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Sources, fate and geochemical dynamics of nitrate in an oligotrophic lake

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Abstract

To trace the fate of atmospheric nitrate deposited into a oligotrophic lake and to clarify the geochemical dynamics of nitrate in a oligotrophic environment, the stable isotopic compositions of nitrate, including the ^{17}O anomalies (Δ^{17}O), were determined twice in one year (June and August 2007) in the water column of Lake Mashu, Japan, which is a crater lake with a depth of 211 m. The highest transparency of the lake (41.6 m) was recorded in 1931, although the transparency has decreased substantially in recent years. While the total inventory of nitrate in the lake water decreased from 4.2 to 2.1 Mmol (Mmol = 10^6 mol) during the period between the observations, the average Δ^{17}O nitrate values were uniform at +2.5‰, which corresponded to an average mixing ratio of atmospheric nitrate to total nitrate of 9.7 ± 0.8%. Using the total mass of the atmospheric nitrate deposited onto the entire catchment area of the lake during a period of 2 months (0.047 Mmol), we estimated that 0.52 ± 0.34 Mmol of the remineralized nitrate was fed into the water column through nitrification, while 2.6 ± 0.4 Mmol of nitrate was simultaneously removed from the water column by assimilation. The lake water dissolved nitrate was characterised by rapid removal through assimilation during summer until it was almost completely removed from the euphotic layer, as well as continuous feeding into the lake through nitrification and deposition, regardless of the seasons, which corresponds to a gross annual flux of 3.2 ± 0.3 Mmol a^{-1} and 0.35 ± 0.2 Mmol a^{-1}, respectively. The ^{15}N-depleted nitrogen isotopic compositions of nitrate were as low as −6.5‰ in June, which also indicates that in-lake nitrification is the major source of nitrate in the lake, and suggests that there is low potential for denitrification in and around the lake. These results indicate that atmospheric nitrate deposited into the lake will be assimilated quickly having a mean residence time of 1.2 ± 0.1 years. Besides, more than 90% of the assimilated nitrate will be remineralized to nitrate and assimilated again via active nitrogen cycling in the lake.

1 Introduction

Anthropogenic activities have led to increased emissions of fixed nitrogen from land to the atmosphere. Indeed, the amount of emitted nitrogen has almost doubled globally, with much greater increases occurring in some regions, and this fixed-nitrogen flux is expected to double again by 2030 (Galloway et al., 2008). The increase in NO_x emissions in eastern Asia has been particularly dramatic over the last decade (Akimoto, 2003; Zhang et al., 2007; Uno et al., 2007) owing to rapid growth in both industry and the number of automobiles. Most of the emitted NO_x is transported eastward by the Asian monsoon and deposited in the western north Pacific, which includes Japan, as atmospheric nitrate ($NO^-_{3\ \mathrm{atm}}$) (Akimoto, 2003; Uno et al., 2007).

Excess fixed-nitrogen input is linked to various environmental problems such as forest decline (e.g. Fenn et al., 1998), degradation of groundwater quality (e.g. Murdoch and Stoddard, 1992; Williams et al., 1996), eutrophication of coastal and open ocean (e.g. Paerl, 1997; Duce et al., 2008), and shifts in biodiversity (e.g. Tilman et al., 1996). Besides, significant declining trends in clarity have been detected in some highly transparent oligotrophic lakes, such as Danish lake in Europe (Riis and Sand-jensen, 1998), Lake Tahoe in north America (Jassby et al., 1999), and Lake Mashu in eastern Asia (CGER NIES et al., 2004). Atmospheric input of excess fixed-nitrogen nutrients, rather than local human activities, could be highly responsible for the decline in clarity, through the increase in phytoplankton in water column (Riis and Sandjensen, 1998; Jassby et al., 1994; Fukazawa, 2008).

Although the water chemistry has been routinely measured in the lakes, our understanding of the nitrogen cycle in such oligotrophic lakes is limited, especially with respect to the fate of deposited atmospheric nitrate. Indeed, the fate of atmospheric nitrate deposited onto hydrosphere is determined through an interplay of several processes including (1) dilution through nitrification, (2) uptake by phytoplankton, periphyton, or microbes, and (3) decomposition through denitrification. Moreover, assimilated nitrate can be remineralized to nitrate again via nitrification. As a result, tracing the fate of atmospheric nitrate deposited into lakes has been difficult.

Michalski and Thiemens (2006) recently determined the natural stable isotopic compositions of nitrate in Lake Tahoe and estimated that the average mixing ratio of $NO_{3\ atm}^-$ to the total nitrate in the lake ($NO_{3\ total}^-$) was 13%. The natural stable isotopic compositions of nitrate consists of $\delta^{15}N$, $\delta^{17}O$ and $\delta^{18}O$, where $\delta^{18}O = R_{sample}/R_{standard} - 1$ and R is the $^{18}O/^{16}O$ ratio (or the $^{17}O/^{16}O$ ratio in the case of $\delta^{17}O$ or the $^{15}N/^{14}N$ ratio in the case of $\delta^{15}N$) of the sample and each international standard. While the remineralized nitrate ($NO_{3\ re}^-$), the oxygen atoms of which are derived from either terrestrial O_2 or H_2O through nitrification, undergo mass-dependent relative variations between $\delta^{17}O$ and $\delta^{18}O$, $NO_{3\ atm}^-$ displays an anomalous enrichment in ^{17}O reflecting oxygen atom transfers from ozone during the conversion of NO_x to $NO_{3\ atm}^-$ (Michalski et al., 2003). As a result, the $\Delta^{17}O$ signature defined by the following equation (Miller, 2002; Kaiser et al., 2007) can be used to distinguish $NO_{3\ atm}^-$ ($\Delta^{17}O > 0$) from the other nitrate ($NO_{3\ re}^-$) ($\Delta^{17}O = 0$).

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

where the constant β is 0.5247 (Miller, 2002; Kaiser et al., 2007). In addition, $\Delta^{17}O$ is stable in the mass-dependent isotope fractionation processes; therefore, we can use $\Delta^{17}O$ as a novel conserved tracer of $NO_{3\ atm}^-$ to determine the $NO_{3\ atm}^-/NO_{3\ total}^-$ ratio ($[NO_{3\ atm}^-]/[NO_{3\ total}^-]$) irrespective of partial removal through denitrification and/or assimilation subsequent to deposition using the following equation:

$$\frac{[NO_{3\ atm}^-]}{[NO_{3\ total}^-]} = \frac{[NO_{3\ atm}^-]}{[NO_{3\ re}^-] + [NO_{3\ atm}^-]} = \frac{\Delta^{17}O_{lake}}{\Delta^{17}O_{atm}}, \tag{2}$$

where $\Delta^{17}O_{atm}$ denotes the average $\Delta^{17}O$ value of $NO_{3\ atm}^{-}$, and $\Delta^{17}O_{lake}$ denotes the average $\Delta^{17}O$ value of nitrate dissolved in the lake. Using both the estimated $NO_{3\ atm}^{-}/NO_{3\ total}^{-}$ ratio and the rate at which $NO_{3\ atm}^{-}$ is deposited into the lake, Michalski and Thiemens (2006) also succeeded in estimating average nitrification rate in the lake.

The average $\delta^{15}N$, $\delta^{18}O$ and $\Delta^{17}O$ values of atmospheric nitrate deposited onto the eastern Asian region have been determined through continuous monitoring at Rishiri Island (Fig. 1; Tsunogai et al., 2010), which is located in the same prefecture as Lake Mashu in Japan. Accordingly, the results will be applicable to atmospheric nitrate deposited onto Lake Mashu. Thus, in this study, we determined both the concentrations and stable isotopic compositions of nitrate in Lake Mashu, including the $\Delta^{17}O$ values, to quantify the $NO_{3\ atm}^{-}/NO_{3\ total}^{-}$ ratio of nitrate in the water column and clarify the geochemical dynamics of nitrate in the water column, with a special emphasis on the fate of atmospheric nitrate deposited into the ultra oligotrophic environment. In addition to evaluating the distribution, we also determined their temporal variation during summer stratification of the lake to enable accurate quantification of the geochemical dynamics of nitrate in the water column, including their seasonal variations. These results will be useful to clarify the reasons for the significant declines in clarity detected in the highly transparent oligotrophic lakes. Also, they will be useful to clarify the fate of atmospheric nitrate deposited onto hydrosphere in general.

2 Experimental section

2.1 Site description

Lake Mashu is a dimictic crater lake located in the northern part of Japan (43°35′ N, 144°32′ E; Fig. 1), situated in the subalpine area 355 m above sea level encircled by a steep caldera wall. The surface of the lake is covered with ice and snow from December to April. The autumn overturn occurs around December to January, while the spring overturn occurs around April to May (Nojiri et al., 1990).

The lake has a catchment area of about 32.4 km^2, most of which is occupied by the lake surface, which comprises 19.6 km^2 (Fig. 1; Horiuchi et al., 1985). The maximum depth of the lake is 211.5 m and the lake bottom is almost flat and covered with pumice deposits. There are no perennial streams entering or exiting the lake, and the surface level is approximately in a state of equilibrium, suggesting that the lake water supplied as rain water to the catchment area seeps out through the porous bottom, in addition to being lost via evaporation. The mean residence time of the lake water is around 110 years (Ambe et al., 1988).

The lake water is ultra-oligotrophic, showing PO_4^{3-} concentrations less than 0.1 µM (µM = µmol L^{-1}) and NO_3^- concentrations less than 3 µM at the epilimnion (CGER NIES et al., 2004). In addition, the transparency (Secchi depth) of the lake was 41.6 m on 31 August 1931, which was the highest ever recorded (Yoshimura, 1932), surpassing that of Lake Baikal (40.5 m). However, the transparency has decreased to less than 30 m in recent years (Fig. 2; CGER NIES et al., 2004), which has probably occurred due to increased levels of phytoplankton in the water column (Haga et al., 1994; Fukazawa, 2008). The phytoplankton in the lake is primarily composed of diatoms and dinoflagellates, such as *Fragilaria lapponica*, *Ceratium hirundinella*, *Dinobryon sertularia* and *Dinobryon* sp. (Haga et al., 1994). The maximum chlorophyll-*a* concentrations in the water column are around 1 µg L^{-1}, being observed in the subsurface zone at depths ranging from 10 to 75 m in summer (Fig. 3; CGER NIES et al., 2004). The lake water is well oxygenated, regardless of depth or season, showing dissolved oxygen (DO) saturation ratios of 100 ± 5% (Fig. 3).

The entire catchment area of the lake has been designated as a specially restricted zone of the Akan National Park of Japan since 1934 to preserve the lake in its pristine form so that atmospheric input of nutrients, rather than local human activities, are likely responsible for the increase in phytoplankton in the water column (Fukazawa, 2008). On the other hand, trespassing on the lakeshore have been strictly prohibited. As a result, while the lake has been studied to evaluate background levels of environmental pollutants in the hydrosphere over the last 30 years (CGER NIES et al., 2004), the chances for lake water sampling have been limited, usually to just a few days in summer. Furthermore, all equipment must be carried to the lake, which has resulted in lake water sampling/monitoring that requires heavy instruments or high power electricity being difficult.

2.2 Sampling

Water samples were collected on 25 June and 24 August 2007 from the central sampling station of the lake (43°35′02 N, 144°32′03″ E; Fig. 1) using a 2.0 L Niskin sampler (Nishimura et al., 1999) at depths of 0, 20, 50, 100, 150 and 200 m (Table 1). Each sample was transferred into 250 mL polyethylene bottles on the boat subsequent to being rinsed at least twice with the sample itself and then filtered through a pre-combusted Whatman GF/F filter (0.7 µm pore size) within a few hours of collection, after which it was stored in a refrigerator until analysis. The seston that collected on the filter was washed with pure water, placed in a plastic case and then stored in a deep freezer to analyse particulate organic nitrogen (PON) in the lake water.

2.3 Analysis

The concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) were measured using a UV-detector HPLC by introducing 200 µl of the sample solution (Ito et al., 2005). To determine the stable isotopic compositions, the sample nitrate was chemically converted to N_2O using a method originally developed to determine $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotope ratios of seawater and freshwater nitrate (McIlvin and Altabet, 2005), with a slight modification (Tsunogai et al., 2008, 2010). A summary of the procedures is presented below.

Aliquots of 45 ml were pipetted into 60 mL clean screw-cap vials with butyl rubber septum caps. Next, 2 g of spongy cadmium was added, followed by 450 µl of a 1 M $NaHCO_3$ solution, which resulted in a pH of approximately 8.5. The samples were then shaken for 20–30 h on a horizontal shaker at a rate of 2 cycles s^{-1}. Subsequently, 30 mL aliquots of the samples were decanted into clean 60 ml vials and then capped tightly with Teflon-lined septum caps. After evacuating the air from the head space and the sample solution for 20 min, 1.2 ml of azide/acetic acid buffer was added to each vial via a syringe and the mixture was shaken vigorously. Owing to the volatility and toxicity of HN_3, all reactions were conducted using an original automatic reaction line. In addition, all the exhaust was vented via a fume hood. After 30 min, the solution was made basic by adding 0.6 mL of 6 M NaOH with a syringe and shaking to prevent residual HN_3 from escaping into the laboratory during subsequent isotopic analysis.

The stable isotopic compositions of N_2O were determined using our Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system (Tsunogai et al., 2008; Hirota et al., 2010), which consists of an original helium purge and trap line, a gas chromatograph (Agilent 6890) and a Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified Combustion III interface (Tsunogai et al., 2000, 2002, 2005). First, approximately 5 ml of head space gas in each vial was sampled in a gas-tight syringe and injected into a helium sparging line via an injection port (Ijiri et al., 2003). The sample was then sent through a purification port packed with Ascarite II and magnesium perchlorate to remove both the CO_2 and residual H_2O, after which it was collected into a final stainless steel trap immersed in liquid nitrogen. After changing the flow path, the liquid nitrogen bath was then removed to introduce the sample into a PoraPLOT-Q analytical capillary column (0.32 mm i.d. × 50 m) at a column oven temperature of +30 °C, where the N_2O was separated from any remaining CO_2. The eluted N_2O was carried continuously into a Finnigan MAT 252 isotope-ratio-monitoring mass spectrometer with a specially designed multicollector system via an open split interface to monitor isotopologues of N_2O^+ at m/z ratios of 44, 45 and 46 to determine δ^{45} and δ^{46}. Each analysis was calibrated using a machine-working reference gas (99.999% N_2O gas in a cylinder) introduced into the mass spectrometer via an open split interface according to a defined schedule to correct for sub-daily temporal variations in the mass spectrometry. In addition, a working-standard gas mixture containing N_2O of a known concentration (ca. 1000 ppm N_2O in air) that was injected from a sampling loop attached to the sparging unit was analysed in the same way as the samples at least once a day to correct for daily temporal variations in the mass spectrometry.

After the analyses based on the N_2O^+ monitoring were conducted, another aliquot of head space was introduced into the same purge and trap line to determine the $\Delta^{17}O$ for N_2O (Komatsu et al., 2008). Using the same procedures as those used in the N_2O^+ monitoring mode, purified N_2O eluted from the PoraPLOT-Q analytical capillary column was introduced into our original gold tube unit (Komatsu et al., 2008), which was held at 780 °C for the thermal decomposition of N_2O to N_2 and O_2. The produced O_2 purified from N_2 through separation using a MolSieve 5A PLOT column was subjected to CF-IRMS to determine the δ^{33} and δ^{34} by simultaneous monitoring of O_2^+ isotopologues at m/z ratios of 32, 33 and 34. Each analysis was calibrated with a machine-working reference gas (99.999% O_2 gas in a cylinder) that was introduced into the mass spectrometer via an open split interface according to a defined schedule to correct sub-daily temporal variations in the mass spectrometry. In addition, a working-standard gas mixture containing N_2O of known concentration (ca. 1000 ppm N_2O in air) that was injected from a sampling loop attached to the sparging unit was analysed in the same way as the samples at least once a day to correct for daily temporal variations in the mass spectrometry.

All δ values are expressed relative to air (for nitrogen) and VSMOW (for oxygen) in this paper. To calibrate the δ values of the sample nitrate on an international scale, as well as correcting for both the isotope fractionation during the chemical conversion to N_2O and the progress of the oxygen isotope exchange between the nitrate-derived reaction intermediate and water (ca. 20%), the obtained values of the sample-derived N_2O were compared with those of international standards. We used both USGS-34 ($\delta^{15}N = -1.8‰$, $\delta^{18}O = -27.93‰$, $\Delta^{17}O = +0.04‰$) and USGS-35 ($\delta^{15}N = +2.7‰$, $\delta^{18}O = +57.5‰$, $\Delta^{17}O = +20.88‰$), the internationally distributed isotope reference materials for nitrate, for the primary isotopic scale normalization (Böhlke et al., 2003; Kaiser et al., 2007). In addition, several local laboratory standards that had been calibrated using the internationally distributed isotope reference materials were also used for routine calibration purposes by measuring them in the same manner in which we measured the samples.

In calculating δ^{15}N, δ^{18}O or Δ^{17}O, we first determine those of N_2O (Komatsu et al., 2008). Then the δ^{15}N and Δ^{17}O values were simply calibrated using calibration lines obtained from the measurements of N_2O derived from the nitrate standards. In the case of calculating δ^{18}O for NO_3^-, we also corrected the oxygen exchange between the δ^{18}O of NO_3^- and water to consider cases in which the δ^{18}O of the water that dissolved the standards differed from the δ^{18}O of the sample water. Thus, we used the following formula to correct for δ^{18}O,

$$\delta^{18}\mathrm{O}_{\mathrm{nitrate}} = \frac{\left(\delta^{18}\mathrm{O}_{\mathrm{N2O}} - b\right) - (1-m) \times \Delta^{18}\mathrm{O}_{\mathrm{water}}}{m}, \tag{3}$$

where b is the intercept of the standards, m is the slope obtained using the standards and $\Delta^{18}\mathrm{O}_{\mathrm{water}}$ is the δ^{18}O difference between the sample water and standard water. Most of samples had nitrate concentrations of more than 1 µM, which corresponds to nitrate quantities of more than 30 nmol in a 30 ml sample and is sufficient to determine δ^{15}N, δ^{18}O and Δ^{17}O values with high precision. Even for the nitrate-depleted samples showing concentrations of less than 1 µM, we attained similar high precisions through repeated measurements using another aliquot of water samples. Thus, all isotopic data presented in this study have an error better than ±0.3‰ for δ^{15}N, better than ±0.5‰ for δ^{18}O and better than ±0.2‰ for Δ^{17}O.

Because we used the more precise power law (Eq. 1) for calculating Δ^{17}O, the estimated Δ^{17}O values are somewhat different from those estimated based on the traditional linear approximation (Michalski et al., 2002). Please note that our Δ^{17}O values would be 0.02 ± 0.02‰ lower for the lake water nitrate.

When we use the chemical conversion method to determine the stable isotopic compositions of nitrate (NO_3^-), nitrite (NO_2^-) in the samples also interfere in the final N_2O produced (McIlvin and Altabet, 2005), so that we have to correct for the contribution to determine accurate stable isotopic compositions of sample nitrate. Because the lake water samples analysed in this study contained little NO_2^- showing concentrations less than the detection limit which corresponded to the NO_2^-/NO_3^- ratios less than 10%, we saw that the interference must be minimum and used the results without any corrections.

The concentrations and δ^{15}N values of PON on the GF/F filters were analysed using the method developed by Tsunogai et al. (2008). In this method, organic nitrogen on the filter is oxidized to nitrate using persulfate. Subsequent processes to determine δ^{15}N values of nitrate were generally the same as the nitrate analyses described above. To calibrate the value to the international scale, several local laboratory standards that had been calibrated using the internationally distributed isotope reference materials (IAEA N1, USGS 25, and IAEA N2; Böhlke and Coplen, 1995), were used for routine calibration purposes by measuring them in the same manner in which we measured the samples. The data of PON presented in this study have an error of ± 0.3‰ for δ^{15}N.

3 Results and discussion

3.1 Distribution and temporal variation of nitrate in the water column

The concentrations and δ^{15}N, δ^{18}O and Δ^{17}O values of nitrate in the water column are presented in Table 1 and Fig. 4. The δ^{15}N values of nitrate in Lake Mashu were characterised by ^{15}N-depletion. Indeed, the δ^{15}N values in June corresponded to one of the lowest ever reported for nitrate in temperate large lakes ($> 10\,km^2$) worldwide (Ostrom et al., 1998). The reasons for these findings are discussed in detail in Sect. 3.5. Moreover, all of the water samples in Lake Mashu had small but positive Δ^{17}O values ranging from +1.6 to +4.3‰, suggesting that the lake water contained some $NO_{3\ atm}^-$.

In June, the concentrations of nitrate were almost uniform at 1.6 ± 0.1 µM, suggesting that the water was vertically well mixed during the spring overturn in 2007. These findings are supported by the vertically uniform δ^{15}N, δ^{18}O and Δ^{17}O values that were observed in June, except for the surface (0 m). Conversely, the nitrate decreased to less than 1.3 µM in August, especially for the top 100 m layer, suggesting that the active removal of nitrate occurred through assimilation by phytoplankton/periphyton during the summer stratification. These results are supported by the ^{15}N and ^{18}O enrichment of the isotopic compositions of nitrate in August when compared with the values in June, because partial removal of nitrate by phytoplankton/periphyton results in the residual nitrate being enriched with ^{15}N and ^{18}O.

To verify these findings quantitatively, we estimated the total inventory of nitrate in the lake water ($\mathrm{N_{total}}$) and their average δ^{15}N, δ^{18}O and Δ^{17}O values (δ_{avg}) from the data describing their vertical distributions in the water column and the vertical distribution of area in the caldera (Horiuchi et al., 1985) using the following equations (Tsunogai et al., 2000):

$$\mathrm{N_{total}} = \sum_{z=0}^{200} (C_z \times A_z \times \Delta z), \tag{4}$$

$$\delta_{\mathrm{avg}} = \frac{\sum_{z=0}^{200} (\delta_z \times C_z \times A_z \times \Delta z)}{\mathrm{N_{total}}}, \tag{5}$$

where C_z, δ_z and A_z denote nitrate concentration, each isotopic value (δ^{15}N, δ^{18}O and Δ^{17}O), and the area of the caldera at a depth z, respectively. Assuming that the data obtained at 0, 20, 50, 100, 150, and 200 m represented the average value from 0 to 10 m, 10 to 40 m, 40 to 90 m, 90 to 140 m, 140 to 190 m and 190 m to the bottom, respectively, we obtained the $\mathrm{N_{total}}$, $\delta^{15}\mathrm{N_{avg}}$, $\delta^{18}\mathrm{O_{avg}}$ and $\Delta^{17}\mathrm{O_{avg}}$ for each observation in Tables 1 and 2.

From June to August, the total nitrate inventory decreased from 4.2 to 2.1 Mmol (Mmol = 10^6 mol) in the lake (Table 2). In accordance with this decrease, both δ^{15}N and δ^{18}O increased from −6.5 to −2.5‰ (δ^{15}N) and from +3.4 to +7.6‰ (δ^{18}O), while the Δ^{17}O values were almost uniform around +2.5‰ (Table 1).

The extent to which a biological unidirectional transformation fractionates the values of δ^{15}N and δ^{18}O is given by the kinetic isotope effects, $^{15}\varepsilon$ and $^{18}\varepsilon$, respectively. These are functions of the ratio of the reaction rate constants (k) for the molecules containing the two isotopes:

$$^{15}\varepsilon = \frac{^{14}k}{^{15}k} - 1, \tag{6}$$

$$^{18}\varepsilon = \frac{^{16}k}{^{18}k} - 1, \tag{7}$$

where ^{14}k and ^{15}k denote reaction rate constants of the ^{14}N and ^{15}N isotopologues of nitrate, respectively, and ^{16}k and ^{18}k denote reaction rate constants of the ^{16}O and ^{18}O isotopologues of nitrate, respectively. The nitrate N and O isotope discrimination during nitrate assimilation have been quantified by mono-cultures of marine unicellular eukaryotic algae (Granger et al., 2004) and unicellular prokaryotic and eukaryotic plankton cultures (Granger et al., 2010). While the range of N and O isotope effect amplitudes that were observed for cultures of assimilators ($^{15}\varepsilon = 5$–20‰) was large, O isotope effects were always equivalent to the corresponding N isotope effect ($^{15}\varepsilon = {^{18}\varepsilon}$). Thus, nitrate N and O isotope ratios appear to co-vary linearly with a constant ratio of 1 (i.e., $\Delta\delta^{18}\mathrm{O} = \Delta\delta^{15}\mathrm{N}$) during nitrate assimilation. This biological imprint is also reflected in marine environments, with $\Delta\delta^{18}\mathrm{O}/\Delta\delta^{15}\mathrm{N}$ ratios of 1 being associated with nitrate assimilation on the surface of the ocean (Casciotti et al., 2002). In contrast, $^{15}\varepsilon$:$^{18}\varepsilon$ ratios observed for denitrification in freshwater environments, such as lakes or groundwater, are generally around 0.6 (Bottcher et al., 1990; Lehmann et al., 2003). The $^{15}\varepsilon$:$^{18}\varepsilon$ ratio during molecular diffusion of nitrate, the fractionation factor of which must be a function of the square root of molecular mass, should be around 0.5. Thus, we can differentiate the fractionation processes based on the relationship between $^{15}\varepsilon$ and $^{18}\varepsilon$.

In Lake Mashu, the observed variations in the δ^{15}N and δ^{18}O of nitrate between June and August corresponded to $^{15}\varepsilon = 6.0‰$ and $^{18}\varepsilon = 6.1‰$. Both the $^{15}\varepsilon$ and $^{18}\varepsilon$ values, as well as the $^{15}\varepsilon$:$^{18}\varepsilon$ ratios were reasonable with respect to commonly observed values during assimilation by eukaryotic phytoplankton (Granger et al., 2004, 2010), such as diatoms or chlorophytes. Based on these findings, we can approximate the lake water to be a closed system for nitrate from external/internal sources. In addition, the assimilation of nitrate by phytoplankton (or by periphyton to some extent) in the lake was the major process that controlled temporal variation of nitrate in the lake during the observation period. The uniform average Δ^{17}O values between June and August also support this conclusion because Δ^{17}O values of nitrate must be stable during partial removal through assimilation.

While the in lake assimilation was the major process that controlled both concentration and isotopic compositions of nitrate in the lake, we found evidence of new nitrate contribution from internal/external sources as well. Evaluation of the variations in the δ^{15}N and δ^{18}O values of nitrate at each depth revealed that the $^{15}\varepsilon$:$^{18}\varepsilon$ relationship was somewhat different from 1 (Fig. 5). For example, the $^{15}\varepsilon$:$^{18}\varepsilon$ was 3 at a depth of 20 m. Conversely, the $^{15}\varepsilon$:$^{18}\varepsilon$ was around 0.3 at 200 m (Fig. 5). These were partly due to vertical mixing with the surrounding layer. In other words, each layer was not as rigidly closed as we assumed when calculating $^{15}\varepsilon$ and $^{18}\varepsilon$ for each depth, but vertically mixed with the surrounding layer through eddy diffusion and/or advection. The δ^{15}N and δ^{18}O values of nitrate at 20 m in August, which were close to those observed at 0 m, can be explained by the contribution of the 0 m nitrate to that at 20 m. Most of the original nitrate at the 20 m depth in June might have been exhausted until August through active assimilation at that depth, so that the relative contribution ratio of nitrate from the surrounding layer might become significant in the 20 m layer.

In addition to vertical mixing, a slight contribution of new nitrate must have been responsible for the deviations in the $^{15}\varepsilon$:$^{18}\varepsilon$ ratios from 1. The maximum Δ^{17}O value, which was obtained at the surface in August, was substantially higher than that obtained in June, suggesting that $NO_{3\ \mathrm{atm}}^{-}$ had been deposited from the atmosphere onto the lake surface during summer stratification. Conversely, the minimum $\Delta^{17}O$ value obtained at the bottom (200 m) in August was lower than that obtained in June, suggesting that the accumulated $NO_3^-{}_{re}$ remineralized from sinking organic nitrogen via nitrification at the bottom during summer stratification. Except for samples collected from the surface and the bottom, the $\Delta^{17}O$ values were almost uniform around +2.5 ± 0.4‰. Thus, while the lake assimilation of nitrate was the major process that controlled the distribution and temporal variation of nitrate in the lake, deposition of $NO_3^-{}_{atm}$ to the surface and production of $NO_3^-{}_{re}$ through nitrification in and around the lake and accumulation to the bottom also contributed to the distribution and temporal variation of nitrate in the lake. The magnitude of each flux is discussed in Sect. 3.3 and 3.6.

3.2 Mixing ratio of $NO_3^-{}_{atm}$ to nitrate

The estimated average $\Delta^{17}O$ values of $NO_3^-{}_{atm}$ were uniform at +2.5‰ for both June and August (Table 1), suggesting that the observed average $\Delta^{17}O$ values represented the average $\Delta^{17}O$ value of nitrate in the lake. By using the $\Delta^{17}O$ value of nitrate as $\Delta^{17}O_{lake}$ and the annual average $\Delta^{17}O$ value of $NO_3^-{}_{atm}$ estimated in Rishiri Island (+26.2‰; Tsunogai et al., 2010) as $\Delta^{17}O_{atm}$ in Eq. (2), we estimated the average mixing ratio of $NO_3^-{}_{atm}$ to the total nitrate ($NO_3^-{}_{total}$) in the lake to be 9.7 ± 0.8%. Based on these findings, we concluded that 9.7 ± 0.8% of the total nitrate in the lake originates directly from the atmosphere, therefore, the remainder of nitrate that was of remineralized origin ($NO_3^-{}_{re}$) was produced through nitrification in and around the lake.

The average mixing ratio of $NO_3^-{}_{atm}$ to $NO_3^-{}_{total}$ in Lake Mashu corresponds to those estimated for Lake Tahoe (13%) based on the same $\Delta^{17}O$ method (Michalski and Thiemens, 2006). These findings indicate that the mixing ratios observed in the present study can be considered representative mixing ratios of $NO_3^-{}_{atm}$ to $NO_3^-{}_{total}$ in oligotrophic lakes. In Lake Superior, which is an oligotrophic lake in North America, atmospheric deposition was assumed to be the primary source of nitrate in the lake water column based on the ^{15}N-depleted δ^{15}N values of nitrate (average of −4.1‰; Ostrom et al., 1998). Similar ^{15}N-depleted δ^{15}N values were observed in Lake Mashu as well (Table 1). However, the results of the present study suggest that it is difficult to assume atmospheric nitrate is the primary source of nitrate. The same conclusion was suggested based on the δ^{18}O values of nitrate in Lake Superior as well (Finlay et al., 2007). Thus, we must assume that other sources and/or isotopic fractionation processes led to the ^{15}N-depleted nitrate in the lakes. These processes are discussed in detail in Sect. 3.5.

3.3 Quantification of gross nitrate cycling rate during the observations

Because diazotroph was in minute amounts in Lake Mashu (Haga et al., 1994), primary production in the lake requires either nitrate or ammonium to be supplied to the euphotic layer of the lake as the source of nitrogen. The nitrate in the euphotic zone of the lake must be supplied through nitrification in and around the lake, as well as through atmospheric deposition onto the catchment area of the lake (Fig. 6). While the average mixing ratio of $\mathrm{NO_{3\ atm}^-}$ to $\mathrm{NO_{3\ total}^-}$ was almost uniform in the lake water for the period, we already found evidence of the accumulation of $\mathrm{NO_{3\ atm}^-}$ deposited from the atmosphere at the lake surface, as well as the accumulation of $\mathrm{NO_{3\ re}^-}$ from sinking organic nitrogen within the bottom water during the summer stratification. These findings indicate that $\mathrm{NO_{3\ re}^-}$ must have been produced at a rate that could compensate for the deposition of $\mathrm{NO_{3\ atm}^-}$ at the surface and keep the mixing ratio of $\mathrm{NO_{3\ atm}^-}$ to $\mathrm{NO_{3\ total}^-}$ uniform in the lake water (Fig. 6).

By applying Eq. (2), the total inventory of $\mathrm{NO_{3\ atm}^-}$ in the lake water ($\mathrm{N_{atm}}$) in the initial and temporal observations can be estimated from the following equations:

$$\frac{(\mathrm{N_{atm}})_0}{(\mathrm{N_{total}})_0} = \frac{\left(\Delta^{17}\mathrm{O_{lake}}\right)_0}{\Delta^{17}\mathrm{O_{atm}}}, \tag{8}$$

$$\frac{(\mathrm{N_{atm}})_\mathrm{t}}{(\mathrm{N_{total}})_\mathrm{t}} = \frac{\left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}}{\Delta^{17}\mathrm{O_{atm}}}, \tag{9}$$

where $(\mathrm{N_{atm}})_0$ and $(\mathrm{N_{atm}})_\mathrm{t}$ denote the initial and temporal inventory of $\mathrm{NO^-_{3\ atm}}$ in the lake, $(\mathrm{N_{total}})_0$ and $(\mathrm{N_{total}})_\mathrm{t}$ denote the initial and temporal inventory of $\mathrm{NO^-_{3\ total}}$ in the lake, and $(\Delta^{17}\mathrm{O_{lake}})_0$ and $(\Delta^{17}\mathrm{O_{lake}})_\mathrm{t}$ denote the initial and temporal average $\Delta^{17}\mathrm{O_{lake}}$ values of $\mathrm{NO^-_{3\ total}}$ in the lake, respectively. In addition, $\Delta^{17}\mathrm{O_{atm}}$ denotes the $\Delta^{17}\mathrm{O}$ value of $\mathrm{NO^-_{3\ atm}}$. Adopting the inventory of $\mathrm{NO^-_{3\ total}}$ in the lake water in June as $(\mathrm{N_{total}})_0$ and that in August as $(\mathrm{N_{total}})_\mathrm{t}$ results in $(\mathrm{N_{atm}})_0$ and $(\mathrm{N_{atm}})_\mathrm{t}$ being 0.41 Mmol and 0.20 Mmol, respectively (Table 2).

Because the $\Delta^{17}\mathrm{O}$ value of nitrate is stable during the partial uptake of nitrate through assimilation or denitrification, $(\Delta^{17}\mathrm{O_{lake}})_\mathrm{t}$ can be expressed by the gross nitrate feeding rate through both atmospheric deposition ($\Delta\mathrm{N_{atm}}$) and nitrification in and around the lake ($\Delta\mathrm{N_{nit}}$) to the initial inventories of nitrate in the lake ($(\mathrm{N_{atm}})_0$ and $(\mathrm{N_{total}})_0$),

$$\frac{(\mathrm{N_{atm}})_0 + \Delta\mathrm{N_{atm}}}{(\mathrm{N_{total}})_0 + \Delta\mathrm{N_{atm}} + \Delta\mathrm{N_{nit}}} = \frac{\left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}}{\Delta^{17}\mathrm{O_{atm}}}. \tag{10}$$

By using the Eqs. (8) and (10), we can obtain $\Delta\mathrm{N_{nit}}$ as follows:

$$\Delta\mathrm{N_{nit}} = \frac{\left(\Delta^{17}\mathrm{O_{lake}}\right)_0 - \left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}}{\left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}} \times (\mathrm{N_{total}})_0 + \frac{\Delta^{17}\mathrm{O_{atm}} - \left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}}{\left(\Delta^{17}\mathrm{O_{lake}}\right)_\mathrm{t}} \times \Delta\mathrm{N_{atm}}. \tag{11}$$

The uptake rate of nitrate through assimilation ($\Delta\mathrm{N_{up}}$) can then be estimated from $\Delta\mathrm{N_{nit}}$ using the following equation:

$$\Delta\mathrm{N_{up}} = (\mathrm{N_{total}})_0 + \Delta\mathrm{N_{atm}} + \Delta\mathrm{N_{nit}} - (\mathrm{N_{total}})_\mathrm{t}. \tag{12}$$

The nearby background monitoring station of atmospheric deposition, Cape Ochiishi (43°9′34″ N, 145°30′50″ E), was located on the southeastern side of the lake at a distance of 90 km from the lake centre. The monthly wet deposition rate of $NO_{3\ atm}^{-}$ measured at the station was 0.62 Mmol m^{-2} for July 2007, and 0.53 Mmol m^{-2} for August 2007 (EANET, 2008). Because the dry deposition rate of nitrate consists of 21% of the total (wet + dry) deposition rate in the northern district of Japan (Ministry of the Environment, 2009), we estimated that 0.047 Mmol of $NO_{3\ atm}^{-}$ had been deposited onto the entire catchment area of the lake (32.4 km^2) during the approximately two month period between observations. Using the $NO_{3\ atm}^{-}$ deposition rate as ΔN_{atm} and the annual average $\Delta^{17}O_{atm}$ as $\Delta^{17}O_{atm}$ in Eqs. (11) and (12), we estimated that 0.52 ± 0.34 Mmol of remineralized nitrate had been fed into the lake water through nitrification, while 2.6 ± 0.4 Mmol of nitrate had been removed from the lake water through assimilation during the period between sampling events (Table 2).

3.4 Comparison with primary production rates

To determine if the estimated gross nitrate uptake rate in summer (2.6 ± 0.4 Mmol per 2 months) was reasonable, we compared it with the rate of primary production measured in the lake. The rates of gross primary production measured in the lake water column at depths from 0 to 70 m on 2 September 1985 using the ^{13}C-tracer method (Hama et al., 1983) ranged from 0.81 to 2.50 mg C m^{-3} d^{-1} (CGER NIES et al., 2004). Integrating the gross primary production rates to the entire water column shallower than 70 m gave a gross primary production rate of 0.14 Mmol C d^{-1} for the lake water. Assuming a C:N ratio of 6.6 (Redfield ratio) for primary production, this is equivalent to an N uptake rate of 0.022 Mmol N per day and 1.3 Mmol N per 2 months. The N uptake rate was similar to the nitrate uptake rate of 2.6 ± 0.4 Mmol per 2 months that was estimated based on $\Delta^{17}O$.

However, further corrections are necessary to convert the gross N uptake rate to the gross nitrate uptake rate in the lake, such as (1) reducing the primary production using ammonium and urea, (2) reducing the primary production using atmospheric N_2 (N_2 fixation), (3) adding the primary production that occurs at depths greater than

70 m, (4) correcting temporal changes and seasonal variations in primary production, (5) adding the nitrate uptake by those other than phytoplankton in the water column, such as periphyton (Axler and Reuter, 1996), and (6) correcting uncertainties in the C:N ratio during assimilation in oligotrophic freshwater environments (Axler et al., 1982). Among these possible corrections, important corrections for the present study might be Nos. (1), (5) and (6). If the parameters estimated in Castle Lake (a meso-oligotrophic subalpine lake in California, USA; Axler et al., 1996) were applied to Lake Mashu, the assimilation rate would have to be reduced by a 1/2 through correction (1), but then multiplied by approximately 10 according to correction (5) (Axler and Reuter, 1996), and then reduced by a 1/5 following correction (6) (Axler et al., 1982). Thus, adding the corrections results in a similar rate as that obtained without the correction. Although it is impossible to estimate the exact nitrate uptake rate from primary production data alone, the data regarding primary production also supports that our nitrate uptake rate is highly reliable for the studied lake.

3.5 Processes to produce ^{15}N-depleted nitrate

As described in Sect. 3.1, the δ^{15}N values of nitrate in Lake Mashu were characterised by ^{15}N-depletion, which was especially low in June (−6.1‰ on the average; Table 1). However, comparably ^{15}N-depleted δ^{15}N-NO_3^- values as low as −4.1‰ have been found in Lake Superior (Ostrom et al., 1998), which is an undisturbed oligotrophic lake in North America. Moreover, in the case of oceanic nitrate, δ^{15}N values as low as −6.5‰ (Conception Bay, Newfoundland, Canada; Ostrom et al., 1997) and −8.5‰ (Ise Bay, Japan; Sugimoto et al., 2008) have been found in some coastal water columns. All of these sites were well oxygenated when these depleted ^{15}N nitrate levels were found, which suggests that dissolved nitrate is produced through nitrification, while denitrification plays a minor role in nitrogen cycling.

Nitrification is accompanied by significant fractionations in nitrogen isotopes (Wada et al., 1975; Mariotti et al., 1981), therefore, nitrogen cycling in a water column in which most nitrate is derived from NH_4^+ through nitrification typically results in lower

δ^{15}N-NO$_3^-$ levels than δ^{15}N-NH$_4^+$ levels. For example, in the case of Lake Michigan, the δ^{15}N-NO$_3^-$ is 9‰ lower than the δ^{15}N-NH$_4^+$ level and 2‰ lower than the δ^{15}N-PON level (McCusker et al., 1999). Thus, the observed δ^{15}N values are reasonable for those produced through nitrification under the condition in which the δ^{15}N values of the total fixed-nitrogen pool were low, less than 0‰. To determine if the total fixed-nitrogen pool in Lake Mashu is depleted in ^{15}N, we additionally determined the δ^{15}N values of PON in samples collected from the upper 50 m of the lake in August 2007 (Table 1). The ^{15}N-depleted δ^{15}N values of PON in August (around −2‰; Table 1) suggested that the total fixed-nitrogen pool were low in Lake Mashu. Thus, the primary source of the observed ^{15}N-depleted nitrate in Lake Mashu was nitrification under the condition in which the δ^{15}N values of the total fixed-nitrogen pool were low, around −2‰.

Progress of denitrification in the lake, however, results in ^{15}N-enrichment of residual nitrate through isotopic fractionation during denitrification (Wada et al., 1975; Lehmann et al., 2003; Granger et al., 2008). Thus, not only (1) nitrate production through nitrification under total δ^{15}N values of the fixed-nitrogen pool of around −2‰, but also (2) low potential for the denitrification process in and around the lake, could be responsible for the ^{15}N-depleted δ^{15}N values of nitrate that were observed in the lake water column. The low potential for denitrification in the lake is supported by the low rates of carbon burial in the lake sediment (CGER NIES et al., 2004), as well as the apparent low potential for denitrification due to oxygen enrichment of the water column (Fig. 3). The ^{15}N-depleted feature in the total fixed-nitrogen pool in comparison with other lakes can also be explained by the low potential for denitrification in the lake because active denitrification during nitrogen cycling results in ^{15}N-enrichment of the residual total fixed-nitrogen pool through isotopic fractionation during denitrification.

The δ^{18}O values of nitrate in June (+3.4‰) also indicate that nitrification is the major source of nitrate in the lake. By using the linear Δ^{17}O–δ^{18}O relationship observed in samples of well oxygenated groundwater ($n = 19$) eluted from the background forested watersheds of Rishiri Island, Japan, Tsunogai et al. (2010) estimated the average δ^{18}O value of remineralized nitrate produced through nitrification within the forest ecosystem to be −4.2 ± 2.4‰ by extrapolating the linear relationship to the endmember having $\Delta^{17}O = 0$. While the exchange of oxygen atoms between nitrification intermediates and the ambient water is the dominant process that determines the $\delta^{18}O$ value of nitrate produced through nitrification (Casciotti et al., 2002), we can expect similar $\delta^{18}O$ values for nitrate produced through nitrification in the lake because Lake Mashu is located at almost the same latitude as Rishiri Island (Fig. 1). As a result, similar $\delta^{18}O$ values in ambient water during nitrification can be expected in Lake Mashu and Rishiri Island.

Although the $\delta^{18}O$ values of nitrate in June (+3.4‰) were higher than those estimated for nitrate produced through nitrification (−4.2 ± 2.4‰), this discrepancy can be explained by the contribution of atmospheric nitrate. This is because the lake water nitrate contained 9.7 ± 0.8% of atmospheric nitrate, as estimated in Sect. 3.2. Atmospheric nitrate can be characterised by significant ^{18}O-enrichment (Durka et al., 1994; Burns and Kendall, 2002), as well as ^{17}O. Thus, we can anticipate slight ^{18}O-enrichment in nitrate when compared with pure nitrate produced through nitrification. The quantitative relationship is shown in Fig. 7, in which the average $\delta^{18}O$ value of nitrate in the lake water in June was plotted as a function of $\Delta^{17}O$, together with the hypothetical mixing line between atmospheric nitrate having $\Delta^{17}O$ and $\delta^{18}O$ values of +26.2‰ and +87.1‰, respectively (Tsunogai et al., 2010), and the nitrification-derived nitrate having $\Delta^{17}O$ and $\delta^{18}O$ values of 0‰ and −4.2‰, respectively (Tsunogai et al., 2010). The average $\delta^{18}O$ value of nitrate in the lake water in June (Fig. 7) shows that ca. 10% contribution of atmospheric nitrate to remineralized nitrate produced through nitrification reasonably explains the $\delta^{18}O$ value of lake water nitrate. As shown in Fig. 7, the $\delta^{18}O$ value of surface nitrate (0 m) in the lake water in June was also plotted on the mixing line at the side closer to the endmember of atmospheric nitrate. These results also suggest that, prior to starting assimilation in the lake, the mixing ratio of the atmospheric nitrate to the remineralized nitrate governed the $\delta^{18}O$ value of nitrate in the lake and that an approximately 10% contribution of the atmospheric nitrate to remineralized nitrate produced through nitrification can reasonably explain the $\delta^{18}O$ value of nitrate in the lake water column. Moreover, the hypothetical endmember values of both atmospheric nitrate ($NO_{3\ atm}^-$) and remineralized nitrate ($NO_{3\ re}^-$) represented those in Lake Mashu well.

Such mixing of atmospheric nitrate of around 10% does not contradict the finding that nitrification is the primary source of the ^{15}N-depleted nitrate because the atmospheric nitrate is depleted in ^{15}N as well. The annual average δ^{15}N value has been estimated to be −1.1‰ in eastern Asia (Tsunogai et al., 2010). Accordingly, a mixing ratio of atmospheric nitrate to remineralized nitrate of around 10% with remineralized nitrate could increase δ^{15}N by only 0.5‰ under the conditions in which remineralized nitrate has δ^{15}N values around −6‰. Thus, we conclude that the isotopic fractionation during nitrification reaction, from which ca. 90% of nitrate in the lake was derived, was responsible for the ^{15}N-depletion in the lake. In addition to the δ^{15}N values, the δ^{18}O values of nitrate in Lake Superior (around +2.4‰; Finlay et al., 2007) resembled those of Lake Mashu, suggesting that nitrification in the lake might be the major source of nitrate as well, with atmospheric nitrate serving as the minor source.

Unfortunately, it has been difficult to determine δ^{15}N values of trace nitrate present at concentrations lower than 5 µM using traditional analytical methods (Bremner and Edwards, 1965; Amberger and Schmidt, 1987; Sigman et al., 1997; Silva et al., 2000). Thus, past δ^{15}N data describing nitrate in lakes have primarily been limited to mesotrophic/eutrophic lakes in which nitrate is abundant in the water column (more than 5 µM). These mesotrophic/eutrophic lakes can be characterised by active denitrification in their water column and/or within the sedimentary layer, so that most δ^{15}N data describing nitrate in lakes indicate values greater than 0‰. Recent advances in analytical methods have enabled determination of trace levels of nitrate less than 1 µM (Sigman et al., 2001; Casciotti et al., 2002; McIlvin and Altabet, 2005; Tsunogai et al., 2010). Additional δ^{15}N data describing nitrate in oligotrophic lakes could reveal similar nitrification nitrate having similar low δ^{15}N values around −6‰.

3.6 Annual variation in nitrate in Lake Mashu

When an infinitely long duration for the period for accumulation of both atmospheric nitrate (ΔN_{atm}) and remineralized nitrate produced through nitrification (ΔN_{nit}) is considered in Eq. (10), the initial nitrate in the lake – $(N_{atm})_0$ and $(N_{total})_0$ – can be removed from the equation. Because the observed average Δ^{17}O value of nitrate in the lake represented the average $NO_{3\ atm}^-/NO_{3\ total}^-$ ratio of the entire amount of nitrate that was dissolved in the lake at the point of observation, the observed average Δ^{17}O value in June ($\overline{\Delta^{17}O_{lake}}$) reflected the long range mean value in the relationship between ΔN_{atm} and ΔN_{nit} that was fed into the lake prior to the observation. Thus, we can estimate the average feeding flux of nitrate through nitrification (F_{nit}) using the average feeding flux of nitrate through atmospheric deposition (F_{atm}) from the following equation:

$$\frac{F_{atm}}{F_{atm} + F_{nit}} = \frac{\overline{\Delta^{17}O_{lake}}}{\Delta^{17}O_{atm}}, \tag{13}$$

where $\Delta^{17}O_{atm}$ denotes the Δ^{17}O value of $NO_{3\ atm}^-$.

Based on the average annual wet deposition flux of $NO_{3\ atm}^-$ measured on Cape Ochiishi over the last 5 years (EANET, 2008), the annual deposition flux of $NO_{3\ atm}^-$ onto the entire catchment area of the lake (F_{atm}) is $0.35 \pm 0.2\,\mathrm{Mmol\,a^{-1}}$, so that the annual feeding flux of nitrate through nitrification (F_{nit}) becomes $3.2 \pm 0.3\,\mathrm{Mmol\,a^{-1}}$ in the lake.

The estimated flux is greater than the lower boundary condition for the annual feeding flux of nitrate in the lake to compensate for the net loss of nitrate during the observations in 2007 (2.1 Mmol). Furthermore, the estimated nitrification fluxes coincided with those obtained by applying the estimated instantaneous feeding rate during summer (0.57 ± 0.33 Mmol per 2 months; Table 2) to annual rate ($3.4 \pm 2.0\,\mathrm{Mmol\,a^{-1}}$), suggesting that the estimated annual flux is highly reliable and that the nitrification rate in the lake is almost stable, regardless of the season.

Conversely, if the estimated instantaneous gross uptake rate in summer (2.6 ± 0.4 Mmol per 2 months) would be the same even after the observations at the end of August, all of the residual nitrate in the lake (2.1 Mmol) would be exhausted by the end of October. In the case of the nitrate in the euphotic layer, the assimilation would be completed much earlier, within early September. Thus, the uptake rate in other seasons should be much smaller than the instantaneous rate during summer. Assuming steady state annual variation in nitrate in Lake Mashu, the estimated total annual feeding flux ($F_{\text{atm}} + F_{\text{nit}} = 3.6 \pm 0.4\,\text{Mmol a}^{-1}$) also corresponds to the annual uptake flux of nitrate in the lake through assimilation (Fig. 6). Thus, the rapid instantaneous uptake rate (2.6 ± 0.4 Mmol per 2 months) can be expected to last only another 3 weeks in the lake. That is to say, all of the nitrate assimilation will be completed by the middle of September at the latest. During continuous monitoring using a mooring system from 2008, the annual maximum in the chlorophyll-*a* concentration at 20 m was observed in August, after which it suddenly decreased to the background level in the beginning of September (Tanaka et al., 2009). Although no data for 2007 is available, the temporal variation in chlorophyll-*a* concentration seemed reasonable when compared with our estimation. In conclusion, while both atmospheric deposition and nitrification fed nitrate into the lake at a similar rate regardless of seasons, nitrate removal through assimilation was concentrated in summer, occurring for less than 3 months.

The estimated annual gross uptake mass of nitrate (3.6 ± 0.4 Mmol) was similar to the total nitrate inventory in the upper 100–120 m in June of 2007. Thus, the primary production in the lake appears to be complete when all of the nitrate in the upper euphotic layer is exhausted. Although we have little direct information regarding the limiting nutrients in the lake, the observed annual variation of nitrate in Lake Mashu implies that nitrogen could be a limiting nutrient. Past data describing the distribution of nitrate revealed nitrate concentrations less than 0.1 µM in August or September in the epilimnion (CGER NIES et al., 2004), which also supports this suggestion.

Dividing the total mass of nitrate in June (4.2 Mmol) by the annual average uptake/feeding rate ($3.6 \pm 0.4\,\text{Mmol a}^{-1}$) gave an approximate residence time of nitrate in the lake of 1.2 ± 0.1 years, suggesting that nitrate in Lake Mashu has an ephemeral property. Thus, we can expect atmospheric nitrate deposited into the lake to be rapidly assimilated.

3.7 Implications for the loss of transparency in Lake Mashu

Because nitrogen appears to be one of the nutrients limiting primary production in the lake, an increase in the deposition rate could result in a direct increase in primary production. However, as revealed in this study, atmospheric nitrate is not the only source of nitrate assimilation in the lake through primary production. Rather, it comprises only ca. 10% of the entire nitrate assimilated, therefore, any direct increase in the rate would be only ca. 10% of the increase in the deposition rate. In other words, even if the annual nitrate deposition rate suddenly increased by 20%, the direct increase in primary production using nitrate would be only 2% and, therefore, difficult to detect. Nevertheless, this does not mean that atmospheric nitrate will not have a significant effect on the primary production in the lake.

The present study also revealed a rapid turnover of nitrate in the summer euphotic layer through assimilation, as well as active remineralization of assimilated nitrogen to nitrate, which was probably due to the absence of denitrification processes within the well oxygenated water environment (Fig. 6). Thus, an increase in the deposition flux of atmospheric nitrate (and ammonium, probably) will increase the primary production indirectly in the lake, by increasing the entire fixed nitrogen pool cycling in the lake (Fig. 6). Because the indirect effects that occur through accumulation of fixed nitrogen are long lasting, such an indirect effect would be more important for primary production in the lake.

It should be noted that this conclusion is based on the assumption that nitrogen is always the limiting nutrient in the lake. While Fe is abundant in the lake water (ca. 40 nM; Nojiri, 1992.), dissolved inorganic phosphate is always present at levels below the detection limit (less than 0.1 µM) in the lake water (CGER NIES et al., 2004). As a result, phosphorous components may also be limiting nutrients, especially when nitrogen accumulates in the lake water. To clarify the potential for the effects of phosphorous components available in the lake, the distribution of their concentration, as well as their temporal variations must be determined using highly sensitive analytical methods (Patey et al., 2008). Some incubation experiments to clarify the limiting nutrients are also needed. Furthermore, there is little knowledge available regarding the dynamics of ammonium in the lake, especially how much primary production occurs due to the presence of ammonium, which must be important to evaluate future primary production in the lake. Accordingly, further studies are needed to clarify the geochemical dynamics of nutrients in the lake.

3.8 Comparison with the traditional ^{15}N-labeled tracer method

In most studies that have been conducted to date, the gross uptake rate of nitrate has been estimated by incubation experiments using ^{15}N tracer techniques. In this conventional approach, which was originally introduced by Dugdale and Goering (1967), ^{15}N labeled NO_3^- is added into bottles or mesocosms that simulate in situ conditions, which leads to the production of particulate organic-^{15}N (PO^{15}N) through assimilation over a known incubation period of several hours to several days. This PO^{15}N is then gathered and quantified by mass spectrometry. However, these experimental procedures are generally costly, complicated, and time consuming, especially those that employ mesocosms. Furthermore, the ^{15}N tracer method has several problems with determining accurate nitrate uptake rates. For example, the usual ^{15}N tracer method does not include assimilated nitrogen released to dissolved organic-^{15}N (DO^{15}N) during incubation within the estimated uptake rates, which results in the rates being underestimated (Slawyk et al., 1998). Additionally, incubation itself could also result in the production of artifacts by changing the physical/chemical environments. Finally, it is difficult to simulate nitrate uptake by periphyton or microbes on the lake floor through typical incubation in bottles (Axler and Reuter, 1996).

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

The gross nitrate uptake rate estimated using Δ^{17}O values of NO_3^- (Δ^{17}O method) overcomes the aforementioned problems inherent in the conventional ^{15}N-labelled tracer methods. Accordingly, the Δ^{17}O method can be an alternative to standard techniques for the determination of accurate gross nitrate uptake rates in lakes that contain detectable quantities of atmospheric nitrate within the total nitrate.

In addition to determining the gross nitrate uptake rate, this study clearly demonstrated that Δ^{17}O can be a powerful tracer describing the fate of $NO_{3\ \mathrm{atm}}^-$ deposited onto an oligotrophic hydrosphere, even if it has only a small fixed-nitrogen input. By measuring the Δ^{17}O data describing nitrate in various hydrosphere, we can increase our understanding of the fixed-nitrogen processing and fixed-nitrogen retention efficiencies in hydrospheric ecosystems that are subjected to the input of atmospheric fixed-nitrogen.

4 Conclusions

The nitrification is the major source of nitrate in Lake Mashu. The average mixing ratio of atmospheric nitrate to total nitrate was only 9.7 ± 0.8% in the lake. Besides, there is low potential for denitrification in and around the lake. The lake water dissolved nitrate was characterised by rapid removal through assimilation during summer until it was almost completely removed from the euphotic layer, as well as continuous feeding into the lake through nitrification and deposition, regardless of the seasons, which corresponds to a gross annual flux of 3.2 ± 0.3 Mmol a^{-1} and 0.35 ± 0.2 Mmol a^{-1}, respectively. Thus, atmospheric nitrate deposited into the lake will be assimilated quickly having a mean residence time of 1.2 ± 0.1 years. Besides, more than 90% of the assimilated nitrate will be remineralized to nitrate and assimilated again via active nitrogen cycling in the lake, as schematically shown in Fig. 6.

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Table 1. Concentration and stable isotopic compositions (δ^{15}N, δ^{18}O and Δ^{17}O) of nitrate and PON in lake water samples.

	25 June 2007				24 August 2007					
Depth (m)	[NO_3^-] (µM)	δ^{15}N (‰)	δ^{18}O (‰)	Δ^{17}O (‰)	[NO_3^-] (µM)	δ^{15}N (‰)	δ^{18}O (‰)	Δ^{17}O (‰)	[PON] (µM)	δ^{15}N (‰)
0	1.6	−6.3	+7.3	+3.7	0.6	−1.2	+11.0	+4.3	0.9	−2.3
20	1.5	−5.4	+3.1	+2.4	0.4	−2.5	+11.1	+2.7	0.8	−2.8
50	1.5	−6.9	+3.2	+2.4	0.8	−3.7	+7.2	+2.8	1.2	−0.5
100	1.5	−7.6	+3.2	+2.4	0.8	−2.7	+7.3	+2.4	–	–
150	1.6	−6.2	+3.0	+2.6	1.2	−1.6	+7.3	+2.1	–	–
200	1.5	−5.5	+3.1	+2.7	1.3	−1.7	+4.3	+1.6	–	–
Avg.	1.6	−6.5	+3.4	+2.5	0.8	−2.5	+7.6	+2.5	–	–

– = not determined

Table 2. Variations in the total inventory of nitrate (N_{total}) in Lake Mashu, together with those of atmospheric nitrate (N_{atm}) and remineralized nitrate (N_{re}). Each net and gross variation are presented as well, together with gross variation in atmospheric nitrate through depositions (ΔN_{atm}), remineralized nitrate through nitrification (ΔN_{nit}), and nitrate uptake through assimilation (ΔN_{up}) in the lake during the observations. All units are in Mmol N.

	Inventory		Net variation	Gross variation	
	25 June	24 August		Feed	Remove
N_{atm}	0.41	0.20	−0.20	+0.047[*] (ΔN_{atm})	−0.26
N_{re}	3.8	1.9	−1.9	+0.52 (ΔN_{nit})	−2.4
N_{total}	4.2	2.1	−2.1	+0.57	−2.6 (ΔN_{up})

[*] EANET (2008)

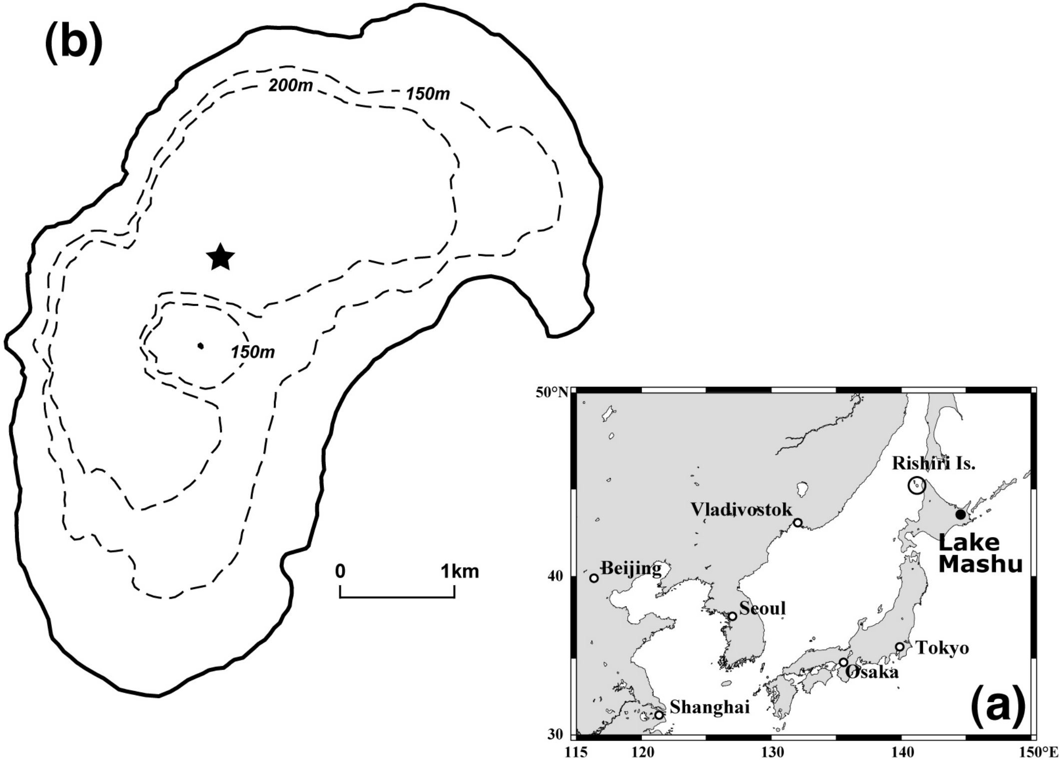

Fig. 1. Maps showing the location of Lake Mashu in eastern Asia **(a)** and the sampling station (asterisk) plotted on the bathymetry of Lake Mashu **(b)**.

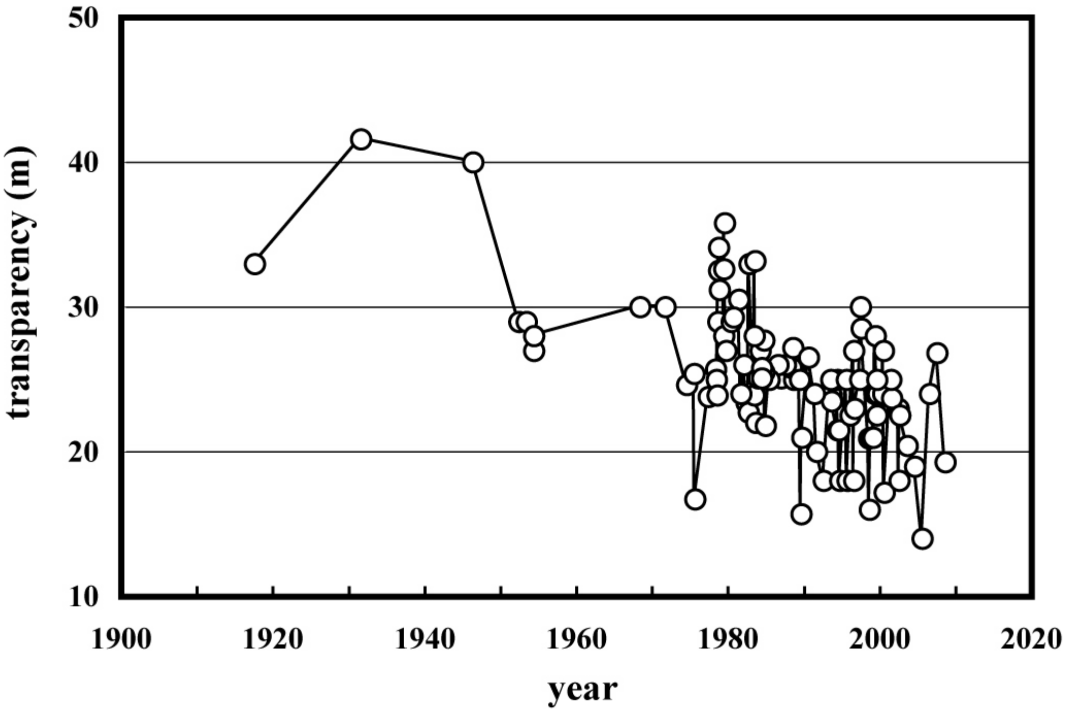

Fig. 2. The trend for transparency (Secchi depth; in metres) measured at the central station of Lake Mashu. Data prior to 2003 are cited from the compiled data set in CGER NIES et al. (2004). Recent data were provided by the UNEP GEMS/Water Programme.

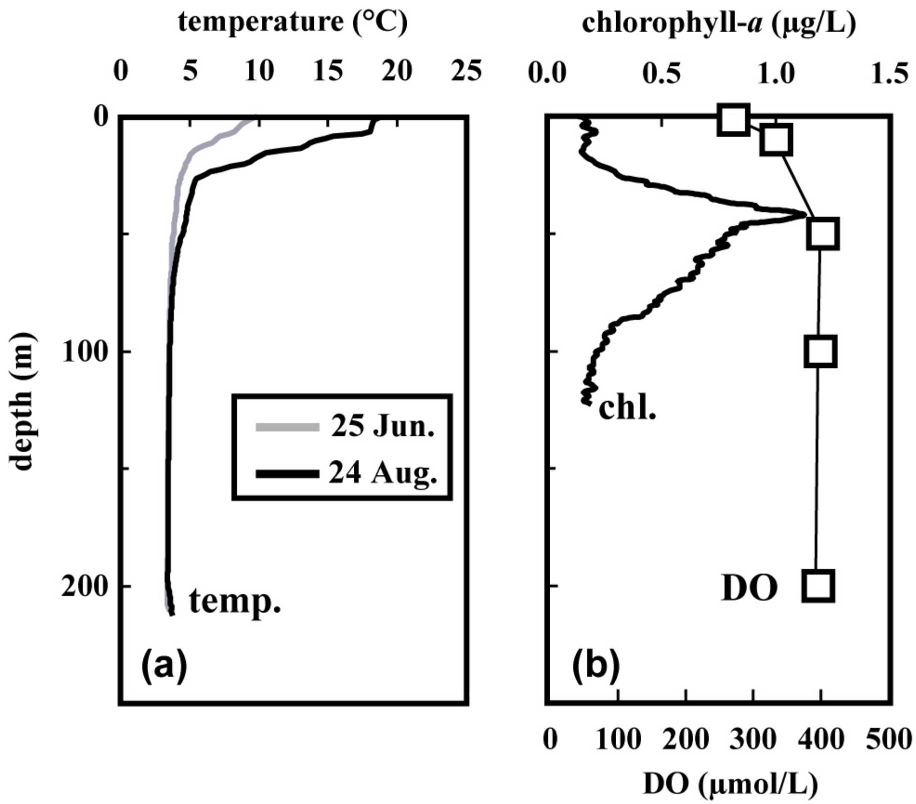

Fig. 3. Vertical distribution of temperature in June 2007 (grey line) and August 2007 (bold line) **(a)**, chlorophyll-*a* concentration (bold line) and dissolved oxygen concentration (DO; white square) in August 2007 **(b)** at Lake Mashu.

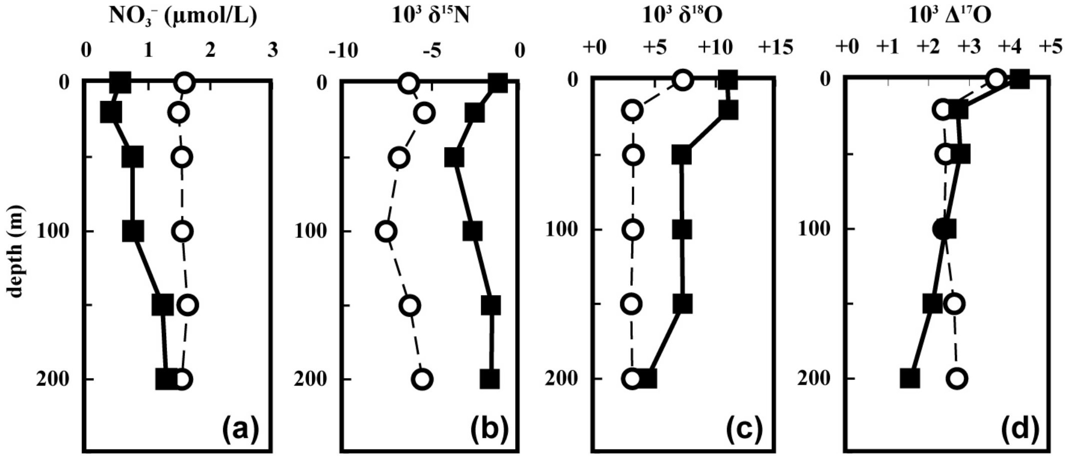

Fig. 4. Vertical distribution of concentration **(a)**, δ^{15}N **(b)**, δ^{18}O **(c)**, and Δ^{17}O **(d)** of nitrate at Lake Mashu in June (white circle) and August (black square) 2007.

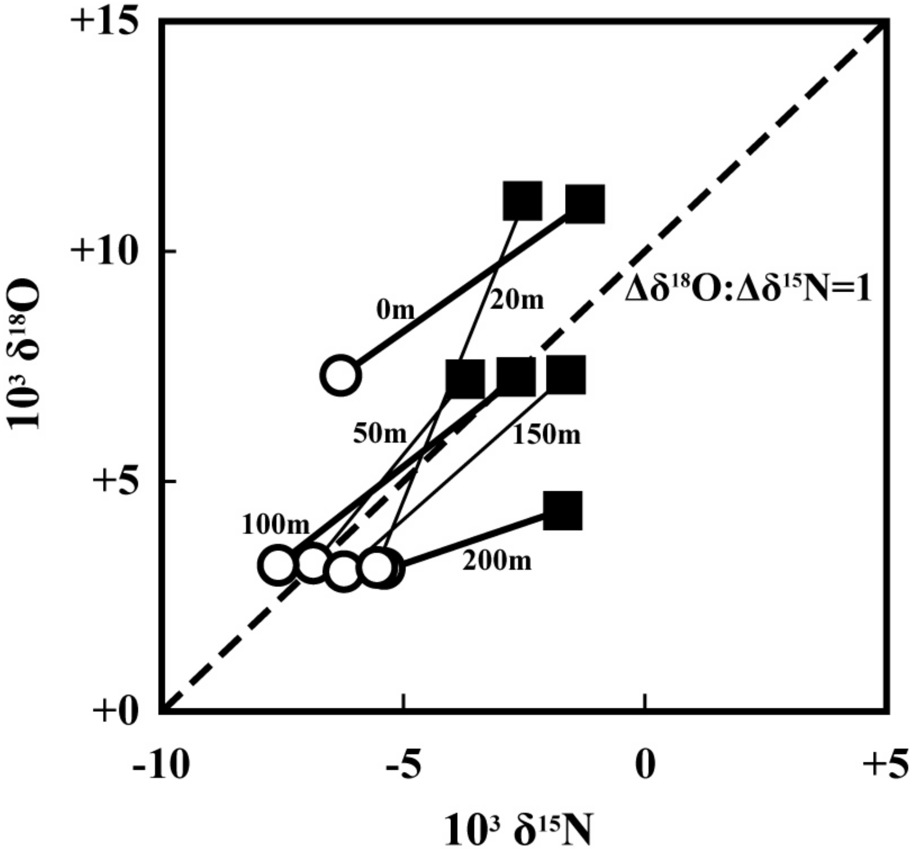

Fig. 5. Temporal changes in the δ^{18}O and δ^{15}N values of nitrate at each depth during the period between observations. The symbols are the same as those in Fig. 4.

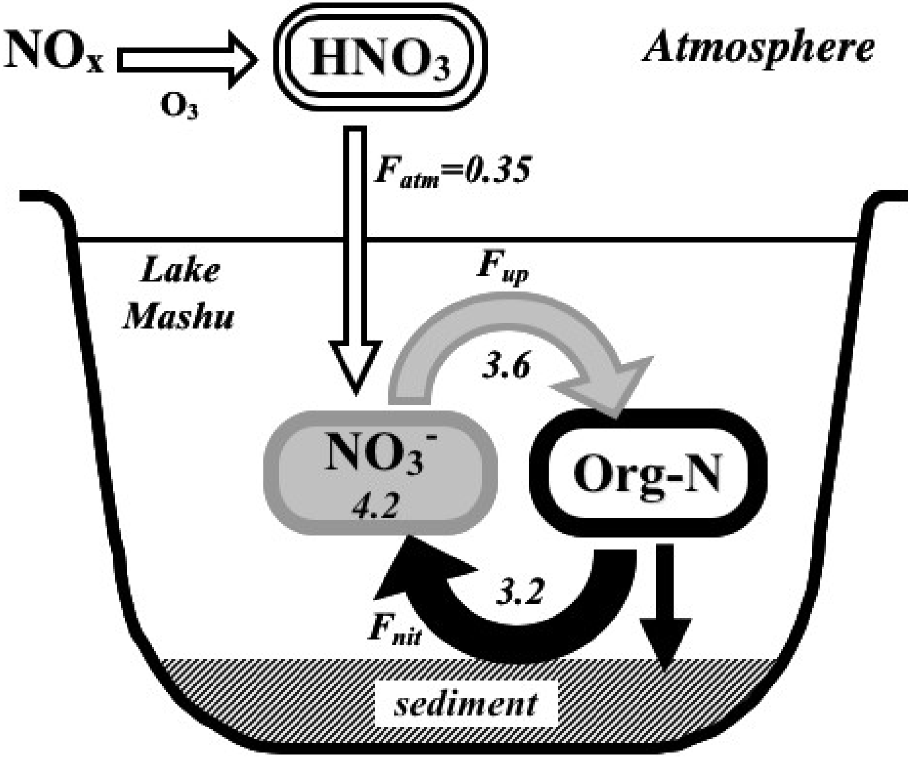

Fig. 6. Schematic diagram showing the nitrate cycling dynamics in Lake Mashu. The magnitude of the nitrate reservoir in June is presented (unit: Mmol), together with the estimated annual fluxes between each reservoir (unit: Mmol N a^{-1}).

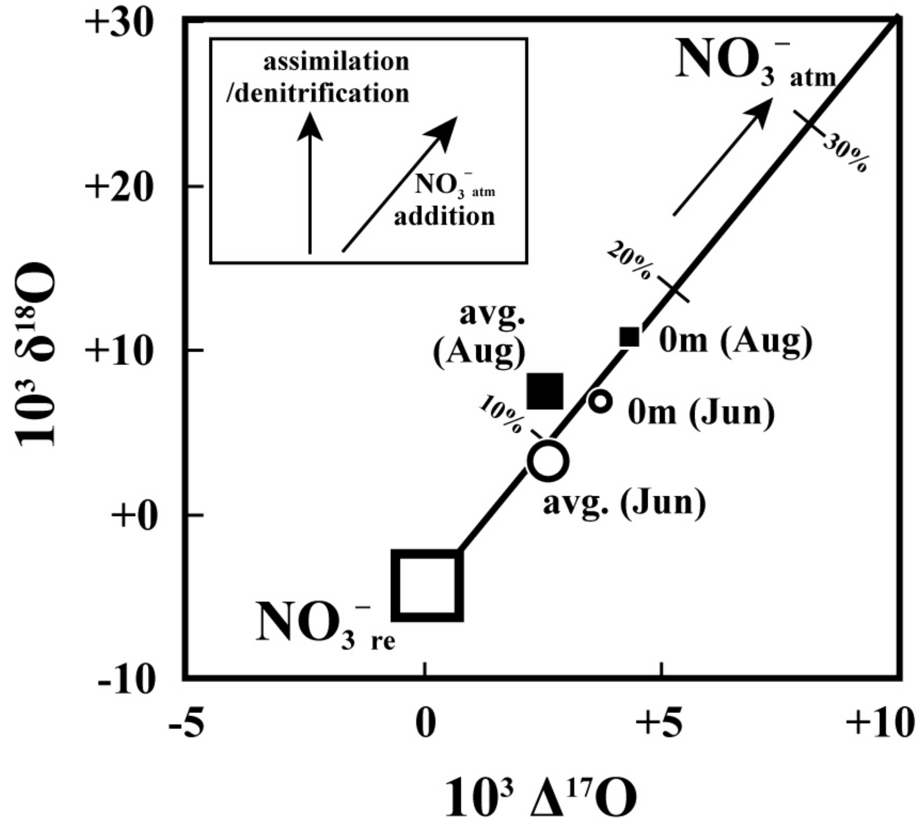

Fig. 7. Relationship between the values of $\Delta^{17}O_{avg}$ and $\delta^{18}O_{avg}$ of $NO^-_{3\ total}$ in the lake water (June: large white circle, August: large black square), as well as those of NO_3^- in the surface (0 m) water (June: small white circle, August: small black square). A hypothetical mixing line between atmospheric nitrate ($NO^-_{3\ atm}$) and remineralized nitrate ($NO^-_{3\ re}$) is shown, together with the mixing ratios of $NO^-_{3\ atm}$ on the line and the endmember value of $NO^-_{3\ re}$ (large white square).