



# Effects of simulated N deposition on foliar nutrient status, N metabolism and photosynthetic capacity of three dominant understory plant species in a mature tropical forest



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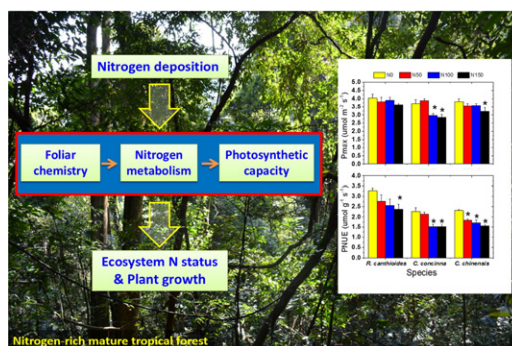
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## HIGHLIGHTS

- It remains unclear how N deposition affects plant growth in N-rich tropical forest.
- To evaluate impacts of N inputs on understory plants, foliar traits were measured.
- Excess N accumulates as soluble protein or free amino acid, but not as chlorophyll.
- Nitrogen inputs induced nutrient imbalance and lower photosynthetic capacity.
- PNUE (Photosynthetic nitrogen use efficiency) is a sensitive indicator to N status.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Anthropogenic increase of nitrogen (N) deposition has threatened forest ecosystem health at both regional and global scales. In N-limited ecosystems, atmospheric N input is regarded as an important nutrient source for plant growth. However, it remains an open question on how elevated N deposition affects plant growth in N-rich forest ecosystems. To address this question, we used a simulated N deposition experiment in an N-rich mature tropical forest of southern China, with N addition levels as 0 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Control), 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Low-N), 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Middle-N) and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> (High-N), respectively. We measured foliar nutrient element status (e.g., N, P, K, Ca and Mg), N metabolism and photosynthesis capacity of three dominant understory plant species (*Cryptocarya concinna* and *Cryptocarya chinensis* as medium-light species; and *Randia canthioides* as shade tolerant species) in this forest. Results showed that two years of N addition greatly increased foliar N content, but decreased the content of nutrient cations (e.g., K, Ca and Mg). Nitrogen addition also increased N accumulation as organic forms as soluble protein and/or free amino acid (FAA), but not as chlorophyll in all three species. We further found that the photosynthesis capacity (Pmax) of *C. concinna* and *C. chinensis* decreased significantly with elevated N addition, with no effects on *R. canthioides*. However, photosynthetic nitrogen use efficiency (PNUE) significantly declined with N addition for all three species, with significantly negative relationships between PNUE/Pmax and foliar N content. These findings suggest that excess N inputs can accelerate nutrient imbalance, and inhibit photosynthetic capacity of understory plant species, indicating continuous high N deposition can threaten understory plant growth in N-rich tropical forests in the future. Meanwhile, PNUE can be used as a sensitive indicator to assess ecosystem N status under chronic N deposition.

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## 1. Introduction

Human activities have altered the global and regional cycles of nitrogen (N) more than for any other elements (Galloway et al., 2008; Erisman et al., 2013). Accelerating industrialization and excess use of N fertilizer now make N deposition significant not only in densely populated and highly developed regions (e.g., Europe and North America), but also in other parts of the world (e.g., Asia and Latin America) (Galloway et al., 2003; Adams et al., 2004). Of all N fertilizers, 40%–60% has been used in the tropics and subtropics (Galloway et al., 2003). At the same time, fossil fuel usage is expected to increase by several hundred percent in many areas of the tropics and subtropics over the coming decades (Galloway et al., 2008).

Enhanced atmospheric N deposition dramatically alters forest ecosystem properties and processes (such as net primary production and nutrient cycling), especially when inputs are large and continuous (Aber et al., 1989; Högberg et al., 2006; Liu et al., 2011), and has threatened the health of ecosystems in North America and Europe (Matson et al., 2002; Aber et al., 2003; Magill et al., 2004; Högberg et al., 2006; Bobbink et al., 2010; Binkley and Hogberg, 2016). Understory plants, as an important component of forest ecosystems, were undoubtedly affected by enhanced atmospheric N deposition. Many studies on the effects of N deposition on forest plants have been reported (Van Dijk and Roelofs, 1988; Aber et al., 1995; Pitcairn et al., 1998; Bauer et al., 2000, 2004; Nakaji et al., 2002; Magill et al., 2004; McGrath et al., 2005; Elvir et al., 2006; Lu et al., 2010; Phoenix et al., 2012). In these studies, there have been increasing concerns regarding changes in foliar nutrient status, N metabolism and photosynthetic capacity.

In N-limited forests, N deposition can satisfy plant demand for N and improve nutrient conditions, which will increase photosynthetic capacity and simulate plant growth. However, excess N inputs in forest ecosystems may result in nutrient imbalance in trees (Boxman and Roelofs, 1988; Whytemare et al., 1997; Magill et al., 2000; Nakaji et al., 2001) and reduce net photosynthesis (Bauer et al., 2004; Elvir et al., 2006). One of the most consistent responses of forests to high N inputs is higher foliar N concentrations, suggesting that N accumulates in plants when excess atmospheric N is absorbed (Van Dijk and Roelofs, 1988; Ericsson et al., 1993; Näsholm et al., 1994; Magill et al., 2000; Talhelm et al., 2011). To mitigate inorganic N toxicity, plants always regulate the N metabolism level by allocating substantially N to protein, chlorophyll, and/or amino acids (Näsholm et al., 1994; Richter et al., 1995; Bauer et al., 2004; Bubier et al., 2011). Until now, most studies on the effects of enhanced N deposition on foliar nutrient status, N metabolism and photosynthesis of forest trees have been conducted in N-limited temperate/boreal forests (Van Dijk and Roelofs, 1988; Richter et al., 1995; Nakaji et al., 2001; Bauer et al., 2004; Elvir et al., 2006; Talhelm et al., 2011; Bubier et al., 2011). However, it remains unclear how forest trees respond to excess N deposition in tropical and subtropical areas, where forest ecosystems are often N rich, but more phosphorus (P) deficient (Vitousek and Sanford, 1986; Matson et al., 1999; Lu et al., 2010; Cleveland et al., 2011; Santiago et al., 2012).

In Asia, from 1961 to 2000, the reactive N from anthropogenic sources increased from 14.4 Tg N yr<sup>-1</sup> to 67.7 Tg N yr<sup>-1</sup>, and is predicted to be 105.3 Tg by the year of 2030 (Zheng et al., 2002). Currently, this leads to high atmospheric N deposition (30–73 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in some forests of southern China (Ren et al., 2000; Zhou and Yan, 2001; Xie et al., 2010; Huang et al., 2012). For example, the amount of N deposition from rainfall to the tropical forests of Dinghushan Biosphere Reserve was 34.4 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 2009–2010 (Lu et al., 2013). This value is comparable to the highest levels of N deposition occurring in Europe and USA (MacDonald et al., 2002; Aber et al., 2003), causing N saturation of forest ecosystems. There have been increasing concerns about the effects of enhanced N deposition on forest ecosystems in southern China (Mo et al., 2006; Fang et al., 2008; Liu et al., 2011; Lu et al., 2010, 2015).

Changes in vegetation can be observed before ecosystem processes are greatly affected by N deposition (Bobbink et al., 2010; Phoenix

et al., 2012), and are considered to be the first signs of N saturation (Gundersen, 1991). The understory vegetation is predicted to play an important role in forest ecosystems (Kondo et al., 2005; Gilliam, 2007), and can be used as an indicator of excess N. We have previously reported that the mature forest in the Dinghushan Nature Reserve of southern China has been N saturated due to both long-term high N deposition in the region and the age of the ecosystem (Mo et al., 2006; Fang et al., 2008). In present study, we selected three representative understory plants in this mature forest: one shade tolerant species *Randia canthioides*, and two medium-light species *Cryptocarya concinna* and *Cryptocarya chinensis*. To evaluate the impacts of N deposition on plant growth, the main leaf parameters were measured: nutrient status, N metabolism, and photosynthetic capacity. We aim to test the following hypothesis: short-term N additions will not affect foliar nutrient status, N metabolism, and photosynthetic capacity of these three understory plant species, considering that this mature forest has been N saturated, and additional N inputs have minor effects on plant growth.

## 2. Material and methods

### 2.1. Study site

This study was conducted at Dinghushan Biosphere Reserve, an UNESCO/MAB site. The reserve is located in the central area of Guangdong Province in southern China (112°10' E and 23°10' N) and occupies an area of approximately 1200 ha. About 20% of the reserve area is covered by undisturbed monsoon evergreen broadleaf forest (mature forest), that represents the climax forest type of lower subtropics in China (Wang et al., 1982; Mo et al., 2003). We have established research site in the mature forest, at 250–300 m above sea level, which has been protected from human impacts for >400 years.

The reserve experiences a typical monsoon climate. The average annual rainfall is 1927 mm, having a distinct seasonal pattern with 75% of it falling from March to August, and 6% from December to February (Huang and Fan, 1982). Annual average relative humidity is 80%, and the mean annual temperature is 21.0 °C. The reserve has been experiencing high atmospheric N deposition in precipitation (>30 kg N ha<sup>-1</sup> yr<sup>-1</sup>) since 1990s (Zhou and Yan, 2001; Lu et al., 2013). In 2009–2010, the total dry N deposition was 14.21 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Long, 2010).

A survey conducted in June 2003 (before the start of N addition experiment) showed that the major species in the mature forest were *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, *Syzygium rehderianum* in the canopy and sub-canopy layers, which represented up to 80% of total basal area. The plants in the understory layers were mainly consisted of woody plant with diameters at breast high (DBH) often below 2.5 cm. The dominant understory trees were *C. concinna*, *C. chinensis*, *R. canthioides*, and *Blastus cochinchinensis*. The soil in the study site is lateritic red earth formed from sandstone (oxisols) with a soil depth deeper than 60 cm (Mo et al., 2003). Mineral soil properties of mature forest are shown in Table 1.

### 2.2. Experimental design and sample collection

A nitrogen addition experiment was initiated in 2003 (Mo et al., 2006) including four N addition treatments (in three replicates): 0 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Control), 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Low-N), 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Middle-N) and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> (High-N). The N addition gradient was based on both present N deposition level and its further increase in the future. Totally, twelve 20 × 10 m plots were established in a completely randomized design. Each plot was surrounded by at least a 10 m wide buffer strip to the next plot. For the N addition, NH<sub>4</sub>NO<sub>3</sub> salt was weighed, dissolved with 20 L of water, and applied monthly on the floor of each plot (usually below the canopy of the studied understory trees) by using a backpack sprayer from July 2003. The Control plots only received the same amount of

**Table 1**  
Mineral soil properties in the Control plots of a mature forest of Dinghushan in subtropical China.

Depth	pH (H <sub>2</sub> O)	Total C (%)	Total N (%)	C/N	Available P (mg kg <sup>-1</sup> )	Soil bulk density (g cm <sup>-3</sup> )
0–10 cm	3.76(0.01)	3.2 (0.3)	0.3 (0.02)	12.8 (2.3)	5.0 (0.2)	0.98(0.06)
10–20 cm	3.86(0.01)	2.1 (0.4)	0.1 (0.01)	16.4 (1.5)	2.6 (0.1)	1.15(0.08)

\*Data are cited from Mo et al., 2006; Values are the mean ± S.E. ( $n = 3$  for all samples; measured in July 2004).

water. The study objects were three dominant understory plant species (*R. canthioides*, *C. concinna*, and *C. chinensis*) chosen to have similar eco-physiological characteristics and environmental conditions. Their structural characteristics are shown in Table 2. One-year-old mature leaves, lacking visible signs of senescence, were selected for the measurement.

### 2.3. Photosynthesis measurements

In June 2005, light-saturated net photosynthesis rate (P<sub>max</sub>) was measured on fully developed leaves at the top of each plant using a Li-6400 CO<sub>2</sub>/H<sub>2</sub>O portable photosynthesis system with integrated LED light source (Li-Cor, Lincoln, NE, USA). The chamber CO<sub>2</sub> concentration was maintained at ambient level (about 370 μmol mol<sup>-1</sup>), and leaf temperature was kept at ~25 °C. To obtain light-saturated rates of photosynthesis, the leaf in the chamber was illuminated at 1000 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density (PPFD), under adjusted internal conditions at vapour pressure deficits between 0.5 and 1.2 k Pa, and air-flow rate at 500 μmol s<sup>-1</sup>. Preliminary experiments showed that the PPFD level chosen was sufficient to light saturate photosynthesis. All photosynthesis measurements were conducted on 6–8 individual leaves per tree, and 2 trees of each species were selected in the same measuring date to create an averaged value for each plot level.

After photosynthesis measurements, leaf sampling was carried out. Leaves were put into a sealed plastic bag with silica gel, placed in an ice box, and then transported to the laboratory and stored at 4 °C until further analysis. Leaf mass per area (LMA) was determined as the ratio of leaf dry weight over the leaf fresh area by cutting disks ( $n > 30$ ) of known area with the aid of stainless steel templates.

### 2.4. Foliar nutrient status

A fraction of the collected leaves were used for element analysis. Dried leaves (70 °C for >48 h) were ground to a fine powder with a vibrating sample mill. The total N concentration was determined with Kjeldahl digestion method (Ryan et al., 2007), while total P concentration was analyzed colorimetrically based on the Molybdate-blue reaction. To measure the leaf contents of K, Ca, Na, Mg, and Al, the powder was digested with sulphuric and perchloric acid (Dong et al., 1996). The element concentrations were determined by ICP optical emission spectrometer (Perkin Elmer, USA).

### 2.5. Free amino acid determination

Amino acid extraction followed the procedure of Barnett (1966) and Sun et al. (2000). A number of fresh leaves were homogenized in liquid N using a mortar and pestle. Five ml of 5% salicylic acid were added to

the homogenized sample (Approximately 100 mg). After 15 min of extraction, the samples were centrifuged at 15000 r min<sup>-1</sup> for 30 min, and the supernatant collected. The remaining pellets were re-extracted one more time and the supernatants combined. The FAA content was quantified based on an external standard, and measured with amino acid auto-analyzer (HITACHI 835-50).

### 2.6. Leaf soluble proteins and pigment quantification

The total soluble proteins were extracted adopting the method described by Xu and Zhou (2006). About 1 g of leaves was homogenized with 10 ml of 50 mM sodium phosphate, pH 7.8, containing 2 mM EDTA and 80 mM L-ascorbic acid. After the homogenate was centrifuged at 15,000g for 20 min, the supernatants were used to determine soluble protein. The protein concentration was determined according to Bradford (1976). Calibration curves were made with bovine serum albumine (BSA) as the standard. The photosynthetic pigments (chlorophyll/Chl, Chl *a*, Chl *b*) in leaves were extracted in 80% acetone and centrifuged twice at 5000g for 15 min. The concentrations of chlorophylls and carotenoids were determined spectrophotometrically and calculated per unit fresh mass basis according to the equations of Lichtenthaler (1987). Subsamples of leaves were dried to 105 °C to determine the fresh-weight to dry-weight ratio, and all results are reported on 105 °C basis.

### 2.7. Statistics

One-way analysis of variance (ANOVA) with LSD test was performed to test the effects of N treatment on nutrient status, N metabolism, photosynthetic capacity of the three understory species. Significant differences between treatments and among species were determined by using multiple comparisons of means. Linear regression analyses were performed to establish the strength and significance of relationships between two different variables for PNUE and foliar N per mass (N<sub>mass</sub>, or foliar N content), and P<sub>max</sub> and N<sub>mass</sub>, respectively. Spearman's rho correlation coefficients were calculated to show the relationship between N treatments, leaf mass per unit leaf area (LMA), N<sub>mass</sub>, foliar nitrogen per unit leaf area (N<sub>area</sub>), P<sub>max</sub> and PNUE. All analyses were conducted using SPSS software package (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA). Statistical significant differences were recognized at  $p < 0.05$  unless otherwise stated.

## 3. Results

### 3.1. Foliar element chemistry

In the Control plots, *R. canthioides* had significantly higher foliar K, Ca, and Mg concentrations, and Ca/Al ratios than the other two species, and *C. concinna* had higher foliar N concentrations (Table 3). Generally, elevated N additions greatly increased foliar N concentrations, but decreased foliar base cations (K, Ca and Mg) in all three species. Nitrogen addition significantly increased foliar Al concentration in *C. concinna*, and decreased the ratios of Ca/Al in *C. concinna* and *C. chinensis*. There were no responses of foliar P and Na to N addition in any species.

**Table 2**  
Structural characteristics of three dominant understory species in the mature forest. Survey areas were 2400 m<sup>2</sup> for the research site.

Species	Mean height (m)	Mean stem base diameter (cm)	Tree density (tree ha <sup>-1</sup> )	Mean age (years)
<i>R. canthioides</i>	1.32	1.05	2761	8
<i>C. concinna</i>	1.08	1.07	3422	7
<i>C. chinensis</i>	1.36	1.43	893	8



### 3.2. Photosynthetic parameters

In the Control plots, there were no significant differences in Pmax among the three species (Fig. 1a). *R. canthioides* had significantly higher PNUe than *C. concinna* and *C. chinensis* (Fig. 1d). However, both LMA and Narea in *R. canthioides* were significantly ( $p < 0.001$ ) lower than those in *C. concinna* and in *C. chinensis* (Fig. 1b and c).

In the N treatment plots, the photosynthesis capacity significantly decreased with elevated N addition in *C. concinna* and *C. chinensis*, but *R. canthioides* did not respond (Fig. 1a). Nitrogen addition greatly increased foliar N content per area (Fig. 1b), and decreased PNUe in all three species (Fig. 1d). There were no N treatment effects on LMA for any species (Fig. 1c). Moreover, there were significantly negative relationships between PNUe/Pmax and foliar N content in three species across all sampled plants, especially for PNUe (Fig. 2).

### 3.3. Soluble protein, chlorophyll and free amino acid (FAA)

In the Control plots, *C. concinna* had the lowest values of soluble protein and the highest values of FAA compared with *R. canthioides* and *C. chinensis*. There were no significant differences between *R. canthioides* and *C. chinensis* in values of soluble protein and FAA.

In general, N addition significantly increased concentrations of foliar soluble protein in *C. concinna* and *C. chinensis* (Fig. 3a), but only had marginally significant increase in Middle-N treatment for *R. canthioides* ( $p = 0.06$ ). For foliar chlorophyll, there were generally no response to N addition for any of the species (Fig. 3b). Total FAA increased greatly in *R. canthioides* and *C. chinensis* under Low-N and/or Middle-N treatments (Fig. 3c). Nitrogen additions had no effect on FAA in *C. concinna*.

## 4. Discussion

### 4.1. Responses of foliar elements and N metabolites

Contrary to our expectation, our results showed that elevated N additions greatly increased foliar N contents in all three studied species (Fig. 1b) in this already N-rich forest ecosystem, suggesting a potentially priming effect under short-term (2 years) high N inputs, in spite of ecosystem N status. These results are consistent with results of numerous studies in temperate forests (Magill et al., 2000; Bauer et al., 2004; Elvir et al., 2006; Talhelm et al., 2011), where N was usually limited. However, the present study suggested that short-term N addition could lead to foliar N accumulation in N-rich tropical forests. This result was further supported by a latter  $^{15}\text{N}$  isotope study, which found plants in the same N saturated tropical forest could use the “new” added N (Gurmesa et al., 2016). In present study, consistent increases of foliar N in three species demonstrate that plants in N-rich ecosystem still have capacity to retain the external N inputs of inorganic N in a short term. However, it merits a further study to track the effects of long-

term N addition on foliar N contents in the future, before a clear conclusion can be reached.

We further found that elevated N additions decreased foliar base element (K, Ca and Mg) of all three species (Table 3), suggesting that nutrient imbalance may arise. In fact, a number of reports on higher foliar N and lower Ca and Mg occurred in the situation with more access to N resource (Whytemare et al., 1997; Elvir et al., 2006; Wortman et al., 2012). The decreases in foliar K, Ca and Mg could be the result of either exchange with  $\text{NH}_4^+$  from the leaves or depletion in soil. Considering that N application affects mainly on the ground layer, foliar loss through exchanges seems not to be the main reason for the decrease of foliar K, Ca, and Mg. Thus, it could result from N induced soil acidification and subsequent leaching loss of the base cations. Indeed, another study in our forest had confirmed that soil base cations were exhausted. After 26 month N application, for example, this forest exhibited significant negative symptoms such as soil acidification, Al mobilization and leaching of base cations from the soil (Lu et al., 2009), and the situation became even worse in the long-term (Lu et al., 2014). Soil acidification resulted from nitrification of  $\text{NH}_4^+$  and subsequent  $\text{NO}_3^-$  leaching may trigger base cations leaching, exacerbating cations depletion. In addition, decreased root uptake of K, Ca and Mg may arise from ion competition with  $\text{NH}_4^+$  (Schulze, 1989). Declines in the contents of K, Ca and Mg resulted from N and/or acid deposition have been frequently reported in temperate ecosystem (Elvir et al., 2006). However, Ca and Mg depletion in plants induced by increasing N deposition are rarely addressed in tropical forests, where forests may be particularly susceptible to nutrient imbalance (Matson et al., 1999; Lu et al., 2014). Our findings suggest that the N-induced soil base cation depletion could affect nutrient supply to plants, which provides a new insight into nutrient imbalance in tropical forests.

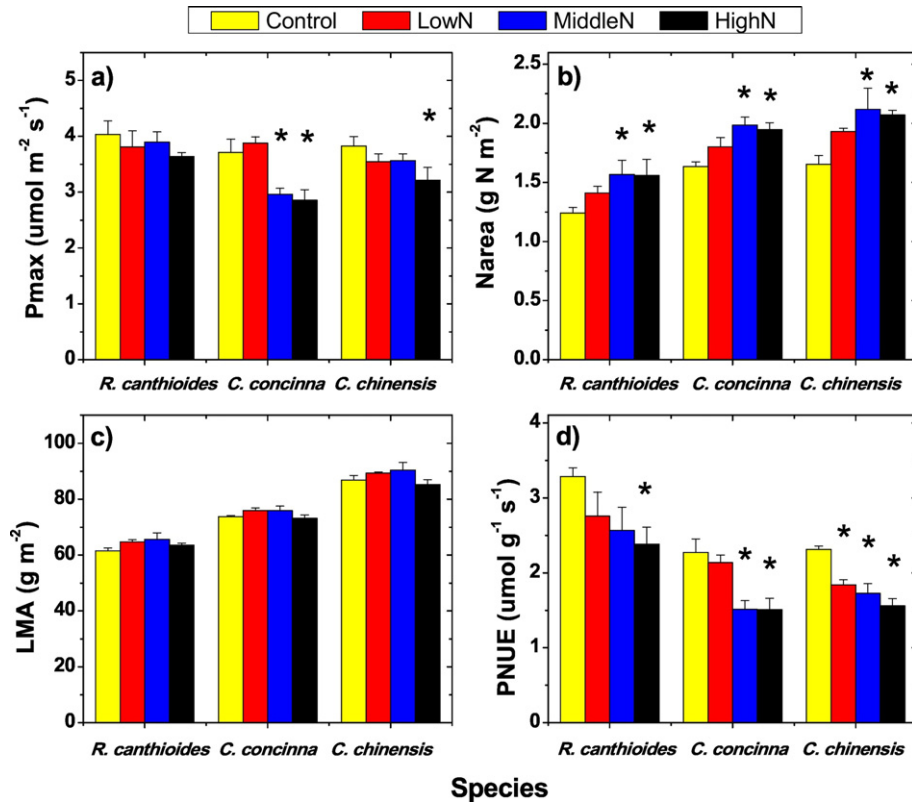
Similar to the change of foliar N content, foliar N allocated to protein or free amino acids increased correspondingly with N addition (Fig. 3), but chlorophyll content showed no response. Plants are known to respond to various forms of abiotic and biotic environmental stress though alteration in the N metabolite levels. Increases in foliar N concentration in trees resulting from high N deposition are typically accompanied by increases in individual amino acids, total free amino acids, and/or protein in temperate forests (Bauer et al., 2000; Ericsson et al., 1993; Van Dijk and Roelofs, 1988; Bubier et al., 2011). So far, less is known about the N metabolism in tropical forests. Our results were in line with findings in temperate forests. Moreover, the consistent increase of foliar N compounds suggests that the increased foliar N partially accumulate as soluble protein or FAA rather than in chlorophyll in these understory species in this mature tropical forest.

### 4.2. Responses of foliar photosynthetic capacity

In contrast to our hypothesis, the photosynthesis capacity decreased under high N addition in the medium-light species (*C. concinna* and *C.*

**Table 3**  
Foliar element concentrations ( $\text{mg g}^{-1}$  dry weight) and ratios of N/P and Ca/Al of *Randia canthioides*, *Cryptocarya concinna* and *Cryptocarya chinensis* understory tree in a mature tropical forest in southern China. Values shown are the mean  $\pm$  S.E. ( $n = 3$ ). The different lowercase letters and capital letters indicate significant differences at  $p < 0.05$  and  $p < 0.1$  level among N treatments within a single species, respectively.

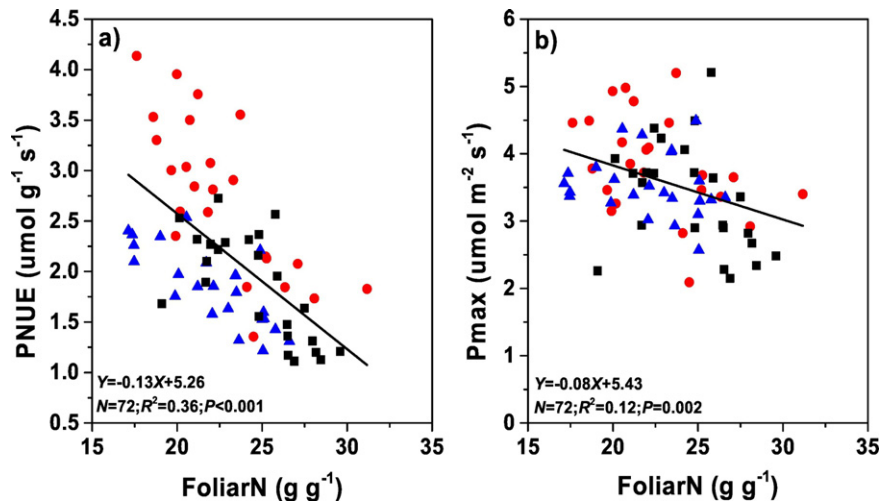
		N	P	K	Ca	Na	Mg	Al	N/P	Ca/Al
<i>R. canthioides</i>	Control	20.1 (0.5)A	0.86 (0.09)	18.6 (0.5)a	14.6 (0.4)a	0.22 (0.04)	7.98 (0.04)a	0.36 (0.02)	23.90 (1.88)a	44.50 (4.65)
	Low N	21.8 (0.7)AB	0.81 (0.03)	16.5 (0.8)ab	12.5 (0.9)b	0.20 (0.04)	6.66 (0.26)b	0.33 (0.04)	27.32 (1.81)ab	41.61 (7.94)
	Middle-N	23.0 (1.9)AB	0.81 (0.02)	15.4 (1.3)b	11.7 (0.2)b	0.18 (0.03)	6.00 (0.53)b	0.38 (0.03)	29.74 (1.96)ab	32.28 (3.31)
	High-N	24.6 (2.4)B	0.75 (0.00)	14.7 (0.5)b	11.7 (0.3)b	0.19 (0.04)	5.89 (0.27)b	0.39 (0.03)	32.98 (3.37)b	31.71 (1.43)
<i>C. concinna</i>	Control	22.2 (0.6)a	0.96 (0.03)a	7.7 (0.4)a	6.3 (0.3)a	0.72 (0.13)	1.87 (0.03)a	0.25 (0.05)a	23.12 (0.82)	28.03 (6.23)a
	Low-N	23.7 (0.8)ab	1.01 (0.02)ab	7.5 (0.4)ab	5.8 (0.2)ab	0.61 (0.10)	1.72 (0.05)a	0.28 (0.05)a	23.40 (0.67)	22.36 (4.65)ab
	Middle-N	26.1 (0.4)b	1.01 (0.05)ab	6.4 (0.3)bc	4.9 (0.4)b	0.64 (0.04)	1.45 (0.02)b	0.31 (0.02)ab	25.98 (1.46)	16.77 (0.90)ab
	High-N	26.7 (0.9)b	1.06 (0.03)b	6.1 (0.3)c	4.7 (0.3)b	0.74 (0.11)	1.41 (0.13)b	0.40 (0.03)b	25.18 (1.35)	12.93 (2.12)b
<i>C. chinensis</i>	Control	19.1 (1.1)a	0.86 (0.14)	5.3 (0.5)a	4.6 (0.1)a	0.24 (0.02)	1.09 (0.06)a	0.24 (0.05)	23.01 (2.36)	22.87 (4.95)a
	Low-N	21.6 (0.3)ab	0.83 (0.05)	4.8 (0.7)ab	4.0 (0.1)b	0.22 (0.06)	1.13 (0.07)ab	0.25 (0.06)	26.36 (2.02)	18.25 (4.89)ab
	Middle-N	23.4 (1.5)b	0.93 (0.08)	4.9 (0.4)ab	4.3 (0.1)ab	0.21 (0.05)	0.95 (0.07)ab	0.24 (0.04)	25.36 (0.70)	21.38 (3.52)ab
	High-N	24.3 (0.0)b	1.00 (0.18)	3.7 (0.3)b	3.5 (0.3)b	0.19 (0.03)	0.88 (0.03)b	0.32 (0.01)	26.06 (5.07)	11.58 (1.34)b



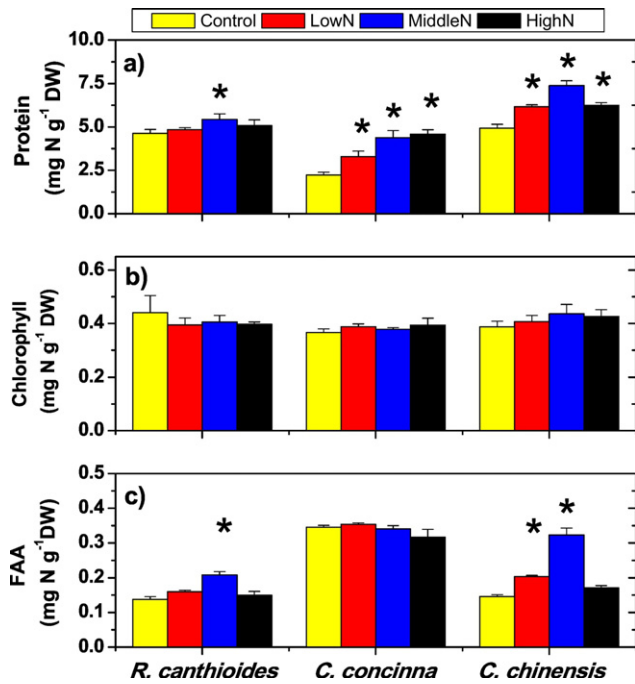
**Fig. 1.** Effects of N treatments on foliar photosynthetic rate at light saturation (Pmax, a), foliar nitrogen per unit leaf area (Narea, b), leaf mass per unit leaf area (LMA, c), and photosynthetic nitrogen-use efficiency (PNUE, d) for *Randia canthioides*, *Cryptocarya concinna* and *Cryptocarya chinensis* in a mature tropical forest. Notes: Values shown are the mean ± S.E. (n = 3); The asterisks (\*) indicate significant differences at p < 0.05 level between the Control and the N-treatment plots within a single species.

*chinensis*), but did not change in the shade tolerant species (*R. canthioides*). These results also contrasted to the findings in N-limited temperate forests (Brown et al., 1996; Clement et al., 2000; Nakaji et al., 2002), where the photosynthetic capacity generally increased with low level of N addition. However, these results were comparable to those results from former nursery experiments (Li et al., 2004; Mo et al., 2008), which suggested that low levels of N promoted Pmax and growth of *Schima superba* and *C. concinna* (dominant tree species in this mature forest), but high levels of N suppress their Pmax and growth. The reduction in photosynthetic capacity of *C. concinna* and *C. chinensis* may be caused by the following two reasons.

First, the overwhelming availability of N over other elements resulted in nutrient imbalances. NH<sub>4</sub><sup>+</sup> uptake by roots may have an antagonistic effect on the uptake of base cations (Schulze, 1989), and the anionic components of N treatments (NO<sub>3</sub><sup>-</sup>) likely leached cations from soils and reduced potentials for Ca<sup>2+</sup> and Mg<sup>2+</sup> uptake (Reuss and Johnson, 1986). Also the N-induced soil acidification in our research site increased the proportion of Al<sup>3+</sup> in the soil solution (Lu et al., 2009), and then increased competition with Al<sup>3+</sup> could act to limit Ca<sup>2+</sup> and Mg<sup>2+</sup> uptake (Reuss and Johnson, 1986). These changes can eventually dilute other nutrient concentrations in the tree tissues (Aber et al., 1995; Ericsson et al., 1995). The above processes are



**Fig. 2.** Relationships between PNUE and Pmax, and foliar N per mass across all plants for *Randia canthioides*, *Cryptocarya concinna* and *Cryptocarya chinensis* in a mature tropical forest (*R. canthioides*, ●; *C. concinna*, □; *C. chinensis*, ▲). Notes: each point indicates one plant sample, and there are two samples in each plot and 24 samples per species across all plots.



**Fig. 3.** Effects of N treatments on content of soluble protein (a), chlorophyll (a + b) (b) and total free amino acid (c) in leaves of *R. canthioides*, *C. concinna* and *C. chinensis*. Notes: Values shown are the mean  $\pm$  S.E. ( $n = 3$ ); The asterisks (\*) indicate significant differences at  $p < 0.05$  level between the Control and the N-treatment plots within a single species.

supported by our findings, showing that N addition increased foliar N concentrations but decreased the concentrations of K, Ca and Mg (Table 3), resulted in significant increase in ratios of N/base cations for both species (data not shown). Numerous studies have shown that photosynthetic capacity is reduced by declines in foliar Ca and Mg (Subrahmanyam and Pandley, 1986; McLaughlin et al., 1990; Whytemare et al., 1997; Fischer, 1997; Laing et al., 2000; Elvir et al., 2006).

Reductions in net photosynthesis of *C. concinna* and *C. chinensis* were also accompanied by increases in foliar Al (Table 3). Further correlation analysis revealed a positive relationship between the net photosynthetic rate and the ratios of Ca/Al (data not shown). So increasing foliar Al, decreasing Mg, Ca, and Ca/Al ratios may reduce photosynthetic capacity (Reich et al., 1994; Cronan and Grigal, 1995). In addition, the significant reduction of foliar K may be also responsible for the decline of photosynthesis capacity, because allocation of N to Rubisco is plastic and modified by the supply of K (McGrath et al., 2005). Interestingly, we found no significant changes in N/P ratios in N-treatment plots, which could be related to slight increase in foliar P. This finding is different from some others, where imbalance in N/P ratio could reduce net photosynthesis and dry matter production of the seedlings (Nakaji et al., 2001).

Second, high leaf N concentrations could also result in a high fraction of non-protein or non-photosynthetic protein N. In this study, increased N as organic N forms only account for a moderate proportion of totally increased foliar N (Fig. 3 and Table 3). Hence, with excess N uptake and transport to foliage, inorganic N toxicity may happen directly. In that case, excess nitrogen was diverted away from photosynthesis causing a decoupling in the functional allocation of photosynthetically used nitrogen. As a result, photosynthetic capacity was down regulated, because the excess N could not be invested into the primary processes of carboxylation (Bauer et al., 2004).

Another question arose from the divergent responses of photosynthesis capacity between the two medium-light species (*C. concinna* and *C. chinensis*) and the shade tolerant species *C. concinna*. We suspect that higher foliar K, Ca, and Mg concentrations may play an important role in alleviating excessive N toxicity in *R. canthioides*, because the

ratios of N/K, N/Mg, and N/Ca were significantly lower than for the other two plants (data not shown). Possibly, Al toxicity could happen in *C. concinna* and *C. chinensis* with the increase of Al concentrations and decrease of the Ca/Al ratios. At the same time, the inhibition of organic N synthesis in High-N treatments may induce inorganic N toxicity. Accordingly, *C. concinna* and *C. chinensis* may lack a better strategy to utilize excess N, and thus increase the risk of nutrient imbalance. Hence, the shade tolerant species *R. canthioides*, which grows in understory all the life, could respond much better than the other two species in high N deposition. These results suggest that medium-light species could be more sensitive to high N inputs than shade tolerant species in tropical forests.

As expected, with increased foliar N content, PNUE of all species decreased significantly. There was a significantly negative correlation between PNUE and N<sub>mass</sub> across all plots (Fig. 2). But there was no significant correlation between PNUE and LMA. Hence high N concentrations were the most important reason for the decrease in PNUE. Lower PNUE and a lack of increase in net photosynthesis with foliar N might indicate that the diffusion of CO<sub>2</sub> to RUBISCO active sites in the chloroplast and/or partitioning of foliar N to the photosynthetic apparatus are less at a given foliar N concentration (Lloyd et al., 1992; Wullschlegel, 1993). In the N addition plots, photosynthetic capacity was not simulated by increased foliar N concentrations, suggesting that additional foliar N did not contribute to increasing photosynthetic rates. Consequently, PNUE could be a more sensitive indicator than foliar N and P<sub>max</sub> to assess status of plants persisting under high N inputs. In other studies, however, above a minimum N concentration in the leaf, PNUE could increase up to a maximum and then decline again (Lambers et al., 1989). If the leaf N concentration is far above the optimal level, trees will show serious nutrient imbalance, growth disturbance and chlorotic foliage (Van Dijk and Roelofs, 1988; Kaupenjohann et al., 1989; Fangmeier et al., 1994).

## 5. Conclusions

The present study examined foliar nutrient status, N metabolites and photosynthetic capacity of three dominant understory species in an N-rich mature tropical forest. We found that two years of N addition increased foliar N content, but decreased the content of nutrient cations (e.g., K, Ca and Mg). Nitrogen addition also increased N accumulation, mainly as soluble protein and/or free amino acid, but not as chlorophyll in all three species. We further found that the photosynthesis capacity of medium-light species *C. concinna* and *C. chinensis* was more sensitive to N addition than that of the shade tolerant species *R. canthioides*. However, PNUE significantly declined with N addition for all three species, with significantly negative relationships between PNUE/P<sub>max</sub> and foliar N content. These findings suggest that excess N inputs can promote nutrient imbalances, and inhibit photosynthetic capacity of understory plant species, indicating high N deposition can threaten understory plant growth in tropical forests in the future. Meanwhile, PNUE can be used as a sensitive indicator to assess ecosystem N status under high N deposition.

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## References

- Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. *Bioscience* 39 (6), 378–386.



- Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air Soil Pollut.* 85, 1665–1670.
- Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.L., Magill, A.H., Martin, M.E., Hallett, R.A., Stoddard, J.L., 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? *Bioscience* 53, 375–389.
- Adams, M.A., Ineson, P., Binkley, D., Cadisch, G., Tokuchi, N., Scholes, M., Hicks, K., Chadwick, M., 2004. Soil functional responses to excess N inputs at global scales. *Ambio* 33, 530–536.
- Barnett, N.M., 1966. Amino acid and protein metabolism in Bermuda grass during water stress. *Plant Physiol.* 41, 1222–1230.
- Bauer, G.A., Persson, H., Persson, T., Mund, M., Hein, M., Kummert, E., Matteucci, G., van Oene, H., Scarascia-Mugnozza, G., Schulze, E.D., 2000. Linking plant nutrition and ecosystem processes. In: Schulze, E.D. (Ed.), *Carbon and Nitrogen Cycling in European Forests*. Springer, Berlin, pp. 63–98.
- Bauer, G.A., Bazzaz, F.A., Minocha, R., Long, S., Magill, A.H., Aber, J.D., Berntson, G.M., 2004. Effects of chronic N additions on tissue chemistry, photosynthetic capacity, and carbon sequestration potential of a red pine (*Pinus resinosa* Ait.) stand in the NE United States. *For. Ecol. Manag.* 196, 173–186.
- Binkley, D., Hogberg, P., 2016. Tamm review: revisiting the influence of nitrogen deposition on Swedish forests. *For. Ecol. Manag.* 368, 222–239.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Ciederby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20 (1), 30–59.
- Boxman, A.W., Roelofs, J.G., 1988. Some effects of nitrate versus ammonium nutrition on the nutrient fluxes in *Pinus sylvestris* seedlings. Effects of mycorrhizal infection. *Can. J. Bot.* 66 (6), 1091–1097.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dyebinding. *Anal. Biochem.* 72, 248–254.
- Brown, K.R., Thompson, W.A., Camm, E.L., 1996. Effects of N addition rates on the productivity of *Picea Sitchensis*, *Thuja plicata*, and *Tsuga heterophylla* seedlings II. Photosynthesis,  $^{13}\text{C}$  discrimination and N partitioning in foliage. *Trees* 10, 198–205.
- Bubier, J.L., Smith, R., Juutinen, S., Moore, T.R., Minocha, R., Long, S., Minocha, S., 2011. Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. *Oecologia* 167, 355–368.
- Clement, J.M.A.M., de Boer, M., Venema, J.H., Van Hasselt, P.R., 2000. There is no direct relationship between N-status and frost hardness in needles of  $\text{NH}_3$ -exposed Scots pine seedlings. *Phyton-Ann. Rei Bot. A* 40, 21–33.
- Cleveland, C.C., Townsend, A.R., Taylor, P., Alvarez-Clare, S., Bustamante, M.M.C., Chuyong, G., Dobrowski, S.Z., Grieron, P., Harms, K.E., Houlton, B.Z., Marklein, A., Parton, W., Porder, S., Reed, S.C., Sierra, C.A., Silver, W.L., Tanner, E.V.J., Wieder, W.R., 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecol. Lett.* 14, 939–947.
- Cronan, C.S., Grigal, D.F., 1995. Use of calcium aluminum ratios as indicators of stress in forest ecosystems. *J. Environ. Qual.* 24, 209–226.
- Dong, M., Wang, Y.F., Kong, F.Z., Jiang, G.M., Zhang, Z.B., 1996. Standard Methods for Observation and Analysis in Chinese Ecosystem Research Network-Survey, Observation and Analysis of Terrestrial Biocommunities. Standards press of China, Beijing, pp. 152–162.
- Elvir, J.A., Wiersma, G.B., Greenwood, M., Day, M., Fernandez, I., 2006. Effects of enhanced nitrogen deposition on foliar chemistry and physiological processes of forest trees at the Bear Brook Watershed in Maine. *For. Ecol. Manag.* 221 (1–3), 207–214.
- Ericsson, A., Norden, L.G., Nasholm, T., Walheim, M., 1993. Mineral nutrient imbalances and arginine concentrations in needles of *Picea abies* (L.) Karst. from two areas with different levels of airborne deposition. *Trees* 8, 67–74.
- Ericsson, A., Walheim, M., Norden, L.G., Naesholm, T., 1995. Concentrations of mineral nutrients and arginine in needles of *Picea abies* trees from different areas in southern Sweden in relation to nitrogen deposition and humus form. *Ecol. Bull. (Copenhagen)* 44, 147–157.
- Erisman, J.W., Galloway, J.N., Seitzinger, S., Bleeker, A., Dise, N.B., Petrescu, A.M.R., Leach, A.M., de Vries, W., 2013. Consequences of human modification of the global nitrogen cycle. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 368 (1621).
- Fang, Y., Zhu, W., Gundersen, P., Mo, J., Zhou, G., Yoh, M., 2008. Large loss of dissolved organic nitrogen from nitrogen-saturated forests in subtropical China. *Ecosystems* 12, 33–45.
- Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden, L.J., Jäger, H.J., 1994. Effects of atmospheric ammonia on vegetation: a review. *Environ. Pollut.* 86, 43–82.
- Fischer, E.S., 1997. Photosynthetic irradiance response curves of *Phaseolus vulgaris* under moderate or severe magnesium deficiency. *Photosynthetica* 33, 385–390.
- Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. *Bioscience* 53, 341–356.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892.
- Gilliam, F.S., 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *Bioscience* 57, 845–858.
- Gundersen, P., 1991. Nitrogen deposition and the forest nitrogen cycle: role of denitrification. *For. Ecol. Manag.* 44, 15–28.
- Gurmesa, G.A., Lu, X., Gundersen, P., Mao, Q., Zhou, K., Fang, Y., Mo, J., 2016. High retention of  $^{15}\text{N}$ -labeled nitrogen deposition in a nitrogen saturated old-growth tropical forest. *Glob. Chang. Biol.* 22, 3608–3620.
- Högberg, P., Fan, H.B., Quist, M., Binkley, D., Tamm, C.O., 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Glob. Chang. Biol.* 12, 489–499.
- Huang, Z.F., Fan, Z.G., 1982. The climate of Ding Hu Shan. *Trop. Subtrop. For. Ecosyst.* 1, 11–23 (in Chinese).
- Huang, L., Zhu, W., Ren, H., Chen, H., Wang, J., 2012. Impact of atmospheric nitrogen deposition on soil properties and herb-layer diversity in remnant forests along an urban-rural gradient in Guangzhou, southern China. *Plant Ecol.* 213, 1187–1202.
- Kaupenjohann, M., Döhler, H., Bauer, M., 1989. Effects of N-immissions on nutrient status and vitality of *Pinus sylvestris* near a hen-house. *Plant Soil* 113, 279–282.
- Kondo, M., Muraoka, H., Uchida, M., Yazaki, Y., Koizumi, H., 2005. Refixation of respired  $\text{CO}_2$  by understorey vegetation in a cool-temperate deciduous forest in Japan. *Agric. For. Meteorol.* 134, 110–121.
- Laing, W., Greer, D., Sun, O., Beets, P., Lowe, A., Payn, T., 2000. Physiological impacts of Mg deficiency in *Pinus radiata*: growth and photosynthesis. *New Phytol.* 146, 47–57.
- Lambers, H., Freijesen, N., Poorter, H., Hirose, T., Van der Werf, A., 1989. Analyses of growth based on net assimilation rate and nitrogen productivity. Their physiological background. Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. SPB Academic Publishing, Hague, pp. 1–17.
- Li, D.J., Mo, J.M., Fang, Y.T., Cai, X.A., Xue, J.H., Xu, G.L., 2004. Effects of simulated nitrogen deposition on growth and photosynthesis of *Schima superba*, *Castanopsis chinensis* and *Cryptocarya concinna* seedlings. *Acta Ecologica Sinica* 24, 876–882 (in Chinese).
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids-pigments of photosynthetic biomembranes. *Methods Enzymol.* 148, 350–382.
- Liu, X., Duan, L., Mo, J., Du, E., Shen, J., Lu, X., Zhang, Y., Zhou, X., He, C., Zhang, F., 2011. Nitrogen deposition and its ecological impact in China: an overview. *Environ. Pollut.* 159, 2251–2264.
- Lloyd, J., Syvertsen, J.P., Kriedemann, P.E., Farquhar, G.D., 1992. Low conductances for  $\text{CO}_2$  diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant Cell Environ.* 15, 873–899.
- Long, X.J., 2010. Analysis of Factors Affecting the Characteristics of Atmospheric Organic Acids and Nitrogen Deposition: A Case Study over DingHu Mountain and Guang Zhou City. (in Chinese, Master's Thesis). pp. 79–80.
- Lu, X., Mo, J., Gundersen, P., Zhu, W., Zhou, G., Li, D., Zhang, X., 2009. Effect of simulated N deposition on soil exchangeable cations in three forest types of subtropical China. *Pedosphere* 19, 189–198.
- Lu, X., Mo, J., Gilliam, F.S., Zhou, G., Fang, Y., 2010. Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. *Glob. Chang. Biol.* 16, 2688–2700.
- Lu, X., Gilliam, F.S., Yu, G., Li, L., Mao, Q., Chen, H., Mo, J., 2013. Long-term nitrogen addition decreases carbon leaching in a nitrogen-rich forest ecosystem. *Biogeosciences* 10, 3931–3941.
- Lu, X., Mao, Q., Gilliam, F.S., Luo, Y., Mo, J., 2014. Nitrogen deposition contributes to soil acidification in tropical ecosystems. *Glob. Chang. Biol.* 20, 3790–3801.
- Lu, X., Mao, Q., Mo, J., Gilliam, F.S., Zhou, G., Luo, Y., Zhang, W., Huang, J., 2015. Divergent responses of soil buffering capacity to long-term N deposition in three typical tropical forests with different land-use history. *Environ. Sci. Technol.* 49, 4072–4080.
- Macdonald, J.A., Dise, N.B., Matzner, E., Armbruster, M., Gundersen, P., Forsius, M., 2002. Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Glob. Chang. Biol.* 8, 1028–1033.
- Magill, A.H., Aber, J.D., Berntson, G.M., McDowell, W.H., Nadelhoffer, K.J., Melillo, J.M., Steudler, P., 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. *Ecosystems* 3, 238–253.
- Magill, A.H., Aber, J.D., Currie, W.S., Nadelhoffer, K.J., Martin, M.E., McDowell, W.H., Melillo, J.M., Steudler, P., 2004. Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *For. Ecol. Manag.* 196, 7–28.
- Matson, P.A., McDowell, W.H., Townsend, A.R., Vitousek, P.M., 1999. The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* 46, 67–83.
- Matson, P.A., Lohse, K.A., Hall, S.J., 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio* 31, 113–119.
- McGrath, J.F., Warren, C.R., Adams, M.A., 2005. Differential effects of N, P and K on photosynthesis and partitioning of N in *Pinus pinaster* needles. *Ann. For. Sci.* 62 (2), 1–8.
- Mclaughlin, S.B., Anderson, C.P., Edwards, N.T., Roy, W.K., Layton, P.A., 1990. Seasonal patterns of nitrogen deposition and respiration of red spruce sapling from two elevations in declining southern Appalachian stands, North Carolina, USA. *Can. J. For. Res.* 20, 485–495.
- Mo, J.M., Brown, S., Peng, S.L., Kong, G.H., 2003. Nitrogen availability in disturbed, rehabilitated and mature forests of tropical China. *For. Ecol. Manag.* 175, 573–583.
- Mo, J., Brown, S., Xue, J., Fang, Y., Li, Z., 2006. Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant Soil* 282, 135–151.
- Mo, J., Li, D., Gundersen, P., 2008. Seedling growth response of two tropical tree species to nitrogen deposition in southern China. *Eur. J. For. Res.* 127, 275–283.
- Nakaji, T., Fukami, M., Dokiya, Y., Izuta, T., 2001. Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. *Trees* 15, 453–461.
- Nakaji, T., Takenaga, S., Kuroha, M., Izuta, T., 2002. Photosynthetic response of *Pinus densiflora* seedlings to high nitrogen load. *Environ. Sci.* 9, 269–282.
- Nasholm, T., Edfast, A., Ericsson, A., Norden, L., 1994. Accumulation of amino acids in some boreal forest plants in response to increased nitrogen availability. *New Phytol.* 126, 137–143.
- Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N.B., Helliwell, R., Jones, L., Leake, J.R., Leith, I.D., Sheppard, L.J., Sowerby, A., Pilkington, M.G., Rowe, E.C., Ashmore, M.R., Power, S.A., 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Glob. Chang. Biol.* 18, 1197–1215.
- Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J., Sutton, M.A., Fowler, D., Munro, R.C., Tang, S., Wilson, D., 1998. The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environ. Pollut.* 102 (S1), 41–48.
- Reich, P.B., Oleksyn, J., Tjoelker, M.G., 1994. Relationship of aluminum and calcium to net  $\text{CO}_2$  exchange among diverse Scots pine provenances under pollution stress in Poland. *Oecologia* 97, 82–92.
- Ren, R., Mi, F., Bai, N., 2000. A chemometrics analysis on the data of precipitation chemistry of China. *J. Beijing Polytech. Univ.* 26, 90–95 (in Chinese).
- Reuss, J.O., Johnson, D.W., 1986. *Acid Deposition and the Acidification of Soils and Waters*. Springer-Verlag, New York, p. 119.

- Richter, C.M., Kranig, S., Wild, A., 1995. Contents of free amino acids in needles of Norway spruce trees in relation to novel forest decline. Studies on trees from a site in the northern Black Forest. *Environ. Pollut.* 87, 303–312.
- Ryan, J., Estefan, G., Rashid, A., 2007. Soil and Plant Analysis Laboratory Manual. ICARDA, Aleppo, pp. 55–133.
- Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N., Turner, B.L., 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *J. Ecol.* 100, 309–316.
- Schulze, E.D., 1989. Air pollution and forest decline in a spruce (*Picea abies*) forest. *Science* 244, 776–783.
- Subrahmanyam, K., Pandley, R.K., 1986. Effect of sodium, potassium and calcium on photosynthesis and translocation of carbon-14 photosynthate in black gram (*Vigna mungo* L. Hepper). *J. Nucl. Agric. Biol.* 15, 202–206.
- Sun, G.R., Guan, Y., Yan, X.F., 2000. Effect of sodium carbonate stress on amino acid contents of *Puccinellia tenuiflora* seedlings. *Bull. Bot. Res.* 20 (1), 69–72 (in Chinese).
- Talhelm, A.F., Pregitzer, K.S., Burton, A.J., 2011. No evidence that chronic nitrogen additions increase photosynthesis in mature sugar maple forests. *Ecol. Appl.* 21, 2413–2424.
- Van Dijk, H.F.G., Roelofs, J.G.M., 1988. Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. *Physiol. Plant.* 73, 494–501.
- Vitousek, P.M., Sanford, R.L., 1986. Nutrient cycling in moist tropical forest. *Annu. Rev. Ecol. Syst.* 17, 137–167.
- Wang, Z., He, D., Song, S., Chen, S., Chen, D., Tu, M., 1982. The Vegetation of Dinghushan Biosphere Reserve. Tropical and Subtropical Forest Ecosystem vol. 1. Science Press, Guangzhou, pp. 77–141 (in Chinese).
- Whytemare, A.B., Edmonds, R.L., Aber, J.D., Lajtha, K., 1997. Influence of excess nitrogen deposition on a white spruce (*Picea glauca*) stand in southern Alaska. *Biogeochemistry* 38, 173–187.
- Wortman, E., Tomaszewski, T., Waldner, P., Schleppei, P., Thimonier, A., Eugster, W., Buchmann, N., Sievering, H., 2012. Atmospheric nitrogen deposition and canopy retention influences on photosynthetic performance at two high nitrogen deposition Swiss forests. *Tellus Ser. B Chem. Phys. Meteorol.* 64.
- Wullschlegel, S.D., 1993. Biochemical limitations to carbon assimilation in C3 plants—a retrospective analysis of the A/Ci curves from 109 species. *J. Exp. Bot.* 44, 907–920.
- Xie, Y., Zhang, S., Feng, W., Zhao, X., Guo, T., 2010. Review of atmospheric nitrogen deposition research. *Chin. J. Eco-Agric.* 18, 897–904 (in Chinese).
- Xu, Z., Zhou, G., 2006. Nitrogen metabolism and photosynthesis in *Leymus chinensis* in response to long-term soil drought. *J. Plant Growth Regul.* 25, 252–266.
- Zheng, X., Fu, C., Xu, X., Yan, X., Huang, Y., Han, S., Hu, F., Chen, G., 2002. The Asian nitrogen cycle case study. *Ambio* 31, 79–87.
- Zhou, G., Yan, J., 2001. The influence of region atmospheric precipitation characteristics and its element inputs on the existence and development of Dinghushan forest ecosystems. *Acta Ecologica Sinica* 21, 2002–2012 (in Chinese).