# 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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# 19 Abstract

Temporal variations in the stable isotopic compositions of nitrate dissolved in stream water 20 eluted from a cool-temperate forested watershed (8 ha) were measured to quantify the 21 22 biogeochemical effects of clear-cutting of trees and subsequent strip-cutting of the understory 23 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  $\Delta^{17}$ O values of 24 nitrate. A significant increase in stream nitrate concentration to 15 µmol L<sup>-1</sup> in spring of 2004 25 was correlated with a significant increase in the  $\Delta^{17}$ O values of nitrate. Additionally, the high 26  $\Delta^{17}$ O values of +14.3‰ suggest that the direct drainage of atmospheric nitrate accounted for 27 28 more than 50% of total nitrate exported from the forested watershed peaking in spring.

Similar increases in both concentrations and  $\Delta^{17}$ O values were also found in spring of 2005. 1 2 Conversely, low  $\Delta^{17}$ O values less than +1.5% were observed in other seasons, regardless of increases in stream nitrate concentration, indicating that the majority of nitrate exported from 3 4 the forested watershed during seasons other than spring was remineralized nitrate: those 5 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 6 7 annual export of atmospheric nitrate and remineralized nitrate increased more than 16-fold 8 and 4-fold, respectively, in 2004, and more than 13-fold and 5-fold, respectively, in 2005. The 9 understory vegetation (Sasa) was particularly important to enhancing biological consumption of atmospheric nitrate. 10

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# 12 **1** Introduction

#### 13 **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 14 15 understanding nitrogen cycles within the watershed. In addition, the nitrate concentration in 16 stream water is important to primary production downstream. Increased nitrate in stream 17 water can degrade stream habitats. However, nitrate concentrations in stream water eluted 18 from forested watersheds are determined through a complicated interplay of several processes including (1) deposition of atmospheric nitrate ( $NO_{3 atm}$ ), (2) production of remineralized 19 nitrate  $(NO_3^{-}re)$  through nitrification, (3) uptake by plants or microbes, and (4) reduction 20 21 through denitrification. As a result, interpretation of the processes regulating nitrate 22 concentration in stream water is not always straightforward.

Clear-cutting of plants in forested watersheds often leads to nitrate increasing to levels as high 23 as 1000  $\mu$ mol L<sup>-1</sup> in stream water eluted from the watersheds (Likens et al., 1970; Swank et 24 al., 2001), as well as acidification (Likens et al., 1970; Swank et al., 2001; Vitousek and 25 Melillo, 1979). Enhancement of the production of fresh remineralized nitrate within soils due 26 27 to disturbances and/or hindrance of the uptake of such remineralized nitrate in soils might 28 play a large role in increases in nitrate in streams. Moreover, previous studies of forested 29 catchments have offered considerable insight into the link between atmospheric nitrate 30 deposition and nitrate discharge to streams (Grennfelt and Hultberg, 1986; Williams et al., 31 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.

4 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 5 6 vegetation (mainly Sasa senanensis) were measured in a forested watershed in the Teshio Experimental Forest, Hokkaido University (Fig. 1) in northern Japan (Fukuzawa et al., 2006). 7 8 In that study, an approximately 50% decrease in fine root biomass due to understory 9 vegetation cutting was found to induce an increase in the maximum nitrate concentration in stream water from 3  $\mu$ mol L<sup>-1</sup> to ca. 15  $\mu$ mol L<sup>-1</sup> and that in soil solution from 30  $\mu$ mol L<sup>-1</sup> to 10 more than 100  $\mu$ mol L<sup>-1</sup>. These results implied that nitrogen uptake by the understory 11 vegetation was important to preventing nitrogen leaching after tree-cutting, and that the 12 13 decline of this nitrogen uptake by removal of understory vegetation led to marked nitrate 14 leaching to stream water (Fukuzawa et al., 2006). However, the importance of atmospheric 15 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 16 17 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 18 discharge of NO<sub>3 atm</sub> deposited onto surface ecosystems (Durka et al., 1994; Ohte et al., 2004; 19 Costa et al., 2011; Nakagawa et al., 2013). Thus, in this study, we conducted further isotope 20 21 analysis of archived stream water samples to clarify the source of increased nitrate.

# 22 **1.2** Triple oxygen isotopic compositions of nitrate

The natural stable isotopic composition of nitrate have been widely applied in the 23 24 determination of the sources of nitrate in natural freshwater systems (Wada et al., 1975; 25 Durka et al., 1994; Williard et al., 2001; Burns and Kendall, 2002; Campbell et al., 2002; Michalski et al., 2004; Ohte et al., 2004; Hales et al., 2007; Barnes et al., 2008; Burns et al., 26 2009; Tsunogai et al., 2010; Tobari et al., 2010; Ohte et al., 2010; Barnes and Raymond, 27 2010; Tsunogai et al., 2011; Nestler et al., 2011; Curtis et al., 2011; Costa et al., 2011; 28 Pellerin et al., 2012; Dejwakh et al., 2012; Yue et al., 2013; Ohte, 2013; Lohse et al., 2013; 29 30 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 31 32  $(NO_{3}^{-}atm)$ . While remineralized nitrate  $(NO_{3}^{-}re)$ , the oxygen atoms of which are derived from either terrestrial O<sub>2</sub> or H<sub>2</sub>O through microbial processing (i.e., nitrification), always shows mass-dependent relative relation between <sup>17</sup>O/<sup>16</sup>O ratios and <sup>18</sup>O/<sup>16</sup>O ratios, NO<sub>3</sub><sup>-</sup><sub>atm</sub> displays an anomalous enrichment in <sup>17</sup>O reflecting oxygen atom transfers from atmospheric ozone (O<sub>3</sub>) during the conversion of NO<sub>x</sub> to NO<sub>3</sub><sup>-</sup><sub>atm</sub> (Michalski et al., 2003; Morin et al., 2008; Alexander et al., 2009). Using the  $\Delta^{17}$ O signature defined by the following equation (Miller, 2002; Kaiser et al., 2007) enables NO<sub>3</sub><sup>-</sup><sub>atm</sub> ( $\Delta^{17}$ O > 0) to be distinguished from NO<sub>3</sub><sup>-</sup><sub>re</sub> ( $\Delta^{17}$ O = 0):

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

9 where the constant  $\beta$  is 0.5247 (Miller, 2002; Kaiser et al., 2007),  $\delta^{18}O = R_{\text{sample}}/R_{\text{standard}} - 1$ 10 and *R* is the <sup>18</sup>O/<sup>16</sup>O ratio (or the <sup>17</sup>O/<sup>16</sup>O ratio in the case of  $\delta^{17}O$  or the <sup>15</sup>N/<sup>14</sup>N ratio in the 11 case of  $\delta^{15}N$ ) of the sample and each standard reference material. In addition,  $\Delta^{17}O$  is stable 12 during mass-dependent isotope fractionation processes within surface ecosystems. As a result, 13 while the atmospheric  $\delta^{15}N$  or  $\delta^{18}O$  signature can be overprinted by biogeochemical processes, 14 we can use  $\Delta^{17}O$  as a conserved tracer of NO<sub>3<sup>-</sup>atm</sub> and trace NO<sub>3<sup>-</sup>atm</sub>, regardless of its partial 15 removal through denitrification and/or uptake subsequent to deposition.

In our previous study, we determined the  $\Delta^{17}$ O values of nitrate in aerobic groundwater 16 worldwide to trace the fate of  $NO_{3 \text{ atm}}^{-}$  that had been deposited onto and passed through 17 natural background watersheds (Nakagawa et al., 2013). The results of that study revealed 18 that nitrate in groundwater had small  $\Delta^{17}$ O values ranging from -0.2‰ to +4.5‰; therefore, 19 we estimated the average mixing ratio of  $NO_{3}^{-}$  atm to total nitrate in the groundwater samples 20 to be 3.1%. Moreover, the concentrations of  $NO_{3atm}$  ranged from less than 0.1 µmol L<sup>-1</sup> to 21 8.5  $\mu$ mol L<sup>-1</sup>, with lower NO<sub>3 atm</sub> concentrations being obtained for those recharged in 22 23 forested areas with high coverage of vegetation. Based on these findings, we concluded that most NO<sub>3<sup>-</sup>atm</sub> deposited onto healthy forested watersheds had been removed by plants and/or 24 microbes subsequent to deposition. 25

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  $NO_{3-atm}$  being deposited into the forest ecosystem. Specifically, the  $\Delta^{17}O$  tracer was used to quantify temporal variations in the 1 concentration of NO<sub>3<sup>-</sup>atm</sub> in stream water to gain insight into the processes controlling the fate 2 and transport of NO<sub>3<sup>-</sup>atm</sub> deposited onto the forested watershed. The results presented herein 3 will increase our understanding of fixed-nitrogen processing and fixed-nitrogen retention 4 efficiencies within forest ecosystems as well.

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# 6 2 Experimental Section

#### 7 2.1 Site description and management

8 The study site has been described in detail by Fukazawa et al. (2006) and Takagi et al. (2009). 9 Clear-cutting of trees and subsequent strip-cutting of understory vegetation were conducted in 10 a cool-temperate forested watershed in the Teshio Experimental Forest of Hokkaido 11 University in northern Japan (Fig. 1; 45°03' N, 142°06' E). Prior to clear-cutting, the 12 predominant overstory species were fir (Abies sachalinensis), birch (Betula ermanii and 13 Betula platyphylla var. japonica) and Mongolian oak (Quercus mongolica var. grosserrata). 14 The forest floor of the study site is covered with dense understory vegetation primarily 15 consisting of dwarf bamboo (mainly Sasa senanensis in flat areas and Sasa kurilensis on steep 16 riparian slopes). The bedrock underlying the site consists of sedimentary rock of the 17 Cretaceous period. The air temperature in the region varies from  $-35^{\circ}$ C to  $+35^{\circ}$ C, with an 18 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. 19

20 To evaluate the effects of clear-cutting on CO<sub>2</sub> exchange in the forest, a monitoring tower was 21 established in 2001 at the central part of the area (Fig. 1) and net ecosystem production over 22 the forest stands has been monitored as part of a project known as the Carbon Cycle and 23 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., 24 25 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 26 27 left in the basin. The Sasa spp. were then strip-cut into 4-m rows by crushing and spreading in 28 October 2003. The area in which the Sasa spp. were strip-cut accounted for ca. 50% of the 29 total tree-cut area in the watershed. Larch seedlings were planted in the Sasa spp. strip-cut 30 line immediately after cutting.

#### 1 2.2 Water sampling

2 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 3 4 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 5 6 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 7 membrane filter in the laboratory, the concentrations of major anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) and 8 cations (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) were measured by ion chromatography (DX-500, Dionex 9 Inc., USA). Samples were analyzed within 6 months of sampling and then sealed in 30 mL 10 polyethylene bottles for further analyses, including measurement of the isotopes reported in 11 this study. The longest storage period between bottling and isotope analysis was 7 years. The 12 ion concentrations of samples collected from June 2002 to December 2004 have been 13 14 presented in our previous study (Fukuzawa et al., 2006).

# 15 **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  $\mu$ mol L<sup>-1</sup>). 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 22 N<sub>2</sub>O using the chemical method originally developed to determine the  ${}^{15}N/{}^{14}N$  and  ${}^{18}O/{}^{16}O$ 23 ratios of seawater and freshwater nitrate (McIlvin and Altabet, 2005), with slight 24 modifications (Tsunogai et al., 2008; Tsunogai et al., 2010; Konno et al., 2010; Tsunogai et 25 al., 2011; Yamazaki et al., 2011; Nakagawa et al., 2013). Then, the stable isotopic 26 27 compositions of N<sub>2</sub>O were determined using a Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system (Tsunogai et al., 2008; Hirota et al., 2010). This system 28 29 consists of an original helium purge and trap line, a gas chromatograph (Agilent 6890) and a 30 Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified 31 Combustion III interface (Tsunogai et al., 2000; Tsunogai et al., 2002; Nakagawa et al., 2004;

Tsunogai et al., 2005) and a specially designed multicollector system (Komatsu et al., 2008). 1 For analysis, aliquots of N<sub>2</sub>O were introduced, purified, and then carried continuously into the 2 mass spectrometer via an open split interface, where the isotopologues of  $N_2O^+$  at m/z ratios 3 of 44, 45 and 46 were monitored to determine  $\delta^{45}$  and  $\delta^{46}$ . Each analysis was calibrated using 4 a machine-working reference gas (99.999% N<sub>2</sub>O) that was introduced into the mass 5 spectrometer via an open split interface according to a definite schedule to correct for sub-6 7 daily temporal variations in the mass spectrometry. In addition, a working-standard gas 8 mixture containing a known concentration of N<sub>2</sub>O (ca. 1000 ppm N<sub>2</sub>O in air) that was injected 9 from a sampling loop was analyzed in the same way as the samples at least once a day to 10 correct for daily temporal variations in the mass spectrometry.

Following analyses based on  $N_2O^+$  monitoring, another aliquot of  $N_2O$  was introduced to 11 determine the  $\Delta^{17}$ O of N<sub>2</sub>O (Komatsu et al., 2008). Using the same procedures as those used 12 in the  $N_2O^+$  monitoring mode, purified  $N_2O$  was introduced into our original gold tube unit 13 14 (Komatsu et al., 2008), which was held at 780°C for the thermal decomposition of N<sub>2</sub>O to N<sub>2</sub> and O<sub>2</sub>. The produced O<sub>2</sub> purified from N<sub>2</sub> was then subjected to CF-IRMS to determine the 15  $\delta^{33}$  and  $\delta^{34}$  by simultaneous monitoring of  $O_2^+$  isotopologues at m/z ratios of 32, 33 and 34. 16 Each analysis was calibrated with a machine-working reference gas (99.999% O<sub>2</sub> gas in a 17 cylinder) that was introduced into the mass spectrometer via an open split interface according 18 19 to a definite schedule to correct for sub-daily temporal variations in the mass spectrometry. In addition, a working-standard gas mixture containing N<sub>2</sub>O of known concentration (ca. 1000 20 21 ppm N<sub>2</sub>O in air) was analyzed in the same way as the samples at least once a day to correct 22 for daily temporal variations in the mass spectrometry.

The values of  $\delta^{15}$ N,  $\delta^{18}$ O and  $\Delta^{17}$ O for N<sub>2</sub>O derived from the nitrate in each sample were 23 24 compared with those derived from our local laboratory nitrate standards that had been 25 calibrated using the internationally distributed isotope reference materials (USGS-34 and 26 USGS-35) (Böhlke et al., 2003; Kaiser et al., 2007) to calibrate the  $\delta$  values of the sample nitrate to an international scale, as well as to correct for both isotope fractionation during the 27 chemical conversion to N<sub>2</sub>O and the progression of oxygen isotope exchange between the 28 nitrate-derived reaction intermediate and water (ca. 20%). All  $\delta$  values are expressed relative 29 30 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  $\delta^{15}$ N,  $\delta^{18}$ O or  $\Delta^{17}$ O values of nitrate. Specifically, we added each

of the nitrate standard solutions (containing ca. 10 mmol L<sup>-1</sup> nitrate with known  $\delta^{15}$ N,  $\delta^{18}$ O or 1 2  $\Delta^{17}$ 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<sub>2</sub>O and determined the values of 3  $\delta^{15}$ N,  $\delta^{18}$ O or  $\Delta^{17}$ O in a similar manner as was used for each pure sample. After correcting for 4 the contribution of N<sub>2</sub>O from the nitrate in each sample, we obtained the stable isotopic 5 compositions for N<sub>2</sub>O derived from our laboratory nitrate standards. Next, the  $\delta^{15}N$ ,  $\delta^{18}O$  and 6  $\Delta^{17}$ O values in the samples were simply calibrated using curves generated from the N<sub>2</sub>O 7 derived from the nitrate standards. 8

9 The samples had nitrate concentrations of more than 0.8  $\mu$ mol L<sup>-1</sup>, corresponding to nitrate 10 quantities greater than 20 nmol in a 30 ml sample, which is sufficient to determine  $\delta^{15}N$ ,  $\delta^{18}O$ 11 and  $\Delta^{17}O$  values with high precision. Thus, all isotopic data presented in this study have an 12 error better than  $\pm 0.3\%$  for  $\delta^{15}N$ ,  $\pm 0.5\%$  for  $\delta^{18}O$  and  $\pm 0.2\%$  for  $\Delta^{17}O$ .

Because we used the more precise power law shown in the equation (1) to calculate  $\Delta^{17}$ O, the estimated  $\Delta^{17}$ 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  $0.9 \pm 0.2\%$  for the  $\Delta^{17}$ O values of NO<sub>3<sup>-</sup>atm</sub>. When using the linearly approximated  $\Delta^{17}$ O values of NO<sub>3<sup>-</sup>atm</sub> available in the literature, we recalculated the  $\Delta^{17}$ O values based on the power law.

Nitrite (NO<sub>2</sub><sup>-</sup>) in the samples also interferes with the final N<sub>2</sub>O produced from nitrate (NO<sub>3</sub><sup>-</sup>), because the chemical method also converts NO<sub>2</sub><sup>-</sup> to N<sub>2</sub>O (McIlvin and Altabet, 2005). Therefore, it was necessary to correct for the contribution of NO<sub>2</sub><sup>-</sup>-derived N<sub>2</sub>O to accurately determine the stable isotopic compositions of the sample nitrate. However, all samples analyzed in this study contained NO<sub>2</sub><sup>-</sup> at concentrations below the detection limit (0.05 µmol  $L^{-1}$ ), which corresponded to NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> ratios less than 10%; thus, the results were used without any corrections.

The  $\delta^{18}$ O values of H<sub>2</sub>O 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  $\pm 0.1\%$ . Both VSMOW and VSLAP were used to calibrate the values to the international scale.

#### 29 2.4 Deposition rate of atmospheric nitrate

30 Continuous monitoring of the deposition rate of atmospheric nitrate was conducted from April

31 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 1 collected for every 3 weeks from the monitoring tower at a height of 30 m (Fig. 1) using the 2 filter pack method (flow rate = 4 L min<sup>-1</sup>) and a PM2.5 impactor (Noguchi et al., 2007b). 3 4 Nitrogen oxides (NO<sub>2</sub> 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 5 chromatography (Dionex ICS-2000/1500) at the laboratory of the Institute of Environmental 6 7 Science, Hokkaido Research Organization, and the results were used to estimate the dry 8 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). 9

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# 11 **3 Results and Discussion**

#### 12 **3.1** Temporal variations in stream water nitrate

The average stream nitrate concentration was 0.9  $\mu$ mol L<sup>-1</sup> in 2002 (June to December) and 13 0.7  $\mu$ mol L<sup>-1</sup> in 2003 (annual average), while the maximum nitrate concentration was 2.7 14 umol  $L^{-1}$  in 2002 and 3.1 umol  $L^{-1}$  in 2003. The maximum nitrate concentration was much 15 16 lower than the average nitrate concentration of wet deposition in a background area of eastern Asia (around 10  $\mu$ mol L<sup>-1</sup>) (EANET, 2013). The low and stable stream nitrate concentration 17 during 2002–2003 implied that atmospheric nitrate had been effectively removed from the 18 forested watershed, and that rain or snow events had little direct impact on the stream nitrate 19 concentration. However, as discussed in Fukuzawa et al. (2006), a significant increase in 20 stream nitrate concentration was observed in 2004, probably in response to strip-cutting of the 21 understory dwarf bamboo, S. senanensis, in October 2003 (Fig. 2). The average nitrate 22 concentration increased to 3.8  $\mu$ mol L<sup>-1</sup> in 2004 (annual average) and 3.8  $\mu$ mol L<sup>-1</sup> in 2005 23 (annual average). The maximum nitrate concentration also increased to 15  $\mu$ mol L<sup>-1</sup> in 2004 24 and 12  $\mu$ mol L<sup>-1</sup> in 2005 (Fig. 2). These findings indicate that strip-cutting had significant 25 impacts on nitrate dynamics in the forest ecosystem from 2004 until at least the end of 2005. 26

27 Temporal variations in the values of  $\delta^{15}$ N,  $\delta^{18}$ O, and  $\Delta^{17}$ O of nitrate in accordance with the 28 variations in nitrate concentration since January 2003 are presented in Fig. 3. The arithmetic 29 average and 1 $\sigma$  variation for the  $\delta^{15}$ N and  $\delta^{18}$ O values of nitrate were +1.3±3.3‰ and 30 +3.4±11.1‰, respectively (Fig. 3). While the average values of  $\delta^{15}$ N and  $\delta^{18}$ O were typical of 31 nitrate in natural stream water, the range of  $\delta^{18}$ O values was one of the largest ever reported in

- 1 natural stream water during continuous monitoring (Burns and Kendall, 2002; Campbell et al.,
- 2 2002; Ohte et al., 2004; Hales et al., 2007; Barnes et al., 2008; Burns et al., 2009; Tobari et al.,
- 3 2010; Ohte et al., 2010; Barnes and Raymond, 2010; Nestler et al., 2011; Curtis et al., 2011;
- 4 Pellerin et al., 2012; Yue et al., 2013; Ohte, 2013; Lohse et al., 2013; Thibodeau et al., 2013).
- 5 The arithmetic average and maximum  $\Delta^{17}$ O values of nitrate were +2.2±3.5‰ and +14.3‰,
- 6 respectively (Fig. 3). The  $\Delta^{17}$ O value of +14.3‰ corresponds to the highest  $\Delta^{17}$ O value ever
- 7 reported for dissolved nitrate in natural stream water (Michalski et al., 2004; Tsunogai et al.,
- 8 2010; Dejwakh et al., 2012; Liu et al., 2013), as well as that in soil solution (Michalski et al.,
- 9 2004; Costa et al., 2011).

One of the striking features of the large temporal variations of  $\delta^{18}$ O and  $\Delta^{17}$ O was the 10 enhancement of both  $\delta^{18}$ O and  $\Delta^{17}$ O in spring, especially in the years following strip-cutting. 11 12 Enrichment of nitrate concentration was detected in spring of 2004 and 2005, probably in 13 response to the spring snowmelt after strip-cutting (Fig. 2). The results of the present study 14 using stable isotopes revealed that these enriched nitrate levels were accompanied by elevated values of both  $\delta^{18}$ O and  $\Delta^{17}$ O. Atmospheric nitrate is characterized by elevated values of both 15  $\delta^{18}$ O and  $\Delta^{17}$ O to up to +110‰ (Durka et al., 1994; Kendall, 1998; Savarino et al., 2007; 16 Morin et al., 2008) and +45‰ (Savarino et al., 2007; McCabe et al., 2007; Morin et al., 2008), 17 18 respectively. In addition, atmospheric nitrate is currently the only source of nitrate that shows  $\Delta^{17}$ O values larger than 0‰. Accordingly, atmospheric nitrate might be the source of nitrate 19 enrichment during the spring snowmelt. However, the temporal variations in  $\delta^{15}$ N values were 20 independent from the variations in  $\delta^{18}$ O and  $\Delta^{17}$ O (Fig. 3). Overall, these findings indicate 21 that the major process controlling the  $\delta^{15}N$  values appears to be different from those 22 controlling the  $\delta^{18}$ O and  $\Delta^{17}$ O values. 23

Conversely, temporal variations in the  $\delta^{18}$ O values of H<sub>2</sub>O was small and independent from 24 variations in the  $\delta^{18}$ O and  $\Delta^{17}$ O values of nitrate, with an arithmetic average and  $1\sigma$  variation 25 26 of -11.0±0.7‰, which is typical of stream water in the area (Mizota and Kusakabe, 1994). The annual flow volume of the stream was stable at around  $8 \times 10^8$  L year<sup>-1</sup> every year as well 27 (Fig. 2), which corresponds to more than 80% of the total precipitation in the catchment. 28 Considering the evaporative loss of water from the catchment area, water loss via 29 30 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 31 32 later discussions.

# 1 3.2 $\delta^{18}$ O and $\delta^{15}$ N values of atmospheric nitrate

To further verify that NO<sub>3 atm</sub> was responsible for the elevated  $\Delta^{17}$ O values in the samples by 2 up to +14.3% in spring 2004 and 2005, the  $\delta^{15}N$  and  $\delta^{18}O$  values of nitrate in the samples 3 were plotted as a function of  $\Delta^{17}$ O (Fig. 4). Because NO<sub>3</sub> atm is enriched in both <sup>18</sup>O and <sup>17</sup>O 4 simultaneously (Michalski et al., 2003; Tsunogai et al., 2010), <sup>18</sup>O-enrichment was expected 5 for samples showing elevated  $\Delta^{17}$ O values if NO<sub>3 atm</sub> was responsible for the increased levels. 6 As shown in Fig. 4(b), the  $\delta^{18}$ O values in the samples showed strong linear correlation with 7 the  $\Delta^{17}$ O values (r<sup>2</sup> = 0.92). Additionally, when we extrapolated the linear correlation to the 8  $\Delta^{17}$ O value of NO<sub>3 atm</sub> obtained through continuous monitoring on Rishiri Island (+26± 3‰; 9 Tsunogai et al., 2010), which is located 50 km northwest of the study site (Fig. 1) (Noguchi et 10 al., 2007a; Tsunogai et al., 2010), we obtained  $\delta^{18}O = +79 \pm 20\%$ , which correspond to the 11  $\delta^{18}$ O values for NO<sub>3 atm</sub> (Durka et al., 1994; Morin et al., 2009; Alexander et al., 2009; 12 Tsunogai et al., 2010). These findings indicated that an increase in the export flux of NO<sub>3<sup>-</sup>atm</sub> 13 was primarily responsible for nitrate enrichment in stream water during spring of 2004 and 14 2005. The  $\delta^{15}N$  values of nitrate also supported this conclusion. While the variation in  $\delta^{15}N$ 15 values showed little correlation with  $\Delta^{17}$ O (Fig. 4), the  $\delta^{15}$ N values of those showing  $\Delta^{17}$ O 16 values more than +10% were plotted around  $-1\pm1\%$ , which almost corresponds with the 17 annual average  $\delta^{15}$ N value of NO<sub>3 atm</sub> determined in Rishiri Island (-1.1%; Tsunogai et al., 18 2010). 19

# 20 3.3 $\delta^{18}$ O and $\delta^{15}$ N values of remineralized nitrate

The average  $\delta^{18}$ O value of NO<sub>3</sub><sup>-</sup><sub>re</sub> produced through nitrification in the forested watershed was 21 determined to be  $-3.6 \pm 0.7\%$  based on the intercept ( $\Delta^{17}O = 0$ ) of the plot of  $\Delta^{17}O$  and  $\delta^{18}O$ 22 shown in Fig. 4(b). A similar  $\delta^{18}$ O value of  $-4.2 \pm 2.4\%$  was obtained for NO<sub>3</sub><sup>-</sup><sub>re</sub> produced 23 through nitrification in a forested watershed on nearby Rishiri Island, where the H<sub>2</sub>O showed 24  $\delta^{18}$ O values of around -13‰ based on the linear relationship between the  $\Delta^{17}$ O and  $\delta^{18}$ O of 25 nitrate dissolved in both groundwater and stream water on the island (Tsunogai et al., 2010). 26 Conversely, Spoelstra et al. (2007) proposed much higher  $\delta^{18}$ O values of +3.1 to +10.1% with 27 a mean value of +5.2‰ for nitrate produced through nitrification in soils based on in vitro 28 incubation experiments using soils containing H<sub>2</sub>O with  $\delta^{18}$ O values around -10%. Similar 29 high  $\delta^{18}$ O values of nitrate were also obtained for nitrate produced through nitrification in 30 soils in several past studies based on in vitro soil-incubation experiments (Burns and Kendall, 31 32 2002) and calculations (Durka et al., 1994).

During the conversion of ammonium to nitrate by chemolithoautotrophic bacteria, two 1 oxygen atoms originate from H<sub>2</sub>O and one from O<sub>2</sub> (Aleem et al., 1965; Andersson and 2 Hooper, 1983; Kumar et al., 1983). In recent laboratory studies, the kinetic isotope effects 3 4 during incorporation of O atoms were estimated to be +20.4±2.3‰ for ammonia oxidation (O<sub>2</sub> plus H<sub>2</sub>O incorporation) and +8.6±2.3‰ for incorporation of H<sub>2</sub>O during nitrite oxidation 5 (Buchwald et al., 2012). Furthermore, the equilibrium isotope effect during abiotic O atom 6 exchange between nitrite and H<sub>2</sub>O was estimated to be  $+12.5\pm1.5\%$  (Casciotti et al., 2007). 7 Based on the  $\delta^{18}$ O value of atmospheric O<sub>2</sub> (+23.5‰) and the average  $\delta^{18}$ O value of the 8 stream water in the study area (-11‰), a  $\delta^{18}$ O value of -3.4±5.8‰ for NO<sub>3</sub><sup>-</sup><sub>re</sub> was anticipated, 9 which corresponds with the value obtained. We concluded that values around -3.6%10 represented the  $\delta^{18}$ O value of NO<sub>3</sub><sup>-</sup><sub>re</sub> produced through nitrification in the forest ecosystem, 11 where  $H_2O$  showed  $\delta^{18}O$  values around -11%. 12

Either the slight contribution of NO<sub>3<sup>-</sup>atm</sub> or environmental differences between in vitro and in situ samples might be responsible for the higher  $\delta^{18}$ 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  $\delta^{18}$ O and  $\Delta^{17}$ O of nitrate eluted from various watersheds and generated through soilincubation experiments are warranted.

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

To clarify the major process controlling the  $\delta^{15}$ N values of NO<sub>3</sub><sup>-</sup><sub>re</sub>, we estimated the endmember  $\delta^{15}$ N and  $\delta^{18}$ O values of NO<sub>3</sub><sup>-</sup><sub>re</sub> ( $\delta^{15}$ N<sub>re</sub> and  $\delta^{18}$ O<sub>re</sub>) for each sample by correcting the contribution of NO<sub>3</sub><sup>-</sup><sub>atm</sub> using each  $\Delta^{17}$ O value, as shown in equations (2), (3), and (4):

$$30 \qquad \frac{C_{atm}}{C_{total}} = \frac{\Delta^{17}O}{\Delta^{17}O_{atm}},$$
(2)

$$1 \qquad \delta^{15} N_{re} = \frac{C_{total} \times \delta^{15} N - C_{atm} \times \delta^{15} N_{atm}}{C_{total} - C_{atm}}, \qquad (3)$$

2 
$$\delta^{18}O_{re} = \frac{C_{total} \times \delta^{18}O - C_{atm} \times \delta^{18}O_{atm}}{C_{total} - C_{atm}}.$$
 (4)

3 where  $C_{atm}$  and  $C_{total}$  denote the concentration of  $NO_{3\ atm}$  and  $NO_{3\ brack}$  in each water sample, 4 respectively, and  $\delta^{15}N_{atm}$ ,  $\delta^{18}O_{atm}$ , and  $\Delta^{17}O_{atm}$  denote  $\delta^{15}N$ ,  $\delta^{18}O$ , and  $\Delta^{17}O$  values of  $NO_{3\ atm}$ , 5 respectively. As for the values of  $\delta^{15}N_{atm}$ ,  $\delta^{18}O_{atm}$ , and  $\Delta^{17}O_{atm}$ , we used the annual average 6 values obtained through continuous monitoring on Rishiri Island ( $\delta^{15}N_{atm} = -1.1\%$ ,  $\delta^{18}O_{atm} =$ 7 +87.1‰, and  $\Delta^{17}O_{atm} = +26.2\%$ ; Tsunogai et al., 2010).

While most samples showed positive  $\Delta^{17}$ O values, three showed negative  $\Delta^{17}$ O values as low 8 as -0.2‰, which prevented estimation of  $C_{atm}$  using equation (2). Because the  $\Delta^{17}$ O value of 9 tropospheric  $O_2$  is around -0.2% (Luz and Barkan, 2000), the contribution of oxygen atoms 10 derived from tropospheric O<sub>2</sub> during the production of NO<sub>3</sub><sup>-</sup><sub>re</sub> from ammonium or organic 11 nitrogen could be partly responsible for the observed  $\Delta^{17}$ O values less than 0‰. However, 12 even if the contribution was significant, the possible  $\Delta^{17}$ O value of produced NO<sub>3</sub><sup>-</sup><sub>re</sub> would 13 include 0‰ within the error of our analytical precision ( $\pm 0.2\%$ ). Accordingly, 0‰ was used 14 for the  $\Delta^{17}$ O value of NO<sub>3</sub><sup>-</sup><sub>re</sub> and observed  $\Delta^{17}$ O values less than 0‰ are considered to be 0‰ 15 16 for the remainder of this paper.

The relationship between the estimated  $\delta^{15}N_{re}$  and  $\delta^{18}O_{re}$  is presented in Fig. 5. It should be 17 noted that all estimated  $\delta^{15}N_{re}$  values were nearly identical to the observed  $\delta^{15}N$  values owing 18 to small differences between  $\delta^{15}N_{re}$  and  $\delta^{15}N_{atm}$ . The primary goal of estimating  $\delta^{18}O_{re}$  is to 19 discuss the reason for large variations in  $\delta^{15}N_{re}$  (and thus  $\delta^{15}N$ ) of nitrate in stream water. 20 Additional determinations on the  $\Delta^{17}$ O values of nitrate together with  $\delta^{18}$ O enable us to 21 correct the contribution of NO<sub>3 atm</sub> from the determined values of  $\delta^{18}$ O and to use the 22 corrected values ( $\delta^{18}O_{re}$ ) for discussing the behavior of NO<sub>3</sub><sup>-</sup><sub>re</sub>. Unlike  $\Delta^{17}O_{atm}$ , the values of 23  $\delta^{18}O_{atm}$  used in the calculation could have been altered within the forest ecosystem subsequent 24 to deposition; therefore, we should consider errors up to 20% (as presented in section 3.2) in 25 the values of  $\delta^{18}O_{atm}$ . While the errors in the calculated  $\delta^{18}O_{re}$  values were small for the 26 samples showing low  $\Delta^{17}$ O values, the errors were large for those having high  $\Delta^{17}$ O values. 27 As a result, those having high  $\Delta^{17}$ O values of more than +10‰ are shown in parentheses to 28 denote that they were excluded from subsequent discussions. 29

As clearly presented in the figure,  $\delta^{15}N_{re}$  and  $\delta^{18}O_{re}$  were linearly correlated with a slope of 1 +1.23±0.45 ( $r^2 = 0.31$ ). As a result, both  $\delta^{15}N_{re}$  and  $\delta^{18}O_{re}$  varied simultaneously in the stream 2 water samples. Partial removal of nitrate through denitrification has been shown to be a 3 representative process that enhances both  $\delta^{15}N$  and  $\delta^{18}O$  in residual nitrate simultaneously 4 (Amberger and Schmidt, 1987). Previous studies showed that partial removal of nitrate 5 through assimilation by plants and/or microbes could be an alternative process leading to 6 enrichment of both  $\delta^{15}$ N and  $\delta^{18}$ O in residual nitrate, while the fractionation was found to be 7 small or negligible in general (Högberg, 1997; Kendall, 1998). Denitrification is a more 8 plausible cause of the observed variation in both  $\delta^{15}N_{re}$  and  $\delta^{18}O_{re}$ . Theoretical and laboratory 9 studies have suggested that denitrification results in 2:1 fractionation of  $\delta^{15}$ N:  $\delta^{18}$ O (Amberger 10 and Schmidt, 1987; Aravena and Robertson, 1998), but recent studies proposed a 1:1 ratio as 11 well (Granger et al., 2008). Thus, although other minor factors could have changed  $\delta^{15}N_{re}$ 12 and/or  $\delta^{18}O_{re}$ , the linear correlation between  $\delta^{15}N_{re}$  and  $\delta^{18}O_{re}$  in Fig. 5 implies that  $\delta^{15}N_{re}$  (and 13 thus  $\delta^{15}N$  of nitrate in stream water) primarily represented the progress of denitrification in 14 soils prior to elution into stream water. 15

As a result, temporal variations in the values of both  $\delta^{15}N_{re}$  (and thus  $\delta^{15}N$  of nitrate in stream water) can be a tracer to quantify the effects of strip-cutting on the progress of denitrification in soils of the watershed. However, we did not observe any significant variations in  $\delta^{15}N_{re}$ values in accordance with strip-cutting in the present study. This was likely because only five  $\delta^{15}N_{re}$  data points were available prior to strip-cutting (n=5). Accordingly, additional studies generating more nitrate  $\delta^{15}N_{re}$  data should be conducted to determine if strip-cutting impacts the progression of denitrification in soils.

Conversely, we observed clear depletion of the  $\delta^{15}N_{re}$  values in summer (June, July, and August) when compared with the other seasons (Fig. 3). Specifically, the average  $\delta^{15}N_{re}$  value was  $-2.5\pm1.6\%$  in summer (n=7), while it was  $+2.2\pm3.0\%$  (n=34) during the other seasons (p < 0.001, t-value=8.0). 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 within the total nitrate pool in soils.

# **3.4 Quantifying the effects of strip-cutting on nitrate dynamics**

2 As discussed in section 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 3 dwarf bamboo, S. senanensis, in October 2003. In the present study, the  $\Delta^{17}$ O tracer of nitrate 4 revealed that strip-cutting in October 2003 had a significant impact on C<sub>atm</sub> as well. While the 5 maximum stream  $C_{atm}$  was only 0.53  $\mu$ mol L<sup>-1</sup> in 2003, a significant increase in  $C_{atm}$  to 8.2 6  $\mu$ mol L<sup>-1</sup> was observed in spring of 2004, probably in response to strip-cutting. A similar 7 increase in stream  $C_{atm}$  up to 3.9 µmol L<sup>-1</sup> was also observed in spring of 2005. To quantify 8 9 the effects of the strip-cutting on processes regulating the elution of  $NO_{3-atm}^{-}$ , the daily elution rate of NO<sub>3<sup>-</sup>atm</sub> (F<sub>atm</sub>) was calculated for each day on which the  $\Delta^{17}O$  value of nitrate was 10 determined from each concentration of  $NO_{3 \text{ atm}}$  (C<sub>atm</sub>) and the daily flow rate of stream water 11 12 (V) by applying equation (5):

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

14 There were only four  $C_{atm}$  data points for 2003 because most of the  $C_{total}$  in 2003 were too low 15 (less than 0.1 µmol L<sup>-1</sup>) to determine the  $\Delta^{17}O$  values (Fig. 2). However, if the  $C_{total}$  is less 16 than 0.1 µmol L<sup>-1</sup>, the associated  $C_{atm}$  must be less than 0.1 µmol L<sup>-1</sup> as well, regardless of the 17  $\Delta^{17}O$  values. To estimate the upper limit of  $C_{atm}$  and thus the upper limit of  $F_{atm}$  for 2003, we 18 applied the maximum  $\Delta^{17}O$  value of nitrate in stream water observed in this study ( $\Delta^{17}O =$ 19 +14.3‰) as the maximum  $\Delta^{17}O$  value of nitrate for samples showing  $C_{total}$  less than 0.1 µmol 20 L<sup>-1</sup> 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 equations (6) and (7):

- 24  $F_{\text{total}} = C_{\text{total}} \times V$  (6)
- $25 F_{\rm re} = F_{\rm total} F_{\rm atm} (7)$

The temporal variation of  $F_{atm}$  and the  $F_{total}$  are plotted in Fig. 3(d). As shown in the figure, enrichment of  $F_{atm}$  occurred during spring from 2003 to 2005. More than 90% of NO<sub>3</sub><sup>-</sup><sub>atm</sub> eluted in March, April, and May each year. Direct contribution of NO<sub>3</sub><sup>-</sup><sub>atm</sub> 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  $\delta^{18}$ O of nitrate in runoff (Kendall et al., 1995; Ohte et al., 2004; Piatek et al., 2005; Pellerin et al., 2012). While spring F<sub>atm</sub> 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 µmol s<sup>-1</sup> in 2003 to 88.6 µmol s<sup>-1</sup> in 2004 and 93.3 µmol s<sup>-1</sup> in 2005. Additionally, maximum  $F_{re}$ increased from 13.0 µmol s<sup>-1</sup> in 2003 to 77.8 µmol s<sup>-1</sup> in 2004 and 161.5 µmol s<sup>-1</sup> in 2005.

5 Conversely,  $F_{atm}$  was always small during the other seasons, even after strip-cutting. Most of 6 the nitrate being exported from the watershed during seasons other than spring was  $NO_{3}^{-}re$ : 7 those retained in the forested ecosystem as either organic-N or ammonium and then been 8 converted to nitrate via microbial nitrification.  $F_{atm}$  was especially low during summer.  $F_{re}$ 9 was reduced during summer as well (Fig. 3).

As discussed above,  $\delta^{15}N_{re}$  depletion implied active nitrification during summer. The combination of both active nitrification in soil and active nitrate consumption through assimilation by plants and/or microbes resulted in both a reduction and rapid turnover of the nitrate pool in soil, and thus a reduction in the elution rate of both NO<sub>3<sup>-</sup> atm</sub> (mostly) and NO<sub>3<sup>-</sup></sub> re (partly) during summer. When compared with summer, a slight increase in F<sub>atm</sub> was observed in fall and winter. The decrease in nitrification and nitrate consumption in soils increased the direct drainage rate of NO<sub>3<sup>-</sup> atm</sub>.

We can obtain the annual export flux of  $NO_{3\ atm}^{-}$  per unit area of the catchment (M<sub>atm</sub>) by integrating the F<sub>atm</sub> values for each year of the observation using the equation (8).

19 
$$M_{atm} = \frac{\sum F_{atm}(t) \times \Delta t}{S}$$
 (8)

where S denote the total catchment area (8 ha). We can obtain the annual export flux for  $NO_3^-$ (M<sub>total</sub>) and  $NO_3^-$  (M<sub>re</sub>) by integrating F<sub>re</sub> and F<sub>total</sub> for each year of the observation using equations (9) and (10).

23 
$$M_{\text{total}} = \frac{\sum F_{\text{total}}(t) \times \Delta t}{S}$$
 (9)

24 
$$M_{re} = \frac{\sum F_{re}(t) \times \Delta t}{S}$$
 (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 (mmol m<sup>-2</sup> year<sup>-1</sup>) 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±0.04 (mmol m<sup>-2</sup> year<sup>-1</sup>) 1 in 2003 to 2.6 in 2004 and 2.1 in 2005.  $M_{re}$  also increased from 0.88±0.04 (mmol m<sup>-2</sup> year<sup>-1</sup>) 2 in 2003 to 3.7 in 2004 and 4.8 in 2005.

3 The observed increases in M<sub>atm</sub> and M<sub>re</sub> in accordance with the Sasa-cutting in October 2003 4 suggest that Sasa is important to prevention of nitrogen leaching from soil and enhancement 5 of biological consumption of NO<sub>3 atm</sub> before being exported from forest ecosystems, 6 especially when significant quantities of NO<sub>3 atm</sub> were added to the forest floor through the 7 spring snowmelt. Although both M<sub>atm</sub> and M<sub>re</sub> increased in response to strip-cutting, the 8 relative increase in M<sub>atm</sub> was much higher than the relative increase in M<sub>re</sub>. These results 9 imply that the major impact of strip-cutting was on the biological consumption processes of 10  $NO_{3}^{-}atm$ , rather than the production processes of  $NO_{3}^{-}re$  in soils.

11 While the annual average M<sub>atm</sub>/M<sub>total</sub> ratio was less than 16% in 2003 (Table 1), it increased to 12 41% in 2004 in response to strip-cutting, then slightly decreased to 31% in 2005. The Matm/Mtotal ratios after strip-cutting were much higher than those determined for normal 13 14 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., 15 16 2013), as well as that dissolved in soil solution of temperate forest in northern Michigan (9% 17 on average)(Costa et al., 2011) and that dissolved in an oligotrophic lake water column in 18 Japan  $(9.7 \pm 0.8\%)$  (Tsunogai et al., 2011). As a result, we can easily differentiate the ratios observed after strip-cutting from other normal M<sub>atm</sub>/M<sub>total</sub> ratios in stream water using the 19  $\Delta^{17}$ O values of nitrate, indicating that they can serve as a useful and powerful tracer for 20 quantification of artificial alternations in forested watersheds. 21

#### 22 **3.5** Quantifying the effects of strip-cutting on atmospheric nitrate dynamics

23 If biological consumption processes of NO<sub>3 atm</sub> were fully destroyed in the watershed owing 24 to strip-cutting, the annual export flux via stream water (Matm) would be the same as that 25 deposited throughout the catchment area. Therefore, we determined the annual deposition flux 26 of NO<sub>3<sup>-</sup>atm</sub> (D<sub>atm</sub>) at the monitoring tower of the CC-LaG project adjacent to the catchment 27 area (Fig. 1) to compare M<sub>atm</sub> with D<sub>atm</sub>. The data coverage of the obtained daily deposition 28 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 determined the average 29 daily deposition rate for each year based only on the obtained data set and then estimated the 30 annual deposition flux (D<sub>atm</sub>) for each year assuming the same daily deposition rate with the 31

average for those lacking data. The annual deposition flux of NO<sub>3<sup>-</sup>atm</sub> (D<sub>atm</sub>) was nearly stable
at around 18.6±2.7 (mmol m<sup>-2</sup> y<sup>-1</sup>), and wet deposition accounted for 81±3% of the total NO<sub>3<sup>-</sup></sub>
atm 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).

6 When compared with the  $D_{atm}$  estimated in this study, the annual export flux of  $NO_{3atm}$  via 7 stream water (M<sub>atm</sub>) 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  $\Delta^{17}O$  of nitrate as a tracer, we 8 9 estimated that direct drainage accounts for  $8.8\pm4.6\%$  of NO<sub>3<sup>-</sup>atm</sub> that has been deposited onto the island on average, and that the residual portion has undergone biological processing 10 11 before being exported from the surface ecosystem based on comparison of the inflow (deposition of atmospheric nitrate) and outflow (atmospheric nitrate in groundwater) 12 (Tsunogai et al., 2010). The present study revealed that the studied forest ecosystem removed 13 NO<sub>3<sup>-</sup>atm</sub> more effectively in 2003 than that on Rishiri Island, while the removal efficiency was 14 15 worse than that of Rishiri Island in 2004 owing to strip-cutting.

16 Both surface vegetation and the related ecosystems in soils must play a significant role in the consumption of NO<sub>3 atm</sub> (Nakagawa et al., 2013). The area in which Sasa was strip-cut only 17 accounted for 50% of the total watershed. Additionally, larch seedlings were immediately 18 19 planted in the Sasa strip-cut line. Although the removal processes of NO<sub>3</sub> atm by plants and/or microbes in the forested soils were damaged by strip-cutting, the results of the present study 20 21 demonstrated that the majority of these processes were still active, even after strip-cutting. 22 These findings will be useful in future to develop strategies for both clear-cutting and strip-23 cutting in forested ecosystems without increasing nitrate elution from watersheds.

24

#### 25 4 Summary and conclusions

To quantify the biogeochemical effects of clear-cutting of trees and subsequent strip-cutting of the understory vegetation in a cool-temperate forested watershed, temporal variations in the origin of nitrate dissolved in stream water eluted from the watershed were determined by using the  $\Delta^{17}$ O values of nitrate as tracers, with special emphasis on changes in the fate of atmospheric nitrate that had been deposited into the watershed. When compared with the values prior to strip-cutting, the annual export of atmospheric nitrate and remineralized nitrate increased by more than 13-fold and 4-fold, respectively. These findings indicate that the

understory vegetation is important to the biological consumption of atmospheric nitrate, 1 2 especially when significant quantities of nitrate were added to the forest floor through the 3 spring snowmelt. Additionally, the major impact of strip-cutting was on the biological consumption processes of atmospheric nitrate, rather than the production processes of 4 remineralized nitrate in soils. Nevertheless, the annual export flux of atmospheric nitrate 5 corresponds to less than 14% of atmospheric nitrate deposited into the watershed. Although 6 the removal processes of atmospheric nitrate in the forested soils were damaged by strip-7 8 cutting, the majority of these processes were still active after strip-cutting. This study clearly demonstrates that temporal variations in the  $\Delta^{17}$ O values of nitrate in stream water can be a 9 10 powerful tracer for quantification of artificial alternations in forested watersheds. Moreover, additional measurements of the  $\Delta^{17}$ O values of nitrate together with  $\delta^{15}$ N and  $\delta^{18}$ O enable 11 correction of the contribution of atmospheric nitrate from the determined values and use of 12 the corrected  $\delta^{15}$ N and  $\delta^{18}$ O values for evaluation of the behavior of remineralized nitrate in 13 14 soils.

15

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27

Table 1 Temporal variations in the export flux per unit area of the catchment (mmol  $m^{-2}$  year<sup>-1</sup>) of atmospheric nitrate (M<sub>atm</sub>), together with those of remineralized nitrate (M<sub>re</sub>), total nitrate (M<sub>total</sub>), and M<sub>atm</sub>/M<sub>total</sub> ratio. Changes relative to 2003 are presented in parentheses. 

	2003	2004	2005
M <sub>atm</sub>	0.13±0.04	2.6	2.1
	(1)	(16–30)	(13–24)
M <sub>re</sub>	0.88±0.04	3.7	4.8
	(1)	(4)	(5–6)
M <sub>total</sub>	1.0	6.4	7.0
	(1)	(6.3)	(6.9)
$M_{atm}/M_{total}$	9–16%	41%	31%

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Table 2 Annual deposition rate of atmospheric nitrate at the monitoring tower (mmol  $m^{-2}$ 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



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. 



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).





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



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



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