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Leaf nitrogen assimilation and partitioning differ among subtropical forest plants in response to canopy addition of nitrogen treatments

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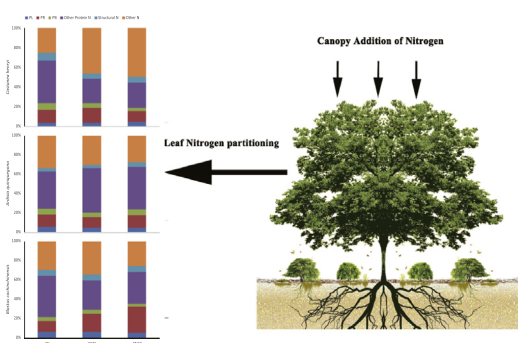
HIGHLIGHTS

- N addition decreased photosynthesis and metabolic protein allocation of a canopy tree.
- Both understory plants could acclimate to N addition but by different mechanisms.
- Dominant forest plants with large canopies may be susceptible to N deposition.
- Specific differences in nitrogen metabolism may explain subtropical forest degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

Global increases in nitrogen deposition may alter forest structure and function by interfering with plant nitrogen metabolism (e.g., assimilation and partitioning) and subsequent carbon assimilation, but it is unclear how these responses to nitrogen deposition differ among species. In this study, we conducted a 2-year experiment to investigate the effects of canopy addition of nitrogen (CAN) on leaf nitrogen assimilation and partitioning in three subtropical forest plants (*Castanea henryi*, *Ardisia quinquegona*, and *Blastus cochinchinensis*). We hypothesized that responses of leaf nitrogen assimilation and partitioning to CAN differ among subtropical forest plants. CAN increased leaf nitrate reductase (NR) activity, and leaf nitrogen and chlorophyll contents but reduced leaf maximum photosynthetic rate (A_{max}), photosynthetic nitrogen use efficiency (PNUE), ribulose-1,5-bisphosphate carboxylase (Rubisco) activity, and metabolic protein content of an overstory tree species *C. henryi*. In an understory tree *A. quinquegona*, CAN increased NR activity and glutamine synthetase activity and therefore increased metabolic protein synthesis (e.g., Rubisco) in leaves. In the shrub *B. cochinchinensis*, CAN increased A_{max}, PNUE, Rubisco content, metabolic protein content, and Rubisco activity in leaves. Leaf nitrogen assimilation and partitioning results indicated that *A. quinquegona* and *B. cochinchinensis* may better acclimate to CAN than *C. henryi* and that the acclimation mechanism differs among the species. Results from this study suggest that long-term elevated atmospheric nitrogen deposition has contributed to the ongoing transformation of subtropical forests into communities dominated by small trees and shrubs.

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1. Introduction

Global atmospheric nitrogen deposition has increased dramatically due to increases in the combustion of fossil fuels, in the use of nitrogen in agriculture, and in industrial activities (Galloway et al., 2008). As estimated by Galloway et al. (2004), the reactive nitrogen deposited on the earth's surface increased from 34 Tg N year^{-1} in 1860 to 100 Tg N year^{-1} in 1995, and is projected to be 200 Tg N year^{-1} by 2050. China has experienced substantial nitrogen deposition over the past decades, especially in its rapidly developing central and southeastern regions, and nitrogen deposition in China is predicted to increase dramatically in the future (Liu et al., 2011; Liu et al., 2013; Jia et al., 2014). As a driver of global change, nitrogen deposition has the potential to affect forest ecosystems, and researchers have attempted to understand its long-term effects on forest structure and function (Magill et al., 2004; Galloway et al., 2008; Lu et al., 2010; Talhelm et al., 2013). To date, studies on the effects of nitrogen deposition on forest ecosystems have mainly concentrated on deciduous broad-leaved forests and coniferous forests in the temperate zone (Takashima et al., 2004; Gradowski and Thomas, 2006; Högberg, 2007; Lehmann and Johansson, 2010), and few studies have focused on tropical and subtropical evergreen broad-leaved forests, especially in China (Lu et al., 2010, 2014).

As natural nitrogen passes through the forest canopy layer in the process of being deposited, water soluble NH_4^+, NO_3^-, and gaseous nitrogen (NH_3 and HNO_3) become immediately available to trees (Rose, 1996; Wortman et al., 2012). Canopy leaves, shoots, and branches may intercept and retain from 8 to 70% of the natural nitrogen deposition (Gaige et al., 2007; Staelens et al., 2008; Dail et al., 2009). Most experiments that have simulated nitrogen deposition in forests have sprayed nitrogen on the understory or applied it to the soil (Warren et al., 2003; Högberg, 2007; Lu et al., 2014). These experiments exclude the effects of the canopy and thus may not indicate the true effects of nitrogen deposition on forest ecosystems (Zhang et al., 2015).

Plants require nitrogen for the synthesis of amino acids, proteins, chlorophylls, nucleic acids, lipids, and a variety of other metabolites (Kusano et al., 2011). Nitrate (NO_3^-) and ammonium (NH_4^+) are the main sources of inorganic nitrogen for plant growth, and plants integrate several potential signals of internal nitrogen status to regulate nitrogen uptake and assimilation in order to match plant demand. Following its uptake, NO_3^- is reduced and incorporated into cells. In the first step, NO_3^- is converted into nitrite (NO_2^-) by nitrate reductase (NR) in the cytosol. NO_2^- can be further reduced to NH_4^+ via the enzyme nitrite reductase (NiR) in chloroplasts or plastids. NH_4^+, derived either from NO_3^- reduction or from direct uptake, is first converted to glutamine (Gln) by glutamine synthetase (GS) and then to glutamate (Glu) by glutamate synthase (GOGAT) (Sanchez-Rodriguez et al., 2011; Liu et al., 2014).

The increased deposition of nitrogen has caused a series of ecological problems in forests, including an imbalance in the partitioning of nitrogen within forest plants (Warren et al., 2003). Some forest studies have indicated that the addition of nitrogen increased leaf biomass and ribulose-1,5-bisphosphate carboxylase (Rubisco, the photosynthesizing enzyme) content and thereby increased CO_2 assimilation and photosynthesis per unit area of forest (Warren et al., 2003; Högberg, 2007). According to other studies, the increased input of nitrogen may drive chlorophyll degradation (Shi et al., 2017), disorder cellular carbon metabolism (Bauer et al., 2004), increase free amino acid content (Strengbom et al., 2003), decrease forest viability, and increase tree death (Liu et al., 2011). As a result, nitrogen deposition could significantly change the species composition and structure of forests (Nordin et al., 2005; Lu et al., 2010; Gilliam et al., 2006). It is still unclear, however, how nitrogen deposition interferes with the nitrogen metabolism of different tree species and how intensified atmospheric nitrogen deposition may affect the species composition of diverse forest types.

In this study, we determined the effects of canopy addition of nitrogen (CAN) on nitrogen assimilation and partitioning in three woody species (an overstory tree, an understory tree, and a shrub) in a subtropical forest. We tested the following two hypotheses: (1) Overstory trees with large canopies are more sensitive than understory trees to CAN in terms of leaf nitrogen metabolism; and (2) Changes in leaf nitrogen assimilation and allocation in response to CAN differ among woody plants with different ecological characteristics. Finally, we consider the potential effects of CAN on the species composition of subtropical forests.

2. Materials and methods

2.1. Study site

CAN was used to realistically simulate natural nitrogen deposition in the current study. The CAN experiment was conducted at the Shimentai forest site, which is located in Shimentai National Nature Reserve (24°22′-24°31′ N, 113°05′-113°31′ E), Guangdong Province, South China. The site has a subtropical monsoon climate with a wet season (April–September) and dry season (November–March). The annual rainfall is 2364 mm, and the mean annual temperature is 20.8 °C. The site has a latosolic red soil with pH from 5.0 to 5.5. The average total soil phosphorus content was 0.37 g/kg, and while the total soil nitrogen content was 1.92 g/kg; the system is phosphorus limited. The study site is covered by a broad-leaved evergreen forest. The main overstory tree is *Castanea henryi* (Skam) Rehd; the main understory tree is *Ardisia quinquegona* Blume; and the main shrubs are *Blastus cochinchinensis* Lour., *Lasianthus chinensis* (Champ.) Benth, and *Symplocos ramosissima* Wall. ex G. Don.

Nitrogen was applied with a canopy spraying system located in the center of each CAN treatment plot (Fig. S1). The system was composed of a tank for nitrogen solution storage, connecting pipes, a supporting tower, four sprinklers, and a central computer controller. 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; approximated 7–10% of the precipitation was intercepted by the forest canopy and the rest penetrated through. The treatments were applied monthly from April to October (seven times per year) in 2013, 2014, and 2015. Nitrogen addition events were completed within 1 h and were conducted in the morning or evening on days when sunlight radiation was minimal and wind speed was <1 m s^{-1}. The total solution addition was 21 mm per year, which accounted for <1% of the total annual precipitation of the forest site such that, the confounding effect of water addition was negligible. Our previous study showed that the nitrogen deposition in rainfall in Shimentai National Nature Reserve was 34.1 kg N ha^{-1} year^{-1} (Zhang et al., 2015). Thus, 25 kg N ha^{-1} year^{-1} was selected as the medium level, and 50 kg N ha^{-1} year^{-1} was selected as the high level of nitrogen addition. The experiment had a full factorial design with two levels of nitrogen application, including 50 kg N ha^{-1} year^{-1} (CN50) and 25 kg N ha^{-1} year^{-1} (CN25), and the control (CK, 0 kg N ha^{-1} year^{-1}). There were four blocks, and the three treatments were randomly assigned to three circular plots within each block. Each circular plot had a radius of 17 m, an area of 907 m^2, and a central area of 400 m^2 for plant and soil sampling. Between-plot nitrogen contamination was minimized by a 20-m buffer zone and the placement of polyvinylchloride boards between adjacent plots.

2.2. Plant species

Three native and dominant woody species of subtropical broad-leaved forest were selected for this study. *Castanea henryi* is a fast-growing tree with a straight, symmetrical trunk. It is a light-preferring pioneer species that can grow up to 30 m tall. *Ardisia quinquegona* is a shade-tolerant species. It is a non-pioneer native tree that can attain a height of 6 m. *Blastus cochinchinensis* is a mesophytic small tree or