



Quantifying the
effects of clear- and
strip-cutting

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Quantifying the effects of clear-cutting and strip-cutting on nitrate dynamics in a forested watershed using triple oxygen isotopes as tracers

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Received: 7 April 2014 – Accepted: 30 April 2014 – Published: 21 May 2014

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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Abstract

Temporal variations in the stable isotopic compositions of nitrate dissolved in stream water eluted from a cool-temperate forested watershed (8 ha) were measured to quantify the biogeochemical effects of clear-cutting of trees and subsequent strip-cutting of the understory vegetation, dwarf bamboo (*Sasa senanensis*), with special emphasis on changes in the fate of atmospheric nitrate that had been deposited onto the watershed based on $\Delta^{17}\text{O}$ values of nitrate. A significant increase in stream nitrate concentration to $15\ \mu\text{mol L}^{-1}$ in spring of 2004 was correlated with a significant increase in the $\Delta^{17}\text{O}$ values of nitrate. Additionally, the high $\Delta^{17}\text{O}$ values of $+14.3\text{‰}$ suggest that the direct drainage of atmospheric nitrate accounted for more than 50 % of total nitrate exported from the forested watershed. Similar increases in both concentrations and $\Delta^{17}\text{O}$ values were also found in spring of 2005. Conversely, low $\Delta^{17}\text{O}$ values less than $+1.5\text{‰}$ were observed in other seasons, regardless of increases in stream nitrate concentration, indicating that the majority of nitrate exported from the forested watershed during seasons other than spring was remineralized nitrate: those retained in the forested ecosystem as either organic-N or ammonium and then been converted to nitrate via microbial nitrification. When compared with the values prior to strip-cutting, the annual export of atmospheric nitrate and remineralized nitrate increased more than 16-fold and 4-fold, respectively, in 2004, and more than 13-fold and 5-fold, respectively, in 2005. The understory vegetation (*Sasa*) was particularly important to enhancing biological consumption of atmospheric nitrate.

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1 Introduction

1.1 Effects of clear-cutting and strip-cutting on nitrate in stream water

Investigation of nitrate in stream water eluted from a forested watershed is important to understanding nitrogen cycles within the watershed. In addition, the nitrate concentration in stream water is important to primary production downstream. Increased nitrate in stream water can degrade stream habitats. However, nitrate concentrations in stream water eluted from forested watersheds are determined through a complicated interplay of several processes including (1) deposition of atmospheric nitrate ($\text{NO}_{3\text{atm}}^-$), (2) production of remineralized nitrate ($\text{NO}_{3\text{re}}^-$) through nitrification, (3) uptake by plants or microbes, and (4) reduction through denitrification. As a result, interpretation of the processes regulating nitrate concentration in stream water is not always straightforward.

Clear-cutting of plants in forested watersheds often leads to nitrate increasing to levels as high as $1000 \mu\text{mol L}^{-1}$ in stream water eluted from the watersheds (Likens et al., 1970; Swank et al., 2001), as well as acidification (Likens et al., 1970; Swank et al., 2001; Vitousek and Melillo, 1979). Enhancement of the production of fresh remineralized nitrate within soils due to disturbances and/or hindrance of the uptake of such remineralized nitrate in soils might play a large role in increases in nitrate in streams. Moreover, previous studies of forested catchments have offered considerable insight into the link between atmospheric nitrate deposition and nitrate discharge to streams (Grennfelt and Hultberg, 1986; Williams et al., 1996; Tietema et al., 1998; Durka et al., 1994). As a result, disturbances to forested watersheds can also increase direct drainage of atmospheric nitrate in stream water subsequent to deposition by hindering biological uptake processes of atmospheric nitrate within forested watersheds.

Temporal variations in stream and soil solution chemistry, fine root biomass, and soil nitrogen processing in accordance with clear-cutting of trees and subsequent strip-cutting of understory vegetation (mainly *Sasa senanensis*) were measured in a forested

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watershed in the Teshio Experimental Forest, Hokkaido University (Fig. 1) in northern Japan (Fukuzawa et al., 2006). In that study, an approximately 50 % decrease in fine root biomass due to understory vegetation cutting was found to induce an increase in the maximum nitrate concentration in stream water from $3 \mu\text{mol L}^{-1}$ to ca. $15 \mu\text{mol L}^{-1}$ and that in soil solution from $30 \mu\text{mol L}^{-1}$ to more than $100 \mu\text{mol L}^{-1}$. These results implied that nitrogen uptake by the understory vegetation was important to preventing nitrogen leaching after tree-cutting, and that the decline of this nitrogen uptake by removal of understory vegetation led to marked nitrate leaching to stream water (Fukuzawa et al., 2006). However, the importance of atmospheric nitrate as the source of increased nitrate in the stream water has not been evaluated to date. Quantitative evaluation of the source of increased nitrate in stream water subsequent to artificial clear-cutting and strip-cutting will improve our understanding of N cycling in forested soils prior to artificial alternations, as well as the mechanisms that regulate the direct discharge of $\text{NO}_3^-_{\text{atm}}$ deposited onto surface ecosystems (Durka et al., 1994; Ohte et al., 2004; Costa et al., 2011; Nakagawa et al., 2013). Thus, in this study, we conducted further isotope analysis of archived stream water samples to clarify the source of increased nitrate.

1.2 Triple oxygen isotopic compositions of nitrate

The natural stable isotopic compositions of nitrate have been widely applied in the determination of the sources of nitrate in natural freshwater systems (Wada et al., 1975; Durka et al., 1994; Williard et al., 2001; Burns and Kendall, 2002; Campbell et al., 2002; Michalski et al., 2004; Ohte et al., 2004, 2010; Hales et al., 2007; Barnes et al., 2008; Burns et al., 2009; Tsunogai et al., 2010, 2011; Tobari et al., 2010; Barnes and Raymond, 2010; Nestler et al., 2011; Curtis et al., 2011; Costa et al., 2011; Pellerin et al., 2012; Deiwakh et al., 2012; Yue et al., 2013; Ohte, 2013; Lohse et al., 2013; Thibodeau et al., 2013; Nakagawa et al., 2013). In particular, triple oxygen isotopic compositions of nitrate have been shown to be a conservative tracer of atmospheric nitrate ($\text{NO}_3^-_{\text{atm}}$). While remineralized nitrate ($\text{NO}_3^-_{\text{re}}$), the oxygen atoms of which are derived from either

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terrestrial O₂ or H₂O through microbial processing (i.e., nitrification), always shows mass-dependent relative relation between ¹⁷O/¹⁶O ratios and ¹⁸O/¹⁶O ratios, NO_{3atm}⁻ displays an anomalous enrichment in ¹⁷O reflecting oxygen atom transfers from atmospheric ozone (O₃) during the conversion of NO_x to NO_{3atm}⁻ (Michalski et al., 2003; Morin et al., 2008; Alexander et al., 2009). Using the Δ¹⁷O signature defined by the following equation (Miller, 2002; Kaiser et al., 2007) enables NO_{3atm}⁻ (Δ¹⁷O > 0) to be distinguished from NO_{3re}⁻ (Δ¹⁷O = 0):

$$\Delta^{17}\text{O} = \frac{1 + \delta^{17}\text{O}}{(1 + \delta^{18}\text{O})^\beta} - 1, \quad (1)$$

where the constant β is 0.5247 (Miller, 2002; Kaiser et al., 2007), δ¹⁸O = R_{sample}/R_{standard} - 1 and R is the ¹⁸O/¹⁶O ratio (or the ¹⁷O/¹⁶O ratio in the case of δ¹⁷O or the ¹⁵N/¹⁴N ratio in the case of δ¹⁵N) of the sample and each international standard. In addition, Δ¹⁷O is stable during mass-dependent isotope fractionation processes within surface ecosystems. As a result, while the atmospheric δ¹⁵N or δ¹⁸O signature can be overprinted by biogeochemical processes, we can use Δ¹⁷O as a conserved tracer of NO_{3atm}⁻ and trace NO_{3atm}⁻, regardless of its partial removal through denitrification and/or uptake subsequent to deposition.

In our previous study, we determined the Δ¹⁷O values of nitrate in aerobic ground-water worldwide to trace the fate of NO_{3atm}⁻ that had been deposited onto and passed through natural background watersheds (Nakagawa et al., 2013). The results of that study revealed that nitrate in groundwater had small Δ¹⁷O values ranging from -0.2‰ to +4.5‰; therefore, we estimated the average mixing ratio of NO_{3atm}⁻ to total nitrate in the groundwater samples to be 3.1 %. Moreover, the concentrations of NO_{3atm}⁻ ranged from less than 0.1 μmol L⁻¹ to 8.5 μmol L⁻¹, with lower NO_{3atm}⁻ concentrations being obtained for those recharged in forested areas with high coverage of vegetation. Based on these findings, we concluded that most NO_{3atm}⁻ deposited onto healthy forested watersheds had been removed by plants and/or microbes subsequent to deposition.

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In this study, we measured temporal variations in the stable isotopic compositions of nitrate in stream water eluted from the forested watershed in the Teshio Experimental forest in accordance with clear-cutting and strip-cutting to quantify the biogeochemical effects of these activities. In particular, this study focused on the fate of NO_3^- being deposited into the forest ecosystem. Specifically, the $\Delta^{17}\text{O}$ tracer was used to quantify temporal variations in the concentration of NO_3^- in stream water to gain insight into the processes controlling the fate and transport of NO_3^- deposited onto the forested watershed. The results presented herein will increase our understanding of fixed-nitrogen processing and fixed-nitrogen retention efficiencies within forest ecosystems as well.

2 Experimental section

2.1 Site description and management

The study site has been described in detail by Fukazawa et al. (2006) and Takagi et al. (2009). Clear-cutting of trees and subsequent strip-cutting of understory vegetation were conducted in a cool-temperate forested watershed in the Teshio Experimental Forest of Hokkaido University in northern Japan (Fig. 1; $45^\circ 03' \text{N}$, $142^\circ 06' \text{E}$). Prior to clear-cutting, the predominant overstory species were fir (*Abies sachalinensis*), birch (*Betula ermanii* and *Betula platyphylla* var. *japonica*) and Mongolian oak (*Quercus mongolica* var. *grosserrata*). The forest floor of the study site is covered with dense understory vegetation primarily consisting of dwarf bamboo (mainly *Sasa senanensis* in flat areas and *Sasa kurilensis* on steep riparian slopes). The bedrock underlying the site consists of sedimentary rock of the Cretaceous period. The air temperature in the region varies from -35°C to $+35^\circ \text{C}$, with an annual mean of 5.6°C . The annual mean precipitation is 1170 mm, 30 % of which is snow. As a result, the site is covered with dense snow from November to March every year.

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To evaluate the effects of clear-cutting on CO₂ exchange in the forest, a monitoring tower was established in 2001 at the central part of the area (Fig. 1) and net ecosystem production over the forest stands has been monitored as part of a project known as the Carbon Cycle and Larch Growth experiment (CC-LaG) (Takagi et al., 2009). Clear-cutting of trees surrounding the tower with an area of 13.7 ha was conducted from January to March 2003 (Takagi et al., 2009; Fukuzawa et al., 2006). Following clear-cutting, logs were transported outside of the basin, while *Sasa* spp. were conserved and detritus (including shoots, twigs and leaves) was left in the basin. The *Sasa* spp. were then strip-cut into 4 m rows by crushing and spreading in October 2003. The area in which the *Sasa* spp. were strip-cut accounted for ca. 50 % of the total tree-cut area in the watershed. Larch seedlings were planted in the *Sasa* spp. strip-cut line immediately after cutting.

2.2 Water sampling

Stream water was sampled at a weir located on the outlet (Yatsume-zawa River) of the watershed (Fig. 1) every 2 weeks from June 2002 to December 2005. The total catchment area of the stream was 8 ha, all of which was the clear-cutting area of CC-LaG project, except for the riparian area and slope, which had a width of about 13 m from the stream. After measurement of the pH using a glass electrode, water samples were filtered through a 0.7 µm GF/F filter and kept at 4 °C for further analysis. Following additional filtering using a 0.2 µm membrane filter in the laboratory, the concentrations of major anions (Cl⁻, NO₃⁻, SO₄²⁻) and cations (Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺) were measured by ion chromatography (DX-500, Dionex Inc., USA). Samples were analyzed within 6 months of sampling and then sealed in 30 mL polyethylene bottles for further analyses, including measurement of the isotopes reported in this study. The longest storage period between bottling and isotope analysis was 7 years. The ion concentrations of samples collected from June 2002 to December 2004 have been presented in our previous study (Fukuzawa et al., 2006).

2.3 Isotope analysis

Prior to isotope analyses, we excluded samples (1) having a residual water volume less than 10 mL, or (2) having nitrate concentrations below the detection limit in this study ($0.8 \mu\text{mol L}^{-1}$). In addition, the nitrate concentration of each stream water sample was determined again by ion chromatography to exclude samples that had been altered during storage. Following screening, a total of one, four, 15 and 18 samples from 2002, 2003, 2004, and 2005, respectively, were analyzed for stable isotopic compositions.

The stable isotopic compositions were determined by converting the nitrate in each sample to N_2O using the chemical method originally developed to determine the $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of seawater and freshwater nitrate (McIlvin and Altabet, 2005), with slight modifications (Tsunogai et al., 2008, 2010, 2011; Konno et al., 2010; Yamazaki et al., 2011; Nakagawa et al., 2013). Then, the stable isotopic compositions of N_2O were determined using a Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system (Tsunogai et al., 2008; Hirota et al., 2010). This system consists of an original helium purge and trap line, a gas chromatograph (Agilent 6890) and a Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified Combustion III interface (Tsunogai et al., 2000, 2002, 2005; Nakagawa et al., 2004) and a specially designed multicollector system (Komatsu et al., 2008). For analysis, aliquots of N_2O were introduced, purified, and then carried continuously into the mass spectrometer via an open split interface, where the isotopologues of N_2O^+ at m/z ratios of 44, 45 and 46 were monitored to determine δ^{45} and δ^{46} . Each analysis was calibrated using a machine-working reference gas (99.999 % N_2O) that was introduced into the mass spectrometer via an open split interface according to a definite schedule to correct for sub-daily temporal variations in the mass spectrometry. In addition, a working-standard gas mixture containing a known concentration of N_2O (ca. 1000 ppm N_2O in air) that was injected from a sampling loop was analyzed in the same way as the samples at least once a day to correct for daily temporal variations in the mass spectrometry.

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Following analyses based on N_2O^+ monitoring, another aliquot of N_2O was introduced to determine the $\Delta^{17}\text{O}$ of N_2O (Komatsu et al., 2008). Using the same procedures as those used in the N_2O^+ monitoring mode, purified N_2O was introduced into our original gold tube unit (Komatsu et al., 2008), which was held at 780°C for the thermal decomposition of N_2O to N_2 and O_2 . The produced O_2 purified from N_2 was then subjected to CF-IRMS to determine the δ^{33} and δ^{34} by simultaneous monitoring of O_2^+ isotopologues at m/z ratios of 32, 33 and 34. Each analysis was calibrated with a machine-working reference gas (99.999 % O_2 gas in a cylinder) that was introduced into the mass spectrometer via an open split interface according to a definite schedule to correct for sub-daily temporal variations in the mass spectrometry. In addition, a working-standard gas mixture containing N_2O of known concentration (ca. 1000 ppm N_2O in air) was analyzed in the same way as the samples at least once a day to correct for daily temporal variations in the mass spectrometry.

The values of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ for N_2O derived from the nitrate in each sample were compared with those derived from our local laboratory nitrate standards that had been calibrated using the internationally distributed isotope reference materials (USGS-34 and USGS-35) (Böhlke et al., 2003; Kaiser et al., 2007) to calibrate the δ values of the sample nitrate to an international scale, as well as to correct for both isotope fractionation during the chemical conversion to N_2O and the progression of oxygen isotope exchange between the nitrate-derived reaction intermediate and water (ca. 20 %). All δ values are expressed relative to air (for nitrogen) and VSMOW (for oxygen) in this paper.

In this study, we adopted the internal standard method (Nakagawa et al., 2013) for accurate calibrations to determine the $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ or $\Delta^{17}\text{O}$ values of nitrate. Specifically, we added each of the nitrate standard solutions (containing ca. 10 mmol L^{-1} nitrate with known $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values) to additional aliquots of the samples until the nitrate concentration was three to five times larger than the original. Then we converted it to N_2O and determined the values of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ or $\Delta^{17}\text{O}$ in a similar manner as was used for each pure sample. After correcting for the contribution of N_2O

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from the nitrate in each sample, we obtained the stable isotopic compositions for N_2O derived from our laboratory nitrate standards. Next, the $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values in the samples were simply calibrated using curves generated from the N_2O derived from the nitrate standards.

The samples had nitrate concentrations of more than $0.8 \mu\text{mol L}^{-1}$, corresponding to nitrate quantities greater than 20 nmol in a 30 mL sample, which is sufficient to determine $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values with high precision. Thus, all isotopic data presented in this study have an error better than $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$, $\pm 0.5\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.2\text{‰}$ for $\Delta^{17}\text{O}$.

Because we used the more precise power law shown in the Eq. (1) to calculate $\Delta^{17}\text{O}$, the estimated $\Delta^{17}\text{O}$ values were somewhat different from those estimated based on traditional linear approximation (Michalski et al., 2002). While the differences were insignificant for most stream water samples evaluated in this study, the differences would be $0.9 \pm 0.2\text{‰}$ for the $\Delta^{17}\text{O}$ values of $\text{NO}_{3\text{atm}}^-$. When using the linearly approximated $\Delta^{17}\text{O}$ values of $\text{NO}_{3\text{atm}}^-$ available in the literature, we recalculated the $\Delta^{17}\text{O}$ values based on the power law.

Nitrite (NO_2^-) in the samples also interferes with the final N_2O produced from nitrate (NO_3^-), because the chemical method also converts NO_2^- to N_2O (McIlvin and Altabet, 2005). Therefore, it was necessary to correct for the contribution of NO_2^- -derived N_2O to accurately determine the stable isotopic compositions of the sample nitrate. However, all samples analyzed in this study contained NO_2^- at concentrations below the detection limit, which corresponded to $\text{NO}_2^-/\text{NO}_3^-$ ratios less than 10 %; thus, the results were used without any corrections.

The $\delta^{18}\text{O}$ values of H_2O in the samples were analyzed using Cavity Ring-Down Spectroscopy (Picarro L2120-i with an A0211 vaporizer and auto sampler), which had an error of $\pm 0.1\text{‰}$. Both VSMOW and VSLAP were used to calibrate the values to the international scale.

2.4 Deposition rate of atmospheric nitrate

Continuous monitoring of the deposition rate of atmospheric nitrate was conducted from April 2008 to March 2012 (FY2008 to FY2011). Wet deposition samples were collected weekly at a height of 2.5 m using a wet only sampler. Nitrate aerosol, nitric acid and nitrous acid were collected for every 3 weeks from the monitoring tower at a height of 30 m (Fig. 1) using the filter pack method (flow rate = 4 L min^{-1}) and a $\text{PM}_{2.5}$ impactor (Noguchi et al., 2007b). Nitrogen oxides (NO_2 and NO) were collected monthly (every 3 or 6 weeks) from a height of 1.5 m using an Ogawa passive sampler. These components were measured by ion chromatography (Dionex ICS-2000/1500) at the laboratory of the Institute of Environmental Science, Hokkaido Research Organization, and the results were used to estimate the dry deposition rates of nitrate by the inferential method using a mean tree height of 3.0 m for FY2008-09 and 4.0 for FY2010-11 (Noguchi et al., 2011).

3 Results and discussion

3.1 Temporal variations in stream water nitrate

As discussed in Fukuzawa et al. (2006), a significant increase in stream nitrate concentration was observed in 2004, probably in response to strip-cutting of the understory dwarf bamboo, *S. senanensis*, in October 2003 (Fig. 2). However, the clear-cutting of trees in March 2003 had little direct impact on stream nitrate concentration. Specifically, the average nitrate concentration was only $0.9 \mu\text{mol L}^{-1}$ in 2002 (average from June to December) and $0.7 \mu\text{mol L}^{-1}$ in 2003 (annual average), while it increased to $3.8 \mu\text{mol L}^{-1}$ in 2004 (annual average) and $3.8 \mu\text{mol L}^{-1}$ in 2005 (annual average). The maximum nitrate concentration also increased from $2.7 \mu\text{mol L}^{-1}$ in 2002 and $3.1 \mu\text{mol L}^{-1}$ in 2003 to $15 \mu\text{mol L}^{-1}$ in 2004 and $12 \mu\text{mol L}^{-1}$ in 2005 (Fig. 2). These

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findings indicate that strip-cutting had significant impacts on nitrate dynamics in the forest ecosystem from 2004 until at least the end of 2005.

Temporal variations in the values of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ of nitrate in accordance with the variations in nitrate concentration since January 2003 are presented in Fig. 3.

The arithmetic average and 1σ variation for the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate were $+1.3 \pm 3.3\text{‰}$ and $+3.4 \pm 11.1\text{‰}$, respectively (Fig. 3). While the average values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were typical of nitrate in natural stream water, the range of $\delta^{18}\text{O}$ values was one of the largest ever reported in natural stream water during continuous monitoring (Burns and Kendall, 2002; Campbell et al., 2002; Ohte et al., 2004, 2010; Hales et al., 2007; Barnes et al., 2008; Burns et al., 2009; Tobari et al., 2010; Barnes and Raymond, 2010; Nestler et al., 2011; Curtis et al., 2011; Pellerin et al., 2012; Yue et al., 2013; Ohte, 2013; Lohse et al., 2013; Thibodeau et al., 2013). The arithmetic average and maximum $\Delta^{17}\text{O}$ values of nitrate were $+2.2 \pm 3.5\text{‰}$ and $+14.3\text{‰}$, respectively (Fig. 3). The $\Delta^{17}\text{O}$ value of $+14.3\text{‰}$ corresponds to the highest $\Delta^{17}\text{O}$ value ever reported for dissolved nitrate in natural stream water (Michalski et al., 2004; Tsunogai et al., 2010; DeJwakh et al., 2012; Liu et al., 2013), as well as that in soil solution (Michalski et al., 2004; Costa et al., 2011).

One of the striking features of the large temporal variations of $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ was the enhancement of both $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ in spring, especially in the years following strip-cutting. Enrichment of nitrate concentration was detected in spring of 2004 and 2005, probably in response to the spring snowmelt after strip-cutting (Fig. 2). The results of the present study using stable isotopes revealed that these enriched nitrate levels were accompanied by elevated values of both $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$. Atmospheric nitrate is characterized by elevated values of both $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ to up to $+110\text{‰}$ (Durka et al., 1994; Kendall, 1998; Savarino et al., 2007; Morin et al., 2008) and $+45\text{‰}$ (Savarino et al., 2007; McCabe et al., 2007; Morin et al., 2008), respectively. In addition, atmospheric nitrate is currently the only source of nitrate that shows $\Delta^{17}\text{O}$ values larger than 0‰ . Accordingly, atmospheric nitrate might be the source of nitrate enrichment during the spring snowmelt. However, the temporal variations in $\delta^{15}\text{N}$ values

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were independent from the variations in $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ (Fig. 3). Overall, these findings indicate that the major process controlling the $\delta^{15}\text{N}$ values appears to be different from those controlling the $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values.

Conversely, temporal variations in the $\delta^{18}\text{O}$ values of H_2O was small and independent from variations in the $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values of nitrate, with an arithmetic average and 1σ variation of $-11.0 \pm 0.7\text{‰}$, which is typical of stream water in the area (Mizota and Kusakabe, 1994). The annual flow volume of the stream was stable at around $8 \times 10^8 \text{ L year}^{-1}$ every year as well (Fig. 2), which corresponds to more than 80 % of the total precipitation in the catchment. Considering the evaporative loss of water from the catchment area, water loss via groundwater flow must be very low for the watershed. Thus, we assumed that the studied stream was the only channel through which nitrate was eluted from the catchment area for later discussions.

3.2 $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of atmospheric nitrate

To further verify that $\text{NO}_{3\text{atm}}^-$ was responsible for the elevated $\Delta^{17}\text{O}$ values in the samples by up to $+14.3\text{‰}$ in spring 2004 and 2005, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in the samples were plotted as a function of $\Delta^{17}\text{O}$ (Fig. 4). Because $\text{NO}_{3\text{atm}}^-$ is enriched in both ^{18}O and ^{17}O simultaneously (Michalski et al., 2003; Tsunogai et al., 2010), ^{18}O -enrichment was expected for samples showing elevated $\Delta^{17}\text{O}$ values if $\text{NO}_{3\text{atm}}^-$ was responsible for the increased levels. As shown in Fig. 4b, the $\delta^{18}\text{O}$ values in the samples showed strong linear correlation with the $\Delta^{17}\text{O}$ values ($r^2 = 0.92$). Additionally, when we extrapolated the linear correlation to the $\Delta^{17}\text{O}$ value of $\text{NO}_{3\text{atm}}^-$ obtained through continuous monitoring on Rishiri Island ($+26 \pm 3\text{‰}$; Tsunogai et al., 2010), which is located 50 km northwest of the study site (Fig. 1) (Noguchi et al., 2007a; Tsunogai et al., 2010), we obtained $\delta^{18}\text{O} = +79 \pm 20\text{‰}$, which correspond to the $\delta^{18}\text{O}$ values for $\text{NO}_{3\text{atm}}^-$ (Durka et al., 1994; Morin et al., 2009; Alexander et al., 2009; Tsunogai et al., 2010). These findings indicated that an increase in the export flux of $\text{NO}_{3\text{atm}}^-$ was primarily responsible for nitrate enrichment in stream water during spring of 2004 and

2005. The $\delta^{15}\text{N}$ values of nitrate also supported this conclusion. While the variation in $\delta^{15}\text{N}$ values showed little correlation with $\Delta^{17}\text{O}$ (Fig. 4), the $\delta^{15}\text{N}$ values of those showing $\Delta^{17}\text{O}$ values more than +10‰ were plotted around -1 ± 1 ‰, which almost corresponds with the annual average $\delta^{15}\text{N}$ value of $\text{NO}_3^-_{\text{atm}}$ determined in Rishiri Island (−1.1‰; Tsunogai et al., 2010).

3.3 $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of remineralized nitrate

The average $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{re}}$ produced through nitrification in the forested watershed was determined to be -3.6 ± 0.7 ‰, based on the intercept ($\Delta^{17}\text{O} = 0$) of the plot of $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ shown in Fig. 4b. A similar $\delta^{18}\text{O}$ value of -4.2 ± 2.4 ‰ was obtained for $\text{NO}_3^-_{\text{re}}$ produced through nitrification in a forested watershed on nearby Rishiri Island, where the H_2O showed $\delta^{18}\text{O}$ values of around −13‰ based on the linear relationship between the $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate dissolved in both ground-water and stream water on the island (Tsunogai et al., 2010). Conversely, Spoelstra et al. (2007) proposed much higher $\delta^{18}\text{O}$ values of +3.1 to +10.1‰ with a mean value of +5.2‰ for nitrate produced through nitrification in soils based on in vitro incubation experiments using soils containing H_2O with $\delta^{18}\text{O}$ values around −10‰. Similar high $\delta^{18}\text{O}$ values of nitrate were also obtained for nitrate produced through nitrification in soils in several past studies based on in vitro soil-incubation experiments (Burns and Kendall, 2002) and calculations (Durka et al., 1994).

During the conversion of ammonium to nitrate by chemolithoautotrophic bacteria, two oxygen atoms originate from H_2O and one from O_2 (Aleem et al., 1965; Andersson and Hooper, 1983; Kumar et al., 1983). In recent laboratory studies, the kinetic isotope effects during incorporation of O atoms were estimated to be $+20.4 \pm 2.3$ ‰ for ammonia oxidation (O_2 plus H_2O incorporation) and $+8.6 \pm 2.3$ ‰ for incorporation of H_2O during nitrite oxidation (Buchwald et al., 2012). Furthermore, the equilibrium isotope effect during abiotic O atom exchange between nitrite and H_2O was estimated to be $+12.5 \pm 1.5$ ‰ (Casciotti et al., 2007). Based on the $\delta^{18}\text{O}$ value of atmospheric O_2

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(+23.5‰) and the average $\delta^{18}\text{O}$ value of the stream water in the study area (−11‰), a $\delta^{18}\text{O}$ value of $-3.4 \pm 5.8\text{‰}$ for $\text{NO}_{3\text{re}}^-$ was anticipated, which corresponds with the value obtained. We concluded that values around −3.6‰ represented the $\delta^{18}\text{O}$ value of $\text{NO}_{3\text{re}}^-$ produced through nitrification in the forest ecosystem, where H_2O showed $\delta^{18}\text{O}$ values around −11‰.

Either the slight contribution of $\text{NO}_{3\text{atm}}^-$ or environmental differences between in vitro and in situ samples might be responsible for the higher $\delta^{18}\text{O}$ values of nitrate produced through nitrification in soils obtained in past estimates. Differences in some environmental parameters of soils in the watersheds investigated in this study from those used in past experiments could also be responsible. Accordingly, studies using additional data describing the values of both $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ of nitrate eluted from various watersheds and generated through soil-incubation experiments are warranted.

The average $\delta^{15}\text{N}$ value of $\text{NO}_{3\text{re}}^-$ was determined to be $+1.5 \pm 9\text{‰}$ from the intercept ($\Delta^{17}\text{O} = 0$) of the plot shown in Fig. 4a, which was much greater variance than that of $\delta^{18}\text{O}$. While samples with high $\Delta^{17}\text{O}$ values ($\Delta^{17}\text{O} > +3\text{‰}$) had $\delta^{15}\text{N}$ values that showed little dispersion around the linear fit line, samples with low $\Delta^{17}\text{O}$ values ($\Delta^{17}\text{O} < +3\text{‰}$) showed large dispersions (Fig. 4a). The presence of highly variable $\delta^{15}\text{N}$ values only in low $\Delta^{17}\text{O}$ stream nitrate implied that the $\delta^{15}\text{N}$ values of $\text{NO}_{3\text{re}}^-$ produced in the studied watershed were highly variable.

To clarify the major process controlling the $\delta^{15}\text{N}$ values of $\text{NO}_{3\text{re}}^-$, we estimated the end-member $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{NO}_{3\text{re}}^-$ ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$) for each sample by

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correcting the contribution of NO_3^- using each $\Delta^{17}\text{O}$ value, as shown in Eqs. (2–4):

$$\frac{C_{\text{atm}}}{C_{\text{total}}} = \frac{\Delta^{17}\text{O}}{\Delta^{17}\text{O}_{\text{atm}}}, \quad (2)$$

$$\delta^{15}\text{N}_{\text{re}} = \frac{C_{\text{total}} \times \delta^{15}\text{N} - C_{\text{atm}} \times \delta^{15}\text{N}_{\text{atm}}}{C_{\text{total}} - C_{\text{atm}}}, \quad (3)$$

$$\delta^{18}\text{O}_{\text{re}} = \frac{C_{\text{total}} \times \delta^{18}\text{O} - C_{\text{atm}} \times \delta^{18}\text{O}_{\text{atm}}}{C_{\text{total}} - C_{\text{atm}}}. \quad (4)$$

where C_{atm} and C_{total} denote the concentration of NO_3^- and NO_3^- in each water sample, respectively, and $\delta^{15}\text{N}_{\text{atm}}$, $\delta^{18}\text{O}_{\text{atm}}$, and $\Delta^{17}\text{O}_{\text{atm}}$ denote $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of NO_3^- , respectively. As for the values of $\delta^{15}\text{N}_{\text{atm}}$, $\delta^{18}\text{O}_{\text{atm}}$, and $\Delta^{17}\text{O}_{\text{atm}}$, we used the annual average values obtained through continuous monitoring on Rishiri Island ($\delta^{15}\text{N}_{\text{atm}} = -1.1\text{‰}$, $\delta^{18}\text{O}_{\text{atm}} = -87.1\text{‰}$, and $\Delta^{17}\text{O}_{\text{atm}} = +26.2\text{‰}$; Tsunogai et al., 2010).

While most samples showed positive $\Delta^{17}\text{O}$ values, three showed negative $\Delta^{17}\text{O}$ values as low as -0.2‰ , which prevented estimation of C_{atm} using Eq. (2). Because the $\Delta^{17}\text{O}$ value of tropospheric O_2 is around -0.2‰ (Luz and Barkan, 2000), the contribution of oxygen atoms derived from tropospheric O_2 during the production of NO_3^- from ammonium or organic nitrogen could be partly responsible for the observed $\Delta^{17}\text{O}$ values less than 0‰ . However, even if the contribution was significant, the possible $\Delta^{17}\text{O}$ value of produced NO_3^- would include 0‰ within the error of our analytical precision ($\pm 0.2\text{‰}$). Accordingly, 0‰ was used for the $\Delta^{17}\text{O}$ value of NO_3^- and observed $\Delta^{17}\text{O}$ values less than 0‰ are considered to be 0‰ for the remainder of this paper.

It should be noted that the estimated $\delta^{18}\text{O}_{\text{re}}$ values sometimes differed greatly from the observed $\delta^{18}\text{O}$ values, especially for samples with $\Delta^{17}\text{O}$ values greater than $+1\text{‰}$. However, all estimated $\delta^{15}\text{N}_{\text{re}}$ values were nearly identical to the observed $\delta^{15}\text{N}$ values

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owing to small differences between $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{15}\text{N}_{\text{atm}}$. The primary goal of estimating $\delta^{18}\text{O}_{\text{re}}$ is to discuss the reason for large variations in $\delta^{15}\text{N}_{\text{re}}$ (and thus $\delta^{15}\text{N}$) of nitrate in stream water. Additional determinations on the $\Delta^{17}\text{O}$ values of nitrate together with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ enable us to correct the contribution of $\text{NO}_{3\text{atm}}^-$ from the determined values ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) and to use the corrected values ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$) for discussing the behavior of $\text{NO}_{3\text{re}}^-$.

The relationship between the estimated $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ is presented in Fig. 5. Extraordinarily high $\Delta^{17}\text{O}$ values (more than +10‰) are shown in parentheses to denote that they were excluded from subsequent discussions because they could result in estimated $\delta^{18}\text{O}_{\text{re}}$ values with large errors. As clearly presented in the figure, $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ were linearly correlated with a slope of $+1.23 \pm 0.45$ ($r^2 = 0.31$). As a result, both $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ varied simultaneously in the stream water samples. Partial removal of nitrate through denitrification has been shown to be a representative process that enhances both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in residual nitrate simultaneously (Amberger and Schmidt, 1987). Previous studies showed that partial removal of nitrate through assimilation by plants and/or microbes could be an alternative process leading to enrichment of both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in residual nitrate, while the fractionation was found to be small or negligible in general (Högberg, 1997; Kendall, 1998). Denitrification is a more plausible cause of the observed variation in both $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$. Theoretical and laboratory studies have suggested that denitrification results in 2 : 1 fractionation of $\delta^{15}\text{N}$: $\delta^{18}\text{O}$ (Amberger and Schmidt, 1987; Aravena and Robertson, 1998), but recent studies proposed a 1 : 1 ratio as well (Granger et al., 2008). Thus, although other minor factors could have changed $\delta^{15}\text{N}_{\text{re}}$ and/or $\delta^{18}\text{O}_{\text{re}}$, the linear correlation between $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ in Fig. 5 implies that $\delta^{15}\text{N}_{\text{re}}$ (and thus $\delta^{15}\text{N}$ of nitrate in stream water) primarily represented the progress of denitrification in soils prior to elution into stream water.

As a result, temporal variations in the values of both $\delta^{15}\text{N}_{\text{re}}$ (and thus $\delta^{15}\text{N}$ of nitrate in stream water) can be a tracer to quantify the effects of strip-cutting on the progress

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of denitrification in soils of the watershed. However, we did not observe any significant variations in $\delta^{15}\text{N}_{\text{re}}$ values in accordance with strip-cutting in the present study. This was likely because only five $\delta^{15}\text{N}_{\text{re}}$ data points were available prior to strip-cutting. Accordingly, additional studies generating more nitrate $\delta^{15}\text{N}_{\text{re}}$ data should be conducted to determine if strip-cutting impacts the progression of denitrification in soils.

Conversely, we observed clear depletion of the $\delta^{15}\text{N}_{\text{re}}$ values in summer (June, July, and August) when compared with the other seasons (Fig. 3). Specifically, the average $\delta^{15}\text{N}_{\text{re}}$ value was $-2.5 \pm 1.6\text{‰}$ in summer ($n = 7$), while it was $+2.2 \pm 3.0\text{‰}$ ($n = 34$) during the other seasons ($p < 0.001$). A significant positive relationship between soil temperature and gross nitrification rates was observed in previous studies (Breuer et al., 2002; Zaman and Chang, 2004; Hoyle et al., 2006). Active nitrification during summer might reduce the relative progress of denitrification in soils.

3.4 Quantifying the effects of strip-cutting on nitrate dynamics

As discussed in Sect. 3.1, a significant increase in stream nitrate concentration was observed in spring of 2004 and 2005, probably in response to the strip-cutting of understory dwarf bamboo, *S. senanensis*, in October 2003. In the present study, the $\Delta^{17}\text{O}$ tracer of nitrate revealed that strip-cutting in October 2003 had a significant impact on C_{atm} as well. While the maximum stream C_{atm} was only $0.53 \mu\text{mol L}^{-1}$ in 2003, a significant increase in C_{atm} to $8.2 \mu\text{mol L}^{-1}$ was observed in spring of 2004, probably in response to strip-cutting. A similar increase in stream C_{atm} up to $3.9 \mu\text{mol L}^{-1}$ was also observed in spring of 2005. To quantify the effects of the strip-cutting on processes regulating the elution of NO_3^- , the daily elution rate of NO_3^- (F_{atm}) was calculated for each day on which the $\Delta^{17}\text{O}$ value of nitrate was determined from each concentration of NO_3^- (C_{atm}) and the daily flow rate of stream water (V) by applying Eq. (5):

$$F_{\text{atm}} = C_{\text{atm}} \times V \quad (5)$$

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There were only four C_{atm} data points for 2003 because most of the C_{total} in 2003 were too low (less than $0.1 \mu\text{mol L}^{-1}$) to determine the $\Delta^{17}\text{O}$ values (Fig. 2). However, if the C_{total} is less than $0.1 \mu\text{mol L}^{-1}$, the associated C_{atm} must be less than $0.1 \mu\text{mol L}^{-1}$ as well, regardless of the $\Delta^{17}\text{O}$ values. To estimate the upper limit of C_{atm} and thus the upper limit of F_{atm} for 2003, we applied the maximum $\Delta^{17}\text{O}$ value of nitrate in stream water observed in this study ($\Delta^{17}\text{O} = +14.3\text{‰}$) as the maximum $\Delta^{17}\text{O}$ value of nitrate for samples showing C_{total} less than $0.1 \mu\text{mol L}^{-1}$ in 2003 ($n = 9$).

The daily elution fluxes of NO_3^- (F_{total}) and NO_3^- (F_{re}) were also calculated from both the NO_3^- concentration (C_{total}) and the daily average flow rate of the stream water (V) by applying Eqs. (6) and (7):

$$F_{\text{total}} = C_{\text{total}} \times V \quad (6)$$

$$F_{\text{re}} = F_{\text{total}} - F_{\text{atm}} \quad (7)$$

The temporal variation of F_{atm} and the F_{total} are plotted in Fig. 3d. As shown in the figure, enrichment of F_{atm} occurred during spring from 2003 to 2005. More than 90 % of NO_3^- eluted in March, April, and May each year. Direct contribution of NO_3^- from snow pack to the stream must be responsible for this phenomenon. Similar spring enrichment of F_{atm} due to snowmelt has been observed through continuous monitoring of $\delta^{18}\text{O}$ of nitrate in runoff (Kendall et al., 1995; Ohte et al., 2004; Piatek et al., 2005; Pellerin et al., 2012). While spring F_{atm} enrichment was observed from 2003 to 2005, regardless of strip-cutting, the levels became much higher after strip-cutting. The maximum F_{atm} increased from $5.3 \mu\text{mol s}^{-1}$ in 2003 to $88.6 \mu\text{mol s}^{-1}$ in 2004 and $93.3 \mu\text{mol s}^{-1}$ in 2005. Additionally, maximum F_{re} increased from $13.0 \mu\text{mol s}^{-1}$ in 2003 to $77.8 \mu\text{mol s}^{-1}$ in 2004 and $161.5 \mu\text{mol s}^{-1}$ in 2005.

Conversely, F_{atm} was always small during the other seasons, even after strip-cutting. Most of the nitrate being exported from the watershed during seasons other than spring was NO_3^- : those retained in the forested ecosystem as either organic-N or ammonium

and then been converted to nitrate via microbial nitrification. F_{atm} was especially low during summer. F_{re} was reduced during summer as well (Fig. 3).

As discussed above, $\delta^{15}\text{N}_{\text{re}}$ depletion implied active nitrification during summer. The combination of both active nitrification in soils and active nitrate consumption through assimilation by plants and/or microbes resulted in rapid turnover of nitrate in soils and thus a reduction in the elution rate of both $\text{NO}_3^-_{\text{atm}}$ (mostly) and $\text{NO}_3^-_{\text{re}}$ (partly) during summer. When compared with summer, a slight increase in F_{atm} was observed in fall and winter. The decrease in nitrification and nitrate consumption in soils increased the direct drainage rate of $\text{NO}_3^-_{\text{atm}}$.

We can obtain the annual export flux of $\text{NO}_3^-_{\text{atm}}$ per unit area of the catchment (M_{atm}) by integrating the F_{atm} values for each year of the observation using the Eq. (8).

$$M_{\text{atm}} = \frac{\sum F_{\text{atm}}(t) \times \Delta t}{S} \quad (8)$$

where S denote the total catchment area (8 ha). We can obtain the annual export flux for NO_3^- (M_{total}) and $\text{NO}_3^-_{\text{re}}$ (M_{re}) by integrating F_{re} and F_{total} for each year of the observation using Eqs. (9) and (10).

$$M_{\text{total}} = \frac{\sum F_{\text{total}}(t) \times \Delta t}{S} \quad (9)$$

$$M_{\text{re}} = \frac{\sum F_{\text{re}}(t) \times \Delta t}{S} \quad (10)$$

The estimated M_{atm} , M_{re} , and M_{total} for 2003 to 2005 are presented in Table 1. While M_{total} was $1.0 \text{ (mmol m}^{-2} \text{ year}^{-1})$ in 2003, it increased to 6.4 in 2004 and to 7.0 in 2005. In accordance with the increase in M_{total} , M_{atm} also increased from $0.13 \pm 0.04 \text{ (mmol m}^{-2} \text{ year}^{-1})$ in 2003 to 2.6 in 2004 and 2.1 in 2005. M_{re} also increased from $0.88 \pm 0.04 \text{ (mmol m}^{-2} \text{ year}^{-1})$ in 2003 to 3.7 in 2004 and 4.8 in 2005.

The observed increases in M_{atm} and M_{re} in accordance with the *Sasa*-cutting in October 2003 suggest that *Sasa* is important to prevention of nitrogen leaching from

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soil and enhancement of biological consumption of NO_3^- before being exported from forest ecosystems, especially when significant quantities of NO_3^- were added to the forest floor through the spring snowmelt. Although both M_{atm} and M_{re} increased in response to strip-cutting, the relative increase in M_{atm} was much higher than the relative increase in M_{re} . These results imply that the major impact of strip-cutting was on the biological consumption processes of NO_3^- , rather than the production processes of NO_3^- in soils.

While the annual average $M_{\text{atm}}/M_{\text{total}}$ ratio was less than 16 % in 2003 (Table 1), it increased to 41 % in 2004 in response to strip-cutting, then slightly decreased to 31 % in 2005. The $M_{\text{atm}}/M_{\text{total}}$ ratios after strip-cutting were much higher than those determined for normal natural discharges, such as 3.1 to 7.7 % in southern California (Michalski et al., 2004), 7.4 ± 2.6 % on Rishiri Island (Tsunogai et al., 2010), and 0 to 7 % in the Yellow River (Liu et al., 2013), as well as that dissolved in soil solution of temperate forest in northern Michigan (9 % on average) (Costa et al., 2011) and that dissolved in an oligotrophic lake water column in Japan (9.7 ± 0.8 %) (Tsunogai et al., 2011). As a result, we can easily differentiate the ratios observed after strip-cutting from other normal $M_{\text{atm}}/M_{\text{total}}$ ratios in stream water using the $\Delta^{17}\text{O}$ values of nitrate, indicating that they can serve as a useful and powerful tracer for quantification of artificial alternations in forested watersheds.

3.5 Quantifying the effects of strip-cutting on atmospheric nitrate dynamics

If biological consumption processes of NO_3^- were fully destroyed in the watershed owing to strip-cutting, the annual export flux via stream water (M_{atm}) would be the same as that deposited throughout the catchment area. Therefore, we determined the annual deposition flux of NO_3^- (D_{atm}) at the monitoring tower of the CC-LaG project adjacent to the catchment area (Fig. 1) to compare M_{atm} with D_{atm} . The data coverage of the obtained daily deposition rate was 94 % in FY2008, 89 % in FY2009, 95 % in FY2010, and 82 % in FY2011. To complement the lacking data of the daily deposition rate, we first

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determined the average daily deposition rate for each year based only on the obtained data set and then estimated the annual deposition flux (D_{atm}) for each year assuming the same daily deposition rate with the average for those lacking data. The annual deposition flux of NO_3^- (D_{atm}) was nearly stable at around 18.6 ± 2.7 ($\text{mmol m}^{-2} \text{year}^{-1}$), and wet deposition accounted for 81 ± 3 % of the total NO_3^- deposition (Table 2). The estimated deposition flux corresponds with that observed in the forested watershed prior to clear-cutting (Fukuzawa et al., 2006) as well as that deposited in a background area in eastern Asia (EANET, 2013).

When compared with the D_{atm} estimated in this study, the annual export flux of NO_3^- via stream water (M_{atm}) corresponds to less than 1 % in 2003, about 14 % in 2004, and about 12 % in 2005. In our previous study on nearby Rishiri Island using $\Delta^{17}\text{O}$ of nitrate as a tracer, we estimated that direct drainage accounts for 8.8 ± 4.6 % of NO_3^- that has been deposited onto the island on average, and that the residual portion has undergone biological processing before being exported from the surface ecosystem based on comparison of the inflow (deposition of atmospheric nitrate) and outflow (atmospheric nitrate in groundwater) (Tsunogai et al., 2010). The present study revealed that the studied forest ecosystem removed NO_3^- more effectively in 2003 than that on Rishiri Island, while the removal efficiency was worse than that of Rishiri Island in 2004 owing to strip-cutting.

Both surface vegetation and the related ecosystems in soils must play a significant role in the consumption of NO_3^- (Nakagawa et al., 2013). The area in which *Sasa* was strip-cut only accounted for 50 % of the total watershed. Additionally, larch seedlings were immediately planted in the *Sasa* strip-cut line. Although the removal processes of NO_3^- by plants and/or microbes in the forested soils were damaged by strip-cutting, the results of the present study demonstrated that the majority of these processes were still active, even after strip-cutting. These findings will be useful in future to develop strategies for both clear-cutting and strip-cutting in forested ecosystems without increasing nitrate elution from watersheds.

Acknowledgements. Both the clear-cutting and subsequent strip-cutting experiments were conducted as part of a collaboration among Hokkaido University, the National Institute for Environmental Studies (NIES), and Hokkaido Electric Power Co. through the CC-LaG project. We thank the staff of the Teshio Experimental Forest for their long-term water sampling. Additionally, we are grateful to the members of the Biogeochemistry Group, Graduate School of Environmental Studies, Nagoya University, for their valuable support throughout this study. This work was supported by a Grant-in-Aid of Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan under grant numbers 22651001, 23241001, 24651002, and 25121506.

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Table 1. Temporal variations in the export flux per unit area of the catchment ($\text{mmol m}^{-2} \text{year}^{-1}$) of atmospheric nitrate (M_{atm}), together with those of remineralized nitrate (M_{re}), total nitrate (M_{total}), and $M_{\text{atm}}/M_{\text{total}}$ ratio.

	2003	2004	2005
M_{atm}	0.13 ± 0.04	2.6	2.1
M_{re}	0.88 ± 0.04	3.7	4.8
M_{total}	1.0	6.4	7.0
$M_{\text{atm}}/M_{\text{total}}$	9–16 %	41 %	31 %

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Table 2. Annual deposition rate of atmospheric nitrate at the monitoring tower ($\text{mmol m}^{-2} \text{ year}^{-1}$).

	FY2008	FY2009	FY2010	FY2011	Average
Wet deposition	11.9	17.4	13.9	17.2	15.1 ± 2.7
Dry deposition	3.2	3.0	3.9	3.7	3.5 ± 0.4
Total	15.1	20.4	17.8	20.9	18.6 ± 2.7

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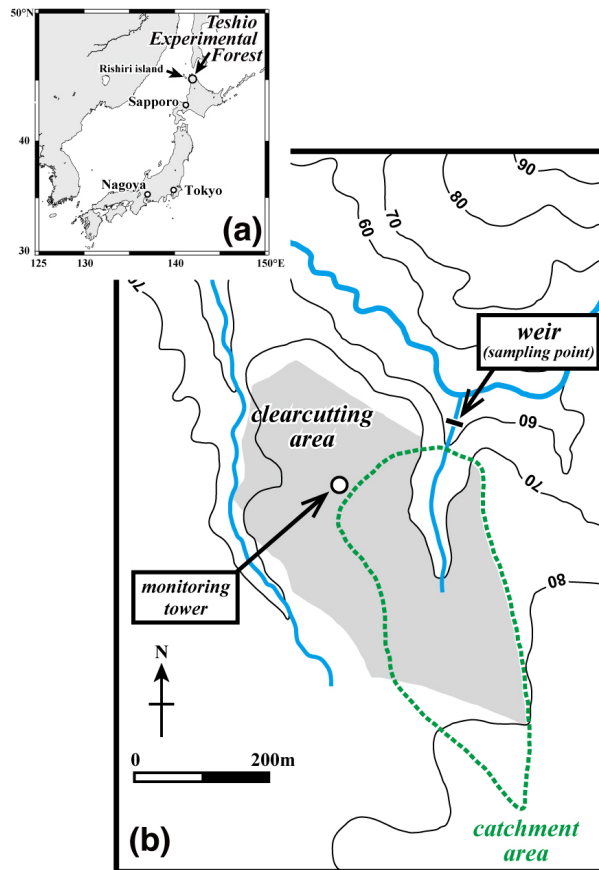


Figure 1. Map showing the location of Teshio Experimental Forest in northern Japan (a), and a contour map showing the water sampling point (weir) in the forest (b), together with both the catchment area shown by a dotted line and the clear-cutting area of the CC-LaG project shown by the hatched region. The white circle denotes the location of the monitoring tower.

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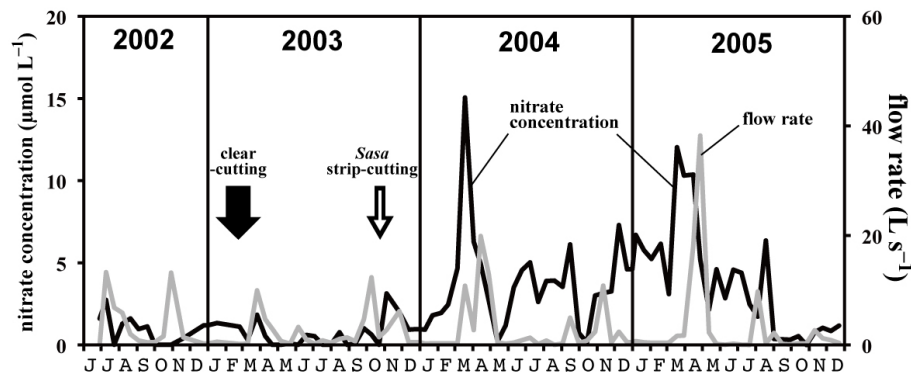


Figure 2. Temporal variations in nitrate concentrations in stream water (black line), together with those of flow rate of the stream water (grey line). Solid and open arrows denote the period of clear-cutting of trees and strip-cutting of *Sasa*, respectively. The temporal variations in nitrate concentration from June 2002 to the end of 2004 were previously presented by Fukuzawa et al. (2006).

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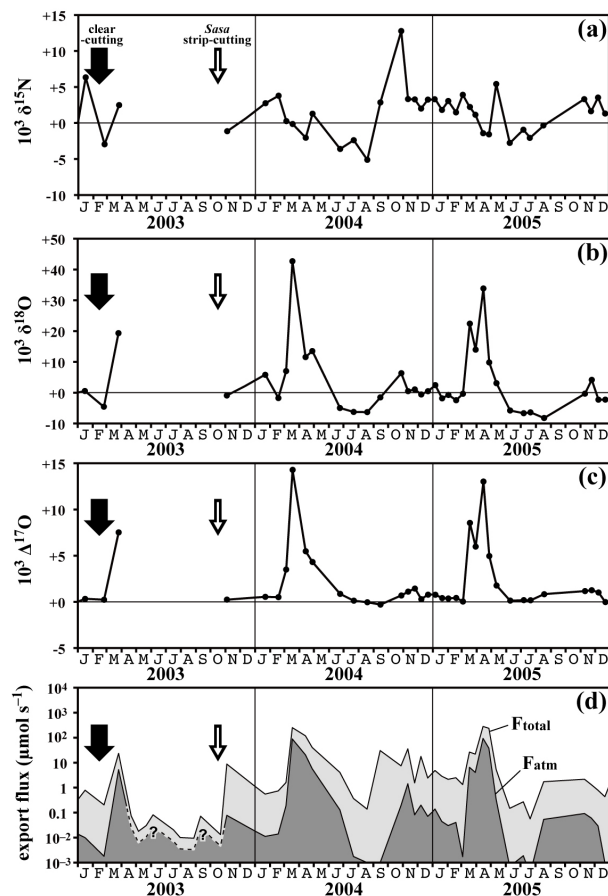


Figure 3. Temporal variations in the values of $\delta^{15}\text{N}$ (a), $\delta^{18}\text{O}$ (b), and $\Delta^{17}\text{O}$ (c) of nitrate in the stream water, together with those in the export fluxes of nitrate (F_{total}) and atmospheric nitrate (F_{atm}) on a logarithmic scale (d). Solid and open arrows denote the period of clear-cutting of trees and strip-cutting of *Sasa*, respectively.

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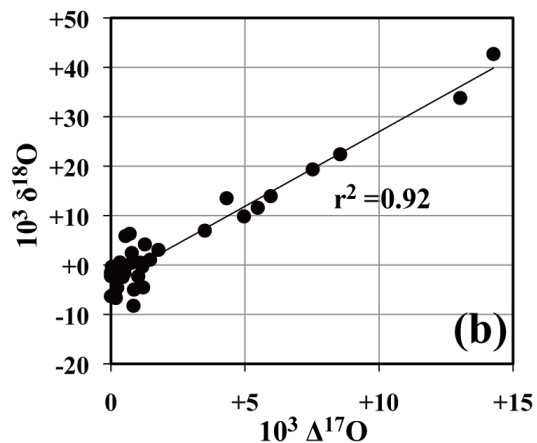
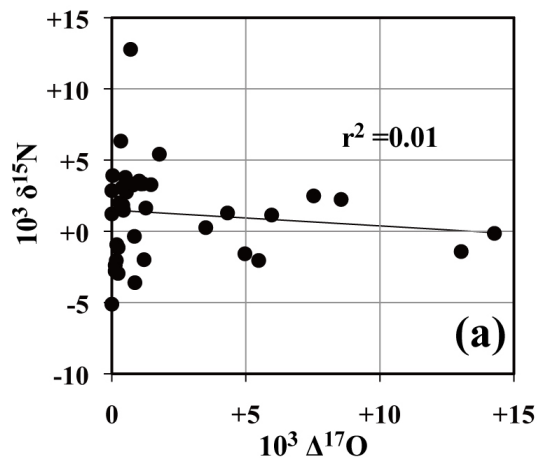


Figure 4. Relationship between $\Delta^{17}\text{O}$ and $\delta^{15}\text{N}$ of nitrate **(a)** and $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate **(b)** in stream water samples.

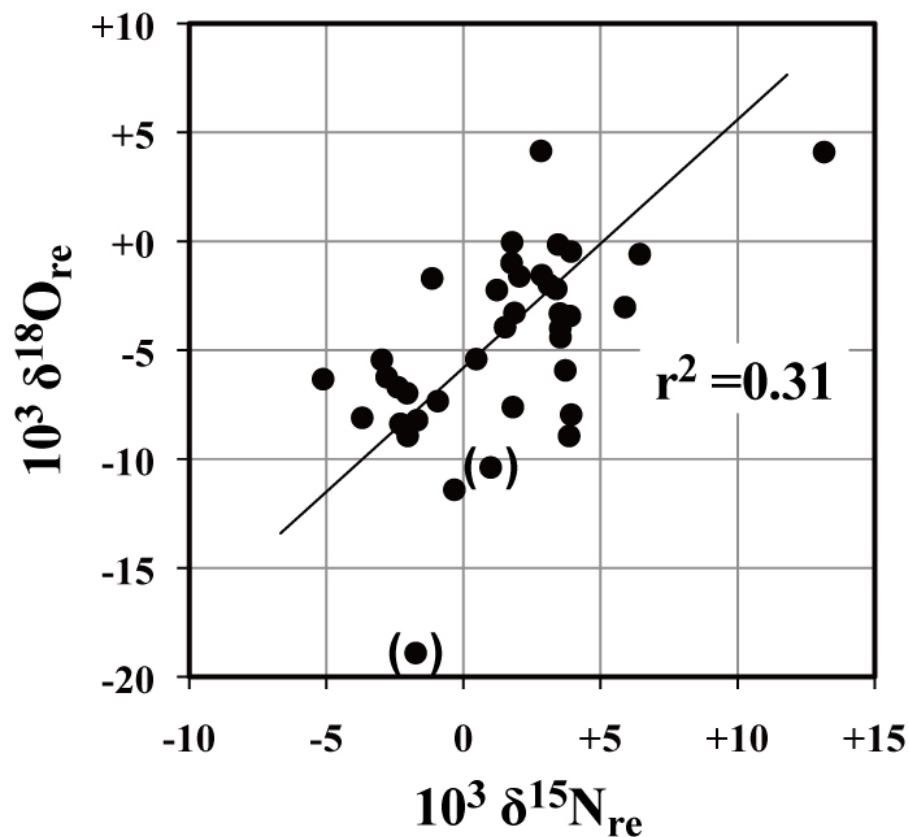


Figure 5. Relationship between estimated $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of remineralized nitrate in stream water samples ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$, respectively). See text for the detailed processes used to obtain the values. Data points obtained from samples with high $\Delta^{17}\text{O}$ values ($> +10\text{‰}$) are shown in parentheses to indicate that they could include large errors.

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