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Vertical Changes in the Flux of Atmospheric Nitrate From a Forest Canopy to the Surface Soil Based on $\Delta^{17}O$ Values

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Key Points:

- The vertical profiles of the proportion of atmospheric NO_3^- to total NO_3^- were different from those of atmospheric NO_3^- flux
- The forest canopy retained a large portion of the deposited atmospheric NO_3^-
- The retention rates of atmospheric NO_3^- were different among different canopy types

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract

To better understand the factors that control N retention and N export in forested watersheds, it is necessary to understand the relationships among atmospheric nitrogen (N) deposition, internal N cycling within plant-soil systems, and N leaching. The relative contributions of atmospheric nitrate ($NO_3^-{}_{atm}$) and remineralized nitrate produced by microbial nitrification to total nitrate (NO_3^-) in stream water have been investigated in many studies. However, the dynamics of these two types of NO_3^- from the forest canopy to the soil are not well understood. Therefore, we determined the changes in the NO_3^- flux and the ^{17}O excess ($\Delta^{17}O$) of NO_3^-, a robust tracer of $NO_3^-{}_{atm}$, from bulk deposition to the soil water beneath oak and spruce trees as well as dwarf bamboo-dominated canopy gaps in a natural coniferous-broadleaved mixed forest in northern Japan. The $\Delta^{17}O$ values in NO_3^- dramatically decreased after passing through the forest floor, indicating that the dominant source of NO_3^- leaching is nitrification in the forest floor. In contrast, a large decrease in $NO_3^-{}_{atm}$ flux was observed between bulk deposition and throughfall, especially for oak and spruce, suggesting that the forest canopy is an important sink for deposited $NO_3^-{}_{atm}$. The retention of $NO_3^-{}_{atm}$ by the canopy was higher for oak (86.3 ± 10.1%) and spruce (87.7 ± 8.8%) than for *Sasa* in the canopy gap (49.9 ± 26.6%). Our study demonstrated that the $\Delta^{17}O$ value of NO_3^- is a promising tool for quantifying the atmospheric nitrate dynamics in complex forest N cycling.

Plain Language Summary

Excess atmospheric nitrogen (N) deposition in forests can increase N leaching to streams, which may induce eutrophication in downstream ecosystems. Therefore, understanding the relationships among atmospheric N deposition, internal N cycling within plant-soil systems, and N leaching is necessary to better understand the factors that control N export in forested watersheds. Triple oxygen isotopes of nitrate (NO_3^-) are useful tracers of atmospheric nitrate ($NO_3^-{}_{atm}$). Therefore, we examined the proportion of $NO_3^-{}_{atm}$ to total NO_3^- and the flux of $NO_3^-{}_{atm}$ in waters that pass through forest canopies and surface soils in a natural coniferous-broadleaved mixed forest by using triple oxygen isotopes of NO_3^-. The results showed that the dominant source of NO_3^- in the water samples switched from $NO_3^-{}_{atm}$ to NO_3^- produced by nitrification when the water passed through the forest floor. This is in line with previous findings. In contrast, the $NO_3^-{}_{atm}$ flux largely decreased from rainfall to throughfall, especially for oak and spruce, suggesting that the forest canopy is an important sink for deposited $NO_3^-{}_{atm}$. Our study demonstrated that the $\Delta^{17}O$ value of NO_3^- is a promising tool for quantifying the atmospheric nitrate dynamics in complex forest N cycling.

1. Introduction

The deposition of atmospheric nitrogen (N) has increased globally since industrialization began (Galloway et al., 2004). Reductions in atmospheric N deposition have occurred over the last four decades in some parts of the world, such as Europe, the Indo-Pacific, and the northeastern United States, due to reductions in N emissions from anthropogenic activity. In other regions though, significant increases have occurred, such as East Asia and southern Brazil, offsetting the reduction, and an increase is still seen at a global scale (Ackerman et al., 2019). Chronically high N deposition in forests can shift the ecosystem from being N limited to being N saturated (Aber et al., 1989, 1998). In N-saturated forests, the atmospheric N input to the ecosystem exceeds the N assimilation capacity of the plants and soil microbes, resulting in increased nitrification and nitrate (NO_3^-) leaching into stream water (Aber et al., 1989, 1998). Excess NO_3^- leaching, in turn, may induce eutrophication in downstream aquatic ecosystems (Aber et al., 1989, 1998; Vitousek et al., 1997).

Many studies have examined the relationship between atmospheric N deposition and N export from forest ecosystems (Aber et al., 2003; Campbell et al., 2004; Dise & Wright, 1995; Dise et al., 2009; Grigal, 2012; Mitchell et al., 1997; Rose et al., 2015; Sebestyen et al., 2019; Tsunogai et al., 2014). Some of these studies reported that N export increases with increasing N deposition (Dise & Wright, 1995; Dise et al., 2009; Grigal, 2012; Mitchell et al., 1997), while other studies reported substantial variations in N export with similar N deposition rates (Aber et al., 2003; Campbell et al., 2004; Grigal, 2012). Early studies on the relationship between atmospheric N deposition and N export from forest ecosystems mostly assumed that significant NO_3^- exports to streams occur after chronically high N supply to forests through deposition that exceeded the N demand of vegetation and soil microbes (Aber et al., 2003; Campbell et al., 2004; Dise & Wright, 1995; Dise et al., 2009; Grigal, 2012; Mitchell et al., 1997). However, Lovett and Goodale (2011) found an immediate increase in NO_3^- leaching after the onset of a long-term N addition experiment in an oak forest in the northeastern USA and proposed that the added N can flow simultaneously to all sinks and losses in the system. They suggested that N leaching loss is controlled by not only biological factors such as retention by vegetation and soil and N transformations by microbes but also by hydrologic factors such as water flow through the soil. Indeed, increases in the direct leaching of atmospheric NO_3^- ($NO_3^-{}_{atm}$) to streams during snowmelt and stormflow events have been reported (Goodale et al., 2009; Michalski et al., 2004; Sebestyen et al., 2008; Williard et al., 2001). These findings highlight the importance of understanding how atmospheric N moves within the watershed after its deposition and how both atmospheric N dynamics and internal N cycling, such as assimilation, decomposition, and inorganic N production within the plant-soil system, affect N export to streams.

In addition to input-output studies conducted at a catchment scale, stable isotope studies have provided insights into the retention, transformation, and loss of N in forest ecosystems. For instance, a study that applied enriched ^{15}N isotope tracers directly to a mature coniferous forest canopy in central Maine, demonstrated that the aboveground parts of the trees were the most important short-term N sink (Dail et al., 2009). Additionally, the natural stable isotopic composition of NO_3^-, especially $\delta^{15}N$ and $\delta^{18}O$, has been used to understand the relative importance of atmospheric and microbial sources of NO_3^- in stream water and groundwater (e.g., Durka et al., 1994; Kendall et al., 2007). NO_3^- formed in the atmosphere ($NO_3^-{}_{atm}$) has a high $\delta^{18}O$ value (+63‰ to +94‰), while remineralized NO_3^- produced through nitrification ($NO_3^-{}_{re}$) has a low $\delta^{18}O$ value (−10‰ to +10‰) (Kendall et al., 2007). By using this distinctive difference in the $\delta^{18}O$ value between $NO_3^-{}_{atm}$ and $NO_3^-{}_{re}$, the fraction of $NO_3^-{}_{atm}$ in total NO_3^- in sample water (f_{atm}) can be calculated as

$$f_{atm} = \frac{\delta^{18}O - NO_{3\,sample}^- - \delta^{18}O - NO_{3\,re}^-}{\delta^{18}O - NO_{3\,atm}^- - \delta^{18}O - NO_{3\,re}^-} \times 100 \quad (1)$$

where the subscripts sample, re, and atm refer to NO_3^- in the water sample (e.g., stream water and groundwater), one from the nitrification end-member, and one from the atmospheric end-member, respectively (Durka et al., 1994). Many studies have examined the f_{atm} of stream water in forests and revealed that microbial nitrification is the dominant source of stream water NO_3^- and that the proportion of $NO_3^-{}_{atm}$ is small regardless of the atmospheric N deposition flux (Curtis et al., 2011; Rose et al., 2015). Durka et al. (1994) are among the few researchers who quantified the fraction of NO_3^- input that is discharged to streams without biological processing. By using the $\delta^{18}O$ value of NO_3^-, they found that 16%–30% of the deposited NO_3^- reached spring water in relatively healthy forests, while almost all of the deposited NO_3^- was directly leached to the spring water in severely damaged forests. However, our understanding of what proportion of the deposited $NO_3^-{}_{atm}$ is directly leached from forested watersheds without biological uptake and transformation is still limited.

Although the $\delta^{18}O$ value of NO_3^- has been measured in many studies, it should be interpreted with caution. To estimate the proportion of $NO_3^-{}_{atm}$ relative to total NO_3^- using the $\delta^{18}O$ value of NO_3^-, we must obtain the $\delta^{18}O$ value of NO_3^- for both $NO_3^-{}_{atm}$ and $NO_3^-{}_{re}$ in each sample (Durka et al., 1994). While the $\delta^{18}O$ value of $NO_3^-{}_{atm}$ can be measured, several assumptions are needed to calculate the $\delta^{18}O$ value of $NO_3^-{}_{re}$ (Kendall et al., 2007). In addition, the $\delta^{18}O$ value of NO_3^- in a sample is known to increase during biological processes such as plant and microbial assimilation of NO_3^- and denitrification (Granger et al., 2008, 2010). Since the source differentiation of NO_3^- in a sample is based on the fact that the $\delta^{18}O$ value of $NO_3^-{}_{atm}$ is distinctively higher than that of $NO_3^-{}_{re}$, the increase in the $\delta^{18}O$ value caused by these biological processes leads to the

overestimation of the proportion of the $NO_3^-{}_{atm}$ to the total NO_3^-. Hence, the accurate quantification of the source differentiation of NO_3^- in a sample using the $\delta^{18}O$ value of NO_3^- is difficult.

To overcome the limitation of using $\delta^{18}O$ for the source differentiation of NO_3^-, ^{17}O excess ($\Delta^{17}O$) of NO_3^- have been increasingly used as a conservative tracer of $NO_3^-{}_{atm}$ in stream water, soil water, and groundwater (Costa et al., 2011; Michalski et al., 2004; Nakagawa et al., 2013, 2018; Tsunogai et al., 2010, 2016). $NO_3^-{}_{atm}$ is produced photochemically and has positive $\Delta^{17}O$ values (+20 to +35‰ in temperate regions, Tsunogai et al., 2010, 2016), whereas $NO_3^-{}_{re}$ has a $\Delta^{17}O$ value of 0‰; therefore, positive $\Delta^{17}O$ values in a sample indicate that some portion of the NO_3^- is $NO_3^-{}_{atm}$ (Michalski et al., 2003, 2004). Moreover, $\Delta^{17}O$ is stable during mass-dependent isotope fractionation processes such as assimilation and denitrification (Michalski et al., 2004). These characteristics of the $\Delta^{17}O$ value of NO_3^- enable the proportion of $NO_3^-{}_{atm}$ to NO_3^- to be quantified in each sample.

Many studies have investigated the proportion of $NO_3^-{}_{atm}$ in the total NO_3^- in stream water and groundwater of forested watersheds using $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^-, and much fewer studies have used the $\Delta^{17}O$ value of NO_3^- (Rose et al., 2015; Sebestyen et al., 2019; Tsunogai et al., 2014). However, the dynamics of $NO_3^-{}_{atm}$ and its relationship with $NO_3^-{}_{re}$ from the forest canopy to the mineral soil are considerably understudied, despite highly dynamic N cycling. During the passage of rainwater through the canopy, forest floor, and mineral soil, some of the $NO_3^-{}_{atm}$ in the water is consumed, while $NO_3^-{}_{re}$ is added to the water (Osaka et al., 2010; Shi et al., 2014). Thus, quantifying these two types of NO_3^- is crucial to understanding the relationships among atmospheric N deposition, internal N cycling, and N leaching. Furthermore, this quantification will improve our understanding of how NO_3^- dynamics differ among different ecosystems and how these dynamics are related to various factors in the system; this understanding will in turn contribute to the development of a predictive model that describes forest N cycling.

This study quantitatively evaluated the dynamics of atmospheric and remineralized NO_3^- from the canopy to the surface soil using the $\Delta^{17}O$ value of NO_3^- in a cool-temperate conifer-broadleaved mixed natural forest. We conducted observations beneath three representative plant species of cool-temperate natural forests of northern Japan, oak, spruce, and an understory dwarf bamboo, as N cycling is known to differ among plant species (Augusto et al., 2015; Binkley & Giardina, 1998; Hobbie et al., 1992). In this study, we use the term "retention" to mean assimilation and transformation by organisms, adsorption to a plant surface, and gaseous loss.

2. Methods

2.1. Study Site

This study was conducted in the Uryu Experimental Forest (UREF) of Hokkaido University (44°21′ N, 142°16′ E), a core site of the Japan Long-Term Ecological Research Network, located in northern Hokkaido, Japan (Figure 1a). The soil is a Cambisol (IUSS Working Group WRB 2006) that is situated on Pleistocene sedimentary rock. The organic horizon mainly consists of an Oe/Oa horizon with a relatively thin Oi horizon. According to the meteorological data recorded in the UREF from 2000 to 2009, the mean annual temperature is approximately 4 °C, and the mean annual precipitation is approximately 1,150 mm. The forest floor is usually covered by snow from November to May, with a maximum snow depth of approximately 200 cm. The annual wet N deposition for 2014 was 4.5 kg N ha^{-1} yr^{-1} (Environmental Laboratories Association, 2016).

Coniferous-broadleaved mixed forest is a major type of cool-temperate forest. The dominant coniferous species in the study area are *Abies sachalinensis* and *Picea glehnii*. The dominant deciduous broadleaved species in the UREF are *Quercus crispula*, *Acer mono*, and *Betula ermanii* (Noguchi & Yoshida, 2004). *Sasa senanensis,* dwarf bamboo, is a dominant understory species and usually covers the forest floor exclusively in areas where tree densities are low. Within a 3 ha coniferous-broadleaved mixed forest watershed, we selected four mature individuals of *Q. crispula* (hereafter oak) and *P. glehnii* (hereafter spruce) and four canopy gaps with dense *Sasa senanensis* (hereafter *Sasa*) to collect throughfall, forest floor leachate, and soil water (Figure 1b). We selected trees whose canopies did not overlap with others or overlapped with the same species as much as possible to avoid collecting throughfall from other tree species. The diameter at breast height of the trees ranged from 39 to 105 cm; the height of *Sasa* ranged from 40 to 140 cm, depending

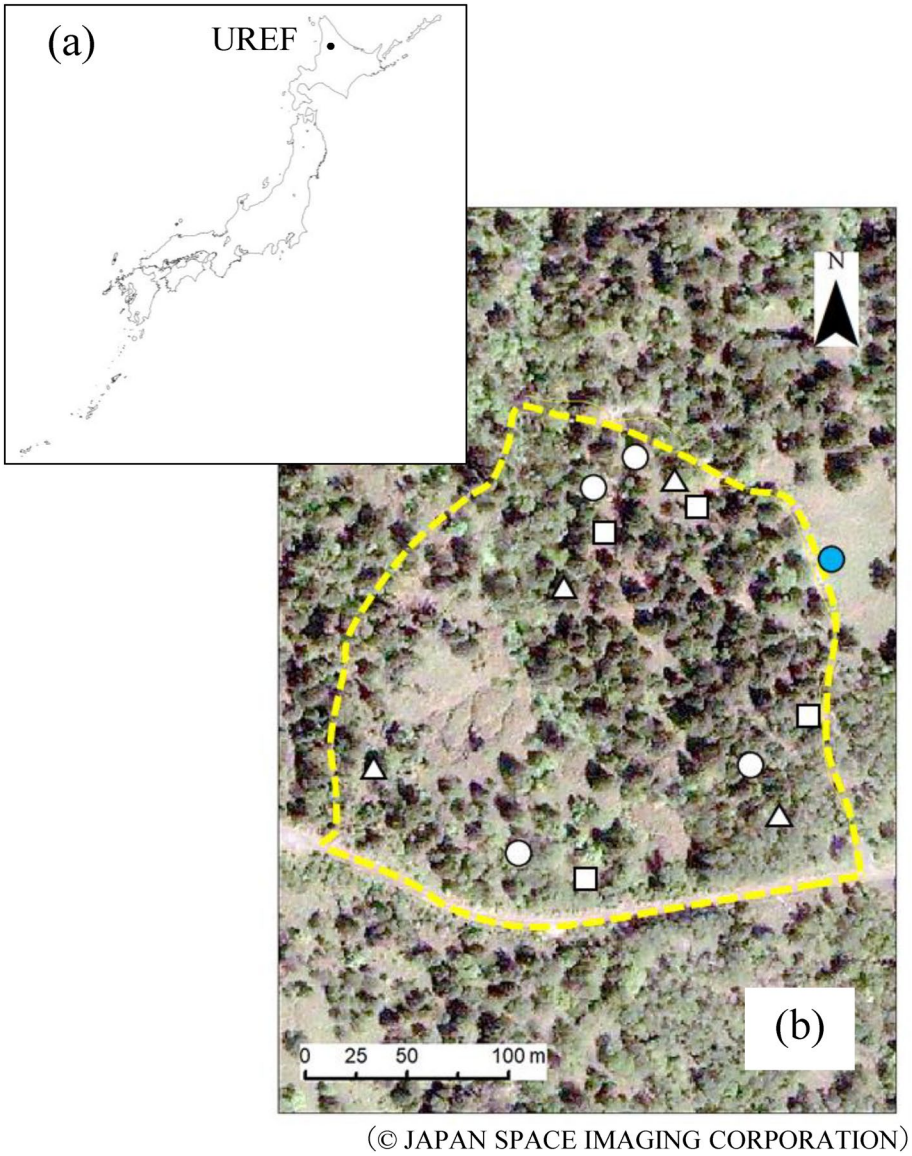

Figure 1. Location of the study site. (a) Location of the Uryu Experimental Forest (UREF); (b) Satellite image of the study forest (coniferous-broadleaved mixed forest; canopy trees are located in the areas of rough surface with dark or light green color, and *Sasa*-dominated canopy gaps are located in the areas of smooth surface with a pale green area in the image). The dotted line shows the watershed boundary and the sampling locations for oak (circle), spruce (triangle), *Sasa* in canopy gaps (square), and bulk deposition (blue circle).

on the light conditions; and the area of the canopy gaps ranged from 113 to 1,019 m^2. The age of the canopy gaps is at least 10 years. The watershed has a gentle slope. The forest floor beneath the oak trees is covered by *Sasa,* although its biomass beneath the oak trees is usually lower than that in the canopy gaps. *Sasa* is sparse beneath the spruce trees.

2.2. Sample Collection

Bulk deposition was collected at one location in a large canopy gap next to the study watershed (Figure 1b) using a polyethylene funnel (diameter: 30 cm) attached to a polyethylene plastic bottle; the funnel was placed above the height of surrounding *Sasa*. We assumed that the quantity and quality of bulk deposition would be the same across our study site. Throughfall was collected using a polyvinyl chloride (PVC) rain gutter (10 × 180 cm) attached to a polyethylene plastic bottle. The throughfall collector for all canopy types

was set at a height of approximately 30 cm above the ground. The throughfall samplers for oak and spruce were oriented from the tree trunk toward the edge of the canopy to take the spatial variation of throughfall into account. In the canopy gaps, the throughfall samplers were set in the locations where throughfall from surrounding trees did not reach. Bulk deposition and throughfall collectors were both covered with a 1 mm mesh screen to prevent litterfall and insects from entering the plastic bottles. Tension-free lysimeters made of a PVC column (5 × 20 cm) cut in half and attached to a polyethylene plastic bottle were installed at the bottom of the Oa layer and at a 10 cm depth of the mineral soil to collect two types of leaching water, that is, forest floor leachate and soil water. The lysimeters were installed at the halfway point between the tree trunk and the canopy edge or in the middle of the canopy gap. One throughfall collector, three lysimeters for forest floor leachate, and three lysimeters for soil water were installed beneath each tree canopy and in each canopy gap. The sampling bottles for bulk deposition and throughfall collection were installed under thick *Sasa* shade, and those for forest floor leachate and soil water were installed below ground to minimize increases in water temperature.

Sample collection was conducted during rain events (four to seven rainy days) once in June, September, and October 2014, representing spring, late summer, and autumn, respectively, at the study site. To reduce the impact of collector installation on water quality, tension-free lysimeters were installed well before the first sampling (from late August to early September 2013). The water samples were transported to the laboratory within 3 days of the last rainfall to minimize the changes in water quality and evaporation. After each sampling event, the rain and throughfall collectors were washed with tap water and installed again immediately before the next sampling. The sampling bottles were prewashed by soaking in 0.5 M HCl and were rinsed with pure water. The samples were filtered through precombusted glass microfiber filters (filter porosity: 0.7 μm, Whatman GF/F, GE Healthcare UK Ltd., Buckinghamshire, England) within 24 h after collection and stored in a freezer at −15 °C until analysis.

We did not measure stemflow because we assumed that the contribution of stemflow to the NO_3^- inputs to the forest floor was relatively small. Ozawa (2004) examined the NO_3^- flux for throughfall and stemflow in a coniferous-broadleaved mixed forest stand located in the UREF and reported that the NO_3^- flux in throughfall was 9.1 mmol m^{-2} $period^{-1}$, while that in stemflow was 0.06 mmol m^{-2} $period^{-1}$ despite the similar NO_3^- concentrations in throughfall and stemflow. Studies in other boreal and temperate forests have also reported that the amount of stemflow relative to rainfall was very low (<15%), while the amount of throughfall relative to rainfall was higher than 63% (Barbier et al., 2009; Llorens & Domingo, 2007).

2.3. Analysis

The NO_3^- concentrations of the water samples were analyzed by ion chromatography (HIC-20A Super; Shimadzu Corp., Japan). The stable isotopic compositions of NO_3^- were determined using a method that chemically converts NO_3^- into N_2O, according to Tsunogai et al. (2018). Approximately 10 mL of each sample solution was pipetted into a vial with a septum cap. Then, 0.5 g of spongy cadmium was added, followed by 150 μL of a 1 M $NaHCO_3$ solution. The sample was then shaken for 18–24 h at a rate of 2 cycles s^{-1}. Then, the sample solution was decanted into a different vial with a septum cap. After purging the solution using high-purity helium, 0.4 mL of azide/acetic acid buffer was added. After 45 min, the solution was made basic by adding 0.2 mL of 6 M NaOH.

Then, the stable isotopic compositions ($\delta^{15}N$, $\delta^{18}O$, and $\delta^{17}O$) of N_2O in each vial were determined by using a continuous-flow isotope ratio mass spectrometry (CF-IRMS) system, which consists of an original helium purge, a trap line and a Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified Combustion III interface and an Agilent 6890 gas chromatograph. The isotopic ratios of the N and O values of NO_3^- in the sample are expressed as:

$$\delta^{15}\mathrm{N}\left(\text{or } \delta^{18}\mathrm{O} \text{ or } \delta^{17}\mathrm{O}\right) = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \tag{2}$$

where R is the $^{15}N/^{14}N$ (or $^{18}O/^{16}O$ or $^{17}O/^{16}O$) ratio of a sample (R_{sample}) or standard material ($R_{standard}$). Then, the $\Delta^{17}O$ value of NO_3^- in the sample was calculated by the following equation:

$$\Delta^{17}O = \frac{\left(1+\delta^{17}O\right)}{\left(1+\delta^{18}O\right)^{\beta}} - 1 \quad (3)$$

where the constant β is 0.5279 (Kaiser et al., 2007).

To calibrate the δ values of the sample NO_3^- relative to the international scale, as well as to correct for both the isotope fractionation during the chemical conversion to N_2O and the progress of oxygen isotope exchange between the NO_3^--derived reaction intermediate and water (ca. 20%), the $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of the sample-derived N_2O were compared with those derived from the local laboratory NO_3^- standards. The local laboratory NO_3^- standards were calibrated using USGS-34 ($\delta^{15}N = -1.8‰$, $\delta^{18}O = -27.93‰$, $\Delta^{17}O = +0.04‰$) and USGS-35 ($\delta^{15}N = +2.7‰$, $\delta^{18}O = +57.5‰$, $\Delta^{17}O = +20.88‰$) (Böhlke et al., 2003; Kaiser et al., 2007). In this study, the internal standard method (Nakagawa et al., 2013; Tsunogai et al., 2014) was adopted for the calibration of sample NO_3^-. All values in this study are expressed relative to air (for N) and Vienna Standard Mean Ocean Water (for oxygen). The analyses of the $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of NO_3^- for each sample were repeated at least three times to attain high precision. As a result, all isotopic data presented in this study have an error (standard error of the mean) within ±0.2‰ for $\delta^{15}N$, ±0.3‰ for $\delta^{18}O$, and ±0.1‰ for $\Delta^{17}O$.

To determine whether the samples had deteriorated or were contaminated during storage and whether the conversion rate from NO_3^- to N_2O was sufficient, the concentrations of NO_3^- in the samples were determined each time we analyzed the isotopic compositions using CF-IRMS based on the N_2O^+ or O_2^+ outputs. The $\delta^{15}N$, $\delta^{18}O$, or $\Delta^{17}O$ values were adopted only when concentrations measured by CF-IRMS correlated with those measured by ion chromatography immediately after sampling within a difference of 10%. For the samples that did not meet the criterion, NO_3^- in the samples was converted to N_2O and analyzed for stable isotopic compositions again. None of the samples showed conversion efficiencies lower or higher than the criterion during the second analysis, indicating that none of the samples had deteriorated or were contaminated during storage.

In this study, water samples that did not contain enough water volume or high enough NO_3^- concentration for the stable isotope analysis were not included in the following data analysis. Additionally, samples with nitrite (NO_2^-) to NO_3^- ratios higher than 10% were excluded from further analysis because the chemical conversion method converts not only NO_3^- but also NO_2^- to N_2O (McIlvin & Altabet, 2005), and high NO_2^- concentrations may mislead the interpretation of the results.

2.4. Data Analysis

We calculated the concentrations and fluxes of $NO_3^-{}_{atm}$ from bulk deposition to soil water, the canopy retention of NO_3^-, and the proportion of $NO_3^-{}_{atm}$ flux in the soil water to the atmospheric deposition flux. A schematic diagram of these variables along the canopy-soil continuum is shown in Figure 2. The proportions of $NO_3^-{}_{atm}$ to the total NO_3^- (% $NO_3^-{}_{atm}$) were calculated by the following equation:

$$\%NO_{3\,atm}^{-} = \frac{\Delta^{17}O_{sample}}{\Delta^{17}O_{atm}} \times 100 \quad (4)$$

where $\Delta^{17}O_{sample}$ is the $\Delta^{17}O$ value of the sample (i.e., throughfall, forest floor leachate, and soil water), and $\Delta^{17}O_{atm}$ is the $\Delta^{17}O$ value of the bulk deposition.

Then, the $NO_3^-{}_{atm}$ concentrations (C_{atm}) were calculated as:

$$C_{atm} = C_{total} \times \%NO_{3\,atm}^{-} / 100 \quad (5)$$

where C_{total} is the total NO_3^- concentration. The total NO_3^- fluxes (F_{total}) and the $NO_3^-{}_{atm}$ fluxes (F_{atm}; arrows 1–4 in Figure 2) were calculated as:

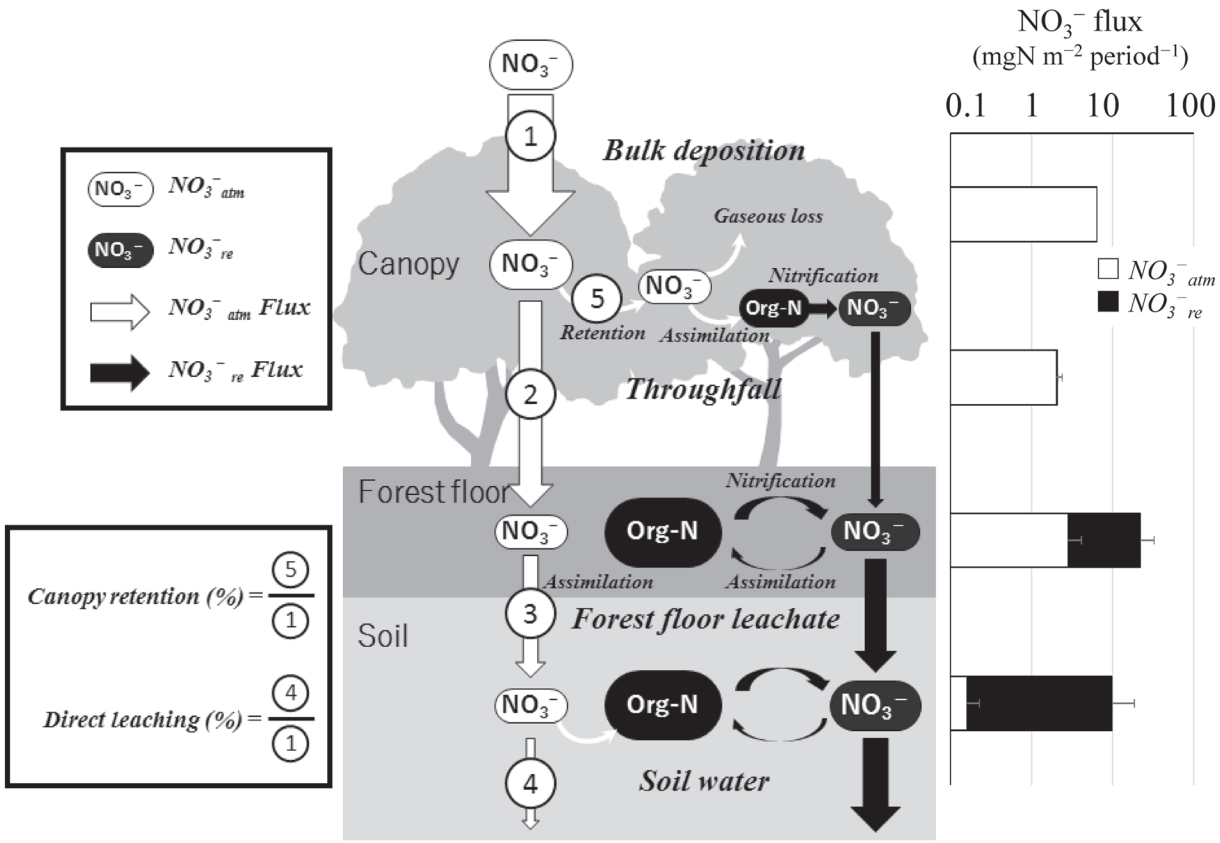

Figure 2. Schematic diagram of the fluxes of atmospheric NO_3^- ($NO_3^-{}_{atm}$) and of remineralized NO_3^- ($NO_3^-{}_{re}$), canopy retention of $NO_3^-{}_{atm}$ (canopy retention), and the proportion of the $NO_3^-{}_{atm}$ flux in the soil water to the atmospheric deposition flux (direct leaching) in the canopy–soil continuum. The bar chart shows the vertical changes in the NO_3^- flux with the fractions of $NO_3^-{}_{atm}$ and $NO_3^-{}_{re}$ for *Sasa* in the canopy gap for October 2014 in this study.

$$F_{\text{total}} = C_{\text{total}} \times \text{water volume} \tag{6}$$

$$F_{\text{atm}} = F_{\text{total}} \times \text{NO}_{3\text{atm}}^- / 100 \tag{7}$$

Then, we calculated the proportion of $NO_3^-{}_{atm}$ flux in the soil water to the atmospheric deposition flux (% direct leaching: Figure 2) by:

$$\%\ \text{direct leaching} = \frac{F_{\text{atm}}\ \text{in soil water}}{F_{\text{atm}}\ \text{in bulk deposition}} \times 100 \tag{8}$$

Additionally, the retention of NO_3^- by the canopy (canopy retention: Figure 2) was calculated by the following equation:

$$\text{Canopy}\text{NO}_3^- \text{ retention}\,(\%) = \frac{F_{\text{atm}}\ \text{in bulk deposition} - F_{\text{atm}}\ \text{in throughfall}}{F_{\text{atm}}\ \text{in bulk deposition}} \times 100 \tag{9}$$

We also calculated the $\delta^{15}N$ and $\delta^{18}O$ values of $NO_3^-{}_{re}$ ($\delta^{15}N_{re}$ and $\delta^{18}O_{re}$, respectively) by excluding the contribution of $NO_3^-{}_{atm}$ from the $\delta^{15}N$ and $\delta^{18}O$ values of total NO_3^- (Dejwakh et al., 2012; Tsunogai et al., 2016). Dual-isotope plots of $\delta^{15}N$ versus $\delta^{18}O$ were used previously to assess the occurrence of denitrification and assimilation (Granger et al., 2004, 2008). Assimilation of NO_3^- causes $\delta^{15}N$ and $\delta^{18}O$ to increase in a 1:1 ratio, and denitrification of NO_3^- causes $\delta^{15}N$ and $\delta^{18}O$ to increase in a ratio between 1:1 and 2:1 (Granger et al., 2004, 2008).

Table 1
The Proportions of Atmospheric Nitrate to Total Nitrate in Bulk Deposition, Throughfall, Forest Floor Leachate, and Soil Water During Each Sampling Event

		Oak		Spruce		*Sasa*	
Sampling month	Sample type	Mean ± se	*n*	Mean ± se	*n*	Mean ± se	*n*
June 2014	BD	100	1	100	1	100	1
	TF	NA	–	76.3	1	91.8 ± 1.9	3
	FF	19.8 ± 11.6	2	NA	–	6.2 ± 0.5	3
	SW	4.1 ± 1.7	3	NA	–	8.6 ± 3.2	3
September 2014	BD	100	1	100	1	100	1
	TF	82.5 ± 3.5	4	86.0 ± 4.7	2	100 ± 0.0	3
	FF	1.5 ± 1.2	2	1.1 ± 0.7	3	3.9 ± 0.1	2
	SW	5.2 ± 1.8	4	NA	–	2.9 ± 0.5	2
October 2014	BD	100	1	100	1	100	1
	TF	96.5	1	100	1	97.7 ± 1.2	3
	FF	5.4 ± 1.9	3	4.5 ± 0.5	2	19.9 ± 7.2	4
	SW	4.3 ± 3.9	3	NA	–	11.8 ± 11.3	2

Abbreviations: BD, bulk deposition; FF, forest floor leachate; *n*, sample size; NA, not available; se, standard error; SW, soil water at 10 cm depth; TF, throughfall.

However, because $\delta^{18}O$ values of $NO_3^-{}_{atm}$ are higher than those of $NO_3^-{}_{re,}$ while $\delta^{15}N$ values of $NO_3^-{}_{atm}$ are similar to those of $NO_3^-{}_{re}$, the influence of $NO_3^-{}_{atm}$ on the $\delta^{15}N$ versus $\delta^{18}O$ plots results in an interpretation with low accuracy. Therefore, we calculated $\delta^{15}N_{re}$ and $\delta^{18}O_{re}$ by using the following equations (Riha et al., 2014; Tsunogai et al., 2014):

$$\delta^{15}N_{re} = \frac{C_{total} \cdot \delta^{15}N - C_{atm} \cdot \delta^{15}N_{atm}}{C_{total} - C_{atm}} \quad (10)$$

$$\delta^{18}O_{re} = \frac{C_{total} \cdot \delta^{18}O - C_{atm} \cdot \delta^{18}O_{atm}}{C_{total} - C_{atm}} \quad (11)$$

Before the statistical analyses, the observed and calculated values for the forest floor and soil water samples were averaged over the lysimeter replicates for each tree and each canopy gap (see Table 1 for sample sizes for each measurement). Statistical analyses were performed with log-transformed data in R software (version 4.0.0; R Core Team, 2020). When the original data contained negative values, the original values were used. Vertical changes in the variables for each plant species were analyzed using all data across the three sampling periods (i.e., data on bulk deposition, throughfall, forest floor leachate, and soil water in June, September, and October) because the small number of replications made sampling event-based analysis difficult. For the differences between the bulk deposition and throughfall, we used a pairwise t-test because bulk deposition was collected at a single location, and we assumed that the bulk deposition characteristics were the same across all sampling locations. For the differences between the throughfall and the forest floor leachate and between the forest floor leachate and the soil water, we used Welch's two-sample *t*-test because it was not possible to analyze pairs due to missing data. The differences in the canopy retention of NO_3^- among the plant species were analyzed for each sampling event using a linear model with Tukey's multiple comparisons with the multcomp package (version 1.4-8).

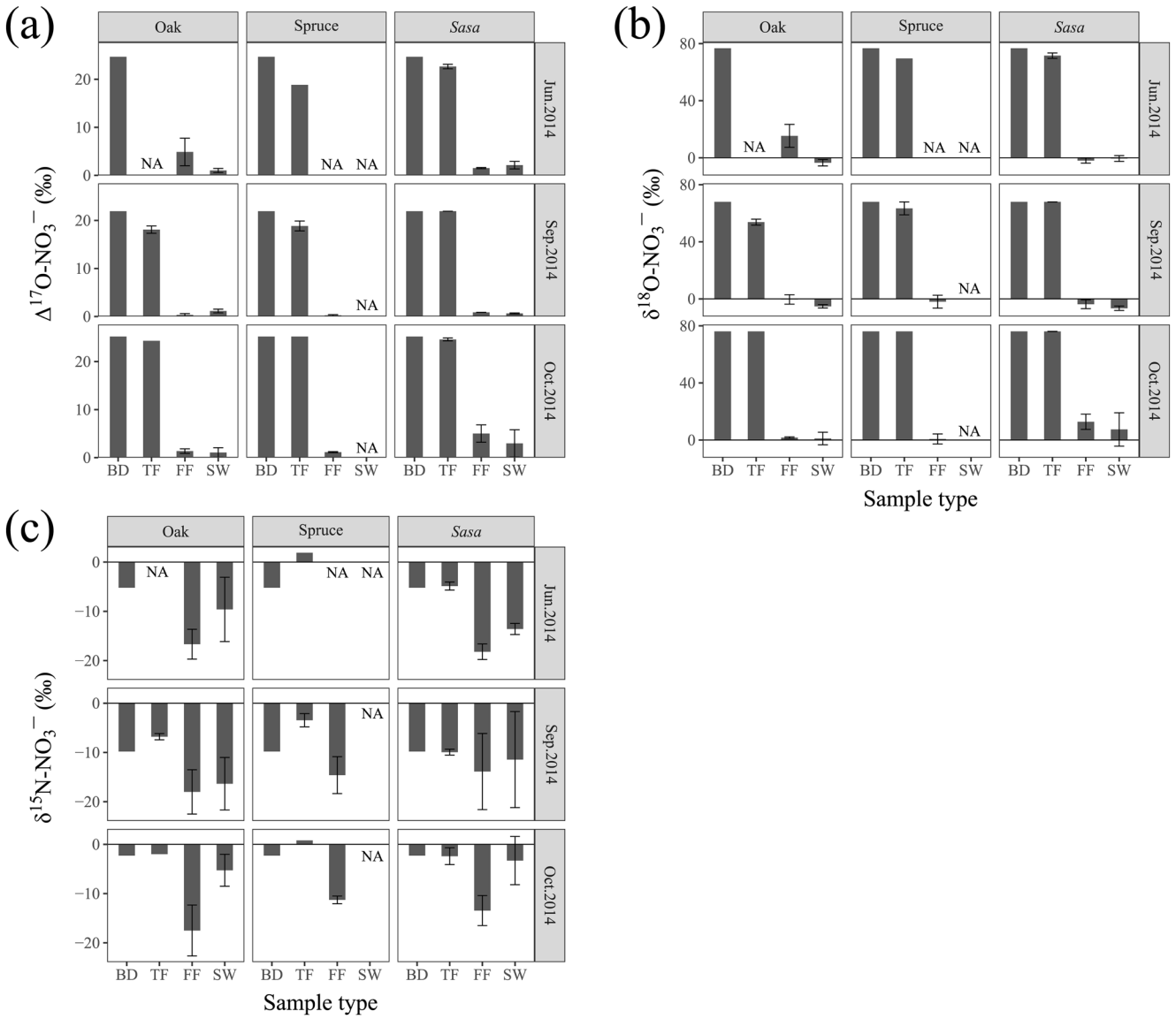

Figure 3. Vertical trend of mean $\Delta^{17}O$ (a), $\delta^{18}O$ (b), and $\delta^{15}N$ (c) values of nitrate for each plant species during each sampling event (June, September, and October 2014). Error bars show standard errors. BD, bulk deposition; FF, forest floor leachate; NA, not available; SW, soil water at 10 cm depth; TF, throughfall.

3. Results and Discussion

3.1. Change in the Proportion of $NO_3^-{}_{atm}$ to Total NO_3^- From Bulk Deposition to Surface Soil Water

The $\Delta^{17}O$ value of NO_3^- significantly decreased from bulk deposition to throughfall for oak, spruce, and *Sasa* in the canopy gap (Figure 3a and Table S1). The $\delta^{18}O$ values of NO_3^- also decreased from bulk deposition to throughfall (Figure 3b); a significant decrease was found only for oak (Table S2).

The measured $\Delta^{17}O$ values of NO_3^- in water samples collected during the three sampling events were comparable with the values reported in previous studies. For bulk deposition, the average $\Delta^{17}O$ value of NO_3^- in this study was +23.9 ± 1.8‰ (n = 3), while the annual average value for Rishiri Island, located approximately 110 km northwest of the study site, was reported to be +26.2‰ (n = 32, Tsunogai et al., 2010). The 3-year average $\Delta^{17}O$ value of the bulk deposition on Sado Island, central Japan, was also similar to that on Rishiri Island: +26.3‰ (n = 196, Tsunogai et al., 2016). It has been reported that the $\Delta^{17}O$ value of bulk deposition is low (approximately +20‰) in mid-summer and high (approximately +30‰) in mid-winter (Tsunogai

et al., 2010, 2016). Therefore, it is reasonable that the average $\Delta^{17}O$ value of our samples collected in late spring to early autumn was a few ‰ points lower than the reported annual average values. For throughfall, the $\Delta^{17}O$ value in this study ranged from +16.1‰ to +25.2‰ ($n = 18$), while the $\Delta^{17}O$ values measured in forests in the United Kingdom ranged from +10.1‰ to +21.7‰ ($n = 4$, Guerrieri et al., 2015).

The observed decline in the $\Delta^{17}O$ values of NO_3^- from bulk deposition to throughfall suggests the occurrence of nitrification in the canopy (Figure 3a). The average % $NO_3^-{}_{atm}$ in the throughfall for each sampling event for oak, spruce, and *Sasa* in the canopy gap ranged from 82.5% to 96.5%, from 76.3% to 100%, and from 91.8% to 100%, respectively (Table 1). In other words, on average, 14.7 ± 3.9% ($n = 5$), 12.9 ± 5.2% ($n = 4$), and 3.5 ± 1.4% ($n = 9$) of the total NO_3^- in the throughfall was remineralized NO_3^- for oak, spruce, and *Sasa* in the canopy gap, respectively. There are several reports on nitrification in forest canopies (Guerrieri et al., 2015; Shi et al., 2014). For example, in the United Kingdom, Guerrieri et al. (2015) reported that 17% and 59% of NO_3^- in throughfall was derived from nitrification in the canopies of Scots pine and beech, respectively, using the $\Delta^{17}O$ value of NO_3^-. Shi et al. (2014) also reported decreases in the $\delta^{18}O$ value of NO_3^- from bulk deposition to throughfall and stem flow in a *Quercus acutissima* stand and a *Cryptomeria japonica* stand in Japan and concluded that the decreases were caused by nitrification in the canopies. Furthermore, Watanabe et al. (2016) detected genes of ammonia-oxidizing archaea from the throughfall and leaf surface of Japanese cedar trees in Japan.

Although the decrease in the $\Delta^{17}O$ value of NO_3^- from the bulk deposition to the throughfall suggests the occurrence of nitrification in the canopy, the result needs to be interpreted with caution. First, there is a possibility that the reduction in the $\Delta^{17}O$ value of NO_3^- from the bulk deposition to the throughfall was caused by the washing off of HNO_3 and particulate NO_3^- that had deposited in the forest canopy as dry deposition. Previous studies suggested that dry deposition significantly contributes to the total oxidized N deposition in some locations (Sickles & Shadwick, 2007). Additionally, Rose et al. (2019) noted that interactions between biogenic NO_x emitted from soils and hydroxyl and organo-peroxy radicals could result in the accumulation of gaseous HNO_3 and particulate NO_3^- with low $\Delta^{17}O$ values within the forest canopy between rainfall events and the washout of this biogenically sourced atmospheric NO_3^- during subsequent rainfall events. Therefore, if the dry deposition of HNO_3 and particulate NO_3^- exhibits lower $\Delta^{17}O$ values than wet NO_3^- deposition, our results do not necessarily indicate the occurrence of nitrification in the canopy. However, a previous report on the $\Delta^{17}O$ value of NO_3^- of dry deposition suggests that such an influence is unlikely. Nelson et al. (2018) investigated the $\Delta^{17}O$ value of wet and dry NO_3^- deposition in urban and rural sites along the western coast of Hokkaido, Japan. They found no significant difference in the $\Delta^{17}O$ value of NO_3^- between wet and dry deposition in a rural area where spruce- and fir-dominated forests and *Sasa*-dominated shrublands were the main land cover type; in contrast, the $\Delta^{17}O$ value of dry NO_3^- deposition in the urban site was lower than that of wet deposition in the urban site and those of wet and dry deposition in the rural site. As our study site is located in a rural area, we can assume that the impact of dry deposition on the $\Delta^{17}O$ value of NO_3^- in the throughfall is not significant in this study. Second, washing off NO_3^-, which is released from leaves, might have lowered the $\Delta^{17}O$ value. Recent studies have shown that leaf NO_3^- consists of both atmospheric NO_3^- and soil NO_3^- (Bourgeois et al., 2019; Liu et al., 2018). Therefore, if the NO_3^- released from leaves contains NO_3^- originating from nitrification in soils, the $\Delta^{17}O$ value of throughfall NO_3^- would become lower than that of bulk deposition. Last, nitrification might have occurred in the sampling bottle between rainfall events and sample collection. Although we placed the sampling bottles for throughfall under the shade of thick *Sasa* leaves to minimize the impact of sunlight and temperature rise and collected the bottles within three days after the last rain event, we cannot prove that there was no nitrification within the sampling bottles. To the best of our knowledge, none of the previous studies have adequately excluded the possible contribution of processes other than canopy nitrification to the decrease in $\Delta^{17}O$ or $\delta^{18}O$ during the passage through forest canopies. This needs to be clarified in future research to demonstrate the occurrence of canopy nitrification, especially in quantitative examinations.

The $\delta^{15}N$ of NO_3^- significantly increased from bulk deposition to throughfall for oak and spruce (Figure 3c and Table S3). Dry deposition of HNO_3 gas and particulate NO_3^- generally exhibits higher values of $\delta^{15}N$ than NO_3^- in wet deposition (Craine et al., 2015; Elliott et al., 2009; Heaton et al., 1997) The increase in the $\delta^{15}N$ value after passing through the canopies of oak and spruce therefore suggests that dry deposition was washed off during rainfall events. It has been suggested that forest edges can trap horizontally driven

particles and gases more than the forest interior and open fields (Weathers et al., 2001). As seen in Figure 1b, canopy gaps (areas of the smooth surface with pale green color in Figure 1b) were distributed across the study site with various sizes, which means that relatively isolated groups of trees may function as forest edges. In this study, many of the oak and spruce trees are located near canopy gaps (Figure 1b), which might have resulted in a higher capture of the dry deposition by oak and spruce and the subsequent higher wash-off in the rain events compared with *Sasa* in the canopy gaps. This could be a common phenomenon in forests with a *Sasa* understory because canopy gaps tend to exist for a long time due to the inhibition of tree regeneration by *Sasa* (Hiura et al., 1996). Overall, the changes in the isotopic composition of NO_3^- from bulk deposition to throughfall imply that throughfall water contains NO_3^- that originates from wet and dry deposition as well as nitrification in the canopy.

From the throughfall to the forest floor leachate, the $\Delta^{17}O$ value of NO_3^- dramatically decreased from +21.4 ± 2.8‰ (n = 18) to +2.1 ± 2.6‰ (n = 21) (Figure 3a and Table S1). It did not significantly change (+1.4 ± 1.5‰, n = 17) between the forest floor leachate and the soil water (Figure 3 and Table S1). These values are comparable with reported values of extracts from the forest floor (+3.6 ± 2.4‰; n = 4) and surface soil (0–30 cm; +2.0 ± 1.1‰; n = 16) in a temperate forest in northern lower Michigan, USA (Costa et al., 2011) and those of the soil water at a depth of 20 cm (from +0.1‰ to +5.7‰) in a coniferous forest in Niigata prefecture, Japan (Nakagawa et al., 2018). The average % $NO_3^-{}_{atm}$ decreased from 76.3%–100% for the throughfall to 1.1%–11.8% for the soil water (Table 1). The low % $NO_3^-{}_{atm}$ in the soil water indicates that the NO_3^- that leached to the deeper soil horizon consisted mainly of $NO_3^-{}_{re}$. This is consistent with the results of previous studies. Dramatic decreases in the $\delta^{18}O$ or $\Delta^{17}O$ value of NO_3^- from throughfall to soil water have been reported, and these decreases were attributed to the mixing of large amounts of $NO_3^-{}_{re}$ with $NO_3^-{}_{atm}$ (Hattori et al., 2019; Nakagawa et al., 2018; Osaka et al., 2010; Shi et al., 2014). The large decreases in $\delta^{15}N$ from throughfall to forest floor leachate (Figure 3c and Table S1) also suggest the occurrence of nitrification in the forest floor as nitrification produces $\delta^{15}N$-depleted NO_3^- (Högberg, 1997). The analysis of the relationship between $\delta^{15}N$ and $\delta^{18}O$ after removing the influence of $NO_3^-{}_{atm}$ ($\delta^{15}N_{re}$ and $\delta^{18}O_{re}$, respectively) did not imply that there was denitrification during the passage from the forest floor to the mineral soil at 10 cm depth (Figure S1)

3.2. How $NO_3^-{}_{atm}$ Flux Changes Along the Canopy-Soil Continuum

Water flux is an important factor in the estimation of NO_3^- flux. The water flux decreased significantly from bulk deposition to throughfall for all plant species (Figure 4a and Table S2). However, the water fluxes of the forest floor leachate and the soil water were larger than that of the throughfall in many cases and sometimes larger than that of bulk deposition (Figure 4a). There are several possible reasons for the larger fluxes of the forest floor leachate and the soil water than that of the throughfall. First, spatial variation in throughfall flux is a possible reason, especially for oak and spruce. Beier et al. (1993) found that the amount of throughfall water is lower near tree trunks than at locations farther from tree trunks beneath canopies. In this study, throughfall was collected by rain gutters that were oriented from the tree trunk toward the edge of the canopy, while forest floor leachate and soil water were collected by tension-free lysimeters installed at the halfway point between the tree trunk and the canopy edge. This resulted in throughfall being collected in areas of high and low water fluxes, while forest floor leachate and soil water were collected only in the areas that receive high throughfall water flux. Therefore, the amount of throughfall per unit area may have been lower than those of forest floor leachate and soil water. Second, although we assumed that the contribution of stemflow is low for canopy trees, it may not be as low for the *Sasa* in the canopy gaps, which could lead to an underestimation of input water flux to the forest floor by measuring only throughfall. Taniguchi (1995) investigated the throughfall and stemflow of *Sasa* grown in an open space in a small watershed located near our study site and reported that the relative amount of stemflow to bulk deposition ranged from 18% to 57%. These values are larger than the amount for canopy trees, that is, less than 15% (Barbier et al., 2009; Llorens & Domingo, 2007). Last, preferential flow in the forest floor and mineral soil might have resulted in the observed larger water flux in the forest floor leachate and soil water because the occurrence of preferential flow in soils is a common phenomenon (Flury et al., 1994).

The F_{atm} showed a significant and large decrease from bulk deposition to throughfall for oak and *Sasa* in the canopy gap (Figure 4b and Table S5), indicating that the canopies retained $NO_3^-{}_{atm}$. On average, the

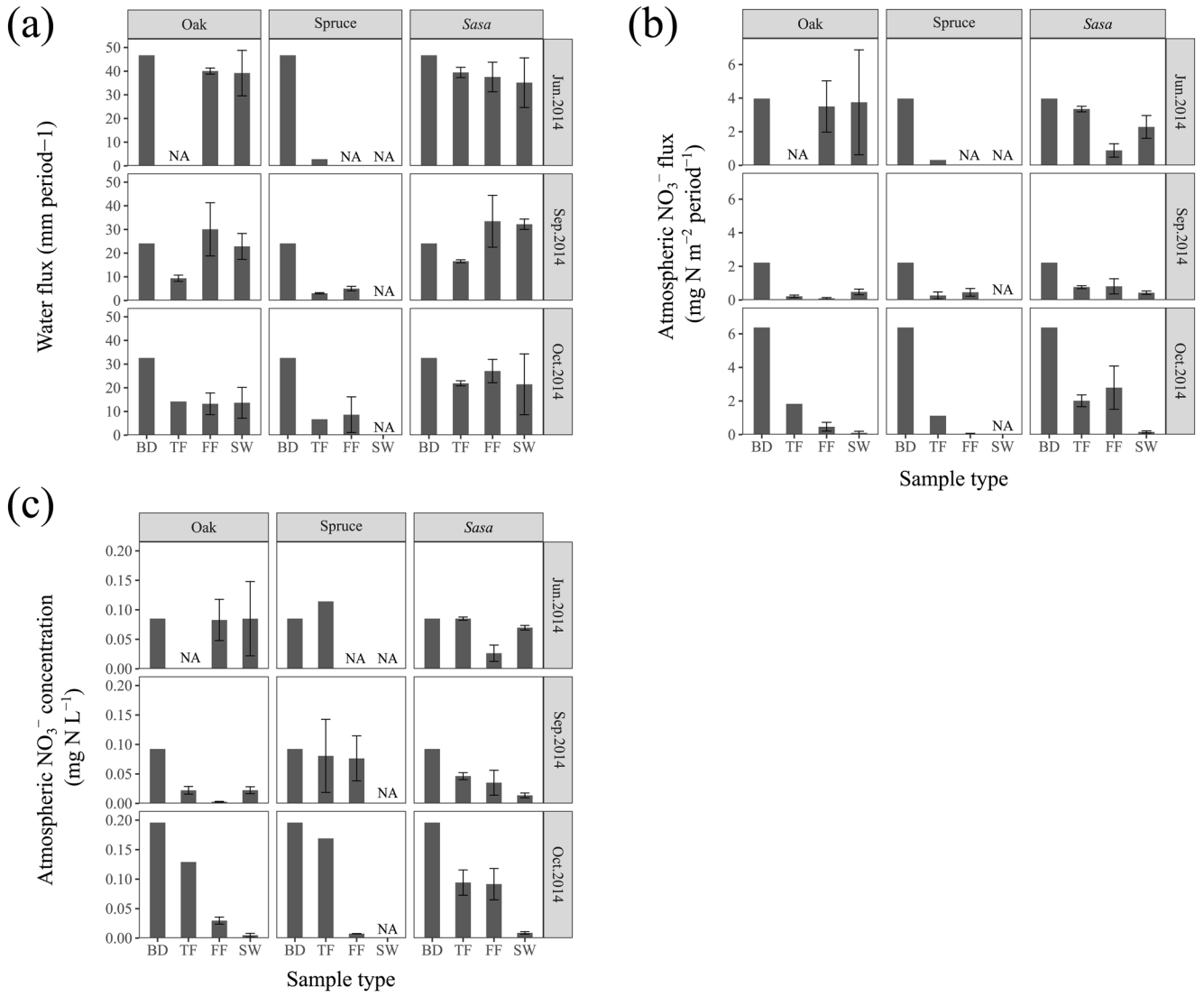

Figure 4. Vertical change in the mean water flux (a), the mean atmospheric nitrate flux (F_{atm}) (b), and the mean atmospheric nitrate concentration (c) for each plant species during the different sampling events (June, September, and October 2014). Error bars show standard errors. BD, bulk deposition; FF, forest floor leachate; NA, not available; SW, soil water at 10 cm depth; TF, throughfall.

canopies of oak, spruce, and *Sasa* in the canopy gap retained 86.3 ± 10.1%, 87.7 ± 8.8%, and 49.9 ± 26.6%, respectively. Our result is consistent with the findings of previous studies. By applying an NH_4NO_3 solution directly to the canopy of a mature spruce-hemlock forest, Gaige et al. (2007) found that the canopy retained 58%–75% of experimental NO_3^- inputs. Lovett and Lindberg (1993) examined wet and dry deposition, throughfall, and stemflow in 12 forested sites across North America and Europe and found that forest canopies retained 10%–90% of the deposited NO_3^-. In their study, the degree of retention was higher in sites with lower atmospheric N deposition than in sites with higher atmospheric N deposition. Some studies used ^{15}N-labeled fertilizer to understand canopy retention. Dail et al. (2009) found that 59% of the applied $^{15}NO_3^-$ was recovered in the aboveground part of the tree (foliage, branches, and sapwood and bark of the trunk) in a whole-forest canopy N-fertilization experiment. By using a different approach from these previous studies, this study demonstrated that the forest canopy is an important retention location for atmospheric N deposition.

We found that the canopy retention of $NO_3^-{}_{atm}$ was significantly higher for oak and spruce than for *Sasa* in the canopy gap in September 2014 ($P < 0.05$, Figure 5). The spruce canopy also showed higher retention than *Sasa* in the canopy gaps in June 2014 ($P < 0.1$, Figure 5). The difference in the canopy retention of

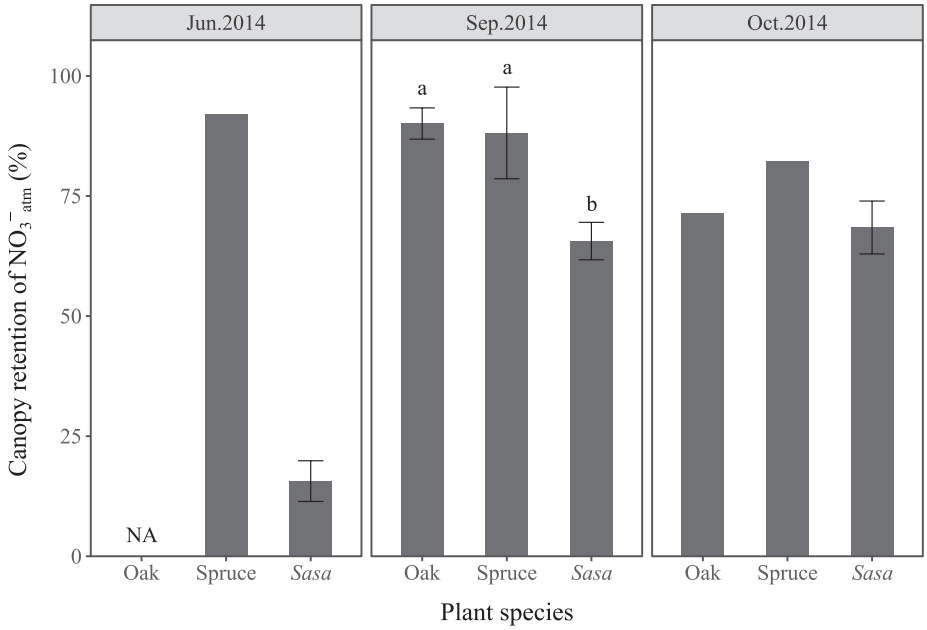

Figure 5. Mean canopy retention of deposited atmospheric nitrate for each plant species during different sampling events (June, September, and October 2014). Error bars show standard errors. NA, not available. Different lowercase letters indicate significant differences among the plant species at $P < 0.05$. In June 2014, spruce had a higher value than *Sasa* in the canopy gap ($P < 0.1$).

$NO_3^-{}_{atm}$ may be due to the difference in the tree canopy structure between the canopy trees and the canopy gap. Nadkarni and Sumera (2004) found that rainfall interception by the canopy increases with increasing amounts of foliage, branches, and epiphytes. Llorens and Domingo (2007) found that an increase in certain canopy structure characteristics, such as basal area, height, and LAI, increases the interception of rainfall. These canopy structure properties were larger in mature oak and spruce than in *Sasa* in the canopy gap in the present study, suggesting that such differences in the canopy structures resulted in the difference in the interception of rainfall and hence the retention of $NO_3^-{}_{atm}$. Additionally, leaf fall had started at the time of our October sampling according to the phenological data collected by the UREF in 2014. Therefore, the lack of significant differences among the plant species in the October sample may be explained by the lower retention capacity of the oak canopy due to the reduced amount of canopy leaves.

The vertical profiles from throughfall to soil water of mean F_{atm} did not show clear decreasing trends and varied among plant species and sampling events (Figure 4b). Experiments that have examined the fate of ^{15}N tracers applied in forests have shown that soil and litter retain the majority of applied N in the short term (Templer et al., 2012). Osaka et al. (2010) found a drastic decrease in the $\delta^{18}O$ of NO_3^- with increasing soil depth without an accompanying decrease in NO_3^- concentration in a forest in central Japan. They attributed this finding to the immediate consumption of atmospheric NO_3^- and production of nitrified NO_3^-. Based on the findings of these studies, one could expect that the flux or concentration of atmospheric NO_3^- would decrease from throughfall to soil water. However, a clear decrease in F_{atm} along the canopy-soil continuum was found only for oak in October 2014, and the other cases of our study did not show a clear continuous decrease in F_{atm} from throughfall to soil water; some showed larger F_{atm} for forest floor leachate than that for throughfall, and others showed larger F_{atm} for soil water than that for forest floor leachate, but all of the trends seem to be buried in a large range of the F_{atm} (Figure 4b). Such high variability in the vertical trend of F_{atm} is partly due to the high variability in the water flux (discussed earlier in this section). If all the variation could be explained by the variability in the water flux and the water evaporation is negligible, the atmospheric NO_3^- concentration should show a constant decrease from throughfall to soil water. However, this was not the case in some of our results; *Sasa* in the canopy gap in June and oak in September showed higher atmospheric NO_3^- concentrations in soil water than in forest floor leachate (Figure 4c). A possible explanation for this pattern is as follows: tension-free lysimeters for collecting soil water tend to collect

Table 2
Proportions of the Atmospheric Nitrate Flux in the Soil Water to the Nitrate Deposition Flux (%)

	Oak		Spruce		*Sasa*	
Sampling month	Mean ± se	*n*	Mean ± se	*n*	Mean ± se	*n*
June 2014	94.4 ± 78.6	3	NA	–	57.6 ± 17.0	3
September 2014	21.5 ± 7.5	4	NA	–	19.4 ± 4.5	2
October 2014	1.6 ± 1.4	3	NA	–	2.5 ± 1.0	2

Abbreviations: *n*, sample size; NA, not available; se, standard error.

rapid water flows in the mineral soil horizon. Rapid water flows transport atmospheric NO_3^- downwards at a rate faster than the rate at which the ecosystem can retain the supplied N. As a result, the atmospheric NO_3^- concentration in the soil water was sometimes higher than that in the forest floor leachate when the lysimeter below the organic horizon collected slow water flows. Tension-free lysimeters tend to collect macropore flow, whereas tension lysimeters are more efficient at collecting micropore water (Fares et al., 2009). Toosi et al. (2014) compared element concentrations, including the concentration of NO_3^-, in soil solutions collected by tension-free lysimeters with those collected by tension lysimeters in a forest and found that NO_3^- concentration decreased with increasing depth only for those solutions collected with tension lysimeters. They attributed the lack of decrease in NO_3^- concentration with increasing soil depth in samples collected by tension-free lysimeters to the reduced opportunities for retention of the dissolved elements resulting from the rapid hydrologic flow paths.

Similarly, the proportion of F_{atm} in the soil water to F_{atm} in the bulk deposition (% direct leaching) was highly variable in time and space (Table 2). As a result, the sum of canopy retention and % direct leaching sometimes exceeded 100% (oak in September 2014). Additionally, we observed a decrease in the % direct leaching from June to October 2014 (Table 2). Seasonal variation in uptake by plant roots and immobilization by soil microorganisms could be a possible reason; however, the general seasonal patterns of these processes have yet to be established. Huang and Schoenau (1997) estimated plant uptake by using ion-exchange resins in a boreal aspen forest and found high NO_3^- uptake in the later growing period, whereas Socci and Templer (2011), using the in situ depletion and ex situ ^{15}N tracer methods, found that uptake by sugar maple and red spruce was higher in the early to the middle part of the growing season than in the later growing period. No seasonal pattern was reported in a whole-ecosystem ^{15}N tracer-addition study in a deciduous forest catchment (Goodale et al., 2015). Likewise, seasonal patterns in microbial immobilization vary among studies (Wardle, 1998). Another possible reason for the observed variability in this study is the difference in rainfall intensity among the sampling events. Greater rainfall intensity is known to increase the leaching of surface-applied solutes (Jarvis, 2007). The volume of bulk deposition decreased in the order June, October, and September 2014 (Figure 4a), whereas the % direct leaching decreased in the order June, September, and October (Table 2); they do not match perfectly. Other factors, such as the initial soil moisture content and the saturated hydraulic conductivity of the soil matrix (Jarvis, 2007), may need to be taken into account to understand the effect of rainfall on the seasonal difference in % direct leaching.

The average % direct leaching for oak and *Sasa* in the canopy gap was larger than the values calculated from stream water $NO_3^-{}_{atm}$ in previous studies. Nakagawa et al. (2018), for instance, determined the $\Delta^{17}O$ value of NO_3^- in stream water in forested watersheds in central Japan and estimated that 2.6%–9.4% of the NO_3^- input to the watershed was directly leached. If we assume that a similar magnitude of direct leaching of $NO_3^-{}_{atm}$ to a stream as that observed by Nakagawa et al. (2018) occurred in our study watershed, then the decline in the direct leaching of $NO_3^-{}_{atm}$ from soil water to stream water suggests significant NO_3^- consumption in deeper soil and groundwater through plant and microbial assimilation or denitrification. This conclusion is supported by the findings of recent studies that used natural isotopes in NO_3^- to examine denitrification in forested catchments. Yu et al. (2016) observed a sharp decline in the NO_3^- concentration and a simultaneous increase in both the $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- along the hydrological flow path in the groundwater discharge zones in a subtropical forested catchment. These authors attributed this to NO_3^- removal due to denitrification. Additionally, by using the $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of NO_3^-, Fang et al. (2015) estimated the denitrification rates across forest sites in southern China and central Japan and found that denitrification, on average, accounted for 65% of the total NO_3^- loss from the sites. Our quantitative understanding of NO_3^- assimilation and denitrification in deep soils and groundwater, however, is still limited; thus, further study is necessary to better understand terrestrial N balances in forested watersheds.

4. Conclusions and Implications

We quantified the vertical changes in the proportion of atmospheric NO_3^- to total NO_3^- and those in the atmospheric NO_3^- fluxes from bulk deposition to soil water using natural triple oxygen isotopes of NO_3^- as tracers in a coniferous-broadleaved mixed forest. The $\Delta^{17}O$ value of NO_3^- dramatically decreased from throughfall to forest floor leachate, indicating that the dominant source of NO_3^- leaching changed from atmospheric NO_3^- to remineralized NO_3^- produced by nitrification when the water passed through the forest floor. This is in line with the findings of previous studies and shows that forest floor and mineral soil play an important role in NO_3^- leaching. In contrast, the atmospheric NO_3^- dynamics suggested that forest canopies play an important role in retaining the deposited atmospheric NO_3^-. We observed a large decrease in the atmospheric NO_3^- flux between bulk deposition and throughfall. On average, oak and spruce canopies retained more than 85% of the deposited atmospheric NO_3^-. The canopy retention of atmospheric NO_3^- seemed higher in the tree canopies than in the canopy of *Sasa* growing in canopy gaps. However, we were not able to adequately assess whether this apparent difference in canopy retention and thus the difference in throughfall input of atmospheric NO_3^- to the ground, were sustained in the soil. This was due to the low number of replicates and the high spatial variability in the atmospheric NO_3^- flux of the forest floor leachate and soil water. Spatially highly variable water flow paths in the organic and mineral soil horizons may have contributed to the large spatial variations in both water flux and atmospheric NO_3^- concentration in soil water. Therefore, more accurate estimation of water flux in forest floor leachate and soil water is warranted for a quantitative evaluation of both atmospheric and remineralized NO_3^- dynamics in soil to understand the relationships among atmospheric N deposition, internal N cycling, and N export from forested watersheds. The use of a combination of tension-free lysimeters and tension lysimeters to collect fast and slow water flows with a large number of replicates would be an option (Fares et al., 2009).

Data Availability Statement

Our data sets are available from the Japan Long-Term Ecological Research Network database (http://db.cger.nies.go.jp/JaLTER/metacat/metacat?action=read&qformat=jalter&sessionid=0&docid=JaLTER-Hokkaido-Kita.326.3).

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