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Alterations in leaf nitrogen metabolism indicated the structural changes of subtropical forest by canopy addition of nitrogen

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ABSTRACT

Globally, nitrogen deposition increment has caused forest structural changes due to imbalanced plant nitrogen metabolism and subsequent carbon assimilation. Here, a 2 consecutive-year experiment was conducted to reveal the effects of canopy addition of nitrogen (CAN) on nitrogen absorption, assimilation, and allocation in leaves of three subtropical forest woody species (*Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis*). We hypothesized that CAN altered leaf nitrogen absorption, assimilation and partitioning of different plants in different ways in subtropical forest. It shows that CAN increased maximum photosynthetic rate (A_{max}), photosynthetic nitrogen use efficiency (PNUE), and metabolic protein content of the two understory species *A. quinquegona* and *B. cochinchinensis*. By contrary, for the overstory species, *C. henryi*, A_{max}, PNUE, and metabolic protein content were significantly reduced in response to CAN. We found that changes in leaf nitrogen metabolism were mainly due to the differences in enzyme (e.g. Ribulose-1,5-bisphosphate carboxylase, nitrate reductase, nitrite reductase and glutamine synthetase) activities under CAN treatment. Our results indicated that *C. henryi* may be more susceptible to CAN treatment, and both *A. quinquegona* and *B. cochinchinensis* could better adapt to CAN treatment but in different ways. Our findings may partially explain the ongoing degradation of subtropical forest into a community dominated by small trees and shrubs in recent decades. It is possible that persistent high levels of atmospheric nitrogen deposition will lead to the steady replacement of dominant woody species in this subtropical forest.

1. Introduction

Forests account for 80% of Earth's plant biomass and 75% of the gross primary productivity of the Earth's biosphere (Pan et al., 2013). However, the world's forests declined from 4128 million ha in 1990 to 3999 million ha in 2015, and about 3.3 million ha of forests have been converted to degraded land or other land uses annually between 2010 and 2015 (FAO, 2015). Such huge forest declines have caused a series of ecological and environmental issues, and resulted in alterations of forest stand structure (Cohen et al., 2016), loss of biodiversity (Lu et al., 2010), enhanced soil erosion and land degradation (Ren et al., 2007). Apart from direct deforestation, global climate change has also had a disruptive influence in many ecosystems and contributed to forest decline (Anderegg et al., 2015; Cohen et al., 2016; Chen et al., 2018). Changes in precipitation and temperature regimes (Zhou et al., 2014), frequent extreme climatic events (Lloret et al., 2012), as well as increased rates of nitrogen deposition (Lu et al., 2010) continue to impact forest ecosystems in multiple ways.

Globally, nitrogen deposition has increased drastically due to human activities, such as increases in fossil fuel combustion, agricultural fertilization, and industrial pollution (Galloway et al., 2008). The reactive nitrogen deposited on the Earth's surface has increased from 34 Tg N yr^{-1} in 1860 to 100 Tg N yr^{-1} in 1995, and is going to reach 200 Tg N yr^{-1} by 2050 (Galloway et al., 2004). As a driver of global change, nitrogen deposition has been proved to cause forest degradation, soil acidification and land degradation, but much of the

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long-term effects on forest structure and function (Magill et al., 2004; Galloway et al., 2008; Lu et al., 2010, 2014; Talhelm et al., 2013) remains to be investigated.

Nitrogen is largely required by plants in the synthesis of amino acids, proteins, chlorophylls, nucleic acids, lipids, and other metabolites, and plants adjust internal nitrogen status to regulate nitrogen uptake and assimilation in order to match plant demand (Kusano et al., 2011). Thus, the increased deposition of nitrogen may be a disruptive influence in natural ecosystems, causing an imbalance in the absorption, assimilation and allocation of nitrogen within plants (Warren et al., 2003). Some studies have found that the nitrogen addition could increase leaf biomass and ribulose-1,5-bisphosphate carboxylase (Rubisco, the key enzyme in photosynthesis) content so as to increase carbon assimilation forest (Warren et al., 2003; Högberg, 2007). However others have found that the nitrogen addition may cause the degradation of chlorophyll (Shi et al., 2017), disorder of carbon metabolism (Bauer et al., 2004), increase in free amino acid content (Strengbom et al., 2003), decrease in forest viability (Liu et al., 2011). As a result, nitrogen deposition was found to change the species composition and function of forests (Nordin et al., 2005; Lu et al., 2010; Gilliam et al., 2006).

China has experienced substantial nitrogen deposition, especially in its rapidly developing central and southeastern regions, and the rates of nitrogen deposition are predicted to increase dramatically in the future (Liu et al., 2011, 2013; Jia et al., 2014). Till date however, studies on the effects of nitrogen deposition on forest have mainly focused on coniferous or deciduous broad-leaved forests in the temperate zone (Takashima et al., 2004; Gradowski and Thomas, 2006; Högberg, 2007; Janssens et al., 2010), and few studies have concentrated on tropical and subtropical evergreen broad-leaved forests (Lu et al., 2010, 2014).

Worldwide, there are only a few nitrogen addition experiments, and even they have resorted to spraying nitrogen on understory plants (Warren et al., 2003; Högberg, 2007; Lu et al., 2014). These experiments on understory plants do not therefore account for the effects of nitrogen deposition on canopy-associated biota and processes, which may not reflect the full range of effects of nitrogen deposition in forest ecosystems (Zhang et al., 2015; Guerrieri et al., 2015).

In this study, we determined the effects of canopy addition of nitrogen (CAN) on nitrogen absorption, assimilation and partitioning in three woody species in a subtropical forest. We focused on the potential effects of CAN on species composition of subtropical forest and the modifications to leaf nitrogen metabolism as a driver of forest degradation. We tested the hypotheses that CAN changes nitrogen absorption, assimilation, and partitioning in canopy leaves, and that these changes would differ among tree species. Finally, we consider tree species selection in forest restoration and development.

2. Materials and methods

2.1. Study site

For the realistic simulation of natural nitrogen deposition in the forest ecosystems, the CAN experiment was conducted at the Shimentai Experimental Station, which is located in Shimentai National Nature Reserve (24°22'–24°31' N, 113°05'–113°31' E), Guangdong Province, China. The study site is covered by broad-leaved evergreen forest. The CAN experiment is a full factorial design with 3 different levels of treatments (Fig. 1), including 25 kg N ha^{-1} yr^{-1} (CN25), 50 kg N ha^{-1} yr^{-1} (CN50), and 0 kg N ha^{-1} yr^{-1} (CK). Four blocks was set up in the forest site and each treatment was replicated once at each of the four blocks. Three treated plots were randomly assigned in each block and a total of 12 plots were established corresponding to three treatments with four replications. Nitrogen was applied with a canopy spraying system located in the center of each CAN treatment plot. Each circular plot has a 17 m semi diameter with an area of 907 m^2, leaving the central core area of 400 m^2 for plant sampling (3–4 individuals per species per plot). Contamination of nitrogen solution between each plot was minimal as they were separated by at least 20 m buffer zone, and polyvinylchloride boards were inserted between two adjacent plots when necessary. A nitrogen solution (NH_4NO_3) of the designated concentration was made by mixing the salt with surface lake water. Each application of nitrogen solution was equivalent to 3 mm of rainfall, with 30–40% of the precipitation intercepted by the forest canopy and the rest penetrating through. The treatments were applied monthly from April to October (seven times per year) from year 2013 to 2016. The total solution addition was 21 mm per year, accounting for less than 1% of total annual precipitation of the forest site, so the confounding effect caused by water addition was negligible (Zhang et al., 2015).

2.2. Plant species

Three native woody species were chosen for this study. *Castanea henryi* (Skan) Rehd. et Wils. is the representative tree species of subtropical broad-leaved forests. The tree can grow up to 30 m tall with a straight, symmetrical trunk. *Ardisia quinquegona* Bl. is a tree in the Myrsinaceae family and can attain a height of 6 m. *Blastus cochinchinensis* Lour. is a small tree or shrub with heights ranging from 0.6 to 3.0 m (Ren et al., 2010). At this study site, the importance values for *C. henryi*, *A. quinquegona*, and *B. cochinchinensis* were 0.3197, 0.2219, and 0.1128, respectively. In June 2015 and 2016, the current-year leaves were collected from the outer branches of each species at similar heights (2–3 m) in each plot. Leaves were taken for photosynthetic measurements while they were still attached to the branch. After that, they were collected for determination of other properties in lab.

2.3. Measurement on gas exchange

Leaf photosynthesis was measured using a portable photosynthesis system (LI-6400, Li-Cor, USA) equipped with a fluorometer leaf chamber (6400-40). The light-saturated net photosynthetic rate (A_{max}) of leaves was obtained at 1500 μmol m^{-2} s^{-1} PPFD, before leaf photosynthetic responses to varying substomatal CO_2 concentrations (A-Ci curve) were measured. Each A-Ci curve was measured in nine steps, starting from 400 and decreasing to 200, 100, and 50 μmol m^{-2} s^{-1}, and then increasing to 300, 500, 700, 1000, and 1400 μmol m^{-2} s^{-1}. Before data were logged, leaves were equilibrated for at least 3 min at each step (Misson et al., 2010). The maximum carboxylation rate (V_{cmax}) and the maximum electron transport rate (J_{max}) were estimated by A-Cc curves (transferred from A-Ci curves). The curves were fitted by the equation of the Farquhar model (Farquhar et al., 1980).

2.4. Measurements on specific leaf area, chlorophyll, and nitrogen content

Leaves were removed from branches, and leaf areas were measured using a portable area meter (LI-3000, LI-Cor, USA) after gas exchange was measured. Leaves were then dried for 72 h at 65 °C and weighed to calculate specific leaf area (SLA) as leaf area per dry mass. Leaf nitrogen content was determined on the dried leaves using the Kjeldahl method. And then photosynthetic nitrogen use efficiency (PNUE) was calculated as the ratio of A_{max} to leaf nitrogen content. Another set of fresh leaves was frozen in liquid nitrogen and stored at − 80 °C for biochemical analyses. Chlorophyll was extracted from a 0.1-g fresh leaf sample with 10 mL of 80% acetone, and the extracts were measured by a spectrophotometer at 663 and 645 nm for the determination of leaf chlorophyll content (Lin et al., 1984).

2.5. Determination in Rubisco and leaf protein

Rubisco concentration was assayed from frozen leaf samples. A 0.5-g leaf sample was ground in liquid nitrogen and homogenized. The homogenate was then centrifuged, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the gels

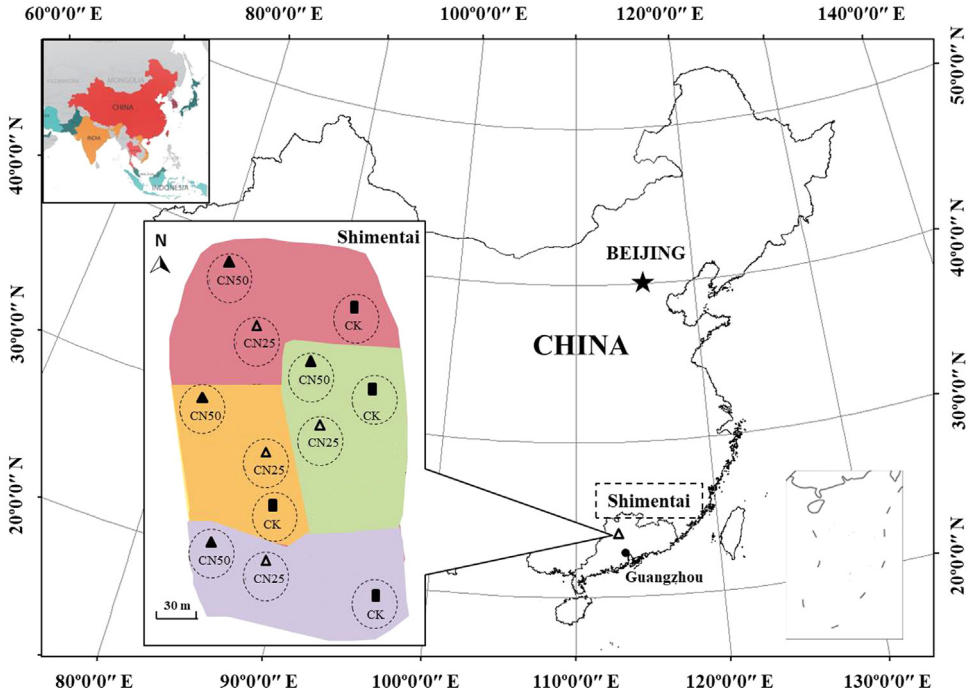

Fig. 1. The location of Shimentai forest experimental site and the layout of treatment plots.

were stained with Coomassie Brilliant Blue R-250 and the bands of the large and small subunits of Rubisco were extracted with formamide for spectrophotometric determination of Rubisco (Hikosaka et al., 1998; Guan and Wen, 2011).

Leaf proteins were separated as water-soluble, detergent-soluble, and detergent-insoluble fractions (Takashima et al., 2004). The frozen leaf samples were homogenized and centrifuged. The supernatant was regarded as the water-soluble fraction. A phosphate buffer containing 3% (w/v) SDS was then added to the pellet and heated for centrifugation again. All of the supernatants were collected as the SDS-soluble fraction of proteins. The final pellet was considered as the SDS-insoluble fraction of protein (Guan and Wen, 2011). The quantities of nitrogen in structural nitrogen (SDS-insoluble fraction) and metabolic nitrogen (water-soluble and SDS-soluble fractions) were estimated assuming 16% nitrogen in proteins.

The specific activity of Rubisco was calculated as the V_{cmax}/Rubisco content. Nitrogen in bioenergetics (P_B, Calvin cycle enzymes, ATP synthase, and electron carriers) was assessed as proportional to J_{max} (Niinemets et al., 2005). Nitrogen factions in Rubisco (P_R) were estimated assuming a nitrogen concentration in Rubisco of 16% (Hikosaka and Terashima, 1995). Nitrogen in the light-harvesting complex (P_L) was estimated assuming 37.1 mol mol^{-1} Chl (Evans and Seemann, 1989). Other protein nitrogen was estimated as the difference between metabolic protein and (P_R + P_L + P_B). Other nitrogen was calculated as leaf nitrogen minus nitrogen partitioned into protein.

2.6. Determination in nitrogen assimilation enzyme activity

Nitrate Reductase (NR) activity was measured according to Foyer et al. (1998), Kaiser and Brendle-Behnisch (1991), Zhang et al. (2009) and Liu et al. (2014) with modifications. The enzyme was determined by homogenization of 0.1 g of frozen leaf sample in 1 mL of phosphate buffer (pH 8.7) containing 10 mM cysteine and 1 mM EDTA. The homogenate was centrifuged at 8000*g* for 10 min, and the supernatant was mixed with 20 mM KNO_3 and 10 mM NADH. After 30 min, the reaction was terminated by addition of sulfanilamide and N(1-naphthyl) ethylene diamine dihydrochloride. Absorbance at 540 nm was determined with a spectrophotometer.

To analyze nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT) activities, frozen leaves (0.1 g) were extracted in 1 mL of Tris-HCl (pH 7.8) containing 1 mM EDTA, 15% glycerol, 14 mM 2-mercaptoethanol, and 0.1% Triton X-100. The homogenate was centrifuged at 8000*g* for 10 min. NiR activity was measured by determining the decrease of NO_2^- at 520 nm (Lillo, 1984). GS activity was determined by measuring the formation of glutamyl hydroxamate in the supernatant at 540 nm after reaction with acidified ferric chloride (Kaiser and Lewis, 1984). GOGAT activity was determined by detecting the oxidation of NADH in the supernatant at 340 nm (Tang, 1999; Liu et al., 2014).

2.7. Statistical analyses

Two-way repeated-measures ANOVA was performed to compare the effects of treatment, species and their interaction on each variables. To test homogeneity of variance, Levene's test was applied. Variables were log-transformed to meet model assumptions when necessary. Pearson correlation analysis (two-tailed) and logistic regression were applied to test the relationships between paired variables. Statistical significance was considered as $P < 0.05$. R version 3.4.1. and SPSS 17.0 (SPSS Inc.) were used for data analyses.

3. Results

3.1. Photosynthesis

Compared to the CKs, the A_{max} of *A. quinquegona* and *B. cochinchinensis* increased but that of *C. henryi* decreased in response to CN25 and CN50 in both 2015 and 2016 (Fig. 2A, B). The leaf nitrogen content in *C. henryi* was elevated significantly in response to CN25 and CN50, but that in the understory species *A. quinquegona* and *B. cochinchinensis* was not statistically changed by CAN (Fig. 2C, D). Therefore, PNUE decreased for *C. henryi* but increased for *A. quinquegona* or *B. cochinchinensis* in both 2015 and 2016 under CAN treatment (Fig. 2E, F).

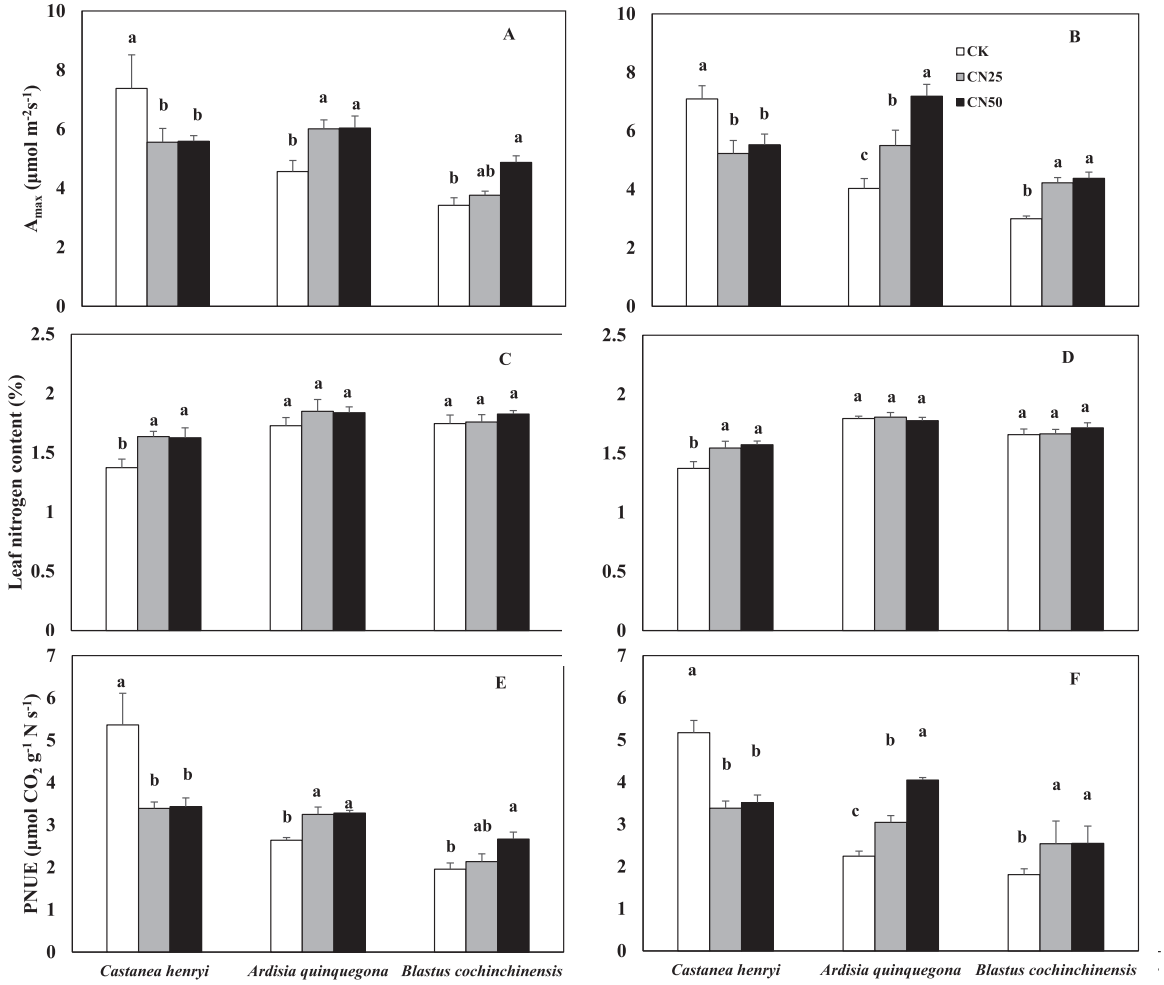

Fig. 2. Maximum photosynthetic rate (A_{max}), leaf nitrogen content, and photosynthetic nitrogen use efficiency (PNUE) of *Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis* leaves as affected by two levels of canopy nitrogen addition (CN50, and CN25) in 2015 (left panels) and 2016 (right panels). Data are mean + SE. Data For each groups of three bars, different lowercase letters indicate significant differences among the CAN treatments ($P < 0.05$).

3.2. Chlorophyll content, Rubisco content and Rubisco activity

For years 2015 and 2016, canopy leaf chlorophyll content was increased in *C. henryi* but not in *A. quinquegona* or *B. cochinchinensis* under CAN treatment (Fig. 3A, B). Compared to Cks, Rubisco content in *A. quinquegona* was increased in CN25 and CN50 plots in 2015 (Fig. 3C), which was significantly elevated but the tendency changed for this species in 2016 (Fig. 3D). We found that Rubisco content was not significantly altered by the treatments in the other species. In both years, the specific activity of Rubisco (V_{cmax}/Rubisco) was lowered in *C. henryi* and *A. quinquegona* but elevated in *B. cochinchinensis* in response to CAN (Fig. 3E, F).

3.3. Leaf nitrogen partitioning

The metabolic protein content of *C. henryi* was significantly reduced in response to CAN in both years (Fig. 4A, B). By contrary, the content of metabolic protein in *A. quinquegona* and *B. cochinchinensis* leaves were significantly elevated by CN50 in both years. CN25 and CN50 decreased P_B and other protein and structural protein fractions but increased other leaf nitrogen fractions in *C. henryi* in both years (Fig. 5A, B). In leaves of *A. quinquegona*, in contrast, other nitrogen fractions mainly decreased while P_R greatly increased in response to CN25 and CN50 treatment (Fig. 5C, D). For *B. cochinchinensis* leaves, nitrogen fractions decreased under both CN25 and CN50 but other protein fractions increased for both 2015 and 2016 (Fig. 5E, F). P_L fractions did not greatly differ among the three treatments for any of the three species in 2014 or 2015 (Fig. 5).

3.4. Nitrogen assimilation enzyme activities

In both 2015 and 2016, NR activities of *C. henryi* were significantly elevated, but the changes were not significant in the other two species (Fig. 6A, B). NiR activities were elevated by CAN for *A. quinquegona* but not for *C. henryi* or *B. cochinchinensis* (Fig. 6C, D). Activities of GS in leaves of *A. quinquegona* was significantly increased by CN25 and CN50 in both 2015 and 2016 (Fig. 6E, F), while leaf GOGAT activities were not significantly changed by CAN treatments for any of the three species in either year (Fig. 6G, H).

3.5. Leaf biochemistry and nitrogen partitioning relationships

There was a negative correlation between P_B and leaf nitrogen content in each species (Fig. 7A, B). Rubisco relative activity decreased as the Rubisco content in leaves increased, resulting in a negative correlation between V_{cmax}/Rubisco and Rubisco content (Fig. 7C, D). For each species, PNUE was positively correlated with P_B in both years (Fig. 7E, F). It means that a high partitioning of nitrogen to bioenergetics (other Calvin cycle enzymes, ATP synthase, and electron carriers) results in high PNUE in the leaves of all three species.

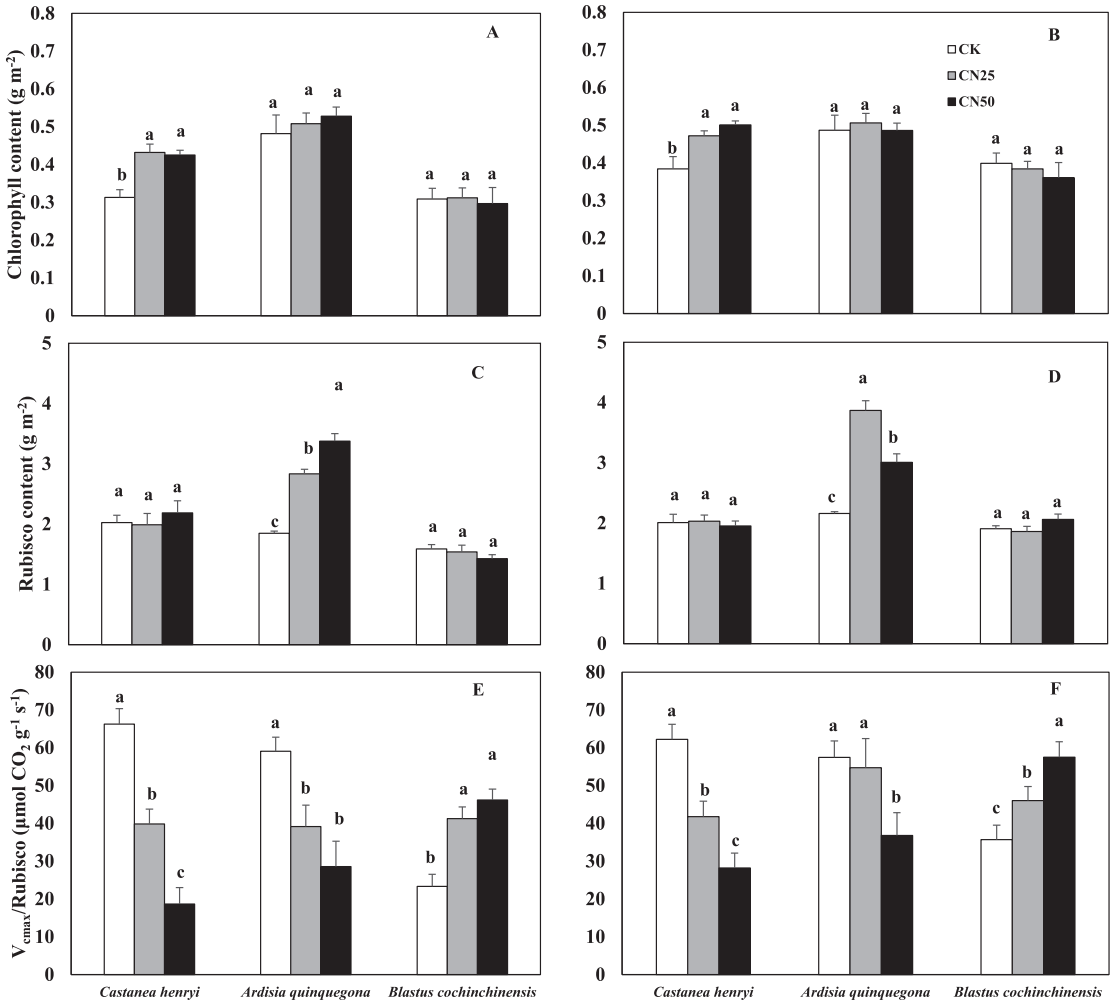

Fig. 3. Chlorophyll content, Rubisco content, and V_{cmax}/Rubisco of *Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis* leaves as affected by two levels of canopy nitrogen addition (CN50, and CN25) in 2015 (left panels) and 2016 (right panels). Data are mean + SE. For each groups of three bars, different lowercase letters indicate significant differences among the CAN treatments ($P < 0.05$).

4. Discussion

Nitrogen retention by the forest canopy is important to understand the effects of nitrogen deposition on forest (Wortman et al., 2012; Zhang et al., 2015). The nitrogen retained by the forest canopy may be absorbed by canopy leaves, twigs, barks, epiphytes, and microorganisms as well as decaying leaves or dead organic matter in the canopy (Hanson and Lindberg, 1991; Sparks, 2009; Matson et al., 2014). In our study site, the proportion of nitrogen retained by the forest canopy was around 30–50% (unpublished data), causing an increase of leaf nitrogen content in *C. henryi*. A significant percentage of the added nitrogen was retained by the dominant tree species, i.e., *C.*

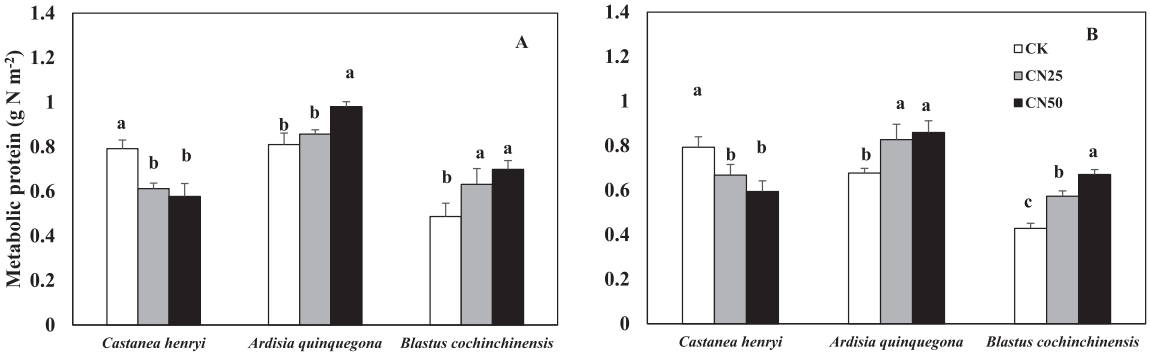

Fig. 4. Metabolic protein content of *Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis* leaves as affected by two levels of canopy nitrogen addition (CN50, and CN25) in 2015 (A) and 2016 (B). Data are mean + SE. For each groups of three bars, different lowercase letters indicate significant differences among the CAN treatments ($P < 0.05$).

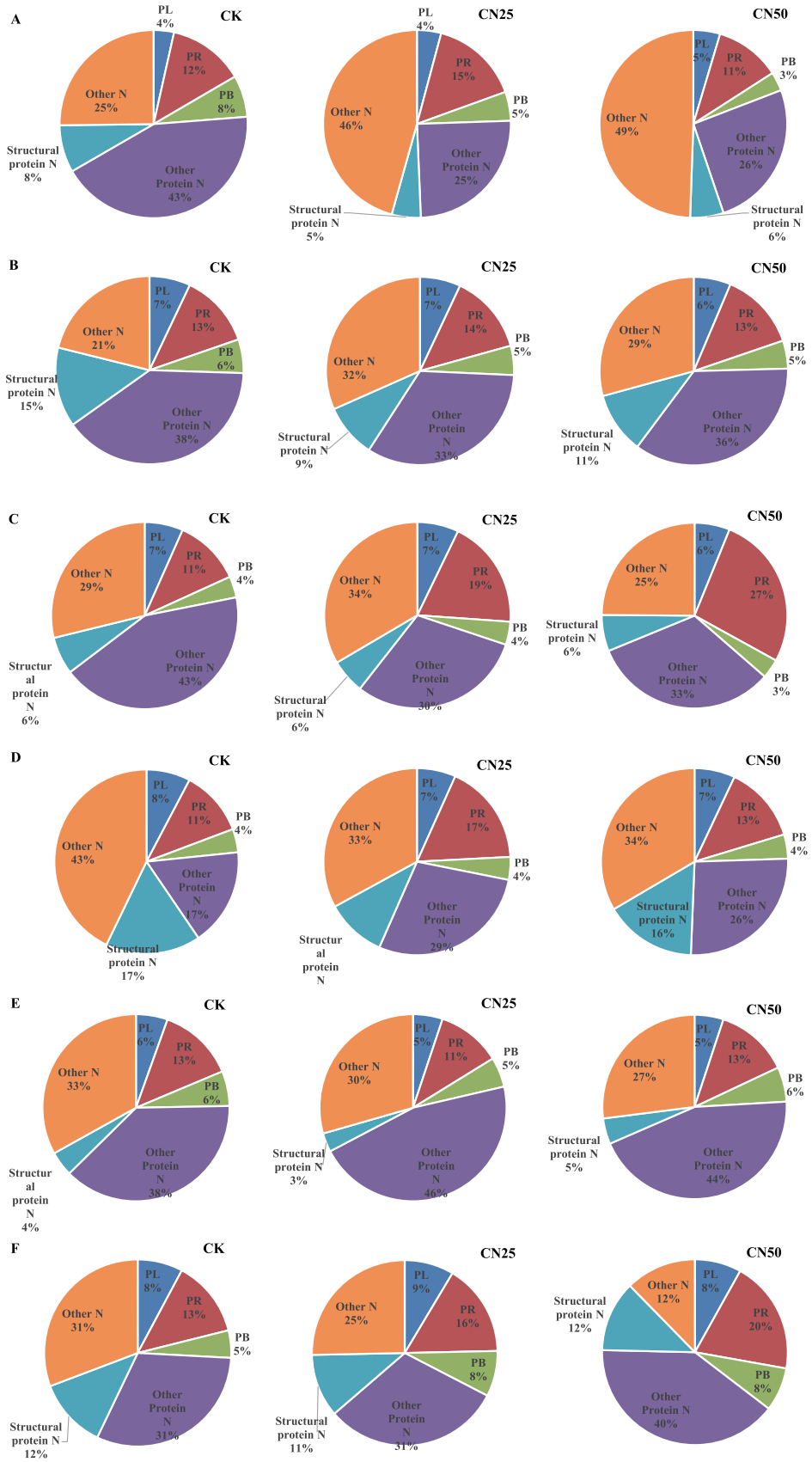

Fig. 5. Nitrogen partitioning in leaves of *Castanea henryi* (A, B), *Ardisia quinquegona* (C, D), and *Blastus cochinchinensis* (E, F) as affected by two levels of canopy nitrogen addition (CN50, and CN25) in 2015 (A, C, E) and 2016 (B, D, F). Nitrogen partitioned into light harvesting (P_L), Rubisco (P_R), bioenergetics (P_B), or metabolic protein minus nitrogen partitioned into photosynthesis, i.e., $P_R + P_L + P_B$ (other protein N). Other N: the difference between leaf nitrogen and nitrogen partitioned into protein.

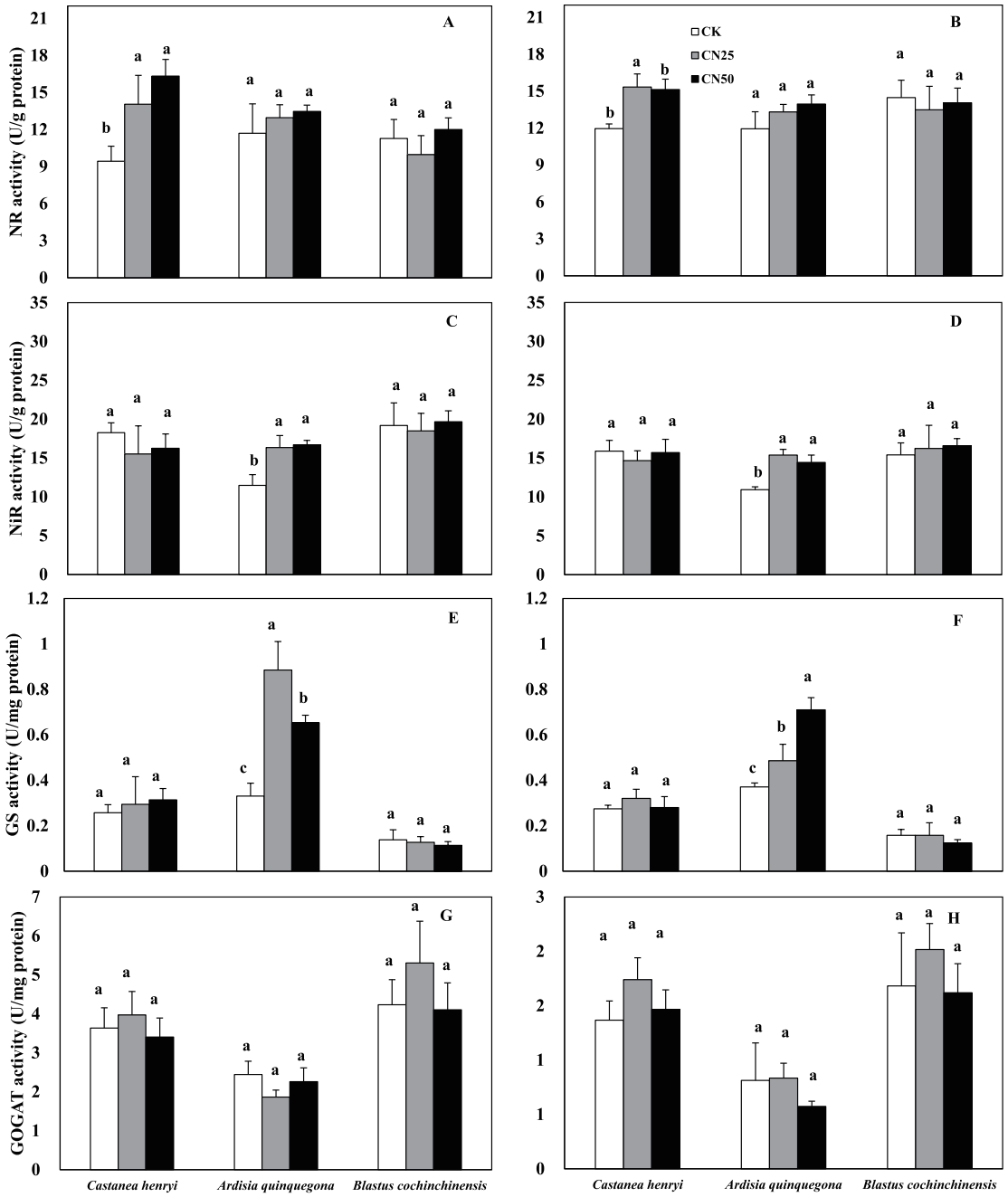

Fig. 6. Nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT) activities in leaves of *Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis* as affected by three levels of canopy nitrogen addition (CN50, CN25, and CK) in 2015 (left panels) and 2016 (right panels). Data are mean + SE. For each groups of three bars, different lowercase letters indicate significant differences among the CAN treatments ($P < 0.05$).

henryi, and only the penetrated nitrogen solution could reach the understory tree *A. quinquegona* or the shrub *B. cochinchinensis*. With the fact that *C. henryi* protected lower species with its branches and leaves from nitrogen supply by nitrogen spraying system, the input of additional nitrogen may have had little effect on nitrogen absorption by leaves of the understory species *A. quinquegona* and *B. cochinchinensis*.

In the canopy leaves of *C. henryi* there was a consistent increase in leaf nitrogen, and a concomitant increase in leaf chlorophyll content. This finding is in consistent with previous studies that photosystem and light harvesting complex were accumulated under added nitrogen treatment (Warren et al., 2003; Ji et al., 2015). Our results, however, showed a decreased Rubisco activity together with an increased leaf nitrogen content and increased chlorophyll content in response to CAN. Such a pattern has been reported earlier where Rubisco activation and A_{max} were found to decrease with increasing leaf nitrogen. This most likely explanation is that Rubisco catalytic function might be suppressed, causing a reduced CO_2 assimilation efficiency (Manter et al., 2005; Guan and Wen, 2011). The CN25 and CN50 treatments increased A_{max}, Rubisco content, or V_{cmax}/Rubisco, and thus elevated PNUE for the two understory tree species, *A. quinquegona* and *B. cochinchinensis*. The increased A_{max} was also in accordance with the increased distribution of nitrogen to the photosynthetic apparatus ($P_L + P_R + P_B$) in the understory species.

In our study, NR activity in the canopy leaves of *C. henryi* was higher in response to CAN in both years, indicating positive responses to NO_3^- addition. For the two understory species, *A. quinquegona* and *B.*

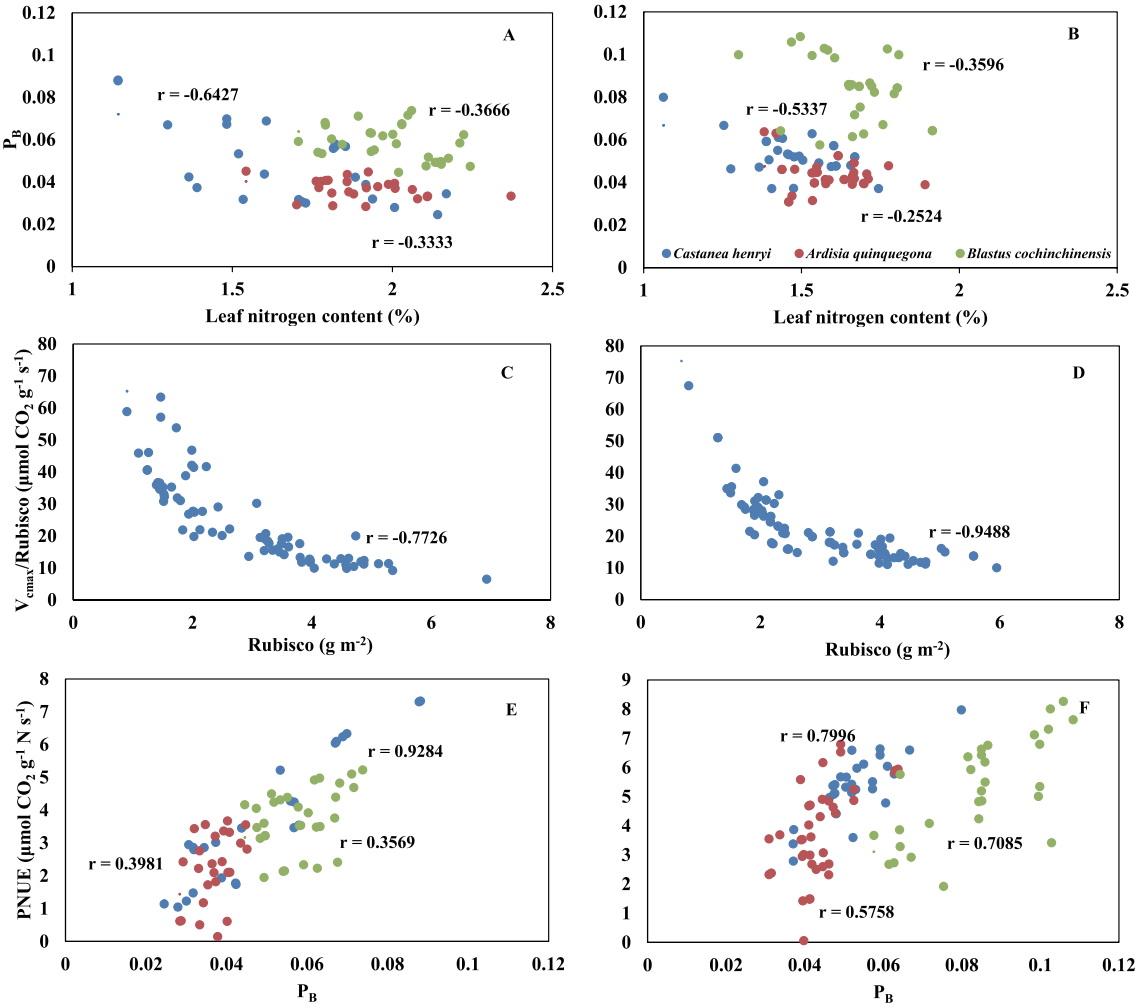

Fig. 7. Correlations between nitrogen partitioned into bioenergetics (PB) vs. leaf nitrogen content (A, B), V_{cmax}/Rubisco vs. Rubisco content (C–D), and photosynthetic nitrogen use efficiency (PNUE) vs. PB (E, F) in 2015 (left panels) and 2016 (right panels).

cochinchinensis, NR activities were unaffected in the CN25 and CN50 treatments. It is possible that most of the solar radiation is accumulated by the highest *C. henryi*, thus this species gathers more energy than the two lower species to produce NADH and ferredoxin which are necessary for nitrogen assimilation as electron donors for NR. In our study, NR and GS activities of *A. quinquegona* were significantly elevated in the CN25 and CN50 treatments. This is in partial agreement with a previous study conducted on moss species under nitrogen addition condition (Liu et al., 2016).

Our results showed that even though CAN activated NR, increased the foliar nitrogen content, and increased the chlorophyll content in *C. henryi*, CAN reduced the leaf A_{max} of *C. henryi* mainly by decreasing P_B and Rubisco activity and by decreasing the partitioning of nitrogen into metabolic protein. In response to CAN, however, *A. quinquegona* increases NiR and GS activities and thus increases the syntheses of metabolic protein, e.g., Rubisco and light harvesting proteins. *B. cochinchinensis*, in contrast, was not able to increase its nitrogen assimilation enzyme activity in response to an increase in the canopy nitrogen supply but was able to greatly increase Rubisco carboxylation activity, resulting in higher potentials in carbon and nitrogen assimilation.

5. Conclusion

Overall, these results supported our initial hypothesis that woody species may use different strategies to cope with an increase in nitrogen supply by balancing how the assimilated nitrogen is used in photophosphorylation, ATP and NADPH syntheses, and CO_2 fixation during photosynthesis. We show that dominant trees with large canopies (e.g., *C. henryi*) are more likely to be susceptible in terms of nitrogen assimilation and utilization, while small tree and shrub (e.g., *A. quinquegona* and *B. cochinchinensis*) are more likely to be stimulated by an increase in the quantity of nitrogen contacting the canopy.

Similarly, our previous studies also showed that *Pinus massoniana*, another dominant canopy tree species in the subtropical forest, was sensitive to air pollution, showing imbalanced nitrogen and energy metabolisms in response to nitrogen and acid pollutions. By contrast, later successional tree species in this forest, *Schima superba*, *Castanopsis fissa* and *Acmena acuminatissima*, were more adaptive to high levels of such pollution (Liu et al., 2009; Guan and Wen, 2011). Alterations in nitrogen metabolism of different plants may possibly result in changes in carbon sequestration and biomass accumulation of subtropical forest under elevated nitrogen deposition in the near future. We believe that the patterns that dominant species are sensitive to additional nitrogen input have implications at large spatial scales, as nitrogen deposition is a regional and continental scale phenomenon.

We argue that our findings are useful in deciding on species life histories for reforestation and restoration efforts keeping in mind future environmental changes. We bring forward a novel and crucial viewpoint that changes in leaf nitrogen metabolism (absorption,

assimilation and partitioning) can be tested and easily used in estimating the sensitivities of different species under nitrogen deposition. We suggest that before the initiation of forest construction in subtropical regions, nitrogen sensitive tree species should be properly utilized.

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None.

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