Biogeosciences Discuss., doi:10.5194/bg-2016-416, 2016
Manuscript under review for journal Biogeosciences
Published: 4 October 2016

Biogeosciences
Discussions
Open Access

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

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Atmospheric nitrogen (N) deposition has been a noteworthy aspect of global change. Previous observational studies in temperate and tropical forests have focused on the effects of N deposition on tree growth. Here we asked how trees and other plant growth forms respond to experimental N deposition in a subtropical forest in China. We conducted a four-year N fertilization experiment in a subtropical evergreen forest in southeastern China with three treatment levels applied to 9 20×20 m plots and replicated in three blocks. We classified the plants to trees, saplings, shrubs(including tree seedlings) and ground-cover plants (ferns) according to the growth forms, then we measured the absolute and relative basal area increments of trees and saplings, and the aboveground biomass of understory shrubs and ferns. In addition, we grouped individuals of the dominant tree species *Castanopsis eyrei* into three size classes and analyzed their growth responses to N fertilization separately. Although the total tree growth on plot level did not show a significant response to the N fertilization, the small trees with DBH (diameter at breast height) values of 5-10 cm were hindered by N fertilization, while the growth of large trees with DBH>10 cm showed neutral or weakly positive responses to N fertilization. Small trees, saplings and particularly understory shrubs and ground-cover ferns suppressed seriously by increasing N fertilization. The proportions of plant mortality in N-fertilized plots were higher than in unfertilized plots and most of the dead individuals were small trees, saplings, shrubs and ferns. N deposition potentially leads to increased growth of larger plant individuals at tree layer in the forest community and suppresses the growth and survival of other individuals at understory and ground-cover layers. Therefore, differences in the growth responses of different plant growth forms and individual sizes should be taken into account when evaluating the effects of N deposition on the functioning of these forest ecosystems, including their potential for carbon storage.

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 more and more serious 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 4.78% 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 been shown to reduce 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 to explore the responses of plant growth to nitrogen deposition (e.g., Wright & Tietema 1995; Fowler *et al*. 2015). The majority of these studies have been carried out in boreal forests and focused on the growth response of dominant tree species because of their key role in C storage, with results showing that most trees have a positive growth response and therefore higher potential C storage to N fertilization (e.g., Thomas *et al*. 2010; BassiriRad *et al*. 2015). Recently, fertilization experiments in tropical forests have shown different growth responses of trees among individual size levels (Alvarez-Clare *et al*. 2013), understory shrubs and tree seedlings which contrasted with the ones found for trees in the previously described experiments (Pasquini & Santiago 2012; Santiago *et al*. 2012).

In general, the responses of shrubs and understory plants to N fertilization have rarely been considered in studies of forest ecosystems, although there are abundant investigations of the responses of herbaceous species to N fertilization in other terrestrial ecosystems (Stevens *et al*. 2004; Simkin *et al*. 2016). However, shrubs and understory plants are also an important component in most tropical and subtropical forests, contributing to their species richness and ecosystem functioning, including C storage. Furthermore, as most of the nutrient fertilization experiments have focused on boreal forests and tropical forests, few studies have investigated subtropical forests despite their broad distribution throughout the world (Zhou *et al*. 2013; Huang *et al*. 2015)

To close this knowledge gap about the response of subtropical forests and of different plant growth forms to N deposition, we carried out a 4-year N fertilization experiment with three treatment levels applied to 20 ×20 m plots and replicated in three blocks in a subtropical forest in south-eastern China. We attempt to address the following questions: (1) will N fertilization accelerate plant growth in subtropical forest? (2) do different plant growth forms (trees, saplings, shrubs and seedlings, and ferns) respond differently to N fertilization?

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 (GNJ) in Anhui Province, southeastern China. As a commendable representative of the typical subtropical broadleaved evergreen forest, the GNJ experimental site was an important part of the NEECF (Network of Nutrient Enrichment Experiments in China’s Forests) project (Du *et al*. 2013). The study area has an annual average precipitation of 1,700 mm, an average annual temperature of 14.9 ℃, and a humid climate with strong summer monsoons. 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 (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 growths, species composition and site conditions 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 these 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. NH_4NO_3 was applied each month (not on rainy days) at regular intervals. NH_4NO_3 was dissolved in 15 L of fresh water and then sprayed uniformly in each plot 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 and then calculated tree DBH according to the following equation:

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

In this equation, 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 at the end and 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 (trees that were dead, broken, had shrunk or did not have DBH changes 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's method (2013):

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

In this equation, 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 *C. eyrei* from the other tree species and grouped the *C. eyrei* individuals into three classes based on their DBH values (i.e., 5-10 cm, 10-30 cm and >30 cm) to explore 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 recognized and recorded. Then, we calculated the increments of tree aboveground biomass 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 2014. Then, basal area increments, annual basal area growth rate, RGR, aboveground biomass increments and dead biomass proportion 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 shrubs/seedlings and ferns were distributed homogeneously among the three treatments before N fertilization in March 2011, we could identify the effects of N fertilization by comparing the aboveground biomass of shrubs/seedlings and ferns in 2014 among the different treatments.

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. See detail description at the ‘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 increments, dead biomass proportion of trees and saplings, 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. 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

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). The absolute and relative annual increase rates of basal area in the control plots were 2.36 ±0.18 and 0.10 ±0.03 m^2 ha^{-2} yr^{-1}, respectively, whereas the two variables decreased to 1.97 ±0.75 and 0.06 ±0.02 m^2 ha^{-2} yr^{-1} in the plots with N100 treatment, respectively. Moreover, the increase rates of total aboveground biomass showed no significant responses to N addition (Fig. 1b), whereas the proportions of dead aboveground biomass under fertilized plots relative to the control plots were much higher (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-2b, $p<0.05$). *C. eyrei* individuals with

DBH>30 cm had higher absolute annual increments of basal area than young individuals with DBH of 5-10 cm and median individuals with DBH of 10-30 cm (Fig. 2a, $p<0.05$), while RGR exhibited the opposite trend (Fig. 2b). N fertilization decreased the absolute basal area increments of young trees (Fig. 3a, $p<0.05$) but exerted no significant effects on median trees (Fig. 3b, $p=0.82$). Nonetheless, the average basal area increments of larger individuals with DBH>30 cm were much higher than those smaller trees in N-fertilized treatments, especially in the treatments of N50 (Fig. 3c). Similarly, the RGRs of young individuals showed negative responses, whereas the median and large individuals did not show significant or positive responses to N fertilization (Fig. 3e-3f, $p=0.17$-0.83).

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

The annual absolute increments of basal area and the aboveground biomass of saplings showed no significant responses to N fertilization (Fig. 4a-4b, $p=0.44$-0.47), whereas RGR declined and the proportion of mortality increased severely in the plots with N fertilization (Fig. 4c, 4f; $p=0.07$). The mortality rates of saplings in N50 and N100 treatments were 222 ±158% and 175 ±128% higher than that in the control treatment, respectively.

Similarly, the aboveground biomass of seven predominant shrubs/seedlings drastically decreased, from 404.8 ±86.2 (control) to 137.9 ±64.2 (N50) and 96.2 ±53.7 kg ha^{-1} (N100), indicating that they dropped by 69.4 ±8.3% (N50) and 79.1 ±7.6% (N100) compared with those in the control plots (Fig. 5a, $p<0.01$). The aboveground biomass of ground-cover ferns significantly declined, from 12.94 ±4.57 (control) to 0.88 ±0.31 (N50) and 0.62 ±0.17 kg ha^{-1} (N100), a dramatic decline rate of 92.4 ±3.4% and 93.4 ±2.6%, respectively (Fig. 5b, $p<0.01$).

4 Discussion

4.1 Growth responses of trees

Contrary to our expectation, we did not observe strong positive growth responses of trees to N fertilization at GNJ (Figs. 1 and 3), which contrasts with previous studies in boreal forests but is consistent with studies in tropical forests with high species diversity (e.g., Magnani *et al*. 2007; Fowler *et al*. 2015; Santiago *et al*. 2012; Alvarez-Clare *et al*. 2013). Our findings also suggest that different species may respond to N fertilization in various ways. We expected positive growth responses of plants of all four growth forms exposed to N fertilization because N availability in the soil would be enhanced by N fertilization and the N limitation of plants in the forest ecosystem could be alleviated. However, we only observed negative responses of small trees (5-10 cm DBH) (Fig. 3a, 3d) and saplings (Fig. 4c, 4f) and minimal positive responses of larger trees (>10 cm DBH) (Fig. 3) to N fertilization. Although the growth of juvenile trees has been reported to be promoted by N fertilization in temperate forests (Bedison & McNeil 2009), results from other N fertilization experiments conducted in tropical forests could not confirm the pattern that N fertilization accelerates tree growth (Wright *et al*. 2011; Alvarez-Clare *et al*. 2013).

The effects of N fertilization on tree growth can be influenced by site conditions, the age characteristics of trees, and many other factors (Ryan *et al*. 2004; Li *et al*. 2012; Gao *et al*. 2016). The mature evergreen broad-leaved forest in this study has a high stand density. For trees, biomass can be preferentially allocated to aboveground parts (e.g., leaves, branches or stems) to develop a broader crown for obtaining abundant sunlight (Schroth *et al*. 2015; Schoonmaker *et al*. 2016). The N fertilization promoted the growth of overstory trees with large DBH and height, whereas it inhibited the growth of lower-canopy trees because the increasing shading of the canopy greatly prevented the lower-canopy trees from receiving sunlight (Dirnböck *et al*. 2014). In our research, the mortality proportions of trees and saplings in fertilized plots were almost twice as high as in the control plots (Figs. 1d and 4d); young dead trees accounted for 70.2% of the total dead trees, and the average height of dead trees was 6.4 m, which means that most of the dead trees were in the lower part of the tree layer. These results indicate that N deposition potentially leads to increased growth of larger plant individuals in the forest community and thus suppresses the growth and survival of other individuals, although the increased self- and alien-thinning does not result in increased total aboveground stand biomass.

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

Previous studies involving N fertilization experiments have suggested that the abundance, diversity and productivity of understory plants are suppressed by N fertilization (Lu *et al*. 2010; Bobbink 2010; Dirnböck *et al*. 2014). We also observed a remarkable adverse effect of N fertilization on understory saplings, seven dominant shrubs/seedlings and ferns (Fig. 5a-5b). Comparing the growth of young trees (5-10 cm DBH) with understory saplings, shrubs/seedlings and ferns, the latter were more sensitive to light availability because it is difficult for understory plants to absorb, transport, and invest soil mineral resources to stimulate growth in an environment with a light deficiency (Alvarez-Clare *et al*. 2013). In terms of light availability, the mechanisms resulting in high tree mortality rate and the decrease of understory plant biomass when exposed to N fertilization may be similar.

Additionally, although ferns have been reported to benefit from low-level atmospheric N deposition (Holub 2010; Jones *et al*. 2011), ground plants, such as ferns, mosses and lichens, showed remarkable negative responses to N fertilization; therefore, these ground plants have often been used as indicators of the health of forest ecosystems (Rainey *et al*. 1999; Lu *et al*. 2010; Du *et al*. 2014). During the N fertilization experiment, we observed that many shrubs/seedlings and fern leaves yellowed and even withered, which visually demonstrates that understory plants sustained more stress than upper-layer trees.

4.3 Influences of changing soil conditions induced by N fertilization on the growth responses

The N fertilization in our experiment led to soil acidification (Fig. S1c) which may have caused base cation leaching, Al_3^+ accumulation and nutrient loss, threatening the health of soil and plants (Lu *et al*. 2014; Huang *et al*. 2015; Yang *et al*. 2015). Moreover, phosphorus (P) is commonly regarded as a limited nutrient in tropical and subtropical forests (Crous *et al.* 2015). The total N content of soil was enhanced by N fertilization, whereas total P content was potentially diminished in our study (Fig. S1a-1b). The detection of N and P concentration in plant leaves and fine roots showed that N concentration increased significantly. However, the P concentration in the fine roots of shrubs/seedlings declined considerably despite the P concentration of leaves being stable (Figs. S2 and S3). Similar results were obtained in the Dinghushan subtropical evergreen forest ecosystem (Liu *et al*. 2012). Therefore, we predict that plants grown in our site will be severely affected by P limitation as a result of N fertilization, especially the understory plants, which receive lower quantities of light and mineral nutrients and exhibit slower photosynthesis and growth rates (Pasquini & Santiago 2012).

We conducted a two-year P fertilization experiment in another subtropical forest near the GNJ experiment site to test the hypothesis of P limitation on plant growth. We applied 50 kg ha^{-1} yr^{-1} P to the forest and then measured the growth of the dominant tree species (*C. sclerophylla*) following the same steps presented in the 'Materials and methods' section. As expected, we found significant positive responses to P fertilization in the annual absolute basal area increments and relative basal area increase of *C. sclerophylla* (Fig. 6) as well as understory plants. These findings further indicate that plant growth in subtropical forest ecosystems is highly limited by P.

5. Conclusion

Contrasting growth responses among four plant growth forms to nitrogen fertilization were present in this mature subtropical evergreen forest. Trees at the plot level showed no significant responses to the N fertilization; however, when the dominant tree species *C. eyrei* was separated from the other species and grouped into three DBH classes, the small individuals with a DBH of 5-10 cm experienced seriously negative effects of N fertilization, whereas the growth of larger individuals with DBH>10 cm showed positive but insignificant responses to the N fertilization. The growths of understory saplings, shrubs/seedlings and ground-cover ferns were suppressed by the N fertilization. Moreover, individual growth characteristics, life strategies of different plant growth forms, resource limitation conditions, the variation of soil conditions induced by N fertilization and many other factors influenced the growth of plants. N fertilization will potentially aggravate the P-limited status in the mature subtropical forest and amplify the negative impacts on plant growth. Therefore, we should pay attention to the contrasting effects of N fertilization on the growth of different plant growth forms and individual sizes, especially in subtropical forests with high species diversity. This emphasis will help to maintain ecosystem biodiversity and the stable development of forest communities from an ecosystem sustainability perspective.

Funding: This study was funded by the National Natural Science Foundation of China (31321061 and 31330012).

*Acknowledgements.*We wish to thank Bernhard schmid, Gianalberto Losapio, Lilian Dutoit, Peter Schmid and Jessica Baby for their helpful suggestions on the manuscript. We thank the Sino-German Center for Research Promotion for the participation in a summer school in Jingdezhen (GZ1146).

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, 53-63, 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.

Bobbink, R., Hornung, M., and Roelofs, J.G.: The effects of air‑borne nitrogen pollutants on species diversity in natural and semi‑natural European vegetation, J. Ecol., 86, 717-738, 1998.

Crous, K.Y., Ósvaldsson, A., and Ellsworth, D.: Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilisation stimulates stem growth, Plant and soil, 391, 293-305, 2015.

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.

Gao, S., Zhou, T., Zhao, X., Wu, D.H., Li, Z., Wu, H., Du, L., and Luo, H.: Age and climate contribution to observed forest carbon sinks in East Asia, Environ. Res. Lett., 11, doi:10.1088/1748-9326/11/3/034021, 2016.

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.

Holub, P., and Tůma, I., The effect of enhanced nitrogen on aboveground biomass allocation and nutrient resorption in the fern *Athyrium distentifolium*, Plant Ecol., 207, 373-380, 2010.

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.

Jones, M.E., Fenn, M.E., and Paine, T.D.: The effect of nitrogen additions on bracken fern and its insect herbivores at sites with high and low atmospheric pollution, Arthropod-Plant Inte., 5, 163-173, 2011.

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.

Li, H., Li, M.C., Luo, J., Cao, X., Qu, L., Gai, Y., Jiang, X.N., Liu, T.X., Bai, H., Janz, D., Polle, A., Peng, C.H., and Luo, Z.B.: N‐fertilization has different effects on the growth, carbon and nitrogen physiology, and wood properties of slow–and fast–growing Populus species, J. Exp. Bot., 63, 6173-6185, 2012.

Liu, J.X., Zhang, D.Q., Zhou, G.Y., and Duan, H.L.: Changes in leaf nutrient traits and photosynthesis of four tree species: effects of elevated [CO2], N fertilization and canopy positions, J. Plant Ecol., 5, 376-390, 2012.

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.

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.

Ryan, M.G., Dan, B., Fownes, J.H., Giardina, C.P., and Senock, R.S.: An experimental test of the causes of growth decline with stand age, Ecol. Monogr., 74, 393-414, 2004.

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.

Schoonmaker, A.S., Lieffers, V.J., and Landhäusser, S.M.: Viewing forests from below: fine root mass declines relative to leaf area in aging lodgepole pine stands, Oecologia, 181, 733-747, 2016.

Schroth, G., Mota, M.d.S.S.da., Elias, and M.E.de.Assis.: Growth and nutrient accumulation of Brazil nut trees (Bertholletia excelsa) in agroforestry at different fertilizer levels, J. Forest. Res., 26, 347-353, 2015.

Simkin, S.M., Allen, E.B., Bowman, W.D., Clark, C.M., Belnap, J., Brooks, M.L., Cade, B.S., Collins, S.L., Geiser, L.H., and Gilliam, F.S.: Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States, Proc. Natl. Acad. Sci., 15, 4086-4091, 2016.

Stevens, C.J., Dise, N.B., Mountford, J.O., and Gowing, D.J.: Impact of nitrogen deposition on the species richness of grasslands, Science, 303, 1876-1879, 2004.

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.

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.

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.

Yang, Y.H., Li, P., He, H.L., Zhao, X., Datta, A., Ma, W.H., Zhang, Y., Liu, X.J., Han, W.X., Wilson, M.C., and Fang, J.Y.: Long‐term changes in soil pH across major forest ecosystems in China, Geophys. Res. Lett., 42, 933-940, 2015.

Table 1 Growth measurement statistics for four plant growth forms in this study

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	*Castanopsis 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		

Numbers in the tables represent means (or mean ± (standard error)). TBA: total basal area of trees. DBH: diameter at breast height (~1.3 m). Basal diameter: diameter at 10 cm above the ground.

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Published: 4 October 2016

Fig.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) mortality proportion of all trees. Mortality proportion 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}).

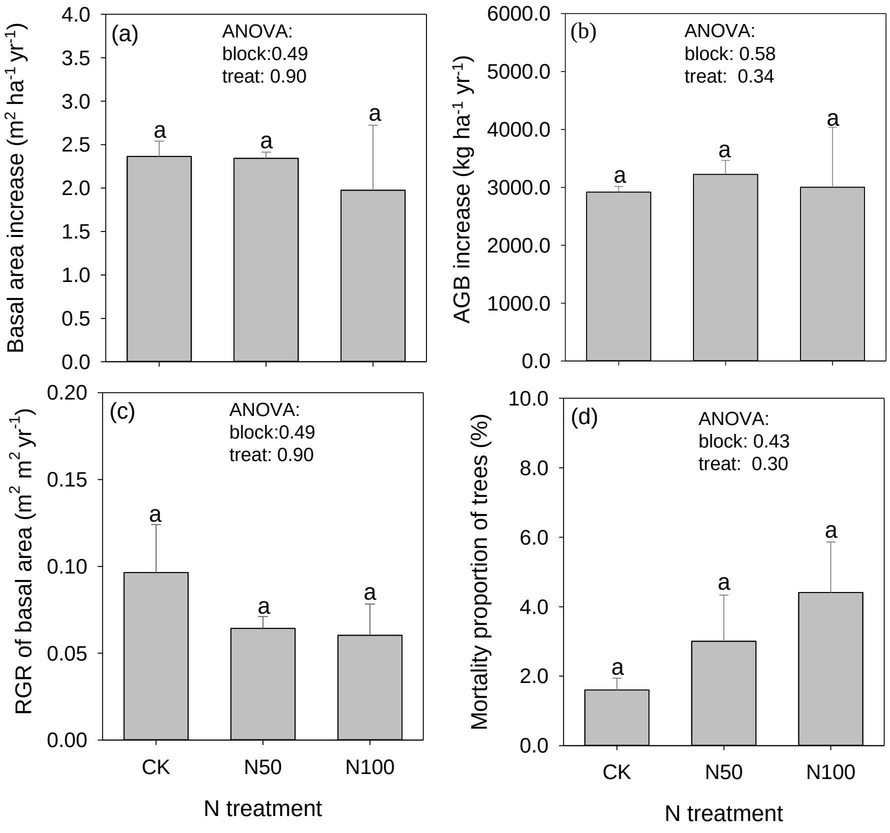
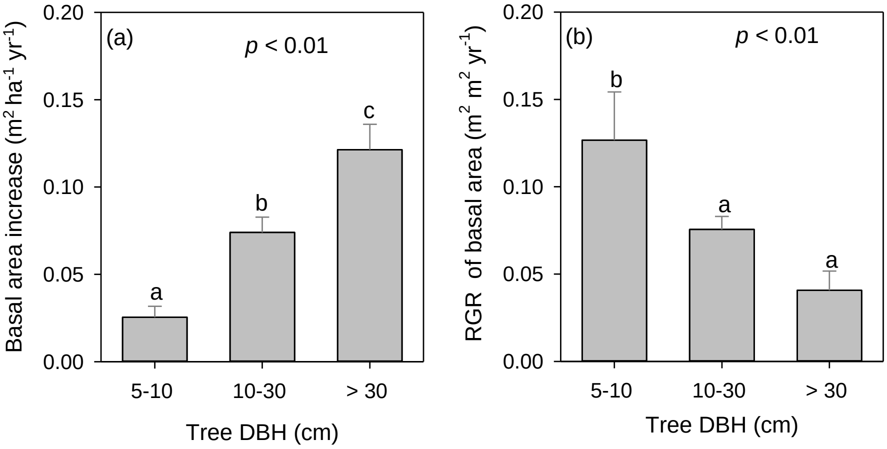


Fig.2. Growth of *C. eyrei* individuals in three DBH classes (mean ± se). (a) Absolute basal area increase of *C. eyrei* individuals; (b) relative basal area growth rate of *C. eyrei* individuals. Only data from the unfertilized plots were analysed. Means with different letters are significantly different (p<0.05).



382 **Fig.3.** Effects of N fertilization on the growth (mean ± se) of *C. eyrei* by DBH classes (5-10 cm, 10-30 cm 383 and >30 cm). (a-c) Absolute basal area increase and (d-f) relative growth increase rate of basal area. 384 Numbers in these figures indicate the results of ANOVA.

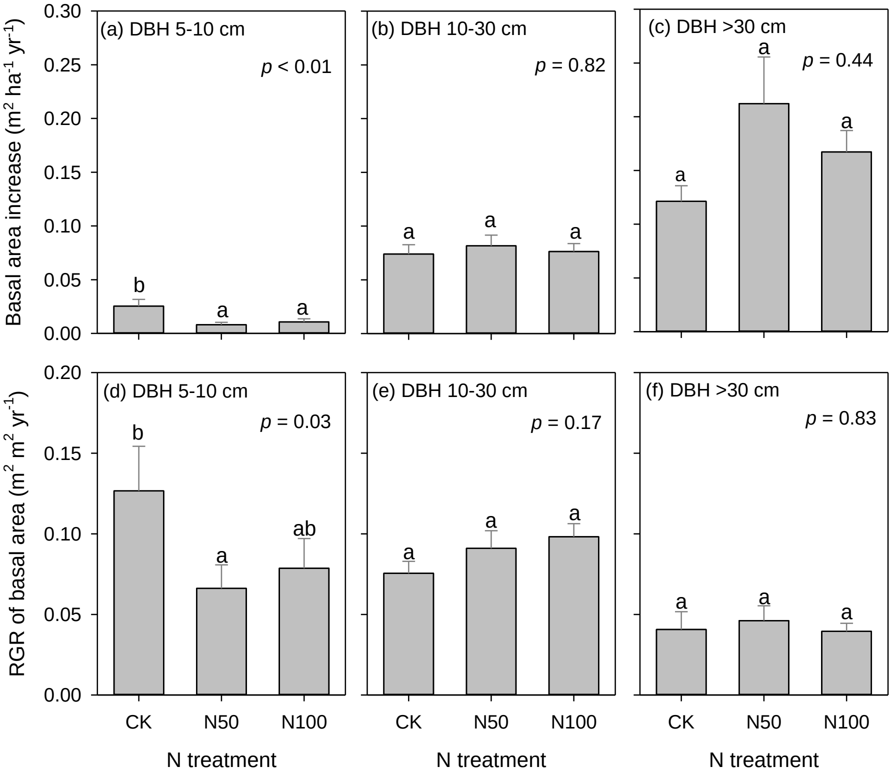


Fig.4. Effects of N fertilization on the growth and mortality of saplings (mean ± se). (a) Absolute basal area increase; (b) aboveground biomass increase; (c) relative basal area growth rate; and (d) mortality proportion of saplings. Mortality proportion was calculated using the aboveground biomass of all dead saplings during the experiment divided by the total aboveground biomass of all saplings in 2014. Numbers in these figures indicate the results of ANOVA.

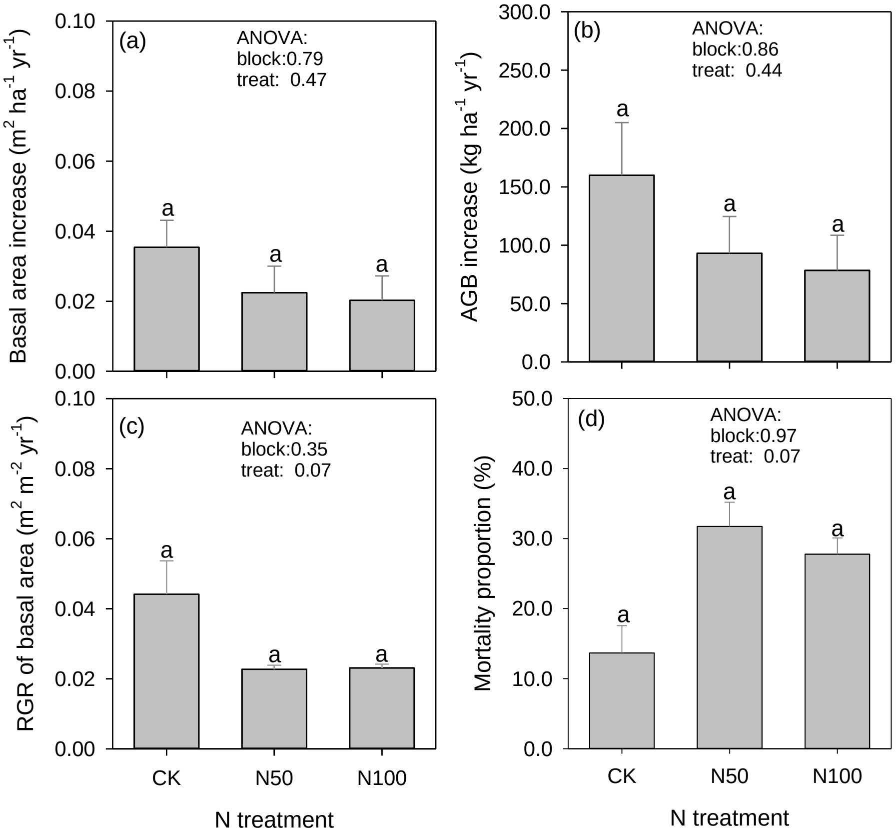
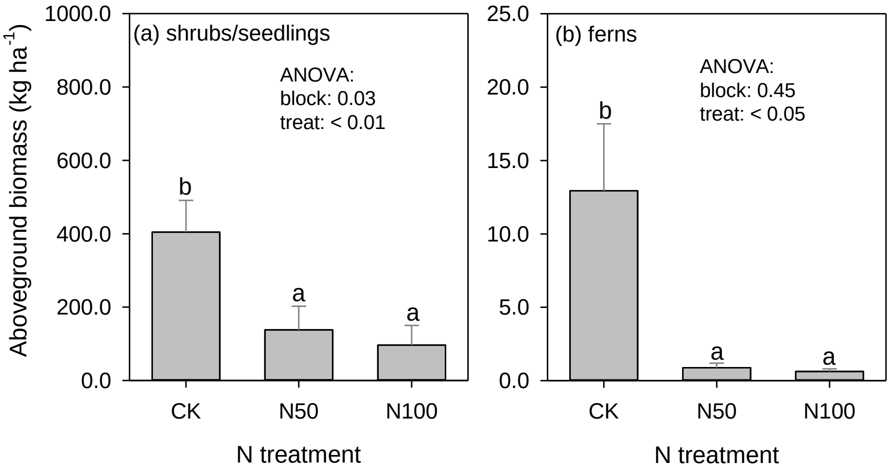


Fig.5. Effects of N fertilization on the aboveground biomass of shrubs, seedlings and ferns. Bars show the aboveground biomass of (a) shrubs/seedlings and (b) ferns (mean ± se). Numbers in these figures indicate the results of ANOVA.



Biogeosciences Discuss., doi:10.5194/bg-2016-416, 2016
Manuscript under review for journal Biogeosciences
Published: 4 October 2016

Fig.6. Effects of P fertilization on the growth of *C. sclerophylla*. (a) Absolute basal area increase; and (b) relative growth rate of basal area. Numbers in these figures indicate the results of ANOVA.