



## Fates of atmospheric deposited nitrogen in an Asian tropical primary forest

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### ABSTRACT

The impacts of increasing nitrogen (N) deposition on forest ecosystems, including on carbon (C) sequestration, largely depend on the extent to which forests are N-limited and so whether and where deposited N is retained within the ecosystem. The <sup>15</sup>N tracer method can provide excellent insight into the ecosystem fates of N, but while it has been extensively used in temperate forests it has yet to be sufficiently employed in tropical forests, which are often thought not to be N-limited. Here, we used stable isotope <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracers applied as solutions to the forest floor to examine the fates of different forms of N in a tropical montane primary forest with low background atmospheric N deposition (6 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in China. We found that a substantial amount of <sup>15</sup>N was assimilated by plants over time and significantly more <sup>15</sup>N was recovered following <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition than following <sup>15</sup>NH<sub>4</sub><sup>+</sup> addition: 7% and 16% of <sup>15</sup>N were recovered three months after the respective <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracer additions and 11% and 29% respectively after one year. In contrast to plants, the organic layer was only an important short-term sink for deposited N: while 21% and 12% of the <sup>15</sup>N from <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions were accumulated in the organic layer after three months, more than half of the retained <sup>15</sup>N was lost after one year. Mineral soil was the largest sink for deposited N, and the <sup>15</sup>N retained in soil was relatively stable over time for both N forms, with 39% and 32% of the initial <sup>15</sup>N input recovered after one year for <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracer additions, respectively. Overall, the total ecosystem <sup>15</sup>N recovery one year after the <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracer additions was large (60% and 66% respectively), and not significantly different from total recovery after three months, suggesting that a large proportion of deposited N could be retained in the longer term. Based on the measured fate of <sup>15</sup>N one year after labeling and the C:N ratios of different plant components, this tropical forest's carbon sequestration efficiency is calculated to be 17 kg C per kg N added, comparable to the values reported for temperate and boreal forests in Europe and North America and indicating substantial N limitation of this tropical forest. Our results suggest that anthropogenic N input in moderate levels may contribute to enhance C sequestration in some tropical forests, without significant long-term loss of N to the environment.

### 1. Introduction

Human activities have been substantially affecting the global nitrogen cycle, with potential wide-ranging and profound impacts on climate, ecosystems, and biodiversity. For example, forest ecosystems worldwide have experienced strongly increased N deposition over recent decades as a result of anthropogenic emissions of reactive N from fossil fuel combustion and modern agriculture (Galloway et al., 2008).

In forests, increased deposited N could alleviate N limitation and stimulate plant growth (LeBauer and Treseder, 2008; Thomas et al., 2010; Niu et al., 2016), but excessive N might also bring negative effects, including nitrate leaching, soil acidification, nutrient imbalance, and forest decline, with the magnitude and timing of the effects depending strongly on ecosystem N status (Gundersen et al., 1998; Aber et al., 2003; Xia and Wan, 2008).

The global C cycle has also been significantly altered, and

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understanding changes of C cycle and their interactions with N is of critical scientific importance because they have consequences for the global greenhouse gas burden and hence for global climate. A substantial body of research is concerned with the effects of N deposition on forest C sequestration (e.g., Luo et al., 2004; Gruber and Galloway, 2008; Thomas et al., 2010; De Vries et al., 2014). These impacts depend ultimately on the fate of deposited N (Lovett and Goodale, 2011; Templer et al., 2012; Niu et al., 2016). Nitrogen deposition may increase tree growth and thereby increase C sequestration if deposited N is taken up by plants. However, N deposition may not increase C sequestration if deposited N is initially retained in the soil, and then lost through gas emission or leaching (Aber et al., 2003; Lovett and Goodale, 2011).

Many studies based on N input-output budgets or N addition experiments have been conducted to quantify N cycling of forest ecosystems and its response to increased N deposition (MacDonald et al., 2002; Campbell et al., 2004; Magill et al., 2004; Fang et al., 2008; Lu et al., 2011), but it remains challenging to identify how the deposited N is distributed among different ecosystem components. The stable isotope  $^{15}\text{N}$  tracer method provides an excellent approach to study the retention and the fates of deposited N (Currie et al., 2002; Templer et al., 2012; Niu et al., 2016). By applying N-compounds enriched in  $^{15}\text{N}$  (but without substantially altering the quantity of N input), it is possible to track cohorts of N input into different ecosystem pools and to determine the fates of deposited N across different time scales (Currie and Nadelhoffer, 1999). To date, however, only limited studies have been conducted in tropical or subtropical forests (Templer et al., 2012), which may be due to the high cost in  $^{15}\text{N}$  tracer studies and the fact that most of tropical and subtropical forests are located in developing countries. So far, world-wide, the fate of deposited N using the  $^{15}\text{N}$  tracer approach has only been investigated for two subtropical lowland forests (Dinghushan, Sheng et al., 2014; Gurmesa et al., 2016; Tieshanping, Liu et al., 2017). These two subtropical forests are somewhat unusual in terms of their N status: both forests are N saturated, caused by high chronic N deposition ( $21\text{--}38\text{ kg N ha}^{-1}\text{ yr}^{-1}$  in Dinghushan and  $54\text{ kg N ha}^{-1}\text{ yr}^{-1}$  in Tieshanping, respectively). In general, tropical lowland forests are considered as N-enriched and limited instead by other nutrients including phosphorus (P) (e.g. Quesada et al., 2009; Mercado et al., 2011), while tropical montane forests are more likely to be N-limited (Matson et al., 1999), but these inferences on tropical forest N status largely remain to be tested experimentally. These considerations, and the findings from the subtropical N-saturated forests, highlight the need for more research into the fate of deposited N in tropical forests, especially those with low N deposition.

In this study, we used both  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  tracers to examine the different fates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  deposition over time in a tropical montane primary forest in China. This site has experienced a relatively low rate of N deposition, at  $6.1\text{ kg N ha}^{-1}\text{ yr}^{-1}$  (Wang et al., 2014). Previous results from a nutrient addition experiment indicate that this forest might be N-limited (Zhou, 2013). Our objectives in the present study were as follows: (1) to determine the fates of deposited N in this tropical forest and thereby the potential effect of N deposition on ecosystem C sequestration; (2) to examine the mechanisms affecting the fates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  to plants, organic layer, and soil pools; and (3) to explore the temporal variation of the retention of deposited N (after three months vs. one year). We hypothesized that: (1) vegetation would be an important N sink in this tropical forest due to a relative thin organic layer, and the proportion of  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  assimilated by plants will be different; (2) most of the added  $^{15}\text{N}$  would be retained in mineral soil, not the organic layer; (3) total ecosystem N retention would be greater than in N-saturated subtropical forests of China, but lower than in temperate and boreal forests world-wide; (4)  $^{15}\text{N}$  retained in the organic layer and mineral soil would be lost over time due to fast turnover under the humid tropical climate.

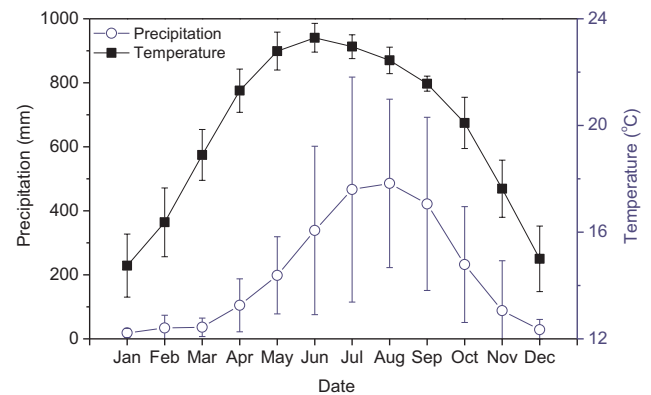


Fig. 1. Mean annual precipitation and mean annual temperature of the study site (climatology based on measurements over a 26-year period from 1980 to 2005).

## 2. Materials and methods

### 2.1. Study site

The study site is an undisturbed tropical montane primary forest located in the Jianfengling National Natural Reserve, southern China ( $18^{\circ}23'\text{--}18^{\circ}50'\text{ N}$ ,  $108^{\circ}36'\text{--}109^{\circ}05'\text{ E}$ ,  $893\text{ m a.s.l.}$ ). The climate is tropical monsoon, characterized by high mean annual temperature ( $19.8 \pm 0.08^{\circ}\text{C}$ ), humidity ( $88 \pm 0.2\%$ ), and precipitation ( $2449 \pm 123.5\text{ mm yr}^{-1}$ , with more than 80% falling during May–October) (climatology based on measurements over a 26-year period from 1980 to 2005, Fig. 1). The forest experiences low rates of atmospheric N deposition ( $6.1\text{ kg N ha}^{-1}\text{ yr}^{-1}$ , roughly half as  $\text{NH}_4^+$  and half as  $\text{NO}_3^-$ ) and no fertilization has ever been applied. Dominant species in this forest include *Livistona saribus*, *Pinanga baviensis*, *Alseodaphne hainanensis*, *Mallotus hookerianus*, *Gironniera subaequalis*, *Cryptocarya chinensis*, *Cyclobalanopsis patelliformis* and *Nephelium topengii* (Fang et al., 2004; Chen et al., 2010). The site has a relative thin organic layer consisting of mainly undecomposed plant materials ( $< 2\text{ cm}$  and averaged  $5.9\text{ Mg ha}^{-1}$  for the biomass, Jiang and Lu, 1991). The soil is acidic (pH 4.1) and is classified as lateritic yellow soil with 57.1% sand, 18.2% silt, and 24.7% clay; the soil is well-drained and its porosity exceeds 50% (Luo et al., 2005).

### 2.2. Experimental design

In August 2014, three separate plots ( $20\text{ m} \times 20\text{ m}$  each) were randomly selected within the forest, each at least  $100\text{ m}$  apart from one other. Each plot was divided into two subplots ( $10\text{ m} \times 20\text{ m}$  each); one subplot received a solution of  $^{15}\text{NH}_4\text{NO}_3$ , and another subplot a solution of  $\text{NH}_4^{15}\text{NO}_3$ . These  $200\text{ m}^2$  subplots contained on average 42 tree species and 86 individual trees. The solutions were made of 99.14 atom %  $^{15}\text{NH}_4\text{NO}_3$  or 99.21 atom %  $\text{NH}_4^{15}\text{NO}_3$ . For each subplot,  $27.234\text{ g }^{15}\text{NH}_4\text{NO}_3$  or  $27.215\text{ g NH}_4^{15}\text{NO}_3$  were dissolved in  $200\text{ L}$  water and then the solutions were sprayed directly on the forest floor using backpack sprayers (equal to  $1\text{ mm}$  precipitation) at the beginning of the rainy season (April 2015). Each subplot was walked four times to achieve the uniformity of application. There was no visible sign of lateral surface runoff when the tracers were applied. The quantity of the  $^{15}\text{N}$  tracer applied to each subplot equaled  $0.25\text{ kg }^{15}\text{N ha}^{-1}$ , which has been typically used in forest  $^{15}\text{N}$  tracer experiments (e.g., Zogg et al., 2000; Liu et al., 2016). In this study forest, N deposition mainly concentrates on the rainy season (accounting for 85% of the total N deposition). Therefore, the added  $^{15}\text{N}$  tracer ( $0.25\text{ kg }^{15}\text{N ha}^{-1}$ ) plus the equal amount of  $^{14}\text{N}$  was approximately equal to the N deposition of two weeks during the rainy season. Furthermore, the content of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in mineral soils ( $0\text{--}40\text{ cm}$ ) equaled  $14.0\text{ kg N ha}^{-1}$  (Wang and Fang, unpublished data). Thus the added  $^{15}\text{N}$  tracer substantially

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