



1 **Accurate and precise quantification of atmospheric nitrate**
2 **in streams draining land of various uses by using triple**
3 **oxygen isotopes as tracers**

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16

17 **Abstract**

18 ¹⁷O anomalies were used to quantify the influence of changes in land use and population
19 density between each catchment area on the fate of atmospheric nitrate by determining the
20 areal distribution and seasonal variation in stable isotopic compositions including the ¹⁷O
21 anomalies ($\Delta^{17}\text{O}$) of nitrate for more than 30 streams within the same watershed. Those in
22 precipitation (wet deposition; $n = 213$) sampled at Sado-seki monitoring station were
23 determined for three years as well. The deposited nitrate showed similar large ¹⁷O anomalies
24 with those already reported for mid-latitudes: $\Delta^{17}\text{O}$ values ranged from +18.6‰ to +32.4‰
25 with a three-year average of +26.3‰. However, nitrate in each inflow stream showed small
26 annual average $\Delta^{17}\text{O}$ values ranging from +0.5‰ to +3.1‰, which corresponds to the mixing
27 ratios of unprocessed atmospheric nitrate to total nitrate from $1.8 \pm 0.3\%$ to $11.8 \pm 1.8\%$, with
28 $5.1 \pm 0.5\%$ as the average of all inflow streams. Although the annual average $\Delta^{17}\text{O}$ values



1 tended to be smaller in accordance with the increase in annual average nitrate concentration
2 from 12.7 to 106.2 $\mu\text{mol L}^{-1}$, the absolute concentrations of unprocessed atmospheric nitrate
3 in the streams were almost stable at $2.3 \pm 1.1 \mu\text{mol L}^{-1}$ irrespective of the changes in
4 population density and land use in each catchment area. We conclude that changes in
5 population density and land use between each catchment area had little impact on the
6 concentration of atmospheric nitrate. Thus, the total nitrate concentration originated primarily
7 from additional contribution of remineralized nitrate from both natural sources, having values
8 of $+4.4 \pm 1.8\%$ and $-2.3 \pm 0.9\%$ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively, and anthropogenic sources
9 having values of $+9.2 \pm 1.3\%$ and $-2.2 \pm 1.1\%$ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively. In addition,
10 both the uniform absolute concentration of atmospheric nitrate and the low and uniform $\delta^{18}\text{O}$
11 values of the remineralized portion of nitrate in the streams imply that in-stream removal of
12 nitrate through assimilation or denitrification had small impact on the concentrations and
13 stable isotopic compositions of nitrate in the streams, except for a few streams in summer
14 having catchments of urban/suburban land uses. Additional measurements of the $\Delta^{17}\text{O}$ values
15 of nitrate together with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ enabled us to exclude the contribution of unprocessed
16 atmospheric nitrate from the determined $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of total nitrate and to use the
17 corrected $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values to evaluate the source and behaviour of the remineralized
18 portion of nitrate in each stream.

19

20 **1 Introduction**

21 Nitrate (NO_3^-) in stream water can be an important clue for understanding the biogeochemical
22 cycles within its catchment area (Durka et al. 1994; Likens et al. 1970; Swank et al. 2001). In
23 addition, the nitrate concentration in stream water is important to primary production and thus
24 eutrophication downstream, including lakes, estuaries, and oceans (Mcisaac et al. 2001; Paerl
25 2009). Nitrate concentrations in stream water, however, are determined through a complicated
26 interplay of several processes within its catchment area including (1) the addition of
27 atmospheric nitrate ($\text{NO}_3^-_{\text{atm}}$) through deposition, (2) the production of remineralized nitrate
28 ($\text{NO}_3^-_{\text{re}}$) through microbial nitrification, and (3) the removal of nitrate through assimilation
29 and denitrification by plants and microbes. In addition to natural processes, anthropogenic
30 processes could have a significant impact on the nitrate dynamics within each catchment area,
31 particularly for those eluted from urban or agricultural catchment zones. Therefore,



1 interpretation of the processes regulating nitrate concentration in stream water is not always
2 straightforward.

3 The $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of nitrate have been widely applied in the determination of the
4 sources and behaviours of nitrate in stream water worldwide (Barnes et al. 2008; Barnes and
5 Raymond 2010; Burns et al. 2009; Burns and Kendall 2002; Campbell et al. 2002; Campbell
6 et al. 2006; Costa et al. 2011; Curtis et al. 2011; Durka et al. 1994; Hales et al. 2007;
7 Johannsen et al. 2008; Kaushal et al. 2011; Lohse et al. 2013; Mayer et al. 2002; Nestler et al.
8 2011; Ohte 2013; Ohte et al. 2004; Ohte et al. 2010; Pellerin et al. 2012; Silva et al. 2002;
9 Thibodeau et al. 2013; Tobarí et al. 2010; Wankel et al. 2006; Williard et al. 2001; Yue et al.
10 2013; Zeng and Wu 2015). By combining the two isotopic ratios, the relative mixing ratios
11 among various nitrate sources such as atmospheric (unprocessed), fertilizer, manure, and
12 sewage plants can be quantified through a simple isotope mass balance approach. Partial
13 removal of nitrate through either assimilation or denitrification, however, results in residual
14 nitrate being enriched with ^{15}N and ^{18}O (Böttcher et al. 1990; Granger et al. 2010), which
15 complicates their interpretation beyond that of the simple isotope mass balance approach. In
16 addition, trace contributions of unprocessed $\text{NO}_3^-_{\text{atm}}$ could have a significant impact on the
17 $^{18}\text{O}/^{16}\text{O}$ ratios of the total nitrate in stream water (Durka et al. 1994; Kendall 1998; Mayer et
18 al. 2001; Michalski et al. 2004; Tsunogai et al. 2010). Therefore, $^{18}\text{O}/^{16}\text{O}$ ratios are used as
19 tracers based on assumptions such as (1) the $^{18}\text{O}/^{16}\text{O}$ ratios of nitrate in stream water simply
20 reflect the mixing ratio of unprocessed $\text{NO}_3^-_{\text{atm}}$ within total nitrate (Barnes et al. 2008; Burns
21 et al. 2009; Campbell et al. 2006; Durka et al. 1994; Ohte et al. 2004; Ohte et al. 2010; Zeng
22 and Wu 2015), and (2) the mixing ratios of unprocessed $\text{NO}_3^-_{\text{atm}}$ within total nitrate are
23 minimum or uniform for whole or specific stream water samples (Johannsen et al. 2008;
24 Mayer et al. 2002; Wankel et al. 2006). To verify the reliability of these assumptions and to
25 utilize the $^{18}\text{O}/^{16}\text{O}$ ratios for the quantification of the accurate and precise mixing ratios
26 among various nitrate sources based on the isotope mass balance approach, the mixing ratio
27 of $\text{NO}_3^-_{\text{atm}}$ within the total nitrate in stream water must be better understood; otherwise, the
28 processes regulating nitrate concentration in stream water will be ambiguous even when
29 adding the data of $^{18}\text{O}/^{16}\text{O}$ ratios of nitrate.

30 To overcome the limitation in using the $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ ratios, the $^{17}\text{O}/^{16}\text{O}$ ratios of
31 nitrate have been used as an additional tracer of $\text{NO}_3^-_{\text{atm}}$ in stream water in recent studies
32 (Dejwakh et al. 2012; Michalski et al. 2004; Riha et al. 2014; Rose et al. 2015; Tsunogai et al.



1 2010; Tsunogai et al. 2014). The $^{17}\text{O}/^{16}\text{O}$ ratios were used because, whereas $\text{NO}_3^-_{\text{re}}$, the
 2 oxygen atoms of which are derived from either terrestrial O_2 or H_2O through nitrification,
 3 shows mass-dependent relative variations between $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios, unprocessed
 4 $\text{NO}_3^-_{\text{atm}}$ displays an anomalous enrichment in ^{17}O from the mass-dependent relative variations,
 5 reflecting oxygen atom transfers from ozone during the conversion of NO_x to $\text{NO}_3^-_{\text{atm}}$
 6 (Michalski et al. 2003; Morin et al. 2008). By using the $\Delta^{17}\text{O}$ signature defined by the
 7 following equation (Kaiser et al. 2007; Miller 2002), we can distinguish $\text{NO}_3^-_{\text{atm}}$ ($\Delta^{17}\text{O} > 0$)
 8 from $\text{NO}_3^-_{\text{re}}$ ($\Delta^{17}\text{O} = 0$):

$$9 \quad \Delta^{17}\text{O} = \frac{1 + \delta^{17}\text{O}}{(1 + \delta^{18}\text{O})^\beta} - 1, \quad (1)$$

10 where the constant β is 0.5247 (Kaiser et al. 2007; Miller 2002), $\delta^{18}\text{O} = R_{\text{sample}}/R_{\text{standard}} - 1$,
 11 and R is the $^{18}\text{O}/^{16}\text{O}$ ratio of the sample (or the $^{17}\text{O}/^{16}\text{O}$ ratio in the case of $\delta^{17}\text{O}$ or the $^{15}\text{N}/^{14}\text{N}$
 12 ratio in the case of $\delta^{15}\text{N}$) and each standard reference material. In addition, $\Delta^{17}\text{O}$ is stable
 13 during the mass-dependent isotope fractionation processes within surface ecosystems.
 14 Therefore, although the atmospheric $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ signature can be overprinted by
 15 biogeochemical processes subsequent to deposition, $\Delta^{17}\text{O}$ can be used as a robust tracer of
 16 unprocessed $\text{NO}_3^-_{\text{atm}}$ to reflect the mixing ratio of unprocessed $\text{NO}_3^-_{\text{atm}}$ within total NO_3^-
 17 regardless of biogeochemical removal processes subsequent to deposition by using the
 18 following equation:

$$19 \quad \frac{C_{\text{atm}}}{C_{\text{total}}} = \frac{\Delta^{17}\text{O}}{\Delta^{17}\text{O}_{\text{atm}}}, \quad (2)$$

20 where C_{atm} and C_{total} denote the concentration of $\text{NO}_3^-_{\text{atm}}$ and NO_3^- in each water sample,
 21 respectively, and $\Delta^{17}\text{O}_{\text{atm}}$ and $\Delta^{17}\text{O}$ denote the $\Delta^{17}\text{O}$ values of $\text{NO}_3^-_{\text{atm}}$ and nitrate (total) in
 22 each water sample, respectively.

23 Moreover, additional measurements of the $\Delta^{17}\text{O}$ values of nitrate together with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$
 24 enable us to exclude the contribution of $\text{NO}_3^-_{\text{atm}}$ in the determined $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values and
 25 to estimate the corrected $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$, respectively) for accurate
 26 evaluation on the source and behaviour of $\text{NO}_3^-_{\text{re}}$ (Dejwakh et al. 2012; Liu et al. 2013; Riha
 27 et al. 2014; Tsunogai et al. 2011; Tsunogai et al. 2010; Tsunogai et al. 2014).

28 Previous studies have successfully applied the $\Delta^{17}\text{O}$ tracer of nitrate to those eluted from
 29 arid/semi-arid watersheds (Dejwakh et al. 2012; Michalski et al. 2004; Riha et al. 2014),



1 forested watersheds (Rose et al. 2015; Tsunogai et al. 2010; Tsunogai et al. 2014), and a large
2 river basin (Liu et al. 2013) to determine the accurate and precise mixing ratios of
3 unprocessed $\text{NO}_3^-_{\text{atm}}$ in total nitrate in addition to the fate of the $\text{NO}_3^-_{\text{atm}}$ that had been
4 deposited into each watershed. However, relative changes in the source and fate of $\text{NO}_3^-_{\text{atm}}$ in
5 accordance with the changes in land use of catchments have not been studied thus far by using
6 the $\Delta^{17}\text{O}$ tracer of nitrate.

7 In this study, we measure the concentrations and the stable isotopic compositions of nitrate
8 including $\Delta^{17}\text{O}$ values for more than 30 streams flowing into a lake in Japan with catchments
9 of widely varying land uses within the same watershed, which includes urban, suburban,
10 agricultural (mostly rice paddies), and forested catchments. By using the $\Delta^{17}\text{O}$ tracer, we
11 quantify both areal and temporal variations in the concentrations of both $\text{NO}_3^-_{\text{atm}}$ and $\text{NO}_3^-_{\text{re}}$
12 in streams across the land use settings to gain insight into the processes controlling the source,
13 transport, and fate of $\text{NO}_3^-_{\text{atm}}$ and $\text{NO}_3^-_{\text{re}}$ (Fig. 1). Although $\text{NO}_3^-_{\text{re}}$ increases during
14 nitrification within each catchment area, for instance, $\text{NO}_3^-_{\text{atm}}$ is stable, so that we can
15 evaluate the progress of nitrification within each catchment area by using the changes in the
16 concentrations of both $\text{NO}_3^-_{\text{atm}}$ and $\text{NO}_3^-_{\text{re}}$. Besides to the streams, we determine those values
17 in precipitation (wet deposition) for comparison to obtain accurate and precise mixing ratios
18 of both $\text{NO}_3^-_{\text{atm}}$ and $\text{NO}_3^-_{\text{re}}$ within nitrate (total) in each stream. Moreover, we exclude the
19 contribution of $\text{NO}_3^-_{\text{atm}}$ in the determined $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values to estimate the corrected $\delta^{15}\text{N}$
20 and $\delta^{18}\text{O}$ values for accurate evaluation of the source and behaviour of $\text{NO}_3^-_{\text{re}}$. Further, we
21 determine those values in an outflow stream of the same lake to evaluate the influences of
22 flow stagnation into the lake on nitrate by using the differences between inflows and outflows
23 (Fig. 1). The results presented herein will increase our understanding of the fate of $\text{NO}_3^-_{\text{atm}}$
24 deposited onto land, particularly those deposited onto urban/suburban and forested
25 catchments (Fig. 1).

26

27 **2 Experimental Section**

28 **2.1 Site description**

29 **2.1.1 Lake Biwa watershed basin**



1 Lake Biwa, located in the central part of the Japanese Islands, is the largest freshwater lake in
2 Japan (Fig. 2). It has a surface area of 670.4 km² and a total catchment area of 3174 km² with
3 annual precipitation of around 2000 mm. More than 120 streams flow into the lake, whereas
4 the Seta River (No. 33 in Fig. 2(b)) at the southern end, also known as the Yodo River, is the
5 only natural outflow. The average residence time of water in the lake is 5.5 years.

6 Similar to many lakes throughout the world, Lake Biwa has experienced eutrophication in the
7 past. Urbanization near the lake beginning in the 1960s, particularly on the southern and
8 eastern shore, likely caused an increase in nutrient loading. Blooms of *Uroglena americana*
9 and cyanobacteria have occurred since 1977 and 1983, respectively (Hsieh et al. 2011). To
10 clarify the pathways and sources of nitrate that were fed into the lake, the stable isotopic
11 compositions ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of dissolved nitrate were determined in the major streams
12 flowing into the lake (Ohte et al. 2010). Based on the $\delta^{15}\text{N}$ values of nitrate showing positive
13 correlation with population densities of each catchment area, it was concluded that sewage
14 effluent was the dominant source contributing to the increase in the $\delta^{15}\text{N}$ values of nitrate.

15 2.1.2 Sado-seki monitoring station

16 Sado-seki National Acid Rain Monitoring Station (38°14'59"N, 138°24'00"E) was established
17 on Sado Island (Fig. 2(a)), at 110 m above sea level, as a monitoring observatory of the Acid
18 Deposition Monitoring Network in East Asia (EANET) representing the central Japan area
19 (EANET 2014). The monitoring has shown that the observatory received 24.5 mmol NO₃⁻
20 m⁻²yr⁻¹ and 17.1 mmol NH₄⁺ m⁻²yr⁻¹ on average from FY2009 to 2011 (EANET 2014),
21 which corresponds to a total fixed N deposition rate of 5.8 kg of N ha⁻¹yr⁻¹.

22 2.2 Sampling

23 Stream water samples were collected near the mouths of each stream during base flow periods
24 four times in 2013 on March 15, June 17, August 5, and October 21 from 33 inflow streams
25 and 1 outflow river (Seta River) of Lake Biwa (Table 1; Fig. 2(b)) except for stream Nos. 3
26 and 28 in June, which became dry arroyos at that time. The catchments of the studied inflow
27 streams occupied 70% of the entire Lake Biwa basin area. The streams were selected to cover
28 those in which the concentrations and stable isotope compositions of nitrate, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$,
29 had already been determined in 2004–2006 (Ohte et al. 2010). The categories of locations
30 classified by Ohte et al. (2010) were also used in this study to classify the location of the



1 streams (Table 1). Either a bucket or dipper was used to collect samples as far from the bank
2 as possible. Each sample was transferred into a dark polyethylene bottle that was pre-rinsed at
3 least twice with the sample itself and stored under refrigeration. Then, the samples were
4 filtered through a pre-combusted Whatman GF/F filter with a 0.7 μm pore size within a few
5 hours after collection, and the filtrate was stored in a different dark polyethylene bottle under
6 refrigeration at 4°C until analysis.

7 To calculate the annual influx/efflux of nitrate via each stream to/from Lake Biwa as well as
8 its seasonal variation, we used the sampling number n , where $n = 1, 2, 3$, and 4, to represent
9 the sampling in March, June, August, and October, respectively. In addition, we used one
10 more hypothetical sampling number ($n = 5$) set just one year later than the $n = 1$ date to
11 quantify the annual influx/efflux. Accordingly, we assumed that secular change was minimum
12 in the streams and that the streams returned back to the initial state one year later.
13 Furthermore, we rated the interval between $n = 1$ and 2 as spring, $n = 2$ and 3 as summer, $n =$
14 3 and 4 as autumn, and $n = 4$ and 5 as winter for the streams in this study.

15 Samples of wet deposition were taken at the Sado-seki National Acid Rain Monitoring Station
16 by using standard methods for evaluating acid deposition in Japan from April 2009 to March
17 2012. An automatic wet deposition sampler (US-420, Ogasawara) was used in the collection.
18 All of the deposition samples were introduced and stored in 1 L polyethylene bottles under
19 refrigeration until daily recovery. After measuring both the conductivity and pH, the
20 recovered samples were filtered through a 0.2 μm pore-size membrane filter (Dismic-25CS,
21 ADVANTEC) and stored in a refrigerator until analysis. The annual wet deposition rate of
22 nitrate was 19.3 $\text{mmol m}^{-2}\text{y}^{-1}$ for FY2009, from April 2009 to March 2010; 28.0 $\text{mmol m}^{-2}\text{y}^{-1}$
23 for FY2010, from April 2010 to March 2011; and 27.0 $\text{mmol m}^{-2}\text{y}^{-1}$ for FY2011, from April
24 2011 to March 2012 (EANET 2014).

25 **2.3 Analysis**

26 The concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) in each filtrate sample were measured
27 by ion chromatography (Prominence HIC-SP, Shimadzu, Japan) within a few days after each
28 sampling. The $\delta^{18}\text{O}$ values of H_2O in the samples were analysed by using the cavity ring-
29 down spectroscopy method by employing the Picarro L2120-I instrument equipped with an
30 A0211 vaporizer and auto sampler; the error in this method was $\pm 0.1\%$. Both Vienna



1 Standard Mean Ocean Water (VSMOW) and Standard Light Antarctic Precipitation (SLAP)
2 were used to calibrate the values to the international scale.

3 To determine the stable isotopic compositions, nitrate in each filtrate sample was chemically
4 converted to N₂O by using a method originally developed to determine the ¹⁵N/¹⁴N and
5 ¹⁸O/¹⁶O ratios of seawater and freshwater nitrate (Mcilvin and Altabet 2005) which was later
6 modified (Konno et al. 2010; Nakagawa et al. 2013; Tsunogai et al. 2011; Tsunogai et al.
7 2008; Tsunogai et al. 2010; Yamazaki et al. 2011). Then, the stable isotopic compositions of
8 N₂O were determined by using a continuous-flow isotope ratio mass spectrometry (CF-IRMS)
9 system in Nagoya University (Hirota et al. 2010; Komatsu et al. 2008). The analytical
10 procedures are the same as those detailed in previous research (Nakagawa et al. 2013;
11 Tsunogai et al. 2014).

12 To determine whether samples were deteriorated or contaminated during storage and whether
13 the conversion rate from nitrate to N₂O was sufficient, concentrations of nitrate in the samples
14 were determined each time we analysed isotopic compositions using MS based on the N₂O⁺
15 or O₂⁺ outputs. We adopted the δ¹⁵N, δ¹⁸O, or Δ¹⁷O values only when concentrations
16 measured by MS correlated with those measured by ion chromatography just after the
17 sampling within 10% differences.

18 We repeated the analyses on the δ¹⁵N, δ¹⁸O, and Δ¹⁷O values of nitrate at least three times for
19 each sample to attain high precision. Most of the samples had nitrate concentrations of more
20 than 5.0 μmol L⁻¹, which corresponded to nitrate quantities greater than 50 nmol in a 10 mL
21 sample. This amount was sufficient for determining the δ¹⁵N, δ¹⁸O, and Δ¹⁷O values with high
22 precision. The sample volume was increased to 30 mL for those having nitrate concentrations
23 less than 5.0 μmol L⁻¹; the number of analyses was also increased in such cases. Thus, all
24 isotopic data presented in this study have an error better than ±0.2‰ for δ¹⁵N, ±0.3‰ for δ¹⁸O,
25 and ±0.1‰ for Δ¹⁷O.

26 Because the more precise power law shown in Eq. (1) was used to calculate Δ¹⁷O, the
27 estimated Δ¹⁷O values were somewhat different from those estimated based on traditional
28 linear approximation (Michalski et al. 2002). Please note that our Δ¹⁷O values of NO₃⁻_{atm}
29 would be 0.9±0.1 ‰ higher if we use the linear approximation. However, these differences
30 were insignificant for most of the stream water samples evaluated in this study.

31 Nitrite (NO₂⁻) in the samples interferes with the final N₂O produced from nitrate (NO₃⁻)
32 because the chemical method also converts NO₂⁻ to N₂O (Mcilvin and Altabet 2005).



1 Therefore, it is sometimes necessary to correct for the contribution of NO_2^- -derived N_2O to
 2 accurately determine the stable isotopic compositions of the sample nitrate. However, all
 3 samples analysed for stable isotopic compositions in this study showed $\text{NO}_2^-/\text{NO}_3^-$ ratios of
 4 less than 5%; thus, the results were used with no corrections.

5 **2.4 Calculating average concentration and isotopic compositions in each** 6 **stream**

7 To quantitatively clarify the chemical and isotopic characteristics of each stream, we
 8 determined both the flow-weighted annual average concentration (\bar{C}_{total}) and flow-weighted
 9 annual average $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values ($\bar{\delta}$) of nitrate for each stream by using Eqs. (3),
 10 (4), and (5):

$$11 \quad q = \sum_{n=1}^4 (f_n \cdot \Delta t_n), \quad (3)$$

$$12 \quad \bar{C}_{\text{total}} = \frac{\sum_{n=1}^4 (C_n \cdot f_n \cdot \Delta t_n)}{q}, \quad (4)$$

$$13 \quad \bar{\delta} = \frac{\sum_{n=1}^4 (\delta_n \cdot C_n \cdot f_n \cdot \Delta t_n)}{\sum_{n=1}^4 (C_n \cdot f_n \cdot \Delta t_n)}, \quad (5)$$

14 where C_n and δ_n denote the concentration (C_{total} in Eq. (2)) and isotopic values ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, or
 15 $\Delta^{17}\text{O}$) of nitrate in each stream during each observation n , respectively; f_n denotes the flow
 16 rate of each stream during each observation n ; and Δt_n denotes the time interval between the
 17 observation n and the next observation $n+1$. When possible, we used the flow rate of each
 18 stream which was determined monthly by the Shiga Prefecture (Shiga_Prefecture 2015) for f_n .
 19 For small streams with no data for the flow rate, we used a small and stable flow rate of 0.1
 20 m^3/s for f_n .

21 **2.5 Calculating $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of remineralized nitrate**

22 To exclude the contribution of $\text{NO}_3^-_{\text{atm}}$ from the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate and to clarify
 23 the source and behaviour of $\text{NO}_3^-_{\text{re}}$ by using both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ as tracers, we estimated the
 24 end-member $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the remineralized nitrate portion, $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$, by



1 excluding the contribution of $\text{NO}_3^-_{\text{atm}}$ in each nitrate (Dejwakh et al. 2012; Liu et al. 2013;
 2 Riha et al. 2014; Tsunogai et al. 2011; Tsunogai et al. 2010; Tsunogai et al. 2014) by using
 3 Eqs. (6) and (7):

$$4 \quad \delta^{15}\text{N}_{\text{re}} = \frac{C_{\text{total}} \cdot \delta^{15}\text{N} - C_{\text{atm}} \cdot \delta^{15}\text{N}_{\text{atm}}}{C_{\text{total}} - C_{\text{atm}}}, \quad (6)$$

$$5 \quad \delta^{18}\text{O}_{\text{re}} = \frac{C_{\text{total}} \cdot \delta^{18}\text{O} - C_{\text{atm}} \cdot \delta^{18}\text{O}_{\text{atm}}}{C_{\text{total}} - C_{\text{atm}}}, \quad (7)$$

6 where C_{atm} and C_{total} denote the concentration of $\text{NO}_3^-_{\text{atm}}$ and nitrate in each water sample,
 7 respectively, and $\delta^{15}\text{N}_{\text{atm}}$, $\delta^{18}\text{O}_{\text{atm}}$, and $\Delta^{17}\text{O}_{\text{atm}}$ denote the $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of
 8 $\text{NO}_3^-_{\text{atm}}$ in each sample, respectively. The actual values of $\delta^{15}\text{N}_{\text{atm}}$, $\delta^{18}\text{O}_{\text{atm}}$, and $\Delta^{17}\text{O}_{\text{atm}}$ used
 9 in this study will be determined in section 3.1.

10

11 3 Results and Discussion

12 3.1 Atmospheric Nitrate

13 The triple oxygen isotopic compositions ($\Delta^{17}\text{O}$) of atmospheric nitrate ($\text{NO}_3^-_{\text{atm}}$) are plotted in
 14 Fig. 3(c) as a function of the sampling day (local time, UT +9:00), together with the $\delta^{15}\text{N}$ and
 15 $\delta^{18}\text{O}$ of $\text{NO}_3^-_{\text{atm}}$ in Figs. 3(a) and 3(b). The atmospheric nitrate at Sado-seki monitoring
 16 station showed large ^{17}O anomalies with $\Delta^{17}\text{O}$ values from +18.6‰ to +32.4‰.

17 Moreover, a clear normal correlation with $\delta^{18}\text{O}$ was shown (Supplement Fig. S1). A similar
 18 trend has been reported in atmospheric nitrate aerosols collected for a one-year period in La
 19 Jolla, California (32.7°N, 117.2°W) (Michalski et al. 2003), and in other areas worldwide
 20 (Kaiser et al. 2007; Morin et al. 2009). Michalski et al. (2003) interpreted that the linear
 21 correlation corresponds to the mixing line between the tropospheric ozone and tropospheric
 22 H_2O , and thus tropospheric OH radicals, with $\Delta^{17}\text{O} = 0\text{‰}$ and $\delta^{18}\text{O} = -5\text{‰}$. The $\text{NO}_3^-_{\text{atm}}$ data
 23 obtained at Sado-seki monitoring station, however, showed a somewhat different trend in the
 24 $\Delta^{17}\text{O}$ – $\delta^{18}\text{O}$ plot between summer, from May to October, and winter, from November to April
 25 (Supplement Fig. S1). Although the linearly fitted line to the summer data showed a slope of
 26 2.21 ± 0.22 and an intercept of $+19.7 \pm 5.1\text{‰}$ in the $\Delta^{17}\text{O}$ – $\delta^{18}\text{O}$ plot, that to the winter data
 27 showed a statistically significant larger slope of 2.89 ± 0.38 and the smaller intercept of $+3.0$
 28 $\pm 9.2\text{‰}$; all errors were in the 2σ range. Although the winter data included an intercept of



1 -5% as the end member $\delta^{18}\text{O}$ value of the tropospheric OH radical within the possible error
 2 range, as reported by Michalski et al. (2003), the intercept of summer data deviated strongly
 3 from the value. Because the monitoring station is located in the Asian monsoon area, the
 4 major air mass that arrived at the station was seasonally different; Pacific air originated from
 5 the south-eastern direction in summer whereas continental air originated from north-western
 6 direction in winter. The present results imply seasonal and regional changes in the $\delta^{18}\text{O}/\Delta^{17}\text{O}$
 7 ratios of tropospheric ozone and in the OH radical.

8 On the basis of both the temporal variation in the depositional flux of NO_3^- and the $\Delta^{17}\text{O}$
 9 value, we estimated the average $\Delta^{17}\text{O}$ value of NO_3^- ($\Delta^{17}\text{O}_{\text{avg}}$) deposited at Sado-seki
 10 monitoring station as $+25.5\%$ for FY2009, $+27.2\%$ for FY2010, $+25.7\%$ for FY2011, and
 11 $+26.3\%$ for the three years by using

$$12 \quad \Delta^{17}\text{O}_{\text{avg}} = \frac{\sum_k (C_k \cdot V_k \cdot \Delta^{17}\text{O}_k)}{\sum_k (C_k \cdot V_k)}, \quad (8)$$

13 where C_k denotes the concentration of nitrate in each wet deposition sample, and V_k denotes
 14 the total water volume of each wet deposition sample. Substituting $\Delta^{17}\text{O}$ with $\delta^{15}\text{N}$ ($\delta^{18}\text{O}$) in
 15 Eq. (8), we estimated $\delta^{15}\text{N}_{\text{avg}}$ ($\delta^{18}\text{O}_{\text{avg}}$) as -4.4 ‰ ($+78.5\%$) for FY2009, -3.8 ‰ ($+81.8\%$)
 16 for FY2010, -4.4 ‰ ($+78.6\%$) for FY2011, and -4.2% ($+79.8\%$) for the three years.

17 To apply the $\Delta^{17}\text{O}_{\text{avg}}$ values obtained at Sado-seki monitoring station as for $\Delta^{17}\text{O}$ of NO_3^-
 18 deposited into the studied watershed (i.e. $\Delta^{17}\text{O}_{\text{atm}}$ in Eq. (2)), however, additional corrections
 19 could be needed because the $\Delta^{17}\text{O}$ value of NO_3^- is a function of the NO_x oxidation
 20 channels in the atmosphere that shift depending on the intensity of sunlight, temperature, and
 21 oxidant levels (e.g. Alexander et al. 2009; Kunasek et al. 2008; Michalski et al. 2003; Morin
 22 et al. 2012; Morin et al. 2008; Savarino et al. 2013). The latitudinal difference between Sado-
 23 seki monitoring station ($38^\circ 15' \text{N}$, $138^\circ 24' \text{E}$; Fig. 2) and the watershed studied ($35^\circ 15' \text{N}$,
 24 $136^\circ 5' \text{E}$; Fig. 2) could change the intensity of sunlight and thus the NO_x oxidation channel.
 25 Moreover, Tsunogai et al. (2010) reported that nitrate in polluted air masses derived directly
 26 from megacities in winter showed slightly larger $\Delta^{17}\text{O}$ values than those in the same seasons
 27 owing likely to the relative increase in the reaction via NO_3 radicals within the entire NO_3^-
 28 production channel to produce NO_3^- in the polluted air mass. The annual average $\Delta^{17}\text{O}$
 29 values determined in this study was the lowest value in FY2009 when the deposition rate was
 30 the smallest, at $19.3 \text{ mmol m}^{-2} \text{ y}^{-1}$ (EANET 2014), whereas the annual average $\Delta^{17}\text{O}$ value



1 was the highest value in FY2010 when the deposition rate was the largest, at 28.0 mmol
2 m⁻²y⁻¹ for nitrate, within the three years of observation. These results also imply that we must
3 correct for the difference in arrival frequency of polluted air mass as well.

4 Nevertheless, both the annual average and the seasonal variation range of $\Delta^{17}\text{O}$ correlated
5 strongly with those determined at Rishiri monitoring station (45°07'11"N, 141°12'33"E) in
6 FY2008, at +26.2‰ (Tsunogai et al. 2010), where the wet deposition rate of $\text{NO}_3^-_{\text{atm}}$ was an
7 average 40% smaller than that at Sado-seki monitoring station from 2000 to 2013 (EANET 2014).
8 Moreover, the values also coincided with those reported for mid-latitudes, such as at La Jolla,
9 at 33°N (Michalski et al. 2003) and Princeton, at 40°N (Kaiser et al. 2007). We concluded that
10 by allowing an appropriate range of errors presented later, the obtained $\Delta^{17}\text{O}_{\text{avg}}$ value of
11 $\text{NO}_3^-_{\text{atm}}$ can represent those deposited at middle latitudes worldwide, including the Lake Biwa
12 watershed basin.

13 In addition, the actual $\Delta^{17}\text{O}_{\text{atm}}$ values of $\text{NO}_3^-_{\text{atm}}$ in each stream water sample can differ from
14 the $\Delta^{17}\text{O}_{\text{avg}}$ owing to the seasonal variation in the $\Delta^{17}\text{O}$ values of $\text{NO}_3^-_{\text{atm}}$. In correcting for the
15 seasonal variation, however, it is not adequate to use the $\Delta^{17}\text{O}$ values determined for the
16 seasons of sampling, as $\Delta^{17}\text{O}_{\text{atm}}$ in Eq. (2), because the duration of the recession period is
17 longer than a few months for most of the forest catchments in Japan with a humid temperate
18 climate (Ohte et al. 2010; Takimoto et al. 1994). That is, nitrate in base flow stream water had
19 been stored in subsurface runoff and groundwater, of which seasonal $\Delta^{17}\text{O}$ changes have not
20 been found thus far (Nakagawa et al. 2013; Tsunogai et al. 2010).

21 In summary, we used the obtained $\Delta^{17}\text{O}_{\text{avg}}$ value of $\text{NO}_3^-_{\text{atm}}$ as $\Delta^{17}\text{O}_{\text{atm}}$ in Eq. (2) to estimate
22 C_{atm} in the streams of the Lake Biwa watershed basin by allowing the error range of 3.0‰,
23 considering the whole factor change of $\Delta^{17}\text{O}_{\text{atm}}$ from $\Delta^{17}\text{O}_{\text{avg}}$. About 65% of the all $\Delta^{17}\text{O}$ data
24 of $\text{NO}_3^-_{\text{atm}}$ obtained at Sado-seki monitoring station were included in this range of +26.3 ±
25 3.0‰.

26 In the case of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, the values of $\text{NO}_3^-_{\text{atm}}$ in each stream water sample (i.e. $\delta^{15}\text{N}_{\text{atm}}$
27 and $\delta^{18}\text{O}_{\text{atm}}$ in Eqs. (6) and (7)) differed further from $\delta^{15}\text{N}_{\text{avg}}$ and $\delta^{18}\text{O}_{\text{avg}}$ owing to isotopic
28 fractionation during partial removal subsequent to deposition. As a result, while using the
29 $\delta^{15}\text{N}_{\text{avg}}$ and $\delta^{18}\text{O}_{\text{avg}}$ values as $\delta^{15}\text{N}_{\text{atm}}$ and $\delta^{18}\text{O}_{\text{atm}}$, we assumed much larger error range on the
30 values; i.e. ± 10‰ for both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. We will further discuss the appropriateness of
31 these error ranges section 3.3. Because of the small $C_{\text{atm}}/C_{\text{total}}$ ratios of stream water at



1 generally less than 7‰ (section 3.2), the error propagated to $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ was generally
2 small, less than 1‰ and 2‰, respectively, for most of the data presented in this study.

3 **3.2 Stream nitrate overview**

4 The concentrations (C_{total}) and $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of nitrate in the stream water
5 samples determined for each observation ($n = 1, 2, 3,$ and 4) are presented in Fig. 4. The
6 annual average concentration ($\overline{C_{\text{total}}}$) and annual average $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values ($\overline{\delta^{15}\text{N}}$,
7 $\overline{\delta^{18}\text{O}}$, and $\overline{\Delta^{17}\text{O}}$, respectively) in each stream estimated by using Eqs. (3), (4), and (5) are
8 shown in the figure as black bars. In this figure, each stream was plotted on the x-axis in the
9 order of location beginning from stream No. 31, which lies southwest of all of the streams
10 (Fig. 2), and proceeding in a clockwise direction. The spatially continuous variation in the
11 values of $\overline{\delta^{15}\text{N}}$, $\overline{\delta^{18}\text{O}}$, and $\overline{\Delta^{17}\text{O}}$ imply that the values may represent land use changes in each
12 catchment area.

13 Although the $\Delta^{17}\text{O}$ values presented significant areal and temporal variation from +0.0‰ to
14 +6.8‰, the range of the $\overline{\Delta^{17}\text{O}}$ values from +0.5‰ to +3.1‰ was typical for nitrate in natural
15 stream water (Liu et al. 2013; Michalski et al. 2004; Rose et al. 2015; Tsunogai et al. 2010;
16 Tsunogai et al. 2014). These results correspond to the mixing ratios of unprocessed $\text{NO}_3^-_{\text{atm}}$
17 to total nitrate from $1.8 \pm 0.3\%$ to $11.8 \pm 1.3\%$, obtained by using Eq. (2).

18 By using the concentration (C_{total}) and $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of nitrate, the $\text{NO}_3^-_{\text{atm}}$
19 concentration (C_{atm}) and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the remineralized portion of nitrate
20 ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$) in the samples were calculated by using Eqs. (2), (6), and (7) and are
21 plotted in Fig. 5. In addition, the annual average concentration of $\text{NO}_3^-_{\text{atm}}$ ($\overline{C_{\text{atm}}}$) and annual
22 average values of $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$ ($\overline{\delta^{15}\text{N}_{\text{re}}}$ and $\overline{\delta^{18}\text{O}_{\text{re}}}$, respectively) in stream nitrate were
23 calculated and are presented in Fig. 5 as black bars. Because of the large $\delta^{18}\text{O}$ differences of
24 about 80‰ between nitrate in streams and $\text{NO}_3^-_{\text{atm}}$, the $\delta^{18}\text{O}_{\text{re}}$ values were a few ‰ lower
25 than each original $\delta^{18}\text{O}$ value in total nitrate. On the contrary, owing to the small $\delta^{15}\text{N}$
26 differences of less than 15‰ between the total nitrate in streams and $\text{NO}_3^-_{\text{atm}}$, as well as the
27 small $C_{\text{atm}}/C_{\text{total}}$ ratios in the streams, most of the $\delta^{15}\text{N}_{\text{re}}$ values showed small deviations of
28 less than 1‰ from each original $\delta^{15}\text{N}$ value in the total nitrate in most of the streams.

29 To verify possible secular changes, the estimated $\overline{C_{\text{total}}}$, $\overline{\delta^{15}\text{N}}$, and $\overline{\delta^{18}\text{O}}$ for each stream were
30 compared with those determined by Ohte et al. (2010) in which annual average concentration



1 and annual average $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate (total) were determined for the same
2 streams in 2004 to 2006 (Supplement Fig. S2). Although both concentrations and $\delta^{15}\text{N}$ and
3 $\delta^{18}\text{O}$ values in the streams showed significant areal and temporal variations during 2013, as
4 presented in Fig. 4, the annual average values almost correlated with the values determined in
5 2004 to 2006. We concluded that secular changes were minimal for nitrate in the streams, at
6 least for the most recent 10-year period of observations.

7 3.3 Relationship between $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$

8 One of the features in the areal variation shown in Fig. 4 is the positive correlation between
9 $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$. As clearly presented in the relationship between $\overline{\Delta^{17}\text{O}}$ and $\overline{\delta^{18}\text{O}}$ (Fig. 6), these
10 values showed linear correlation with the r^2 value of 0.88. Because $\text{NO}_3^-_{\text{atm}}$ is characterized
11 by highly elevated values of both $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ (Fig. 3), changes in the mixing ratio of
12 unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool must be strongly responsible for the positive
13 correlation between $\overline{\Delta^{17}\text{O}}$ and $\overline{\delta^{18}\text{O}}$ for nitrate in the streams.

14 The slope value of the least-squares-fitted line between $\overline{\Delta^{17}\text{O}}$ and $\overline{\delta^{18}\text{O}}$ (Fig. 6) also supports
15 this hypothesis. By extrapolating the least-square-fitted line to the region of $\text{NO}_3^-_{\text{atm}}$ having a
16 $\Delta^{17}\text{O}$ value of +26.3‰, we obtained $\delta^{18}\text{O} = +86 \pm 7\%$, which also corresponds with the
17 average $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{atm}}$ of +79.8‰ obtained in section 3.1. We concluded that $\overline{\delta^{18}\text{O}}$
18 values primarily reflect the mixing ratio of $\text{NO}_3^-_{\text{atm}}$ within nitrate as well.

19 By extrapolating the linear correlation between $\overline{\Delta^{17}\text{O}}$ and $\overline{\delta^{18}\text{O}}$ to $\overline{\Delta^{17}\text{O}} = 0\%$, we obtained the
20 $\delta^{18}\text{O}$ value of $-2.9 \pm 1.2\%$ as the average $\delta^{18}\text{O}$ value of the remineralized portion of nitrate
21 ($\text{NO}_3^-_{\text{re}}$) in the streams. Although the $\delta^{18}\text{O}$ value was substantially ^{18}O -depleted compared
22 with that obtained through in vitro incubation experiments in past studies (Burns and Kendall
23 2002; Mayer et al. 2001; Spoelstra et al. 2007), it correlated strongly with the $\delta^{18}\text{O}$ value of
24 $\text{NO}_3^-_{\text{re}}$ determined recently by using the linear relationship between $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$. This
25 correlation occurred in the groundwater of cool-temperate forested watersheds at $-4.2 \pm 2.4\%$,
26 where the $\delta^{18}\text{O}(\text{H}_2\text{O})$ was around -13% (Tsunogai et al. 2010) and in stream water in a cool-
27 temperate forested watershed at $-3.6 \pm 0.7\%$, where the $\delta^{18}\text{O}(\text{H}_2\text{O})$ was around -11%
28 (Tsunogai et al. 2014). Moreover, the $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{re}}$ obtained in this study, $-2.9 \pm$
29 1.2% , is close to the possible lowermost $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{re}}$ produced through nitrification
30 under H_2O of $-7.8 \pm 1.0\%$ (Buchwald et al. 2012). Furthermore, the $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{re}}$



1 correlates strongly with that obtained through in vitro incubation experiments in recent studies
2 that simulated temperate forest soils (Fang et al. 2012). We concluded that the $\delta^{18}\text{O}$ value of
3 $\text{NO}_3^-_{\text{re}}$ produced through nitrification in the temperate watershed having $\delta^{18}\text{O}(\text{H}_2\text{O})$ values of
4 $-7.8 \pm 1.0\text{‰}$ was $-2.9 \pm 1.2\text{‰}$ and that we should use such a low $\delta^{18}\text{O}$ value as for that
5 produced through nitrification in the watershed. Understanding the relationship between $\Delta^{17}\text{O}$
6 and $\delta^{18}\text{O}$ of nitrate shown in Fig. 6 is highly useful for determining the $\delta^{18}\text{O}$ value of $\text{NO}_3^-_{\text{re}}$
7 in each watershed (Tsunogai et al. 2010).

8 Although the $\Delta^{17}\text{O}$ values of nitrate were stable during the biogeochemical processing such as
9 partial removal through assimilation or denitrification, the $\delta^{18}\text{O}$ values of nitrate could vary
10 through the isotopic fractionation processes within each catchment area. Nevertheless, the
11 $\overline{\delta^{18}\text{O}}$ values of nitrate in the streams were plotted on the mixing line between the $\text{NO}_3^-_{\text{atm}}$ that
12 had been deposited into the watershed and $\text{NO}_3^-_{\text{re}}$ having $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values close to those
13 produced through nitrification in the catchments. We concluded that the range of isotopic
14 fractionations owing to partial removal through assimilation or denitrification subsequent to
15 deposition of $\text{NO}_3^-_{\text{atm}}$ or production of $\text{NO}_3^-_{\text{re}}$ within each catchment area was generally small
16 for the major portion of nitrate eluted from the watershed. This result also supports our
17 assumption in section 3.1 such that the actual $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{NO}_3^-_{\text{atm}}$ in each stream
18 water sample ($\delta^{15}\text{N}_{\text{atm}}$ and $\delta^{18}\text{O}_{\text{atm}}$ in Eqs. (6) and (7)) correlate with the $\delta^{15}\text{N}_{\text{avg}}$ and $\delta^{18}\text{O}_{\text{avg}}$
19 estimated at Sado-seki monitoring station within an error of $\pm 10\text{‰}$.

20 **3.4 $\delta^{15}\text{N}$ values of remineralized nitrate in streams**

21 To trace the source of the ^{18}O -depleted $\text{NO}_3^-_{\text{re}}$ eluted from the watershed into the lake, the
22 annual average $\delta^{15}\text{N}$ values of the remineralized portion of nitrate ($\overline{\delta^{15}\text{N}_{\text{re}}}$) in each inflow
23 stream were estimated and are plotted as a function of population density in Fig. 7(c), together
24 with $\overline{\delta^{18}\text{O}_{\text{re}}}$ and $\overline{\delta^{18}\text{O}}$ in Fig. 7(d). Although the annual average $\overline{\delta^{18}\text{O}_{\text{re}}}$ values were low and
25 almost uniform from -4.0‰ to -0.1‰ , as implied in the linear correlation between $\overline{\Delta^{17}\text{O}}$ and
26 $\overline{\delta^{18}\text{O}}$ in Fig. 6, $\overline{\delta^{15}\text{N}_{\text{re}}}$ showed larger variation from $+1.7\text{‰}$ to $+10.9\text{‰}$. Moreover, $\overline{\delta^{15}\text{N}_{\text{re}}}$
27 showed positive correlation with the population density (Fig. 7). A similar trend was reported
28 for the $\delta^{15}\text{N}$ values of total nitrate in past studies in this watershed (Ohte et al. 2010) and
29 others (Mayer et al. 2002). We further verified that the remineralized portion of nitrate
30 ($\text{NO}_3^-_{\text{re}}$) was responsible for the positive correlation between the $\delta^{15}\text{N}$ values of total nitrate
31 and population density.



1 Both the concentrations and the isotopic compositions shown in Fig. 7 clearly demonstrate
2 that most portions of the nitrate eluted from the catchments with lower population densities of
3 less than 100 km^{-2} were produced through nitrification in naturally occurring soil organic
4 matter (Kendall et al. 1995; Ohte et al. 2010), showing $\delta^{15}\text{N}$ values of $+4.4 \pm 1.8\text{‰}$ and $\delta^{18}\text{O}$
5 values of about $-2.3 \pm 0.9\text{‰}$. In this section, we discuss the source of the ^{15}N -enriched $\text{NO}_3^-_{\text{re}}$
6 eluted from the catchments with higher population densities of more than 1000 km^{-2} , showing
7 $\delta^{15}\text{N}$ values of $+9.2 \pm 1.3\text{‰}$ or more and $\delta^{18}\text{O}$ values of about $-2.2 \pm 1.1\text{‰}$.

8 Denitrification in riverbed sediments adjacent to riparian zones or groundwater bodies
9 (McMahon and Böhlke 1996) can increase the $\delta^{15}\text{N}$ value of stream nitrate. However, if such
10 post-production alteration were responsible for the ^{15}N enrichment of $\text{NO}_3^-_{\text{re}}$ and thus the
11 total nitrate, the values of $\delta^{18}\text{O}_{\text{re}}$ in addition to those of $\delta^{15}\text{N}_{\text{re}}$ should increase (Granger et al.
12 2008). Moreover, the absolute concentration of $\text{NO}_3^-_{\text{atm}}$ (C_{atm}) should decrease in accordance
13 with the progress of denitrification. The low and uniform $\delta^{18}\text{O}_{\text{re}}$ values (Fig. 7(d)) as well as
14 the uniform C_{atm} irrespective of the population densities (Fig. 7(b)) imply that the
15 denitrification in riverbed sediments was minor for nitrate in the streams. Rather, the $\text{NO}_3^-_{\text{re}}$
16 must be enriched in ^{15}N from its initial production through nitrification within the catchments
17 with high population densities. In addition, the small differences in $\delta^{18}\text{O}$ values of $\text{NO}_3^-_{\text{re}}$
18 between those values irrespective of the population densities in the catchment area (Fig. 7)
19 imply that the essential parameters for determining the $\delta^{18}\text{O}$ values of nitrate during
20 nitrification, such as the $\delta^{18}\text{O}$ values of H_2O and pH of soils (Buchwald et al. 2012; Fang et al.
21 2012), should be similar between them.

22 Based on the $\delta^{15}\text{N}$ values of total nitrate eluted from catchments with high population
23 densities, as well as the positive correlation between the $\delta^{15}\text{N}$ values of total nitrate and
24 population densities, Ohte et al. (2010) proposed sewage effluent as the dominant source
25 contributing to the increase in the $\delta^{15}\text{N}$ values of total nitrate eluted from such catchments.
26 The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{NO}_3^-_{\text{re}}$ newly estimated in this study, $+9.2 \pm 1.3\text{‰}$ or more and
27 $-2.2 \pm 1.1\text{‰}$, respectively, also imply that the dominant source contributing to the increase in
28 the $\delta^{15}\text{N}$ values of total nitrate had been produced through nitrification in which the source N
29 of the nitrate had already been enriched in ^{15}N . Although the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{NO}_3^-_{\text{re}}$
30 eluted from the high population density catchments, $\delta^{15}\text{N}_{\text{re}} = +9.2 \pm 1.3\text{‰}$ and $\delta^{18}\text{O}_{\text{re}} = -2.2 \pm$
31 1.1‰ , were a few ‰ lower than the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of total nitrate in the sewage
32 effluent determined in past studies (Aravena et al. 1993; Wankel et al. 2006; Widory et al.



1 2005; Xue et al. 2009), the slight deviations in the reported $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values from our
2 results can be explained by the following factors: (1) a slight contribution of $\text{NO}_3^-_{\text{atm}}$, and (2)
3 the progress of denitrification subsequent to production. We concluded that sewage effluent
4 was the most probable pollution source of nitrate to explain the observed concentrations and
5 isotopic compositions of nitrate eluted from the catchments with high population densities,
6 particularly for those more than 1000 km^{-2} .

7 3.5 Seasonal variation

8 Although the annual average values of $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ in each river, $\overline{\Delta^{17}\text{O}}$ and $\overline{\delta^{18}\text{O}}$,
9 respectively, showed linear correlation as presented in Fig. 6, the same results were not
10 always attained for those in each season. Particularly for those obtained during June and
11 August (i.e. summer), some of the streams showed significant deviations in $\delta^{18}\text{O}$ of more than
12 5‰ from the hypothetical mixing line between $\text{NO}_3^-_{\text{atm}}$ ($\Delta^{17}\text{O} = +26.3 \text{ ‰}$ and $\delta^{18}\text{O} = 79.8 \text{ ‰}$)
13 and $\text{NO}_3^-_{\text{re}}$ ($\Delta^{17}\text{O} = 0 \text{ ‰}$ and $\delta^{18}\text{O} = -2.9 \text{ ‰}$; Fig. 6). Even though the values of $\Delta^{17}\text{O}$ and
14 $\delta^{18}\text{O}$ of $\text{NO}_3^-_{\text{atm}}$ showed seasonal variation, as presented in Fig. 3, it could not explain the
15 large deviations from the mixing line based on the seasonal changes in $\text{NO}_3^-_{\text{atm}}$. Rather, we
16 must assume some seasonal changes in the biogeochemical nitrogen cycles within each
17 catchment area to explain the relationship because different from that of the $\Delta^{17}\text{O}$ values, the
18 $\delta^{18}\text{O}$ values of nitrate can vary during biogeochemical processing within each catchment area.
19 As a result, we can evaluate the seasonal changes in the biogeochemical processing within
20 each catchment area by using the seasonal changes in the relationship between $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$
21 shown in Fig. 8.

22 The increases in the number of data plotted on the highly ^{18}O -enriched region of more than a
23 few ‰ in $\delta^{18}\text{O}$ from the lines imply that partial nitrate removal through assimilation or
24 denitrification was active within each catchment area in June and August. The areal
25 differences in the ^{18}O enrichment also support this hypothesis. As presented in Fig. 8, ^{18}O
26 enrichments were common in samples obtained at the southern streams having high
27 population densities in each catchment area, as shown in the figure by white squares. We can
28 anticipate elevated loading of both nutrients and organic matter of anthropogenic origin in
29 these catchments, both of which naturally enhance both assimilation and denitrification.

30 On the contrary, most samples obtained during March and October were distributed on the
31 hypothetical mixing line between $\text{NO}_3^-_{\text{atm}}$ and $\text{NO}_3^-_{\text{re}}$ as presented in Fig. 8. We concluded



1 that in winter, the range of isotopic fractionation subsequent to production, such as partial
2 removal through assimilation or denitrification, was generally small for the major portion of
3 nitrate eluted from the watershed and fed into the lake. Therefore, the annual average values
4 (i.e. $\overline{\delta^{18}\text{O}}$ and $\overline{\Delta^{17}\text{O}}$) of streams were distributed on the hypothetical mixing line, as shown in
5 Fig. 6, because the nitrate influx in winter occupied a major portion of the annual nitrate
6 influx. Active removal of nitrate from the streams through denitrification/assimilation in
7 summer was also responsible for the small relative importance of nitrate influx into the lake in
8 summer. In conclusion, the relationship between $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate eluted from a
9 catchment area is a useful indicator for evaluating the biogeochemical processing within the
10 catchment area, including the seasonal change.

11 3.6 Areal and temporal $\Delta^{17}\text{O}$ variation

12 By using the $\delta^{18}\text{O}$ values of nitrate as tracers, Ohte et al. (2010) found that the mixing ratios
13 of unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool were high in the northern streams of the
14 watershed in winter, from November to late April. Our present results further verified the past
15 results shown in Fig. 4, which adds more robust evidence through the use of the $\Delta^{17}\text{O}$ tracer
16 for $\text{NO}_3^-_{\text{atm}}$.

17 Based on the high accumulation rate of snow in the catchment zones of the northern streams,
18 Ohte et al. (2010) concluded that high loading of unprocessed $\text{NO}_3^-_{\text{atm}}$ via snow in the
19 catchment zones increased the stored unprocessed $\text{NO}_3^-_{\text{atm}}$ in the snowpack, which was
20 subsequently released into the streams during the melting seasons. This process enhanced the
21 mixing ratio of unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool during the melting season,
22 which was also reported for streams worldwide (Kendall et al. 1995; Ohte et al. 2004; Ohte et
23 al. 2010; Pellerin et al. 2012; Piatek et al. 2005; Tsunogai et al. 2014). However, the
24 contribution of nitrate from anthropogenic sources could be smaller in this area owing to
25 lower population densities in the catchments (Table 1). Because a major portion of possible
26 anthropogenic nitrate in the catchments must be occupied by $\text{NO}_3^-_{\text{re}}$ (Ohte et al. 2010), a
27 lower $\text{NO}_3^-_{\text{re}}$ supply from anthropogenic sources in each catchment area could elevate the
28 mixing ratio of unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool even if absolute
29 concentration of $\text{NO}_3^-_{\text{atm}}$ (C_{atm}) was uniform in the streams.

30 To determine the C_{atm} variability among the streams, the C_{atm} values estimated in this study
31 were plotted as a function of population densities in Fig. 7(b). The C_{atm} was almost uniform at



1 $2.3 \pm 1.1 \mu\text{mol L}^{-1}$ irrespective of changes in the population density of each catchment area.
2 However, a clear C_{total} enrichment trend was noted in accordance with the increase in
3 population densities of the catchments (Fig. 7(a)). Similar C_{total} enrichment trends have been
4 reported in previous studies (Ohte et al. 2010).

5 The northern streams such as Nos. 3, 4, and 5 were enriched in C_{atm} , showing C_{atm} annual
6 average values of 5.3 ± 0.9 , 2.9 ± 0.6 , and $4.3 \pm 0.8 \mu\text{mol L}^{-1}$, respectively, and $\Delta^{17}\text{O}$ values
7 of +3.1‰, +1.9‰, and +2.9‰, respectively. These results support the previous observation of
8 the streams determined by using the $\delta^{18}\text{O}$ tracer. Similar C_{atm} enrichment of about $3 \mu\text{mol L}^{-1}$
9 or more, however, was also found in streams in other areas, such as Nos. 14 ($C_{\text{atm}} = 3.3 \pm 0.9$
10 $\mu\text{mol L}^{-1}$), 25 ($3.2 \pm 0.7 \mu\text{mol L}^{-1}$), and 21 ($4.2 \pm 0.8 \mu\text{mol L}^{-1}$), while showing lower $\Delta^{17}\text{O}$
11 values of +0.9‰, +1.5‰, and +2.0‰, respectively, thus showing low mixing ratios of
12 unprocessed $\text{NO}_3^-_{\text{atm}}$ within total nitrate. We concluded that the difference in the addition of
13 anthropogenic nitrate composed of $\text{NO}_3^-_{\text{re}}$ in the catchments was primarily responsible for the
14 difference in the mixing ratio of unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool, as well as
15 C_{total} variation in accordance with the population densities of the catchment area as shown in
16 Fig. 1. That is, a small contribution of anthropogenic nitrate in the catchments in the northern
17 rivers was primarily responsible for the low C_{total} and thus the high mixing ratio of
18 unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate pool, or $C_{\text{atm}}/C_{\text{total}}$ ratios, in the northern streams
19 of the watershed.

20 Although the difference in the accumulation rate of snow between each catchment zone was
21 not the major factor controlling the $C_{\text{atm}}/C_{\text{total}}$ ratios, the concentrated release of $\text{NO}_3^-_{\text{atm}}$
22 stored in the snowpack during the melting seasons should be one of the important factors in
23 determining the C_{atm} variation among the streams. Most of the C_{atm} -enriched streams, such as
24 Nos. 3, 4, 5, 14, and 25, originated from forested catchment in high elevations of more than
25 800 m above sea level; thus, we can anticipate heavy snowpack in winter of each headwater.
26 Moreover, the maximum C_{atm} values in these streams were found in March, which is the
27 season of snowmelt (Fig. 5). On the contrary, most of the C_{atm} -depleted streams such as Nos.
28 29 ($0.9 \pm 0.3 \mu\text{mol L}^{-1}$), 19 ($0.8 \pm 0.2 \mu\text{mol L}^{-1}$), 23 ($0.5 \pm 0.2 \mu\text{mol L}^{-1}$), and 30 (0.6 ± 0.2
29 $\mu\text{mol L}^{-1}$) originated from low elevations having urban and sub-urban catchment areas (Table
30 1). As a result, the concentrated release of stored $\text{NO}_3^-_{\text{atm}}$ in the snowpack to the forest floor
31 in the catchment zone during the melting seasons is strongly responsible for the C_{atm}



1 enrichment for some of the streams, particularly that in the streams during March as presented
2 in Fig. 1.

3 The only exception is stream No. 21, located in the southernmost part of the watershed, which
4 showed a high annual average C_{atm} of $4.2 \pm 0.8 \mu\text{mol L}^{-1}$. This small stream originated from a
5 low elevation of about 200 m having a small catchment area of 4 km^2 . In addition, although
6 the other C_{atm} -enriched streams showed the maximum C_{atm} in March, that in No. 21 was
7 highest in August, showing extraordinarily high C_{atm} of more than $10 \mu\text{mol L}^{-1}$. It is unlikely
8 that the $\text{NO}_3^-_{\text{atm}}$ stored in the snowpack in winter was the major source of $\text{NO}_3^-_{\text{atm}}$ in this
9 stream.

10 The catchment zone of stream No. 21 showed the highest population density within the
11 catchments of the streams studied (Table 1). About one-third of the catchment includes
12 residential areas. Artificial drainage systems in urban or residential areas and agricultural
13 lands in humid temperate regions are usually designed to drain rainwater efficiently into
14 streams (Takimoto et al. 1994). As a result, a significant portion of $\text{NO}_3^-_{\text{atm}}$ deposited into the
15 catchment area was deposited onto paved surfaces and was then drained directly into the
16 stream via storm sewers without penetrating the ground. Thus, no interaction occurred with
17 soils, as presented in Fig. 1. Because biogeochemical interactions within soils are the major
18 sink for $\text{NO}_3^-_{\text{atm}}$ and thus for ^{17}O anomalies of nitrate (Nakagawa et al. 2013; Tsunogai et al.
19 2014), the development of such sewage systems in urban/suburban areas is strongly
20 responsible for the high C_{atm} in stream No. 21. Similar bypassing effects of $\text{NO}_3^-_{\text{atm}}$ from soil
21 contacts by paved surfaces have been suggested in urban/suburban watersheds by using $\delta^{18}\text{O}$
22 values of nitrate as tracers (Burns et al. 2009; Kaushal et al. 2011). We further verified that
23 the sewage systems in urban/suburban catchments changed the fate of the $\text{NO}_3^-_{\text{atm}}$ deposited
24 onto land to some extent.

25 The observed uniform C_{atm} irrespective of population densities and headwater elevations
26 shown in Fig. 7 implies that the influences of snow packs and paved surfaces were still minor
27 in determining the C_{atm} values in the streams. Rather, the observed stable C_{atm} implies that
28 most of $\text{NO}_3^-_{\text{atm}}$ in the streams had been stored in groundwater/subsurface runoff in the
29 watershed having similar C_{atm} concentrations and then gushed to the surface at respective
30 headwater zones of various elevations and various land uses as presented in Fig. 1.

31 When using the $\delta^{18}\text{O}$ tracer, it was difficult to determine the precise absolute concentration of
32 $\text{NO}_3^-_{\text{atm}}$ (C_{atm}) in each stream water as presented in this study and to determine whether the



1 absolute concentration of $\text{NO}_3^-_{\text{atm}}$ was stable among the streams. By using the $\Delta^{17}\text{O}$ values,
2 however, we can determine the precise C_{atm} in each stream for each season; thus, we can
3 clarify the fate of $\text{NO}_3^-_{\text{atm}}$.

4 3.7 Differences in outflows from inflows

5 The concentrations and $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of nitrate in the outflow (Seta River; No.
6 33) are also presented in Fig. 4. In a manner similar to the inflow streams (i.e. by using Eqs.
7 (3) to (5)), we estimated the annual average concentration of total nitrate in the outflow river (
8 \bar{C}_{total}) to be $13.3 \mu\text{mol L}^{-1}$, the annual average $\delta^{15}\text{N}$ values ($\overline{\delta^{15}\text{N}}$) to be $+13.1\text{‰}$, the annual
9 average $\delta^{18}\text{O}$ values ($\overline{\delta^{18}\text{O}}$) to be $+1.5\text{‰}$, and the annual average $\Delta^{17}\text{O}$ values ($\overline{\Delta^{17}\text{O}}$) to be
10 $+0.9\text{‰}$, as presented in Fig. 4. Moreover, in a manner similar to that used for the inflow
11 streams (i.e. by using Eqs. (2), (6), and (7)), we estimated the annual average concentration of
12 $\text{NO}_3^-_{\text{atm}}$ in the outflow river (\bar{C}_{atm}) to be $0.4 \pm 0.1 \mu\text{mol L}^{-1}$, the annual average $\delta^{15}\text{N}_{\text{re}}$ values (
13 $\overline{\delta^{15}\text{N}_{\text{re}}}$) to be $+13.7 \pm 0.2\text{‰}$, and the annual average $\delta^{18}\text{O}_{\text{re}}$ values ($\overline{\delta^{18}\text{O}_{\text{re}}}$) to be $-1.2 \pm 0.6\text{‰}$,
14 as presented in Fig. 5. Similar to that for inflows, the $\Delta^{17}\text{O}$ values were typical for nitrate in
15 the natural stream waters. The striking features of the outflow in comparison with the inflows
16 were the depletions of both C_{total} and C_{atm} as well as the ^{15}N enrichment in the outflow
17 compared with that in the inflow (Figs. 4 and 5). Because the denitrification/assimilation
18 processes remove both nitrate and $\text{NO}_3^-_{\text{atm}}$ and preferentially consumes ^{14}N during the
19 removal, the progress of denitrification/assimilation in the lake water column can be strongly
20 responsible for the removal of both nitrate and $\text{NO}_3^-_{\text{atm}}$, as well as the ^{15}N enrichment of
21 nitrate in the outflow compared with the inflow. If this were the case in Lake Biwa, the total
22 nitrate efflux must have been smaller than the total nitrate influx. To quantitatively verify this
23 hypothesis and to evaluate the influences of the stagnated flow into the lake on nitrate, we
24 estimated the total influx through all of the inflow streams for nitrate and $\text{NO}_3^-_{\text{atm}}$, $\Delta\text{N}_{\text{in}}$, and
25 $\Delta\text{A}_{\text{in}}$, respectively, and the total efflux for nitrate and $\text{NO}_3^-_{\text{atm}}$, $\Delta\text{N}_{\text{out}}$, and $\Delta\text{A}_{\text{out}}$, respectively,
26 as well as the flow-weighted average $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of all inflows and outflows,
27 to discuss their changes in the lake.

28 The $\Delta\text{N}_{\text{in}}$ and $\Delta\text{A}_{\text{in}}$ in each interval between the observation n and the next observation $n + 1$
29 (i.e. each season) and the flow-weighted average $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values of the inflows
30 ($\delta(n)$) during each interval between the observation n and the next observation $n + 1$ were
31 determined by using the following equations:



$$1 \quad \alpha = \frac{Q_{in}}{\sum_i q_i}, \quad (9)$$

$$2 \quad \Delta N_{in}(n) = \sum_i C_i \cdot f_i \cdot \Delta t_i \cdot \alpha, \quad (10)$$

$$3 \quad \delta(n) = \frac{\sum_i \delta_i \cdot C_i \cdot f_i \cdot \Delta t_i}{\sum_i C_i \cdot f_i \cdot \Delta t_i}, \quad (11)$$

$$4 \quad \Delta A_{in}(n) = \Delta N_{in}(n) \cdot \frac{\Delta^{17}O_{in}(n)}{\Delta^{17}O_{atm}}, \quad (12)$$

$$5 \quad \Delta N_{in} = \sum_{n=1}^4 \Delta N_{in}(n), \quad (13)$$

$$6 \quad \Delta A_{in} = \sum_{n=1}^4 \Delta A_{in}(n), \quad (14)$$

$$7 \quad \delta = \frac{\sum_{n=1}^4 \delta(n) \cdot \Delta N_{in}(n)}{\sum_{n=1}^4 \Delta N_{in}(n)}, \quad (15)$$

8 where Q_{in} denotes the annual gross influx of water into the lake; C_i and δ_i denote the
 9 concentration and isotopic values ($\delta^{15}N$, $\delta^{18}O$, or $\Delta^{17}O$) of nitrate on each stream i during each
 10 observation n , respectively; f_i denotes the flow rate of each stream i during each observation
 11 n ; and Δt_n denotes the time interval between the observation n and the next observation $n + 1$.

12 For Q_{in} , we used the annual influx of water estimated by Kunimatsu et al. (1995), in which the
 13 influx via streams and that via groundwater were included. To include the influx of nitrate via
 14 groundwater and the other minor streams not measured in this study during the calculations,
 15 we used the correction factor α in Eq. (9), whereby we assumed that both the average
 16 concentration and average isotopic compositions of the inflows determined in this study
 17 represented those of all inflows into the lake, while assuming an error range of 20% on α .

18 By using the aforementioned equations, we estimated the total influx of nitrate to the lake
 19 (ΔN_{in}) for each interval, together with the average $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values of nitrate
 20 during each interval, as presented in Table 2. Moreover, by using the values of ΔN_{in} during
 21 each interval, as well as their $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$ values, we estimated the total influx of
 22 $NO_3^-_{atm}$ to the lake (ΔA_{in}) and their average $\delta^{15}N_{re}$ and $\delta^{18}O_{re}$ values for each interval, as



1 presented in Table 2, by using Eqs. (12), (6), and (7). Furthermore, we estimated the annual
2 total influx and their annual average values, as shown in Table 2.

3 The estimated annual average $\Delta^{17}\text{O}$ value of inflows, +1.3‰, corresponded to the average
4 mixing ratio of $\text{NO}_3^-_{\text{atm}}$ within total nitrate was $5.1 \pm 0.5\%$. We concluded that about 5% of
5 the total nitrate in the inflows originated directly from the atmosphere; therefore, the
6 remainder of the nitrate was of remineralized origin ($\text{NO}_3^-_{\text{re}}$) likely produced through
7 nitrification within the catchments, as discussed in section 3.4. In addition, we estimated the
8 annual total influx of nitrate to the lake ($\Delta\text{N}_{\text{in}}$) to be 199 ± 40 Mmol and that of $\text{NO}_3^-_{\text{atm}}$
9 ($\Delta\text{A}_{\text{in}}$) to be 10.1 ± 2.0 Mmol.

10 Moreover, we estimated the total efflux of nitrate from the lake via the outflows ($\Delta\text{N}_{\text{out}}$) and
11 that of $\text{NO}_3^-_{\text{atm}}$ ($\Delta\text{A}_{\text{in}}$) for each interval by using Eqs. (9) to (15) in which $\Delta\text{N}_{\text{in}}$ was replaced
12 with $\Delta\text{N}_{\text{out}}$, which is the gross efflux of nitrate from the lake via the streams and groundwater
13 during the interval between the observation n and the next observation $n + 1$. Additionally, Q_{in}
14 was replaced with Q_{out} , which is the annual gross efflux of water. To include the minor efflux
15 of nitrate to $\Delta\text{N}_{\text{out}}$, such as that via canals, we used the correction factor γ instead of α in Eqs.
16 (9) and (10), whereby we assumed that both the concentration and isotopic compositions of
17 the natural outflow determined for each season in this study represented all outflows. For Q_{out} ,
18 we used the annual efflux of water from Lake Biwa estimated by Kunimatsu et al. (1995),
19 which included the efflux via a natural river (Seta River, No. 33) and that via canals.

20 Compared with the annual $\Delta\text{N}_{\text{in}}$, 199 ± 40 Mmol, and annual $\Delta\text{A}_{\text{in}}$, 10.1 ± 2.0 Mmol, both the
21 annual $\Delta\text{N}_{\text{out}}$, 67 ± 13 Mmol, and annual $\Delta\text{A}_{\text{out}}$, 2.2 ± 0.4 Mmol, were significantly smaller by
22 about 34% and 22%, respectively. Hence, Lake Biwa acts as a net sink for both nitrate and
23 $\text{NO}_3^-_{\text{atm}}$, as previously implied from the ^{15}N enrichment in outflows. Considering that nitrate
24 occupied about 70% of the total fixed N pool in the inflows and about 40% of the total fixed
25 N pool in the outflows (Shiga_Prefecture 2015), Lake Biwa also acts as a net sink for fixed N.
26 Similar results had been obtained in past studies that discussed fixed N input/output of the
27 lake (Kunimatsu 1995; Tezuka 1985; Tezuka 1992; Yamada et al. 1996). As implied in the
28 significant ^{15}N enrichment in the remineralized portion of nitrate ($\delta^{15}\text{N}_{\text{re}}$) in the outflow,
29 +13.7 \pm 0.2‰, compared to that in the inflow, +5.6 \pm 0.3‰, partial removal of nitrate through
30 either assimilation or denitrification to result in ^{15}N enrichment of residual nitrate is strongly
31 responsible for the 8.1 \pm 0.5‰ increase in $\delta^{15}\text{N}_{\text{re}}$, as well as the net removal of both nitrate
32 and $\text{NO}_3^-_{\text{atm}}$ from the lake.



1 On the contrary, the $\delta^{18}\text{O}$ differences in the remineralized portion of nitrate ($\delta^{18}\text{O}_{\text{re}}$) between
2 the inflows and outflows were significantly smaller than $\delta^{15}\text{N}_{\text{re}}$, at an annual average of only
3 $1.6 \pm 1.5\text{‰}$ (Table 2). If nitrate in outflows is the residual of assimilation/denitrification in the
4 lake, $\delta^{18}\text{O}_{\text{re}}$ should increase as well (Granger et al. 2008; Granger et al. 2004). The much
5 smaller $\delta^{18}\text{O}_{\text{re}}$ difference implies that nitrate supplied directly from inflows occupied a small
6 portion of nitrate in the outflows and that most of nitrate with high $\delta^{15}\text{N}$ values in the outflows
7 had been produced through nitrification in the lake water column in which the fixed N had
8 previously been enriched in ^{15}N . Isotopic fractionations during fixed N cycling in the lake
9 such as denitrification or assimilation and the subsequent removal of ^{15}N -depleted organic N
10 during sedimentation (Fig. 1) should be responsible for the ^{15}N enrichment of the total fixed N.
11 That is, most of nitrate fed into the lake via the inflows had been removed at least once from
12 the lake water column and been involved into the total fixed N cycling in the lake, in which
13 the ^{15}N -enriched nitrate in the outflow had been produced (Fig. 1). The stagnation of flow in
14 the lake encouraged primary production and thus the net removal of total fixed N through
15 either denitrification or sedimentation, which resulted in ^{15}N enrichment of total fixed N pool
16 compared with that in the inflows. Further studies on N cycling in the lake are needed to
17 verify these results.

18

19 **4 Concluding Remarks**

20 In this study, we applied the $\Delta^{17}\text{O}$ tracer of nitrate to determine accurate and precise mixing
21 ratios of unprocessed $\text{NO}_3^-_{\text{atm}}$ within the total nitrate value for more than 30 streams in the
22 Lake Biwa watershed basin. Although the nitrate concentration changed from 12.7 to 106.2
23 $\mu\text{mol L}^{-1}$ between the inflow streams and the mixing ratio of $\text{NO}_3^-_{\text{atm}}$ within total nitrate also
24 changed from 1.8% to 11.8%, the absolute concentration of $\text{NO}_3^-_{\text{atm}}$ (C_{atm}) in each stream
25 water was almost stable at $2.3 \pm 1.1 \mu\text{mol L}^{-1}$ irrespective of the changes in population
26 density and land use among the catchment areas. We concluded that changes in population
27 density and land use in each catchment area had little impact on C_{atm} , and the total nitrate
28 concentration was determined primarily by the extent of the additional $\text{NO}_3^-_{\text{re}}$ contribution
29 mostly from anthropogenic sources. Relying on only the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ tracers of nitrate, it
30 was difficult to determine the precise C_{atm} in stream water and whether C_{atm} was uniform
31 among the streams. By using the $\Delta^{17}\text{O}$ values, we can estimate accurate and precise C_{atm} in



1 each stream for each season; thus, we can clarify the fate of $\text{NO}_3^-_{\text{atm}}$ deposited into the
2 catchments.

3 Moreover, additional measurements of the $\Delta^{17}\text{O}$ values of nitrate together with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$
4 enabled us to exclude the contribution of $\text{NO}_3^-_{\text{atm}}$ from the determined $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values
5 and to use the corrected $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, $\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$, to evaluate the source and
6 behaviour of $\text{NO}_3^-_{\text{re}}$ in each stream. Based on the correction, we successfully estimated the
7 $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{NO}_3^-_{\text{re}}$ in the streams to be $+4.4 \pm 1.8\text{‰}$ and $-2.3 \pm 0.9\text{‰}$,
8 respectively, for that produced through nitrification in naturally occurring soil organic matter
9 and $+9.2 \pm 1.3\text{‰}$ and $-2.2 \pm 1.1 \text{‰}$, respectively, for that supplied from anthropogenic
10 sources, most of which had been occupied by sewage effluent. In addition, the low and
11 uniform annual average $\delta^{18}\text{O}_{\text{re}}$ values of $\text{NO}_3^-_{\text{re}}$ in the streams implies that the denitrification
12 in the riverbed sediments was minor in the streams.

13 Furthermore, we clarified the seasonal changes in the range of isotopic fractionation through
14 partial nitrate removal via assimilation or denitrification by using the relationship between
15 $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate in the streams. The changes were small in winter in all of the
16 catchment area but large in summer in some catchments. Therefore, the relationship between
17 $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate eluted from a catchment area is a powerful indicator for evaluating
18 the biogeochemical nitrogen cycles within a catchment area, including the seasonal changes.

19 In summary, interpretations on the stable isotopic compositions of nitrate ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$)
20 without $\Delta^{17}\text{O}$ values can be often misleading when assuming (1) $\delta^{18}\text{O}$ values simply reflect
21 the mixing ratio of $\text{NO}_3^-_{\text{atm}}$ within total nitrate, (2) the mixing ratios of $\text{NO}_3^-_{\text{atm}}$ within the
22 total NO_3^- is minimum for whole/specific samples studied, or (3) the mixing ratio of $\text{NO}_3^-_{\text{atm}}$
23 within total nitrate is uniform in whole/specific samples studied. In using the stable isotopic
24 compositions of nitrate in freshwater environments to trace the source and fate of the nitrate,
25 particularly the $\delta^{18}\text{O}$ value, determination of the $\Delta^{17}\text{O}$ values is essential.

26

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8

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- 30



1 Table 1 List of studied streams.

No.	Name	Loc. #	Basin Area* (km ²)	Population Density* (km ⁻²)	Residential* (%)	No.	Name	Loc. #	Basin Area* (km ²)	Population Density* (km ⁻²)	Residential* (%)		
<i>Inflow</i>													
31	Tenjin	West	10	539	7.3	14	Seri	East	74	462	8.1		
30	Mano	West	23	1048	15.6	15	Inukami	East	102	109	2.8		
29	Wani	West	17	186	5.4	16	Ajiki	East	15	1002	20.1		
28	U	West	7	66	0.8	17	Uso	East	84	411	11.0		
1	Kamo	West	47	89	1.2	18	Bunroku	East	14	595	11.7		
2	Ado	West	306	27	0.7	19	Nomazu	East	7	758	20.2		
3	Ishida	North	60	84	2.0	20	Echi	East	211	110	2.5		
4	Momose	North	13	65	0.6	27	Hino	South	226	338	8.4		
5	Chinai	North	51	44	1.8	26	Yanomune	South	42	859	15.7		
6	Ohura	North	39	98	2.7	25	Yasu	South	391	324	6.5		
7	Oh	North	20	55	1.6	24	Yamaga	South	6	2540	33.7		
8	Yogo	North	7	141	3.4	23	Sakai	South	2	979	27.7		
9	Chonoki	North	10	412	13.5	22	Hayama	South	34	2048	29.7		
10	Ta	North	36	301	9.6	34	Kusatsu	South	48	370	30.6		
11	Ane	North	372	61	1.7	21	Nagaso	South	4	3174	31.4		
12	Yone	North	15	2047	34.5	32	Fujinoki	South	4	1805	20.8		
13	Amano	North	111	226	5.8	<i>Outflow</i>							
								33	Seta	South	3848	323	-

2 # Category of location classified by Ohte et al. (2010).

3 * Data source: Ohte et al. (2010).

4

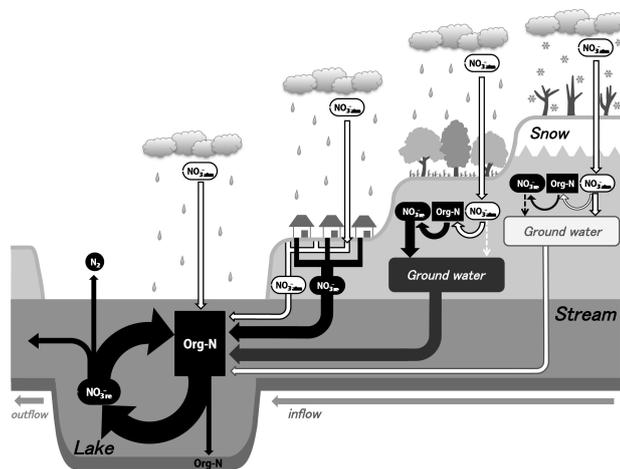


- 1 Table 2 Estimated gross influx/efflux of total nitrate (ΔN) and atmospheric nitrate (ΔA) via
 2 inflows/outflows during each observation interval, together with the average $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and
 3 $\Delta^{17}\text{O}$ values of total nitrate and remineralized portions of nitrate ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$) in the
 4 inflows/outflows during each interval.

	Spring (n=1 to 2)	Summer (n=2 to 3)	Autumn (n=3 to 4)	Winter (n=4 to 5)	Annual (n=1 to 5)
Duration (days)	94	49	77	145	365
Inflow					
ΔN_{in} (10^6 mol)	69 ± 14	3 ± 1	13 ± 3	114 ± 23	199 ± 40
ΔA_{in} (10^6 mol)	6.4 ± 1.3	0.1	0.8 ± 0.2	2.8 ± 0.6	10.1 ± 2.0
$10^3 \delta^{15}\text{N}$	+4.0	+6.8	+5.6	+5.6	+5.1
$10^3 \delta^{18}\text{O}$	+6.1	-0.8	+3.3	-1.5	+1.4
$10^3 \Delta^{17}\text{O}$	+2.5	+0.8	+1.7	+0.6	+1.3
$10^3 \delta^{15}\text{N}_{\text{re}}$	$+4.8 \pm 0.7$	$+7.1 \pm 0.2$	$+6.3 \pm 0.5$	$+5.9 \pm 0.2$	$+5.6 \pm 0.3$
$10^3 \delta^{18}\text{O}_{\text{re}}$	-1.5 ± 1.8	-3.2 ± 0.5	-2.0 ± 1.2	-3.5 ± 0.4	-2.8 ± 0.9
Outflow					
ΔN_{out} (10^6 mol)	24 ± 5	6 ± 1	5 ± 1	32 ± 6	67 ± 13
ΔA_{out} (10^6 mol)	1.4 ± 0.1	0.1	0.2	0.4 ± 0.1	2.2 ± 0.4
$10^3 \delta^{15}\text{N}$	+7.3	+11.4	+10.4	+18.0	+13.1
$10^3 \delta^{18}\text{O}$	+3.4	+4.8	+3.0	-0.7	+1.5
$10^3 \Delta^{17}\text{O}$	+1.6	+0.4	+1.4	+0.4	+0.9
$10^3 \delta^{15}\text{N}_{\text{re}}$	$+8.1 \pm 0.4$	$+11.7 \pm 0.1$	$+11.2 \pm 0.4$	$+18.3 \pm 0.1$	$+13.7 \pm 0.2$
$10^3 \delta^{18}\text{O}_{\text{re}}$	-1.5 ± 1.1	$+3.6 \pm 0.3$	-1.2 ± 0.9	-1.9 ± 0.2	-1.2 ± 0.6

5

6

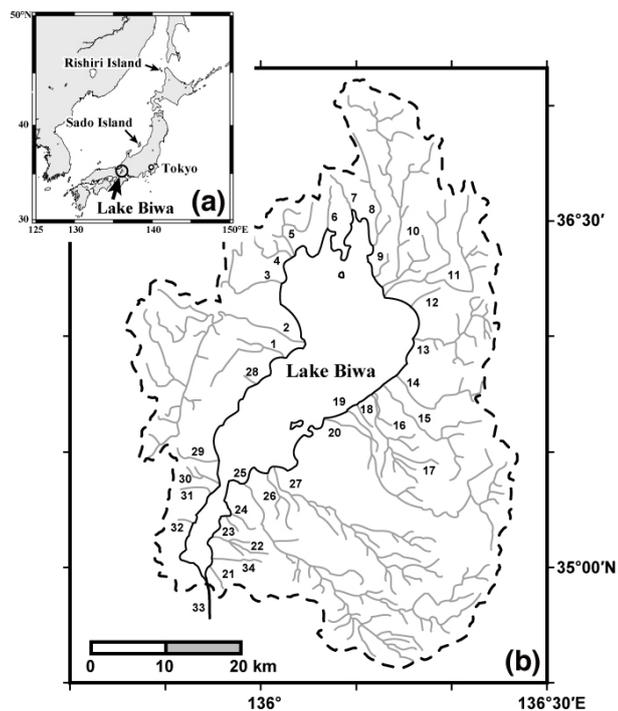


1

2 Figure 1. Schematic diagram showing the biological processing of atmospheric nitrate
3 ($\text{NO}_3^-_{\text{atm}}$) and remineralized nitrate ($\text{NO}_3^-_{\text{re}}$) in the watershed with catchments of varying land
4 uses and in the lake water column.

5

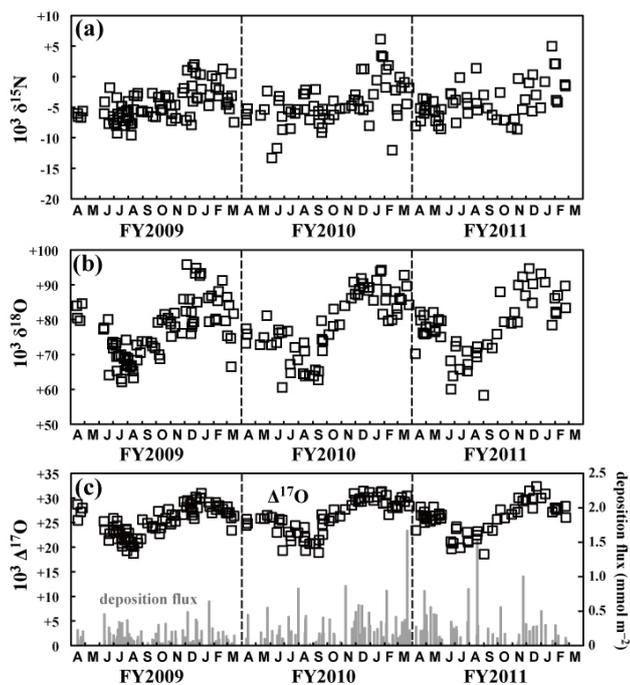
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2 Figure 2. (a) Map showing the location of Lake Biwa watershed basin in Japan and Sado
3 Island, where the Sado-seki National Acid Rain Monitoring Station is located. (b) Map
4 showing the boundary of the Lake Biwa watershed basin (dashed line) and the locations of the
5 inflows (represented by numbers) and outflow (Seta River, No. 33) studied in this paper
6 (modified from Ohte et al. 2010).

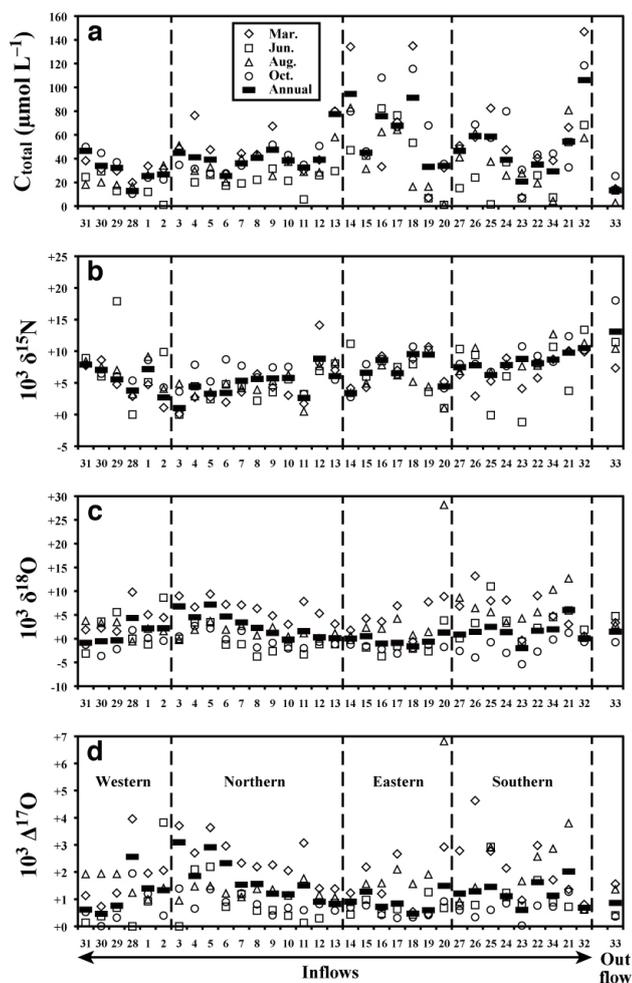
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2 Figure 3. Temporal variations in the values of (a) $\delta^{15}\text{N}$, (b) $\delta^{18}\text{O}$, and (c) $\Delta^{17}\text{O}$ of nitrate in wet
3 deposition recorded at the Sado-seki National Acid Rain Monitoring Station. Total deposition
4 flux of nitrate during each rain event was also presented (c).

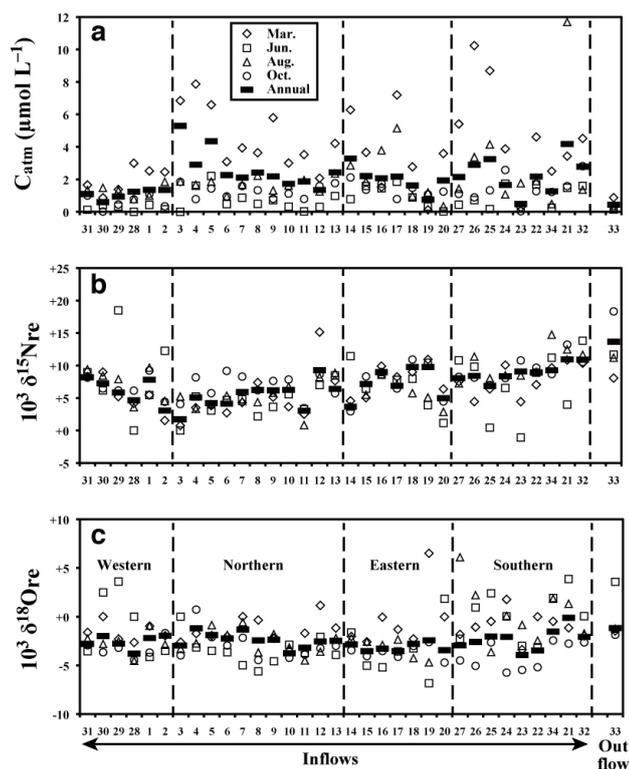
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2 Figure 4. Distribution of (a) total concentrations, (b) $\delta^{15}\text{N}$, (c) $\delta^{18}\text{O}$, and (d) $\Delta^{17}\text{O}$ for nitrate in
3 inflow streams showing various station numbers and the outflow at station No. 33 in Lake
4 Biwa watershed in March (diamonds), June (squares), August (triangles), and October
5 (circles) 2013 together with the annual averages for each river (black bars).

6

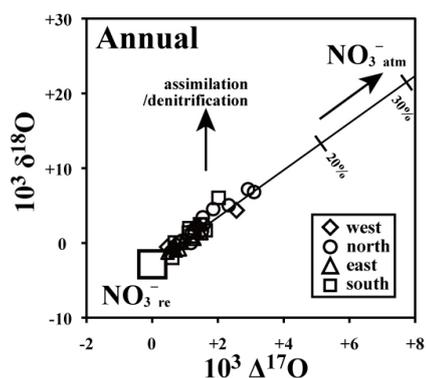


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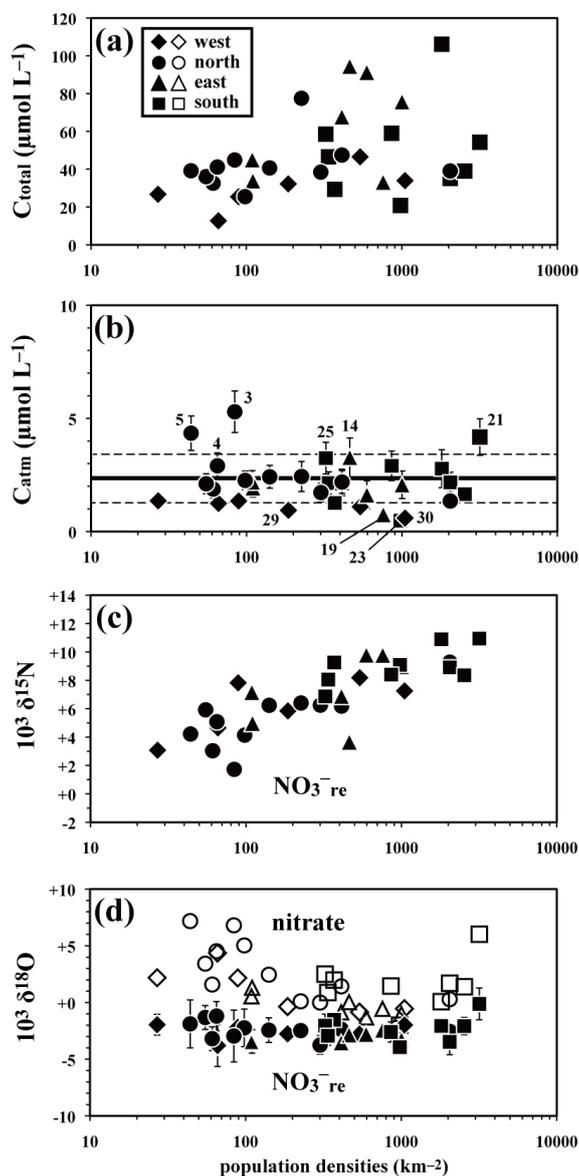
2 Figure 5. Distribution of concentrations of (a) atmospheric nitrate and (b) $\delta^{15}\text{N}$ and (c) $\delta^{18}\text{O}$
 3 for remineralized nitrate ($\delta^{15}\text{N}_{\text{re}}$ and $\delta^{18}\text{O}_{\text{re}}$, respectively) in inflow streams shown by various
 4 station numbers and the outflow at station No. 33 of Lake Biwa watershed in March
 5 (diamonds), June (squares), August (triangles), and October (circles) 2013 together with the
 6 annual averages for each river (black bars).

7

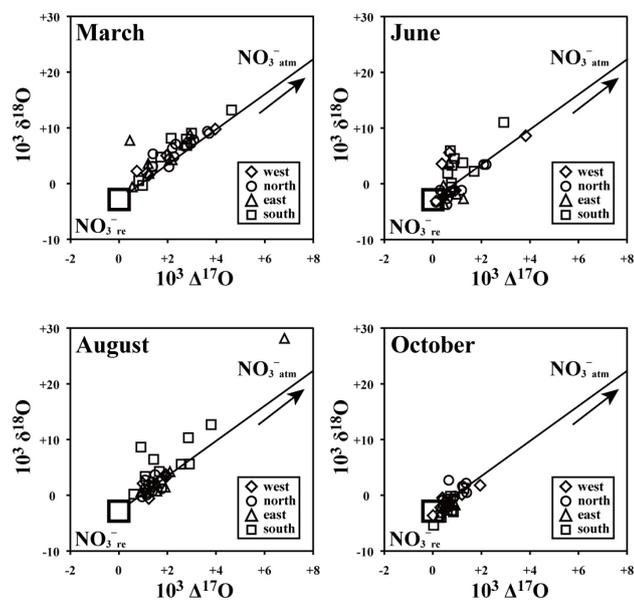
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2 Figure 6. Relationship of the annual average values of $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of NO_3^- in the inflow
3 streams. The symbols represent the location of each river (west: diamonds; north: circles;
4 east: triangles; south: squares). A hypothetical mixing line between atmospheric nitrate
5 ($\text{NO}_3^-_{\text{atm}}$) and remineralized nitrate ($\text{NO}_3^-_{\text{re}}$) is shown together with the end member value of
6 $\text{NO}_3^-_{\text{re}}$ (large white square) and the 20% and 30% mixing ratios of $\text{NO}_3^-_{\text{atm}}$ on the line.
7



1
 2 Figure 7. Annual average concentration of (a) nitrate (C_{total}) and that of (b) $NO_3^-_{atm}$ (C_{atm}) in
 3 each inflow stream plotted as a function of the population density in each catchment, together
 4 with (c) the annual average value of $\delta^{15}N$ and (d) that of $\delta^{18}O$ for remineralized NO_3^- ($NO_3^-_{re}$).
 5 (d) The annual average $\delta^{18}O$ values of nitrate are also presented. The symbols represent the
 6 location of each river (west: diamonds; north: circles; east: triangles; south: squares). The
 7 uncertainties are presented only for those larger than the symbols.
 8



1

2 Figure 8. Temporal changes in the relationship between the values of $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of total
 3 nitrate in the stream water. The symbols are the same as those in Figure 6. A hypothetical
 4 mixing line between atmospheric nitrate ($\text{NO}_3^-_{\text{atm}}$) and remineralized nitrate ($\text{NO}_3^-_{\text{re}}$) is also
 5 shown together with the end member value of $\text{NO}_3^-_{\text{re}}$ (large white square).

6