Global Ecology and Conservation 33 (2022) e01966

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

The retention dynamics of early-spring N input in a temperate forest ecosystem: Implications for winter N deposition

Linna Ma [a,b,*,1], Chaoxue Zhang [a,b,1], Yixia Lv [a,b], Renzhong Wang [a,b,*]

[a] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China
[b] University of Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Keywords:
Forest
^{15}N
N deposition
N dynamic
Snowmelt
Winter

ABSTRACT

In N-limited temperate regions of China, rates of atmospheric N deposition remain high during winter due to industrial development and energy consumption. Winter-deposited N accumulates and is then released after snowmelt. However, little is known about the retention dynamics of early-spring N input in temperate forest ecosystems. We applied ^{15}N isotopic tracer after snowmelt, and then quantified ^{15}N dynamics in litter, soils, microbes and vascular plants over the following growing season in a warm temperate forest of northern China. In early spring (7 days after ^{15}N addition), approximately 80% of applied ^{15}N was retained in the ecosystem. The ^{15}N recovery was the highest in litter, followed by soils and microbes, with only trivial acquisition in vascular plants. After early spring, there was little change in total ^{15}N recovery over the following season, which indicated that the temperate forest ecosystem had high potential for the retention of early-spring N input. The ^{15}N levels gradually declined in litter and microbes, while they were gradually increased in the vascular plants. In late fall, substantial ^{15}N tracer retained in litter and was resorbed from senescing tissues to roots. Evergreen coniferous trees presented higher ^{15}N acquisition than deciduous broad-leaved trees. Our results suggest that substantial early-spring N input can be retained in warm temperate forest ecosystems. The findings highlight the importance of litter and plants in sustaining early-spring exogenous N resources, inferring the need to considering winter N deposition for a better understanding of N cycling in temperate ecosystems.

1. Introduction

During the past several decades, human activities, including industrial development and agricultural activities, have dramatically increased atmospheric nitrogen (N) deposition rates in N-limited ecosystems (Fowler et al., 2013; Yu et al., 2019; IPCC (Intergovernmental Panel on Climate Change), 2021). In the temperate regions, the annual N deposition rate is approximately 1–2 g m^{-2} y^{-1}, and the rate is predicted to reach 5 g N m^{-2} y^{-1} by 2050 (Galloway et al., 2004). Therefore, a huge body of recent research has assessed the fates of N deposition with ^{15}N tracers in arctic, subarctic, and temperate ecosystems in the context of plant growing seasons (Nadelhoffer et al., 1999; Gurmesa et al., 2016; Liu et al., 2017; Li et al., 2019; Wang et al., 2021). There is evidence that wet and dry N deposition during winter remains high due to industrial development and substantial energy consumption (i.e., industrial production

* Corresponding authors at: State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China.
E-mail addresses: maln@ibcas.ac.cn (L. Ma), wangrz@ibcas.ac.cn (R. Wang).
[1] Linna Ma and Chaoxue Zhang contributed equally to this work.

https://doi.org/10.1016/j.gecco.2021.e01966
Received 21 October 2021; Received in revised form 27 November 2021; Accepted 12 December 2021
Available online 20 December 2021

and the combustion of coal, oil, and natural gas), accounting for approximately 15–20% of the annual N deposition fluxes in the temperate ecosystems of China (Wang et al., 2020). The ammonium and nitrate N deposited during the winter accumulate in the snowpack, resulting in a pulse of N release through the snowmelt process in early spring (Ma et al., 2020). The deposited N (mainly NO_X) is vulnerable to leaching losses and volatilization, and the extent to which the early-spring N input is retained in the ecosystem over the following growing seasons may have important implications for plant growth in N-limited ecosystems (Du et al., 2020).

Previous studies of ecosystem N dynamics over winter and spring have focused primarily on an arctic meadow (Jaeger et al., 1999), arctic and alpine tundra (Bilbrough et al., 2000; Liu et al., 2018), subarctic heaths (Grogan et al., 2004; Larsen et al., 2012), temperate old fields (Turner and Henry, 2009; Vankoughnett and Henry, 2014), and a temperate grassland (Ma et al., 2018). The capacity of ecosystems to retain early spring N resources is mainly dependent on the ecosystem type, plant functional type, mycorrhizal colonization rate, and soil properties, as well as the form of ^{15}N applied. For example, the N retention was high during the snowmelt period in arctic and alpine meadows (Jaeger et al., 1999; Bilbrough et al., 2000; Edwards and Jefferies, 2010; Larsen et al., 2012), but very low during the same period in an arctic tundra (Bilbrough et al., 2000). Although studies have shown inconsistent results for the magnitude of early-spring N retention within these ecosystems, there is evidence that early-spring N resources play an important role in ecosystem functioning.

To date, evidence for the retention dynamics of early-spring N input is limited to arctic tundra and alpine and temperate grasslands (Bilbrough et al., 2000; Tye et al., 2005; Ma et al., 2020). These observations have revealed that the N input retention capacity after snowmelt varies among ecosystems. For instance, Joseph and Henry (2009) and Ma et al. (2020) both applied ^{15}N after snowmelt and found that the ^{15}N recovery in a temperate old field was only a small fraction, and the vascular plants were found to be the major sink, while the ^{15}N recovery in a temperate grassland was approximately 85% and the soil and microbes were the most important sinks over the following seasons. Despite strong competitive interaction between plants and microbes for exogenous N pulses, this ecological consequence protects ecosystems from N losses through leaching, volatilization and denitrification. However, to the best of our knowledge, the retention dynamics of early-spring N input within temperate forest ecosystems have not yet been investigated.

It has been proposed that coexisting vascular plants may be able to partition the soil N pool in order to avoid competition for limited resources (Reynolds et al., 2003). The magnitude of N acquisition by different plant species during winter and the snowmelt period varies widely likely because of differences in the root traits, mycorrhizal colonization rate, and phenological period of various species (Andresen and Michelsen, 2005; Larsen et al., 2012; Ma et al., 2021). For example, Andresen and Michelsen (2005) demonstrated that evergreen shrubs had a higher potential for N uptake than deciduous shrubs and graminoids during winter and early spring due to their higher specific root length and mycorrhizal colonization rate in a temperate heath. However, the early-spring N retention capacity of different plant functional types is seldom compared in temperate forests.

To investigate the dynamics of early-spring N input in a temperate forest ecosystem, we conducted a field experiment in which we artificially applied ammonium nitrate-$^{15}N_2$ ($^{15}NH_4{}^{15}NO_3$) after snowmelt to simulate the early-spring N input. We hypothesized that the greatest quantity of early-spring N input would be retained in litter in the short-term, based on the assumption that the litter layer is very important in temperate forests for N retention due to its high absorbance capacity (Liu et al., 2016); (2) the early-spring N would be gradually acquired by vascular plants over the following season, based on the assumption that N often limits temperate forest productivity (Magill et al., 2000); and (3) the broad-leaved trees would acquire higher early-spring exogenous N than the coniferous trees based on the assumption that the broad-leaved trees need to rapidly acquire nutrients during growing season to ensure that they produce enough energy to sustain them during periods when photosynthesis is impossible (Nadelhoffer et al., 1999).

2. Materials and methods

2.1. Study site

This study was conducted at the Beijing Forest Experimental Station of the Institute of Botany, Chinese Academy of Sciences (39.57°N, 115.26°E, 1248–1509 m a.s.l), situated on Dongling Mountain, northern China. The climate at this study site is a typical temperate continental monsoon climate with a mean annual air temperature of 11 °C and mean annual precipitation of 550 mm (http://data.cma.cn). The period from early October to mid-March of the following year is the winter (daily mean air temperatures $\leq$ 0 °C) (Sang et al., 2002). In winter, most of the precipitation falls as snow, and the snowpack lasts for approximately 170 days. The winter precipitation accounts for approximately 12% of annual mean precipitation in the temperate forest (http://data.cma.cn). In late winter, the snow depth is typically 25–30 cm. The period from mid-February to mid-March is characterized as early spring (i.e. the snowmelt period), during which snow completely melts and frozen soils completely thaw. Soil temperatures rapidly increase after snowmelt, and then peak at approximately 20 °C from mid-July to mid-August (the peak biomass period).

The soil is classified as a Eutric Cambisol (FAO-WRB (ISSS-ISRIC-FAO-UNESCO), 1998), with a depth of 60 cm. The soil sand, silt and clay content are approximately 41%, 40% and 19%, respectively. The soil bulk density is 0.9 g cm^{-3} and the pH is 6.8. The contents of soil organic carbon (C) and total N (0–15 cm) are approximately 4% and 0.3%, respectively. The zonal vegetation in the warm temperate forest is highly heterogeneous. We selected a 40-year-old mixed forest of planted evergreen coniferous tree species (*Pinus tabuliformis formis* Carrière) and naturally regenerated broad-leaved tree species (*Quercus wutaishanica* Mayr) for investigation. The dominant deciduous shrub is *Abelia biflora* Turcz. and the dominant herb is *Carex duriusata* C. A. Mey. Detailed information on the investigated vascular plant species investigated is presented in Table S1.

2.2. Experimental design

On 18 September 2018, eight 8 m × 8 m plots, with at least a 6-m buffer between any two adjacent plots were established in the site, covering a total area of 50 m × 60 m. Each plot contained all the investigated plant species. The eight plots comprised four plots with the addition of $^{15}NH_4{}^{15}NO_3$ solution (98 atom% ^{15}N, Shanghai Research Institute of Chemical Industry, China), while four plots were designated as control treatments (injected with water instead of ^{15}N solution). The eight plots were assigned randomly in the study site (two treatments × four replicates).

We added $^{15}NH_4{}^{15}NO_3$ solution three days (15 March 2019) after the final snowmelt and the last of the free standing water had drained as quickly as possible. We sprayed 80 mg ^{15}N L^{-1} of $^{15}NH_4{}^{15}NO_3$ (corresponding to 40 mg ^{15}N m^{-2}) onto the surface of the plots. This amount was chosen because the annual N deposition rate was approximately 2 g m^{-2} y^{-1}, and the winter N deposition accounted for approximately 20% of the annual deposition rate in the temperate forests of China (Wang et al., 2020). Therefore, the total amount of the ^{15}N tracer sprayed onto each plot accounted for approximately 10% of the winter-deposited N, which was within the natural variability of the winter deposited N. The ^{15}N solution was sprayed with a syringe guided using a 1 m × 1 m grid frame with 100 holes and with each hole being sprayed with 5 ml of solution. It was estimated that each plot received a total of 32 L solution, which equaled to approximately 1.5% of the winter precipitation. Therefore, we assumed there was no significant impact caused by the addition of the solution.

2.3. Sampling

Sampling was conducted at 7 days, 53 days, 118 days, 158 days, and 214 days (on 22 March, 7 May, 11 July, 20 August, and 15 October 2019) after the ^{15}N addition; these.

sampling events corresponded with early spring, early summer, mid-summer, early summer, the peak biomass point, and late fall, respectively, according the database of the Beijing Forest Experimental Station. Litter samples were harvested from a randomly-located 1 m × 1 m quadrat in each plot. During each litter sampling, the litter was divided into three layers: the F-layer (fresh litter), the P-layer (partially decomposed litter), and the H-layer (highly decomposed litter). For soil sampling, three samples of each soil layer (0–5 cm, 5–15 cm, or 15–30 cm) were mixed, and this composite sample was sieved through a 2-mm sieve. Two subsamples of the sieved soil were obtained: one was air dried for the analyses of soil properties, and the other was stored at − 80 °C for the analyses of microbial properties.

For tree and shrub samplings, the root excavation was followed the trunk, and then a location was identified along the branch roots of the trunk at a 1.5 m distance. A soil core (20 length × 20 width × 30 cm deep) was excavated in each plot. Trunk samples were collected using an increment corer. For herb sampling, a same volume core was excavated at a random location in each plot. We classified the live roots of each species based on their location, colour, bark trait and branching pattern. More details of root sampling was presented by Ma et al. (2021).

For all the vascular plants, the live root segments were gently separated from the soil cores and then cleaned with deionized water to remove ^{15}N from the surfaces. The root samples of each plant species were divided into fine roots (diameter ≤ 2 mm) and coarse roots (diameter > 2 mm) (Guo et al., 2008). Each root sample of the trees was divided into two subsamples: one was fixed in Formalin-AcetoAlcohol solution (90 ml 50% ethanol, 5 ml 100% glacial acetic acid and 5 ml 37% methanol) and the other was oven-dried at 65 ℃. The aboveground tissues and roots of herbs were also oven-dried at 65 ℃ for 48 h until weights were constant.

2.4. Measurements of abiotic and biotic factors

Soil temperatures at 5 cm, 15 cm, and 30 cm soil depth were automatically measured every 3 h during the study period by ECH_2O sensors (Em50, Decagon, USA). Levels of total N in the litter, soils, microbes and vascular plants were measured using an elemental analyser (Elementar Analyzer, Vario Max CN, Germany). Soil ammonium (NH_4^+) and nitrate N (NO_3^-) were analysed using a flow injection auto-analyser (SANplus segmented flow analyzer, Skalar, The Netherlands). Soil microbial biomass N was analyzed using the fumigation-extraction method (Vance et al., 1987). Soil extracts from fumigated and unfumigated samples were obtained by shaking samples with 0.5 mol/L K_2SO_4 for 30 min within three days after each sampling. Microbial biomass N was calculated from the difference between extractable N contents in the fumigated and the unfumigated samples using conversion factors of 0.45 (Lovell et al., 1995). The allometric equations, which were obtained from the database of the Chinese National Ecosystem Research Network (CNERN) (http://www.cnern.cern.ac.cn/en/), were used to estimate the leaf, branch, trunk and root biomass of the investigated trees and shrubs (Table S2).

Soil N mineralization rate was measured using the intact core method (Raison et al., 1987). In each plot, four PVC tubes (20 cm in length, 5 cm in diameter) were inserted vertically into 15 cm soil layer. Two PVC cores were covered with plastic film and incubated in situ for 14 days. The other two PVC cores were immediately transport to the laboratory and stored at − 20 °C. The cores before and after incubation were extracted with 2 mol/L KCl, and then NH_4^+−N and NO_3^-−N concentrations were determined with a flow injection auto-analyser (FIAstar 5000 Analyzer). Net N mineralization rate (or net nitrification rate) was calculated as the changes in NH_4^+−N (or NO_3^-−N) concentrations between the initial and incubated cores.

The ectomycorrhizal mycorrhizal colonization rate for the trees was calculated by the number of root tips (i.e. first- and second-order roots) colonized by fungi divided by the total number of root segments examined (Guo et al., 2008). The mycorrhizal colonization was confirmed by the presence of fungal mantle and/or Hartig net in a root segment (Brundrett and Tedersoo, 2018).

Phospholipid fatty acids (PLFAs) have been used to quantify live soil microbial biomass (Bossio and Scow, 1998). The separation

and identifification of extracted PLFAs were conducted according to the standard protocol of the Sherlock Microbial Identifification System V4.5 (MIDI) with a Gas Chromatograph (Agilent 6850, USA). Three fatty acids (16: 1ω5c, 18: 2ω6, 9c and 18: 1ω9c) were chosen to represent the fungal group (Frostegård and Bååth, 1996).

2.5. Isotopic analyses

Levels of ^{15}N in the litter, soils, microbes and vascular plants were measured using an elemental analyser (Elementar Analyzer, Vario Max CN, Germany) linked to an isotope-ratio mass spectrometer (IRMS, Finnigan DELTA^{plus} XP, Thermo, USA). Soil microbial ^{15}N was determined using alkaline persulphate oxidation method (Stark and Hart, 1996; Zhou et al., 2003). The microbial ^{15}N was calculated as the difference between the levels of ^{15}N in the fumigated and unfumigated samples (Ma et al., 2018). The soil immobilized ^{15}N was calculated as the ^{15}N in dried soil minus the microbial ^{15}N.

2.6. Calculations and statistics

N isotope ratios of all samples are presented using δ notation (Fry, 2006): $\delta^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$. R_{sample} and $R_{standard}$ are the ratios between ^{15}N and ^{14}N of the sample and the standard, respectively. The atmospheric N_2 is used as a standard with $R_{standard} = 0.003665$. ^{15}N enrichment was calculated as: ^{15}N enrichment (‰) = $[(\delta^{15}N_{sample} - \delta^{15}N_{ambient})/(\delta^{15}N_{ambient} + 1000)] \times 1000$ (Friedrich et al., 2011). $\delta^{15}N_{sample}$ and $\delta^{15}N_{ambient}$ are the isotope enrichment in a labeled sample and in a nonlabeled sample.

^{15}N recovery in the labeled N pools was calculated as: $^{15}N_{recovery} = [(atom\%^{15}N_l - atom\%^{15}N_a) \times N_l\ \% \times M_p]/[(atom\%^{15}N_t - atom\%^{15}N_a) \times N_l\ \% \times M_t] \times 100\%$ (Providoli et al., 2006). Atom% $^{15}N_l$ is the atomic percentage of ^{15}N in the labeled sample; atom% $^{15}N_a$ is the atom% of ^{15}N in the non-labelled sample; atom% $^{15}N_t$ is the atom% of ^{15}N in the applied tracer; N_l % is the N concentration of the labelled sample; M_p is the dry mass of the labeled pool (g m^{-2}); M_t is the mass of ^{15}N in the ^{15}N tracer applied to a plot (g ^{15}N m^{-2}).

One-way analysis of variances (ANOVAs) were used to tested the effects of sampling time on litter N pool, net N mineralization rate, soil total and fungal PLFAs, and microbial biomass N at a significance level of $P < 0.05$. Two-way ANOVAs were used to examine the effects of inorganic N form (or plant functional type) and sampling time on inorganic N content and mycorrhizal colonization rate. Two-way ANOVAs were also used to examine the effects of soil layer (or plant functional type), sampling time, and their interaction on soil, microbial and plant ^{15}N recovery. Stepwise linear regression analyses were used to explore how abiotic (soil temperature, soil moisture, soil inorganic N content, N mineralization rate) and biotic factors (litter mass, soil microbial biomass, fungal biomass and mycorrhizal colonization rate) affected ^{15}N recovery in litter, soils, microbes and plants. All statistical analyses were performed using R (v3.3.1).

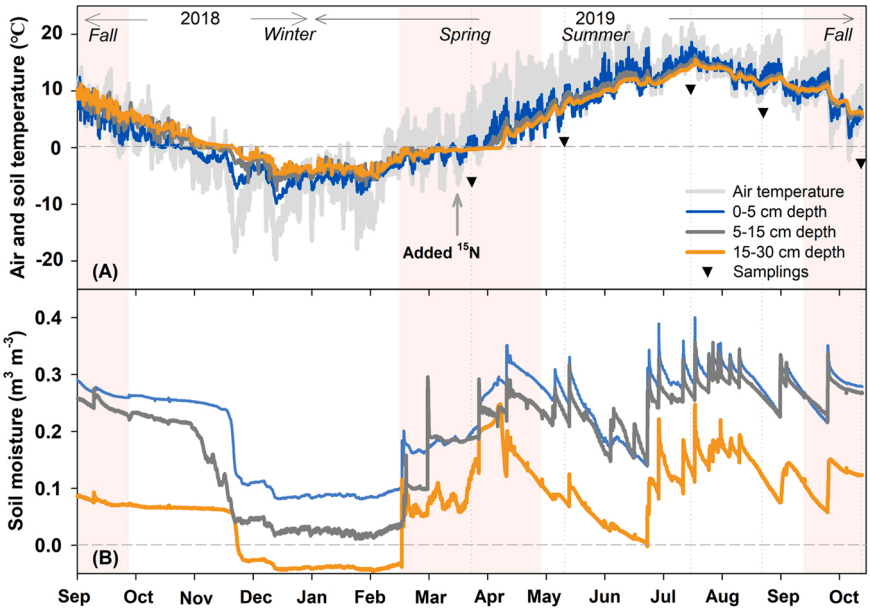

Fig. 1. Air temperature, soil temperature and moisture (at 5, 15 and 30 cm depth) over 18 September 2018 (mid-fall) to 15 October 2019 (late fall); ^{15}N tracer addition and sampling dates are also indicated.

3. Results

3.1. Background environmental conditions

In general, the air temperature, soil temperature, and soil moisture showed pronounced seasonal variations from mid-fall in 2018 to late fall in 2019 in the warm temperate forest (Fig. 1A, B). From mid-September to mid-February (the winter freezing period), the lowest daily soil temperatures decreased to − 6.5 °C, − 4 °C and − 3 °C at the soil depths of 5 cm, 15 cm and 30 cm, respectively. From mid-February to mid-March (early spring), the soil temperatures fluctuated near 0 °C and gradually rose to 5 °C, and most of the snow disappeared and soil thawing was completed by the end of this period. From mid-March to early May (spring), the soil temperature rapidly increased from 5 °C to 10 °C, and then gradually reached to 15 °C in mid-July (the peak biomass period) (Fig. 2A). After mid-February (the snowmelt period), the soil moisture increased rapidly during the following season. The soil moisture in the 0–5 cm and 5–15 cm layers was higher than that in the 15–30 cm soil layer throughout the study period ($P < 0.05$; Fig. 2B).

3.2. Soil, microbial and plant properties

In the warm temperate forest, the litter N pool gradually declined from 1.52 g N m^{-2} in early spring to 0.91 g N m^{-2} in summer, and then increased to 1.72 g N m^{-2} in late fall (Fig. 2A). Mycorrhizal colonization rates were significantly higher in evergreen coniferous trees (35%) than in deciduous broad-leaved trees (27%) ($P < 0.05$; Fig. 2B). During the study period, the net N mineralization and nitrification rates (0–30 cm soil layer) gradually increased from 0.013 and 0.008 g N m^{-2} day^{-1} in early spring, respectively, to 0.039 and 0.028 g N m^{-2} day^{-1} in the peak biomass period, respectively, and then decreased to 0.023–0.024 g N m^{-2} day^{-1} in late fall, respectively (Fig. 2C). The soil NH_4^+-N pool gradually increased, while the soil NO_3^--N pool gradually decreased from early spring to late fall. After early spring, soil NH_4^+-N dominated the soil inorganic N pools and accounted for 58–72% of the soil inorganic N pool (Fig. 2D). Neither total phospholipid fatty acids (PLFAs; i.e. soil microbial biomass) nor fungal PLFAs (i.e. fungal biomass) showed significant seasonal variation (Fig. 2E). However, the soil microbial biomass N gradually increased over the study period (Fig. 2F).

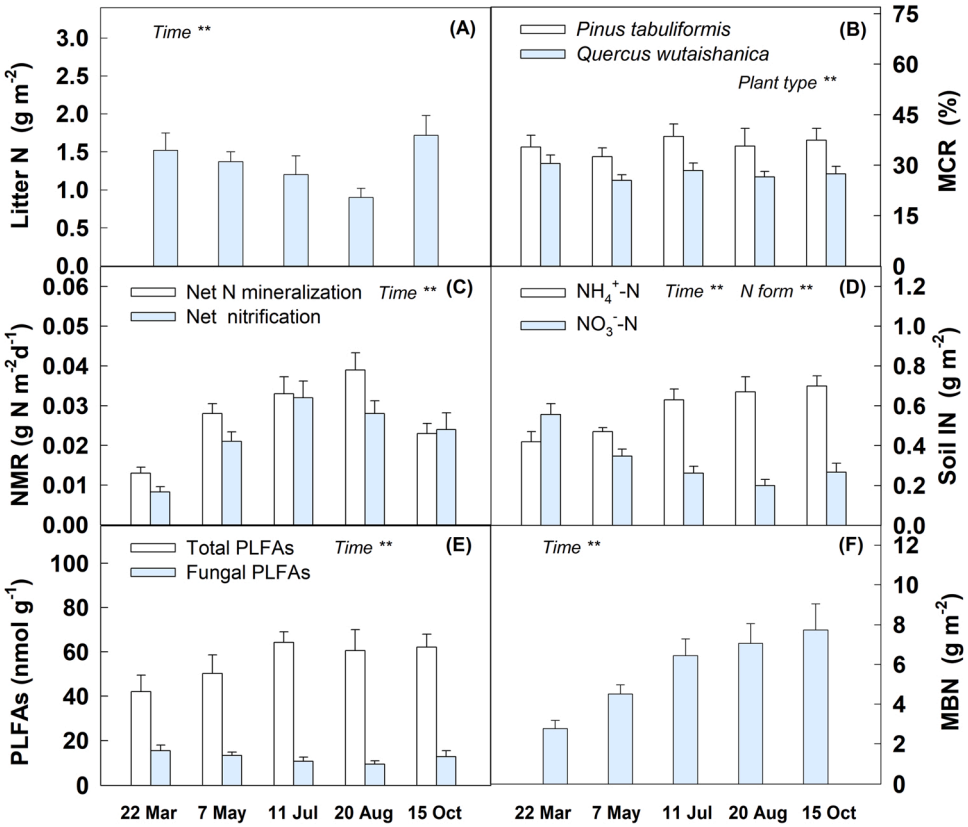

Fig. 2. Seasonal variation of litter N pool (A), mycorrhizal colonization rates (B), N mineralization rates (net N mineralization and nitrification rates) (C), inorganic N (NH_4^+-N and NO_3^--N) content (D), soil total and fungal PLFAs (E), and microbial biomass N (0–15 cm soil layer) (F) in a warm temperate forest of northern China (n = 4). The effects of sampling time on litter N pool, N mineralization rates, soil total and fungal PLFAs, and microbial biomass N were determined using One-way ANOVAs, respectively; the effects of sampling time and N form (or plant functional type) on inorganic N content (or mycorrhizal colonization rates) were determined using Two-way ANOVAs (**, $P < 0.01$).

3.3. ^{15}N dynamics in the ecosystem

In early spring (7 days after ^{15}N addition), the total ^{15}N recovery was approximately 80% in the forest ecosystem. The initial ^{15}N recovery was the highest in litter (35%), followed by microbes (28%) and soils (12%), and minimal recovery in vascular plants (5%) (Fig. 3, Tables S3, S4; Fig. S1). After early spring, the total ^{15}N recovery was maintained at approximately 70% in the ecosystem. During the peak biomass period, the ^{15}N levels rapidly declined to 18% in litter and 9% in soil microbes, but increased to 42% in vascular plants (aboveground plants: 30%, roots: 12%), while the ^{15}N level was maintained at 10% in mineral soils. In late fall, most of the ^{15}N tracer was retained in litter (24%) and roots (24%).

3.4. Plant N acquisition

All the plant species investigated presented significant differences in ^{15}N acquisition during the study period (Fig. 4A–D, Table S4, Fig. S2). During early spring to the peak biomass period, evergreen coniferous trees had the highest ^{15}N recovery, followed by broad-leaved trees and deciduous shrubs, while perennial grasses had the lowest ^{15}N recovery. During the peak biomass period, coniferous trees, broad-leaved trees, shrubs and herbs acquired 17%, 10%, 9%, and 6% of added ^{15}N, respectively. Coniferous trees had higher ^{15}N enrichment in leaves and fine roots than broad-leaved trees during the study period ($P < 0.05$; Fig. 5A, B). However, herbs had the highest ^{15}N enrichment in leaves (52‰) and fine roots (28‰) compared to other functional types ($P < 0.05$; Fig. 5A, B).

3.5. The controlling effects of biotic and abiotic factors on ecosystem ^{15}N recovery

Stepwise multiple regression analysis of the environmental factors controlling ecosystem ^{15}N recovery indicated that litter mass explained 74% of the spatial variation in litter ^{15}N recovery; the soil moisture and net mineralization rate accounted for 71% of the spatial variation in soil ^{15}N recovery; the microbial biomass explained 75% of the spatial variation in microbial ^{15}N retention; and the mycorrhizal colonization rate and fungal biomass together explained 63% of the variation in plant ^{15}N recovery (Table S5).

4. Discussion

4.1. Litter and soil microbes are the initial sinks for early-spring N input

In this study, the initial ^{15}N recovery within the ecosystem was approximately 80% in the early spring (Fig. 3; Table S3), indicating that the warm temperate forest had a considerable capability to retain early-spring exogenous N resources. Consistent with hypothesis 1, litter was the primary sink for early-spring N input during the early growing season. This finding was consistent with the previous field experiments conducted during the growing season, which demonstrated that substantial N deposition was intercepted in the litter layer in the short-term after ^{15}N tracer application (Providoli et al., 2005; Sheng et al., 2014; Liu et al., 2017). Templer et al. (2012) used a meta-analysis to assess the retention dynamics of N inputs and found that approximately 60% of applied ^{15}N was intercepted in the forest litter layer at 48 sites across America and Europe.

Soil microbes acted as a main sink for the added ^{15}N during the early growing season in the temperate forest. This finding supported the previous reports that the soil microbes competed effectively with the plants for available N in heath, grassland, and forest ecosystems (Zak et al., 1990; Andresen and Turner, 2013; Ma et al., 2018). The calculated Michaelis–Menten kinetics were used to analyze 77 studies on the uptake of NH_4^+ and NO_3^- by roots and microbes, revealing that soil microbes had a higher capacity for N

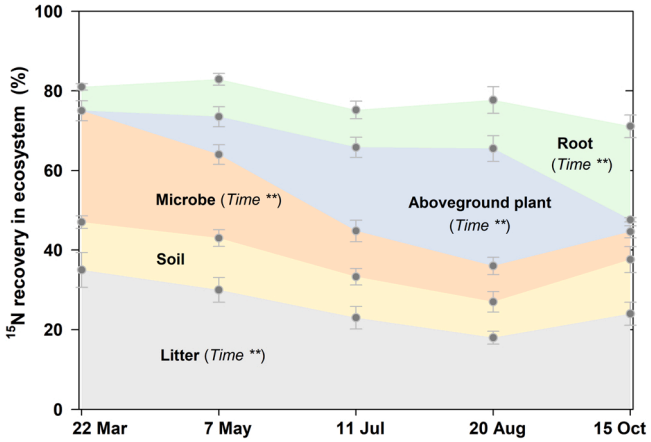

Fig. 3. Recovery (%) of added ^{15}N in litter, soils, microbial biomass and vascular plants during the study period in a temperate forest of northern China. Vertical bars indicate one unit of standard error of the means (n = 4). The effects of sampling time on ^{15}N recovery in each pool were determined using One-way ANOVAs (**, $P < 0.01$).

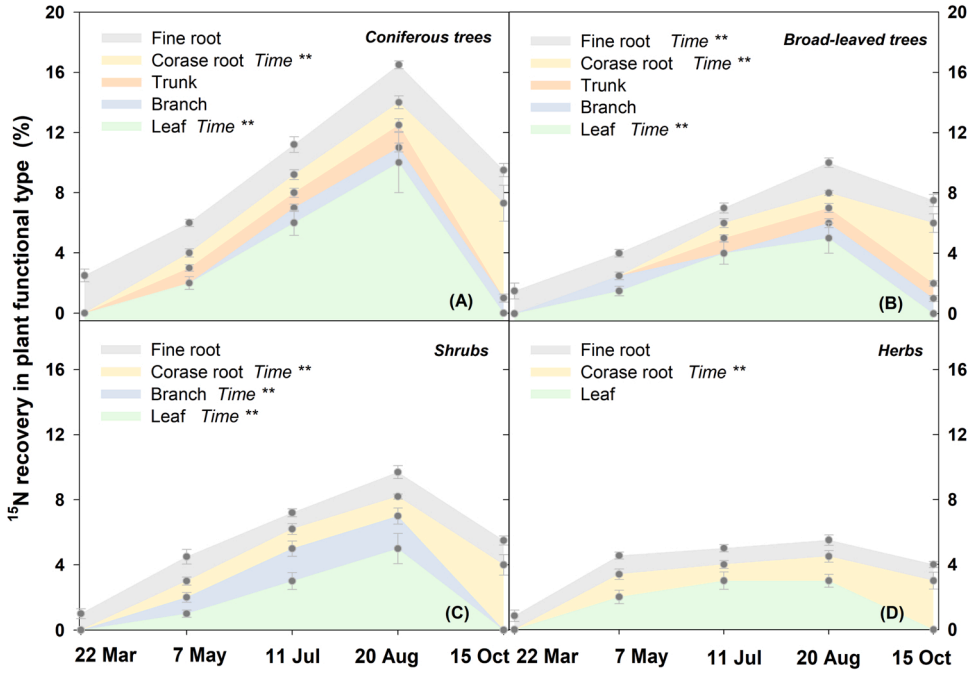

Fig. 4. Recovery (%) of added ^{15}N in evergreen coniferous trees (*Pinus tabuliformis*), deciduous broad-leaved trees (*Quercus liaotungensis*), deciduous shrubs (*Abelia biflora*), and herbs (*Carex duriusata*) during the study period in a temperate forest of northern China. Vertical bars indicate standard errors of the means (n = 4). The effects of sampling time on ^{15}N recovery different plant tissues were determined using One-way ANOVAs (**, $P < 0.01$).

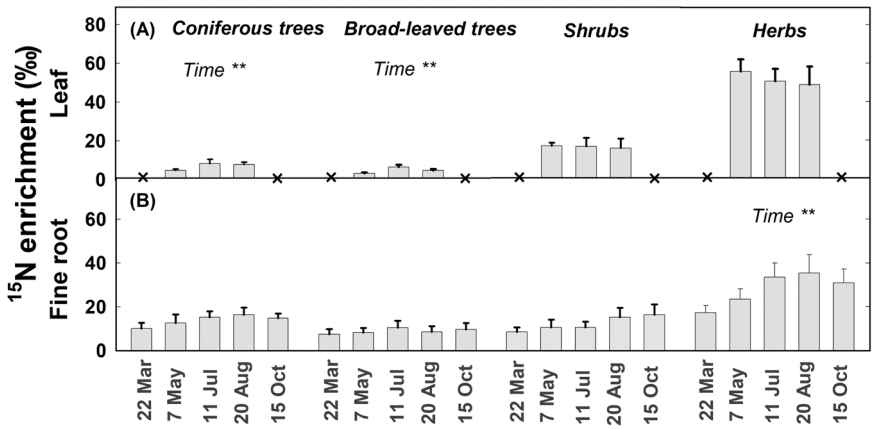

Fig. 5. ^{15}N enrichment (‰) in leaves (A) and fine roots (B) at 7, 53, 118, 158 and 214 days after ^{15}N tracer addition. Vertical bars indicate standard errors of the means (n = 4). The effects of sampling time on ^{15}N recovery in leaves and fine roots were determined using One-way ANOVAs (**, $P < 0.01$).

immobilization compared with roots, and were especially efficient in low-N conditions across shrub, heath, tundra, grassland, and forest ecosystems (Kuzyakov and Xu, 2013).

All the plant species acquired the ^{15}N tracer in early spring, which was supported by the rapid increases in ^{15}N recovery in their fine roots (Fig. 4). The finding was in accordance with previous reports that N acquisition in the temperate regions did occurred during periods of zero (or subzero) air and soil temperatures (Ueda et al., 2015; Ma et al., 2021).

4.2. Plants become the important sink for early-spring N input in the long term

There appeared to be little change in total ^{15}N recovery over the following growing season (Fig. 3, Table S3), which suggested that the temperate forest ecosystem had a high potential for the retention of early-spring exogenous N in the long-term. Consistent with

hypothesis 2, the ^{15}N levels gradually declined in the litter and soil microbes with sampling (Fig. 3). This finding indicated that litter and soil microbes rapidly captured early-spring N and they subsequently slowly relocated it from the litter and soils to the ecosystem, which was consistent with previous findings (Friedrich et al., 2011; Liu et al., 2017). Therefore, the litter layer in our study was served as a buffer against early-spring N input from being lost via leaching or gaseous compounds.

It was also found that the aboveground tissues of the vascular plants gradually acquired added ^{15}N tracer over the following growing season (Fig. 3). This was consistent with previous studies conducted in arctic tundra and temperate forest ecosystems that found high allocation of ^{15}N tracer from the roots to aboveground tissues after early spring (Tye et al., 2005; Ueda et al., 2015), suggesting that early-spring N input was not only supplied to root growth, but also supplied to aboveground tissue growth. In late fall, it was found that the ^{15}N originally retained in the aboveground tissues had been partly resorbed to the roots. At the individual species level, this finding has important implications for plant growth and competitive ability in nutrient-poor ecosystems (Aerts et al., 1992).

It is likely that some of the applied ^{15}N (20–30%) was lost during the study period owing to denitrification rather than leaching in the temperate forest because a small amount of ^{15}N tracer ($< 2\%$) was found in the 30–50 cm soil depth (data not shown). This suggests that a potential increase in winter N deposition may not lead to N enrichment or N pollution in adjacent aquatic ecosystems. We acknowledge the potential limitations of the ^{15}N labelling time and the associated uncertainty in this study. To address this limitation, we applied ^{15}N solution three days after the final snowmelt and the last of the free standing water had drained as quickly as possible. We found net N mineralization rate and microbial N immobilization were very low at early spring. Therefore, we assumed that although the actual early-spring N resource have already started cycling in the ecosystem, the ^{15}N labelling time did not have a significant impact on our results.

4.3. Difference in magnitude of plant N uptake

In contrast to the third hypothesis, we found that the evergreen coniferous trees (*P. tabuliformis*) presented a higher ^{15}N uptake capacity than the deciduous broad-leaved trees (*Q. liaotungensis*) in the temperate forest (Figs. 4, 5). Previous studies that added ^{15}N tracers to trace N deposition during the growing seasons reported that deciduous trees had higher ^{15}N enrichment than coniferous trees (Nadelhoffer et al., 1999; Liu et al., 2017). The differences in these findings are probably attributed to several possible mechanisms. First, the most probable reason may be higher ECM colonization rates in the coniferous trees than the broad-leaved trees in the current study region (Fig. 2B; Table S5). Numerous studies have suggested that higher ECM colonization rates may have a greater ability to promote N uptake because ECM colonization is essential to ecosystem functioning as a result of enhancing host plant N acquisition (Johnson et al., 2012). Second, evergreen coniferous trees require higher amounts of nutrients for survival compared to deciduous trees during harsh climatic conditions. During early spring, evergreen trees do not lose their leaves and need to take up more nutrients to ensure their growth, while deciduous trees have low energy requirements at this time. Third, interspecific divergence in ^{15}N acquisition may be attributed to their phenological asynchrony. From a phenological perspective, the germination and maturation periods of *P. tabuliformis* are earlier than those of *Q. liaotungensis* (Sang et al., 2002); thus *P. tabuliformis* likely acquires more deposited N to meet their growing requirements.

Compared with the trees and shrubs, the herbs had the highest ^{15}N concentrations among the tested species (Fig. 5). This finding was consistent with a study in the temperate grasslands, in which perennial forbs rapidly acquired N during early spring due to their fast-growing traits (Ma et al., 2018, 2020). From a niche perspective, when overstory leaves are absent during winter, the understory receives high light availability before canopy closure in early spring (Grogan and Jonasson, 2003; Miyazawa and Kikuzawa, 2004). Therefore, the ecological consequence may be that herbage layers acquire more N in the short term than other tall trees and shrubs. In contrast, continuous N uptake during the winter may give trees and shrubs an advantage of compensation because of their slower growth rates during growing seasons compared to that of herbs (Das, 2012; Ma et al., 2021). Our findings confirm that early-spring N input is an important N source for plant N uptake (Tye et al., 2005).

4.4. Implications for future study

Given the findings, we identified several future efforts and research directions to assess the contribution of early-spring N input to ecosystem N budgets in temperate regions. First, it is projected that extreme winter warming events are likely to increase in frequency in the temperate regions in the future (IPCC (Intergovernmental Panel on Climate Change), 2021). Such warming events can result in early snowmelt and then the release of snowpack N, potentially altering the ecosystem N cycling in the following seasons (Henry, 2007). Therefore, further field study is needed to explore the retention of increasing winter-deposited N in temperate ecosystems under global climatic changes. Second, the present ecosystem models only consider the effects of N deposition in the growing seasons on N cycling, whereas the effects of winter N deposition on ecosystem processes have not been integrated into models. Given the important contribution of early-spring N input to temperate ecosystems, the findings of this study should be applied to model development to better simulate vegetation growth.

5. Conclusions

We investigated the retention dynamics of early-spring N input in a temperate forest ecosystem over the following growing season using a ^{15}N isotopic technique. Temperate forests have a considerable capability to immobilize early-spring exogenous N resources. Approximately 80% of applied ^{15}N was initially retained in the ecosystem, and litter and soil microbes were the main sinks of the early-spring N input. From the peak biomass period to late fall, vascular plants and litter became the main sinks of the N input. Among the

different plant functional types, coniferous trees showed higher ^{15}N acquisition than broad-leaved trees, likely due to their higher energy requirements and mycorrhizal colonization rate. Data on the retention dynamics of early-spring exogenous N resources within ecosystems adds value to the understanding and prediction of N cycling responses to climate change in temperate forests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the funding provided by the National Natural Science Foundation of China (No. 32071602); the Beijing Forest Experimental Station of the Institute of Botany, Chinese Academy of Sciences for help with logistics and access permission to the study site.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01966.

References

Aerts, R., Walle, B., Malmer, N., 1992. Growth-limiting nutrients in Sphagnum-dominated bogs subject to low and high atmospheric nitrogen supply. J. Ecol. 80, 131–140. https://doi.org/10.2307/2261070.

Andresen, L.C., Michelsen, A., 2005. Off-season uptake of nitrogen in temperate heath vegetation. Oecologia 144, 585–597. https://doi.org/10.1007/s00442-005-0044-1.

Andersen, K.M., Turner, B.L., 2013. Preferences or plasticity in nitrogen acquisition by understorey palms in a Tropical Montane forest. J. Ecol. 101, 819–825. https://doi.org/10.1111/1365-2745.12070.

Bilbrough, C.J., Welker, J.M., Bowman, W.D., 2000. Early spring nitrogen uptake by snow-covered plants: a comparison of arctic and alpine plant function under the snowpack. Arct. Antarct. Alp. Res. 324, 404–411. https://doi.org/10.2307/1552389.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acids profifiles and substrate utilization patterns. Microb. Ecol. 35, 265–278. https://doi.org/10.1007/s002489900082.

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220, 1108–1115. https://doi.org/10.1111/nph.14976.

Das, A., 2012. The effect of size and competition on tree growth rate in old-growth coniferous forests. Can. J. For. Res. 42, 1983–1995. https://doi.org/10.1139/x2012-142.

Du, E., Terrer, C., Pellegrini, A.F.A., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X.H., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. Nat. Geosci. 13, 221–229. https://doi.org/10.1038/s41561-019-0530-4.

Edwards, K.A., Jefferies, R.L., 2010. Nitrogen uptake by *Carex aquatilis* during the winter–spring transition in a low Arctic wet meadow. J. Ecol. 98, 737–744. https://doi.org/10.1111/j.1365-2745.2010.01675.x.

FAO-WRB (ISSS-ISRIC-FAO-UNESCO), 1998. World reference base for soil resources. World Soil Resources Report. FAO.

Friedrich, U., Falk, K., Bahlmann, E., Marquardt, T., Meyer, T., Niemeyer, T., Schemmel, S., Oheimb, G.V., Rdtle, W.H., 2011. Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study. Glob. Change Biol. 17, 1549–1559. https://doi.org/10.1111/j.1365-2486.2010.02322.x.

Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., 2013. The global nitrogen cycle in the twenty-first century. Philos. Trans. R. Soc. B 368, 20130164. https://doi.org/10.1098/rstb.2013.0164.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fert. Soils 22, 59–65. https://doi.org/10.1007/BF00384433.

Fry, B., 2006. Stable Isotope Ecology. Springer-Verlag, New York, NY, USA.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Horwarth, E.W., Seitzinger, S.P., Asner, G.P., 2004. Nitrogen cycles, past, present, and future. Biogeochemistry 70, 152–226. https://doi.org/10.1007/s10533-004-0370-0.

Grogan, P., Jonasson, S., 2003. Controls on annual nitrogen cycling in the understory of a subarctic birch forest. Ecology 84, 202–218. https://doi.org/10.1890/0012-9658(2003)084[0202:COANCI]2.0.CO;2.

Grogan, P., Michelsen, A., Ambus, P., Jonasson, S., 2004. Freeze-thaw regime effects on carbon and nitrogen dynamics in subarctic heath tundra mesocosms. Soil Biol. Biochem. 36, 641–654. https://doi.org/10.1016/j.soilbio.2003.12.007.

Guo, D., Xia, M.X., Wei, X., Chang, W.J., Liu, Y., Wang, Z.Q., 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytol. 180, 673–683. https://doi.org/10.1111/j.1469-8137.2008.02573.x.

Gurmesa, G.A., Lu, X.K., Gundersen, P., Mao, Q.G., Zhou, K.J., Fang, Y.T., Mo, J.M., 2016. High retention of ^{15}N-labeled nitrogen deposition in a nitrogen saturated old-growth tropical forest. Glob. Change Biol. 22, 3608–3620. https://doi.org/10.1111/gcb.13327.

Henry, H.A.L., 2007. Soil freeze–thaw cycle experiments: trends, methodological weak nesses and suggested improvements. Soil Biol. Biochem. 39, 977–986. https://doi.org/10.1016/j.soilbio.2006.11.017.

IPCC (Intergovernmental Panel on Climate Change), 2021. Climate Change 2021: The Science of Climate Change. Cambridge University Press, New York, NY.

Jaeger, C.H., Monson, R.K., Melany, C.F., Schmidt, S.K., 1999. Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology 80, 1883–1891.

Johnson, D., Martin, F., Cairney, W.G., Anderson, I.C., 2012. The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. New Phytol. 194, 614–628. https://doi.org/10.1890/0012-9658(1999)080[1883:SPONBP]2.0.CO;2.

Joseph, G., Henry, H.A.L., 2009. Retention of surface nitrate additions in a temperate old field: implications for atmospheric nitrogen deposition over winter and plant nitrogen availability. Plant Soil 319, 209–218. https://doi.org/10.1007/s11104-008-9862-3.

Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656–669. https://doi.org/10.1111/nph.12235.

Larsen, L.S., Michelsen, A., Jonasson, S., Beier, C., Grogan, P., 2012. Nitrogen uptake during fall, winter and spring differs among plant functional groups in a subarctic heath ecosystem. Ecosystems 15, 927–929. https://doi.org/10.1007/s10021-012-9555-x.

Li, S.L., Gurmesa, G.A., Zhu, W.X., Gundersen, P., Zhang, S.S., Xi, D., Huang, S.N., Wang, A., Zhu, F.F., Zhu, X.J., Fang, Y.T., 2019. Fate of atmospherically deposited NH_4^+ and NO_3^- in two temperate forests in China: temporal pattern and redistribution. Ecol. Appl. 29, e01920 https://doi.org/10.1002/eap.1920.

Liu, J., Peng, B., Xia, Z.W., Sun, J.F., Gao, D.C., Dai, W.W., Bai, E., 2017. Different fates of deposited NH_4^+ and NO_3^- in a temperate forest in northeast China: a ^{15}N tracer study. Glob. Change Biol. 23, 2441–2449. https://doi.org/10.1111/gcb.13533.

Liu, Q., Qiao, N., Xu, X., Xin, X., Han, J.Y., Tian, Y., Ouyang, H., Kuzyakov, Y., 2016. Nitrogen acquisition by plants and microorganisms in a temperate grassland. Sci. Rep. 6, 22642. https://doi.org/10.1038/srep22642.

Liu, X.Y., Koba, K., Koyama, L.A., Hobbie, S.E., Weiss, M.S., Inagaki, Y., Shaver, G.R., Giblin, A.E., Hobara, S., Nadelhoffer, K.J., Sommerkorn, M., Rastetter, E.B., Kling, G.W., Laundre, J.A., Yano, Y., Makabe, A., Yano, M., Liu, C.Q., 2018. Nitrate is an important nitrogen source for Arctic tundra plants. Proc. Natl. Acad. Sci. USA 115, 3398–3403. https://doi.org/10.1073/pnas.1715382115.

Lovell, R.D., Jarvis, S.C., Bardgett, R.D., 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. Soil Biol. Biochem. 27, 969–975. https://doi.org/10.1016/0038-0717(94)00241-R.

Ma, L.N., Liu, G.F., Xu, X.F., Xin, X.P., Bai, W.M., Zhang, L.H., Chen, S.P., Wang, R.Z., 2018. Nitrogen acquisition strategies during the winter-spring transitional period are divergent at the species level yet convergent at the ecosystem level in temperate grasslands. Soil Biol. Biochem. 122, 150–159. https://doi.org/10.1016/j.soilbio.2018.04.020.

Ma, L.N., Gao, X.L., Liu, G.F., Xu, X.F., Lü, X.T., Xin, X.P., Lü, Y.X., Zhang, C.X., Zhang, L.H., Wang, R.Z., 2020. The retention dynamics of N input within the soil–microbe–plant system in a temperate grassland. Geoderma 368, 114290. https://doi.org/10.1016/j.geoderma.2020.114290.

Ma, L.N., Xu, X.F., Zhang, C.X., Lv, Y.X., Liu, G.F., Zhang, Q.B., Feng, J.C., Wang, R.Z., 2021. Strong non-growing season N uptake by deciduous trees in a temperate forest: a ^{15}N isotopic experiment. J. Ecol. https://doi.org/10.1111/1365-2745.13754.

Magill, A.H., Aber, J.D., Berntson, G.M., McDowell, W.H., Nadelhoffer, K.J., Melillo, J.M., Steudler, P., 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems 3, 238–253. https://doi.org/10.1007/s100210000023.

Miyazawa, Y., Kikuzawa, K., 2004. Winter photosynthesis by saplings of evergreen broadleaved trees in a deciduous temperate forest. New Phytol. 165, 857–866. https://doi.org/10.1111/j.1469-8137.2004.01265.x.

Nadelhoffer, K.J., Emmett, B.A., Gundersen, P., Kjùnaas, O.J., Koopmans, C.J., Schleppi, P., Tietemal, A., Wright, R.F., 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398, 145–148. https://doi.org/10.1038/18205.

Providoli, I., Bugmann, H., Siegwolf, R., Buchmann, N., Schleppi, P., 2005. Flow of deposited inorganic N in two Gleysol-dominated mountain catchments traced with $^{15}NO_3^-$ and $^{15}NH_4^+$. Biogeochemistry 76, 453–475. https://doi.org/10.1007/s10533-005-8124-1.

Providoli, I., Bugmann, H., Siegwolf, R., Buchmann, N., Schleppi, P., 2006. Pathways and dynamics of $^{15}NO_3^-$ and $^{15}NH_4^+$ applied in a mountain *Picea abies* forest and in a nearby meadow in central Switzerland. Soil Biol. Biochem. 38, 1645–1657. https://doi.org/10.1016/j.soilbio.2005.11.019.

Raison, R.J., Connell, M.J., Khanna, P.K., 1987. Methodology for studying fluxes of soil mineral-N in situ. Soil Biol. Biochem. 19, 521–530. https://doi.org/10.1016/0038-0717(87)90094-0.

Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291. https://doi.org/10.1890/02-0298.

Sang, W.G., Su, H.X., Chen, L.Z., 2002. Coupling biomass and energy in warm temperate deciduous broad-leaved (*Quercus liaotungensis*) forest ecosystem. Chin. J. Plant Ecol. 26, 88–92.

Sheng, W.P., Yu, G.R., Fang, H.J., Jiang, C.M., Yan, J.H., Zhou, M., 2014. Sinks for inorganic nitrogen deposition in forest ecosystems with low and high nitrogen deposition in China. PLoS One 9, e89322. https://doi.org/10.1371/journal.pone.0089322.

Stark, J.M., Hart, S.C., 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. Am. J. 60, 1846–1855. https://doi.org/10.2136/sssaj1996.03615995006000060033x.

Templer, P.H., Mack, M.C., Chapin, F.S., Christenson, L.M., Compton, J.E., Crook, H.D., Currie, W.S., Dail, D.B., D'Antonio, C.M., Emmett, B.A., Epstein, H.E., Goodale, C.L., Gundersen, P., Hobbie, S.E., Holland, K., Hooper, D.U., Hungate, B.A., Lamontagne, S., Nadelhoffer, K.J., Osenberg, C.W., Perakis, S.S., Schleppi, P., Schimel, J., Schmidt, I.K., Sommerkorn, M., Spoelstra, J., Tietema, A., Wessel, W.W., Zak, D.R., 2012. Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of ^{15}N tracer field studies. Ecology 93, 1816–1829. https://doi.org/10.1890/11-1146.1.

Turner, M.M., Henry, H.A.L., 2009. Interactive effects of warming and increased nitrogen deposition on ^{15}N tracer retention in a temperate old field: seasonal trends. Glob. Change Biol. 15, 2885–2893. https://doi.org/10.1111/j.1365-2486.2009.01881.x.

Tye, A.M., Young, S.D., Crout, N.M.J., West, H.M., Stapleton, L.M., Poulton, P.R., Laybourn-Parry, J., 2005. The fate of ^{15}N added to high Arctic tundra to mimic increased inputs of atmospheric nitrogen released from a melting snowpack. Glob. Change Biol. 11, 1640–1654. https://doi.org/10.1111/j.1365-2486.2005.01044.x.

Ueda, M.U., Tokuchi, N., Hiura, T., 2015. Soil nitrogen pools and plant uptake at sub-zero soil temperature in a cool temperate forest soil: a field experiment using ^{15}N labeling. Plant Soil 392, 205–214. https://doi.org/10.1007/s11104-015-2453-1.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Vankoughnett, M.R., Henry, H.A.L., 2014. Soil freezing and N deposition: transient vs. multi-year effects on extractable C and N, potential trace gas losses and microbial biomass. Soil Biol. Biochem. 77, 170–178. https://doi.org/10.1016/j.soilbio.2014.06.020.

Wang, A., Chen, D.X., Phillips, O.L., Gundersen, P., Zhou, X.L., Gurmesa, G.A., Li, S.L., Zhu, W.X., Hobbie, E.A., Wang, X.Y., Fang, Y.T., 2021. Dynamics and multi-annual fate of atmospherically deposited nitrogen in montane tropical forests. Glob. Change Biol. 27, 2076–2087. https://doi.org/10.1111/gcb.15526.

Wang, Z., Zhang, X.Y., Liu, L., Cheng, M.M., Xu, J.F., 2020. Spatial and seasonal patterns of atmospheric nitrogen deposition in North China. Atmos. Sci. Lett. 13, 188–194. https://doi.org/10.1080/16742834.2019.1701385.

Yu, G., Jia, Y., He, N., Zhu, J., Chen, Z., Wang, Q., Piao, S., Liu, X., He, H., Guo, X., 2019. Stabilization of atmospheric nitrogen deposition in China over the past decade. Nat. Geosci. 424–429. https://doi.org/10.1038/s41561-019-0352-4.

Zak, D.R., Groffman, P.M., Pregitzer, K.S., Christensen, S., Tiedje, J.M., 1990. The vernal Dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology 71, 651–656. https://doi.org/10.2307/1940319.

Zhou, J., Chen, Z., Li, S., 2003. Oxidation efficiency of different oxidants of persulfate method used to determine total nitrogen and phosphorus in solutions. Commun. Soil Sci. Plan. 34, 725–734. https://doi.org/10.1081/CSS-120018971.