N treatments in each block: CK ($0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), N50 (50 kg N
142 $\text{ha}^{-1} \text{ yr}^{-1}$), and N100 ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). As the amount of wet N deposition in this region
143 was $5.9\text{-}7.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, we applied N fertilization at these two levels to simulate the
144 extreme N deposition cases. In total, nine $20 \text{ m} \times 20 \text{ m}$ plots were established with a 5-10 m
145 buffer zone between each plot. The total NH_4NO_3 was divided into 12 dosages and applied to
146 the forest in each month at regular intervals. NH_4NO_3 in dosages of 0.48 kg/plot and 0.95
147 kg/plot were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly in

148 N50 and N100 plots using a back-hatch sprayer. The unfertilized plots (controls) were
149 similarly treated with 15 L of fresh water without NH_4NO_3 .

150

151 **2.2 Sampling and measurement**

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

$$158 \quad \text{DBH} = \text{DBH}_1 + \frac{X_2 - X_1}{3.14 \times 10}$$

159

160 where DBH_1 represents the initial DBH (cm) of trees measured in March 2011, and X_2 and X_1
161 (mm) represent the widths of gaps on the tapes measured in July 2014 and at the beginning of
162 the experiment, respectively.

163

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

$$174 \quad \text{RGR} = \frac{\ln(2014 \text{ BA}) - \ln(2011 \text{ BA})}{3.4}$$

175

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

178

179 Because *C. eyrei* was the only dominant species in the tree layer, we separated it from other
180 tree species and grouped its individuals into three classes based on their DBH values (i.e.,
181 5-10 cm, 10-30 cm and >30 cm) to investigate the effects of N fertilization on the growth of

182 trees after removing the plant species and original size factors. During the monitoring of tree
183 growth, dead trees were recorded. Then, we calculated the aboveground biomass increments
184 of trees and the proportion of dead biomass using allometric equations (see Table S1).

185
186 We examined the effects of N fertilization on understory tree saplings distributed in the plots
187 according to their sizes and characteristics. For small trees with $DBH < 5$ cm and $height > 2$ m
188 (defined as “saplings”), DBH was measured at the beginning of N fertilization and in July
189 2014. Then, annual basal area growth rate and RGR of saplings were calculated based on
190 DBH changes. For very small trees or shrubs with $DBH < 5$ cm and $height < 2$ m (defined as
191 “shrubs/seedlings”), we set two $5\text{ m} \times 5\text{ m}$ subplots in each plot along a diagonal direction
192 and investigated the abundance, dominance, basal diameter (diameter at 10 cm above the
193 ground), height and crown diameters of all shrubs/seedlings inside the subplots at two
194 specific times. The first time was at the beginning of N fertilization (March 2011), and the
195 second was in July 2014. The length, width and number of fern leaves were measured
196 carefully in the above-mentioned subplots, and the allometric equations for seven dominant
197 species were then obtained (Table S1). Because the average aboveground biomass of
198 shrubs/seedlings and ferns showed no significant differences across three N treatments before
199 N fertilization in March 2011, we regarded the distribution of these understory
200 shrubs/seedlings and ferns to be homogeneous among the three treatments. Then we
201 identified the effects of N fertilization by comparing the aboveground biomass of
202 shrubs/seedlings and ferns in 2014 among the different treatments. Meanwhile, to investigate
203 the canopy cover and understory light availability, we used a digital camera (Canon, Japan)
204 with a fisheye lens (Sigma circular fisheye) to take photographs of canopy. In each subplot,
205 we put the camera at 1m above ground and took 5 photos upwards from understory.

206
207 In addition, to further explore the influences of N fertilization on plants' growth from
208 biogeochemical aspect at the Discussion part, we measured soil N, P content and pH (for
209 details, see “Methods of soil sampling and nutrient detection” in the Supplementary
210 Materials).

211

212 **2.3 Statistical analysis**

213 We used an analysis of variance (ANOVA) to evaluate the effects of N fertilization on basal
214 area increments, RGR, aboveground biomass increments, proportion of dead trees, and
215 aboveground biomass of shrubs/seedlings and ferns. Block and N treatment were both

216 regarded as fixed factors in the statistical model. We excluded the interactions between block
217 and N treatment from the model because they do not have ecological meaning. Tukey's
218 honest significant difference (HSD) tests were used to conduct the multi-comparisons among
219 the three N treatments. For the estimation of canopy cover, we followed the detailed
220 procedures of weighted ellipsoidal method using the software of Hemisfer (version 2.16.6) to
221 obtain values of vertical total gap fraction (Fmv) which indicate the proportion of projected
222 light spots to the total projected area (Thimonier *et al.* 2010). Then we obtained the values of
223 [1-Fmv] to indicate canopy cover. All statistical analyses were performed in R.3.2 (R
224 Development Core Team, 2010), and all figures were drawn in SigmaPlot 12 (Systat, 2010).

225

226 **3 Results**

227

228 **3.1 Growth responses of trees to N fertilization**

229 The increments of absolute basal area, aboveground biomass and RGR of all trees at plot
230 level showed no significant response to N fertilization during 3.4-year N fertilization (Fig.
231 1a~c). Compared with the unfertilized plots, N50 and N100 fertilized plots showed a
232 tendency toward higher averaged proportions of dead trees' aboveground biomass despite no
233 significant difference between them (Fig. 1d).

234

235 Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent
236 responses of absolute basal area increments and RGR to N fertilization (Fig. 2a-2f). The
237 small trees with a DBH of 5-10 cm growing under unfertilized plots showed greater basal
238 area increments than those growing under N fertilized plots (Fig. 2a, $p_{treat} < 0.05$). Specifically,
239 the N50 and N100 fertilization decreased the absolute basal area increments of small
240 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
241 indicated that the decreasing degrees of the absolute basal area of small trees reached 66.4%
242 and 59.5% in N50 and N100 plots. The small individual trees also showed a tendency toward
243 lower averaged RGR in N fertilized plots although no significant difference was detected
244 between them (Fig. 2d, $p_{treat} = 0.19$). Inconsistent with the negative responses of small trees to
245 N fertilization, the basal area increment and RGR of median *C. eyrei* individuals with DBH
246 of 10-30 cm and large *C. eyrei* individuals with DBH of >30cm showed no significant
247 responses to N fertilization (Fig. 2b-2c and 2e-2f, $p_{treat} > 0.05$ in all cases).

248

249 **3.2 Growth responses of understory saplings, shrubs/seedlings, and ferns to N** 250 **fertilization**

251 Responses of understory saplings to N fertilization were similar to those of small dominant
252 trees. Although the annual absolute increments of basal area increments of saplings showed
253 no significant response to N fertilization (Fig. 3a, $p=0.72$), the RGR of sapling growing in
254 N50 and N100 plots showed a substantial decrease at rates of $0.021 \text{ m}^2 \text{ m}^{-2} \text{ yr}^{-1}$ and 0.019 m^2
255 $\text{m}^{-2} \text{ yr}^{-1}$, respectively, compared to sapling growing in unfertilized plots (Fig. 3b, $p_{\text{treat}} <$
256 0.001). In addition, a general negative effect of N fertilization also occurred on understory
257 shrubs and ground-cover ferns. The aboveground biomass of seven predominant
258 shrubs/seedlings was drastically decreased by 69.4% and 79.1% in N50 and N100 fertilized
259 plots, respectively, compared with those in the unfertilized plots (Fig. 4a, $p < 0.01$).
260 Remarkably, the aboveground biomass of ground-cover ferns significantly declined by 92.4%
261 and 93.4% in N50 and N100 fertilized plots (Fig. 4b, $p < 0.05$).

262

263 **4 Discussion**

264

265 **4.1 Growth responses of trees to N fertilization**

266 Nutrient limitation was generally determined through evaluating ecosystem feedbacks to
267 nutrient addition (Vitousek 1991; Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013). When the
268 forest ecosystems showed a positive response to added nutrient, e.g., plant growth or rates of
269 physiological processes were promoted, the added nutrient then could be interpreted as
270 limiting to the ecosystem, otherwise, as not limiting to the ecosystem (Santiago 2015). We
271 initially expected positive growth responses of trees exposed to N fertilization in this
272 subtropical forest because N availability in the soil would be enhanced by N fertilization and
273 the potential N limitation of plants in the forest ecosystem could be alleviated. However,
274 contrary to our expectation, we did not observe strong positive growth responses of trees to N
275 fertilization (Figs. 1 and 2). Across individual trees of different sizes and plant growth forms,
276 we only observed substantial negative responses of small trees (5-10 cm DBH; Fig. 2a and 2d)
277 and saplings (Fig. 3a-3b) and weak responses of median and large trees (>10 cm DBH) to N
278 fertilization (Fig. 2b-2c and 2f-2e), which further demonstrated that the growth of trees in this
279 old-aged subtropical forest was not essentially limited by N as hypothesized.

280

281 Contrasted with previous positive responses of trees to N fertilization in boreal and temperate
282 forests which were considered as N limited ecosystems (Högberg *et al.* 2006; Thomas *et al.*
283 2010; BassiriRad *et al.* 2015), our finding of the unchanged responses of trees to N
284 fertilization was partly consistent with observations of trees from tropical forests (e.g.,

285 Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013). Studies from mature tropical forests have
286 revealed that P availability was a critical element shaping tree species distribution and
287 productivity (Santiago 2016; Dalling *et al.* 2016). Given the similar high-weathered soil
288 properties, humid climatic conditions and dominant evergreen broadleaf trees in mature
289 subtropical forest as those in wet tropical forest, we speculated that P limitation rather than N
290 limitation, might have played a key role in influencing growth of plants in subtropical forest.

291

292 As a supplement, we used a P fertilization experiment conducted in another subtropical forest
293 with similar community structure nearby our experiment site to check if P limits plant growth.
294 We applied 50 kg ha⁻¹ yr⁻¹ P (P₂O₅) to the forest and measured the growth of the dominant
295 tree species (*C. sclerophylla*) following the same steps presented in the ‘Materials and
296 methods’ section in this paper. After two years’ P fertilization, we found that the annual
297 absolute basal area increments and relative basal area in P fertilized plots were 56.0% and
298 101.5% higher, respectively, than in unfertilized plots ($p=0.02$ and $p=0.03$, respectively,
299 unpublished data). Our results from N fertilization and the supplementary P fertilization
300 experiments indicate that plant growth in subtropical forest ecosystems might be highly
301 limited by P, although it is in great need for further verification in the next studies. Similarly,
302 limitation of other nutrients, such as K (potassium) which was highlighted in tropical forests,
303 and their combination as well as heterogeneous nutrient limitation of specific species and
304 plant growth forms may warrant further consideration in subtropical forests (Wright *et al.*
305 2011; Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013).

306

307 Moreover, the high spatial heterogeneity in old-aged subtropical forest, similar to tropical
308 forests, could be a possible explanation for the lack of significant responses of plot-averaged
309 basal area growth, RGR, aboveground biomass of trees with a DBH of >5cm and the
310 proportion of dead trees to N fertilization. In eastern China, the distributions of subtropical
311 forest stands are quite topographically fragmented, while relative flat stands are required to
312 avoid N losses and minimize spatial heterogeneity among experimental treatments. The
313 actual distribution and topography of the subtropical forests limited the number of
314 replications in the N fertilization experiment. This limitation might reduce the statistic power
315 of N treatment on plot-averaged plant growth rate which has been pointed out in previous
316 studies (Alvarez-Clare *et al.* 2013). Hence, long-term monitoring of the trees might provide
317 another choice for accurate evaluating of the forest dynamics with N fertilization.

318

319 **4.2 Growth responses of small trees, understory saplings, shrubs/seedlings and ferns to** 320 **N fertilization**

321 Although the positive responses of small or juvenile trees to nutrient fertilization has been
322 reported in boreal, temperate and tropical forest (e.g., Högberg *et al.* 2006; Bedison &
323 McNeil 2009; Alvarez-Clare *et al.* 2013), our results showed a remarkable negative effect of
324 N fertilization on small-sized plants including trees, understory saplings, shrubs/seedlings and
325 ferns. During our field investigation, we also found that the average proportion of dead trees
326 (Fig. 1d) tended to increase in N fertilized plots although the result was not statistically
327 significant ($p_{treat} = 0.50$). Additionally, the ground-cover ferns in N100 plots almost
328 disappeared after 3.4-year N fertilization (personal observation). Given the high stand density
329 in this mature subtropical forest, we suggest that N fertilization might potentially lead to
330 increased self- and alien-thinning of individuals through decreasing understory light
331 availability.

332

333 The pivotal role of light availability in the eco-physiological processes of understory growth
334 forms has been widely recognized (Santiago 2015). Due to the limited light availability,
335 understory plants may not be able to incorporate the added nutrient and promote their
336 photosynthetic rates (Alvarez-Clare *et al.* 2013). Nevertheless, a study conducted in tropical
337 forest with thick canopy showed that photosynthetic process could be enhanced by nutrient
338 addition even under low light availability (Pasquini & Santiago 2012). In a sharp contrast, the
339 study conducted in an Australian rainforest revealed that understory seedlings increased
340 growth when the light availability was high, but showed no significant response to nutrient
341 fertilization in low lights (Thompson *et al.* 1988). These studies, together with our field
342 observations, suggest that the growth of understory plants is largely co-limited by nutrient
343 and light availability in the local environment. Further, our results of forest canopy cover
344 estimated by photographic fisheye showed no significant differences between unfertilized
345 (0.77 ± 0.01) and N fertilized plots (0.76 ± 0.04 and 0.72 ± 0.01 in N50 and N100 plots,
346 respectively), which was consistent with the findings of Lu *et al.* (2010). Although the
347 understory light irradiance fluctuated largely during a day and was very hard to detect
348 precisely, our measurements of forest canopy cover provided a rough evaluation for light
349 availability. The results might indicate that other factors in addition to the low light
350 availability in this old-aged forest had also played a crucial role in influencing understory
351 plants during 3.4 years' N fertilization.

352

353 **4.3 Potential N saturation and plant growth**

354

355 The striking biomass reduction of the understory plants, especially ferns, in response to N
356 fertilization in our study well corroborated the similar findings in an old-aged tropical forest
357 at Mt. Dinghushan in China (Lu et al., 2010). Also, consistent with previous studies obtained
358 from boreal, temperate and tropical forests (Rainey *et al.* 1999; Alvarez-Clare *et al.* 2013;
359 Dirnböck *et al.* 2014), our experiment revealed that understory small-sized plants responded
360 sensitively to nutrient fertilization, which might indicate a possibility of N saturation in the
361 subtropical forest. According to the definition of N saturation addressed by Aber et al. (1989),
362 the drastic decrease of understory ferns, shifted composition of understory plant community,
363 and cation imbalances of understory species after 7 years' chronic N fertilization at Harvard
364 Forest, USA, could be interpreted as useful indicators of N saturation (Rainey *et al.* 1999).
365 Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Mt.
366 Dinghushan also showed signs of N saturation, such as significant increases in nitrate (NO₃⁻)
367 leaching, inorganic N concentration and N₂O emissions of soils, and soil acidification (Lu *et*
368 *al.* 2014; Chen *et al.* 2015). In our experiment, we observed mild soil acidification and
369 increased soil N concentration in high N fertilized plots (Fig. S1). Combined with the
370 negative responses of understory plants, we suggest that the 3.4-year N fertilization in this
371 mature subtropical forest site has potentially caused N saturation, but further observations are
372 required.

373

374 **5. Conclusion**

375

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

387 forest and the limitation of other nutrients might be amplified with increasing N addition.

388

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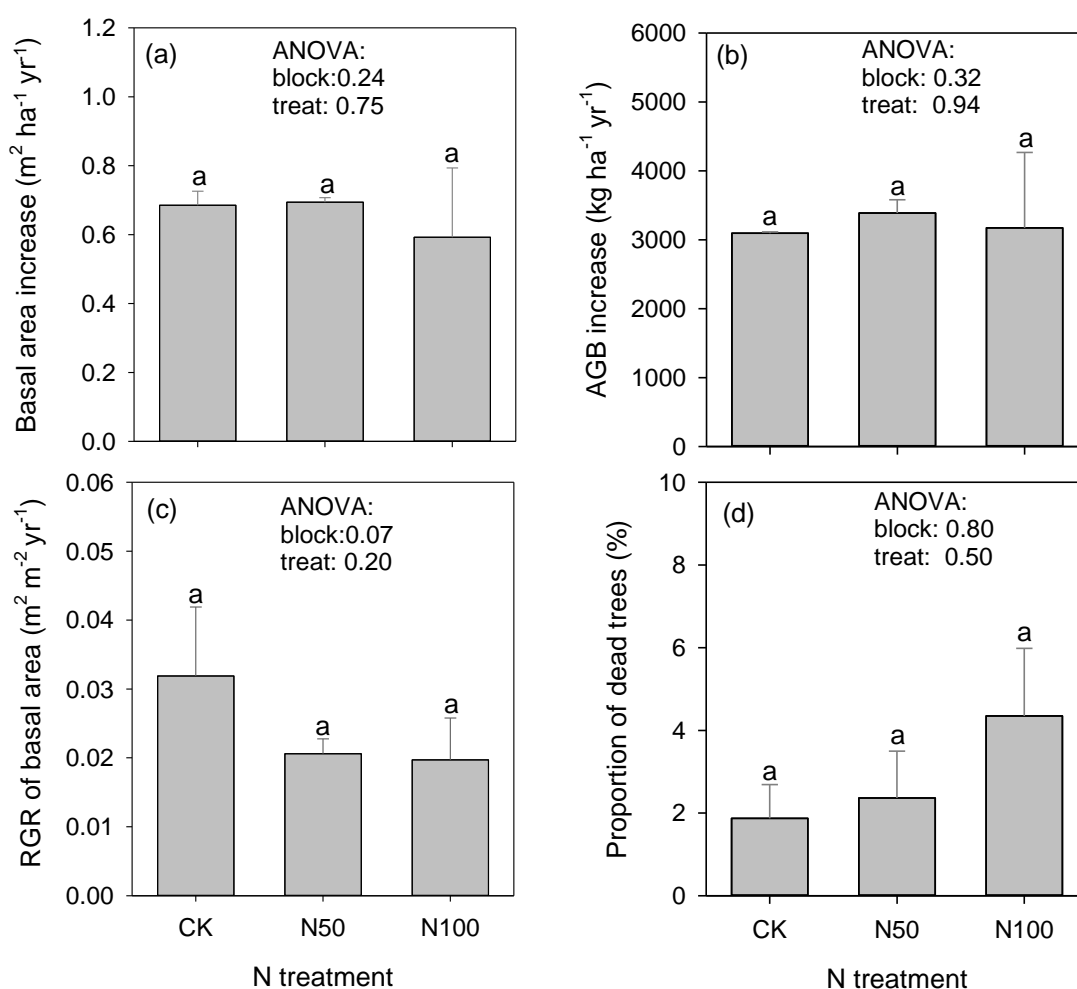
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497 **Table 1** Growth measurements for four plant growth forms in this study before N fertilization.
 498 Numbers in the tables represent means (or mean \pm (standard error), $n=9$) of plants across all
 499 plots. TBA: total basal area of trees;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

Growth forms	Species	Growth variable		
		TBA (m ² 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 & 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</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 ⁻²)		
Ferns	<i>Woodwardia japonica</i>	1.19 \pm 0.23		

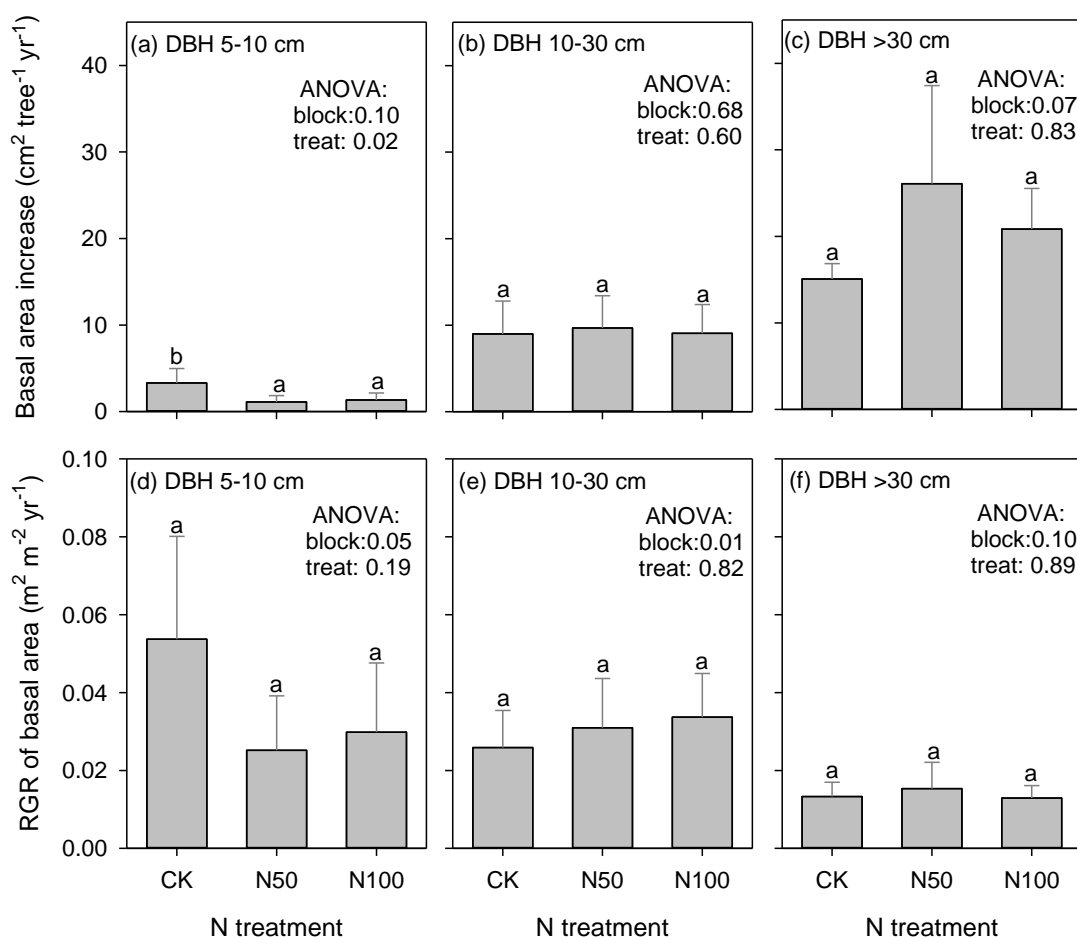
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502 **Figure 1** Effects of N fertilization on the growth and mortality of all trees (mean \pm se). (a)
 503 Absolute basal area increase of all trees; (b) aboveground biomass increase of all trees; (c)
 504 relative growth rate of total tree basal area; and (d) the proportion of all dead trees. The
 505 proportion of dead trees was calculated using the aboveground biomass of all dead trees
 506 during the experiment divided by the total aboveground biomass of all trees in 2014.
 507 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis
 508 represents three levels of N fertilization: CK (0 kg N ha⁻¹ yr⁻¹), N50 (50 kg N ha⁻¹ yr⁻¹) and
 509 N100 (100 kg N ha⁻¹ yr⁻¹).



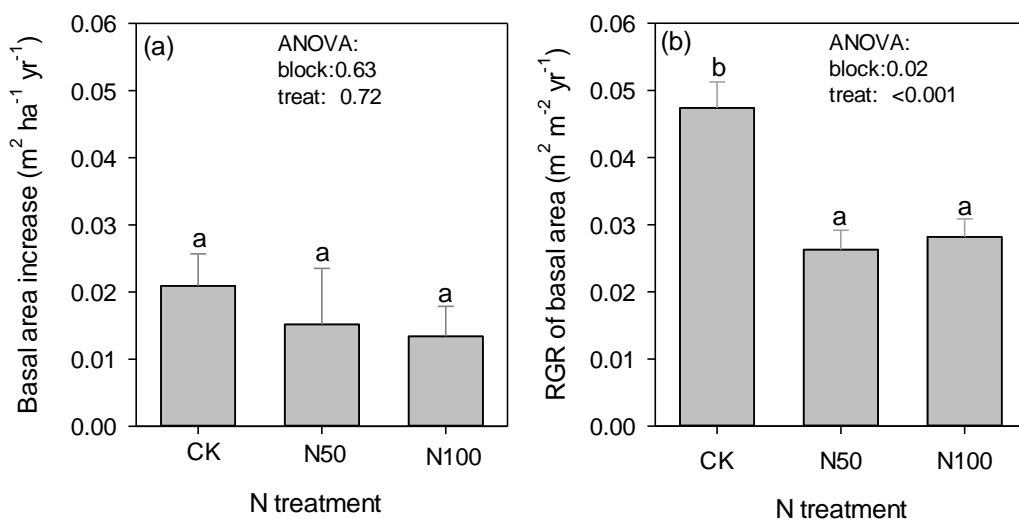
510

511 **Figure 2** Effects of N fertilization on the growth (mean \pm se) of *C. eyrei* by DBH classes
 512 (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth
 513 increase rate of basal area. Numbers in these figures indicate the results of ANOVA. The N
 514 treatment on x-axis represents three levels of N fertilization: CK (0 kg N ha⁻¹ yr⁻¹), N50 (50
 515 kg N ha⁻¹ yr⁻¹), and N100 (100 kg N ha⁻¹ yr⁻¹).



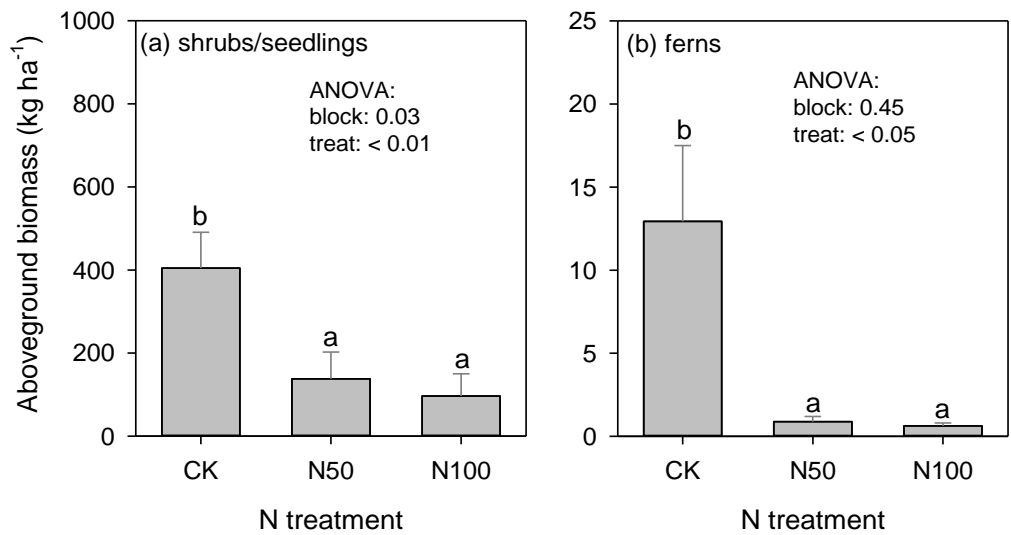
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517 **Figure 3** Effects of N fertilization on the growth of saplings (mean \pm se). (a) Absolute basal
518 area increase and (b) the relative growth rate of basal area. Numbers in these figures indicate
519 the results of ANOVA. The N treatment on x-axis represents three levels of N fertilization:
520 CK (0 kg N ha⁻¹ yr⁻¹), N50 (50 kg N ha⁻¹ yr⁻¹) and N100 (100 kg N ha⁻¹ yr⁻¹).
521



522

523 **Figure 4** Effects of N fertilization on the aboveground biomass of shrubs, seedlings and ferns.
524 Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean \pm se).
525 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis
526 represents three levels of N fertilization: CK (0 kg N ha⁻¹ yr⁻¹), N50 (50 kg N ha⁻¹ yr⁻¹) and
527 N100 (100 kg N ha⁻¹ yr⁻¹).
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529