

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.

Di Tian, tiandi@pku.edu.cn

Jingyun Fang, jyfang@urban.pku.edu.cn

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*.

We appreciate very much your insightful comments which allowed us to improve our manuscript. We have carefully studied your helpful suggestions and rephrased the title, results and discussion in the updated version. Following your comments, we have rephrased the title as "*Growth responses of trees and understory plants to nitrogen fertilization in a subtropical forest in China*" in the revised manuscript. The point-to-point responses and the revised manuscript with changes marked are attached with this letter at the bottom.

We hope that our manuscript is now satisfied with you and look forward to hearing from you soon. Thank you!

On behalf of all the authors,

Di Tian (tiandi@pku.edu.cn)

Jingyun Fang (jyfang@urban.pku.edu.cn)

[Comment] 1. The title focusses the comparison on growth form, whereas in reality, your results mainly give evidence for the differential response between over and understorey, with the latter including different growth forms. This needs to be adequately reflected in title and abstract.

[Reply] Many thanks for your helpful and insightful comment. Following your suggestions, we have rephrased the title as "Growth responses of trees and understory plants to nitrogen fertilization in a subtropical forest in China" in the revised manuscript. Then, we checked the wording in the abstract to adequately reflect different responses of trees and understory plants to nitrogen fertilization.

[Comment] 2. In the methods, you have removed the information on site N deposition, which is however, relevant. I would present this information next to precipitation and temperature estimates earlier in Section 2.1.

[Reply] Thanks. Yes, we presented the information on N deposition next to precipitation and temperature in the revision as follows: The amount of wet N deposition in this region was 5.9-7.3 kg N ha⁻¹ yr⁻¹ [Lines 121-122].

[Comment] 3. New Section 3.1: I think this section needs to be accompanied by a statement as to whether it is possible that the fish eye measurements did not provide evidence for changes in total forest cover, while there still may be a shift between the contribution of overstorey and understory trees to the total forest cover (partly therefore explaining the difference between the response across DBH)?

[Reply] Thanks. We agree with you. Accordingly, we added these statements in the New Section 3.1 as follows: Although the fish eye measurements did not provide evidence for changes in total forest cover with the effects of N fertilization, there still may be a shift between the contribution of overstorey and understory trees to the total forest cover [Lines 239-242]. In addition, we added a statement in the Discussion section as follows: our measurements of forest canopy cover provided a rough evaluation for light availability and a potential shift between the contribution of overstorey and understory trees to the total forest cover which partly explained the difference between the responses across trees in different size (i.e. different DBH classes) [Lines 371-376].

[Comment] 4. Given that the pH at the end of the treatment is 4.1, whereas in Section 2.1 it was introduced as 4.6, this needs more explicit mentioning in Section 3.1 and the Discussion on other limiting factors. This is a change of 0.5 pH within 3.4 years, which in my view cannot be described as "mild".

[Reply] Many thanks for your insightful comment. We have removed the word "mildly" in line 247 in the revision. Due to the seasonal changes of soil pH in the subtropical forest, the value of 4.6 introduced in Section 2.1 represented the averaged value during a whole year of 2011 before the N addition. However, the values of soil pH reported in Section 3.1 showed the difference among three N treatments (i.e. control, N50 and N100) in July 2014 after 3.4 years' of N fertilization. As showed in Figure 1, the soil pH values in these three treatments were 4.35±0.04 (mean±se), 4.21±0.06, 4.09±0.06, respectively.

1 **Contrasting growth responses among trees and understory plants**
2 **growth forms to nitrogen fertilization in a subtropical forest in China**

4 Di Tian¹, Peng Li¹, Wenjing Fang¹, Jun Xu², Yongkai Luo³, Zhengbing Yan¹, Biao Zhu¹,
5 Jingjing Wang², Xiaoniu Xu², Jingyun Fang^{1*}

7 ¹*Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory
8 for Earth Surface Processes of the Ministry of Education, Peking University, Beijing, 100871,
9 China;*

10 ²*Department of Forestry, Anhui Agricultural University, 230036, Hefei, Anhui, China;*

11 ³*State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese
12 Academy of Sciences, Beijing 100093, China*

14 *Correspondence author:

15 Dr. Jingyun Fang

16 Department of Ecology, Peking University

17 Beijing 100871, China

18 E-mail: jyfang@urban.pku.edu.cn

20 **Abstract**

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

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

45

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

48 **1 Introduction**

49

50 Reactive nitrogen (N) increase in the biosphere, especially atmospheric N deposition, is a
51 globally prevalent phenomenon (Galloway *et al.* 2004). It has become a serious
52 environmental issue in China, especially in the southeastern regions, with drastic increase of
53 N entering terrestrial ecosystems which produces considerable ecological effects on the
54 functioning and dynamics of the terrestrial ecosystems (Liu *et al.* 2013; Gu *et al.* 2015). Since
55 the 1990s, the simulated N-fertilization experiments have been conducted in various forest
56 ecosystems to explore the responses of plants and other organisms to the potential high N
57 enrichment and changes of soil N availability (e.g., Wright & Tietema 1995; Bobbink *et al.*
58 2010; Fowler *et al.* 2015). Although a number of studies have reported a general positive
59 effect of N enrichment on plants in N-limited forests and a negative effect of excess N (e.g.,
60 Aber *et al.* 1998; Höglberg *et al.* 2006; Gilliam 2006; Thomas *et al.* 2010), specific responses
61 of plants appeared to be highly growth form-dependent and ecosystem-dependent (LeBauer
62 & Treseder 2008; Bedison & McNeil 2009; Dirnböck *et al.* 2014).

63

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

77

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

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

92

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

101

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

109

110 **2 Materials and methods**

111

112 **2.1 Study site and experimental design**

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

116 broadleaved evergreen forest, the Guniujiang experimental site is an important part of the
117 NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du *et al.*
118 2013), because of its representativeness in both species composition and landscape structure
119 in the subtropical evergreen forest region. The study area has a humid climate with strong
120 summer monsoons with an annual average precipitation of 1,700 mm and an average annual
121 temperature of 14.9 °C. **The amount of wet N deposition in this region was 5.9-7.3 kg N**
122 **ha⁻¹ yr⁻¹**. The soil in this area has been classified as yellow brown earth (Chinese Soil
123 Taxonomic Classification), and the pH_{H2O} value at 0-10 cm soil depth was 4.58±0.05
124 (mean±SE). The total N, P, NH₄⁺-N and NO₃-N content in the soil at 0-10 cm depth were 3.23
125 (0.37), 0.32 (0.02), 0.012 (0.001), and 0.002 (0.0006) mg g⁻¹, respectively (Li *et al.* 2015).

126
127 The study was conducted in a well-protected, mature subtropical evergreen forest (>300 year
128 age) with a three-layered vertical structure: the canopy tree layer (DBH>5 cm and height>5
129 m); the understory layer of saplings, shrubs and seedlings (DBH<5 cm and height<5 m); and
130 the ground-cover layer (ferns and herbs). The average density and basal area of trees were
131 1,219 trees ha⁻¹ and 36.35 m² ha⁻¹, respectively; *Castanopsis eyrei* was the dominant species
132 (which was also an important species at some other sites in subtropical forests) and accounted
133 for 87% of the total aboveground biomass of trees. The understory saplings and shrubs
134 contained several species, including *Cleyera japonica*, *Camellia cuspidata*, *Rhododendron*
135 *ovatum*, *Eurya muricata*, *Cinnamomum japonicum*, *Cinnamomum subavenium*, *Sarcandra*
136 *glabra*, and *C. eyrei*, and other native subtropical evergreen species (Table 1). Two fern
137 species (*Woodwardia japonica* and *Dryopteris hwangshanensis*) and an orchid (*Cymbidium*
138 *tortisepalum* var. *longibracteatum*) appeared on the floor layer, while *W. japonica* exclusively
139 dominated the floor layer with a coverage of 10%-20%.

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

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:

$$158 \quad DBH = DBH_1 + \frac{X_2 - X_1}{3.14 \times 10}$$

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 ($m^2 \text{ ha}^{-2} \text{ year}^{-1}$) 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, $m^2 \text{ m}^{-2} \text{ year}^{-1}$) 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):

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

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

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

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 m × 5 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, we measured soil N, P content and pH. Specifically, we set three
209 subplots randomly within each plot and collected three subsamples of 0-10 cm soil for each
210 subplot using a hand-held steel soil borer (3 cm in diameter), during investigation of the
211 understory plants. Then, the three subsamples were mixed together to form one sample per
212 plot and transported to a laboratory and air dried naturally. After air-dried, soil samples were
213 ground with a ball mill (NM200, Retsch, Haan, Germany) and screened through a 100 mesh
214 sieve. The N concentration of soil was measured using an elemental analyser (2400 Series2
215 CHNS/O Elemental Analyzer, Perkin-Elmer, USA). After acid digestion of the samples, soil P
216 concentrations were measured using a flow injection analysis instrument (AutoAnanlyzer3,
217 Bran+Lubbe City, Germany). Soil pH was measured by dry soil in water suspension with a

218 water:soil ratio of 1:2.5.

219

220 **2.3 Statistical analysis**

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

234

235 **3 Results**

236

237 **3.1 Effects of N fertilization on canopy cover, soil N and P contents and pH**

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

243

244 3.4 years of N fertilization significantly increased the N content of 0-10 cm soil ($p=0.03$),
245 especially in N100 plots (Fig. 1a), but showed no significant effect on soil P content (Fig. 1b,
246 $p>0.05$), thus leading to a significant increase in soil N:P ratio (Fig. 1c, $p=0.02$). Additionally,
247 the N fertilization also decreased **mildly** soil pH and aggravated soil acidification (Fig. 1d,
248 $p=0.05$).

249

250 **3.2 Growth responses of trees to N fertilization**

251 The increments of absolute basal area, aboveground biomass and RGR of all trees at plot
252 level showed no significant response to N fertilization during 3.4-year N fertilization (Fig.

253 2a~2c). Compared with the unfertilized plots, N50 and N100 fertilized plots showed a
254 tendency toward higher averaged proportions of dead trees' aboveground biomass despite no
255 significant difference between them (Fig. 2d).

256
257 Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent
258 responses of absolute basal area increments and RGR to N fertilization (Fig. 3a-3f). The
259 small trees with a DBH of 5-10 cm growing under unfertilized plots showed greater basal
260 area increments than those growing under N fertilized plots (Fig. 3a, $p=0.02$). Specifically,
261 the N50 and N100 fertilization decreased the absolute basal area increments of small
262 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
263 indicated that the decreasing degrees of the absolute basal area of small trees reached 66.4%
264 and 59.5% in N50 and N100 plots. The small individual trees also showed a tendency toward
265 lower averaged RGR in N fertilized plots although no significant difference was detected
266 between them (Fig. 3d, $p>0.05$). As opposed to the negative responses of small trees to N
267 fertilization, the basal area increment and RGR of median *C. eyrei* individuals (DBH of 10-30
268 cm) and large *C. eyrei* individuals (DBH of >30cm) showed no significant response to N
269 fertilization, but the averaged growth rate of large *C. eyrei* individuals in N50 plots almost
270 doubled the value of the corresponding large individuals in unfertilized plots (Fig. 3b-3c and
271 3e-3f, $p>0.05$ in all cases).

272
273 **3.3 Growth responses of understory saplings, shrubs/seedlings, and ferns to N
274 fertilization**
275 Responses of understory saplings to N fertilization were similar to those of small dominant
276 trees. Although the annual absolute increments of basal area increments of saplings showed
277 no significant response to N fertilization (Fig. 4a, $p>0.05$), the RGR of sapling growing in
278 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
279 $\text{m}^{-2} \text{ yr}^{-1}$, respectively, compared to sapling growing in unfertilized plots (Fig. 4b, $p<0.001$). In
280 addition, a general negative effect of N fertilization also occurred on understory shrubs and
281 ground-cover ferns. The aboveground biomass of seven predominant shrubs/seedlings was
282 drastically decreased by 69.4% and 79.1% in N50 and N100 fertilized plots, respectively,
283 compared with those in the unfertilized plots (Fig. 5a, $p<0.01$). Remarkably, the aboveground
284 biomass of ground-cover ferns significantly declined by 92.4% and 93.4% in N50 and N100
285 fertilized plots (Fig. 5b, $p<0.05$).

286

287 **4 Discussion**

288

289 **4.1 Growth responses of trees to N fertilization**

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

304

305 Contrasted with previous positive responses of trees to N fertilization in boreal and temperate
306 forests which were considered as N limited ecosystems (Högberg *et al.* 2006; Thomas *et al.*
307 2010; BassiriRad *et al.* 2015), our finding of the unchanged responses of trees to N
308 fertilization was partly consistent with observations of trees from tropical forests (e.g.,
309 Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013). Studies from mature tropical forests have
310 revealed that P availability was a critical element shaping tree species distribution and
311 productivity (Santiago 2016; Dalling *et al.* 2016). Given the similar high-weathered soil
312 properties, humid climatic conditions and dominant evergreen broadleaf trees in mature
313 subtropical forest as those in wet tropical forest, we speculated that P limitation, rather than N
314 limitation, might have played a key role in influencing growth of plants in subtropical forest.

315

316 The N and P stoichiometry of soil might have objectively provided indicators of P limitation
317 with the effects of N fertilization in this subtropical forest, because soil N contents and N:P
318 ratio in N fertilized plots were remarkably higher than those in unfertilized plots (Fig. 1).
319 Additionally, limitation of other nutrients, such as K (potassium) which was highlighted in
320 tropical forests, and their combination as well as heterogeneous nutrient limitation of specific

321 species, plant growth forms and individuals in different sizes may warrant further
322 consideration in subtropical forests (Wright *et al.* 2011; Santiago *et al.* 2012; Alvarez-Clare *et*
323 *al.* 2013).

324

325

326 Moreover, the high spatial heterogeneity in old-aged subtropical forest, similar to tropical
327 forests, could be a possible explanation for the lack of significant responses of plot-averaged
328 basal area growth, RGR, aboveground biomass of trees with a DBH of >5 cm and the
329 proportion of dead trees to N fertilization. In eastern China, the distributions of subtropical
330 forest stands are quite topographically fragmented, while relative flat stands are required to
331 avoid N losses and minimize spatial heterogeneity among experimental treatments. The
332 actual distribution and topography of the subtropical forests limited the number of
333 replications in the N fertilization experiment. This limitation might reduce the statistic power
334 of N treatment on plot-averaged plant growth rate which has been pointed out in previous
335 studies (Wright *et al.* 2011; Alvarez-Clare *et al.* 2013). Furthermore, our observation of large
336 trees with DBH >30 cm showed that the averaged growth rate of large *C. eyrei* individuals in
337 N50 plots almost doubled the value of the corresponding large individuals in unfertilized
338 plots. Nevertheless, the results of ANOVA showed that the effect was not significant. As the
339 number of large trees in the experiment was relatively less than the small trees, the low
340 replication and high spatial site heterogeneity might have reduced the statistical power of N
341 fertilization on the large trees. Thus, fertilization experiments with more homogeneous plots
342 and more replicates are warranted to further strengthen these findings. Overall, given the
343 negative and potential positive effects of N fertilization on small and large trees, it is of
344 urgent necessity to conduct long-term monitoring of the trees which would provide
345 alternatives for accurately evaluating the forest dynamics under the enhanced global N
346 deposition.

347

348 **4.2 Growth responses of small trees, understory saplings, shrubs/seedlings and ferns to** 349 **N fertilization**

350 Although the positive responses of small or juvenile trees to nutrient fertilization has been
351 reported in boreal, temperate and tropical forest (e.g., Högberg *et al.* 2006; Bedison &
352 McNeil 2009; Alvarez-Clare *et al.* 2013), our results showed a remarkable negative effect of
353 N fertilization on small-sized plants including trees, understory saplings, shrubs/seedlings and
354 ferns. During our field investigation, we also found that the average proportion of dead trees

355 (Fig. 2d) tended to increase in N fertilized plots although the result was not statistically
356 significant ($p = 0.50$). Additionally, the ground-cover ferns in N100 plots almost disappeared
357 after 3.4-year N fertilization (personal observation). Given the high stand density in this
358 mature subtropical forest, we suggest that N fertilization might potentially lead to increased
359 self- and alien-thinning of individuals through decreasing understory light availability.

360
361 The pivotal role of light availability in the eco-physiological processes of understory growth
362 forms has been widely recognized (Santiago 2015). Due to the limited light availability,
363 understory plants may not be able to incorporate the added nutrient and promote their
364 photosynthetic rates (Alvarez-Clare *et al.* 2013). However, a study conducted in tropical
365 forest with thick canopy showed that photosynthetic process could be enhanced by nutrient
366 addition even under low light availability (Pasquini & Santiago 2012). In a sharp contrast, the
367 study conducted in an Australian rainforest revealed that understory seedlings increased
368 growth when the light availability was high, but showed no significant response to nutrient
369 fertilization in low lights (Thompson *et al.* 1988). These studies, together with our field
370 observations, suggest that the growth of understory plants is largely co-limited by nutrient
371 and light availability in the local environment. Further, our results of forest canopy cover
372 estimated by photographic fisheye showed no significant differences between unfertilized and
373 N fertilized plots, which was consistent with the findings of Lu *et al.* (2010). Although the
374 understory light irradiance fluctuated largely during a day and was very hard to detect
375 precisely, our measurements of forest canopy cover provided a rough evaluation for light
376 availability and a potential shift between the contribution of overstory and understory trees to
377 the total forest cover which could partly explain the differences in the responses of trees with
378 different sizes (i.e. different DBH classes). The results might indicate that other factors in
379 addition to the low light availability in this old-aged forest had also played a crucial role in
380 influencing understory plants during 3.4 years' N fertilization.

381

382 **4.3 Potential N saturation and plant growth**

383

384 The striking biomass reduction of the understory plants, especially ferns, in response to N
385 fertilization in our study well corroborated the similar findings in an old-aged tropical forest
386 at Mt. Dinghushan in China (Lu *et al.*, 2010). Also, consistent with previous studies obtained
387 from boreal, temperate and tropical forests (Rainey *et al.* 1999; Alvarez-Clare *et al.* 2013;
388 Dirnböck *et al.* 2014), our experiment revealed that understory small-sized plants responded

389 sensitively to nutrient fertilization, which might indicate a possibility of N saturation in the
390 subtropical forest. According to the definition of N saturation addressed by Aber *et al.* (1998)
391 (i.e., N availability in the forest ecosystem exceeded the demand of plants and microbes), the
392 drastic decrease of understory ferns, shifted composition of understory plant community, and
393 cation imbalances of understory species after 7 years' chronic N fertilization at Harvard
394 Forest, USA, could be interpreted as useful indicators of N saturation (Rainey *et al.* 1999).
395 Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Mt.
396 Dinghushan also showed signs of N saturation, such as significant increases in nitrate (NO₃⁻)
397 leaching, inorganic N concentration and N₂O emissions of soils, and soil acidification (Lu *et*
398 *al.* 2014; Chen *et al.* 2015). In our experiment, the soil acidification and increased soil N
399 concentration in high N fertilized plots (Fig. 1) combined with the negative responses of
400 understory plants suggest that the 3.4-year N fertilization in this mature subtropical forest site
401 has potentially caused N saturation. Nevertheless, further observations are still required to
402 explore the mechanisms underlying the changes of different growth forms with the effects of
403 N enhancement in the subtropical forests.

404

405 5. Conclusion

406

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

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

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

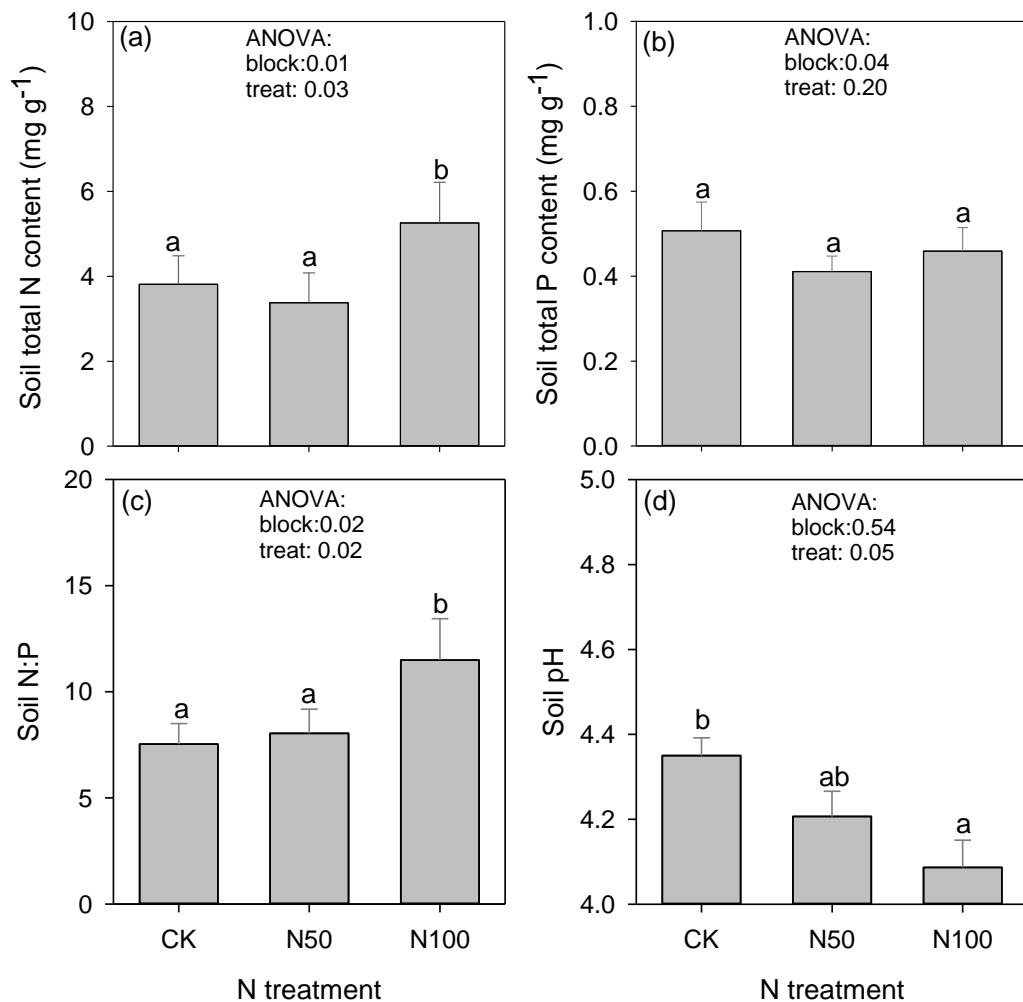
537

538 | Table 2 The indicator of canopy cover (i.e., [1-Fmv]) of the three treatments in our
539 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

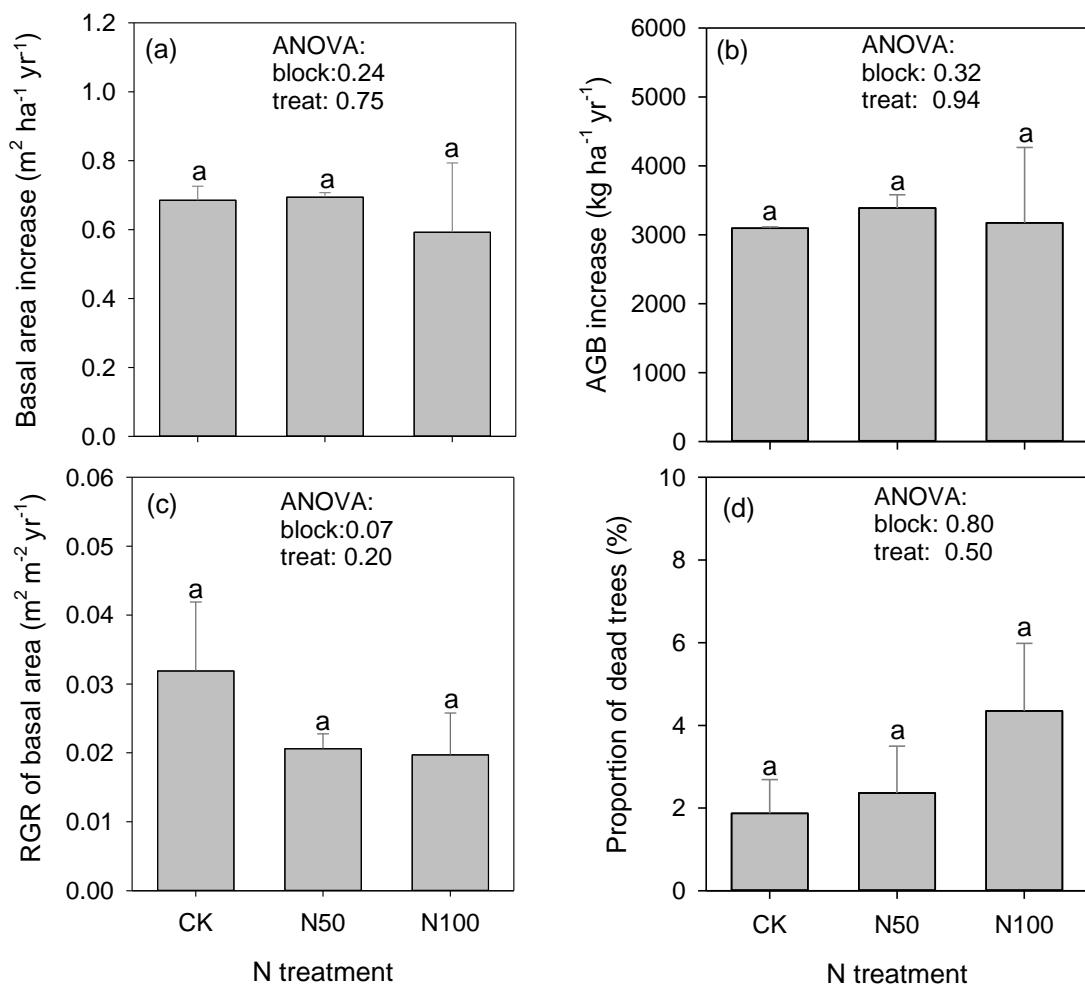
540

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



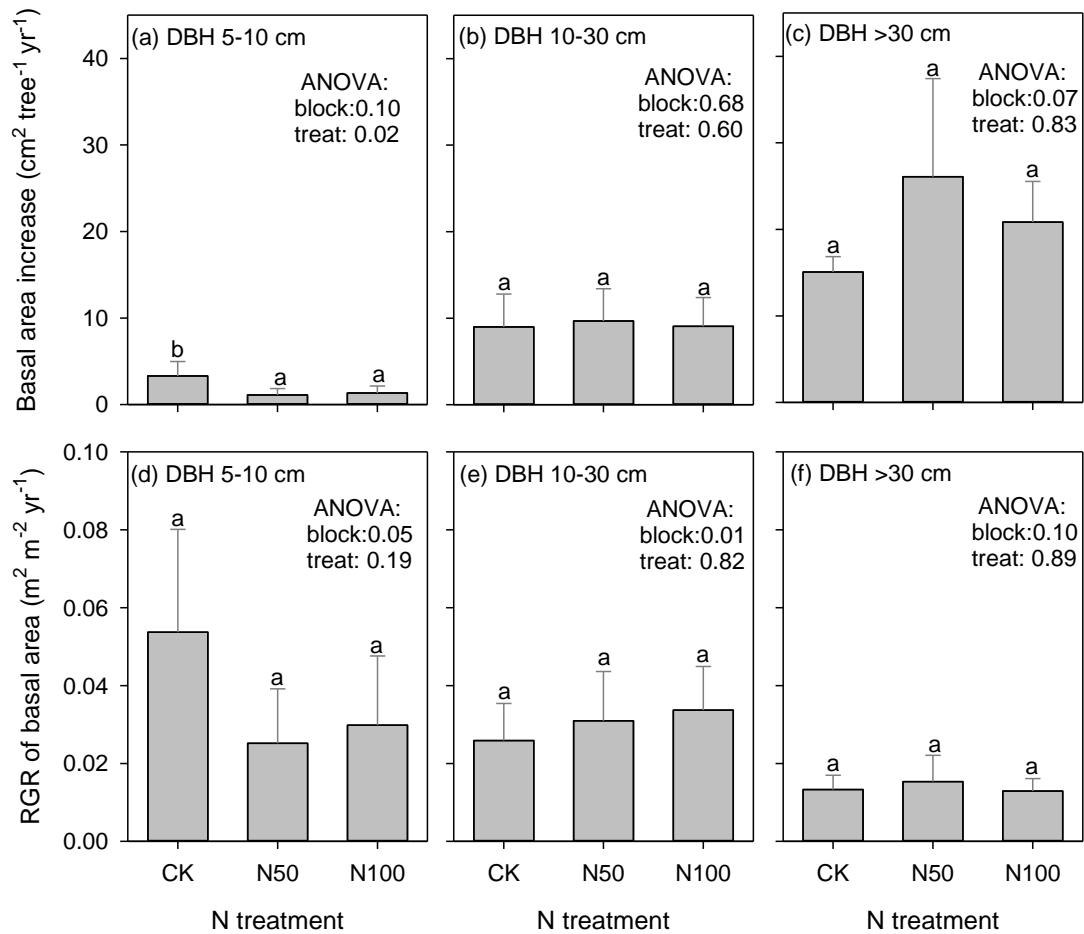
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545 **Figure 2** Effects of N fertilization on the growth and mortality of all trees (mean \pm se). (a)
 546 Absolute basal area increase of all trees; (b) aboveground biomass increase of all trees; (c)
 547 relative growth rate of total tree basal area; and (d) the proportion of all dead trees. The
 548 proportion of dead trees was calculated using the aboveground biomass of all dead trees
 549 during the experiment divided by the total aboveground biomass of all trees in 2014.
 550 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis
 551 represents three levels of N fertilization: CK (0 kg N $\text{ha}^{-1} \text{yr}^{-1}$), N50 (50 kg N $\text{ha}^{-1} \text{yr}^{-1}$) and
 552 N100 (100 kg N $\text{ha}^{-1} \text{yr}^{-1}$).



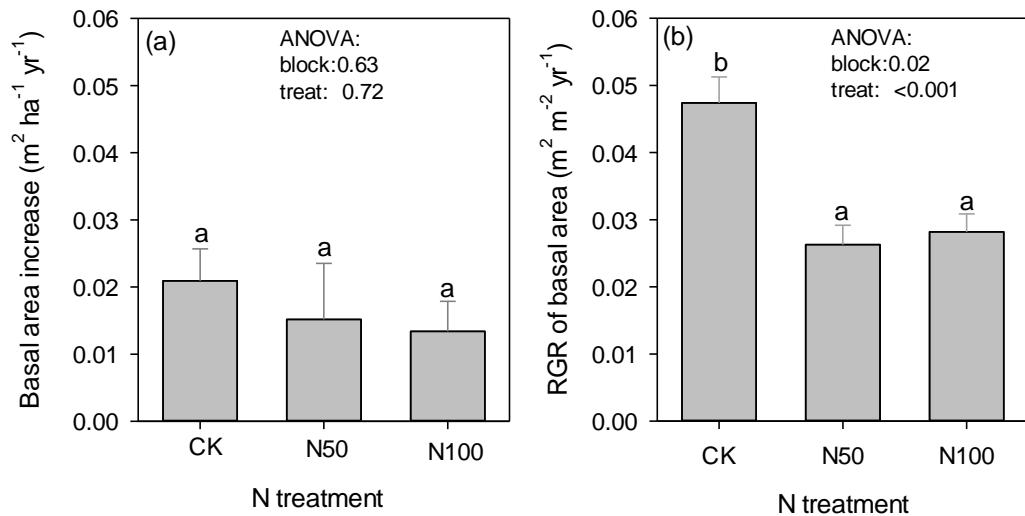
553

554 **Figure 3** Effects of N fertilization on the growth (mean \pm se) of *C. eyrei* by DBH classes
 555 (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth
 556 increase rate of basal area. Numbers in these figures indicate the results of ANOVA. The N
 557 treatment on x-axis represents three levels of N fertilization: CK (0 kg N ha $^{-1}$ yr $^{-1}$), N50 (50
 558 kg N ha $^{-1}$ yr $^{-1}$), and N100 (100 kg N ha $^{-1}$ yr $^{-1}$).



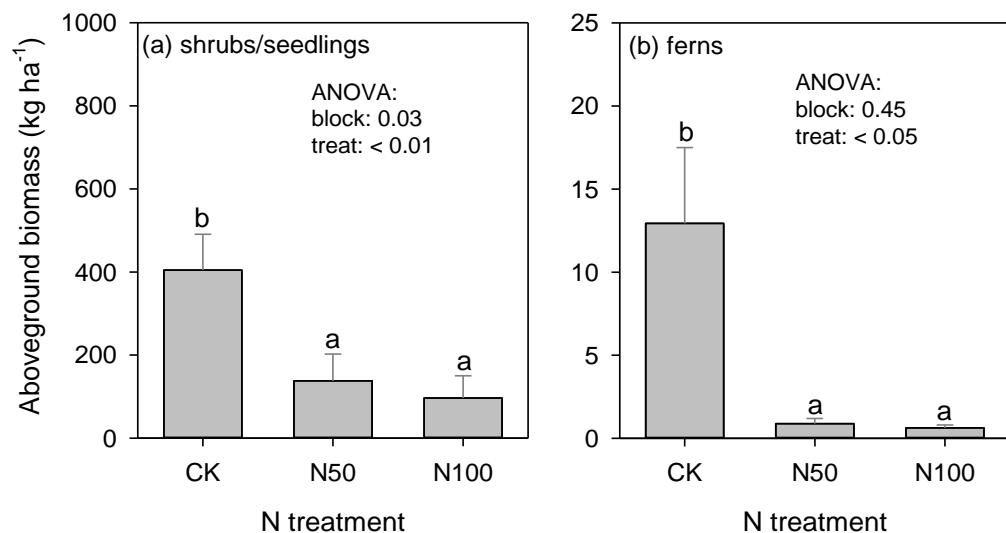
559

560 **Figure 4** Effects of N fertilization on the growth of saplings (mean \pm se). (a) Absolute basal
561 area increase and (b) the relative growth rate of basal area. Numbers in these figures indicate
562 the results of ANOVA. The N treatment on x-axis represents three levels of N fertilization:
563 CK (0 kg N $\text{ha}^{-1} \text{yr}^{-1}$), N50 (50 kg N $\text{ha}^{-1} \text{yr}^{-1}$) and N100 (100 kg N $\text{ha}^{-1} \text{yr}^{-1}$).
564



565

566 **Figure 5** Effects of N fertilization on the aboveground biomass of shrubs, seedlings and ferns.
567 Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean \pm se).
568 Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis
569 represents three levels of N fertilization: CK (0 kg N ha $^{-1}$ yr $^{-1}$), N50 (50 kg N ha $^{-1}$ yr $^{-1}$) and
570 N100 (100 kg N ha $^{-1}$ yr $^{-1}$).
571



572