Authors' response to reviewer' 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.

Dear Dr. Zaehle,

We appreciate your help very much in developing the manuscript and your devotion to find suitable referees. Also, we appreciate the comments from two anonymous referees. The major comments were focused on the N dosages and limited replications, and unclear description of our results, as stated in a separated letter we have written to you. We have carefully studied the comments and rephrased the introduction, results and discussion in the updated version. The point-by-point responses are as follows. We believe that the revised version should be satisfactory to you and the reviewers.

We are looking forward to receiving your decision soon.

Best wishes,
Di Tian and Jingyun Fang

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Inc: Responses to the Referees #1 and #2

To Anonymous Referee #1:

[Major Comments]: This paper describes the results of a 3 year (authors say 4 in the abstract) forest N fertilization study conducted in China. The study focuses on growth of trees, saplings, shrubs and understory growth and mortality. The authors main conclusion is that N fertilization affects the various plant growth forms in different ways, with the smaller plants being most affected. Overall, this paper adds to the growing knowledge regarding N impacts on forest ecosystems, but suffers from many of the limitations that other fertilizer studies have to deal with 1) environmental relevance of the dosage amount and form, 2) short (3 year) period for assessment and 3) no data to support the mechanisms of the observed impact. Further, the study has low replication (3 20 x 20 m plots) per treatment. My suggestion is that in the revised paper - these limitations should be fully addressed and evaluated with respect to the implications for the overall conclusions made by the authors.

[Reply] Many thanks for the helpful and insightful comments regarding our manuscript. We appreciated that the reviewer recognized the unique value of our paper which may add to the growing knowledge regarding N impacts on forest ecosystems, especially in large areas of subtropical forests which are potentially making increasing contribution to carbon storage in China. The reviewer points out two limitations in this study. Firstly, the duration of the fertilization experiment was not accurately described. Data collected from March 2011 to July 2014 (and plants experienced 4 continuous growth seasons) were used in our study, so we briefly described the time scale of N fertilization to be 4 years in abstract. We accept the reviewer's suggestion and rephrased the duration of N fertilization to be 3.4 years in the manuscript.

Secondly, the reviewer pointed out that there were only three replications in each treatment. In fact, that the number of replications in our experiment was only three blocks was because of the actual distribution and topography of the subtropical forests. In eastern China, the distributions of subtropical forest stands are quite topographically fragmented, while relative flat stands are required to avoid N losses and minimize spatial heterogeneity among experimental treatments. Hence, after taking all the environmental conditions into consideration and comparing several evergreen broadleaved forests in subtropical regions, we determined to conduct N fertilization experiment in this forest located in the natural conservation zone of Guniujiang in Anhui Province, eastern China, because both the plant community and the landscape are good representatives of typical subtropical evergreen broadleaved forests. Actually, many of N addition experiments across different sites at boreal, temperate, tropical and subtropical forests have had similar number of replications (Rainey et al., 1999; Magill et al., 2004; Lu et al., 2010). For example, a similar experiment in a subtropical forest at Mt. Dinghushan in south China has a smaller plot size of 20 m × 10 m and 3 replications (Lu et al., 2010). In the Harvard Forest where long-term N fertilization experiments have been conducted for more than 30 years, three replications of three N treatments (control: 0 kg N ha^{-1} yr^{-1}, low N: 50 kg N ha^{-1} yr^{-1}, high N: 100 kg N ha^{-1} yr^{-1}) were settled. That is to say, our experimental treatments (e.g., design of N dosages and replications) are consistent or comparable with those in other regions of forests, which provided a good opportunity to compare results among sites and forest ecosystems globally. Moreover, the experiment introduced in our paper here is an important part of the Network of Nutrient Enrichment Experiments in China's Forest including 8 forests along latitude gradients in eastern China. We have conducted N fertilization experiment to stimulate N deposition simultaneously in 8 forests since 2011.

[Specific comments]

[Comments] 1. Environmental relevance - The application rates of 50 and 100 kg/N/ha are very high and I suspect are found in a few locations in China, but not likely widespread. My experience is that such high dosages almost always produce some effect but 1 year of 50 kg/N/ha is not the same as 5 years at 10 kg/N/ha. The authors should read a very good paper by Lovett and Goodale (2011) Ecosystems - that discusses this issue. Further, if my math is correct the authors are applying 100 kg N in 12 dosages per year, each time in 15 L of water. This makes 8kg N per time - dissolved in 15L, which is about 440g/L. Given the reportedly greater impacts of the treatments on the ground species, I am wondering about the direct effects of this spray? This should be discussed/evaluated.

[Reply] We agree with the reviewer's point that application rates of 50 and 100 kg/N/ha are high and found in a few locations in China. However, with the rapid growth of global population, Nr creation by human beings has increased approximately three times during 1850-2010 (Galloway et al., 2014), of which large amount of reactive N emission lead to serious atmospheric N deposition, especially in eastern North America, Europe, China, India and Brazil (BassiriRad, 2015). In large parts of the non-urban areas across China, the rates of wet N deposition have exceeded 15 kg N $ha^{-1}yr^{-1}$ from 1995 to 2007 (Du et al., 2014). Taking the increasing rates of N deposition in eastern China into consideration, we set the dosages of N fertilization to simulate the potential effects of high N deposition. Moreover, the design of N50 and N100 were kept in accordance with previous studies conducted in boreal forests, temperate forests across Europe and America, tropical forests and subtropical forests (e.g., Rainey et al., 1999; Högberg et al., 2006; Lu et al., 2010; Alvarez-Clare et al., 2013). The consistency of N fertilization provided a good opportunity to compare results among sites.

Regarding the concentration of dosages, total NH_4NO_3 was divided into 12 dosages and applied to the forest in each month at regular intervals during a year. According the design of N treatments (N50: 50 kg N ha^{-1} yr^{-1} and N100: 100 kg N ha^{-1} yr^{-1}) and the size of plots (20 m × 20 m), NH_4NO_3 in dosages of 0.48 kg $plot^{-1}$ $month^{-1}$ and 0.95 kg $plot^{-1}$ $month^{-1}$ were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly in N50 and N100 plots using a back-hatch sprayer. The unfertilized plots were similarly treated with 15 L of fresh water without NH_4NO_3. Therefore, the 0.48 kg and 0.95 kg NH_4NO_3 dissolved in 15 L of fresh water, respectively, represent N concentration of 11.1 g/L and 22.2 g/L in N50 and N100 plots, much lower than high concentration of 440 g/L as the reviewer calculated. For detailed calculation in a case of N100 plots, please see the following:
N concentration (g N L^{-1} $plot^{-1}$ $month^{-1}$) for N100 plots (100 kg N ha^{-1} yr^{-1})
= 285.71 kg NH_4NO_3 ha^{-1} yr^{-1} (please note: 1 kg N = 2.8571 kg NH_4NO_3)
= 0.95 kg NH_4NO_3 $plot^{-1}$ $month^{-1}$
= 0.33 kg N $plot^{-1}$ $month^{-1}$
Therefore, the N concentration for each plot:
= 0.33 kg N /15L
= 22.2 g N L^{-1} (please note: the amount of 0.33 kg N was dissolved into 15 L of fresh water for each plot and each month)
Therefore, to avoid misunderstanding, we described more details about the dosage of N fertilization to make it clear in the revised manuscript (Lines: 127-130).

[Comments] 2. This is a short study (3.4 years - should be consistent throughout which it isn't at present) with relatively low replication. In both instances real changes may be occurring but statistically they are not different among treatments. Throughout the paper the authors refer to differences among treatments - when in fact they are not significant (e.g. Figure 3). Over time or with more replication it could be true - just as equally it may still be noise in the system. The authors are guilty of talking about differences when in fact they statistically the same.

[Reply] We appreciate the reviewer's remind about the statistical result of the data. We described the limitation of the relatively short-term study (3.4 years) and the low replication (n=3) in our experiment in "Materials and methods" of our revised manuscript. Regarding the replications settled in our experiment, the plots were limited by the actual area of the subtropical forests. As we reported above, the distributions of subtropical forests are quite fragmented, while relative flat forests are needed to avoid N losses and minimize spatial heterogeneity among plots. Hence, after comparing several forests in subtropical regions, we conducted N fertilization experiment here because both the plant community and the landscape are very good representatives of typical subtropical evergreen forests. Moreover, a similar experiment in another subtropical forest at Mt. Dinghushan in China has plot size of 20 m ×10 m and replications of 3. Overall, the consistency in the design of N dosages and replications across boreal, temperate, tropical and subtropical forests including ours provided a good opportunity to compare results among sites and forest ecosystems globally. In addition, we carefully checked our description of the results, especially those regarding statistical analysis, and avoided misleading words in the revised manuscript. Please see the detailed revisions of results in Lines 203-220 at Pages 7-8.

[**Comments**] 3. The main argument for the difference in response among growth forms is shading. There is no evidence for this presented in the manuscript (not measured). Equally, there is no evidence for statistical differences in N content among treatments (supplementary info). Thus while the authors present a mechanistic reason behind the differences there is no real statistical evidence to support these claims. Changes in canopy cover were not assessed and N or P (nothing else shown) are not significant among treatments. Soil pH is lower, but Al or Mn are not measured. I found the discussion section (4.3) very misleading for example - "total N content of soil was enhanced by N fertilization and P concentration in plant leaves and in fine roots showed that N concentration increased" - not only is this a poor sentence, it is factually incorrect -N content did not increase in the 50 Kg N treatment nor did N content significantly increase (Figures are actually labeled incorrectly). Similarly there is no evidence of P being lowered by the treatment (soil or plant). Why was nitrate or ammonium not measured?

[**Reply**] Many thanks for these comments. We checked and corrected the wrong labels in the figure. In the Discussion section, we made a substantial revision to discuss potential mechanisms underlying the different responses of different growth forms to N fertilization. First of all, to provide an evidence of shading or light availability, we added the data of canopy cover measured by a digital camera with a fisheye lens [lines: 180-182 at Page 6-7]. We used this results in the discussion as following "Further, our results of forest canopy cover estimated by photographic fisheye showed no significant differences between unfertilized (0.77±0.01) and N fertilized plots (0.76±0.04 and 0.72±0.01 in N50 and N100 plots, respectively), which was consistent with the findings of Lu et al. (2010). Although the understory light irradiance fluctuated largely during a day and was very hard to detect precisely, our measurements of forest canopy cover provided a rough evaluation for light availability" [Lines: 307-309 at Page 10]. Secondly, we deleted the misleading sentences in section 4.3 and focused on the negative effects of potential N saturation on the growth of understory plants [lines: 315-331 at Page 10-11]. Actually, we have measured the changes of nitrate or ammonium of 0-10 cm soil in N fertilized plots (please see the Figure S1). Because the concentrations of nitrate or ammonium were more easily influenced by temperature, moisture (precipitation) and showed seasonal pattern, we did not bring these data into analysis to support our results. Instead, we adopted the soil total N content because not only the general pattern of the responses of soil total N content to N fertilization was similar to soil mineral content, but also was rather stable.

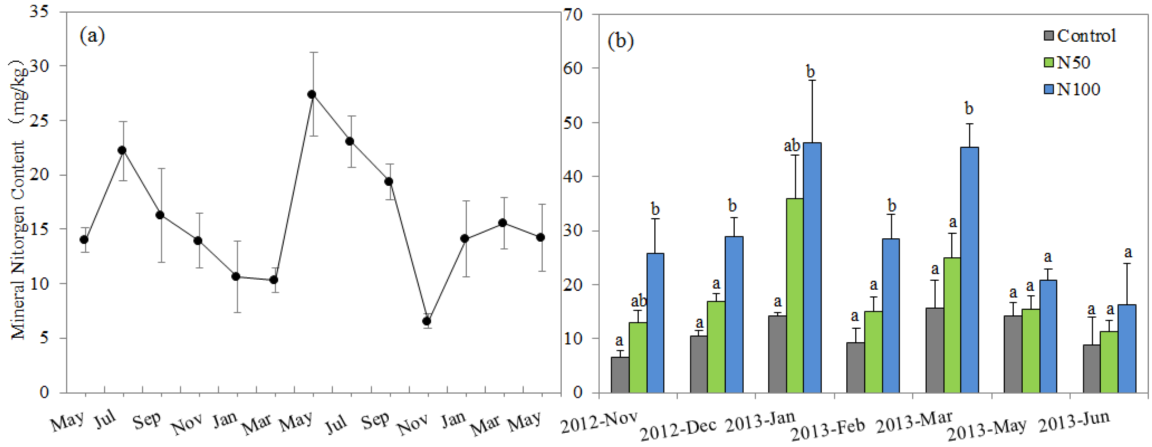


Figure S1. Soil (0-10 cm) mineral nitrogen content (the sum of NH_4 and NO_3-N, mg/kg). (a) Seasonal variation of soil mineral nitrogen content (mean ± se) in unfertilized plots from May 2011 to May 2013, and (b) effects of nitrogen fertilization on soil mineral nitrogen content. Different labels in (b) indicate significant differences among three N treatments in the same month (p<0.05).

[Comments] 4. The P fertilizer study added at the end reads just like an add on and does not help the paper and it should be deleted. Similarly the text on lines 243-249 could be deleted.

[Reply] Thanks. We added results from the P fertilizer study in the manuscript to provide data for the P limitation hypothesis in the subtropical forest. In the revised manuscript, we followed the reviewer's suggestion and deleted the initial Fig. 6 and the text on lines 243-249.

In addition, we mentioned the positive responses of plants to P fertilization in tropical and subtropical forests and included data from this P fertilizer study as a supplementary support [lines: 261-272]: As a supplement, we used a P fertilization experiment conducted in another subtropical forest with similar community structure nearby our experiment site to check if P limits plant growth. We applied 50 kg ha^{-1} yr^{-1} P (P_2O_5) to the forest and measured the growth of the dominant tree species (*C. sclerophylla*) following the same steps presented in the 'Materials and methods' section in this paper. After two years' P fertilization, we found that the annual absolute basal area increments and relative basal area in P fertilized plots were 56.0% and 101.5% higher, respectively, than in unfertilized plots (p=0.02 and p=0.03, respectively, unpublished data). Our results from N fertilization and the supplementary P fertilization experiments indicate that plant growth in subtropical forest ecosystems might be highly limited by P, which is in great need for further verification in the next studies. Similarly, limitation of other nutrients, such as K (potassium) which was highlighted in tropical forests, and their combination as well as heterogeneous nutrient limitation of specific species and plant growth forms may warrant further consideration in subtropical forests (Wright *et al.* 2011; Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013).

[Comments] 5. The data shown in Figure 2 - basal area changes over time by size class are self-evident and this could be deleted. I am much more interested in how size class distribution compared among the study plots at the beginning of the study period. With such low replication (40 trees per plot = 120 trees per treatment, which then get broken down into smaller units - some of these comparisons may be being made on a very few trees). As addressing these comments should alter the paper substantially I will not comment on editorial issues.

[Reply] Many thanks for reviewer's suggestions. We deleted Figure 2 - basal area changes over time by size class in the revised manuscript as suggested.

To Anonymous Referee #2:

[**Major comments**]: My opinion is that the text in large parts of the paper needs to be rephrased. The results needs to be much more carefully described and the authors should make an effort in making it more clear what differences that are statistically supported and what are not. Several of the main results discussed (e.g. that N addition stimulated growth of large trees and suppressed growth by small ones) is not supported by data. I agree that it is likely that the suppression of understory vegetation stems from increased light competition with a denser overstory, and that this was caused by N addition, But this is NOT reflected by any of the data collected by the authors. Perhaps, N addition increased leaf area or canopy cover of the overstory, and by this suppressed light conditions and the growth of the understory? Such effect would over time be expected to be reflected by increased basal area but the limited duration of the experiment (3 and not 4-year as claimed in the text) may have been too short the capture such response. If there are any data on canopy cover or light transmission to the ground level, such data would definitely be worth exploring as it may help explaining the results. The addressed questions could easily be made a bit more sophisticated by asking for differences compared to the known response from other forest systems (e.g. temperate, tropical or boreal forests). This is partly related to how the available knowledge from other systems is described in the introduction (see comments further down). The last part of the abstract can be misleading as the result presented only supports that small trees grow better under ambient N than elevated N and there is actually no support at all for higher growth of large trees under elevated N. The last sentence of the abstract, i.e. the conclusion of the study/implication of the results is extremely vague as the reader is not provided with any clue to why it is important to consider more parts of the vegetation than just the trees. A hint may be given by the results presented, i.e. that large trees responded differently from other parts of the vegetation, but the authors never help the reader describing why this is problematic of what can happen if the response is just evaluated based on the trees (large trees). I miss information on whether the growth in study system in general is N limited. In my opinion this is essential information when it comes to evaluating the response to the N addition. If not, or if the growth in the system is co-limited by other nutrients, a lack of N response should be interpreted a bit differently than if N is the solely or main limiting nutrient. I believe that this is important as the response to the N treatment in general was rather weak and most often non-significant. In fact the additional data presented on P addition (Fig. 6) might suggest that P is co-limiting nutrient.

[Reply] Thanks very much for the constructive suggestions. We have made a substantial revision according to the reviewer's suggestions. First of all, we accept the constructive suggestion that whether the growth in this study system in general is N limited, which is the most important question to answer. Indeed, previous results from boreal and temperate forests have showed that most trees have a positive growth response and therefore higher potential C storage to N fertilization because the status of N limitation was largely alleviated by the increasing N inputs (e.g., Thomas et al., 2010; BassiriRad et al., 2015) [Lines: 58-61]. On the contrary, in addition to the ubiquitous concept that P was a critical element driving plant growth in tropical forests (Vitousek et al., 1991), heterogeneous nutrient limitation concept that the growths of plants were co-limited by multiple nutrients has been proposed recently to explain why diverse plants respond differently to nutrient addition (Wright et al., 2011; Alvarez-Clare et al., 2013; Wurzburger & Wright 2015) [Lines: 74-77]. Therefore, the patterns of specific nutrient limitation and responses of plants to added nutrients among diverse forest ecosystems need further exploration, especially in subtropical forests which were rarely investigated.

Secondly, according to our main focus on answering the question "whether N is limited in this old-aged evergreen subtropical forest" in the revised manuscript, we rewrote the Introduction and Discussion sections with a simple hypothesis: if the subtropical forest is limited by N, a positive response of trees ascribed to enhanced N fertilization but a negative response of understory growth forms to N fertilization due to the potential expansion of canopy crown and limitation of light availability. In the Discussion section, we have added an evidence of canopy cover as following "Further, Our results of forest canopy cover estimated by photographic fisheye showed no significant differences between unfertilized (0.77±0.01) and N fertilized plots (0.76±0.04 and 0.72±0.01 in N50 and N100 plots, respectively), which was consistent with the findings of Lu et al. (2010). Although the understory light irradiance fluctuated largely during a day and was very hard to detect precisely, our measurements of forest canopy cover provided a rough evaluation for light availability. The results might indicate that other factors in addition to the low light availability in this old-aged forest had also played a crucial role in influencing understory plants during 3.4 years' N fertilization". Moreover, We discussed the potential mechanisms underlying the contrasting responses of different plant growth forms to N fertilization, including potential P but not N limitation or heterogeneous nutrient limitation on trees in this subtropical forest as in tropical forests, low light availability for understory plants, and potential N saturation after 3.4 years' N fertilization [lines: 236-331].

[Comments] L. 43-53. The authors seriously exaggerates the lack of knowledge, and I would go so far as saying that the content of this paragraph gives a false picture the available literature on N effects in forested systems. First, studies from boreal areas are not at all limited to tree response. In fact there has been much other work done, both on other plant groups and on other organisms than plants. For a quick overview see the summary paper by Bobbink et al 2010 (that is cited elsewhere in the ms). Second, the authors claim that the response of forest understory communities rarely have been studied, which is simply not true. Just a few examples are van Dobben et al. 1999 (For Ecol Manag 114, 83–95); Strengbom et al 2001 (Funct Ecol 15, 451–457); Gilliam 2006 (Journal of Ecology 94: 1176–1191), and there are many more.

[Reply] Many thanks for reviewer's suggestions. We carefully reviewed available literatures about the effects of N fertilization (or deposition) on plants in boreal, temperate, tropical and subtropical forests. Then, we regrouped the introduction. We recognized many valuable studies conducted in boreal areas focusing not only on trees, but also on other plant growth forms, for example dwarf shrubs, herbaceous species and seedlings. We have synthesized more related literatures in the revised introduction. Please see lines 54-86 at page 3-4.

[Comments] L. 110-118. I can understand why you exclude trees that died, and understand why trees that had decreasing DBH were excluded (but not necessarily agree that they should be excluded, as you then only accept measuring errors in one direction but not the other), but how can you justify excluding trees that showed no change in DBH? I am very worried that by omitting trees that showed no change in DBH may have seriously have influenced the results of your study and risk exaggerating the positive response that the N addition may have had. The authors should in general be much more careful when presenting non-significant differences. If these at all should be mentioned it should be absolutely clear to the reader that these are non-significant differences. Much of the discussion, and even parts of the major conclusions, deals with non-significant differences that are presented as if they were statistically supported (e.g. L. 204-205, 210-212, 252-256).

[Reply] We checked all our data after reading the reviewer's comments. Definitely, our exclusion of trees that were dead, broken, had shrunk or did not have DBH changes, had a risk of exaggerating the positive response of trees to N fertilization. So we re-analyzed our data following the reviewer's and included all the trees in each plot except dead trees. Further, we found no significant difference between N treatments after including all the trees which were excluded at first and the addition of those trees did not change our results. It is likely that most trees that died, were broken, had shrunk or did not have DBH changes were small trees (DBH<5 cm) which earn a relatively small percentage of the total basal area and aboveground biomass. Nevertheless, to better and precisely report the results, we have re-analyzed the data (mainly the saplings, Figure 3) and described our results carefully, especially those showed no significant differences among N treatments. And we re-expressed the effects of N fertilization on the growth (mean ± se) of *C. eyrei* by DBH classes (5-10 cm, 10-30 cm and >30 cm) in Figure 2 in the revision at page 18.

Fig.2 Effects of N fertilization on the growth (mean ± se) of *C. eyrei* by DBH classes (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth increase rate of basal area.

Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N 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}).

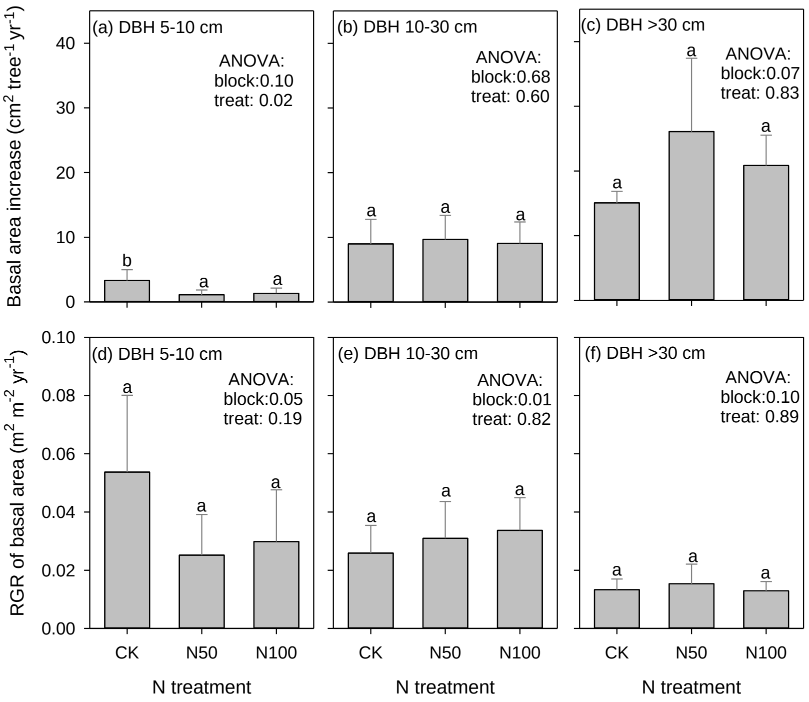
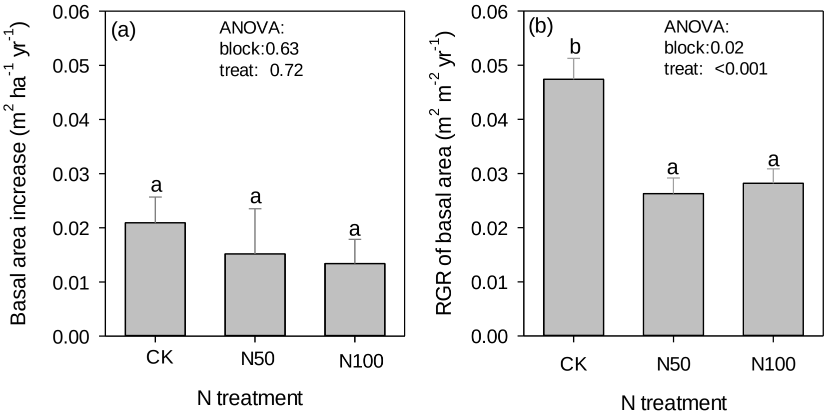


Fig. 3. Effects of N fertilization on the growth of saplings (mean ± se, n=3). (a) Absolute basal area increase, and (b) the relative growth rate of basal area. Numbers in these figures indicate the results of ANOVA.



[Specific comments]

[Comments] L. 22-23. There was no response at all of the larger trees! Avoid bringing up differences that are far from significant in the abstract. This is not just wrong, it is misleading! There is no description on how, when and why P was added in some plots.

[Reply] We appreciate the reviewer's comment. Our initial description focused much on the average values of basal area increment and RGR. In the revised manuscript, we revised the report of our result in abstract as following: Our results showed that the plot-averaged absolute and relative growth rates of basal area and aboveground biomass of trees were not affected by N fertilization. Across the individuals of *C.eyrei*, the small trees with a DBH (diameter at breast height) of 5-10 cm has declined by 66.4% and 59.5%, respectively, in N50 and N100 fertilized plots, while the growth of median and large trees with a DBH of >10 cm has not significantly changed with the N fertilization. The growth rate of small trees, saplings and the aboveground biomass of understory shrubs and ground-cover ferns decreased significantly in the N fertilized plots [lines: 30-36].

The description on how and why P was added in P-fertilized plots was described on lines 261-264 at page 9 as following "As a supplement, we used a P fertilization experiment conducted in another subtropical forest with similar community structure nearby our experiment site to check if P limits plant growth. We applied 50 kg ha^{-1} yr^{-1} P (P_2O_5) to the forest and measured the growth of the dominant tree species (*C. sclerophylla*) following the same steps presented in the 'Materials and methods' section in this paper'.

[Comments] L. 139-140. Do you have pre-treatment measures supporting that the vegetation was homogenous among plots at the initiation of the experiment? If so present these in a simple form. If not you should describe how the homogeneity was assessed.

[Reply] Thanks for the comments. We had a pre-treatment measure in March 2011 and evaluated the aboveground biomass of understory plants among the three N treatments. We presented these results in the revised manuscript in [Lines 194-198] at page 7 as following "Because the average aboveground biomass of shrubs/seedlings and ferns showed no significant differences across the three N treatments, we regarded the distribution of these understory shrubs/seedlings and ferns to be homogeneous among the three treatments before N fertilization in March 2011".

[Comments]: Table 1. It is not clear what the data represents. Are the numbers presented grand mean across all treatments? If so this should be clearly stated in the text explaining the table.

[Reply] We appreciate the reviewer's careful check. The data in Table 1 showed baseline data for four plant growth forms in this study before N fertilization. Numbers in the tables represent grand means (or mean ± standard error, n=9) of plants across all nine plots. We clearly stated these in the revised manuscript.

[Comments]: L. 157-158. I do not understand the results described here "The basal area and RGR of trees at the community level showed no significant response to N fertilization (Fig.1); however, the increase rates of basal area were likely hindered by N fertilization (Fig.1c)"What does this mean? As far as I can see from the statistical results presented and the data presented in Fig 1 there is just simply a lack of N response. Very unclear what you mean when saying that growth was hindered by N addition?

[Reply] We appreciate the reviewer's comments and sorry for the unclear description. We checked our description of the results, especially those with little significance through statistical analysis, and avoided misleading words in the revised manuscript.

In detail, we rephrased the text on lines 205-207 at page 7 as following:
Compared with the unfertilized plots, N50 and N100 fertilized plots showed a tendency toward higher averaged proportions of dead trees' aboveground biomass despite no statistically significant differences between them (Fig. 1d).

We rewrote the text on lines 217-220 at page 8 as following: However, inconsistent with such negative responses of small trees to N fertilization, the basal area increment and RGR of median (DBH of 10-30 cm; see Fig. 2b-2c) and large trees (DBH >30cm; see Fig. 2e-2f) did not show significant responses to N fertilization ($p>0.05$ in all cases).

[Comments]: L. 161-163. Be more careful when presenting non-significant differences. There might be a tendency towards more dead biomass under N addition but the difference is far from significant.

[Reply] We appreciate the reviewer's suggestions. We corrected the description of figure 1(d) as following: "Compared with the unfertilized plots, N 50 and N 100 fertilized plots showed a tendency toward higher averaged proportions of dead trees' aboveground biomass despite no statistically significant differences between them (Fig. 1d)" [Lines 205-207].

[Comments]: L. 164-165. The text here is wrong here. This result has nothing to do with the N treatment. The test and the fig just describes that basal area and RGR differed depending on size among individual trees of this species. This is very important as it seems like part of the conclusion is based on that there is a N effect here.

[Reply] Many thanks to the reviewers' comment. Initially, we aimed at reporting the result that basal area and RGR differed among individual trees with contrasting plant size. Small trees showed higher growth rate while larger trees showed lower growth rate. Then, the figure following this figure indicated different responses of the growth rate of trees in different sizes. In the revised manuscript, we deleted this figure to avoid the ambiguous description.

[Comments]: L. 168-173. The text here is in most parts misleading. The only effects that are sup-ported by the data presented is that the smallest trees growing under no N addition had higher basal area and higher RGR than small trees growing under N addition. All other differences that may or may not be visible in the figure is far from statistically supported and should not be mentioned here in the results.

[Reply] Many thanks for the reviewer's comments. We checked the description and rewrote this part in the section 3.1 in Lines 209-220 at pages 7-8 as followings:

Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent responses of absolute basal area increments and RGR to N fertilization (Fig. 2a-2f). The small trees with a DBH of 5-10 cm growing under unfertilized plots showed greater basal area increments than those growing under N fertilized plots (Fig. 2a, p_{treat} <0.05). Specifically, the N50 and N100 fertilization decreased the absolute basal area increments of small individual trees at rates of 2.2 cm^2 $tree^{-1}$ $year^{-1}$ and 1.98 cm^2 $tree^{-1}$ $year^{-1}$, respectively, which indicated that the decreasing degrees of the absolute basal area of small trees reached 66.4% and 59.5% in N50 and N100 plots. The small individual trees also showed a tendency toward lower averaged RGR in N fertilized plots although no significant difference was detected between them (Fig. 2d, p_{treat} =0.19). Inconsistent with the negative responses of small trees to N fertilization, the basal area increment and RGR of median *C. eyrei* individuals with DBH of 10-30 cm and large *C. eyrei* individuals with DBH of >30cm showed no significant responses to N fertilization (Fig. 2b-2c and 2e-2f, p_{treat} >0.05 in all cases).

[Comments]: L. 175-179. Is the test result presented in the fig correct? According the test results N addition influences RGR and mortality, but form the post hoc test there seem to be difference among the groups. From inspecting the data presented in the fig I wonder if there is some error among the letters indicating the differences among the groups in panel c and d. Results covering the data presented in fig 6 is missing from the result section.

[Reply] Many thanks for the reviewer's comments. Similar to reply before, we have re-analyzed the data of the saplings. The results from *post hoc* test showed that although the annual absolute increments of basal area increments of saplings showed no significant response to N fertilization (Fig. 3a, p_{treat} =0.72), the RGR of sapling growing in N50 and N100 plots relative to the unfertilized plots showed a substantial decrease at rates of 0.021 $m^{-2}m^{-2}yr^{-1}$ and 0.019 $m^{-2}m^{-2}yr^{-1}$, respectively (Fig. 3b, p<0.001) [Lines: 223-227 at page 8].

[Comments]: L. 192-194. What is the rationale for expecting a common positive response for all types of plants? To me this seems a bit na ïve, given that forest plant communities often are size structured communities (see e.g. papers by Peter Grubb), and understory species than can be expected to be light rather than nutrient limited.

[Reply] Thanks for the reviewer's insightful comments. In the revised manuscript, we changed our hypothesis as following: We attempt to explore whether N is a limiting element in the old-aged evergreen subtropical forest. We hypothesize a positive response of trees to N fertilization, but a negative response of understory growth forms to N fertilization due to the expansion of canopy crown and consequent reduction of light availability [Lines: 89-92 at page 4].

[Comments]: L. 204-205. The first part of the sentence (large trees) is NOT supported by the results.

[Reply] Thanks for the reviewer's comment. We rewrote relevant parts in abstract, result and discussion in the revised manuscript to avoid unclear description. The results of large trees were rephrased as following: However, inconsistent with the negative responses of small trees to N fertilization, the basal area increment and RGR of median *C. eyrei* individuals with DBH of 10-30 cm and large *C. eyrei* individuals with DBH of >30cm showed no significant responses to N fertilization (Fig. 2b-2c and 2e-2f, p>0.05 in all cases).

[Minor technical and language errors]

The text is in need of some language edition. I just provide a few examples were the text need some re-phrasing. I have not paid that much attention to text editing as I believe that the paper need to be substantially revised before the paper can reach an acceptable standard.

[Comments]: L. 23-24: Small trees, saplings and particularly understory shrubs and ground-cover ferns suppressed seriously by increasing N fertilization: : : How are the suppressed? I am not very fond of the wording seriously as it is not a neutral wording. Better describe how large the difference was. L 21-24: the small trees with DBH (diameter at breast height) values of 5-10 cm were hindered by N fertilization: : :In what way was the small trees hindered?

[Reply] Thanks for the reviewer's comment. According to the reviewer's suggestions, we revised the text on lines 31-36 in abstract as following "Across the individuals of *C.eyrei*, the small trees with a DBH (diameter at breast height) of 5-10 cm has declined by 66.4% and 59.5%, respectively, in N50 and N100 fertilized plots, while the growth of median and large trees with a DBH of >10 cm has not significantly changed with the N fertilization. The growth rate of small trees, saplings and the aboveground biomass of understory shrubs and ground-cover ferns decreased significantly in the N fertilized plots."

[Comments]: L. 24-24. : : :Proportion of mortality? Here it is better to write either the mortality of plants were: : : or The proportion of plants that died:

[Reply] We appreciate the reviewer's good suggestion. We have changed the description of mortality throughout the whole manuscript as "the proportion of died trees".

[Comments]: L 177. Avoid evaluating your results in the result section by using wording such as severely here. Save that type of wording for the discussion. L. 180-185. There is no need to present mean values in text if these are shown in the figure 5. Do not present data twice, choose either to present then in text or in the fig.

[Reply] We appreciate the reviewer's good suggestion. In the revised manuscript, the description of results had been remarkably changed. The mean values presented in text have been deleted.

References

Alvarez-Clare, S., Mack, M.C., and Brooks, M.: A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest, Ecology, 94, 1540-1551, 2013.

BassiriRad, H.: Consequences of atmospheric nitrogen deposition in terrestrial ecosystems: old questions, new perspectives, Oecologia, 177, 1-3, 2015.

Du, E.Z., and Liu X.J. in Nitrogen deposition, critical loads and biodiversity, eds. Sutton et al. Springer Science+Business Media Dordrecht, pp.49-56, 2014.

Galloway, J. N., Winiwarter. W., Leip, A., Leach, A. M., Bleeker, A., andErisman. J. W.: Nitrogen footprints: past, present and future, Environ. Res. Lett., 9, 115003, 2014.

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

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

Magill, A. H., Aber, J. D., Currie, W. S., Nadelhoffer, K. J., Martin, M. E., McDowell, W. H., Melillo, J. M., and Steudler, P.: Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA., For. Ecol. Manage., 196, 7-28, 2004.

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

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

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

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

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

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

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Abstract

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

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

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

1 Introduction

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

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

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

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

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

2 Materials and methods

2.1 Study site and experimental design

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

NEECF (Network of Nutrient Enrichment Experiments in China's Forests) project (Du *et al*. 2013), because of its representativeness in both species composition and landscape structure in the subtropical evergreen forest region. The study area has a humid climate with strong summer monsoons with an annual average precipitation of 1,700 mm and an average annual temperature of 14.9 ℃. The soil in this area has been classified as yellow brown earth (Chinese Soil Taxonomic Classification), and the pH_{H2O} value at 0-10 cm soil depth was 4.58 ±0.05 (mean ±SE). The total nitrogen, phosphorus, NH_4^+-N and NO_3-N content in the soil at 0-10 cm depth were 3.23 (0.37), 0.32 (0.02), 0.012 (0.001), and 0.002 (0.0006) mg g^{-1}, respectively (Li *et al*. 2015).

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

We began N fertilization in March 2011. A randomized block design was used to avoid spatial heterogeneity. We chose three blocks with similar stand growth, species composition and site condition to establish three N treatments in each block: CK (0 kg N ha^{-1} yr^{-1}), N50 (50 kg N ha^{-1} yr^{-1}), and N100 (100 kg N ha^{-1} yr^{-1}). As the amount of wet N deposition in this region was 5.9-7.3 kg N ha^{-1} yr^{-1}, we applied N fertilization at these two levels to simulate the extreme N deposition cases. In total, nine 20 m × 20 m plots were established with a 5-10 m buffer zone between each plot. The total NH_4NO_3 was divided into 12 dosages and applied to the forest in each month at regular intervals. NH_4NO_3 in dosages of 0.48 kg/plot and 0.95 kg/plot were dissolved in 15 L of fresh water, respectively, and then sprayed uniformly in N50 and N100 plots using a back-hatch sprayer. The unfertilized plots (controls) were similarly treated with 15 L of fresh water without NH_4NO_3.

2.2 Sampling and measurement

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

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

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

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

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

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

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

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

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

2.3 Statistical analysis

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

3 Results

3.1 Growth responses of trees to N fertilization

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

Individuals of the dominant species *C. eyrei* with different initial DBH showed divergent responses of absolute basal area increments and RGR to N fertilization (Fig. 2a-2f). The small trees with a DBH of 5-10 cm growing under unfertilized plots showed greater basal area increments than those growing under N fertilized plots (Fig. 2a, p_{treat} <0.05). Specifically, the N50 and N100 fertilization decreased the absolute basal area increments of small individual trees at rates of 2.2 cm^{2} tree^{-1} year^{-1} and 1.98 cm^{-2} tree^{-1} year^{-1}, respectively, which indicated that the decreasing degrees of the absolute basal area of small trees reached 66.4% and 59.5% in N50 and N100 plots. The small individual trees also showed a tendency toward lower averaged RGR in N fertilized plots although no significant difference was detected between them (Fig. 2d, p_{treat} =0.19). Inconsistent with the negative responses of small trees to N fertilization, the basal area increment and RGR of median *C. eyrei* individuals with DBH of 10-30 cm and large *C. eyrei* individuals with DBH of >30cm showed no significant responses to N fertilization (Fig. 2b-2c and 2e-2f, p_{treat} >0.05 in all cases).

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

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

4 Discussion

4.1 Growth responses of trees to N fertilization

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

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

As a supplement, we used a P fertilization experiment conducted in another subtropical forest with similar community structure nearby our experiment site to check if P limits plant growth. We applied 50 kg ha^{-1} yr^{-1} P (P_2O_5) to the forest and measured the growth of the dominant tree species (*C. sclerophylla*) following the same steps presented in the 'Materials and methods' section in this paper. After two years' P fertilization, we found that the annual absolute basal area increments and relative basal area in P fertilized plots were 56.0% and 101.5% higher, respectively, than in unfertilized plots (p=0.02 and p=0.03, respectively, unpublished data). Our results from N fertilization and the supplementary P fertilization experiments indicate that plant growth in subtropical forest ecosystems might be highly limited by P, although it is in great need for further verification in the next studies. Similarly, limitation of other nutrients, such as K (potassium) which was highlighted in tropical forests, and their combination as well as heterogeneous nutrient limitation of specific species and plant growth forms may warrant further consideration in subtropical forests (Wright *et al.* 2011; Santiago *et al.* 2012; Alvarez-Clare *et al.* 2013).

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

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

N fertilization

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

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

4.3 Potential N saturation and plant growth

The striking biomass reduction of the understory plants, especially ferns, in response to N fertilization in our study well corroborated the similar findings in an old-aged tropical forest at Mt. Dinghushan in China (Lu et al., 2010). Also, consistent with previous studies obtained from boreal, temperate and tropical forests (Rainey *et al.* 1999; Alvarez-Clare *et al.* 2013; Dirnböck *et al.* 2014), our experiment revealed that understory small-sized plants responded sensitively to nutrient fertilization, which might indicate a possibility of N saturation in the subtropical forest. According to the definition of N saturation addressed by Aber et al. (1989), the drastic decrease of understory ferns, shifted composition of understory plant community, and cation imbalances of understory species after 7 years' chronic N fertilization at Harvard Forest, USA, could be interpreted as useful indicators of N saturation (Rainey *et al.* 1999). Moreover, a 6-year N fertilization experiment in an old-aged tropical forest at Mt. Dinghushan also showed signs of N saturation, such as significant increases in nitrate (NO_3-) leaching, inorganic N concentration and N_2O emissions of soils, and soil acidification (Lu *et al.* 2014; Chen *et al.* 2015). In our experiment, we observed mild soil acidification and increased soil N concentration in high N fertilized plots (Fig. S1). Combined with the negative responses of understory plants, we suggest that the 3.4-year N fertilization in this mature subtropical forest site has potentially caused N saturation, but further observations are required.

5. Conclusion

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

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References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems: hypotheses revisited, BioScience, 48, 921-934, 1998.

Alvarez-Clare, S., Mack, M.C., and Brooks, M.: A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest, Ecology, 94, 1540-1551, 2013.

BassiriRad, H., Lussenhop, J.F., Sehtiya, H.L., and Borden, K.K.: Nitrogen deposition potentially contributes to oak regeneration failure in the Midwestern temperate forests of the USA, Oecologia, 177, 1-11, 2015.

Bedison, J.E., and McNeil, B.E.: Is the growth of temperate forest trees enhanced along an ambient nitrogen deposition gradient?, Ecology, 90, 1736-1742, 2009.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., and Dentener, F.: Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis, Ecol. Appl., 20, 30-59, 2010.

Chen, H., Gurmesa, G.A., Zhang, W., Zhu, X.M., Zheng, M.H., Mao, Q.G., Zhang, T., and Mo, J.M.: Nitrogen saturation in humid tropical forests after 6 years of nitrogen and phosphorus addition: Hypothesis testing, Functional Ecol., 30(2), 305-313, 2015.

Dalling, J. W., Heineman, K. Lopez, O. R., Wright, S. J., and Turner, B. L. in : The Paradigm of Phosphorus Limitation, Tropical tree physiology, eds. Goldstein, G., and Santiago, L.S., Springer International Publishing, Switzerland, pp.261-274, 2016.

Dirnböck, T., Grandin, U., Bernhardt‐Römermann, M., Beudert, B., Canullo, R., Forsius, M., Grabner, M.T., Holmberg, M., Kleemola, S., and Lundin, L.: Forest floor vegetation response to nitrogen deposition in Europe, Global Change Biol., 20, 429-440, 2014.

Du, E.Z., Zhou, Z., Li, P., Hu, X.Y., Ma, Y.C., Wang, W., Zheng, C.Y., Zhu, J.X., He, J.S., and Fang, J.Y.: NEECF: a project of nutrient enrichment experiments in China's forests, J. Plant Ecol., 6, 428-435, 2013.

Du, E.Z., Liu, X.Y., and Fang, J.Y.: Effects of nitrogen additions on biomass, stoichiometry and nutrient pools of moss *Rhytidium rugosum* in a boreal forest in Northeast China, Environ. Poll., 188, 166-171, 2014.

Fowler, Z. K., Adams, M. B., and Peterjohn, W. T.: Will more nitrogen enhance carbon storage in young forest stands in central Appalachia?, For. Ecol. Manage., 337, 144-152, 2015.

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

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

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

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

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

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

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

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

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

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P. G., and Kolari, P.: The human footprint in the carbon cycle of temperate and boreal forests, Nature, 447, 849-851, 2007.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 1 Effects of N fertilization on the growth and mortality of all trees (mean ± se). (a) Absolute basal area increase of all trees; (b) aboveground biomass increase of all trees; (c) relative growth rate of total tree basal area; and (d) the proportion of all dead trees. The proportion of dead trees was calculated using the aboveground biomass of all dead trees during the experiment divided by the total aboveground biomass of all trees in 2014. Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N 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}).

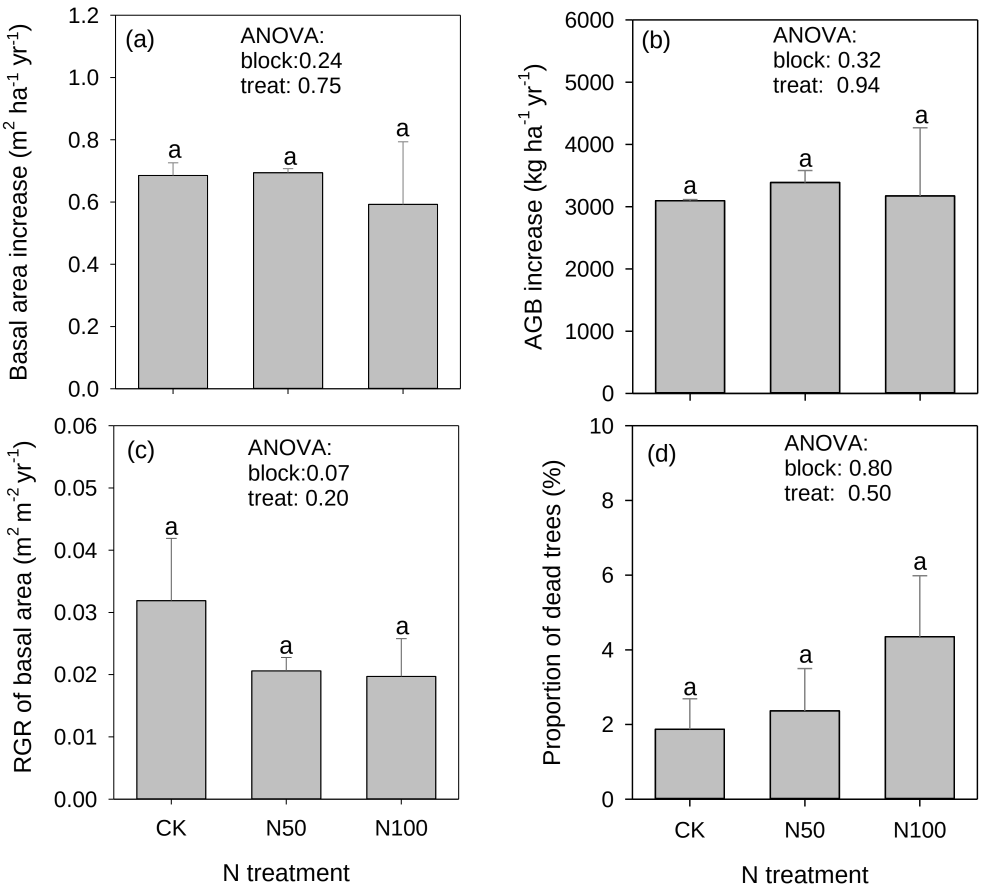

Figure 2 Effects of N fertilization on the growth (mean ± se) of *C. eyrei* by DBH classes (5-10 cm, 10-30 cm and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth increase rate of basal area. Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N 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}).

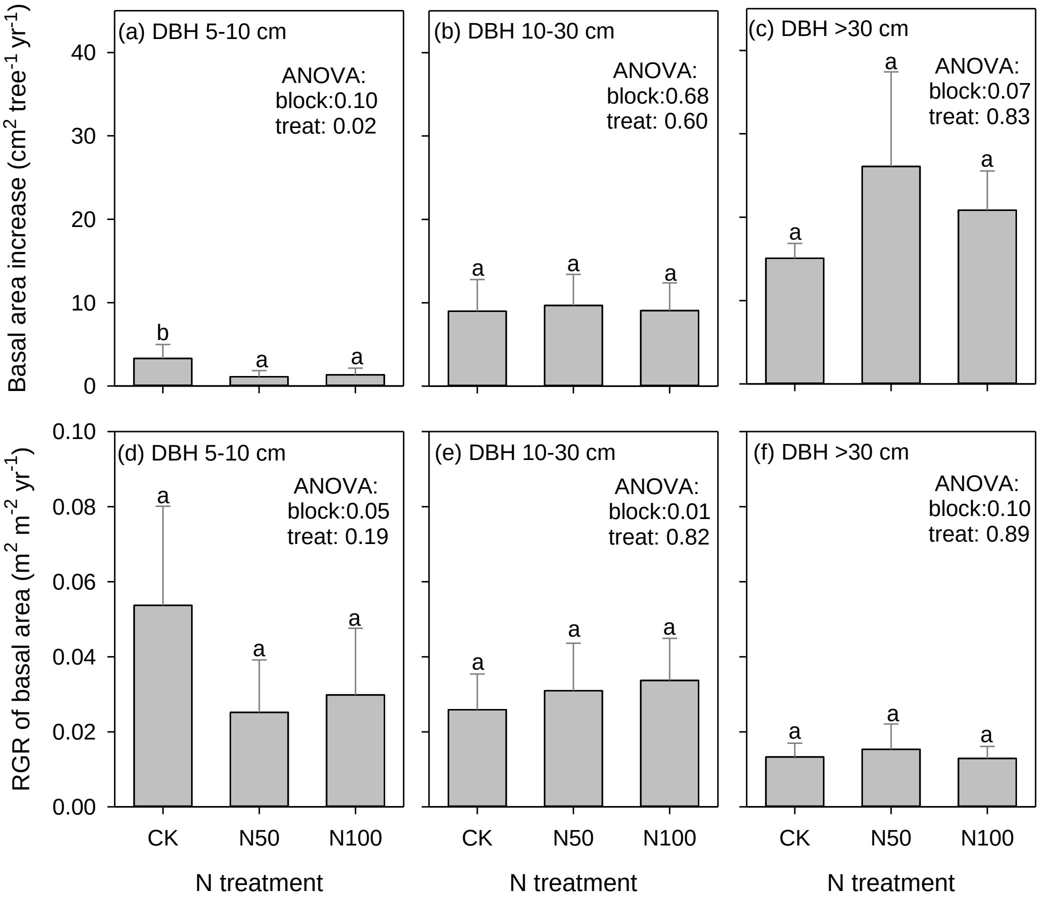

Figure 3 Effects of N fertilization on the growth of saplings (mean ± se). (a) Absolute basal area increase and (b) the relative growth rate of basal area. Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N fertilization: CK (0 kg N ha^{-1} yr^{-1}), N50 (50 kg N ha^{-1} yr^{-1}) and N100 (100 kg N ha^{-1} yr^{-1}).

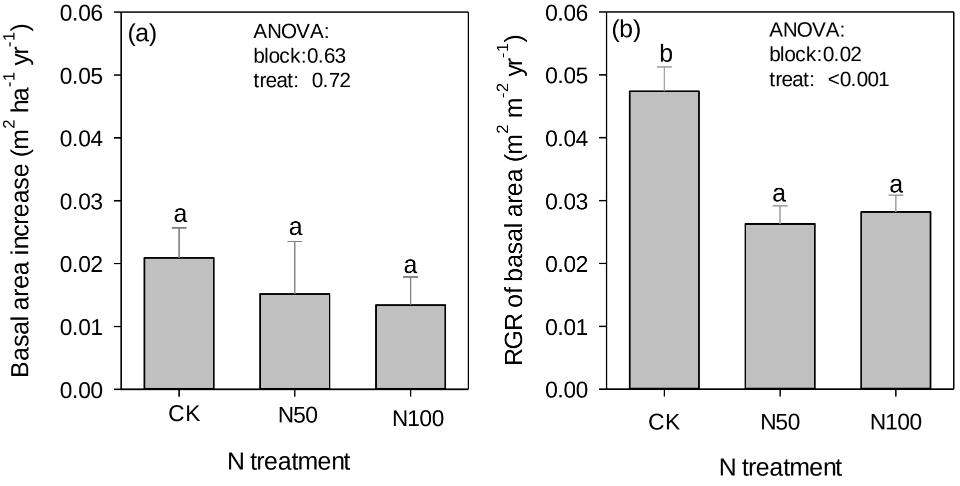

Figure 4 Effects of N fertilization on the aboveground biomass of shrubs, seedlings and ferns. Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean ± se). Numbers in these figures indicate the results of ANOVA. The N treatment on x-axis represents three levels of N fertilization: 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}).