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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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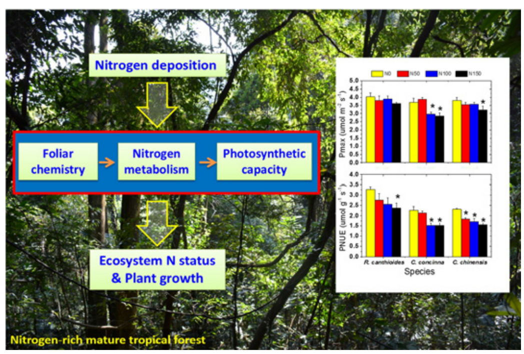
HIGHLIGHTS

- 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^{-1} yr^{-1} (Control), 50 kg N ha^{-1} yr^{-1} (Low-N), 100 kg N ha^{-1} yr^{-1} (Middle-N) and 150 kg N ha^{-1} yr^{-1} (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 threat 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^{-1} to 67.7 Tg N yr^{-1}, 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^{-1} yr^{-1}) 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^{-1} yr^{-1} 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^{-1} yr^{-1}) since 1990s (Zhou and Yan, 2001; Lu et al., 2013). In 2009–2010, the total dry N deposition was 14.21 kg N ha^{-1} yr^{-1} (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^{-1} yr^{-1} (Control), 50 kg N ha^{-1} yr^{-1} (Low-N), 100 kg N ha^{-1} yr^{-1} (Middle-N) and 150 kg N ha^{-1} yr^{-1} (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_4NO_3 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