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Dynamics and fate of atmospherically deposited nitrogen in two tropical montane forests over three years

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Highlights

1. $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers were applied to two tropical montane forests to explore the fates and redistribution of atmospherically deposited N over three years.

2. More than 60% of ^{15}N tracer was retained within the two forests even after three years.

3. Ecosystem ^{15}N retention was not significantly different between two study forests and between two N forms.

4. ^{15}N tracer was redistributed over time from the organic soil layer to plants and

mineral soil.

5. Proportionally more ^{15}N was distributed to mineral soil and plant in tropical forests while more to organic soil layer in temperate forests.

Abstract

The effects of nitrogen (N) deposition on forest ecosystems largely depends on its fates after entering the ecosystems. Several studies have addressed the fates of N deposition using ^{15}N tracers, but long-term fate and redistribution of deposited N in tropical forests remains unknown. In this study, we applied ^{15}N tracers to examine the fates of deposited ammonium (NH_4^+) and nitrate (NO_3^-), separately, over three years in a primary and a secondary tropical montane forests in southern China. Three months after ^{15}N tracer addition, over 60% of ^{15}N was retained in our study tropical forests, and the ecosystem retention did not change significantly over the study period. From three months to three years, the ^{15}N recovery in plants increased from 10% to 19% and 13% to 22% in the primary and secondary forests, respectively, while ^{15}N recovery in the organic soil layer decreased from 16% to 2% and 9% to 2% over time. Mineral soil retained 50% and 35% in the primary and secondary forests of ^{15}N, with retention being stable over time. We found no significant difference in ecosystem retention between two N forms, but plants retained more $^{15}NO_3^-$ than $^{15}NH_4^+$ and the organic layer retained more $^{15}NH_4^+$ than $^{15}NO_3^-$. Mineral soil did not differ in $^{15}NH_4^+$ and $^{15}NO_3^-$ retention. Compared to temperate forests, proportionally more ^{15}N was distributed to mineral soil and plant in our study tropical forests. Overall, our results indicate that atmospherically deposited of both NH_4^+ and NO_3^- is lost within the first three months, and then can be retained steadily over a relatively longer term within the ecosystem, with retained N being redistributed to plants and mineral soil over time from organic soil layer. As a result, we suggest that this N retention may benefit tropical montane forest growth and enhance carbon sequestration from the atmosphere.

Key words: N deposition; N retention and redistribution; Long-term fate; Tropical montane forests; ^{15}N tracer; Ammonium and nitrate

Introduction

Nitrogen (N) is a limiting nutrient that affects primary productivity and ecosystem functions in many terrestrial ecosystems (Vitousek & Howarth, 1991). However, reactive N emitted from human activities such as fossil fuel burning and fertilizer use has tripled N deposition to Earth's terrestrial ecosystems over recent decades (Ackerman et al., 2019; Yu et al. 2019). Increased N deposition could reduce N limitation and promote plant growth in N-limited forest ecosystems. However, once N inputs exceed biotic and abiotic sinks for N, increased N deposition can induce ion imbalances, reduce biodiversity, and acidify soil and water due to losses of nitrate (Gundersen et al., 1998; Aber et al., 2003; Niu et al., 2016; Du et al., 2019). The effects of N deposition on forest ecosystems largely depend on whether and where the deposited N is retained within ecosystems.

The ^{15}N tracer method is the only approach currently available to trace and quantify the fate and (re)distribution of deposited N over multi-year periods in forest ecosystems (Nadelhoffer et al., 1999a; Templer et al., 2012). Many studies have examined the fate of various forms of ^{15}N added to forest ecosystems (Feng et al., 2008; Templer et al., 2012; Gurmesa et al., 2016; Goodale, 2017; Liu et al., 2017a; Liu et al., 2017b; Wang et al., 2018; Li et al., 2019). However, most studies tracked the deposited N for no more than one year and primarily in temperate and boreal forests (see Templer et al., 2012), and they reported that the fate of deposited N varied between temperate and tropical forests, with most added ^{15}N ended up in the organic soil layer in temperate and boreal forests (Feng et al., 2008; Templer et al., 2012; Goodale, 2017; Liu et al., 2017a; Li et al., 2019) whereas plants and mineral soil were more important sinks in tropical and subtropical forests (Gurmesa et al., 2016; Liu et al., 2017b; Wang et al., 2018). Despite these numerous studies, the long-term retention dynamics of deposited N is uncertain since N initially retained in the organic soil layer and mineral soil may redistribute to woody plants (Goodale, 2017), or be lost from the ecosystem (Preston & Mead, 1994; Wessel et al., 2013). To date, only several studies in temperate forests have traced the distribution of deposited N more than two years (Preston & Mead, 1994; Nadelhoffer et al., 2004; Krause et al., 2012; Wessel et al., 2013; Goodale, 2017; Li et al., 2019). However, the long-term retention dynamics of deposited N in topical forest ecosystems are poorly understood. Tropical forests cover approximately 12% of the earth's land area and play a vital role in sustaining global climate and regulating global N and C cycles (Field et al., 1998; Phillips et al., 1998). Nitrogen deposition has substantially increased in the tropics (Galloway et al., 2008; Bejarano-Castillo et al., 2015; Cusack et al., 2016; Ackerman et al., 2019). Thus, it is critical to study the long-term fate of deposited N in tropical forests to predict how these ecosystems respond to N deposition.

Previous short-term ^{15}N tracer studies have suggested that deposited $^{15}NH_4^+$ and $^{15}NO_3^-$ may have different fates since NH_4^+ is more preferable uptake by soil microbes or immobilized in mineral soil while NO_3^- is more prone to leaching and gaseous loss (Providoli et al., 2006; Jacob & Leuschner, 2015; Liu et al., 2017a; Wang et al., 2018). However, most of long-term ^{15}N tracer studies focused on $^{15}NH_4^+$ or $^{15}NO_3^-$ separately or $^{15}NH_4{}^{15}NO_3$ (Providoli et al., 2005; Wessel et al., 2013; Gurmesa et al., 2016; Goodale, 2017), the different fates of deposited NH_4^+ and $^{15}NO_3$ were seldom compared for the same forests over multi-year time scales (Preston et al., 1990; Preston & Mead, 1994; Nadelhoffer et al., 2004; Li et al., 2019), especially in tropical forests. Furthermore, few studies have been carried out on different forests within a given site (Nadelhoffer et al., 2004; Li et al., 2019). There may be differences in the patterns of N retention in forests with different successional status due to different species composition and N status (Li et al., 2019).

In this study, for the first time, we applied $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers to explore the long-term patterns and mechanisms of retention of deposited NH_4^+ versus NO_3^- in tropical forests. Two forests with different species composition and N status (Wang et al., 2014), a primary and a secondary tropical montane forests in southern China, were selected. Our main objectives were to test: 1) the mechanisms and patterns of retention and redistribution of deposited N over three years, and 2) how different N forms (NH_4^+ versus NO_3^-) and forests (primary versus secondary) influence patterns of retention and redistribution of ^{15}N. We hypothesized that: (H1) Tropical montane forests would lose the experimentally added ^{15}N over time due to the rapid N turnover. Even though tropical montane forests are considered to be N-limited (Matson et al., 1999), a previous study shows that in a primary montane forest about 40% of the deposited N was lost during the first year, presumably through rapid hydrologic or gaseous pathways under the hot and humid climate (Wang et al., 2018); (H2) Plants would become a more important sink for N over time as the re-mineralization of organic matter those had initially retained in ^{15}N tracer; (H3) Plants would retain more NO_3^- than NH_4^+ while soil (organic soil layer and mineral soil) retain more NH_4^+ than NO_3^-; (H4) We expected lower ^{15}N retention in the primary forest due to its relatively higher N status than the secondary forest (Wang et al., 2014).

Methods and materials

Site description

Our study was conducted in the Jianfengling National Natural Reserve, on Hainan Island, southern China. The region is characterized by tropical monsoons climate, with a wet season (from May to October) and a dry season (from November to April). Between 2009 and 2018, annual precipitation averages 2414 mm (from 1637 mm to 3458 mm), and the mean annual temperature was 19.7 °C. For this study, we selected two major tropical montane forests: a primary forest (18°43′47″N, 108°53′23″E, elevation 893 m) and a secondary forest (18°44′41″N, 108°50′57″E, elevation 935 m). Total inorganic N deposition in bulk precipitation was 6.7 kg N ha^{-1} yr^{-1}, with the ratio of NH_4^+/NO_3^- being 1, and no fertilization had ever been previously applied.

The primary forest has never been disturbed by human activities and is dominated by *Mallotus hookerianus*, *Gironniera subaequalis*, *Cryptocarya chinensis*, *Nephel iumtopengii* and *Cyclobalanopsis patelliformis*. The secondary forest has developed naturally after a clear-cutting in the 1960s and mainly consists of *Castanopsis tonkinensis*, *Schefflera octophylla*, *Psychotria rubra* and *Blastus cochinchinensis*. The soil is an acidic lateritic yellow, well-drained soil with the porosity of 52% in the primary forest and 47% in the secondary forest. Soil pH was 4 in both forests. Soil texture is similar between the two forests, being sandy clay loam with 57.1% sand, 18.2% silt, and 24.7% clay in the primary forest and with 53.8% sand, 12.1% silt, and 34.1% clay in the secondary forest (Fang et al., 2004; Luo et al., 2005).

Experimental design

In each forest, three separate plots (20 m × 20 m each) were randomly selected. Each plot was divided into two (10 m × 20 m each), with each subplot receiving a solution of either $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$. In April 2015 and 2016, ^{15}N tracer solutions were sprayed directly on the forest floor using backpack sprayers in the primary forest (Wang et al., 2018) and in the secondary forest to simulate N deposition during the rainfall, respectively. In the primary forest, the quantity of applied ^{15}N tracers was 25 mg ^{15}N m^{-2} as 99.14 atom% $^{15}NH_4NO_3$ and 99.21 atom% $NH_4{}^{15}NO_3$. In the secondary forest, the labelling method was similar to that used in the primary forest, but the ^{15}N tracer levels were doubled to 50 mg ^{15}N m^{-2} to increase the ^{15}N signal further above background levels and trace the long-term fate of deposited N. Yet, the added ^{15}N tracer is relatively small compared to N deposition and ecosystem N pool. Thus, the added ^{15}N tracer can substantially increase the concentration of ^{15}N above its natural abundance in all ecosystem pools with minimal disturbance of ecosystem N cycling. The fate of ^{15}N tracer in the first year was reported previously for the primary forest (Wang et al., 2018) and submitted to review for the secondary forest along with the results from 12 other forests (Gurmesa et al., submitted). Here, we reported the fates after three years and compared them with those in the first three months and years and other temperate forests.

Sampling and chemical analysis

Samples were taken away from the edges to minimize edge effects. Major plant components and soil layers were sampled prior to ^{15}N tracer application at three months, one year and three years after ^{15}N tracer application. In the primary forest, samples were also collected at one week and one month after labelling. For plant samples, foliage and branches of trees and shrubs were sampled from common species in each subplot. Bark and wood core (3 cm of an exterior portion) were sampled using an increment corer from trees with a diameter at breast height (DBH) above 5 cm. Herbs and organic soil layer (mainly consisting of undecomposed plant materials on the soil surface) were sampled using a 20 cm × 20 cm iron frame. Six samples taken randomly in each subplot were mixed into one composited sample. 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 soil cores taken randomly in each subplot were mixed into one composite soil by soil depth. Living fine roots (< 2 mm, 0-40 cm) were hand-sorted from separate composite soil samples (taken using an auger of 5 cm inner diameter), and then cleaned by deionized water.

All plant and organic soil layer samples were oven-dried at 65 ºC to constant weight. Mineral soil from each plot was passed through a 2 mm mesh to remove fine roots and coarse fragments and then air-dried at room temperature. All samples were ball-milled and analyzed for ^{15}N abundance and total N and total C concentrations by elemental analyzer-isotope ratio mass spectrometry at the Institute of Applied Ecology (Elementar Analysen Systeme GmbH, Hanau, Germany; IsoPrime100, IsoPrime limited, Stockport, UK). Calibrated D-glutamic, glycine, acetanilide and histidine were used as references. The analytical precision for $\delta^{15}N$ was better than 0.2‰.

Calculation and Statistical analysis

Dry masses of tree or shrub compartments were estimated using allometric equations of mixed-species (Zeng et al., 1997; Chen et al., 2010). Dry masses of herbs, organic layer samples and fine roots were calculated by the weight of the harvested samples. Nitrogen pools of the different tree or shrub tissues, herbs, litters and fine roots were calculated by multiplying dry mass and N concentration of each measured component. Soil N pools were calculated by multiplying soil bulk density at different soil layers, soil depth and the corresponding N concentration.

The ^{15}N tracer recovery in all sampled components of ecosystem was estimated using ^{15}N tracer mass balances as the following (Nadelhoffer & Fry, 1994):

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

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

The carbon sequestration efficiency stimulated by N deposition (NUE_{dep}) was estimated using the ^{15}N recoveries of tree woody biomass (including branch, bark, stem and coarse root of trees) and their corresponding C/N ratios, by the following standard stoichiometry approach of Nadelhoffer et al. (1999b):

$$NUE_{dep} = {}^{15}N_{recovery\ in\ wood} \times (C/N)_{wood}$$

The differences in ^{15}N abundance and ^{15}N recovery between the treatments at each sampling time were tested by the analysis of independent t-tests. Repeated-measures ANOVA was used to test the differences in ^{15}N abundance and ^{15}N recovery over time together with forest types and N forms. All analyses were conducted using the SPSS software (version 19.0; SPSS Inc., Chicago, Illinois, U.S.A.) with significance threshold set at $P \leq 0.05$.

Results

Ecosystem nitrogen pools and $\delta^{15}N$ in the two forests

The total ecosystem N pool was about 7700 kg N ha^{-1} in the primary forest and 7100 kg N ha^{-1} in the secondary forest (excluding soils below 40 cm) (Table 1). The N pools of trees were 2200 kg N ha^{-1} in the primary forest and 1700 kg N ha^{-1} in the secondary forest, accounting for 95% of the total plant N pool in both forests. Total soil N pools down to 40 cm depth were about 5500 kg N ha^{-1} and 5400 kg N ha^{-1} in the primary and the secondary forest, respectively. There were 82 kg N ha^{-1} and 68 kg N ha^{-1} in the forest floor in the primary and the secondary forests, accounting for only about 1% of the total ecosystem N pools. The N pools in the major ecosystem compartments did not differ between the two forests, except that the N pool in 0-10 cm mineral soils in the primary forest was significantly higher than that in the secondary forest (Table 1).

The $\delta^{15}N$ values of different ecosystem compartments did not differ between two forests before labelling, except in tree stems. Plants $\delta^{15}N$ varied from -1.8‰ to 0.3‰ in the primary forest and from -1.7‰ to 0.8‰ in the secondary forest (Figure 1, Table S1 and S2). The $\delta^{15}N$ of organic soil layer $\delta^{15}N$ averaged -0.4‰ in the primary forest and -1.1‰ in the secondary forest, respectively. The $\delta^{15}N$ of mineral soil was always positive and increased with soil depth in both forests (Figure 1).

After the ^{15}N tracer addition, the $\delta^{15}N$ increased in all ecosystem pools of the two forests (Figure 1, Table S1 and S2). However, the temporal patterns of $\delta^{15}N$ in different ecosystem pools varied. The $\delta^{15}N$ of tree components and shrubs increased over time (from -1.8‰ to 48.3‰ in the primary forest and from -1.5‰ to 84.8‰ in the secondary forest) while the $\delta^{15}N$ of herbs, fine roots, and organic soil layer peaked at three months and then decreased (Figure 1, Table S1 and S2). For 0-40 cm mineral soils, $\delta^{15}N$ also increased three months after ^{15}N tracer addition but did not change significantly from three months to three years.

There were major differences in ^{15}N abundance in the receiving ecosystem components between $^{15}NH_4^+$ and $^{15}NO_3^-$. In the two forests, the $\delta^{15}N$ of tree foliage and branches were significantly lower under $^{15}NH_4^+$ labelling (4.2‰ to 18.6‰ in the primary forest and 17.3‰ to 63.3‰ in the secondary forest) than under $^{15}NO_3^-$ labelling (21.4‰ to 38.8‰ in the primary forest and 44.1‰ to 84.8‰ in the secondary forest) from three months to three years (Figure 1, Table S1 and S2). In contrast, the $\delta^{15}N$ of the organic soil layer was consistently higher under $^{15}NH_4^+$ labelling than under $^{15}NO_3^-$ labelling. However, there were no significant differences in $\delta^{15}N$ of herbs and mineral soils between $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling in the primary forest. In the secondary forest, the $\delta^{15}N$ of 0-10 cm mineral soils was significantly higher under $^{15}NH_4^+$ than under $^{15}NO_3^-$ labelling three months to one year after ^{15}N tracer addition, but this difference disappeared at three years after the ^{15}N tracer addition.

Total ecosystem recovery

Three months after ^{15}N tracer addition, the total ecosystem recovery in the primary forest was 60.4% and 59.1% with $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling, respectively, and 63.8% and 48.0% in the secondary forest (Figure 2, Table S3 and S4). One year after ^{15}N tracer addition, the total ecosystem recovery in the primary forest was 58.5% and 64.5% with $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling, respectively, and 60.9% and 59.7% in the secondary forest (Figure 2, Table S3 and S4). Three years after ^{15}N tracer addition, the ^{15}N recovery in the primary forest under $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling was 67.9% and 73.7%, respectively, and 61.0% and 57.1% in secondary forest (Figure 2, Table S3 and S4). The change in the total ecosystem recovery over time was minor considering the uncertainties in estimating the ^{15}N recovery. In addition, neither the tracer form nor the forest type significantly affected the total ecosystem recovery of added ^{15}N (Table S6).

^{15}N tracer redistribution in different ecosystem components

There was no significant difference in plant ^{15}N recovery between two forests over time (Table S6). In both forests, ^{15}N recovery in plants increased from three months to three years after ^{15}N tracer addition. The ^{15}N recovery in plants in the primary forest increased from 6.4% to 12.6% with $^{15}NH_4^+$ labelling and from 13.7% to 26.0% with $^{15}NO_3^-$ labelling (P < 0.05, Table S3). The ^{15}N recovery in plants in the secondary forest increased from 12.1% to 21.3% with $^{15}NH_4^+$ labelling and from 13.8% to 22.1% with $^{15}NO_3^-$ labelling (P < 0.05, Table S4). However, the temporal patterns of ^{15}N recovery differed greatly among different plant components (Figure 3, Table S3, and S4). The ^{15}N recovery in herbs and fine roots decreased with time in both forests, whereas recovery in tree components and shrubs increased with time. Moreover, in the primary forest, significantly more ^{15}N was recovered in plants after the $^{15}NO_3^-$ tracer addition than after $^{15}NH_4^+$ addition at all sampling times. However, ^{15}N recovery in plant compartments in the secondary forest did not differ significantly between $^{15}NO_3^-$ and $^{15}NH_4^+$ labelling.

The temporal pattern of ^{15}N recovery in the organic soil layer differed from the patterns of the plant pools. In both forests, ^{15}N recovery was high in the organic soil layer three months after the ^{15}N tracer addition (21.0% and 11.7% with $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling in the primary forest, and 13.0% and 4.5% in the secondary forest), but declined significantly over time afterward (Figure 2, Table S3 and S4). In addition, ^{15}N recovery in the organic soil layer was higher for $^{15}NH_4^+$ than for $^{15}NO_3^-$ tracer in both forests (Table S6), but the difference between the two decreased over time.

Mineral soil was the dominant sink for the added ^{15}N tracer (Figure 2, Table S3 and S4). In the primary forest, 33.0% and 33.7% of the ^{15}N was found in the mineral soil after three months with $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling, and that recovery was 53.5% and 46.3% after three years. However, soil retention of ^{15}N in the secondary forest did not change significantly over time, with 37.6% and 33.2% of the ^{15}N being retained in the mineral soil after three years under $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling. The recovery of ^{15}N declined with soil depth in two forests, with the highest ^{15}N recovery being observed in the 0-10 cm (26.6% and 21.9% with $^{15}NH_4^+$ and $^{15}NO_3^-$ labelling in the primary forest and 22.9% and 19.2% in the secondary forest). Nevertheless, substantial amounts of ^{15}N were also retained at 10-20 cm and 20-40 cm depth. Overall, ^{15}N recovery in mineral soil did not differ significantly between $^{15}NH_4^+$ and $^{15}NO_3^-$ treatments in either forest (Table S3 and S4).

Discussion

Temporal patterns of total ecosystem recovery compared to temperate forests

Our results indicated that large quantities of ^{15}N (about 40%) were lost only during the first growing season, and from three months to three years, there was no significant change in total ecosystem recovery. These results contradicted our first hypothesis that the amount of ^{15}N retained in tropical montane forests over longer periods would decrease substantially through leaching and gaseous loss. The potential mechanism for the rapid initial losses might be that ^{15}N might be absorbed physically on the litter and surface mineral soil and therefore lost through physical processes such as leaching or erosion caused by heavy rain in the first few months after ^{15}N labelling (Wang et al., 2018; Li et al., 2019). In addition, this pattern of initial losses in the first few months is similar to that observed in several temperate forest experiments (Figure 4), suggesting that total long-term ecosystem retention might be determined by the initial loss. A recent long-term study also suggested that ^{15}N initially retained would remain in temperate forests while newly deposited N would be lost from the system (Veerman et al., 2020). We speculate that ^{15}N initially retained after the first few months might be converted to organic forms and then enters the ecosystem internal N cycling.

The observed total ecosystem recovery of 70% and 60% of ^{15}N in the primary and secondary tropical montane forests three years after the ^{15}N tracer addition (Table S3 and S4) were comparable to the mean recovery reported by long-term ^{15}N studies in temperate forests which are considered to be N-limited (Figure 4, on average 58%, n = 8, t test, $P = 0.935$). Synthesis results of ^{15}N tracer studies have suggested that total ecosystem retention is positively correlated with soil C/N represented ecosystem N status (Vitousek et al., 1998; Templer et al., 2012). The soil C/N (11) in the studied forests being within the range of that in temperate forests (9.7 to 29.6, Table S5), suggesting that the N retention capacity in the studied tropical montane forests is comparable to temperate forests over the long-term. In addition, the net primary production (NPP) in the primary and secondary forests is about 4.5 and 8.3 Mg C ha^{-1} yr^{-1} according to field inventory (Jiang, 2016). If the average C/N of 230 was used, 35 to 64 kg N ha^{-1} yr^{-1} at least was needed to sustain the NPP. Thus, about 80% - 90% N sustaining the NPP comes from soil N pool and is inner-recycled. We, therefore, conclude that both the primary and secondary tropical montane forests we studied have a conservative N cycle, where N is tightly recycled within these ecosystems once after the atmospherically deposited inorganic N was transformed into organic form.

Distribution of ^{15}N in different ecosystem components

The distribution patterns of deposited N in the studied tropical forests differed substantially from temperate and boreal forests, with larger fractions of added ^{15}N found in plants and mineral soils than is the case with temperate and boreal forests where the organic soil layer is a much more important sink (Figure 4, t-test, $P < 0.05$). This difference in the patterns of ^{15}N distribution can be attributed to the differences between tropical and temperate forests in climate (e.g., mean annual temperature and precipitation), mass and decomposition rate of organic soil layers (Templer et al., 2012). The thin organic soil layer and fast decomposition of litter in tropical forests due to high temperature and precipitation might be release the ^{15}N retained and facilitate plant N uptake (Wang et al., 2018). In contrast, thicker organic soil layers (and lower rainfall) hamper the transfer of ^{15}N to mineral soil in temperate and boreal forests (Buchmann et al., 1996; Gundersen, 1998; Koopmans et al., 1996; Nadelhoffer et al., 1999a; Providoli et al., 2006; Liu et al., 2017a; Li et al., 2019). Our results also suggested that the ^{15}N recovery in soil organic layer was positively correlated to the soil organic layer mass ($R^2 = 0.88$, $P < 0.001$) (Figure S1, Table S5), further supporting these mechanisms.

Over a longer time scale, the deposited N is recycled and redistributed among the plant, organic soil layer, and mineral soil in the studied tropical forests. Consistent with our second hypothesis, the ^{15}N recovery increased in plants after three years (Figure 3), indicating that deposited N that was initially retained in mineral soil and organic soil layer over the long term has become available for plant uptake and assimilation (Wessel et al., 2013; Goodale, 2017; Li et al., 2019). Earlier studies have also suggested that ^{15}N tracer immobilized by microorganisms was slowly released to soil solution and then assimilated by plants (Zogg et al., 2000; Zak et al., 2004). In our results, ^{15}N recovery in shrubs and all tree components increased with time, but decreased in herbs and fine roots (Figure 3), suggesting that assimilated N was transferred from active plant pools to stable plant pools (Nadelhoffer et al., 2004; Goodale, 2017; Li et al., 2019).The carbon sequestration efficiency of plants (NUE_{dep}) was estimated to be 14 and 18 kg C per kg N for primary and secondary forest after three years, which is within the range of values in temperate forests (Wang et al., 2018) and higher than the values estimated for tropical forests (9 kg C per kg N, De Vries et al., 2014). According to a nutrient addition experiment in the studied forests (Zhou, 2013), N addition enhanced aboveground biomass carbon pool (NUE_{dep} by 24-35 kg C per kg N and 11 kg C per kg N in the primary and secondary forests, respectively). Together, these results suggest that over time more deposited N will be increasingly retained in high C/N ratio tree components and therefore enhance N deposition-induced carbon sequestration (Nadelhoffer et al., 1999a, b; Goodale, 2017).

In contrast to the pattern in the plants, the organic soil layer ^{15}N recovery declined from three months (4.5% to 21.0%) to three years (1.5% to 2.1%), especially so in the primary forest (Figure 2, Table S3 and S4). We attribute this to fast litter turnover in tropical forests, resulting in the small capacity of organic soil layer to retain the added ^{15}N (Gurmesa et al., 2016; Liu et al., 2017b; Wang et al., 2018). The ^{15}N initially retained in organic soil layer could be transferred to the mineral soil, or released and assimilated by plants (Wessel et al., 2013; Veerman et al., 2020). In numerous studies in temperate forests, ^{15}N recovery decreased over time in the organic soil layer, which was attributed to litter decomposition, physical leaching or downward transport by soil fauna (Nadelhoffer et al., 2004; Goodale, 2017; Li et al., 2019).

In the mineral soils, there was no significant change in ^{15}N recovery from three months to three years (Figure 2 and 4). The mineral soil was still the largest sink for deposited N after three years. The long-term persistence of ^{15}N in mineral soil might be attributed to the incorporation of ^{15}N into stable soil organic matter (SOM) pools (Perakis & Hedin, 2001; Goodale et al., 2015; Veerman et al., 2020). Previous studies have demonstrated that inorganic N could be immobilized in the organic N pool through microbial accumulation, condensation of N in microbial enzymes with phenolic compounds, or abiotic reactions of inorganic N with soil organic matter (Johnson, 1992; Johnson et al., 2000; Lewis & Kaye, 2012; Goodale et al., 2015; Liu et al., 2017a; Fuss et al., 2019), while inputs of high C/N woody debris would also promote the immobilization of ^{15}N by microbes (Lajtha, 2020). Moreover, the deposited N retained in mineral soil may promote soil organic carbon accumulation in the studied forests which have the conservative N cycle (Manzoni et al., 2017; Zhou et al., 2019). The nutrient addition experiment in the studied forests also found that N addition enhanced soil organic carbon pool (Zhou, 2013).

Different fates of $^{15}NH_4^+$ and $^{15}NO_3^-$

In present study, total ecosystem retention did not differ significantly between $^{15}NH_4^+$ and $^{15}NO_3^-$ (Figure 2, Table S6), indicating that deposited NH_4^+ and NO_3^- can be retained equally by the studied forest. Nonetheless, the patterns of ^{15}N distribution within ecosystems (plants, organic soil layer and mineral soil) did differ, which might be the potential reason for the similar total ecosystem ^{15}N recovery. Consistent with many previous ^{15}N-field studies (Nadelhoffer et al., 2004; Feng et al., 2008; Sheng et al., 2014; Liu et al., 2017a; Wang et al., 2018; Li et al., 2019), our results indicate that deposited NO_3^- is more efficiently used by plants compared to NH_4^+. The higher recovery $^{15}NO_3^-$ in plants than $^{15}NH_4^+$ could be attributed to its higher mobility, so that $^{15}NO_3^-$ can move more easily to the root surface and be assimilated by plants (Jacob & Leuschner, 2015). Additionally, NO_3^- is more important in balancing cation uptake (e.g., K^+, Ca^{2+} and Mg^{2+}) than NH_4^+ (Hoffmann et al., 2007). In contrast, NH_4^+ is likely retained on cation exchange sites in soil organic matter and clay particles or preferentially taken up by soil microbes (Gebauer et al., 2000; Providoli et al., 2006; Jacob & Leuschner, 2015; Liu et al., 2017a). Furthermore, plant uptake of NO_3^- would avoid direct competition for NH_4^+ with microbes (Kuzyakov & Xu, 2013). However, these difference in tree components were less prevalent in the long-term, indicating that both deposited $^{15}NH_4^+$ and $^{15}NO_3^-$ are slowly redistributed to stable plant pools over time.

In our results, retention in the organic soil layer was significantly higher for $^{15}NH_4^+$ than for $^{15}NO_3^-$ (Table S6), which is consistent with previous studies (Corre &

Lamersdorf, 2004; Feng et al., 2008; Liu et al., 2017a; Li et al., 2019). Many studies have demonstrated that forest floor microbes prefer NH_4^+ to NO_3^- due to its lower energy cost during assimilation (Recous et al., 1990). Moreover, NO_3^- has greater mobility than NH_4^+ and leaches readily to mineral soils. In the primary forest, the organic soil layer was the major sink for deposited N (46%) under $^{15}NH_4^+$ labelling at one week after ^{15}N tracer addition while 65% of ^{15}N was retained in mineral soil under $^{15}NO_3^-$ labelling (Figure 2), further supporting this mechanism. However, ^{15}N recovery three years after ^{15}N tracer addition in the organic soil layer did not differ between the two N forms. Fast decomposition of litter in tropical forests is a possible mechanism for similar retention patterns after three years. Both deposited NH_4^+ and NO_3^- could over the three years be finally transferred to the mineral soil, or released and assimilated by plants in the growing season (Nadelhoffer et al., 2004; Goodale, 2017; Li et al., 2019).

Surprisingly, ^{15}N recovery in mineral soil did not differ between $^{15}NH_4^+$ and $^{15}NO_3^-$ considering the uncertainties in estimating the mineral soil N pools (Table S3-S4 and S6), in contrast to previous studies (Nadelhoffer et al., 2004; Feng et al., 2008; Sheng et al., 2014; Liu et al., 2017a; Liu et al., 2017b; Li et al., 2019). This might be attributed to the conservative N cycle of the studied tropical montane forests. A previous study also suggested that both $^{15}NH_4^+$ and $^{15}NO_3^-$ can be incorporated into stable soil organic matter and hence resulting in similar long-term "equilibrium" patterns of $^{15}NH_4^+$ and $^{15}NO_3^-$ retention in N-poor forests (Perakis & Hedin, 2001). Added $^{15}NH_4^+$ can be immobilized by soil microbes or incorporated into cation exchange sites in soil organic matter (SOM) and clays (Perakis & Hedin, 2001; Zhu & Wang, 2011; Lewis & Kaye, 2012; Templer et al., 2012). The NO_3^- could also be incorporated to particulate and mineral-associated SOM fractions through abiotic or biotic processes (Matus et al., 2019; Fuss et al., 2019). For example, dissimilatory nitrate reduction to ammonium (DNRA) has been hypothesized to play a key role in the retention of bioavailable N in forests from high rainfall areas (Silver et al., 2001; Huygens et al., 2007; Templer et al., 2008; Gao et al., 2016).

Difference between the two forests

In our study, both tropical montane forests have low N status (Wang et al., 2014), as indicated by low rates of atmospheric N deposition and persistent ecosystem retention (Figure 2), but the primary forest was initially somewhat more N-rich than the secondary forest (Wang et al., 2014). However, there was no significant difference in ecosystem N retention between two forests over time (Table S6), which contradicted our fourth hypothesis that lower ^{15}N retention in the primary forest due to its relatively higher N status than the secondary forest. Although there were differences in labelling time and the amount of ^{15}N tracer between the two forests, the similarity in N retention pattern were not due to the inconsistent experimental designs. The precipitation varied between two years and the precipitation in the first three months after ^{15}N labelling in the primary forest (Year 2015) was larger than that in the secondary forest (Year 2016)

(Figure S2). Moreover, twice as much tracer was applied to the secondary forest (50 mg ^{15}N m^{-2}) as to the primary forest (25 mg ^{15}N m^{-2}). However, our results suggested that about 40% of ^{15}N were lost only during the first three months in the two forests, and from three months to three years, there was no significant change in total ecosystem recovery (Figure 2). Thus, the differences in precipitation and the amount of ^{15}N tracer could not affect the patterns of ^{15}N retention in the two forests. We therefore suggested that succession status did not strongly affect total ecosystem recovery nor the distribution patterns of added ^{15}N in our two tropical forests.

Conclusions

In this study, we presented the first analysis of the fates of deposited NH_4^+ and NO_3^- over three years for two tropical montane forests. More than 60% of ^{15}N was retained in both primary and secondary tropical montane forests one as well as three years after ^{15}N tracer addition, indicating persistent ecosystem retention of deposited N in these forests. Although total ecosystem ^{15}N recovery did not change significantly with time, the deposited N became redistributed within the forests. The retention and retranslocation patterns in plants, organic soil layers, and mineral soil differed between $^{15}NH_4^+$ and $^{15}NO_3^-$ tracer in the two forests. More $^{15}NO_3^-$ than $^{15}NH_4^+$ was retained by plants and the total ^{15}N recovery attributed to plants increased over time. In contrast to long-term retention in plants, the organic soil layer was a transient sink for deposited N and more $^{15}NH_4^+$ was retained here. The mineral soil was the largest ecosystem sink for deposited N. It was surprising that the ^{15}N recovery of mineral soil remained relatively steady in study forests over time and that ^{15}N recovery in mineral soil did not differ between the two N forms. Neither forest types nor N forms significantly affected total ecosystem N retention. Overall, our results suggest that deposited N is redistributed to more stable plant and soil pools over time. Critically, our results indicate that roughly 60% of the deposited N was still retained within tropical montane forests after three years, from which we expect the retained N to benefit tropical forest growth and enhance carbon sequestration.

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Table 1 Dry mass, nitrogen pool, total nitrogen concentration and carbon: nitrogen ratio (C/N) of major ecosystem components before adding ^{15}N tracer in the two tropical montane forests. Values in parentheses are one standard error (n = 3). Different lowercase superscript letters within a row represent statistically significant ($P \leq 0.05$) differences.

Ecosystem components	Dry mass (Mg ha^{-1})		N pool (kg ha^{-1})		%N		C/N	
	Primary	Secondary	Primary	Secondary	Primary	Secondary	Primary	Secondary
Tree								
Foliage	11 (1)	10 (0.2)	188 (24)	167 (3)	1.69 (0.03)	1.72 (0.03)	28.4 (0.5)	28.2 (0.5)
Branch	80 (14)	65 (2)	481 (87)	419 (10)	0.61 (0.02)	0.65 (0.02)	76.6 (2.1)	78.3 (1.8)
Bark	32 (5)	26 (0.5)	189 (29)	188 (4)	0.66 (0.05)	0.71 (0.05)	74.3 (4.1)	82.6 (4.0)
Stem	289 (52)	234 (6)	578 (104)	396 (10)	0.18 (0.01)	0.17 (0.01)	318.9 (24.8)	326.5 (14.8)
Root	167 (37)	130 (4)	670 (146)	530 (17)	0.40 (0.01) *	0.41 (0.01) *	197.7 (13.4) *	202.4 (9.3) *
Subtotal	579 (109)	465 (12)	2105 (390)	1701 (43)				
Shrub	0.4 (0.1)	0.7 (0.1)	5 (0.9)	8 (1)	1.23 (0.1)	1.19 (0.03)	45.9 (3.2)	52.9 (1.4)
Herb	0.1 (0.0)	0.05 (0.01)	2 (0.6)	0.7 (0.2)	1.76 (0.2)	1.51 (0.19)	24.0 (2.0)	27.8 (4.5)
Root								
< 2 mm	5 (1)	3 (0.4)	55 (12)	34 (4)	1.20 (0.1)	1.01 (0.06)	40.8 (2.5)	46.1 (2.8)
2-10mm	8 (0.4)	5 (2)	62 (3)	37 (10)	0.77 (0.1)	0.69(0.04)	64.7 (4.2)	68.9 (4.1)
Plant subtotal	**592 (110)**	**475 (14)**	**2228 (395)**	**1781 (60)**				
Organic soil layer	6 (0.5)	6 (0.4)	82 (7)	68 (5)	1.31 (0.04)	1.21 (0.03)	33.2 (1.0)	36.4 (1.0)
Mineral soil								
0-10 cm	1134 (18)	1085 (18)	2154^{a} (35)	1830^{b} (18)	0.19 (0.01)	0.17 (0.02)	12.0 (0.6)	11.8 (0.5)
10-20 cm	1204 (58)	1106 (21)	1445 (70)	1364 (25)	0.12 (0.02)	0.12 (0.02)	10.9 (0.4)	11.4 (0.2)
20-40 cm	2651 (161)	2397 (80)	1856 (113)	2198 (63)	0.07 (0.01)	0.09 (0.01)	10.0 (0.3)	10.9 (0.2)
Soil subtotal	4995 (208)	4594 (135)	5537 (171)	5395 (115)				
Ecosystem total	**5587 (532)**	**5069 (25)**	**7768 (965)**	**7164 (110)**				

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

Legends for figures

Figure 1 Mean $\delta^{15}N$ values of major ecosystem compartments before and three months after, one year after and three years after ^{15}N addition in two forests. Error bars are standard error of the mean (n = 3).

Figure 2 The ^{15}N recovery in plant, organic soil layer and mineral soil at three months, one year and three years in the two study forests. Labelling date was April 15th, 2015 (primary forest) and April 15th, 2016 (secondary forest). ^{15}N recovery one week and one month after ^{15}N tracer addition was only available for the primary forest and only trees and shrubs were included as plants.

Figure 3 The ^{15}N recovery in different plant compartments three months, one year and three years after ^{15}N labelling in the two study forests. "Tree-woody biomass" includes branch, bark, stem and root of trees. Labelling date was on April 15th, 2015 (primary forest) and April 15th, 2016 (secondary forest). ^{15}N recovery one week and one month after ^{15}N tracer addition was only available for the primary forest and only trees and shrubs were included as plants. ^{15}N recovery in stems and roots of trees was only measured for one year and three years after ^{15}N tracer addition.

Figure 4 The ^{15}N recovery (%) in our two tropical forest ecosystems in comparison with other studies from temperate forests. Only forests for which ^{15}N recovery has been determined for at least three years were analyzed. Details of site characteristics are provided in Table S5. Note: JFL-P and JFL-S represent primary forest and secondary forest in Jianfengling (current study); QY-L and QY-M represent larch forest and mixed forest in Qingyuan (Li et al., 2019); Harvard-H and Harvard-P represent oak forest and red pine forest in Harvard (Nadelhoffer et al., 1999a; Nadelhoffer et al., 2004); Spillimacheen (Preston et al., 1990; Preston & Mead, 1994); Ysselsteyn (only $^{15}NH_4^+$ labelling, Wessel et al., 2013); Arnot (only $^{15}NO_3^-$ labelling, Goodale, 2017); Alptal ($^{15}NH_4{}^{15}NO_3$ labelling, Schleppi et al., 1999; Providoli et al., 2005; Krause et al., 2012).

854 **Figure 1**

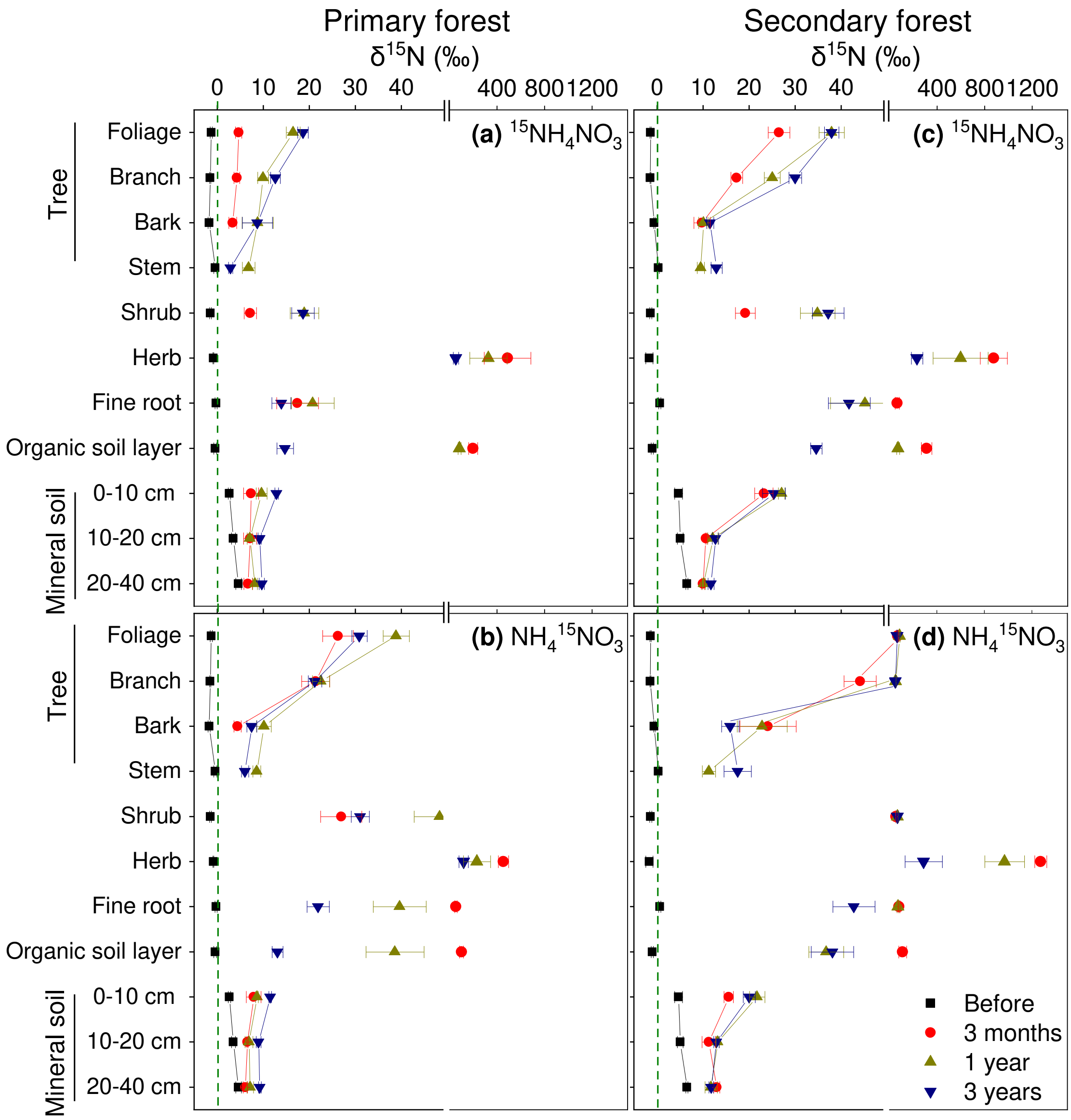

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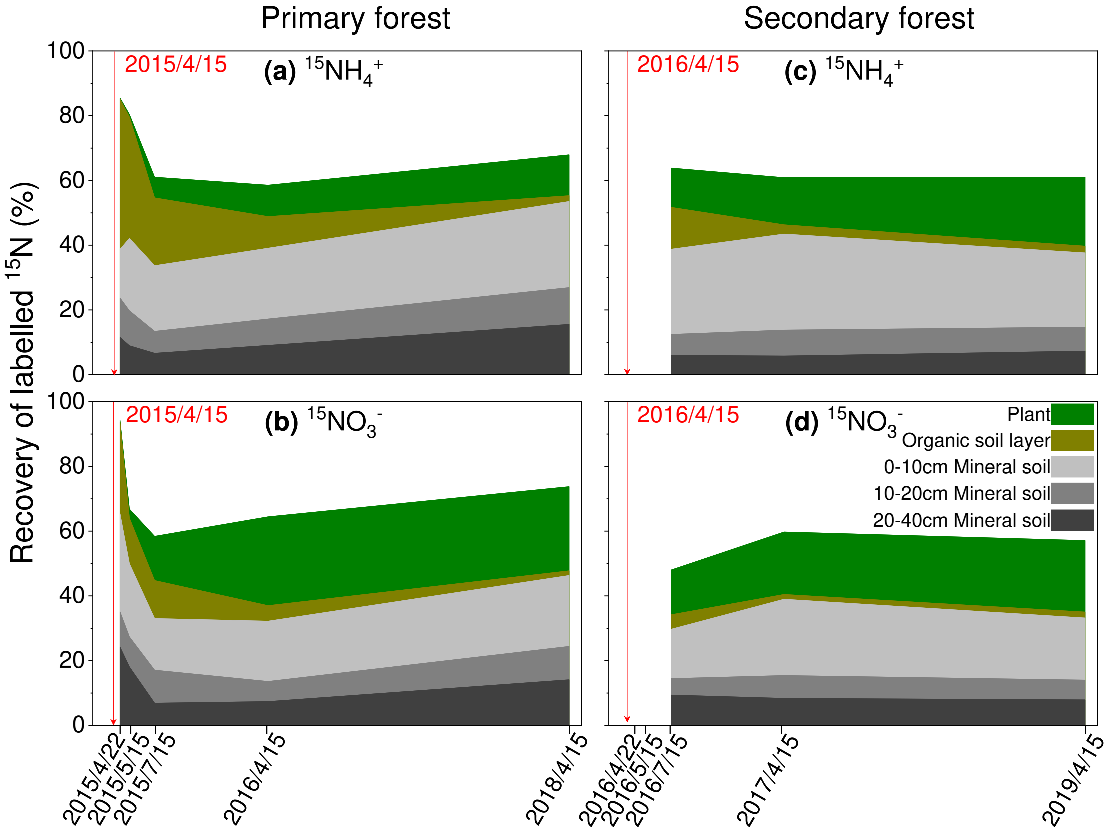
Figure 2



Primary forest
Secondary forest
Recovery of labelled 15N (%)
2015/4/15
(a) 15NH4+
(b) 15NO3-
2016/4/15
(c) 15NH4+
(d) 15NO3-
Plant
Organic soil layer
0-10cm Mineral soil
10-20cm Mineral soil
20-40cm Mineral soil
0
20
40
60
80
100
2015/4/22
2015/5/15
2015/7/15
2016/4/15
2018/4/15
2016/4/22
2016/5/15
2016/7/15
2017/4/15
2019/4/15

858 **Figure 3**

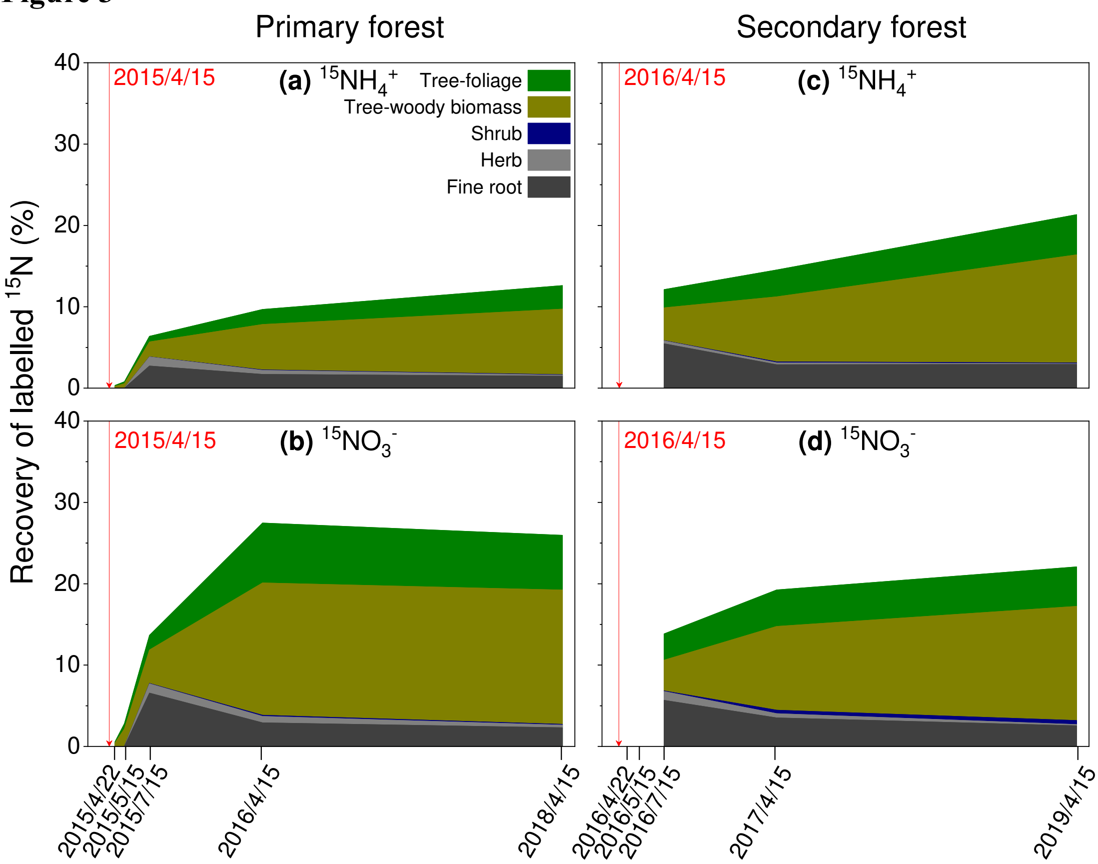


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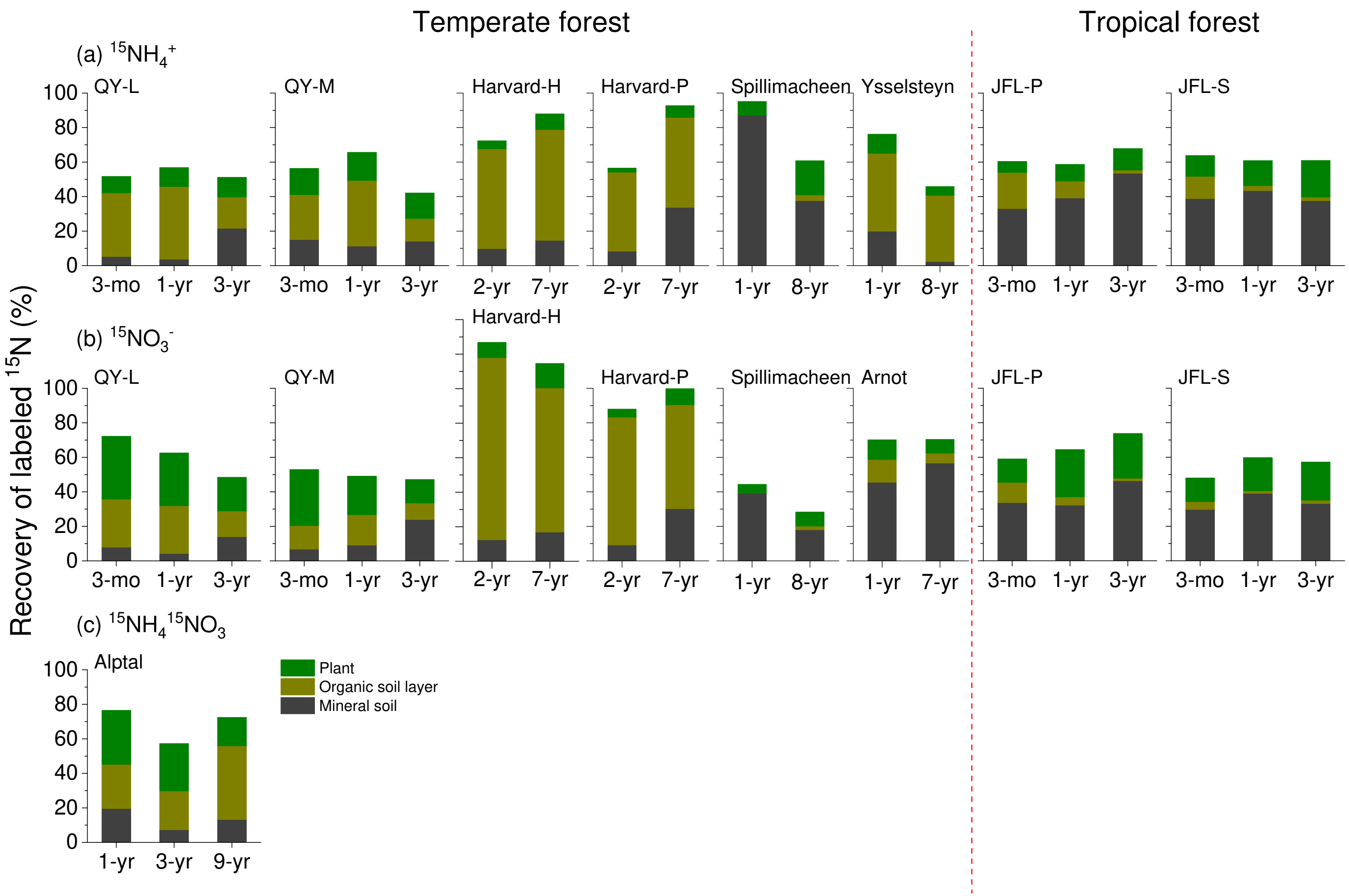
Figure 4


Temperate forest
Tropical forest
(a) 15NH4+
(b) 15NO3-
(c) 15NH415NO3
Recovery of labeled 15N (%)
QY-L
QY-M
Harvard-H
Harvard-P
Spillimacheen
Ysselsteyn
Arnot
JFL-P
JFL-S
Alptal
0 20 40 60 80 100
3-mo 1-yr 3-yr
2-yr 7-yr
1-yr 8-yr
1-yr 7-yr
1-yr 3-yr 9-yr
Plant
Organic soil layer
Mineral soil