Sinks for Inorganic Nitrogen Deposition in Forest Ecosystems with Low and High Nitrogen Deposition in China

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Abstract

We added the stable isotope $^{15}$N in the form of $(^{15}$NH$_4$)$_2$SO$_4$ and K$^{15}$NO$_3$ to forest ecosystems in eastern China under two different N deposition levels to study the fate of the different forms of deposited N. Prior to the addition of the $^{15}$N tracers, the natural $^{15}$N abundance ranging from $-3.4\%$ to $+10.9\%$ in the forest under heavy N deposition at Dingshushan (DHS), and from $-3.92\%$ to $+7.25\%$ in the forest under light N deposition at Daxinganling (DXAL). Four months after the tracer application, the total $^{15}$N recovery from the major ecosystem compartments ranged from 55.3% to 90.5%. The total $^{15}$N recoveries were similar under the $(^{15}$NH$_4$)$_2$SO$_4$ tracer treatment in both two forest ecosystems, whereas the total $^{15}$N recovery was significantly lower in the subtropical forest ecosystem at DHS than in the boreal forest ecosystem at DXAL. The amount of $^{15}$N assimilated into tree biomass represented only 8.8% to 33.7% of the $^{15}$N added to the forest ecosystems. In both of the tracer application treatments, more $^{15}$N was recovered from the tree biomass in the subtropical forest ecosystem at DHS than the boreal forest ecosystem at DXAL. The amount of $^{15}$N assimilated into tree biomass was greater under the K$^{15}$NO$_3$ tracer treatment than that of the $(^{15}$NH$_4$)$_2$SO$_4$ treatment in both forest ecosystems. This study suggests that, although less N was immobilized in the forest ecosystems under more intensive N deposition conditions, forest ecosystems in China strongly retain N deposition, even in areas under heavy N deposition intensity or in ecosystems undergoing spring freezing and thawing melts. Compared to ammonium deposition, deposited nitrate is released from the forest ecosystem more easily. However, nitrate deposition could be retained mostly in the plant N pool, which might lead to more C sequestration in these ecosystems.

Introduction

During the last few decades, atmospheric nitrogen (N) deposition has increased sharply as a result of the consumption of fossil fuels, the emission of industrial waste gases, the excessive application of fertilizers, and the rapid development of animal husbandry [1]. The anthropogenic reactive N emission has increased from 15 Tg N a$^{-1}$ in 1860 to 165 Tg N a$^{-1}$ in early 1990s, and more than 70% of the reactive N deposited back to the terrestrial and aquatic ecosystems [2]. The current global atmospheric deposition of N is approximately 25 to 40 Tg N a$^{-1}$ [3] and is expected to double in the next 25 years [4]. N deposition, as well as increasing concentration of carbon dioxide and ongoing land use/land cover change, has been the well documented issues of global change, altering the biogeochemistry of ecosystems [5].

There has been widespread concern about the effect of increasing N deposition on natural forest ecosystems [6], because of the high sensitivity of biodiversity and productivity of these ecosystems to N input [7]. As one of the most important N sources in forest ecosystem, N deposition increasing might lead to remarkable effects on the forest ecosystem N cycling. Chronic atmospheric N deposition can alter N transmission and transformation processes in the forest ecosystem, such as plant absorption [8], microbe immobilization [9], mineralization [10], nitrification [11], as well as volatilization and leaching losses [12,13].

The increase in available N resulting from atmospheric N deposition could also stimulate carbon (C) sequestration in terrestrial ecosystems and may provide a reasonable explanation for the CO$_2$ “missing sink” [14,15]. The role of N deposition in determining how strongly a forest acts as a sink for CO$_2$ depends on where the deposited nitrogen is immobilized in the ecosystem [16]. The C:N mass ratio in woody tissues is markedly higher than in soil layers, and the turnover time of woody tissues is also longer than that of soils. Therefore, the more deposited N is immobilized in woody tissues, the more C sequestration occurs. In contrast, when more deposited N is immobilized in soil layers, more nitrate leaching and escape of N gases will occur [17,18].
In several studies, the N deposition levels of forest ecosystems were manipulated to evaluate the complex interactions of processes in the N cycle and to measure N cycling within these ecosystems [19,20]. However, in these studies, it is difficult to identify how retention of N deposition is distributed among forest ecosystem components. The N stable isotope tracing method provides a good indicator of the fate and retention of N inputs in forest ecosystems [21,22]. Labeling an N input flux with the stable isotope 15N makes it possible to follow the pathways of N that moves through the ecosystem to assess the fate of N deposition across time scales [23]. Numerous 15N tracing experiments have been conducted in forest ecosystems in Europe and the United States [18].

In China, reactive N emissions have increased more than threefold in the past 30 years, and the N deposition rate shows a higher mean value than is found in the United States and Europe, because of the astonishing economic growth that has occurred since the early 1980s [24,25]. And, there are large spatial differences in the intensity of N deposition among China, owing to the more open N cycle there. We also hypothesized that the recovery of 15N would be higher with the ammonium treatment due to the greater mobility of nitrate.

In this study, our objective was to examine the different fate of N deposition in two old forest ecosystems, by tracing the nitrate and ammonium forms of deposited N by 15N tracers. We hypothesized that less recovery of 15N and lower N assimilation by trees would occur in the N abundant subtropical forest ecosystem than in the N limited boreal forest ecosystem due to the more open N cycle there. We also hypothesized that the recovery of 15N would be higher with the ammonium treatment due to the greater mobility of nitrate.

Materials and Methods

Site Description

We chose two typical old-growth forests to characterize the differences in N deposition residing in these systems and the different contribution of N deposition to C sequestration under different N deposition intensities in eastern China (Table 1). N deposition in the subtropical broad-leaved forest at Dinghushan (DHS) was observed to be as high as 29 kg N ha-1 a-1, and N saturation occurred in this forest ecosystem [19]. In contrast, the boreal coniferous forest at Daxinganling (DXAL) shows low available N, with nitrogen deposition of only 1.3 kg N ha-1 a-1. The predominant species found in the tree layers in the subtropical monsoon evergreen broad-leaved forest are Schima superba, Syzygium jambos, and Castanopsis chinensis [27]. The boreal coniferous forest at DXAL is pure forest in which Larix gmelinii is the predominant tree species [28]. The soils in the two old-growth forests in the south and the north are Greyzems and Ferralsols, respectively. A more extensive description of these sites is given in Table 1.

Experimental Design

In May 2008, six separate plots (9 x 9 m) were randomly distributed at each forest research station, aside the long-term forest ecosystem observation plot, with buffer zones of at least five meters. In three of the plots, a 15N tracer in the form of 99% enriched (15NH4)2SO4 was added. In the other plots, the applied 15N tracer was in the form of 99% enriched K15NO3. The quantity of the 15N tracer applied to each plot was calculated to be 96 mg 15N m-2, thus increasing the ambient 15N concentrations in these ecosystem pools significantly above natural abundance levels while minimizing the disturbance of the soil inorganic N pools. The 15N tracers were dissolved in deionized water and added to each plot in a single application using a portable sprayer.

The control plots for the labeling experiment were situated within the long-term fixed observation plots. Plant and soil samples were collected before and 1 wk, 1 mo, and 4 mo after the addition of 15N. Considering that the intensity of N mobilization is strongest during the spring melt, we collected an additional sample one year after the application of 15N in the boreal forest ecosystem at DXAL.

Sampling and Analysis

By the permissions of Dinghushan Nature Reserve Administration and Daxinganling Hamna Nature Reserve Administration, tree foliage, bark, and wood samples were collected from three each kind of dominant trees within each plot in the two forest ecosystems. Living fine roots (intact and fibrous) were separated from Oi and Oe horizon samples and the 0–10 and 10–20 cm mineral soil layers. Organic soil samples, consisting of the upper Oi horizon and lower Oe+a horizon, were collected from three random (20 x 20 cm) areas within each plot. Three sets of mineral soil profile samples were collected from three depths (0–10, 10–20, and 20–40 cm) with a 5 cm-diameter auger.

Plant and litter samples were dried to a constant weight at 65°C. Mineral soils were air dried at room temperature and then sieved through a 2 mm sieve. All samples were ground into a fine powder with a planetary mill and passed through a no. 100 mesh sieve, then stored in glassware. Plant and soil samples were oven dried at 65°C for 24 h and cooled in an evacuated desiccator immediately prior to analysis.

C and N concentrations and 15N values were determined simultaneously with an automatic online elemental analyzer (Flash EA1112, ThermoFinnigan, Milan, Italy) coupled to an isotope ratio mass spectrometer (IRMS) (Finnigan MAT 253, Thermo Electron, Bremen, Germany). The standard deviation of 10 repeated samples was <0.4%.

Calculations and Statistics

We estimated the movements of the 15N tracers within forest components using an ecosystem N pool size based on field measurements, changes in the 15N contents of the ecosystem pools following tracer addition, and 15N mass recoveries. The percentage of the total amount of tracer applied to the plots recovered within the pools was calculated as follows:

\[ \text{15N} \text{ rec} = \left( \text{atom}\%_{15}\text{N}_{\text{ref}} - \text{atom}\%_{15}\text{N}_{\text{tracer}} \right) \times \frac{N\%_{\text{pool}} \times M_{\text{pool}}}{\left( \text{atom}\%_{15}\text{N}_{\text{tracer}} - \text{atom}\%_{15}\text{N}_{\text{ref}} \right) \times M_{\text{tracer}}} \times 100\% \]

where 15N rec is the percent recovery of the 15N tracer recovered in the labeled N pool (%); atom%15Nref is the atom% of 15N in the labeled N sample; atom%15Ntracer is the atom% of 15N in the reference (pre- or non-labeled) N pool; atom%15Ntracer is the atom% of 15N in the applied tracer; N%pool is the N concentration of the labeled N sample; Mpool is the dry mass of the labeled pool (g m-2); and Mtracer is the mass of 15N in the 15N tracer applied to a plot (g m-2).

The differences in the 15N and N concentrations in the samples were tested through analysis of variance (ANOVA). All analyses were conducted using the SPSS software package (SPSS for
Natural $^{15}$N Abundances and Ecosystem N Pools

Prior to the addition of the $^{15}$N tracers, the natural $^{15}$N abundance in the ecosystem N pools ranged from $-3.4\%$ to $+10.9\%$ in the subtropical forest ecosystem and from $-3.92\%$ to $+7.3\%$ in the boreal forest ecosystem. The natural $^{15}$N abundance exhibited the same increasing tendency in different ecosystem N pools. The $\delta^{15}$N values increased as follows in the tree N pools: wood $>$ roots $>$ foliage $>$ branches and increased from the forest floor to the deep mineral soils (Table 2). While the mean S.E. did not exceed $0.8\%$ in nearly all of the N pools, the natural $^{15}$N abundances in the two forest ecosystems were well defined with respect to their isotopic compositions.

The ecosystem N pool of the subtropical forest ecosystem consisted of $5.76$ Mg N (excluding shrubs, herbage, and soils $>$ 20 cm deep), which was more than twice the size of the N pool found in the boreal coniferous forest (Table 2). The mineral soils contained the greatest amounts of N in both forest ecosystems. The N pool of the 0–20 cm soil layer accounted for $54\%$ of the total N in the subtropical forest ecosystem, and this proportion was more than $80\%$ in the boreal forest ecosystem. In the tree pools, the total N pools were relatively large, accounting for more than $40\%$ of the total N pool in the subtropical forest ecosystem, whereas this proportion was only $11.8\%$ in the boreal forest ecosystem. The forest floor pool accounted for only $5.1\%$ and $5.6\%$ of the total of measured N pools in the subtropical and the boreal forests, respectively.

Total $^{15}$N Recovery

In the subtropical forest ecosystem, the average total $^{15}$N recovery was $71.9\%$ one week after the addition of the $^{15}$NH$_4$SO$_4$ tracer, and the recovery rapidly rose within one month and decreased after the rapid growth season. The same fluctuation of the $^{15}$N recovery was observed following K$^{15}$NO$_3$ treatment (Fig. 1a). The total $^{15}$N recoveries were much higher following treatment with the $^{15}$NH$_4$SO$_4$ tracer than the K$^{15}$NO$_3$ tracer, and significant differences were detected for all of the three sampling times (Fig. 1a).

In the boreal forest ecosystem, the total recovery of the applied $^{15}$N declined a little in the first month, but rose to $90.5\%$ after four months. The total recovery rate decreased back again one year later (Fig. 1b). As in the subtropical forest ecosystem, the fluctuation of $^{15}$N recovery was same in the K$^{15}$NO$_3$ tracer treatment, and there were also significant differences in total $^{15}$N recovery rates between the two tracers' treatments. However, these differences disappeared four months later in the boreal forest ecosystem (Fig. 1b).

N Deposition Retention

Based on the $^{15}$N recovery in each N pool and the N deposition flux measured in the forest ecosystem, the N deposition retention was calculated. More than three quarters of deposited N was retained in the forest ecosystem after the growing season or the rapid growth season, because ammonium immobilization rate was high and a large proportion of N deposition was in the form of ammonium in China (Table 3). Even after the spring freezing and thawing melts, there was still more than half part of deposited N was retained in the forest ecosystem.

$^{15}$N Recovery in each N Pool

The fate of the added $^{15}$N was significantly different between the two tracers' treatments in the subtropical forest ecosystem. Four months after $^{15}$NH$_4$SO$_4$ tracer addition, the organic soil layer (comprising the upper Oi horizon and lower Oe+a horizon) was found to be the largest sink. The second is the mineral soil layer, and tree N pool accounted for less than one third of the total recovered $^{15}$N tracer (Fig. 2). In contrast, following treatment with the K$^{15}$NO$_3$ tracer, the largest sink was the tree N pool, and the

| Sites | Dinghushan (DHS) | Daxinganling (DXAL) |
|-------|----------------|---------------------|
| Forest type | Subtropical evergreen broadleaved forest | Boreal coniferous forest |
| State age (yr) | 400 | 180 |
| Location | 23°10'N, 112°34'E | 50°56'N, 121°30'E |
| Elevation (m) | 300 | 810 |
| Mean annual temperature (°C) | 20.9 | -5.4 |
| Mean annual precipitation (mm) | 1 564 | 500 |
| N deposition (kg N ha$^{-1}$ yr$^{-1}$) | 29.5 | 1.8 |
| Net N mineralization (kg N ha$^{-1}$ yr$^{-1}$) | 164.1 | 71.7 |
| Biomass (Mg C ha$^{-1}$) | 87.7(8.7) | 56.1(4.8) |
| Litter input (Mg C ha$^{-1}$ yr$^{-1}$) | 8.42(0.47) | 2.50(0.27) |
| Gravel (0.002–0.02 mm, %) | 19.65 | 51.76 |
| Sand (0.02–0.2 mm, %) | 19.65 | 27.55 |
| Clay (<0.002 mm, %) | 26.22 | 9.53 |
| SOC density (kg m$^{-2}$) | 8.80(0.58) | 14.62(0.35) |
| Soil pH | 3.80(0.11) | 6.03(0.09) |

Data source: Chinese Ecosystem Research Network (CERN) database.

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recovered $^{15}$N tracer in both organic soil layer and mineral soil layer was less than 40% (Fig. 2).

In the boreal forest, the trees contained the minimum $^{15}$N pool four months after $^{15}$N tracer application in both the $(^{15}$NH$_4)^2$SO$_4$ and K$^{15}$NO$_3$ tracer treatments. The difference between the treatments using the two different tracers was because that the organic soil layer represented the largest $^{15}$N sink in the $(^{15}$NH$_4)^2$SO$_4$ tracer treatment, while the mineral soil layer was the largest $^{15}$N sink in the K$^{15}$NO$_3$ tracer treatment (Fig. 2).

Impact of Spring Melt on $^{15}$N Retention

After the repeated freezing and thawing cycles in spring, total $^{15}$N recovery decreased by 36.7% and 29.8% under the $(^{15}$NH$_4)^2$SO$_4$ and K$^{15}$NO$_3$ tracer treatments, respectively, in the boreal forest ecosystem (Fig. 1). The reduction of retained $^{15}$N from the forest ecosystem mainly occurred in the soil layer (Fig. 3). The amount of reduction in the organic soil and the mineral soil were almost same in the $(^{15}$NH$_4)^2$SO$_4$ tracer treatment, while the mineral soil layer was the largest $^{15}$N sink in the K$^{15}$NO$_3$ tracer treatment (Fig. 2).

Discussion

Natural $^{15}$N Abundances

In our study, $^{15}$N natural abundance in the foliage and branches in the subtropical forest was higher than in the boreal forest. Meta-analysis of $^{15}$N tracer field studies in Europe and North America indicates that foliar $^{15}$N natural abundance is a good indicator of ecosystem N-retention capacities [18]. This point is also supported by our study in eastern China, for the average total $^{15}$N tracer recovery rate was significantly higher in the boreal forest ecosystem than in subtropical forest ecosystem, after the growing season or fast growth season (Fig. 1).

Table 2. Natural $^{15}$N abundance and N mass in the major ecosystem pools in the two old growth forest ecosystems.

| Ecosystem pool | Subtropical forest ecosystem (DHS) | Boreal forest ecosystem (DXAL) |
|----------------|-----------------------------------|--------------------------------|
|                | Natural $^{15}$N abundance (%)     | Mass* (kg/ha) | N (kg/ha) | C/N | Natural $^{15}$N abundance (%) | Mass* (kg/ha) | N (kg/ha) | C/N |
| Tree           | Foliage                           | $-2.26(0.25)$ | 8,483     | 163.9 | 23.8 | $-3.92(0.34)$ | 2,039     | 69.2   | 15.2 |
|                | Branches                          | $-3.08(0.25)$ | 86,699    | 934.7 | 40.3 | $-4.99(0.21)$ | 11,874    | 50.4   | 122.4|
|                | Wood                              | $-1.22(0.38)$ | 196,740   | 469.6 | 183.5| $-1.20(0.56)$ | 86,197    | 66.9   | 705.1|
|                | Roots                             | $0.82(0.44)$  | 59,312    | 787.3 | 33.7 | $9.88(1.46)$  | 22,266    | 112.9  | 108.8|
| Forest floor   | Oi                                | $-2.49(0.26)$ | 7,612     | 145.4 | 26.3 | $-0.93(0.04)$ | 5,526     | 71.8   | 35.0 |
|                | Oa+e                              | $-2.90(0.15)$ | 12,796    | 147.2 | 33.7 | $-1.26(0.18)$ | 5,557     | 74.1   | 35.9 |
| Mineral soil   | 0–10 cm                           | $6.21(0.49)$  | 861,000   | 2,000.0 | 18.8 | $4.34(0.27)$  | 151,333   | 1,057.7 | 30.1 |
|                | 10–20 cm                          | $8.91(0.20)$  | 974,000   | 1,109.4 | 23.3 | $6.41(0.22)$  | 748,400   | 1,024.9 | 21.0 |
|                | Total                             | –                | 5,757.4   | –       | –    | 2,527.8       | –         | –      | –    |

a: From a database of the Chinese Ecosystem Research Network (CERN).
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Figure 1. Changes in total $^{15}$N recovery rates in the subtropical forest ecosystem at DHS (a) and in the boreal forest ecosystem at DXAL (b) in the $(^{15}$NH$_4)^2$SO$_4$ and K$^{15}$NO$_3$ tracer treatments at one week, one month, four months, and one year (sampled only at DXAL) after the addition of the $^{15}$N tracer. An asterisk(*) means that there is a significant difference in $^{15}$N recovery rates between the two tracer treatments ($p$$<$0.05). doi:10.1371/journal.pone.0089322.g001
However, the $^{15}$N natural abundance in roots was much greater in the boreal forest than in the subtropical forest (Table 2). The rate of N-cycling, N losses, and mycorrhizal association are the possible reason for the difference of plant $^{15}$N natural abundance in the terrestrial ecosystems [29]. The inconsistent variation of $^{15}$N natural abundance indicated that the plants survived the low inorganic N available mainly by the existence of mycorrhizal fungi in the boreal forest ecosystem. Through the proliferation of their hyphae, mycorrhizal fungi supply their host plants with organic N via protein degradation [30]. The transfer of N from mycorrhizal fungi to their hosts favors 15N-depletion because of fractionation during metabolic processes and the selective retention of specific N compounds by the fungi, thus the fungal N is enriched by 3–11% relative to the host plant N [29,31]. Therefore, the $^{15}$N natural abundance in the foliage and branches was relatively low, whereas the $^{15}$N natural abundance in infected roots was considerably higher in the boreal forest ecosystem.

N Deposition Retention

In our study, the average total $^{15}$N tracer recovery rate was significantly higher in the boreal forest ecosystem than in subtropical forest ecosystem (Fig. 1, Table 3). The $^{15}$N recovery rate was significantly higher in the $\left( \text{NH}_4 \right)^2\text{SO}_4$ treatment than in the $\text{K}^{15}\text{NO}_3$ treatment, especially at the beginning of the experiment (Fig. 1). At the time N deposition entering the forest ecosystem, ammonium is likely retained on cation exchange sites in soil organic matter and clays, while nitrate is more mobile and prone to uptake by plants and leaching or gaseous losses during denitrification [32,33]. Therefore, more $^{15}$N tracer was recovered in the $\left( \text{NH}_4 \right)^2\text{SO}_4$ treatment, but was mainly stored in the organic and mineral soil layers. However, the less $^{15}$N tracer was recovered in the $\text{K}^{15}\text{NO}_3$ treatment, but more was utilized by trees (Fig. 2). Because the ammonium recovery rate is higher and the N deposition was mainly in ammonium in the forest ecosystem in China [26], more than three quarter of the N deposition could be retained in the forest ecosystem in the growing or fast growth season (Table 3).

Table 3. Nitrogen deposition and retention in the two old growth forest ecosystems during the experimental period.

| Nitrogen deposition* | Immobilization rateb | Nitrogen deposition retention |
|----------------------|----------------------|-----------------------------|
|                      | kg N/(ha a) | (%) | Ammonium | Nitrate | Ammonium | Nitrate | % |
| Subtropical evergreen forest (DHS) | 6.9 | 4.9 | 89.7 | 55.3 | 75.4 |
| Boreal coniferous forest (DXAL) | 0.7 | 0.3 | 90.5 | 85.4 | 88.9 |

*Inorganic nitrogen deposition from May to August 2008, from a database of the Chinese Ecosystem Research Network (CERN).

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**Total $^{15}$N recovery in the ecosystem four months after the application of the $\left( \text{NH}_4 \right)^2\text{SO}_4$ and $\text{K}^{15}\text{NO}_3$ tracers.

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Figure 2. Percentage of recovered $^{15}$N in different ecosystem N pools in the subtropical forest ecosystem at DHS and in the boreal forest ecosystem at DXAL in the $\left( \text{NH}_4 \right)^2\text{SO}_4$ and $\text{K}^{15}\text{NO}_3$ application treatments.

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Substantial capacity to store excess N. However, the heavy forest ecosystem, historically poor in N availability, would have a production and loss of N from the ecosystem [37]. The boreal N, thus stimulating ammonium oxidizers, and leading to elevated ecosystem, and changed the N saturation status of the forest [18]. The deposited N from atmosphere accumulated in the another most consistent and useful indicator of N retention rate soil C:N ratio [18].

Reasons for N Retention Difference in the Two Forest Ecosystem

As we hypothesized, the 15N recovery rate was continuously lower in the tropical forest ecosystem than in the boreal forest ecosystem after the growing season or the fast growth season (Fig. 1). The deposited N partly was absorbed by plants and by microbes, partly was immobilized in the soil through abiotic way, and partly escaped from the ecosystem by leaching and gas emission. Higher temperature in the tropical forest ecosystem might lead to greater rates of N uptake by plants and microbes, while, the rates of soil N-cycling processes, such as mineralization and nitrification, and losses via gas emissions or leaching, were also increased by the higher temperature. And the possible increases in rates of soil N-cycling processes could have more than offset the greater uptake. In the subtropical forest ecosystem, nitrification accounts for close to 80% of the net mineralization, and nitrate is the main N source in this forest ecosystem [34]. Therefore, 15N tracer recovery was relatively low in the treatment involving the K15NO3 tracer application (Fig. 1 Table. 3).

The boreal forest ecosystem was located in northeast China, where microbial activity is restricted by low temperature, and there is far less available N than is needed (Table 1). External N could be tightly fixed in the ecosystem when entering to the ecosystem. The higher soil C:N, which promotes microbial N immobilization and reduces net nitrification, could be another reason contributing to greater 15N recovery rate in the boreal forest. Especially, C:N in the organic soil, strongly influences the nitrate losing in the forest ecosystem [35]. Study across the forest ecosystems of Europe and North America also supports that the total ecosystem retention of applied 15N positively correlates with the main N source in this forest ecosystem [34]. Therefore, 15N accounts for close to 80% of the net mineralization, and nitrate is more mobile than ammonium but also because nitrate is more important in balancing cation uptake [32,33]. The utilization of deposited N by the tree in subtropical forest ecosystem was higher than in boreal forest ecosystem (Fig. 2). The difference in N uptake by tree between the treatments involving the two 15N tracers was much smaller in the boreal forest ecosystem, likely due to the high ammonia absorption rate under N limited conditions [39,40].

However, contrary to our hypothesis, the stimulating effects of external N on tree growth were not obvious in the boreal forest ecosystem, most likely due to the difference in tree species and the competition for N between bacteria and vegetation in this N limited ecosystem [41]. Although N saturation has been observed in the subtropical forest ecosystem [19], the utilization of external N by tree was still higher than that in the boreal forest ecosystem. The role of N deposition in determining how strongly a forest acts as a sink for CO2 depends on where the deposited nitrogen is immobilized and reduces net nitrification, could be another reason contributing to greater 15N recovery rate in the boreal forest ecosystem.

Contribution of Deposited N to C Sequestration

Following the growing season or the rapid growth season, a greater amount of N was utilized by tree following K15NO3 tracer application compared to (15NH4)2SO4 tracer application in both forest ecosystems (Fig. 2). This occurred not only because nitrate is more mobile than ammonium but also because nitrate is more important in balancing cation uptake [32,33]. The utilization of deposited N by the tree in subtropical forest ecosystem was higher than in boreal forest ecosystem (Fig. 2). The difference in N uptake by tree between the treatments involving the two 15N tracers was much smaller in the boreal forest ecosystem, likely due to the high ammonia absorption rate under N limited conditions [39,40].

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Uncertainties

In this study, we explored where deposited N ultimately resides as well as the role that N deposition plays in contributing to C sequestration in the forest ecosystems of China. However, due to the lack of enough technical and capital supports, there are still some shortcomings that need to be overcome in the future. First of all, the 15N tracers were sprayed on the forest floor in this study, giving no considerations to the possibility of deposited N absorption by foliage. Therefore, the utilization of deposited N might have been underestimated in this study, because previous studies show that nitrogen can be absorbed by tree canopies from atmospheric input, and this process relates to both ammonium and nitrate ions [42-44]. Secondly, only foliage, bark, and wood of dominant trees, as well as organic layer and mineral soil above 20 cm were sampled within each plot. Given the possible immobilization of deposited N by other plants and in the deep soil layers, the utilization of deposited N by the forest ecosystems might have been underestimated to some extent in this study. Finally, less sampling frequency and shorter duration of the experiment might also be the source of uncertainty in this study. These all need to be perfected in the future studies.

Conclusions

This study traced atmospheric ammonium and nitrate deposition under different N deposition levels via the application of (15NH4)2SO4 and K15NO3 in two forest ecosystems in China. Prior to 15N tracer application, the natural 15N abundance showed the same pattern in different ecosystem N pools in these two forest ecosystems, with the following order found in the tree N pools:
accounting for 9.8% and 13.4% of the total recovered $^{15}$N pools under (NH$_4$)$_2$SO$_4$ and K$^{15}$NO$_3$ application, respectively. The subtropical forest ecosystem at DHS were 89.7% and 55.3% in areas under a heavy N deposition intensity and in ecosystems, respectively. Our findings suggest that more than half of atmospheric N deposition is immobilized in the forest ecosystems of China, even in areas under a heavy N deposition intensity and in ecosystems undergoing spring freezing and thawing melts. Although deposited nitrogen is readily released from forest ecosystems, nitrate deposition is immobilized more in tree biomass, which means that it would make more contributions to C sequestration than ammonia deposition in the forest ecosystems of China.

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**Author Contributions**

Conceived and designed the experiments: WS GY HF. Performed the experiments: WS JY MZ. Analyzed the data: WS. Contributed reagents/materials/analysis tools: GY. Wrote the paper: WS. Reviewed the manuscript: CJ.

**References**

1. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, et al. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320: 892–895.

2. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, et al. (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153–226.

3. Nadelhoffer KJ, III, NF, Vitousek PM (2000) Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. Frontiers in Ecology and the Environment 1: 205–211.

4. Lamard JP, Kriebel J, Brasseur G, Butler T, Cameron-Smith P, et al. (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. Journal of Geophysical Research: Atmospheres (1984–2012) 110.

5. Vitousek PM (1994) Beyond global warming: ecology and global change. Ecology 75: 1061–1076.

6. Kolocupak JM, Barou MJ, Johnson DW (2006) Potential canopy interception of nitrogen in the Pacific Northwest, USA. Forest ecology and management 234: 344–354.

7. Aber JD, Magill AH (2004) Chronin nitrogen additions at the Harvard Forest (USA) the first 15 years of a nitrogen saturation experiment. Forest Ecology and Management 196: 1–5.

8. Magill AH, Aber JD, Bernston GM, McDowell WH, Nadelhoffer KJ, et al. (2000) Long-term nitrogen additions and nitrogen saturation in two temperate forest ecosystems. Am Nat 155: 239–253.

9. Tietema A (1990) Microbial carbon and nitrogen dynamics in coniferous forest leaf material collected along a European nitrogen deposition gradient. Forest Ecology and Management 29: 101–296.

10. Chen Y, Högberg P (2006) Gross nitrogen mineralization rates still high 14 years after suspension of N input to a N-saturated forest. Soil Biology and Biochemistry 38: 2001–2003.

11. Vente R, Grofman PM, Verchot LV, Magill AH, Aber JD (2004) Gross nitrogen process rates in temperate forest soils exhibiting symptoms of nitrogen saturation. Forest ecology and management 196: 129–142.

12. Fang Y, Gundersen P, Mo J, Zhu W (2009) Nitrogen leaching in response to increased nitrogen inputs in subtropical monsoon forests in southern China. Forest Ecology and Management 257: 332–342.

13. Hoegberg P, Fan H, Qium M, Birkley D, Tamm CO (2006) Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. Global Change Biology 12: 489–499.

14. Gifford K (1994) The global carbon cycle: a viewpoint on the missing sink. Functional Plant Biology 21: 1–15.

15. Luo Y, Hui D, Zhang D (2006) Elevated CO2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87: 53–63.

16. Magnani F, Mencuccini M, Bergatti M, Berbigier P, Berninger F, et al. (2007) The human footprint in the carbon cycle of temperate and boreal forests. Nature 447: 849–851.

17. Nadelhoffer KJ, Emmett BA, Gundersen P, Kjønsaas OJ, Koopmans CJ, et al. (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398: 145–148.

18. Tinner P, Mack III, FC, Christlinder L, Compson J, et al. (2012) Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of 15N tracer field studies. Ecology 93: 1169–1182.

19. Fang Y, Gundersen P, Mo J, Zhu W (2008) Output and input of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution. Biogeosciences 5: 339–352.

20. Wright RF, Rasmussen L (1990) Introduction to the NITREX and EXMAN projects. Forest Ecology and Management 101: 1–7.

21. Schlesinger WH (2009) On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences 106: 203–208.

22. Turner MM, Henry HA (2009) Interactive effects of warming and increased nitrogen deposition on 15N tracer retention in a temperate old field: seasonal trends. Global Change Biology 15: 2885–2893.

23. Currie WS, Nadelhoffer KJ (1995) Original Articles: Dynamic Redistribution of Isotopically Labeled Cohorts of Nitrogen Inputs in Two Temperate Forests. Ecosystems 2: 4–10.

24. Liu X, Zhang Y, Han W, Tang A, Shen J, et al. (2013) Enhanced nitrogen deposition over China. Nature 494: 439–442.

25. Lu C, Tian H (2007) Spatial and temporal patterns of nitrogen deposition in China: Synthesis of observational data. Journal of Geophysical Research: Atmospheres (1984–2012) 112.

26. Sheng W, Yu G, Jiang C, Yan J, Liu Y, et al. (2015) Monitoring nitrogen deposition in typical forest ecosystems along a large transect in China. Environmental monitoring and assessment 185: 833–844.

27. Mo J, Zhang W, Zhu W, Gundersen P, Fang Y, et al. (2008) Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14: 403–412.

28. Fan P, Jiang Y (2010) Nitrogen dynamics differed among the first six root branch orders of Fraxinus mandshurica and Larix gmelinii during short-term decomposition. Journal of plant research 123: 437–438.

29. Hogberg P (1997) Tanley review No 95 - N-15 natural abundance in soil-plant systems. New Physiologist 137: 179–203.

30. Hobbie EA, Hobbie JR (2008) Natural abundance of (15)N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: A review, Ecosystems 11: 815–830.

31. Hogberg P, Hogberg L, Schénkel H, Högberg M, Johannisson C, et al. (1996) N-15 abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108: 207–214.

32. Koha K, Hirose M, Koyama L, Kohau A, Tokuchi N, et al. (2003) Natural N-15 abundance of plants and soil N in a temperate coniferous forest. Ecosystems 6: 457–469.

33. Peri PL, Ladd B, Pepper DA, Bonser SP, Lafijn SW, et al. (2011) Carbon (13C) and nitrogen (15N) stable isotope composition in plant and soil in Southern Patagonia’s native forests. Global Change Biology 18: 1365–2486.

34. Fang HF, Yu GR, Cheng SL, Zhu TH, Wang YS, et al. (2010) Effects of multiple environmental factors on CO2 emission and CH4 uptake from old-growth forest soils. Biogeosciences 7: 395–407.

35. Lovett GM, Christenson LM, Groffman PM, Jones CG, Hart JE, et al. (2002) Insect defoliation and nitrogen cycling in forests. BioScience 52: 335–341.

36. Aber J, McDowell W, Nadelhoffer K, Magill A, Berninger F, et al. (1998) Nitrogen saturation in temperate forest ecosystems. BioScience 48: 921–934.

37. Gundersen P, Schmidt IK, Raauld-Rasmussen K (2006) Leaching of nitrate from temperate forest ecosystems affects of air pollution and forest management. Environmental Reviews 14: 1–57.

38. Aber JD, Goodale CL, Ollinger SV, Smith ML, Magill AH, et al. (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience 53: 375–389.

39. Xu XL, Ouyang H, Richter A, Wanek W, Cao GM, et al. (2011) Spatio-temporal variations determine plant-microbe competition for inorganic nitrogen in an alpine meadow. Journal of Ecology 99: 563–571.

40. Zhang B, Liang C, He HB, Zhang XD (2013) Variations in Soil Microbial Communities and Residues Along an Altitude Gradient on the Northern Slope of Changbai Mountain, China. PloS One 8.
41. Preston CM, Marshall VG, McCallough K, Mead DJ (1990) Fate of 15N-labelled fertilizer applied on snow at two forest sites in British Columbia. Canadian journal of forest research 20: 1583–1592.

42. Garten C, Schwab A, Shirshac T (1998) Foliar retention of 15N tracers: implications for net canopy exchange in low- and high-elevation forest ecosystems. Forest ecology and management 103: 211–216.

43. Garten Jr CT, Hanson PJ (1990) Foliar retention of 15 N-nitrate and 15 N-ammonium by red maple (Acer rubrum) and white oak (Quercus alba) leaves from simulated rain. Environmental and experimental botany 30: 333–342.

44. Stachurski A, Zimka J (2002) Atmospheric deposition and ionic interactions within a beech canopy in the Karkonosze Mountains. Environmental pollution 118: 75–87.