

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 broadleaf forest in
26 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 groundcover 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 growth responses to the N
32 fertilization separately. Our results showed that the plot-averaged absolute and relative
33 growth rates of trees were not affected by N fertilization. Across the individuals of *C. eyrei*,
34 the basal area increment of small trees with a DBH (diameter at breast height) of 5-10 cm has
35 declined by 50% in N fertilized plots, while the growth of median and large trees with a DBH
36 of >10 cm has not significantly changed with the N fertilization. The growth rate of small
37 trees and saplings and the biomass of understory shrubs and groundcover ferns decreased
38 significantly in the N fertilized plots. Our findings suggested that N might not be a limiting
39 nutrient in the mature subtropical forest, and the limitation of other nutrients might be
40 aggravated by the enhanced N deposition resulting in an adverse effect on the development of
41 natural subtropical forest.

42

43 **Key-words:** *Castanopsis eyrei*, N fertilization, plant growth, shrub layer, subtropical forest,
44 tree layer, groundcover fern

45 **1 Introduction**

46

47 Atmospheric nitrogen (N) deposition is a globally prevalent phenomenon (Galloway et al.,
48 2004). It has become a serious issue in China with the drastic increase of nitrogen oxides
49 emissions, producing considerable effects on terrestrial ecosystems (Liu et al., 2013). On the
50 one hand, most forest ecosystems show increased productivity and stand biomass with N
51 deposition (Magnani et al., 2007). A recent study employing a model simulation suggests that
52 N deposition has contributed to a 4.8% increase in the total carbon (C) storage of China's
53 forests between 1981 and 2010 (Gu et al., 2015). On the other hand, N deposition has reduced
54 species richness in terrestrial ecosystems (Lu et al., 2010; Dirnböck et al., 2014) and, in
55 extreme cases, can cause N saturation with negative effects on ecosystem functioning in
56 forest ecosystems (Aber et al., 1998; Lovett & Goodale 2011).

57

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

73

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

88

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

97

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

105

106 **2 Materials and methods**

107

108 **2.1 Study site and experimental design**

109 The N fertilization experiment site was located at 30°01'47" N latitude and 117°21'23" E
110 longitude at an altitude of 375 metres in the natural conservation zone of Guniujiang in Anhui
111 Province, eastern China. As a commendable representative of the typical subtropical
112 broadleaved evergreen forest, the Guniujiang experimental site is an important part of the
113 NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du et al.,
114 2013), because of its representativeness in both species composition and landscape structure
115 in the subtropical evergreen forest region. The study area has a humid climate with strong
116 summer monsoons with an annual average precipitation of 1,700 mm and an average annual
117 temperature of 14.9 °C. The soil in this area has been classified as yellow brown earth
118 (Chinese Soil Taxonomic Classification), and the pH_{H2O} value at 0-10 cm soil depth was 4.58
119 ±0.05 (mean ±SE). The total nitrogen, phosphorus, NH₄⁺-N, and NO₃-N content in the soil
120 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,
121 respectively (Li et al., 2015).

122

123 The study was conducted in a well-protected, mature subtropical evergreen forest (>300 year
124 age) with a three-layered vertical structure: the canopy tree layer (DBH>5 cm and height>5
125 m); the understory layer of saplings, shrubs and seedlings (DBH<5 cm and height<5 m); and
126 the ground-cover layer (ferns and herbs). The average density and basal area of trees were
127 1,219 trees ha⁻¹ and 36.35 m² ha⁻¹, respectively; *Castanopsis eyrei* was the dominant species
128 (which was also an important species at some other sites in subtropical forests) and accounted
129 for 87% of the total aboveground biomass of trees. The understory saplings and shrubs
130 contained several species, including *Cleyera japonica*, *Camellia cuspidata*, *Rhododendron*

131 *ovatum*, *Eurya muricata*, *Cinnamomum japonicum*, *Cinnamomum subavenium*, *Sarcandra*
132 *glabra*, and *C. eyrei*, and other native subtropical evergreen species (Table 1). Two fern
133 species (*Woodwardia japonica* and *Dryopteris hwangshanensis*) and an orchid (*Cymbidium*
134 *tortisepalum* var. *longibracteatum*) appeared on the floor layer, while *W. japonica* exclusively
135 dominated the floor layer with a coverage of 10%-20%.

136

137 We began N fertilization in March 2011. A randomized block design was used to avoid spatial
138 heterogeneity. We chose three blocks with similar stand growth, species composition and site
139 condition to establish three N treatments in each block: CK (0 kg N ha⁻¹ yr⁻¹), N50 (50 kg N
140 ha⁻¹ yr⁻¹), and N100 (100 kg N ha⁻¹ yr⁻¹). As the amount of wet N deposition in this region
141 was 5.9-7.3 kg N ha⁻¹yr⁻¹, we applied N fertilization at these two levels to simulate the
142 extreme N deposition cases. In total, nine 20 m × 20 m plots were established with a 5-10 m
143 buffer zone between each plot. The total NH₄NO₃ was divided into 12 dosages and applied to
144 the forest in each month of a year at regular intervals. NH₄NO₃ in dosages of 0.48 kg/plot and
145 0.95 kg/plot were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly
146 in N50 and N100 plots using a back-hatch sprayer. The unfertilized plots (controls) were
147 similarly treated with 15 L of fresh water without NH₄NO₃.

148

149 **2.2 Sampling and measurement**

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

156

157

$$DBH = DBH_1 + \frac{X_2 - X_1}{3.14 \times 10}$$

158

159 where DBH₁ represents the initial DBH (cm) of trees measured in March 2011, and X₂ and X₁
160 (mm) represent the widths of gaps on the tapes measured at the end and the beginning of the
161 experiment, respectively.

162

163 The basal area is a common indicator for weighing the biomass of trees. Therefore, tree basal
164 area increments were calculated to indicate the responses of tree biomass to the N fertilization.
165 First, to test community-level responses of tree layer to N fertilization, we calculated the sum
166 of total basal area increase (m² ha⁻² year⁻¹) of all trees in a plot after 3.4 years of N
167 fertilization and divided this value by the period of N fertilization (3.4 years) to obtain the
168 annual basal area increase rate of the trees (dead trees were not included). Second, relative
169 annual basal area growth rate (RGR, m² m⁻² year⁻¹) was used to eliminate the conceivable
170 interferential effects resulting from the differences in the number and size of original
171 individuals among plots according to the following equation, similar to Alvarez-Clare et al.'s
172 method (2013):

173

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

174 where RGR represents the relative annual basal area growth rate ($\text{m}^2 \text{m}^{-2} \text{year}^{-1}$), BA indicates
175 the sum of basal area of all trees in each plot, and 3.4 (years) is the N fertilization period
176 (from March 2011 to July 2014).

177

178 Because *C. eyrei* was the only dominant species in the tree layer, we separated it from other
179 tree species and grouped its individuals into three classes based on their DBH values (i.e.,
180 5-10 cm, 10-30 cm and >30 cm) to investigate the effects of N fertilization on the growth of
181 trees after removing the plant species and original size factors. During the monitoring of tree
182 growth, dead trees were recorded. Then, we calculated the aboveground biomass increments
183 of trees and the proportion of dead biomass using allometric equations (see Table S1).

184

185 We examined the effects of N fertilization on understory tree saplings distributed in the plots
186 according to their sizes and characteristics. For small trees with $\text{DBH} < 5$ cm and $\text{height} > 2$ m
187 (defined as “saplings”), DBH was measured at the beginning of N fertilization and in 2014.
188 Then, basal area increments, annual basal area growth rate, RGR, aboveground biomass
189 increments, and dead biomass proportion of saplings were calculated based on DBH changes.
190 For very small trees or shrubs with $\text{DBH} < 5$ cm and $\text{height} < 2$ 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 the three N treatments,
199 we regarded the distribution of these understory shrubs/seedlings and ferns to be
200 homogeneous among the three treatments before N fertilization in March 2011. 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 level above ground and took 5 photos upwards from understory.

206

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

210

211 **2.3 Statistical analysis**

212 We used an analysis of variance (ANOVA) to evaluate the effects of N fertilization on basal
213 area increments, RGR, aboveground increments, dead biomass proportion of trees, and
214 aboveground biomass of shrubs/seedlings and ferns. Block and N treatment were both
215 regarded as fixed factors in the statistical model. We excluded the interactions between block
216 and N treatment from the model because they do not have ecological meaning. Tukey’s

217 honest significant difference (HSD) tests were used to conduct the multi-comparisons among
218 the three N treatments. For the estimation of canopy cover, we followed the procedures of
219 weighted ellipsoidal method using the software Hemisfer (version 2.1.6) to obtain values of
220 vertical total gap fraction (Fmv) which indicates the proportion of projected light spots to the
221 total projected area (Thimonier et al., 2010). Then we obtained the values of [1-Fmv] to
222 indicate canopy cover. All statistical analyses were performed in R.3.2 (R Development Core
223 Team, 2010), and all figures were drawn in SigmaPlot 12 (Systat, 2010).

224

225 **3 Results**

226

227 **3.1 Growth responses of trees to N fertilization**

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

233

234 Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent
235 responses of absolute basal area increments and RGR to N fertilization (Fig. 2a-2f). The
236 small trees with a DBH of 5-10 cm growing under unfertilized plots showed greater basal
237 area increments and RGR than those under N fertilized plots (Fig. 2a and 2d, $p < 0.05$ and
238 $p = 0.03$, respectively). Specifically, the N50 and N100 fertilization decreased the absolute
239 basal area increments of small trees at rates of $0.02 \text{ m}^2 \text{ ha}^{-1} \text{ year}^{-1}$ and $0.39 \text{ m}^2 \text{ ha}^{-1} \text{ year}^{-1}$,
240 respectively. However, inconsistent with such negative responses of small trees to N
241 fertilization, the basal area increment and RGR of median (DBH of 10-30 cm; see Fig. 2b-2c)
242 and large trees (DBH >30cm; see Fig. 2e-2f) did not show significant responses to N
243 fertilization ($p > 0.05$ in all cases).

244

245 **3.2 Growth responses of understory saplings, shrubs/seedlings, and ferns to N 246 fertilization**

247 Responses of understory saplings to N fertilization were similar to those of small dominant
248 trees. Although the annual absolute increments of basal area increments of saplings showed
249 no significant response to N fertilization (Fig. 3a, $p = 0.72$), the RGR of saplings growing in
250 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
251 $\text{m}^{-2} \text{ yr}^{-1}$, respectively, compared to saplings growing in unfertilized plots (Fig. 3b, $p < 0.001$).

252

253 In addition, a general negative effect of N fertilization also occurred on understory shrubs and
254 groundcover ferns. The aboveground biomass of seven predominant shrubs/seedlings was
255 drastically decreased by 69.4% and 79.1% in N50 and N100 fertilized plots, respectively,
256 compared with those in the unfertilized plots (Fig. 4a, $p < 0.01$). Remarkably, the aboveground
257 biomass of groundcover ferns significantly declined by 92.4% and 93.4% in N50 and N100
258 fertilized plots (Fig. 4b, $p < 0.05$).

259

260 4 Discussion

261 262 4.1 Growth responses of trees to N fertilization

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

278
279 Contrasted with previous studies of positive responses of trees to N fertilization in boreal and
280 temperate forests which were considered as N limited ecosystems (Högberg et al., 2006;
281 Thomas et al., 2010; BassiriRad et al., 2015), our finding of the unchanged responses of trees
282 to N fertilization was partly consistent with observations of trees from tropical forests (e.g.,
283 Santiago et al., 2012; Alvarez-Clare et al., 2013). Studies from mature tropical forests have
284 revealed that P availability was a critical element shaping tree species distribution and
285 productivity (Santiago 2016; Dalling et al., 2016). Given the similar high-weathered soil
286 properties, humid climatic conditions, and dominant evergreen species in mature subtropical
287 forest as those in wet tropical forest, we speculated that P limitation, not N limitation, might
288 have played a key role on plants in the subtropical forest. As a supplement, we used data from
289 a P fertilization experiment conducted in another subtropical forest with similar community
290 structure nearby our experiment site to check if P limits plant growth. We applied 50 kg ha⁻¹
291 yr⁻¹ P (P₂O₅) to the forest and measured the growth of the dominant tree species (*C.*
292 *sclerophylla*) following the same steps presented in the ‘Materials and methods’ section in
293 this paper. After two years’ P fertilization, we found that the annual absolute basal area
294 increment and relative basal area in P fertilized plots were 56.0% and 101.5% higher,
295 respectively, than in unfertilized plots ($p=0.02$ and $p=0.03$, respectively, unpublished data).
296 Our results from N fertilization and the supplementary P fertilization experiments indicate
297 that plant growth in subtropical forests might be highly limited by P, but this is in great need
298 for further verification in the future studies. Similarly, limitation of other nutrients, such as K
299 (potassium) which was highlighted in tropical forests, and their combination as well as
300 heterogeneous nutrient limitation of specific species and plant growth forms may warrant
301 further considerations in subtropical forests (Wright et al., 2011; Santiago et al., 2012;
302 Alvarez-Clare et al., 2013).

303

304 **4.2 Growth responses of small trees, understory saplings, shrubs/seedlings and ferns to** 305 **N fertilization**

306 Although the positive responses of small or juvenile trees to nutrient fertilization has been
307 reported in boreal, temperate, and tropical forest (e.g., Högberg et al., 2006; Bedison &
308 McNeil 2009; Alvarez-Clare *et al.* 2013), our results showed a remarkable negative effect of
309 N fertilization on small-size plants including trees, understory saplings, shrubs/seedlings and
310 ferns. During our field investigation, we also found that the average proportion of dead trees
311 (Fig. 1d) and saplings might tend to increase in N fertilized plots although the result was not
312 statistically significant ($p=0.30$). Additionally, the groundcover ferns in N100 plots almost
313 disappeared after 3.4-year N fertilization (personal observation). Given the high stand density
314 in this mature subtropical forest, we suggest that N fertilization might potentially lead to
315 increased self- and alien-thinning of individuals through decreasing understory light
316 availability.

317

318 The pivotal role of light availability in the eco-physiological processes of understory growth
319 forms has been widely recognized (Santiago, 2015). Due to the limited light availability,
320 understory plants may not be able to incorporate the added nutrient and promote their
321 photosynthetic rates (Alvarez-Clare *et al.*, 2013). Nevertheless, a study conducted in a
322 tropical forest with thick canopy showed that photosynthetic process could be enhanced by
323 nutrient addition even under low light availability (Pasquini & Santiago, 2012). In sharp
324 contrast, the study conducted in an Australian rainforest revealed that understory seedlings
325 increased growth when the light availability was high, but showed no significant response to
326 nutrient fertilization in low lights (Thompson et al., 1988). These studies, together with our
327 field observations, suggest that the growth of understory plants is largely co-limited by
328 nutrient and light availability in the local environment.

329

330 Further, Our results of forest canopy cover estimated by photographic fisheye showed no
331 significant differences between unfertilized (0.77 ± 0.01) and N fertilized plots (0.76 ± 0.04
332 and 0.72 ± 0.01 in N50 and N100 plots, respectively), which was consistent with the findings
333 of Lu et al. (2010). Although the understory light irradiance fluctuated largely during a day
334 and was very hard to detect precisely, our measurements of forest canopy cover provided a
335 rough evaluation for light availability. The results might indicate that other factors in addition
336 to the low light availability in this old-aged forest had also played a crucial role in influencing
337 understory plants during 3.4 years' N fertilization.

338

339 **4.3 Potential N saturation and plant growth**

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

346 subtropical forest. According to the definition of N saturation addressed by Aber et al. (1989),
347 the drastic decrease of understory ferns, shifted composition of understory plant community,
348 and cation imbalances of understory species after 7 years' chronic N fertilization at Harvard
349 Forest, USA, could be interpreted as useful indicators of N saturation (Rainey et al., 1999).
350 Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Dinghushan
351 also showed signs of N saturation, such as significant increases in nitrate (NO_3^-) leaching,
352 inorganic N concentration and N_2O emissions of soils, and soil acidification (Lu et al., 2014;
353 Chen et al., 2015). In our experiment, we observed mild soil acidification and increased soil
354 N concentration in high N fertilized plots (Fig. S1). Combined with the negative responses of
355 understory plants, we suggest that the 3.4-year N fertilization in this mature subtropical forest
356 site has potentially caused N saturation, but further observations are required in the future
357 study.

358

359 **5. Conclusion**

360 Contrasting growth responses among plant growth forms to N fertilization were presented in
361 the mature subtropical evergreen forest in this study. Overall growth of trees at the plot level
362 showed no significant responses to the N fertilization; however, if the dominant tree species
363 *C. eyrei* was grouped into three DBH classes, then the basal area increment of small trees
364 with a DBH of 5-10 cm declined by 50% in N fertilized plots, while the growth of large trees
365 with $\text{DBH} > 10$ cm showed weak responses to N fertilization. The growths of understory
366 saplings, shrubs/seedlings, and groundcover ferns showed a negative response to N
367 fertilization. Our results indicated that N might not be a limited nutrient in this subtropical
368 forest and that other nutrients and light availability may potentially co-limit growth of plants
369 with different growth forms. Our data also suggested that even short-term N fertilization
370 might have caused N saturation in this mature subtropical forest and the limitation of other
371 nutrients might be amplified with increasing N addition.

372

373

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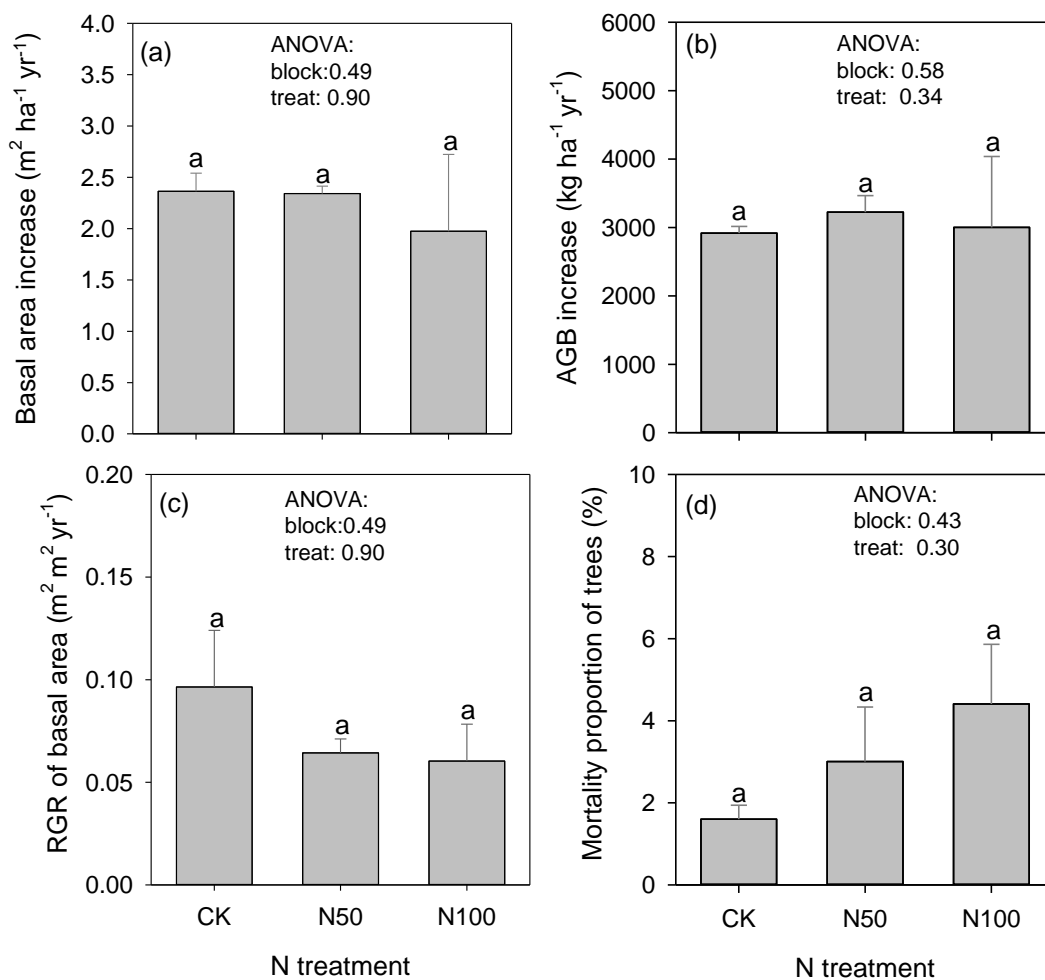
485

486 **Table 1** Growth measurements for four plant growth forms in this study before N fertilization
 487 experiment. Numbers in the tables represent means (or mean \pm (standard error), $n=9$) of
 488 plants across all nine plots. TBA: total basal area of trees; DBH: diameter at breast height
 489 (~1.3 m); Basal diameter: 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)
	<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
Shrubs & Seedlings	<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 ⁻²)		
Ferns	<i>Woodwardia japonica</i>	1.19 \pm 0.23		

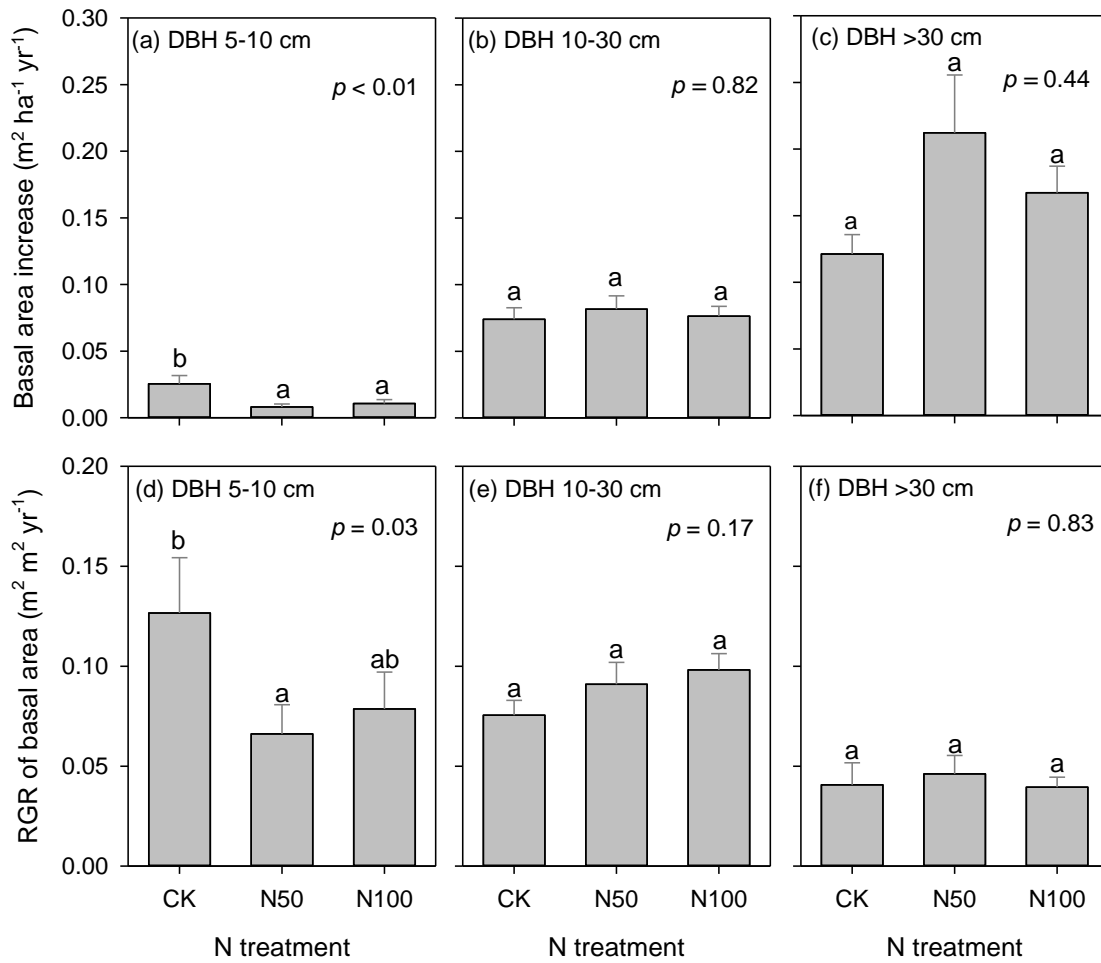
490

491 **Figure 1** Effects of N fertilization on the growth and mortality of all trees (mean \pm se, n=3).
 492 (a) Absolute basal area increase of all trees; (b) aboveground biomass increase of all trees; (c)
 493 relative growth rate of total tree basal area; and (d) the proportion of dead trees. The mortality
 494 proportion of trees was calculated using the aboveground biomass of all dead trees during the
 495 experiment divided by the total aboveground biomass of all trees in 2014. Numbers in these
 496 figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N
 497 fertilization: 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).



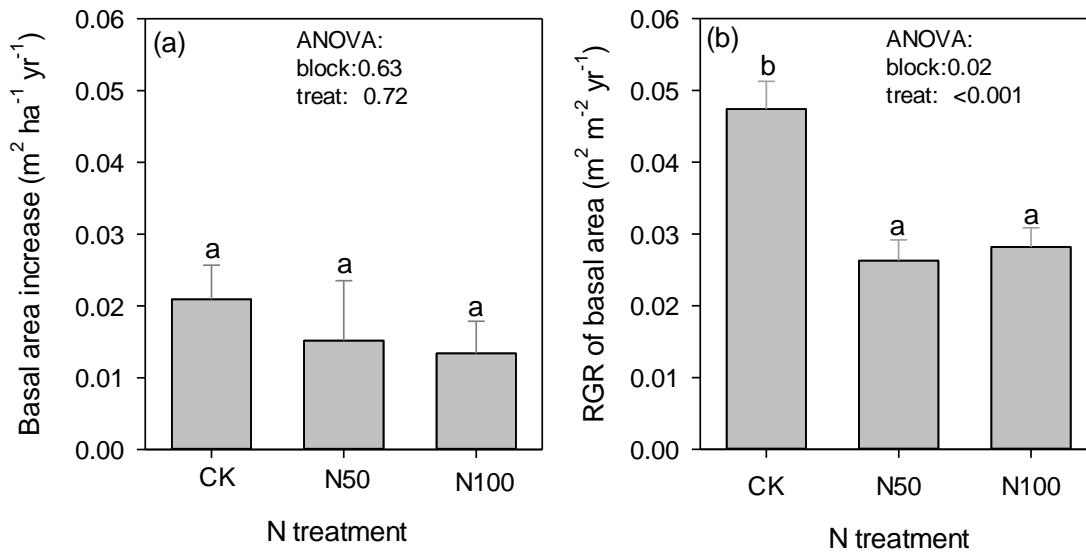
498

499 **Figure 2** Effects of N fertilization on growth (mean \pm se, n=3) of *C. eyrei* by DBH classes
 500 (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase, and (d-f) relative growth
 501 rate of basal area. Numbers in these figures indicate the results of ANOVA.
 502



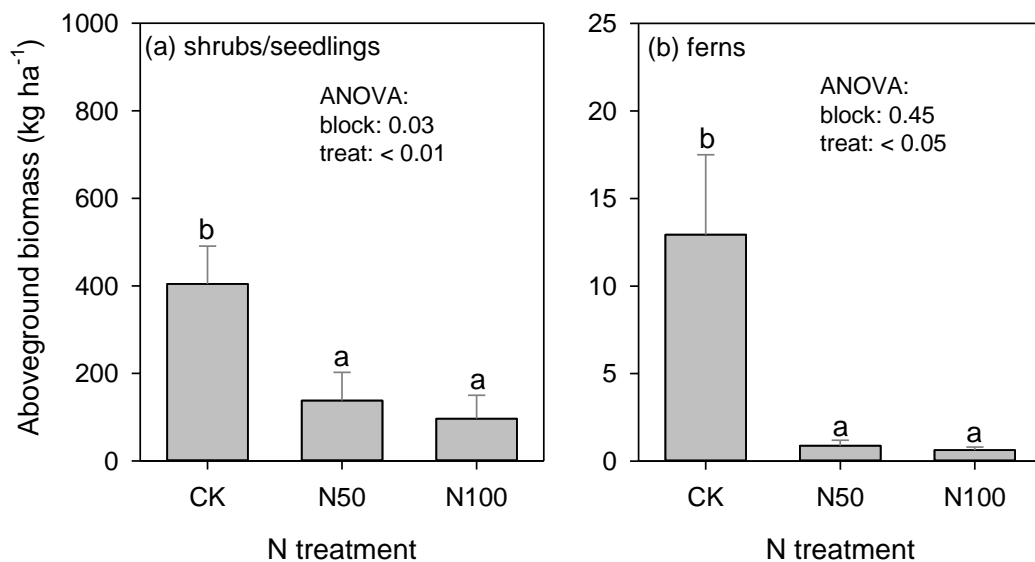
503 **Figure 3** Effects of N fertilization on growth of saplings (mean \pm se, n=3). (a) Absolute basal
504 area increase, and (b) relative growth rate of basal area. Numbers in these figures indicate the
505 results of ANOVA.

506



507

508 **Figure 4** Effects of N fertilization on aboveground biomass of shrubs, seedlings and ferns.
509 Bars show aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean \pm se, n=3).
510 Numbers in these figures indicate the results of ANOVA.



511

512 **Supplementary materials**

513

514 **Methods of soil sampling and nutrient detection**

515 During plant leaf and root sampling, we collected soil samples at a depth of 0-10 cm to detect
 516 soil nutrient availability. We set three subplots randomly within each plot and collected three
 517 subsamples for each subplot using a hand-held steel soil borer (3 cm in diameter). Then, the
 518 three subsamples were mixed together to form one sample per plot and transported to a
 519 laboratory and air dried naturally. After air-dried, soil samples were ground with a ball mill
 520 (NM200, Retsch, Haan, Germany) and screened through a 100 mesh sieve. Soil total N and P
 521 were measured using an elemental analyser (2400 Series2 CHNS/O Elemental Analyzer,
 522 Perkin-Elmer, USA). After acid digestion of the samples, leaf and root P concentrations were
 523 measured using a flow injection analysis instrument (AutoAnalyzer3, Bran+Lubbe City,
 524 Germany). In this study, mass total N and mass total P were used. Soil pH was measured by
 525 dry soil in water suspension with a water:soil ratio of 1:2.5.

526

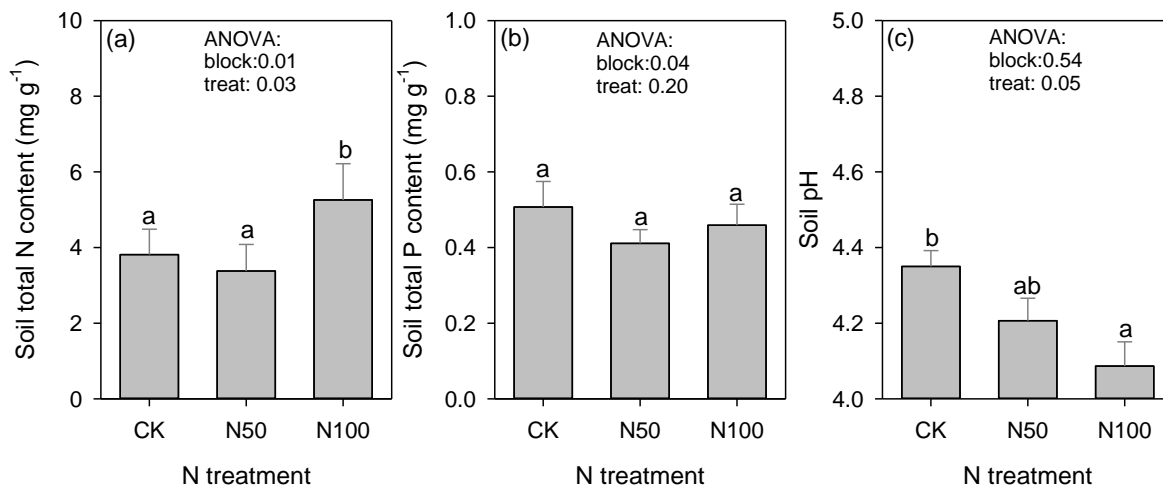
527 **Table S1.** Allometric equations for the aboveground biomass of different plant species in this
 528 study.

Species	Equations	a ₁	a ₂	b	r ²	p
<i>W. japonica</i>	$Y = X_1^{a_1} * X_2^{a_2} * 10^b$	1.703	0.790	-3.418	0.92	<0.001
<i>C. japonica</i>	$Y = a_1 * (BD^2 * H)^b$	0.061		0.707	0.99	<0.001
<i>C. cuspidate</i>	$Y = a_1 * (CV)^b$	0.001		0.829	0.94	<0.001
<i>R. ovatum</i>	$Y = a_1 * (BD^2 * H) + b$	0.004		0.831	0.97	<0.001
<i>E. muricata</i>	$Y = a_1 * (BD^2 * H)^b$	0.028		0.816	0.90	<0.001
<i>C. japonicum</i>	$Y = a_1 * (CV)^b$	0.005		0.716	0.90	<0.001
<i>C. subavenium</i>	$Y = a_1 * (CV)^b$	0.001		0.817	0.93	<0.001
<i>S. glabra</i>	$Y = a_1 * (CV) + b$	0.0002		1.692	0.96	<0.001
<i>C. eyrei</i> ⁽¹⁾	$Y = a_1 * (D^2 * H)^b$	0.065		0.920	0.98	<0.001
Others ⁽²⁾	$Y = a_1 * (D^2 * H)^b$	0.095		0.870	0.91	<0.001

529 Y: Aboveground biomass (g for shrubs; kg for trees/saplings); X₁ (cm): length of fern leaves;
 530 X₂ (cm): width of fern leaves; BD (basal diameter): diameter at 10 cm above the ground; H:
 531 height of plants (cm for shrubs/seedlings, m for trees/saplings); CV (cm³): 3.14*[(canopy
 532 length + canopy width)/2]²*H; D (cm): diameter at breast height (~1.3 m) of tree/saplings.
 533 (1) from Du *et al.* 1987; (2) from Zhang *et al.* 2007.

534

535 **Figure S1** Effects of N fertilization on soil nutrient content and pH (mean \pm se, n=3) at a soil
536 depth of 0-10 cm. (a) Total N content per gram soil; (b) total P content per gram soil; and (c)
537 soil pH. Numbers in these figures indicate the results of ANOVA .



538