

Variations of the nitrate isotopic composition in the St. Lawrence River caused by seasonal changes in atmospheric nitrogen inputs

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Abstract We present 42 dual-isotope nitrate analyses of fresh water samples collected in the St. Lawrence River between June 2006 and July 2008. Measured $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ values correlate negatively, while $\delta^{18}\text{O}\text{-NO}_3^-$ displays no negative correlation with nitrate concentration. This suggests that nitrate uptake and/or elimination by denitrification is not the main driver of observed variations in nitrate concentration and isotopic signature in the St. Lawrence River. In addition, $\delta^{18}\text{O}\text{-NO}_3^-$ is negatively correlated with the seasonally variable $\delta^{18}\text{O}$ of ambient water, indicating that the variation in the isotopic signature of nitrate is barely modulated by in-stream nitrate regeneration (nitrification). It rather is constrained by along-river changes in the external sources of nitrate. Given the distinct nitrogen (N) and oxygen (O) isotopic signature of atmospheric nitrate, we argue that observed seasonal

variations of $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ in the St. Lawrence River are due to variable contributions of snowmelt-derived water. Based on a N and O isotope mass balance, we show that total nitrate loading in the St. Lawrence River is dominated by a N input from the Great Lakes ($47 \pm 28\%$) and from nitrate regeneration of both internal and external N ($48 \pm 22\%$). While temporal nitrate N and O isotope dynamics in the St. Lawrence River are mainly influenced by the atmospheric N input fluctuations, with an increase in atmospheric loading during spring, atmospheric N plays overall a rather insignificant role with regards to the N budget ($5 \pm 4\%$).

Keywords St. Lawrence · Hydrology · Time series · Nitrogen · Isotope · Eutrophication

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Introduction

Riverine transport and processing of nitrogen (N) are major links between terrestrial and marine/estuarine nitrogen cycles, and exert a strong influence on productivity and biodiversity of coastal marine ecosystems (Galloway et al. 2004). Estuaries are particularly sensitive to terrestrial organic matter (OM) and nutrient inputs (Gearing and Pocklington 1990), which can affect primary productivity, OM deposition, and OM remineralization rates. As a major nutrient, fixed (i.e., bio-available) N is partly responsible for

estuarine eutrophication, negatively affecting the health of coastal marine ecosystems (Nixon et al. 1996; Howarth 1998; Howarth et al. 2006). Anthropogenic N inputs from agricultural and fossil fuel sources have increased tenfold globally since the late nineteenth century, adding bio-available N to coastal waters (Galloway et al. 2004).

Recent research in the Laurentian Channel has shown that an hypoxic zone of approximately 1,300 km² has developed in the bottom waters of the Lower St. Lawrence Estuary (LSLE) over the last decades (Gilbert et al. 2005). Climate-driven changes in ocean circulation patterns, which determine the partitioning of water masses that enter the Gulf of St. Lawrence from the North Atlantic through Cabot Strait, may have contributed to the historic decline in dissolved oxygen (DO) (Gilbert et al. 2005; Thibodeau et al. 2010a). However, eutrophication and increased organic particle fluxes may also have caused a decline in DO resulting from enhanced microbial respiration, mostly within the sediments (Benoit et al. 2006; Thibodeau et al. 2006; Lehmann et al. 2009). The observed decline in oxygen content in the LSL bottom waters appears to coincide with a 70 % increase of fertilizer sold within the St. Lawrence watershed between 1970 and 1988 (Thibodeau et al. 2006). Hence, it is reasonable to assume that anthropogenic inputs of N, as well as climatic/oceanographic constraints, both play a role in the generation and maintenance of hypoxic conditions in the LSL (Thibodeau et al. 2010b). While we know that the major sources of nitrate in large catchments are generally from agricultural areas (chemical fertilizers and manure), soils, and atmospheric precipitation (reviewed by Kendall et al. 2007), the contributions of the various N sources to the St. Lawrence system are poorly understood, and their relative contribution can be expected to vary with time. Sufficient measures to preserve the integrity of an aquatic system such as the St. Lawrence River (SLR) and estuary require identification of the main fixed-N sources and the fate of each nitrogen source within this system (i.e., in-stream cycling through assimilation, denitrification, remineralization or nitrification).

Nitrate N isotopes ($\delta^{15}\text{N}-\text{NO}_3^-$; for definition see method section), and more recently nitrate O isotopes ($\delta^{18}\text{O}-\text{NO}_3^-$) have proven to be excellent tracers of nitrate sources. The dual isotope approach is based on the fact that nitrate from different origins have distinct

isotopic signature ranges (see Kendall et al. 2007 for a review). For example, inorganic nitrate fertilizers show significantly higher $\delta^{18}\text{O}$ values compared to most other nitrate sources, whereas their $\delta^{15}\text{N}$ value is generally quite low. In contrast, nitrate derived from organic sources tend to exhibit elevated $\delta^{15}\text{N}$ values, but comparatively low $\delta^{18}\text{O}$ values (Amberger and Schmidt 1987). For simple point-source analyses, these generalizations are useful. However, in riverine systems the situation may be more complex. The isotopic composition of SLR nitrate, for example, is not necessarily only modulated by variable nitrate source loading, but may also depend on isotopic fractionation during in-stream removal processes such as denitrification, assimilation, and in-stream nitrification (i.e., regeneration of nitrate with a different isotopic signature than nitrate from external sources). The dual isotopic analysis of nitrate allows the identification and assessment of coincidental processes such as (1) N-elimination processes (i.e., denitrification and assimilation), (2) nitrification and (3) input from multiple external sources that may have a cancelling effect on the nitrate concentration and $\delta^{15}\text{N}-\text{NO}_3^-$ alone (Burns and Kendall 2002; Mayer et al. 2002; Lehmann et al. 2003, 2004; Kendall et al. 2007; Burns et al. 2009).

Denitrification refers to the dissimilatory reduction of NO_3^- to gaseous products (N_2 , N_2O , or NO) and usually occurs when O_2 concentrations are lower than 20 μM (Kendall et al. 2007). Denitrification causes the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the residual nitrate pool to increase exponentially as nitrate concentration decreases (Kendall et al. 2007). Measured nitrate isotope enrichment factors are variable and range from 10 to 40 ‰, with a nitrate N:O isotope enrichment ratio ($\Delta\delta^{18}\text{O}/\Delta\delta^{15}\text{N}$) of 0.5–1 (Böttcher et al. 1990; Cey et al. 1999; Lehmann et al. 2003; Granger et al. 2004a; Panno et al. 2006). To some parts, the extent of measured fractionation depends on environmental conditions. For example, strong nitrate isotope fractionation is associated with water column denitrification (up to 40 ‰), expressed in the high N and O isotopic composition of residual NO_3^- , whereas denitrification within sediments (0–3 ‰) is limited by diffusive substrate supply so that the expression of the biological N isotope fractionation in the environment is significantly suppressed (Lehmann et al. 2004, 2007; Sigman et al. 2005; Alkhatib et al. 2012). While denitrification in the water column should not play a

role in the SLR at given DO concentrations, closed system conditions are often prevalent in anaerobic soil compartments along the groundwater flow path in aquifers and riparian zones, and occasionally in river sediments (Kellman and Hillaire-Marcel 1998; Sebilo et al. 2003). Nitrate that has undergone partial denitrification in these watershed compartments should, therefore, have elevated $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values compared to input values. Another removal process that is known to discriminate between nitrogen (and O) isotopes is the assimilation of inorganic nitrogen compounds into living organisms via biosynthesis (Altabet et al. 1999). As with dissimilative nitrate reduction by denitrifiers, the nitrate assimilation results in a strongly coupled N and O fractionation (Granger et al. 2004b; Lehmann et al. 2005), with $\Delta\delta^{18}\text{O}/\Delta\delta^{15}\text{N}$ of 1:1 in saltwater environments.

During the regeneration of nitrate by nitrification, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of new nitrate are affected in different ways (Sigman et al. 2005; Bourbonnais et al. 2009). The N-isotopic composition of nitrate produced in-stream by nitrification is principally constrained by the $\delta^{15}\text{N}$ of the OM that is subjected to remineralization. A small isotope fractionation usually accompanies the aerobic mineralization of OM, and results in ammonium with a $\delta^{15}\text{N}$ value 0–4 ‰ lower than the original bulk OM (Lehmann et al. 2002; Dijkstra et al. 2008). Nitrification of ammonium to nitrate is associated with large ^{15}N isotope effects (14–38 ‰; Casciotti et al. 2003). This process may be difficult to observe in river systems, where remineralization and nitrification proceed nearly in parallel and to completion (i.e., with no ammonium accumulation) (Miyajima et al. 2009). Thus, we can expect that nitrate from nitrification will have a $\delta^{15}\text{N}$ slightly lower or equal (of about 0–4 ‰) to the $\delta^{15}\text{N}$ of the particulate OM. For a long time, it has been argued that up to 1/3 of the O atoms in regenerated nitrate derives from DO (Aleem et al. 1965; Hollocher 1984). However, more recent work has shown that ultimately (due to strong nitrifier-catalyzed nitrite-water exchange) the contribution of oxygen atoms in marine and freshwater nitrate from DO is minor or even nil (Casciotti et al. 2002; Wankel et al. 2006, 2007; Knapp et al. 2008; Bourbonnais et al. 2009). Irrespective of the exact mechanisms that determine the $\delta^{18}\text{O}$ of newly-nitrified nitrate, seasonally-driven variation in the $\delta^{18}\text{O}$ of river water should leave its imprint in the $\delta^{18}\text{O}$ of nitrate that has been regenerated in-stream.

There are various potential external sources of nitrate in riverine systems: (1) nitrate derived from natural N_2 -fixation followed by remineralization and nitrification in watershed soils, (2) anthropogenic nitrate derived from manure, inorganic fertilizers, septic and animal waste, and domestic and industrial wastewaters and (3) nitrate derived from atmospheric deposition of nitrogen oxides in the watershed. Each pool of nitrate is characterized by generally distinct values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. While nitrate derived from N_2 -fixation will carry a low- $\delta^{15}\text{N}$ signature, nitrate originating from manure, septic or animal waste, and domestic waste typically display high $\delta^{15}\text{N}$ values (+10 ‰ or higher; McClelland and Valiela 1998; Bedard-Haughn et al. 2003). Since nitrate from these sources are produced via nitrification in the soil, the corresponding $\delta^{18}\text{O}$ should reflect the $\delta^{18}\text{O}$ of the ambient soil water, which may be variably enriched in ^{18}O through evaporation. Synthetic fertilizers are characterized by low $\delta^{15}\text{N}$ - NO_3^- (0 to +5 ‰) and their $\delta^{18}\text{O}$ - NO_3^- depends on whether they are applied in reduced form (ammonium) or directly as nitrate. Nitrification of fertilizer ammonium will lead to a $\delta^{18}\text{O}$ - NO_3^- , that again reflects the $\delta^{18}\text{O}$ of ambient water during nitrification (in the catchment or later in the river), while fertilizer in nitrate form will carry a $\delta^{18}\text{O}$ - NO_3^- that is significantly higher (~20 ‰) (Amberger and Schmidt 1987). However, nitrate fertilizers are used less frequently than ammonium fertilizers by farmers in Québec (Agriculture and Agri-Food Canada, Korol 2002) and are therefore less likely to play a major role in the seasonal variation of $\delta^{18}\text{O}$ - NO_3^- . Nitrate from atmospheric deposition is generally heavily enriched in ^{18}O ($\delta^{18}\text{O} > 60$ ‰; Kendall et al. 2007), and $\delta^{18}\text{O}$ measurements close to the study area (Vermont) averaged +83 ‰ for an annual cycle (Ohte et al. 2004). In the area surrounding the Great Lakes and the SLR watershed, measured $\delta^{15}\text{N}$ - NO_3^- values in precipitation samples ranged from -5.4 to +0.6 ‰ (reported in Kendall et al. 2007). Those estimates are consistent with time-series nitrate isotope data from a site within the Great Lakes watershed, with seasonal means of +65 and +90 ‰ and -6 to 0 ‰, for nitrate $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$, respectively, with the lower values observed in summer (Finlay et al. 2007). Since, on average, nitrate from precipitation displays significantly higher $\delta^{18}\text{O}$ - NO_3^- , and lower $\delta^{15}\text{N}$ - NO_3^- , than the nitrate isotope values observed in streams (Kendall et al. 2007), the

leverage effect can be strong and a concomitant increase in $\delta^{18}\text{O}\text{-NO}_3^-$ and decrease in $\delta^{15}\text{N}\text{-NO}_3^-$ during the spring would suggest a sensitive response of the riverine nitrate pool to an elevated contribution of N from atmospheric precipitation due to the spring snowmelt.

We present 42 dual-nitrate isotope measurements of nitrate sampled from the SLR at its outlet (Québec City) to the estuary, beginning in June 2006 and ending July 2008. We hypothesize that nitrate in the St. Lawrence at Québec City likely derives from three main sources (Great Lakes, atmospheric deposition and external sources ultimately nitrified in-stream or in the catchment) and that variation in the isotopic composition of nitrate are mostly constrained by seasonal changes in the partitioning of these sources. In order to test this hypothesis we first need to evaluate potential N-elimination and nitrification influence on the isotopic composition of nitrate on a seasonal timescale in the SLR, potentially masking external nitrate source signatures. We will compare the nitrate- $\delta^{18}\text{O}$ data to water- $\delta^{18}\text{O}$ data obtained from the same location during the same time interval, in order to assess if and how natural $\delta^{18}\text{O}$ variations of the stream water affect variations in the isotopic composition of the riverine nitrate pool, and what, if any, information on in-stream-nitrate regeneration can be gained from these observations. Finally we will quantitatively validate our hypothesis using an isotopic mass balance model to assess the partitioning between the various N sources that contribute to the St. Lawrence N loading.

Methods

Dissolved nitrate concentrations were determined at the Geochemistry and Geodynamics Research Center (GEOTOP) using a Braan and Luebbe autoanalyzer, with a detection limit of $\sim 0.1 \mu\text{mol L}^{-1}$ (Strickland and Parsons 1972). Stable N and O isotope ratios of dissolved nitrate ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$; where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1,000$, and R refers to the $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$ ratios) were measured using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002). Sample nitrate (and nitrite) was converted to nitrous oxide (N_2O) by denitrifying bacteria that lack N_2O reductase activity (*Pseudomonas chlororaphis* ATCC #43928 or ATCC #13985). The N_2O was purged from the sample vial with helium carrier gas, cryo-

concentrated, and purified using a Micromass Trace-GasTM inlet. The N and O isotopic composition was determined using a Micromass IsoprimeTM universal triple collector isotope ratio mass spectrometer in continuous flow mode. The target sample size was always 20 nmol. Blank contribution was generally lower than 0.3 nmol ($\sim 1.5\%$ of the sample). For $\delta^{18}\text{O}$ isotope analyses, only *P. chlororaphis* ATCC #13985 (subspecies *aureofaciens*) was used. Oxygen isotope exchange with the ambient water during N_2O production resulted in a $\delta^{18}\text{O}$ -scale compression, which was corrected according to Casciotti et al. (2002). The O isotope exchange was never higher than 5%. Based on replicate measurements of laboratory standards and samples (intra- and inter-run), the reproducibility (1σ) of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were better than ± 0.3 and $\pm 0.5\%$, respectively. Isotopic values were normalized using IAEA-N3, which has a $\delta^{15}\text{N}$ value of $+4.72 \pm 0.13\%$ relative to atmospheric N_2 (Gonfiantini et al. 1995) and a $\delta^{18}\text{O}$ value of $+25.6 \pm 0.2\%$ relative to V-SMOW (Bohlke et al. 2003). All isotope measurements are reported in the delta notation in permil (‰), relative to atmospheric N_2 (AIR) for $\delta^{15}\text{N}$ and VSMOW for $\delta^{18}\text{O}$.

The oxygen isotopic composition of water was analyzed at the GEOTOP research center. Water samples were collected in 60 mL plastic bottles, with no headspace, and stored at 4 °C. Unfiltered water (200 μL) was equilibrated with 200 μL of CO_2 at 40 °C. The water/ CO_2 equilibration progressed for 7 h, after which the CO_2 was sampled and measured on a Micromass IsoprimeTM universal triple collector isotope ratio mass spectrometer in dual inlet mode coupled to an AquaprepTM system. The resulting isotopic values were drift and temperature-corrected using two internal laboratory standards previously calibrated on the VSMOW-SLAP scale. The overall analytical uncertainty of the water O-isotope analysis was $\pm 0.05\%$. All reported correlation coefficients (R^2) and two-tailed *P* values were estimated using the Spearman nonparametric correlation between measured variables using the software Prism 6©.

Regional settings

All samples were collected from a water filtration plant located on the south shore of the SLR, near its outlet to the upper St. Lawrence Estuary, close to Québec City (see Hélie and Hillaire-Marcel 2006 for

methods). Here, the SLR water represents a mixture of various water sources originating from the Great Lakes and northern tributaries, such as the Ottawa River (Fig. 1; Table 1). The discharge from Lake Ontario does not display significant seasonal variations. In contrast, discharge from tributaries can be strongly modulated by the seasonal hydrological cycle. During spring snowmelt, outflow rates are three times higher than the mean annual discharge (Environment Canada, unpublished data). Hence, the proportional contribution from Great Lakes water to the total discharge at Québec City varies from >70 % in summer to <55 % in spring (Environment Canada, unpublished data). Base-flow conditions can be

assumed for the summer months. The watershed is composed of different eco- and land use zones, i.e., boreal and mixed forests, as well as agricultural and urban areas, which are located primarily around the Great Lakes and the SLR (Fig. 1).

Results

Nitrate concentrations in the SLR exhibited strong annual cyclicity, with minimal concentrations (<15 $\mu\text{mol L}^{-1}$) in fall and maximum concentrations (>30 $\mu\text{mol L}^{-1}$) in winter/spring (Fig. 2). The $\delta^{15}\text{N-NO}_3^-$ values increased during the summer and

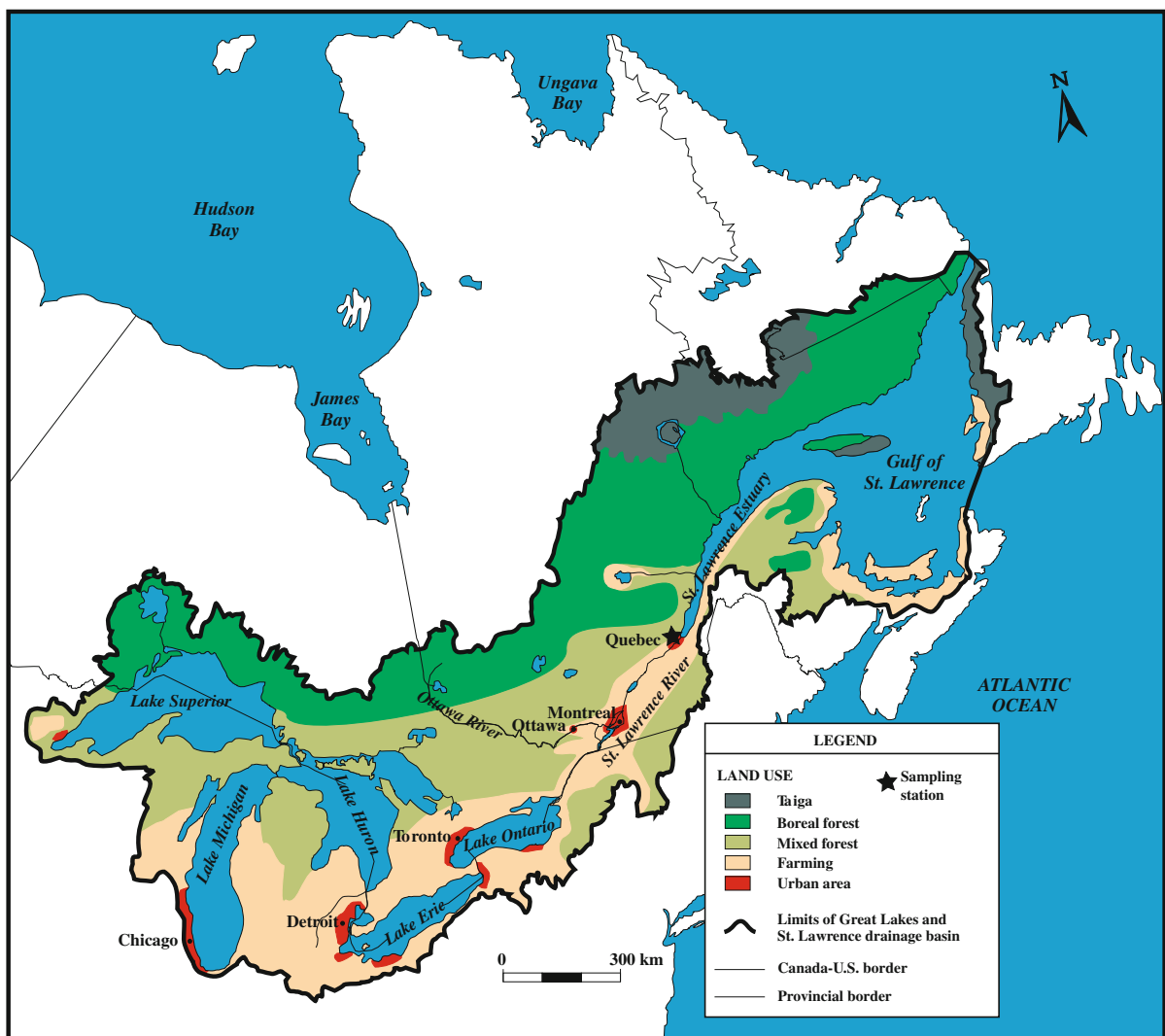


Fig. 1 Map of land use in the Great lakes and the St. Lawrence River watershed (Environment Canada)

Table 1 Annual mean discharge at Québec City and from the Great Lakes (Lake Ontario) and northern tributaries (Ottawa, Saint-Maurice and Richelieu Rivers) (Environment Canada, unpublished data)

Station	Discharge ($\text{m}^3 \text{s}^{-1}$)
Québec City	12,300
Lake Ontario	7,500
Ottawa River	2,000
Saint-Maurice River	660
Richelieu River	360

climaxed in the fall ($>7\%$), then decreased during the winter months to a minimum in April ($<4.5\%$; Fig. 2). Values for $\delta^{18}\text{O}-\text{NO}_3^-$ parallel the nitrate concentration data, with a minimum ($<3\%$) at the end of the summer (August) and a maximum ($>6\%$) in April (Fig. 2). As a logical consequence, $\delta^{18}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NO}_3^-$ show a negative correlation ($R^2 = 0.60$, $P < 0.0001$; Fig. 3). The observed correlation between $\delta^{18}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NO}_3^-$ is similar for every season except fall, when no trend is apparent. Strong intra-seasonal variability is observed in the spring but seems to be of less importance during other seasons (Table 2). Mean $[\text{NO}_3^-]$, $\delta^{15}\text{N}-\text{NO}_3^-$ and

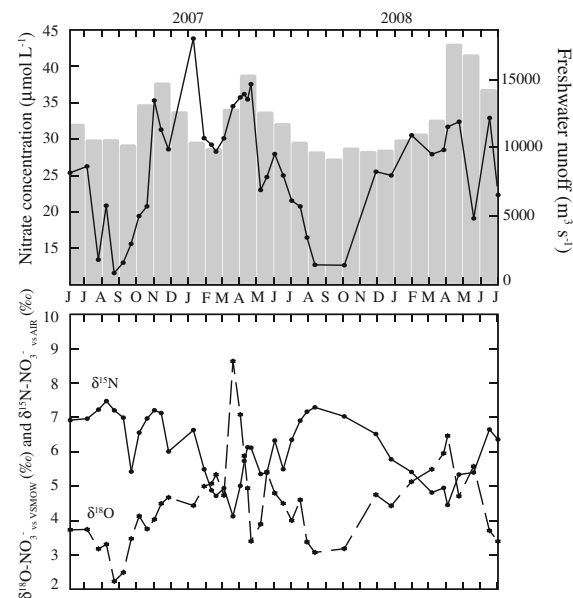


Fig. 2 Nitrate concentrations and histogram of monthly averaged freshwater runoff (upper panel) and nitrate isotope values (lower panel) in the St. Lawrence River (Québec City) ($\delta^{15}\text{N}$: solid line; $\delta^{18}\text{O}$: dashed line) from June 2006 to July 2008

$\delta^{18}\text{O}-\text{NO}_3^-$ show distinct, discernible signatures for each season except between winter and spring, which may be due to the gradual (but irregular) input of snowmelt, as illustrated by the larger standard deviation of $\delta^{18}\text{O}-\text{NO}_3^-$ data in spring. The $\delta^{18}\text{O}-\text{H}_2\text{O}$ at Québec City obeys a seasonal cycle, with ^{18}O -depleted values during the spring during snowmelt, and more ^{18}O enriched values in the fall (Fig. 4).

Discussion

In-stream nitrate removal processes

The present dataset is characterized by a significant negative correlation between $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$, yet the correlation of $\delta^{18}\text{O}-\text{NO}_3^-$ and nitrate concentration is positive ($R^2 = 0.32$, $P < 0.0001$). These observations suggest that the isotopic variations throughout the year cannot be attributed solely to seasonally variable removal processes within the stream water. While the lack of negative correlation between nitrate concentration and the $\delta^{18}\text{O}-\text{NO}_3^-$ could be due to denitrification occurring under diffusion-limiting conditions (i.e., in the sediment), which would likewise limit isotopic fractionation (Lehmann et al. 2007), the negative $\delta^{18}\text{O}-\text{NO}_3^-$ versus $\delta^{15}\text{N}-\text{NO}_3^-$ correlation speaks against denitrification in general as the dominant modulator of nitrate concentrations and isotope ratios. Denitrification within the system cannot totally be excluded, yet, if present at all, the isotopic fractionation linked to nitrate consumption processes appears to be overprinted by stronger signals possibly due to external N loading.

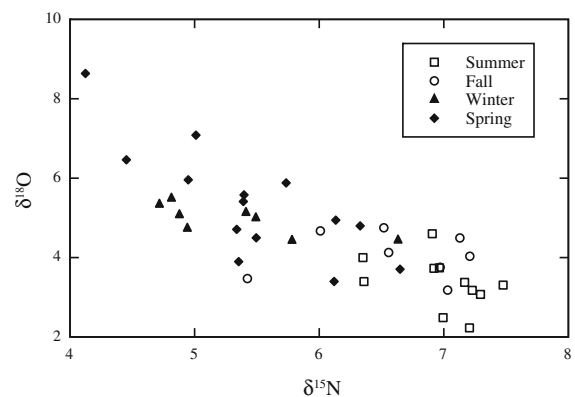
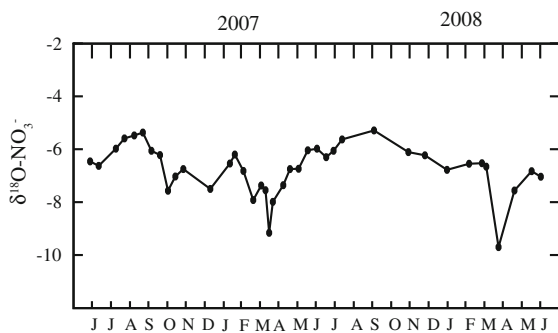


Fig. 3 Riverine nitrate $\delta^{15}\text{N}$ versus $\delta^{18}\text{O}$ for all collected samples

Table 2 Seasonal average of nitrate concentrations (in $\mu\text{mol L}^{-1}$), $\delta^{18}\text{O}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NO}_3^-$ values with the standard deviation (SD)

Season	NO_3^- concentration ($\mu\text{mol L}^{-1}$)	$\delta^{15}\text{N}\text{-NO}_3^-$ (‰)	$\delta^{18}\text{O}\text{-NO}_3^-$ (‰)
Spring	30.4 ± 5.7	5.5 ± 0.7	5.4 ± 1.4
Winter	30.6 ± 5.6	5.3 ± 0.6	5.0 ± 0.4
Fall	23.7 ± 7.9	6.6 ± 0.6	4.1 ± 0.6
Summer	18.6 ± 5.3	7.0 ± 0.4	3.4 ± 0.7

**Fig. 4** Calculated $\delta^{18}\text{O}$ of nitrate produced by nitrification assuming a 3 ‰ difference of nitrified nitrate with respect to the measured $\delta^{18}\text{O}$ of H_2O June 2006–July 2008

In analogy to the argument above, the negative correlation between $\delta^{18}\text{O}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NO}_3^-$ suggests that either nitrate assimilation is not important or, more likely, that its N and O isotopic signature is overprinted by (1) other fractionating N reactions or (2) the input of nitrate sources, which would likely decouple a parallel evolution of the nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. Hélie and Hillaire-Marcel (2006) demonstrated that particulate organic carbon at the sampling station was mainly of autochthonous origin, which confirms the importance of primary productivity and, in turn, implies assimilation of nitrate by algae. Therefore, we conclude that although assimilation is present, its fractionation signature has been overprinted by stronger signals either from in-stream production of nitrate or external inputs. Below we will further consider these two aspects.

In-stream production of nitrate

The observed seasonal pattern in $\delta^{18}\text{O}\text{-H}_2\text{O}$ (with ^{18}O -depleted values during the spring due to snowmelt

compared to high- $\delta^{18}\text{O}$ water during the fall; Fig. 4) is driven by variations observed in the $\delta^{18}\text{O}\text{-H}_2\text{O}$ of precipitation, which is due to the temperature-dependent fractionation occurring during condensation of water in air masses (Dansgaard 1964). Snowmelt events represent an input of ^{18}O -depleted precipitation that was sequestered on land during winter (Myre 2006). Assuming that the $\delta^{18}\text{O}$ of nitrified nitrate is controlled only by the $\delta^{18}\text{O}$ of the ambient water, we would predict it to follow seasonal variations as shown in Fig. 4. In turn, a positive relationship between the $\delta^{18}\text{O}\text{-H}_2\text{O}$ and the measured $\delta^{18}\text{O}\text{-NO}_3^-$ is expected if nitrate from nitrification is a dominant source for the St. Lawrence riverine nitrate pool. There is a statistically significant negative correlation ($R^2 = 0.24$, $P = 0.0016$) between $\delta^{18}\text{O}\text{-H}_2\text{O}$ and the $\delta^{18}\text{O}\text{-NO}_3^-$ (Fig. 5), which essentially excludes in-stream nitrification as the major factor driving the variation in $\delta^{18}\text{O}\text{-NO}_3^-$ (and the incorporation of water O atoms in the regenerated nitrate). Moreover, $\delta^{18}\text{O}\text{-O}_2$ data (collected in 2000–2001 by Hélie, unpublished results) were mostly constant throughout the year ($+22.0 \pm 0.9$ ‰), and the variations in $\delta^{18}\text{O}\text{-NO}_3^-$ originating from nitrification would still follow the seasonal variations of the $\delta^{18}\text{O}\text{-H}_2\text{O}$, even when considering potential incorporation of O atoms from O_2 in the nitrate molecules during nitrification. Hence, irrespective of the O-atom source during nitrate regeneration, the observed variations in $\delta^{18}\text{O}\text{-NO}_3^-$ are not modulated by nitrification. While we thus exclude in-stream nitrate production as an important constraint on observed $\delta^{18}\text{O}\text{-NO}_3^-$ variations, we do not argue that nitrification does not occur at all in the river. Its isotopic traces may simply be overprinted by variations in the external nitrate sources, which in fact may to a large extent originate from nitrification in the catchment, as we will discuss subsequently.

Partitioning of the N-sources

Nitrate in the SLR at Québec City likely derives from three different main sources: (1) input from the Great Lakes reservoir, (2) from atmospheric deposition (3) and from external N sources in the catchment (mainly from soils, ammonium fertilizer, manure, etc.). As discussed in the previous section, N loading from the catchment likely reflects the $\delta^{18}\text{O}$ of ambient water during nitrification (in the catchment, or later in the river). We can thus consider that external reduced N, if

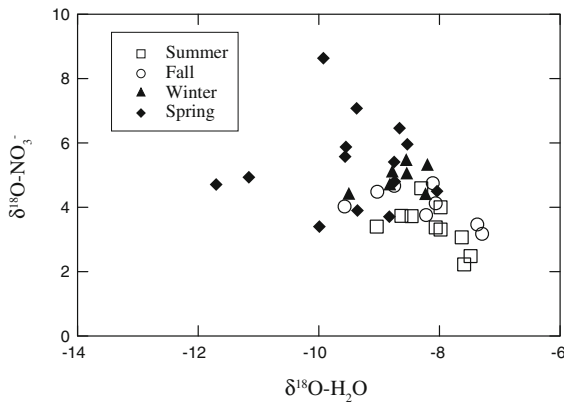


Fig. 5 Nitrate $\delta^{18}\text{O}$ versus water $\delta^{18}\text{O}$. The negative slope suggests decoupling of the nitrate $\delta^{18}\text{O}$ from the water $\delta^{18}\text{O}$

not already oxidized to nitrate during the transfer, is later transformed to nitrate by nitrification occurring in the river between the Great Lakes and Québec City. As discussed previously, while nitrification does not modulate the seasonal O isotopic variations it can still be a significant source of NO_x . The nitrified nitrate pool at Québec City would thus originate from in-stream nitrification in the SLR, as well as nitrate from nitrification of N-inputs from the northern tributaries and within the catchment soils. A dual isotope, three-end-member linear mixing model can be formulated from the following mass balance equations:

$$\delta^{18}\text{O}_{\text{QC}} = f_{\text{G.Lakes}}\delta^{18}\text{O}_{\text{G.Lakes}} + f_{\text{Atm}}\delta^{18}\text{O}_{\text{Atm}} + f_{\text{Nitri}}\delta^{18}\text{O}_{\text{Nitri}} \quad (1)$$

$$\delta^{15}\text{N}_{\text{QC}} = f_{\text{G.Lakes}}\delta^{15}\text{N}_{\text{G.Lakes}} + f_{\text{Atm}}\delta^{15}\text{N}_{\text{Atm}} + f_{\text{Nitri}}\delta^{15}\text{N}_{\text{Nitri}} \quad (2)$$

$$1 = f_{\text{G.Lakes}} + f_{\text{Atm}} + f_{\text{Nitri}} \quad (3)$$

where the subscripts refer to the isotopic composition of nitrate from the different sources (Great Lakes, atmosphere and nitrification) and the observed nitrate isotopic composition at the sampling location (QC; Québec City). The source partitioning is represented by the respective partitioning coefficients f . Calculations for each season were performed according to Phillips and Gregg (2001).

The Great Lakes end-member $\delta^{18}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NO}_3^-$ values were estimated to be around +7 and +9 ‰, respectively (Ostrom et al. 2006; Table 3). Atmospheric $\delta^{18}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NO}_3^-$ values are

variable through the year (+65 to +90 ‰ and -6 to +6 ‰, respectively) (Finlay et al. 2007). Obviously, the N isotopic composition of the various sources of nitrified nitrate likely covers a relatively large range so that it is difficult to pinpoint the mean N isotopic composition with one single $\delta^{15}\text{N}$ value (Fig. 6), so a range of 0 to +6 ‰ was used. The theoretical spectrum of possible $\delta^{15}\text{N}$ values extends to higher $\delta^{15}\text{N}$ when considering sewage and manure. However, $\delta^{15}\text{N}$ value higher than +6 ‰ yielded negative f_{Atm} , which suggests that it is not a possible solution. Thus, the 0 to +6 ‰ range for $\delta^{15}\text{N}$ represent the largest possible range to solve our mass balance equations. The $\delta^{18}\text{O}$ of the nitrified $\delta^{18}\text{O}$, however, is relatively well constrained, defined by the relatively small range of water $\delta^{18}\text{O}$ between -8 and -12 ‰ (average ~ -9 ‰) in the SLR and in regional catchment (~ -10 ‰; Spoelstra et al. 2007). Albeit moderate seasonal variations (Fig. 4), a single average nitrate $\delta^{18}\text{O}$ was assumed based on our SLR data for nitrified nitrate. Given the relatively large difference between the nitrate $\delta^{18}\text{O}$ of the other nitrate sources (Great Lakes and atmospheric precipitation), this simplification does not induce a large error in our isotope budget calculations.

Despite a relatively large uncertainty introduced by the large possible range of $\delta^{15}\text{N}$ values for nitrified nitrate, our results indicate that nitrate input from Great Lakes (47 ± 28 %) and nitrification (48 ± 22 %) are the dominant N-source during all seasons (Table 4). In summer, atmospheric-N only contributes about 0–4 % to the total N-load, while in the spring about 4–11 % of the total N load derives from atmospheric deposition. These numbers are lower than estimates from previous studies, which reported that between 12 and 19 % of the total N loading in the SLR have its ultimate origin in atmospheric deposition (Howarth et al. 1996; Nixon et al. 1996; Prospero et al. 1996). The discrepancy may partly be explained by the fact that our calculation ignores that Great Lakes water feeding the St. Lawrence stream may already contain a relatively large amount of N from the atmosphere and only considered the atmospheric deposition in the catchment between the Great Lakes and Québec City. Deposition of atmospheric N has been estimated to contribute up to 27 % to the total N loading to the Great Lakes (Sternner et al. 2007). Still, while the atmospheric N deposition seems be the main controlling factor with regards to the nitrate

Table 3 Seasonal values for each end-member used in (1)

Season	Québec City ^a		Great Lakes ^b		Atmospheric ^c		Nitrification	
	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}^d$	$\delta^{15}\text{N}$
Summer	3.4	7.0	7	9	68	-6	-5.1	0 to +6
Spring	5.4	5.5	7	9	77	-4	-6.4	0 to +6
Winter	5.0	5.3	7	9	83	0	-5.7	0 to +6
Fall	4.1	6.6	7	9	75	0	-5.3	0 to +6

^a Our data

^b Average value for Lake Erie, in August 2005 (Ostrom et al. 2006)

^c Seasonal average values from Finlay et al. (2007)

^d Our data, $\delta^{18}\text{O} = \delta^{18}\text{O}_{\text{H}_2\text{O}} + 3 \text{‰}$

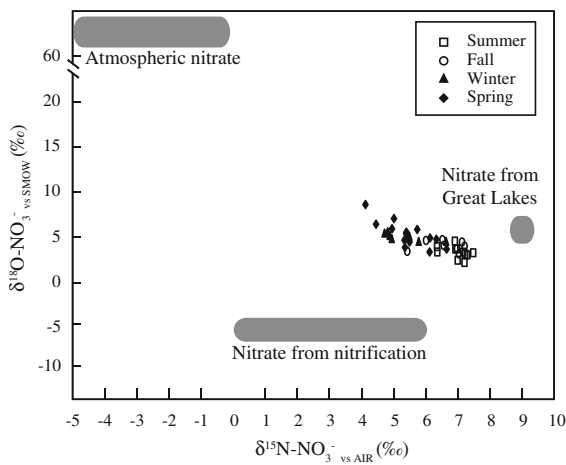


Fig. 6 Plot of nitrate $\delta^{15}\text{N}$ versus $\delta^{18}\text{O}$ for all samples. Expected isotopic compositions of the end-members considered here (atmospheric deposition, nitrification in the catchment and in the river, and nitrate inputs from Great Lakes) are represented by the shaded areas

isotope composition in the river, dominating observed seasonal isotope dynamics because of its very high $\delta^{18}\text{O}$, it does not play a prime role in the SLR N budget in this portion of the SLR.

Summary and concluding remarks

The negative correlation between $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ observed in the SLR at Québec City suggests that the isotopic fractionation associated with removal processes such as in-stream nitrate uptake and denitrification, are not the principal drivers of seasonal variations in the isotopic composition of nitrate in the

Table 4 Partitioning of the different N-sources calculated from (1)

Seasons	Proportion (%)		
	Great Lakes	Atmospheric	Nitrification
Summer ^a	77 ± 4	0 ± 0	24 ± 3
Summer ^b	48 ± 5	4 ± 1	48 ± 5
Spring ^a	63 ± 3	4 ± 1	33 ± 3
Spring ^b	20 ± 5	11 ± 1	69 ± 5
Winter ^a	59 ± 3	4 ± 1	37 ± 3
Winter ^b	1 ± 7	12 ± 1	87 ± 6
Fall ^a	73 ± 4	0 ± 1	26 ± 3
Fall ^b	33 ± 7	7 ± 1	60 ± 6
Average	47 ± 27	5 ± 4	48 ± 22

^a With $\delta^{15}\text{N}$ of nitrified nitrate = 0 ‰

^b With $\delta^{15}\text{N}$ of nitrified nitrate = +6 ‰

river. Moreover, given that nitrate from nitrification should reflect the $\delta^{18}\text{O}$ of the ambient water, the decoupling between observed seasonal variations in $\delta^{18}\text{O}-\text{H}_2\text{O}$ and $\delta^{18}\text{O}-\text{NO}_3^-$ indicates that, although nitrification within the stream must occur to some extent, its O isotopic signature within the nitrate pool is masked by other processes. Nitrate isotope variations observed in the SLR system are mostly influenced by nitrate sources that have not been regenerated within the stream. Fluctuations in the isotopic composition of nitrate in the SLR at the Québec station can be best explained by mixing of variable “external” sources of nitrate, and seasonal variations in the relative nitrate source partitioning modulates the nitrate isotope dynamics in the SLR. The SLR receives greater portions of high- $\delta^{18}\text{O}$ /low-

$\delta^{15}\text{N}$ nitrate in the spring compared to summer (i.e., during baseflow conditions). Spring is characterized by intense snowmelt, and atmospheric nitrate stocked in snowpack added to the riverine nitrate pool can explain the observed nitrate isotope trends. During baseflow conditions, in-stream $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ values reflect the isotopic signatures of soil nitrate, or a mixture of nitrate from agricultural and urban areas. Our N and O isotope mass balance considerations revealed a sixfold increase in the atmospheric N input during spring compared to summer. Yet with less than 10 % of the total N loading, atmospheric precipitation does not play a major role in the St. Lawrence N budget, leaving the N input from the Great Lakes, as well as the N regeneration as the most important fixed N sources. Unfortunately, our approach does not allow more detailed information on the actual source of N that is regenerated, be it from soil-derived or agricultural N, or from in-stream OM remineralization.

Nitrate source tracing using nitrate dual-isotope measurements can be hampered if microbial immobilization and transformation processes have an overprinting effect on the bulk nitrate isotope composition (Aravena et al. 1993; Mengis et al. 2001). Our study demonstrates that in the SLR, in-stream N transformations have a minor impact on observed nitrate isotope dynamics, and nitrate isotope measurements thus allow valuable quantitative insight into temporal variation and N source partitioning in a riverine system. The nitrate isotopic composition in the SLR seems particularly sensitive to the input of atmospheric nitrate.

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