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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 ^{15}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 $^{15}NH_4^+$ and $^{15}NO_3^-$ 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^{-1} yr^{-1}) in China. We found that a substantial amount of ^{15}N was assimilated by plants over time and significantly more ^{15}N was recovered following $^{15}NO_3^-$ addition than following $^{15}NH_4^+$ addition: 7% and 16% of ^{15}N were recovered three months after the respective $^{15}NH_4^+$ and $^{15}NO_3^-$ 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 ^{15}N from $^{15}NH_4^+$ and $^{15}NO_3^-$ additions were accumulated in the organic layer after three months, more than half of the retained ^{15}N was lost after one year. Mineral soil was the largest sink for deposited N, and the ^{15}N retained in soil was relatively stable over time for both N forms, with 39% and 32% of the initial ^{15}N input recovered after one year for $^{15}NH_4^+$ and $^{15}NO_3^-$ tracer additions, respectively. Overall, the total ecosystem ^{15}N recovery one year after the $^{15}NH_4^+$ and $^{15}NO_3^-$ 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 ^{15}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 enhanced C sequestration in some tropical forests, without significant long-term loss of N to the environment.

Keywords: ^{15}N tracer, nitrogen deposition, nitrogen retention, plant uptake, carbon sequestration, total ecosystem recovery

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 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}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}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}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}N tracer approach has only been investigated for two subtropical lowland forests (Dinghushan, Sheng et al., 2014 and 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 (21-38 kg N ha^{-1} yr^{-1} in Dinghushan and 54 kg N ha^{-1} 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 $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers to examine the different fates of NH_4^+ and 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 kg N ha^{-1} 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 NH_4^+ and 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 $^{15}NH_4^+$ and $^{15}NO_3^-$ assimilated by plants will be different; 2) most of the added ^{15}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}N retained in the organic layer and mineral soil would be lost over time due to fast turnover under the humid tropical climate.

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°23′-18°50′ N, 108°36′-109°05′ E, 893 m a.s.l.). The climate is tropical monsoon, characterized by high mean annual temperature (19.8 ± 0.08°C), humidity (88 ± 0.2%), and precipitation (2449 ± 123.5mm yr^{-1}, with more than 80% falling during May-October) (climatology based on measurements over a 26-year period from 1980 to 2005, Figure 1). The forest experiences low rates of atmospheric N deposition (6.1 kg N ha^{-1} yr^{-1}, roughly half as NH_4^+ and half as 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 cm and averaged 5.9 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 m × 20 m each) were randomly selected within the forest, each at least 100 m apart from one other. Each plot was divided into two subplots (10 m × 20 m each); one subplot received a solution of $^{15}NH_4NO_3$, and another subplot a solution of $NH_4{}^{15}NO_3$. These 200 m^2 subplots contained on average 42 tree species and 86 individual trees. The solutions were made of 99.14 atom% $^{15}NH_4NO_3$ or 99.21 atom% $NH_4{}^{15}NO_3$. For each subplot, 27.234 g $^{15}NH_4NO_3$ or 27.215 g $NH_4{}^{15}NO_3$ were dissolved in 200 L water and then the solutions were sprayed directly on the forest floor using backpack sprayers (equal to 1 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}N tracer applied to each subplot equaled 0.25 kg ^{15}N ha^{-1}, which has been typically used in forest ^{15}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}N tracer (0.25 kg ^{15}N ha^{-1}) plus the equal amount of ^{14}N was approximately equal to the N deposition of two weeks during the rainy season. Furthermore, the content of NH_4^+ and NO_3^- in mineral soils (0-40 cm) equaled 14.0 kg N ha^{-1} (Wang and Fang, unpublished data). Thus the added ^{15}N tracer substantially increased the concentration of ^{15}N above its natural abundance in all ecosystem pools without having major impact on ecosystem N pools and fluxes.

2.3. Sampling

Sampling was conducted prior to, three months after, and one year after the addition of ^{15}N tracers. Since there was no buffer zone between the two subplots, we collected the samples from the central part of each subplot to avoid potential edge effects. In each 200 m^2 subplot, foliage and branches of trees and shrubs were sampled from all common species. There were 34 to 63 tree species sampled from each subplot (Table A.1). About 50% of the sampled species were collected from at least three individual trees while others from 1-2 individuals (DBH [diameter at breast height] of sampled trees was above 1 cm). Collected samples were mixed to one composite sample per species. Bark and wood samples were collected using an increment corer from trees with DBH above 5 cm (8 to 18 plant species were sampled from each subplot, Table A.1). Herbs were sampled using a 20 cm × 20 cm iron frame. Six herb samples taken at random locations in each subplot were mixed to one composite sample. The organic layer was sampled using the same frame used for the sampling of the herbs. Mineral soil samples were taken using an auger (2.5 cm inner diameter) and divided into three layers (0-10, 10-20 and 20-40 cm). Six random soil cores in each subplot were composited to one soil sample based on the soil depth. Soil bulk density was estimated using the core (5.0 cm inner diameter) method: soil sample was oven-dried (105ºC for 48h) and bulk density was estimated as the mass of oven-dry soil divided by the volume. Living fine roots (0-40 cm) were hand sorted from another set of composite soil samples (taken in 6 replicates per subplot using a 5.0 cm inner diameter auger) and then cleaned by deionized water.

2.4. Chemical analysis

In the laboratory, all plant and organic layer samples were dried at 60 ºC to constant weight (plant samples were cleaned before oven-dried). Mineral soil was passed through a 2 mm mesh sieve to remove fine roots and coarse fragments, and then air-dried at room temperature. Subsamples of oven-dried foliage, organic layer, and mineral soil were ball-milled and analyzed for ^{15}N natural abundance and total N and total C concentrations by an elemental analyzer-isotope ratio mass spectrometry (Elementar Analysen systeme GmbH, Germany; IsoPrime100, IsoPrime limited, UK). Calibrated DL-alanine ($\delta^{15}N$ = -1.7%), glycine ($\delta^{15}N$ = 10.0%), and histidine ($\delta^{15}N$ =

-8.0%) were used as the internal standards. The analytical precision for $\delta^{15}N$ was 0.2%. The $\delta^{15}N$ of the sample relative to the standard (atmospheric N_2) was expressed as the following equation:

$$\delta^{15}N = [(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{standard} - 1] * 1000 \qquad (1)$$

2.5. Calculation

Tree biomass was estimated by a mixed-species regression model developed by Zeng et al. (1997) for this tropical montane primary forest. The biomass of each individual tree for stem, branch, leaf, bark and root was estimated by the following equations (Zeng et al., 1997; Chen et al., 2010):

$$\text{Stem: } W_t = 0.022816(D^2H)^{0.992674}, \qquad (2)$$

$$\text{Bark: } W_{bk} = 0.006338(D^2H)^{0.902418}, \qquad (3)$$

$$\text{Branch: } W_{br} = 0.005915(D^2H)^{0.999046}, \qquad (4)$$

$$\text{Leaf: } W_l = 0.005997(D^2H)^{0.804661}, \qquad (5)$$

$$\text{Root: } W_r = 0.003612(D^2H)^{1.11527}. \qquad (6)$$

where D represents DBH (cm) and H represents height (m). Tree height was calculated based on the DBH by the following equation (Zeng et al., 1997):

$$\text{Height: } H = 1/(0.026048 + 0.772186/D). \qquad (7)$$

The species-specific biomass of each tree compartment was calculated and then multiplied with the measured N concentration to estimate the compartment N pool, and thereafter compartment N pools were summed to get a plot specific N pool for trees.

Biomass of herbs, organic layer and fine roots were calculated by the weight of the harvested samples. Nitrogen pools of herbs, organic layer and fine roots were calculated by multiplying biomass and N concentration of each measured component. Soil N pools were calculated by multiplying bulk density at each soil layer, soil depth and the corresponding N concentration.

Percent ^{15}N tracer recovery in all sampled components of ecosystem was estimated by ^{15}N tracer mass balance according to the following equation (Nadelhoffer and Fry, 1994):

$$^{15}N_{rec} = \frac{(\text{atom\%}^{15}N_{sample} - \text{atom\%}^{15}N_{ref}) \times N_{pool}}{(\text{atom\%}^{15}N_{tracer} - \text{atom\%}^{15}N_{ref}) \times N_{tracer}} \times 100\% \tag{8}$$

where $^{15}N_{rec}$ = percent of ^{15}N tracer recovered in the labeled N pool; N_{pool} = N pool of each ecosystem compartment; atom% $^{15}N_{sample}$ = atom percent ^{15}N in the labeled sample; atom% $^{15}N_{ref}$ = atom percent ^{15}N in the reference sample (non-^{15}N labeled); and atom% $^{15}N_{tracer}$ = atom percent ^{15}N of added tracer; N_{tracer} = the mass of ^{15}N in the

^{15}N tracer applied to the subplot.

An estimate for carbon sequestration efficiency of plants stimulated by N deposition was derived using the ^{15}N tracer recovery and the C/N ratio of each plant N pool, by the following equation (Nadelhoffer et al., 1999):

$$\mathrm{NUE}_{\mathrm{dep}} = \sum_{i=1}^{n} [{}^{15}\mathrm{N}_{\mathrm{rec,\,i}} \times (\mathrm{C/N})_{\mathrm{i}}] \quad (9)$$

where $\mathrm{NUE}_{\mathrm{dep}}$ = carbon sequestration efficiency stimulated by N deposition; ${}^{15}\mathrm{N}_{\mathrm{rec,\,i}}$ = percent of ^{15}N tracer recovered in each plant pool; $(\mathrm{C/N})_{\mathrm{i}}$ = C/N ratio of each plant pool.

2.6. Statistical analysis

All analyses were conducted using SPSS software (version 19.0; SPSS Inc., Chicago, IL, U.S.A.). The differences in δ^{15}N and ^{15}N recovery between the treatments and sampling time were tested by the independent t-tests. Statistically significant differences were set at the P-value of 0.05 unless otherwise stated.

3. Results

3.1. Ecosystem N pools

The total ecosystem N pool was estimated at 7765 kg N ha^{-1} (Table 1). The plant N pool was 2228 kg N ha^{-1} with trees accounting for about 94.5% of the total plant N

(Table 1). The total soil N pools down to 40 cm depth was 5537 kg N ha^{-1}. There was 82.2 kg N ha^{-1} in the organic litter layer, which accounted for just 1.1% of the total ecosystem N. There were no significant differences between three months and one year in the N pools of herbs, tree foliage and the organic layer (Table A.2).

3.2. δ^{15}N of plants, organic layer and soil pools before and after the ^{15}N tracer addition

Before the ^{15}N tracer addition, the ^{15}N natural abundance (δ^{15}N) ranged from -1.5‰ to 4.6‰ (Figure 2). Plants were depleted in ^{15}N, ranging from -1.5‰ to 0‰. The organic layer was also depleted in ^{15}N, with δ^{15}N averaged -0.4‰. Mineral soil δ^{15}N exhibited an increasing trend with soil depth, ranging from 2.7‰ to 4.6‰.

After the ^{15}N tracer addition, increases in δ^{15}N were detected in all plants, organic layer, and soil pools (Figure 2). The highest increases in δ^{15}N were observed in herbs and organic layer. No significant differences in δ^{15}N of herbs and mineral soils were observed between $^{15}NH_4^+$ and $^{15}NO_3^-$ labeling (Figure 2), but there was a significant difference between the two treatments in the δ^{15}N of organic layer one year after the tracer addition. The δ^{15}N of tree foliage, branches and shrubs were significantly lower under $^{15}NH_4^+$ than under $^{15}NO_3^-$ labeling. From three months to one year, δ^{15}N increased over time in all components of trees (excluding stem, because δ^{15}N of stem was measured only once so that we could not observe the trend of increase), as well as in shrubs, but decreased in herbs, fine roots and organic layer. In addition, δ^{15}N also increased over time in 0-10 cm mineral soils, suggesting a redistribution of the added ^{15}N, plus smaller changes in $\delta^{15}N$ in soils at 10-20 cm and 20-40 cm depths (Figure 2).

3.3. Fates of ^{15}N tracer in plants, organic layer, and soils

The total ecosystem recovery of ^{15}N was 60.9% and 61.1% three months after the ^{15}N tracer addition under $^{15}NH_4^+$ and $^{15}NO_3^-$ labeling, respectively, and 59.8% and 65.5% after one year (Table 2). Thus there was little change of total ecosystem ^{15}N recovery between the three months and one year, indicating that ^{15}N was not being significantly lost from the ecosystem after the initial three months or less.

Three months after the $^{15}NH_4^+$ tracer addition, 6.9% of the ^{15}N was recovered in plant tissues, and this increased to 10.9% after one year. Much more ^{15}N was found in plants after the $^{15}NO_3^-$ tracer addition than after $^{15}NH_4^+$ addition: 15.6% after three months, and 28.5% after one year (Table 2). In this diverse primary tropical forest, tree components, including foliage, branches, bark, and roots, were the dominant ^{15}N sinks, while herbs and shrubs were less significant. With $^{15}NO_3^-$ labeling, ^{15}N recovery in fine roots (of which 95% were tree roots, Table 1) declined from 8.6% after three months to 3.9% after one year, while ^{15}N in aboveground foliage, branches, and bark all increased significantly (Table 2). Significant increases of aboveground ^{15}N pools were also found in foliage and shrubs with $^{15}NH_4^+$ labeling, while the changes of ^{15}N in branches, bark and fine roots were insignificant.

In contrast to plant pools, a large amount of ^{15}N was found in the organic layer three months after the ^{15}N tracer addition (21% under $^{15}NH_4^+$ labeling and 11.7% under $^{15}NO_3^-$ labeling), but that declined by half after one year (9.8% under $^{15}NH_4^+$ labeling and 4.8% under $^{15}NO_3^-$ labeling). There was a significantly higher recovery of ^{15}N with $^{15}NH_4^+$ labeling than with $^{15}NO_3^-$ labeling after one year (Table 2).

Mineral soil was the most important ecosystem pool of recovered ^{15}N tracer, despite smaller increase in $\delta^{15}N$ (Table 2, Figure 2). However, soil retention of ^{15}N did not change significantly over time from three months to one year. With $^{15}NH_4^+$ labeling, 33.0% of the ^{15}N was found in the soil after three months, and that recovery insignificantly increased to 39.2% after one year; with $^{15}NO_3^-$ labeling, 33.7% and 32.2% of the ^{15}N was recovered after three months and one year, respectively. In soils, recovery of ^{15}N was greater close to the surface, even though substantial amounts of ^{15}N were also found in deeper soil layers, at 10-20 cm and 20-40 cm (Table 2). In 0-10 cm soils, with $^{15}NH_4^+$ labeling, ^{15}N recovery increased slightly with time, from average 15.9% after three months to 21.9% after one year, whereas the change was minor with $^{15}NO_3^-$ labeling (20.3% and 18.6%, respectively).

3.4. Carbon sequestration efficiency of plants

According to the recovery of deposited N in different plant components and the measured C:N ratio of each plant component, the carbon sequestration efficiency of plants (NUE_{dep}) was calculated as 9 and 24 kg C per kg N under $^{15}NH_4^+$ and $^{15}NO_3^-$ tracer additions, respectively. This gave an average 17 kg C per kg N for $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers combined.

4. Discussion

4.1. Fates of $^{15}NH_4^+$ and $^{15}NO_3^-$ in plants

As hypothesized, vegetation was an important sink for deposited N in our study, accounting for 10.9% and 28.5% of the initial $^{15}NH_4^+$ and $^{15}NO_3^-$ tracer label, respectively, one year after the tracer addition. Our results for recovery in plants are slightly lower than those of several previous ^{15}N tracer studies in apparently N-saturated forests (Koopmans et al., 1996; Feng et al., 2008; Gurmesa et al., 2016), where large recoveries of ^{15}N in plant biomass (17% to 35%, accounting for 30-48% of total ecosystem recovery) were reported (Table 3). However, the rates of ^{15}N recovery in plants in our study are comparable to, but in the high end of range for, those in many temperate and boreal forests which are considered to be N-limited (plant recoveries 1% to 14% with $^{15}NH_4^+$ labeling and 5% to 25% with $^{15}NO_3^-$ labeling) (Buchmann et al., 1996; Nadelhoffer et al., 2004; Templer et al., 2005; Liu et al., 2016; Table 3). Kuzyakov and Xu (2013) suggested that such differences in plant ^{15}N recovery among different forests were related to different levels of competition for N between plants and microorganisms. The ^{15}N recovery in plants is indicative of ecosystem N status, with high recovery in N-saturated forests and low recovery in N-limited forests. In N-limited forests, trees seem to be less competitive than microorganisms and most deposited N was retained through microbial immobilization. In contrast, the competition between trees and microorganisms may be alleviated in N-rich forests, consequently, increasing the ^{15}N tracer recovery in plants.

In addition, we expected that the thin organic ayer would facilitate the role of vegetation as a sink for deposited N in our N-limited tropical forest. Thus, the thin layer might increase the accessibility of plant roots to deposited N. Also, fast turnover within the organic layer might release the N retained and facilitate plant N uptake. Our results showed that although the organic layer initially retained a considerable amount of ^{15}N, more than half was lost one year after the tracer addition (Table 2, additional discussion in Section 4.2). The ^{15}N recovery in plants increased with time for both $^{15}NH_4^+$ and $^{15}NO_3^-$ treatment. From three months to one year after the tracer addition, the ^{15}N recovery of plants increased from 6.9% to 10.9% and from 15.6% to 28.5% with $^{15}NH_4^+$ and $^{15}NO_3^-$ labeling, respectively (Table 2). Previous studies suggested that immobilization by microorganisms created a rapid initial sink in the short-term; ^{15}N immobilized by microorganisms was released to soil solution at time scales longer than a month and then assimilated by plants (Zogg et al., 2000; Zak et al., 2004). The "temporal niche differentiation" (Kuzyakov and Xu, 2013) protects ecosystems from N losses by leaching or gaseous loss, and also reduces the competition between plants and microorganisms. In addition to this mechanism, we suggest that fast decomposition of litter is another important mechanism for the large ^{15}N recovery in plants. A litter decomposition experiment in our study site has showed that 72% of litter was decayed within one year (Zhou, 2013), much higher than in most temperate and boreal forests (36%-42%, Melillo et al., 1982; Austin and

Vivanco, 2006; Prescott, 2010).

The fates of different forms of deposited N were significantly different in our study, supporting the second part of our hypothesis 1. More of the added $^{15}NO_3^-$ was retained in plants compared to the added $^{15}NH_4^+$, which is consistent with many previous ^{15}N tracer studies (Nadelhoffer et al., 1999; Feng et al., 2008; Sheng et al., 2014; Liu et al., 2016). Although it is more costly for plants to take up NO_3^-, NO_3^- may be more readily available for plant uptake at any given soil concentration because of its higher diffusion efficiency compare to NH_4^+ (Jacob and Leuschner, 2015). This is probably also a strategy of trees to avoid direct competition for NH_4^+ with microbes (Kuzyakov and Xu, 2013). Thus our study suggests that in tropical forests like the one we have studied, plants will constitute an important NO_3^- sink. This is relevant as N deposition increases in the region and the proportion of NO_3^- also increases (Liu et al., 2013).

Among plant pools, the ^{15}N recovery in all tree components increased with time, as well as in shrubs, but decreased in herbs and fine roots, indicating that assimilated N was being redistributed in different plant pools and plant species, and that ^{15}N would be transferred from more active pools (leaves and fine roots) to stable pools (branches, bark, stems, and coarse roots) (Goodale, 2017). These results suggest that more deposited N will be retained in high C:N ratio plant biomass over time and thus likely contribute to long-term C sequestration.

4.2. Fates of $^{15}NH_4^+$ and $^{15}NO_3^-$ in the organic layer

In our study, the organic layer was an important short-term sink for deposited N three months after the tracer addition (supporting our hypothesis 2), and the initial fraction retained in the organic layer (21% and 12% for $^{15}NH_4^+$ and $^{15}NO_3^-$ tracer, respectively, Table 2) is close to the global mean value of 20% (Templer et al., 2012). These results suggest that organic layer in the short-term can serve as a buffer for deposited N, avoiding rapid leaching loss or denitrification. However, the organic layer was not a long-term sink for deposited N in our tropical forest. From three months to one year after the tracer addition, the organic layer lost about half of the ^{15}N retained (Table 2). This may be due to fast litter turnover in the humid climate as mentioned above, resulting in a thin organic layer having limited capacity to retain ^{15}N for the long term (Gurmesa et al., 2016; Liu et al., 2017); ^{15}N tracer could be also transferred to the mineral soil, or released and assimilated by plants in the growing season. That is in direct contrast to findings from many temperate and boreal forest studies (Buchmann et al., 1996; Gundersen, 1998; Koopmans et al., 1996; Nadelhoffer et al., 1999; Templer et al., 2005; Providoli et al., 2006; Liu et al., 2016), where ^{15}N was mainly retained in the organic layer at both the short-term (1-3 months) and long-term (3-18 months) (Table 3). However, there are two studies in temperate forests reporting low ^{15}N recovery (21% and 13%, respectively) in their organic layers, both are thin, one is due to coarse soil texture in a coastal environment (Seely and Lajtha, 1997) and another earthworm’s disturbance (Goodale, 2017).

We found a significantly higher ^{15}N recovery in the organic layer with $^{15}NH_4^+$ labeling than with $^{15}NO_3^-$ labeling, which is consistent with previous studies (Corre and Lamersdorf, 2004; Feng et al., 2008; Liu et al., 2016). The difference in ^{15}N recovery between deposited $^{15}NH_4^+$ and $^{15}NO_3^-$ is affected by their specific characteristics. Deposited NH_4^+ is preferably immobilized by forest floor microbes due to the low energy consumption (Recous et al., 1990). Deposited NO_3^- can also be immobilized via abiotic processes, but this abiotic capacity can be quickly saturated (Davidson et al., 2003). Moreover, NO_3^- has a higher mobility and is prone to leach out to mineral soils.

4.3. Fates of $^{15}NH_4^+$ and $^{15}NO_3^-$ in mineral soils

Consistent with our hypothesis 2, the mineral soil (0-40 cm) was the largest sink of deposited N in our study (39% and 32%, respectively). This result is different from those in many temperate and boreal forests (Buchmann et al., 1996; Koopmans et al., 1996; Gundersen, 1998; Tietema et al., 1998; Nadelhoffer et al., 1999; Templer et al., 2005; Providoli et al., 2006; Liu et al., 2016; also see Table 3), in which the largest proportion of deposited N was retained in the organic layer unless there is a bio-disturbance (e.g., by earthworms, Goodale, 2017) or unusual soil texture (Seely and Lajtha, 1997). Similar results of high soil ^{15}N retention were also found in two subtropical forests in which the organic layer was thin or absent (Gurmesa et al., 2016; Liu et al., 2017). Comparing $^{15}NH_4^+$ with $^{15}NO_3^-$ treatments, we found no significant difference, although less ^{15}N was retained in the mineral soil under $^{15}NO_3^-$ treatments (and more recovered in plants).

The fraction of ^{15}N label retained in the mineral soil was relatively stable over time for both N forms, though $^{15}NH_4^+$ recovery increased slightly from three months to one year (Table 2). Previous studies found that most of the applied $^{15}NH_4^+$ was immobilized immediately in the organic pool or incorporated permanently in the illite clay structure (Gebauer et al., 2000; Providoli et al., 2006; Liu et al., 2016). The elevated ^{15}N pool in the upper (0-10 cm depth) mineral soil layer in this study corresponded closely to the decrease in the organic layer, suggesting that the ^{15}N tracer was transferred from the organic layer to the mineral soil. Under $^{15}NO_3^-$ treatment, ^{15}N recovery changed little from three months to one year in all three soil depths (Table 2). These results imply that the loss of ^{15}N in this tropical forest is minimal after three months of receiving N.

4.4. Total ecosystem ^{15}N recovery

Previous work suggest that our tropical montane forest with low N deposition is N-limited and we therefore expected a greater N retention than recently reported from two N-saturated subtropical forests in South China (Sheng et al., 2014; Gurmesa et al., 2016; Liu et al., 2017). However, our results didn’t fully support our expectation (hypothesis 3). The observed total ecosystem ^{15}N recoveries (in plants, organic layer and mineral soils) of between 60% and 66% (Table 2) were approximately equal to the mean recovery of the two N-saturated subtropical forests (19%-90%, on average

58%, n = 5, Table 3), while somewhat less than the global mean ecosystem recovery of 75% for temperate forest ecosystems (Templer et al., 2012, Table 3). This syggests that tropical forests, even those with low N deposition, may have a rather lower retention capacity to retain deposited N than temperate forests (Table 3), but until more comparable studies are conducted in tropical forests world-wide this will be speculative.

In our study, total ecosystem ^{15}N recovery was 61% (for both N forms) after three months, suggesting a considerable amount of ^{15}N was lost within the first three months. This may be caused by a rapid hydrologic loss under the humid climate (994 mm precipitation in the first three months). Leachate was collected at the depth of 40 cm in each plot by zero tension lysimeters installed before the experiment (methods described in Fang et al. (2009)). Water samples were available only during the rainy season (from April to October), and NH_4^+ and NO_3^- leaching loss were 0.7 and 24.7 kg ha^{-1} in $^{15}NH_4^+$ treatment and 0.9 and 31.3 kg ha^{-1} in $^{15}NO_3^-$ treatment, respectively (Wang et al., unpublished data). The $\delta^{15}N$ of leachate was not determined so the ^{15}N recovery in leachate could not be calculated. In addition, gaseous N loss is a possible explanation for the initial ^{15}N loss. Fang et al. (2015) estimated total denitrification in this forest could be up to 15.4 kg N ha^{-1} yr^{-1}.

Surprisingly, total ecosystem ^{15}N recovery did not change from three months to one year, in spite of the high precipitation in that period (1422 mm precipitation). Thus, our hypothesis 4 is rejected. Our results indicate that after an initial rapid loss, a large proportion of the deposited N is retained in a relatively longer term. In the mineral soil, ^{15}N recovery declined with soil depth (Table 2); however, a significant amount of ^{15}N was found at 20-40 cm soil depth. Yet in all soil layers, ^{15}N retained stayed constant, except a slight increase at 0-10 cm for the $^{15}NH_4^+$ labeling. In plant biomass, recovery of ^{15}N increased over time from three months to one year in the aboveground tree components but declined in the fine roots, which implies that ^{15}N is tightly cycled in the study forest and that this forest is N limited or co-limited by other factors. In the same tropical forest we studied, Zhou (2013) found that N and P addition could enhance tree growth (23% greater growth with N addition, 10%-21% with P addition and 15-32% with N+P addition). However, a multiyear ^{15}N tracer study in an N-limited temperate forest reported persistent ecosystem retention of N deposition even as the deposited N was redistributed, without additional plant uptake over the longer timescale (Goodale, 2017). Thus, follow-up studies on a decadal scale should be conducted to test: 1) whether deposited N can be steadily retained for longer time scale (> 1 year); and 2) whether trees can assimilate more deposited N and enhance C sequestration. For the world's most biodiverse forests these important questions still remain open.

4.5. Implications for carbon sequestration

In our study, a substantial fraction of the ^{15}N tracer addition was assimilated by plants and increasingly so from three months to one year (Table 2). Based on our data, the carbon sequestration efficiency of plants stimulated by N deposition (NUE_{dep}) was estimated at 17 kg C per kg N. This is slightly lower than the 23 kg C per kg N value estimated from chronic N addition experiments in the same forest (Zhou, 2013), which enhanced tree growth and carbon sequestration, and from a N-saturated subtropical forest (23 kg C per kg N, Gurmesa et al., 2016). Compared with temperate forests, NUE_{dep} of this tropical forest is also slightly lower than the mean global value of 26 kg C per kg N (Table 4), but it is markedly higher than one estimated for tropical forests (9 kg C per kg N) (De Vries et al., 2014), and greater than some estimates for temperate forests (Pregitzer et al., 2008; Gundale et al., 2014; Goodale, 2017). Our results therefore indicate potential for a moderate C sequestration in response to increased N deposition in this tropical forest, provided that the N assimilated by plants is actively used for growth and not simply stored in perennial plant tissues.

5. Conclusion

By using $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers, we were able to examine the different fates of deposited NH_4^+ and NO_3^- over time in a tropical primary forest with relatively low background rates of N deposition (6 kg N ha^{-1} yr^{-1}). We found that after an initial loss, a large proportion of added ^{15}N was retained. Moreover, a substantial amount of ^{15}N was recovered in plant biomass, and ^{15}N retention in plant biomass increased from three months to one year. Significantly more ^{15}N was recovered by tropical plants following $^{15}NO_3^-$ addition than $^{15}NH_4^+$ addition. The organic layer was an important transient sink for ^{15}N added; however, about half of the ^{15}N that was retained in the three months was lost after one year. The mineral soil was the largest ecosystem sink for N, and the ^{15}N retained in soil was relatively stable over time for both N forms. The total ecosystem ^{15}N recoveries (60% and 66%), while large, are slightly lower than those reported from many temperate and boreal forests. Furthermore, the pattern of ^{15}N distribution in our tropical forest is substantially different from a majority of temperate and boreal forests, with larger fractions of ^{15}N added being found in plants and mineral soils compared to temperate and boreal forests where the organic layer was a much more important sink. Our results provide new evidence that anthropogenic N input, in moderate levels, may benefit tropical forest growth and consequently enhance C sequestration, without significant long-term loss of N to the environment.

Associated content: Supporting information.

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Table 1 Dry mass, N pool, N content and C/N ratio of major ecosystem components before ^{15}N tracer addition. Values in parentheses are 1 SE (n = 3 plots).

	Mass (Mg ha^{-1})	N pool (kg ha^{-1})	N (%)	C/N
Tree				
Foliage	11.0 (1.4)	187.7 (24.3)	1.7 (0.03)	28.4 (0.5)
Branch	80.1 (14.5)	480.5 (87.3)	0.6 (0.02)	76.6 (2.1)
Bark	31.5 (4.8)	188.8 (29)	0.7 (0.05)	74.3 (4.1)
Stem	289.0 (51.9)	577.9 (103.8)	0.2 (0.01)	318.9 (24.8)
Coarse root	167.4 (36.6)	669.8 (146.2) *	0.4 (0.01) *	197.7 (13.4) *
Subtotal	579.0 (109.2)	2104.6 (389.7)		
Shrub	0.4 (0.1)	4.9 (0.9)	1.2 (0.1)	45.9 (3.2)
Herb	0.1 (0.0)	1.9 (0.6)	1.8 (0.2)	24.0 (2.0)
Fine root				
< 2 mm	4.6 (1.0)	55.0 (11.9)	1.2 (0.1)	40.8 (2.5)
2~10mm	8.0 (0.4)	61.6 (3.3)	0.8 (0.1)	64.7 (4.2)
Plant subtotal	**592.1 (109.5)**	**2227.9 (395.0)**		
Organic layer	6.3 (0.5)	82.2 (6.7)	1.3 (0.04)	33.2 (1.0)
Mineral soil				
0-10 cm	1133.6 (18.5)	2153.8 (35.1)	0.19 (0.01)	12.0 (0.6)
10-20 cm	1203.9 (58.2)	1444.6 (69.9)	0.12 (0.02)	10.9 (0.4)
20-40 cm	2651.1 (160.8)	1855.7 (112.6)	0.07 (0.01)	10.0 (0.3)
Soil subtotal	**4994.9 (207.6)**	**5536.8 (170.9)**		

Notes: *Coarse root of trees was not sampled due to the highly destructive. The N concentration and C/N of coarse root was estimated by the mean value of branch and stem.

Table 2 Mean ^{15}N recovery (%) of ^{15}N tracer in forest ecosystem components at 3 months and 1 year after the ^{15}N tracer addition. Values in parentheses are 1 SE (n = 3 plots).

	3 months		**1 year**		**P values of t-test****	
	$^{15}NH_4NO_3$	$NH_4{}^{15}NO_3$	$^{15}NH_4NO_3$	$NH_4{}^{15}NO_3$	$^{15}NH_4NO_3$	$NH_4{}^{15}NO_3$
Tree						
Foliage	0.7^{a} (0.1)	1.8^{b} (0.3)	1.8^{a} (0.4)	7.4^{b} (1.1)	0.047	0.007
Branch	1.1^{a} (0.5)	3.3^{b} (0.5)	3.2^{a} (1.1)	9.2^{b} (1.2)	0.15	0.011
Bark	0.7^{a} (0.2)	0.8^{a} (0)	1.1^{a} (0.3)	3.6^{b} (1.0)	0.44	0.041
Stem	--	--	0.7^{a} (0.1)	1.9^{b} (0.2)	--	--
Root	--	--	0.6^{a} (0.1) *	1.5^{b} (0.1) *	--	--
Subtotal	2.5^{a} (0.7)	5.9^{b} (0.6)	7.4^{a} (1.7)	23.6^{b} (2.8)	--	--
Shrub	0.01^{a} (0.01)	0.05^{b} (0.01)	0.06^{a} (0.02)	0.14^{b} (0)	0.043	0.002
Herb	1.1^{a} (0.5)	1.1^{a} (0.3)	0.5^{a} (0.1)	0.8^{a} (0.5)	0.28	0.59
Fine root	3.3^{a} (0.1)	8.6^{b} (0.8)	3^{a} (0.5)	3.9^{a} (0.5)	0.54	0.007
Plant subtotal	**6.9^{a} (1.2)**	**15.6^{b} (1.3)**	**10.9^{a} (1.5)**	**28.5^{b} (3.1)**	--	--
Organic layer	21.0^{a} (4.2)	11.7^{a} (3)	9.8^{b} (1.4)	4.8^{a} (0.9)	0.06	0.09
Mineral soil						
0-10 cm	15.9^{a} (6.2)	20.3^{a} (5.5)	21.9^{a} (6.6)	18.6^{a} (1.9)	0.55	0.79
10-20 cm	10.2^{a} (2.1)	6.8^{a} (1.5)	8.2^{a} (2.1)	6.2^{a} (0.6)	0.52	0.74
20-40 cm	6.9^{a} (2.5)	6.6^{a} (3.6)	9.1^{a} (1.4)	7.4^{a} (0.6)	0.49	0.27
Subtotal	33.0^{a} (9.0)	33.7^{a} (2.6)	39.2^{a} (10. 0)	32.2^{a} (1.8)	0.68	0.67
Total	**60.9^{a} (4.2)**	**61.1^{a} (1.5)**	**59.8^{a} (12.7)**	**65.5^{a} (2.6)**	--	--

Notes: -- Stem and coarse root of trees were not sampled at 3months, so the ^{15}N recovery was not calculated.

Different lowercase superscript letters within a row represent statistically significant ($P < 0.05$) differences in recovery between the two N forms at each sampling time.

* Recovery of ^{15}N in coarse root was calculated by the mean δ^{15}N value and N concentration of branch and stem in one year.

** The differences in ^{15}N recovery of different ecosystem components between sampling time were tested by the independent t-tests, with P-values reported.

Table 3 ^{15}N recovery (%) of ^{15}N tracer in forest ecosystems under ambient N deposition.

Site	**Vegetation**	**Climate**				**^{15}N recovery (%)**												**References**
			MAT	MAP	N deposition	Plant			Organic layer			Mineral soil layer			Total			
			(ºC)	(mm)	(kg N ha^{-1}yr^{-1})	$^{15}NH_4$	$^{15}NO_3$	$^{15}NH_4{}^{15}NO_3$	$^{15}NH_4$	$^{15}NO_3$	$^{15}NH_4{}^{15}NO_3$	$^{15}NH_4$	$^{15}NO_3$	$^{15}NH_4{}^{15}NO_3$	$^{15}NH_4$	$^{15}NO_3$	$^{15}NH_4{}^{15}NO_3$	
USA																		
Waquoit Bay	Mixed forest	Temperate	9.8	1150	4.2		1.9			24.7			23.7			50.3		Seely and Lajtha, 1997*
Waquoit Bay	Mixed forest	Temperate	9.8	1150	4.2		1.5			17.2			21.8			40.5		Seely and Lajtha, 1997*
Waquoit Bay	Pitch pine	Temperate	9.8	1150	4.2		1.4			22.8			12			36.2		Seely and Lajtha, 1997*
Harvard Forest	Hardwoods	Temperate	7.0	1120	6.0	4.7	9.0		57.9	105.5		9.8	12.4		72.4	126.9		Nadelhoffer et al., 2004
Harvard Forest	Pines	Temperate	7.0	1120	6.0	2.4	4.7		45.7	74.1		8.4	9.2		56.5	87.9		Nadelhoffer et al., 2004
Catskill Mountain	Beech	Temperate	4.3	1530	11.2	2.9			64.1			2.1			69.1			Templer et al., 2005
Catskill Mountain	Hemlock	Temperate	4.3	1530	11.2	1.4			62.8			1.9			66.1			Templer et al., 2005
Catskill Mountain	Red Oak	Temperate	4.3	1530	11.2	4.0			60.3			10.9			75.2			Templer et al., 2005
Catskill Mountain	Sugar Maple	Temperate	4.3	1530	11.2	5.8			51.1			5.0			61.9			Templer et al., 2005
Arnot Forest	Hardwoods	Temperate	7.8	930	9.0	10.8			13.5			45.5			69.7			Goodale, 2017
Europe																		
Wülfersreuth	Norway spruce	Temperate	5.9	1072	11.8	13.5	24.8		62.6	46.3		24.5	32.6		100.6	103.7		Buchmann et al., 1996
Speuld	Douglas fir	Temperate	9.3	750	23.0	28.8			15.8			21.4			66.0			Koopmans et al., 1996**

Continued

Ysselsteyn	Scots pine	Temperate	9.3	750	33.0	16.7			21.4			15.2			53.3			Koopmans et al., 1996**
Klosterhede	Norway spruce	Temperate	9.0	860	9.0			44.3			25.9			12.0			82.2	Gundersen, 1998
Klosterhede	Coniferous	Temperate	9.0	860	20.0			45.0			26.0			12.0			83.0	Tietema et al., 1998
Aber	Coniferous	Temperate	8.8	1850	51.0			32.0			47.0			1.0			80.0	Tietema et al., 1998
Aber	Coniferous	Temperate	8.8	1850	51.0		32.0			17.0			15.0			64.0		Tietema et al., 1998
Alpta	Norway spruce	Temperate	6.0	2300	42.0			13.0			13.0			63.0			99.0	Schleppi et al., 1999
Alpta	Norway spruce	Temperate	6.0	2300	12.0	31.8	19.5		22.7	58.6		2.7	5.0		57.3	83.2		Providoli et al., 2006
Solling plateau	Norway spruce	Temperate	6.4	1090	32.5	30.0	35.6		64.8	8.0		6.4	34.2		101.0	77.8		Feng et al., 2008
China																		
Changbaishan	Evergreen forest	Temperate	3.6	745	27.0	9.0	23.0		50.0	20.0		25.0	42.0		84.0	85.0		Liu et al., 2016
Tieshanping	Evergreen forest	Subtropical	18.2	1105	54.0	5.0	4.0		10.0	4.9		40.0	9.0		55.0	19.0		Liu et al., 2017
Dinghushan	Mixed forest	Subtropical	21.0	1927	34.4	20.6	34.3		36.0	12.6		33.1	8.4		89.7	55.3		Sheng et al., 2014***
Dinghushan	Mixed forest	Subtropical	21.0	1927	34.4			35.0			0.5			37.0			72.5	Gurmesa et al., 2016***
Jianfengling	Primary forest	Tropical	19.8	2449	6.1	10.9	28.5		9.8	4.8		39.2	32.2		59.8	65.5		This study

Notes: *^{15}N recovery was calculated 6 months after the tracer addition in Seely and Lajtha (1997);

**^{15}N recovery was calculated 18 months after the tracer addition in Koopmans et al. (1996);

***^{15}N recovery was calculated 4 months after the tracer addition in Sheng et al. (2014) and Gurmesa et al. (2016).

Table 4 Estimated ranges in carbon sequestration efficiency (NUE_{dep}) stimulated by N deposition in aboveground biomass in forest.

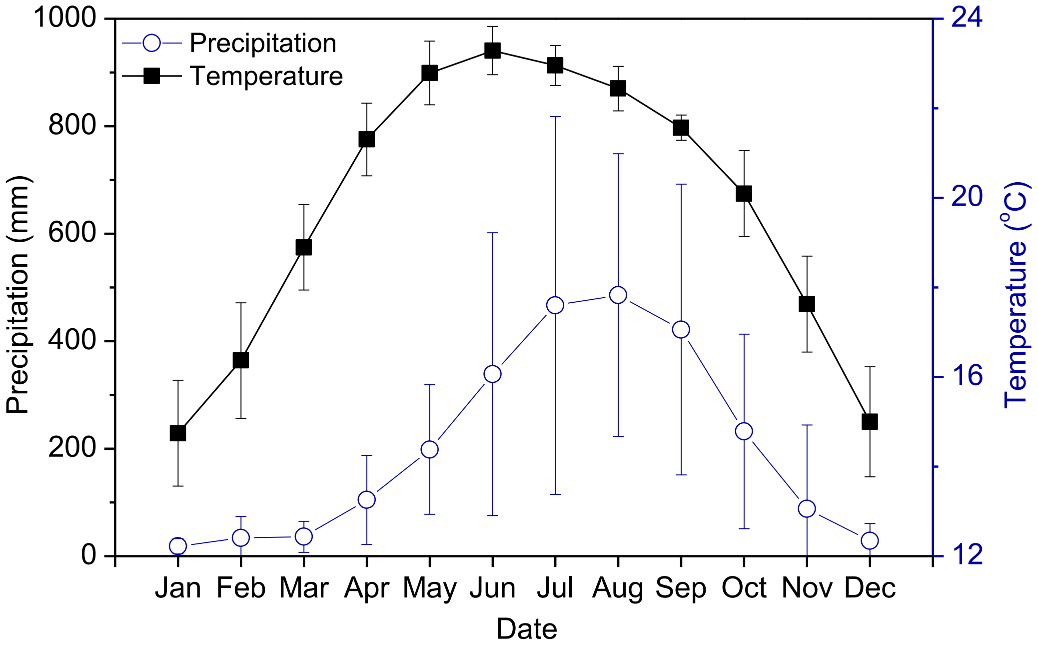
Approach	Country/Region	Climate	NUE_{dep} (kg C/kg N)	Reference
Field inventory	Europe	Temperate	19	Solberg et al., 2009
	Europe	Temperate	21-26	Laubhann et al., 2009
	North America	Temperate	60	Thomas et al., 2010
	Global research	Boreal	33	De Vries et al., 2014
		Temperate	21	
		Tropical	9	
Fertilization	Sweden	Temperate	25	Högberg et al., 2006
	Sweden and Finland	Temperate	25	Hyvönen et al., 2008
	North America	Temperate	17	Pregitzer et al., 2008
	Europe	Temperate	16	Gundale et al., 2014
Model simulations	North America	Temperate	24-67	Pinder et al., 2012
	Netherlands	Temperate	20-30	Wamelink et al., 2009a
	Europe	Temperate	3-12	Wamelink et al., 2009b
	UK	Temperate	15-25	Rehfuess et al., 1999
^{15}N tracer	Sweden	Temperate	30-70	Melin et al., 1983
	Generic average		25	Nadelhoffer et al., 1999
	Europe	Temperate	33	De Vries et al., 2006
	North America	Temperate	12-14	Goodale, 2017
	China	Subtropical	23	Gurmesa et al., 2016
	China	Tropical	17	This study

Legends for figures

Figure 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).

Figure 2 Mean $\delta^{15}N$ values (‰) of all sampled plant (averaged across species) and soil pools before, 3 months after, and 1 year after the ^{15}N tracer addition. Notes: Symbol (**) represent statistically significant ($P < 0.05$) differences between two treatments and symbol (*) represent $P < 0.1$. Stem and coarse root of trees were not sampled after three months, so the $\delta^{15}N$ was not measured.

Figure 1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Temperature
Precipitation (mm)
Temperature (°C)
Date
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600
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Dec

875 **Figure 2**

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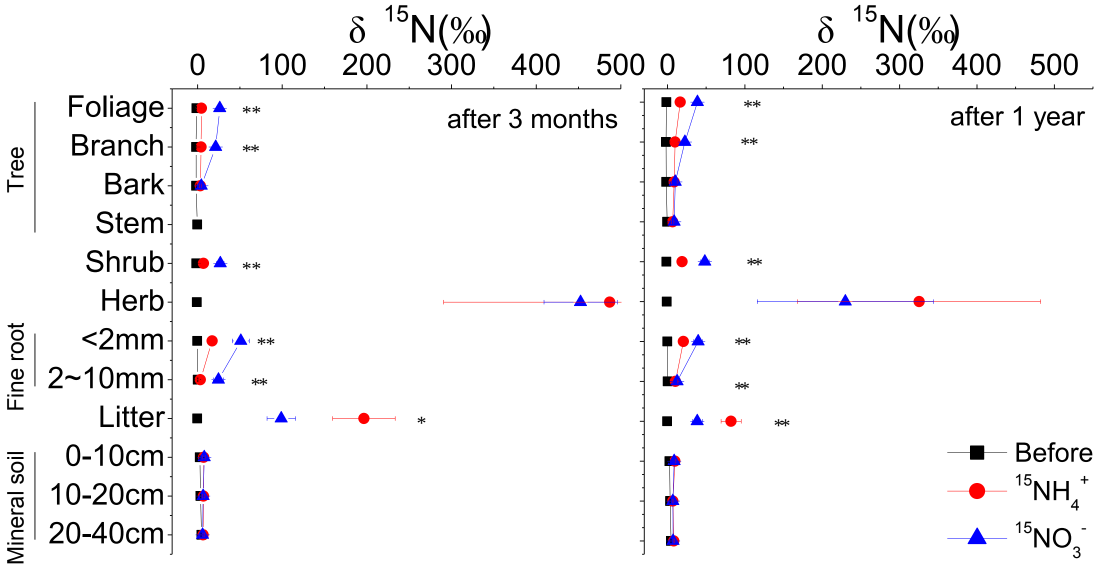

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