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Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints

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Abstract. The feasibility of using nitrogen and oxygen isotope ratios of nitrate (NO_3^-) for elucidating sources and transformations of riverine nitrate was evaluated in a comparative study of 16 watersheds in the northeastern U.S.A. Stream water was sampled repeatedly at the outlets of the watersheds between January and December 1999 for determining concentrations, $\delta^{15}N$ values, and $\delta^{18}O$ values of riverine nitrate.

In conjunction with information about land use and nitrogen fluxes, $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values provided mainly information about sources of riverine nitrate. In predominantly forested watersheds, riverine nitrate had mean concentrations of less than 0.4 mg NO_3^--N L^{-1}, $\delta^{15}N_{nitrate}$ values of less than +5‰, and $\delta^{18}O_{nitrate}$ values between +12 and +19‰. This indicates that riverine nitrate was almost exclusively derived from soil nitrification processes

with potentially minor nitrate contributions from atmospheric deposition in some catchments. In watersheds with significant agricultural and urban land use, concentrations of riverine nitrate were as high as 2.6 mg NO_3^--N L^{-1} with $\delta^{15}N_{nitrate}$ values between +5 and +8‰ and $\delta^{18}O_{nitrate}$ values generally below +15‰. Correlations between nitrate concentrations, $\delta^{15}N_{nitrate}$ values, and N fluxes suggest that nitrate in waste water constituted a major, and nitrate in manure a minor additional source of riverine nitrate. Atmospheric nitrate deposition or nitrate-containing fertilizers were not a significant source of riverine nitrate in watersheds with significant agricultural and urban land use. Although complementary studies indicate that in-stream denitrification was significant in all rivers, the isotopic composition of riverine nitrate sampled at the outlet of the 16 watersheds did not provide evidence for denitrification in the form of elevated $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values. Relatively low isotopic enrichment factors for nitrogen and oxygen during in-stream denitrification and continuous admixture of nitrate from the above-described sources are thought to be responsible for this finding.

Introduction

Human activity has greatly altered the nitrogen (N) cycle in terrestrial and aquatic ecosystems (e.g. Kinzing & Socolow 1994; Vitousek et al. 1997) causing increased nitrogen loads in many rivers (e.g. Paces 1982; Turner & Rabalais 1991; Jaworski & Hetling 1996; Goolsby 2000). According to mass balances, less than 30% of the anthropogenic N inputs to large watersheds are exported to the oceans with surface runoff in rivers and streams (Howarth et al. 1996; Boyer et al. 2002). Consequently, more than 70% of human-controlled N inputs are stored, denitrified, or volatilized in the watersheds. Because of their spatial and temporal variations, the relative importance of these N retention and transformation mechanisms is difficult to quantify on a watershed scale (Van Breemen et al. 2002). It also is difficult to determine the origin of nitrate that is exported from catchments, although there is evidence that different anthropogenic N inputs are differentially retained in large watersheds (Howarth et al. 1996).

Isotopic techniques have been successfully used in numerous case studies to identify nitrogen sources and to describe nitrogen transformations in terrestrial and aquatic ecosystems (e.g. Letolle 1980; Hübner 1986; Nadelhoffer & Fry 1994; Kendall 1998). Nitrogen isotope ratios have proven useful in quantifying the extent of point and non-point nitrogen sources to rivers (e.g. Fogg et al. 1998; Harrington et al. 1998). Nitrate derived from manure or sewage is usually characterized by $\delta^{15}N$ values between +7 and more than +20‰ (Kreitler & Jones 1975; Gormly & Spalding 1979; Kreitler 1979; Kreitler & Browning 1983; Aravena et al. 1993; Wassenaar 1995; Aravena & Robertson 1998). It is therefore isotopically distinct from N in atmospheric deposition (–10 to +8‰), from N in most synthetic fertilizers (0 ± 3‰), from natural soil organic N (–3 to +5‰) and nitrate generated therein by

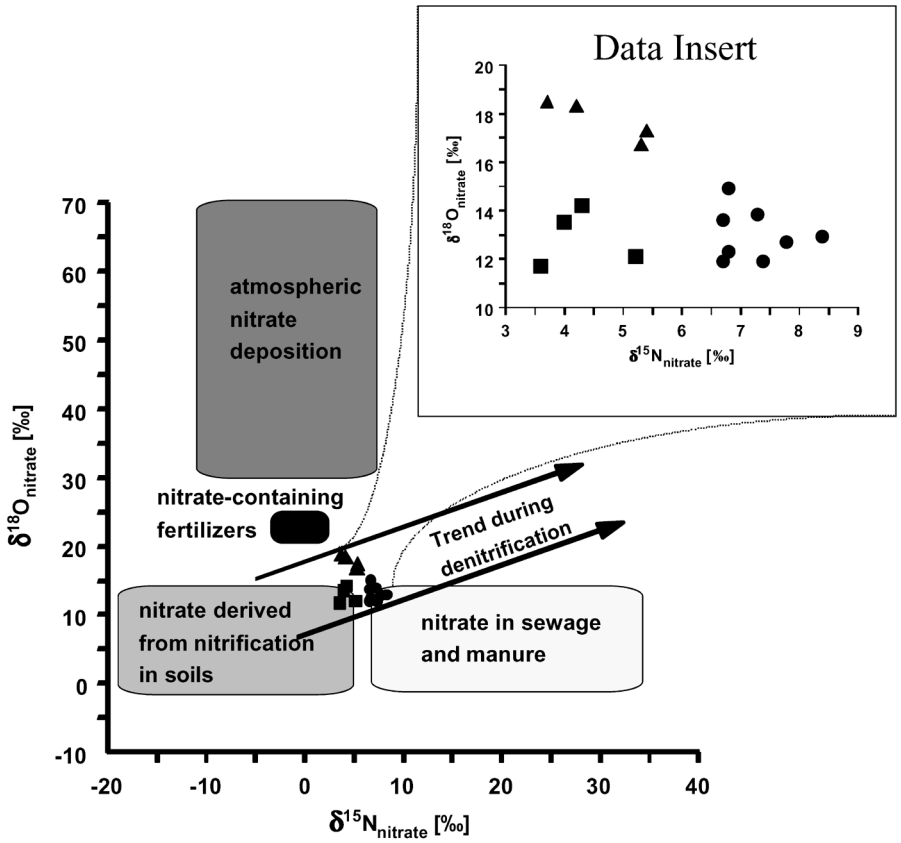

Figure 1. Mean δ^{15}N and mean δ^{18}O values of riverine nitrate collected at the outlet of 16 watersheds located in the Mid-Atlantic and New England states of the U.S.A. (cluster 1 = squares; cluster 2 = triangles; cluster 3 = circles; explanations see text). Ranges of isotopic compositions for four major nitrate sources are indicated by boxes: (a) atmospheric nitrate deposition, (b) nitrate-containing fertilizers, (c) nitrate derived from nitrification e.g. in soils, and (d) nitrate in manure and/or sewage. Also shown is the expected trend for the isotopic composition of residual nitrate undergoing microbial denitrification, assuming that the initial nitrate was derived from soil nitrification processes.

microbial nitrification (e.g. Kendall 1998). Usually, the latter three sources can not be differentiated by nitrogen isotope ratios alone, because of their wide and overlapping ranges of δ^{15}N values (Figure 1).

Recent advances in analytical methodology now allow the measurement of oxygen isotope ratios of nitrate (Amberger & Schmidt 1987; Voerkelius 1990; Wassenaar 1995; Revesz et al. 1997; Chang et al. 1999; Bräuer & Strauch 2000; Silva et al. 2000). Nitrate in atmospheric deposition has positive δ^{18}O values ranging from +25 to more than +70‰ (e.g. Voerkelius 1990; Durka

et al. 1994; Kendall 1998; Mayer et al. 2001). Nitrate-containing synthetic fertilizers typically have $\delta^{18}O$ values near +22 ± 3‰ (Amberger & Schmidt 1987; Voerkelius 1990; Wassenaar 1995). Nitrate derived from microbial soil nitrification processes has $\delta^{18}O$ values between less than 0 and +14‰ depending on the nitrification pathway and the oxygen isotope ratios of the ambient water and O_2 at the site of nitrate formation (Mayer et al. 2001). The few available oxygen isotope ratio determinations for nitrate from manure (Wassenaar 1995) and sewage (Aravena et al. 1993) indicate comparatively low $\delta^{18}O$ values of less than +15‰ for these sources. Hence, the combined analysis of both $\delta^{15}N$ and $\delta^{18}O$ values of nitrate provides a tool for distinguishing between four major nitrate sources: (a) atmospheric deposition of nitrate, (b) nitrate-containing fertilizers, (c) nitrate derived from nitrification e.g. in soils, and (d) nitrate in manure and/or sewage (Figure 1).

The isotopic composition of nitrate is not only a powerful tool to determine its origin, but can also provide clues about nitrogen transformation processes such as ammonia volatilization and denitrification. Volatilization is typically accompanied by isotopic fractionation enriching the lighter isotope ^{14}N in the product ammonia gas (Hübner 1986) causing the remaining nitrogen-bearing compounds such as ammonium and subsequently nitrate to become isotopically enriched in ^{15}N. This has been frequently observed in farmlands after urea and manure applications (Heaton 1986), in sewage treatment plants, and septic systems (Aravena et al. 1993; McClelland et al. 1997; McClelland & Valiela 1998). Another process capable of causing significant alterations to the isotopic composition of nitrate is microbial denitrification, during which the lighter isotopes ^{14}N and ^{16}O are preferentially metabolized by microorganisms and are converted to N_2 and N_2O, causing an enrichment of the heavy isotopes ^{15}N and ^{18}O in the remaining nitrate through kinetic isotope effects (e.g. Blackmer & Bremner 1977; Mariotti et al. 1982; Mariotti et al. 1988; Böttcher et al. 1990). In single source closed system scenarios, microbial denitrification results in progressively increasing $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values as nitrate concentrations decrease. The extent of nitrogen isotope fractionation can be variable and is influenced by several factors including temperature and concentration of the substrate (Mariotti et al. 1982). The increase in $\delta^{15}N_{nitrate}$ values due to microbial denitrification appears to be between 1.5 and 2.0 times that of $\delta^{18}O_{nitrate}$ values in groundwater systems (Böttcher et al. 1990; Aravena & Robertson 1998) and riparian zones (Cey et al. 1999; Mengis et al. 1999). Hence, the remaining nitrate eventually obtains elevated $\delta^{15}N$ and $\delta^{18}O$ values, which are unique for nitrate that has undergone denitrification under closed system conditions (Figure 1). Microbial denitrification may occur in soils, in aquifers, in riparian and hyporheic zones, in river water and sediments, and in sewage treatment

systems, provided that organic carbon or reduced inorganic compounds are available as electron donors and that the appropriate redox conditions are achieved (Knowles 1982).

Current and expected future human alterations to the N balances in catchments make it desirable that we understand how N is cycled through watersheds. For assessing the consequences of increasing anthropogenic nitrogen inputs on issues such as acidification of terrestrial and aquatic ecosystems, nitrate concentrations in aquifers, and eutrophication of surface waters and coastal oceans, a detailed understanding of the fate of N from individual watershed sources is required. These N sources include (a) atmospheric deposition, (b) fertilizers, (c) N fixation in forests and crops, (d) net import of N in food and feedstocks, (e) mineralization of soil organic matter, (f) animal manure, and (g) municipal and industrial waste water (note that a-d represent new N inputs to catchments, whereas e-g can be considered a recycling of watershed-internal N). Nitrogen isotope techniques have proven useful for obtaining information about sources of nitrate or N transformation processes in several compartments of watersheds (e.g. Knowles & Blackburn 1993; Macko & Ostrom 1994; Nadelhoffer & Fry 1994). New analytical capabilities allowing the precise and accurate determination of both δ^{15}N and δ^{18}O values of dissolved nitrate have added a promising tool for elucidating the nitrogen cycle in watersheds.

Here we evaluate the usefulness of the isotopic composition of riverine nitrate in describing N cycling in a comparative study of 16 watersheds in the northeastern U.S.A. Our specific goals were to determine whether the isotopic composition of riverine nitrate can be used to identify its watershed sources and to test whether the isotopic composition of riverine nitrate provides evidence for microbial denitrification in the watersheds. Hence, the objective was to evaluate whether the isotopic composition of riverine nitrate provides source or process information, or a combination of both.

Methods

Sixteen watersheds in the mid-Atlantic and New England states of the U.S.A. with well-constrained N budgets were selected for this study (Figure 2). Related papers in this issue detail watershed characteristics (Boyer et al. 2002), develop nitrogen budgets (Boyer et al. 2002), and quantify nitrogen storage and sinks (Van Breemen et al. 2002) for these basins. The reader is referred to these companion papers for a detailed description of the methods used for assessing nitrogen and nitrate fluxes.

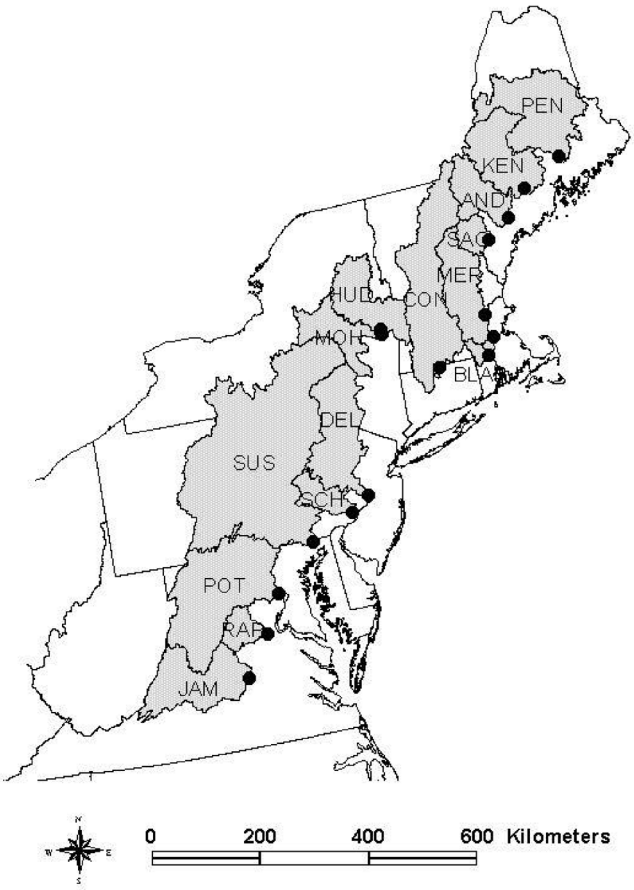

Figure 2. Location of the following 16 watersheds in the in the Mid-Atlantic and New England states of the U.S.A.: Penobscot (PEN), Kennebec (KEN), Androscoggin (AND), Saco (SAC), Merrimack (MER), Charles, Blackstone (BLA), Connecticut (CON), Hudson (HUD), Mohawk (MOH), Delaware (DEL), Schuylkill (SCH), Susquehanna (SUS), Potomac (POT), Rappahannock (RAP), James (JAM). The watershed boundaries shown are delineated upstream of the following USGS stations (black dots), from which streamflow data and water samples for concentration and isotope analyses were obtained: Penobscot River at Eddington, ME; Kennebec River at North Sidney, ME; Androscoggin River near Auburn, ME; Saco River at Cornish, ME; Merrimack River below Concord River at Lowell, MA; Charles River at Dover, MA; Blackstone River at Manville, RI; Connecticut River at Thompsonville, CT; Hudson River above lock 1 near Waterford, NY; Mohawk River at Cohoes NY; Delaware River at Trenton, NJ; Schuylkill River at Philadelphia, PA; Susquehanna River at Conowingo, MD; Potomac River near Washington, DC Lower Falls Pump Station; Rappahannock River near Fredericksburg, VA; James River at Cartersville, VA.

Sample collection

Water from rivers draining the 16 watersheds was sampled up to 7 times between January and December 1999 at USGS stream gaging stations located at the outlet of each basin (Figure 2). Typically, three liters of water were sampled by USGS personnel and shipped in cooled containers by overnight courier to the Isotope Science Laboratory at the University of Calgary.

Nitrate concentrations and fluxes

Nitrate and nitrite concentrations in stream water at the gaging stations, which define the outlet of the 16 watersheds, were determined monthly by various northeastern offices of the USGS Water Resources Division. Daily flows from these gaging stations were obtained from the USGS national watershed information system (USGS 2000).

Riverine fluxes of oxidized inorganic N (defined as the dominant oxidized forms nitrate plus nitrite) were calculated for water year 1999 (October 1, 1998 to September 30, 1999) using an automated implementation of the Beale ratio estimator (Richards & Holloway 1987). This method uses a flow-based stratification to estimate daily and annual loads from the infrequent concentration measurements and daily flow values. For five basins, for which 1999 fluxes could not be calculated due to a lack of concentration or flow data, average annual flux estimates reported for the period 1988–1993 (Boyer et al. this issue) were used as a surrogate. Mean annual concentrations of oxidized inorganic N in each river draining the 16 watersheds were estimated by dividing riverine oxidized inorganic N fluxes by the mean annual discharge (the average of the daily flows observed throughout the water year).

Analysis of nitrogen and oxygen isotope ratios of nitrate

Within two days of collection, water samples were passed through anion exchange resins to retain and store nitrate for subsequent isotopic analyses in the Isotope Science Laboratory at the University of Calgary, Canada. The nitrate was later eluted and converted to $AgNO_3$ using a modified version of a technique described by Silva et al. (2000).

Water samples were passed through a cation exchange resin (2 mL of 50W-X4, H^+-form, Bio-Rad) at a rate of 5 mL min^{-1} to exchange cations with H^+ and simultaneously remove HCO_3^- through acidification. Subsequently, nitrate, sulfate, and phosphate were retained quantitatively on an anion exchange resin (2 mL AG 1-X8 resin, Cl^--form, Bio-Rad). After rinsing with 10 mL deionized water, the anion exchange resins were stored at 5 °C in darkness until further processing.

Nitrate, sulfate, and phosphate were eluted from the anion exchange resins into a beaker by passing 15 mL 3 *M* HCl through the columns. One mL of 0.2 *M* $BaCl_2$ solution was added to the HCl eluate to precipitate sulfate and phosphate as $BaSO_4$ and $Ba_3(PO_4)_2$, respectively. After 24 hours, $BaSO_4$ and $Ba_3(PO_4)_2$ were removed by filtration (0.45 μm membrane filter). Excess Ba^{2+} was removed by passing the sample through a cation exchange resin in H^+-form (2 mL 50W-X4 resin, Bio-Rad). The remaining, almost DOC-free solution containing HNO_3 and HCl was neutralized by adding approximately 7.5 g pure and pre-washed Ag_2O (Merck, Darmstadt, Germany). The resulting AgCl precipitate was removed by membrane filtration (0.45 μm) leaving only Ag^+ and NO_3^- in solution (equation 1).

$$Ag_2O + HNO_3 + HCl \rightarrow Ag^+ + NO_3^- + H_2O + AgCl \downarrow \quad (1)$$

Thereafter, the solution was freeze-dried yielding pure $AgNO_3$. For oxygen isotope analyses on nitrate, 10 mg $AgNO_3$ was mixed with 2 mg pure graphite powder. This mixture was placed in a 6 mm quartz tube, which was evacuated and flame sealed. The mixture was thermally decomposed at 860 °C for 3 hours, followed by slow cooling to ensure complete conversion of the nitrate-oxygen to CO_2 (equation 2):

$$2AgNO_3 + 3C \rightarrow 2Ag + N_2 + 3CO_2 \quad (2)$$

The resulting CO_2 was cryogenically purified and analyzed mass spectrometrically. Accuracy and precision of the measurements was assured by repeated analyses of laboratory internal and international reference materials. The mean $\delta^{18}O_{nitrate}$ value obtained for IAEA-NO-3 was with +23.1 ± 0.7‰ (n = 12) within the range of previously reported $\delta^{18}O$ values for this reference material (e.g. Revesz et al. 1997; Bräuer & Strauch 2000). The reproducibility of nitrate extraction, gas preparation, and mass spectrometric measurement was found to be better than ±1.0‰ for $\delta^{18}O_{nitrate}$, as determined by duplicate analyses.

Nitrogen isotope ratios were determined on N_2 after thermal decomposition of $AgNO_3$ in an elemental analyzer (Carlo Erba NA 1500) and subsequent continuous-flow isotope ratio mass spectrometry (CF-IRMS). $\delta^{15}N$ values for all samples were calibrated against international reference materials (IAEA N1 and N2). The reproducibility of nitrate extraction, gas preparation, and mass spectrometric measurement was better than ±0.3‰ for $\delta^{15}N_{nitrate}$ determinations.

Oxygen isotope ratios for water samples were obtained using standard equilibration techniques (Epstein & Mayeda 1953) with a reproducibility of

better than ±0.2‰. All stable isotope ratios are expressed in the usual delta per mil (‰) notation:

$$\delta_{\text{sample}}(‰) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] * 1000 \quad (3)$$

where R is the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio of the sample and the standard, respectively. $\delta^{15}N$ values are reported with respect to AIR and $\delta^{18}O$ values with respect to Vienna Standard Mean Ocean Water (V-SMOW).

Results

Watershed and land use characteristics and mean annual N inputs and exports (1988–1993) for the 16 watersheds as described by Boyer et al. (2002) are summarized in Table 1. Because river gaging stations were generally located upstream from the major coastal population centers, urban land use constituted less than 5% of the catchment area in most watersheds. Only the watersheds of the Merrimack River (9%), the Schuylkill River (10%), the Blackstone River (18%), and of the Charles River (22%) had significant portions of urban land use. Agricultural land use varied between 2 and 38% among the catchments. Between 48 and 87% of the area of the 16 watersheds was forested, with the remainder being wetlands and water surfaces.

Annual new nitrogen inputs to the 16 watersheds from four major sources – atmospheric NO_y deposition, fertilizers, N fixation in forests and crops, and net import of N in food and feedstocks – ranged from less than 1000 kg N km^{-2} a^{-1} in the predominantly forested watersheds to more than 3000 kg N km^{-2} a^{-1} in catchments with significant agricultural and urban land use (Table 1). Mean annual export of total nitrogen in streamflow ranged from more than 40% of the N input in some predominantly forested watersheds to less than 20% of the total N input in some catchments with significant agricultural and urban land use. On average, 28% of the N inputs to the watersheds were exported in riverine flows (Boyer et al. 2002).

Selected mean annual nitrogen fluxes for the 16 watersheds such as N inputs with atmospheric deposition and fertilizers, and N transfers with human wastewater flows and with animal waste (manure) production, are summarized in Table 2. These data were determined for the period 1988 to 1993 (Boyer et al. 2002), but are believed to be representative for the latter part of the 90's. Nitrate-N inputs with wet and dry deposition varied between less than 400 kg N km^{-2} a^{-1} in the northernmost forested catchment to more than 800 kg N km^{-2} a^{-1} in watersheds receiving significant amounts of industrial emissions. Nitrogen inputs with fertilizers varied markedly from

Table 1. Area, land use, population density, total nitrogen input, and total nitrogen export with riverine flow (1988–1993) for the 16 watersheds in the mid-Atlantic and New England states, U.S.A. (c.f. Boyer et al. 2002)

River/ Watershed	Area [km^2]	Land Area Forested [%]	Land Area Agricultural [%]	Land Area Urban [%]	Population [km^{-2}]	Total N Inputs [kg km^{-2} yr^{-1}]	River N Export [% of N Input]
Penobscot	20109	83.8	1.5	0.4	8	835	38
Kennebec	13994	79.6	5.9	0.9	9	1099	30
Androscoggin	8451	84.6	4.8	1.1	17	1310	31
Saco	3349	87.4	3.6	0.8	16	1233	32
Merrimack	12005	74.7	7.8	8.7	143	2228	22
Charles	475	59.3	8.4	22.2	556	4406	40
Blackstone	1115	63.3	8.1	17.6	276	3407	33
Connecticut	25019	79.0	9.0	4.0	65	2262	24
Hudson	11942	80.8	10.4	2.7	32	1985	25
Mohawk	8935	63.1	28.0	4.7	54	3420	23
Delaware	17560	74.7	16.7	3.3	85	2967	32
Schuylkill	4903	48.1	38.4	10.2	293	5717	31
Susquehanna	70189	66.7	28.5	2.4	54	4173	23
Potomac	29940	60.8	34.6	2.6	63	4689	19
Rappahannock	4134	61.3	35.9	1.4	24	4246	11
James	16206	80.6	15.6	1.4	24	2773	11

Table 2. Selected mean annual N fluxes for the 16 watersheds averaged for the years 1988 to 1993 (c.f. Boyer et al. 2002)

River/ Watershed	Wet & Dry NO_3^--N Deposition [kg km^{-2} yr^{-1}]	Fertilizer N [kg km^{-2} yr^{-1}]	Waste Water N [kg km^{-2} yr^{-1}]	Animal Waste N [kg km^{-2} yr^{-1}]
Penobscot	362	91	25	78
Kennebec	428	54	56	187
Androscoggin	495	80	62	237
Saco	566	42	44	82
Merrimack	606	147	171	219
Charles	674	197	1372	143
Blackstone	707	307	885	333
Connecticut	631	274	123	488
Hudson	658	204	60	439
Mohawk	708	411	110	1261
Delaware	811	527	170	651
Schuylkill	885	1207	618	2147
Susquehanna	816	615	178	1909
Potomac	714	1024	57	2583
Rappahannock	615	1030	35	2234
James	652	361	145	1096

less than 100 kg N km^{-2} a^{-1} in predominantly forested watersheds to more than 1000 kg N km^{-2} a^{-1} in watersheds with intensive agricultural land use. Transfers of N with human wastewater and animal waste were not considered new inputs of N to each watershed, but rather are a recycling of watershed-internal N (Boyer et al. 2002). N transfers in wastewater were correlated with population densities and thus urban land use (Table 1). Waste water inputs ranged from 25 to 178 kg N km^{-2} a^{-1} in watersheds with less than 9% urban land, and from 618 to 1372 kg N km^{-2} a^{-1} in watersheds with more than 10% urban land. Nitrogen transfers in animal waste (manure) ranged from 78 kg N km^{-2} a^{-1} in predominantly forested catchments to 2583 kg N km^{-2} a^{-1} in watersheds with significant agricultural land use.

Average concentrations, fluxes, and isotopic compositions for riverine nitrate determined at the outlet of each of the 16 watersheds are summarized in Table 3. Mean (discharge weighted) annual nitrate concentrations ranged

Table 3. Mean nitrate concentrations, average NO_3^--N fluxes, and mean isotopic composition of nitrate and water-oxygen for the water year 1999 in rivers draining 16 watersheds in the mid-Atlantic and New England states, U.S.A.

River/ Watershed	[NO_3^--N] [mg L^{-1}]	# of [NO_3^-] analyses	# of discharge measurements	NO_3^--N Export [kg km^{-2} yr^{-1}]	$\delta^{15}N_{nitrate}$ [‰]$_{AIR}$	n	$\delta^{18}O_{nitrate}$ [‰]$_{SMOW}$	n	$\delta^{18}O_{water}$ [‰]$_{SMOW}$	n
Penobscot	0.11*	0	0	66*	3.7 ± 2.9	5	18.5	1	–9.5 ± 0.7	5
Kennebec	0.15*	0	0	87*	4.2 ± 1.5	4	18.3	1	–9.5 ± 0.5	4
Androscoggin	0.18*	0	365	112*	4.0 ± 0.9	4	13.5 ± 2.6	3	–9.6 ± 0.6	5
Saco	0.12*	0	365	81*	5.4 ± 1.1	3	17.3 ± 3.2	2	–9.1 ± 0.7	5
Merrimack	0.21	13	365	100 ± 29	6.8 ± 2.4	3	12.3 ± 0.8	2	–7.6 ± 1.2	5
Charles	0.54	19	365	260 ± 18	6.8 ± 2.7	4	14.9 ± 2.0	4	–6.1 ± 1.0	5
Blackstone	0.68	9	365	336 ± 217	7.8 ± 2.2	4	12.7 ± 4.4	4	–6.7 ± 0.8	6
Connecticut	0.31	10	365	153 ± 26	5.3 ± 1.4	6	16.7 ± 1.9	4	–9.3 ± 1.0	7
Hudson	0.36*	0	365	222*	3.6 ± 1.0	2	11.7 ± 0.8	2	–10.9 ± 0.3	2
Mohawk	0.62	12	365	249 ± 16	5.2 ± 0.9	2	12.1 ± 0.8	2	–11.4 ± 0.2	2
Delaware	0.87	13	365	341 ± 41	7.3 ± 1.7	3	13.8 ± 4.1	4	–7.9 ± 1.1	4
Schuylkill	2.57	20	365	1025 ± 64	8.4 ± 2.0	3	12.9 ± 3.8	4	–7.1 ± 0.8	4
Susquehanna	1.10	16	365	319 ± 47	7.4 ± 1.7	5	11.9 ± 2.4	5	–9.0 ± 1.0	5
Potomac	1.07	68	365	162 ± 9	6.7 ± 1.1	5	11.9 ± 2.5	6	–7.4 ± 0.8	7
Rappahannock	0.49	32	365	69 ± 5	6.7 ± 0.5	3	13.6 ± 1.6	2	–6.0 ± 1.2	3
James	0.21	31	365	41 ± 4	4.3 ± 0.3	3	14.2 ± 2.3	3	–7.1 ± 1.0	4

*Data derived from 1988–1993 estimates (Jaworswki, personal communication).

from as low as 0.11 mg NO_3^--N L^{-1} in rivers draining predominantly forested watersheds to 2.57 mg NO_3^--N L^{-1} in the Schuylkill River, which drains considerable areas of urban and agricultural land. Mean annual NO_3^--N fluxes ranged from less than 50 to approximately 225 kg km^{-2} a^{-1} in watersheds with less than 20% combined agricultural and urban land use, and between 100 and 1025 kg km^{-2} a^{-1} in watersheds with more than 20% combined agricultural and urban land use. Mean $\delta^{15}N$ values for riverine nitrate varied from less than +5‰ in predominantly forested watersheds to more than +7‰ in catchments with high percentages of agricultural and/or urban land use. The oxygen isotope ratios of nitrate in 12 of the 16 rivers were uniform with an average $\delta^{18}O_{nitrate}$ value of +13.0 ± 1.0‰. Only in four mainly forested catchments $\delta^{18}O_{nitrate}$ values were markedly higher: between +16.7 and +18.5‰. The oxygen isotope ratios of the river water ranged between –11.4 and –6.0‰ during the observation period. No significant linear relation between the oxygen isotope ratios of water and nitrate was observed for the individual samples from the sixteen watersheds ($r^2 = 0.10$, $p = 0.517$, $n = 49$).

Discussion

The following discussion is based on mean annual concentrations and mean isotopic compositions of riverine nitrate (estimated as described above) from 16 different watersheds. The current database is insufficient to interpret seasonal or flow-dependent variations of nitrate concentrations and isotopic compositions for individual rivers or changes of these parameters along the flowpath within each watershed. Such investigations were beyond the scope of this study, but clearly deserve further attention.

Although the overall range of $\delta^{15}N$ and $\delta^{18}O$ values was comparatively narrow, the isotopic composition of riverine nitrate appeared to be grouped in three different clusters (see data insert Figure 1). At the outlet of the watersheds Androscoggin, Hudson, Mohawk, and James (cluster 1: squares in Figure 1), mean $\delta^{15}N$ values of riverine nitrate were below +5‰ and mean $\delta^{18}O_{nitrate}$ values below +14‰. Samples from Penobscot, Kennebec, Saco, and Connecticut (cluster 2: triangles in Figure 1) had similarly low $\delta^{15}N_{nitrate}$ values, but slightly elevated $\delta^{18}O_{nitrate}$ values (+16 to +19‰). Riverine nitrate from the remaining eight watersheds (cluster 3: circles in Figure 1) had $\delta^{18}O$ values generally below +15‰ and $\delta^{15}N$ values of more than +6‰. The isotopic composition of nitrate in cluster 1 suggests that nitrification processes in soils were the major source of riverine nitrate (Figure 1). The isotopic composition of riverine nitrate in clusters 2 and 3 could be explained by mixing of nitrate from soil nitrification and from other sources (e.g.

atmospheric deposition, fertilizers, sewage and/or manure). Alternately, denitrification of nitrate that was initially formed by nitrification processes in soils, could also result in an isotopic composition similar to that of riverine nitrate in clusters 2 and 3 (see arrows in Figure 1). Combined evaluation of concentration, flux, and isotope data was pursued to gain a better understanding of the somewhat ambiguous information provided by the isotopic composition of riverine nitrate in 12 of the 16 watersheds (clusters 2 and 3).

Mixing of nitrate from various sources or denitrification?

Plotting nitrogen isotope ratios versus nitrate concentrations often reveals whether denitrification or mixing of nitrate from various sources is responsible for increasing $\delta^{15}N_{nitrate}$ values in a given aquatic system. Microbial denitrification typically results in progressively increasing $\delta^{15}N_{nitrate}$ values as nitrate concentrations decrease, whereas mixing of nitrate from two or more sources can result in patterns of increasing $\delta^{15}N$ and concentration values (see schematic inserts in Figure 3). Although we compared data from 16 different rivers, a clear trend of increasing $\delta^{15}N_{nitrate}$ values with increasing nitrate concentrations was evident (Figure 3) with a 2nd degree polynomial regression yielding a r^2 value of 0.78 ($p < 0.001$; $n = 16$). This suggest that riverine nitrate in most watersheds contained contributions from at least two different sources: one nitrate source generating low concentrations and $\delta^{15}N_{nitrate}$ values below +4‰, and another nitrate source with variable but generally high concentrations and a $\delta^{15}N_{nitrate}$ value above +8‰. It appears plausible that the variability of $\delta^{15}N$ values of riverine nitrate at the outlet of the 16 watersheds was governed primarily by mixing of two or more sources of nitrate, rather than by microbial denitrification within the watershed, although the latter can not be excluded based on the presented data set.

Effect of land use

Figure 4 displays a significant positive linear relation ($r^2 = 0.75$, $p = 0.001$, $n = 16$) between increasing $\delta^{15}N$ values of riverine nitrate and percentages of agricultural plus urban land. In watersheds with less than 15% agricultural and urban land, or more than 80% forest cover, $\delta^{15}N_{nitrate}$ values varied between +3.5 and +5.5‰. In watersheds with more than 15% agricultural and urban area, $\delta^{15}N$ values of riverine nitrate were typically above +6‰. Hence, nitrogen isotope ratios of riverine nitrate appear to be directly related to land use practices with higher percentages of urban and/or agricultural land in the watershed causing elevated $\delta^{15}N_{nitrate}$ values.

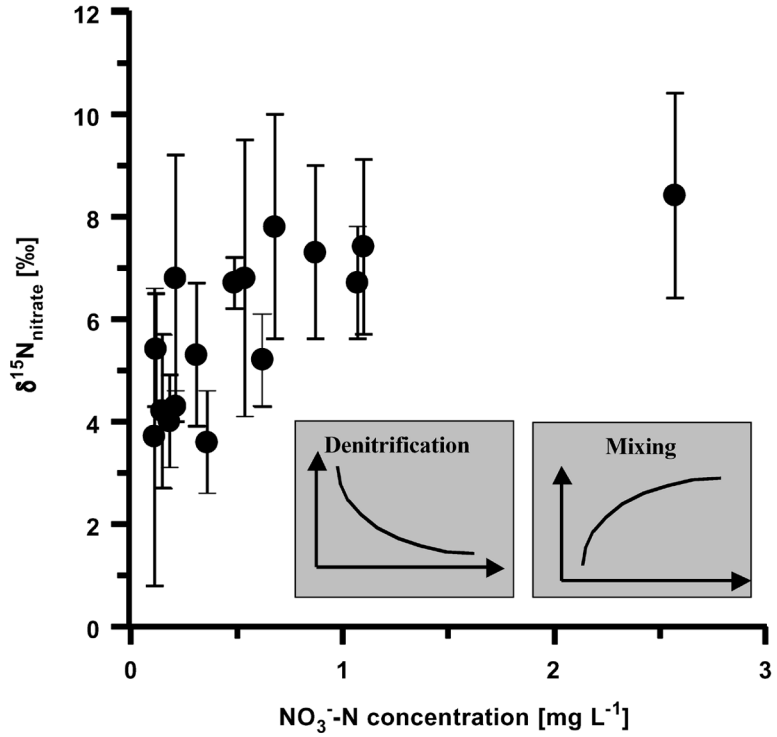

Figure 3. Mean $\delta^{15}N$ values of riverine nitrate versus NO_3^--N concentrations. A 2nd degree polynomial regression yielded a positive relation with a r^2 value of 0.78 ($p < 0.001$; $n = 16$). The left insert shows the expected trend of increasing $\delta^{15}N_{nitrate}$ values with decreasing nitrate concentration typical for denitrification in a single source closed system scenario (no nitrate from other sources added). The right insert displays a trend of increasing $\delta^{15}N_{nitrate}$ values with increasing nitrate concentrations. Such a scenario could be explained as a result of mixing of nitrate from two sources: one with low nitrate concentrations and $\delta^{15}N_{nitrate}$ values and another one with high nitrate concentrations and $\delta^{15}N_{nitrate}$ values.

Nitrogen sources

Figures 3 and 4 provide evidence that the source generating nitrate with low concentrations and $\delta^{15}N$ values of less than +4‰ is located in forested areas. We suggest that this source is mineralization of soil organic matter, which typically causes low nitrate concentrations in seepage water and surface runoff from forested catchments (Sollins & McCorison 1981; Stottlemyer & Troendle 1992; Hedin et al. 1995; Vanderbilt & Lajtha 2000) and $\delta^{15}N_{nitrate}$ values of less than +4‰ (e.g. Durka et al. 1994; Nadelhoffer & Fry 1994). This is presumably true for most first and second order streams in the 16

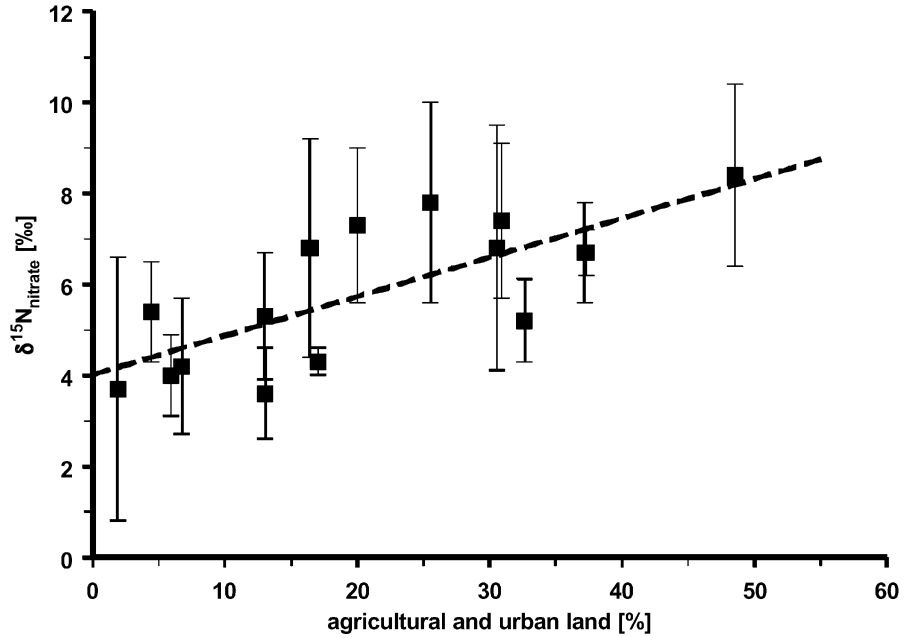

Figure 4. Mean δ^{15}N values of riverine nitrate versus percentage of agricultural plus urban land in the watersheds. A significant positive linear relation with $r^2 = 0.75$ ($p = 0.001$, $n = 16$) was observed.

watersheds, which all have forested headwater portions (Boyer, personal communication).

Combined evaluation of Figures 3 and 4 also suggests that in watersheds with more than 15% agricultural plus urban land use, concentrations and nitrogen isotope ratios of riverine nitrate were markedly higher than in predominantly forested areas. This can in principle result from three different mechanisms: (1) admixture of nitrate with runoff from manured agricultural areas (e.g. Kreitler & Jones 1975; Kreitler 1979); (2) admixture of nitrate from sewage treatment plants or septic systems (e.g. Aravena & Robertson 1998); (3) microbial denitrification (e.g. Farrell et al. 1996; Ostrom et al. 1998). The first two mechanisms typically entail increasing concentrations and δ^{15}N values of nitrate in aquatic systems, since nitrate derived from manure or sewage has usually δ^{15}N values higher than +7‰ (Fogg et al. 1998). The latter process, in contrast, is generally characterized by increasing $\delta^{15}N_{nitrate}$ values and decreasing nitrate concentrations (Böttcher et al. 1990; Aravena et al. 1993; Farrell et al. 1996). Comparison of riverine nitrate from 16 different watersheds revealed that increasing $\delta^{15}N_{nitrate}$ values were accompanied by increasing nitrate concentrations (Figure 3). Also, $\delta^{15}N_{nitrate}$ values

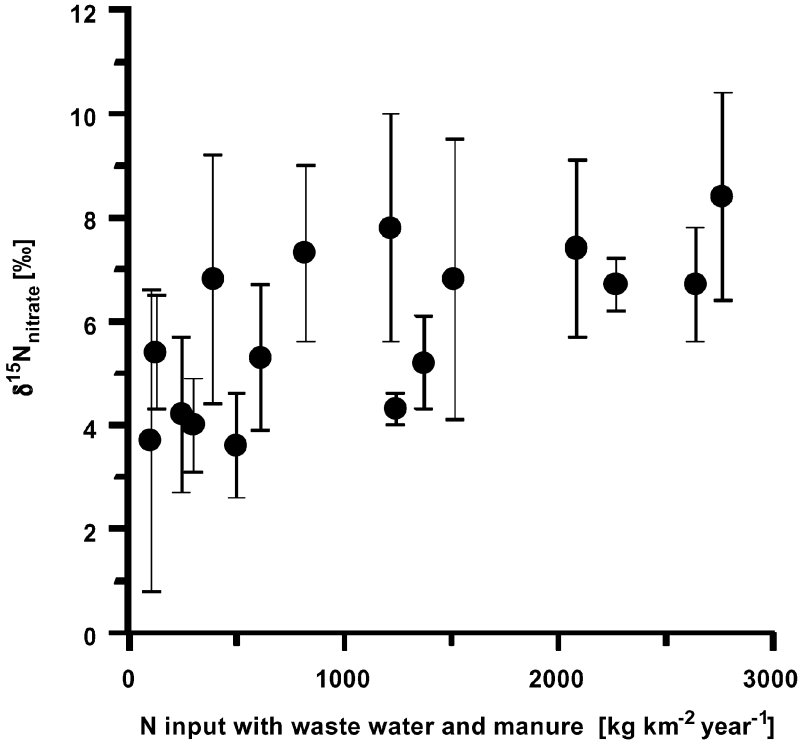

Figure 5. Mean δ^{15}N values of riverine nitrate versus combined N input with waste water and animal waste (manure) in the 16 watersheds. A 2nd degree polynomial regression yielded a significant positive relation with a r^2 value of 0.68 ($p < 0.006$; $n = 16$).

and the annual N fluxes with waste water and manure in the respective watersheds (Figure 5) displayed a significant positive relation with a r^2 value of 0.68 ($p < 0.006$; $n = 16$). We propose, therefore, that elevated $\delta^{15}N_{nitrate}$ values in rivers draining watersheds with significant urban and agricultural land use were caused by nitrate from sewage and/or manure. Since nitrate derived from both of these sources is typically characterized by δ^{15}N values higher than +7‰, we used the Best Regression Algorithm to test a variety of bivariate polynomial regression models to describe the effect of waste water and animal waste on the δ^{15}N values of riverine nitrate. A highly significant polynomial regression of the 2nd degree ($r^2 = 0.70$; $p = 0.002$; $n = 16$) between mean $\delta^{15}N_{nitrate}$ values and the N fluxes with sewage and manure in the watersheds suggests that about 50% of the variability in the δ^{15}N values of riverine nitrate could be attributed to N fluxes in sewage, whereas approximately 20% was caused by manure applied to the agriculturally used portions of the watersheds. Riverine nitrate in watersheds with waste water

N fluxes above 150 kg N km^{-2} a^{-1} had $\delta^{15}N$ values of more than +6.5‰ (Tables 2, 3). In contrast, watersheds with wastewater N fluxes of less than 150 kg N km^{-2} a^{-1} had typically $\delta^{15}N_{nitrate}$ values below +5.5‰. The two exceptions – the Potomac and the Rappahannock River having sewage N fluxes of less than 100 kg km^{-2} a^{-1} in their catchments – had $\delta^{15}N_{nitrate}$ values of +6.7‰. These comparatively high nitrogen isotope ratios were in all likelihood caused by the very high manure applications (>2000 kg N km^{-2} a^{-1}) in these watersheds (Table 2). Sewage, and to a lesser extent manure were probably responsible for the high concentrations and nitrogen isotope ratios of riverine nitrate in watersheds with significant agricultural and urban land use. Lack of information about the spatial and seasonal variation of $\delta^{15}N_{nitrate}$ values of sewage and manure precludes a more quantitative assessment of the contributions of these anthropogenic N fluxes. Nevertheless, the good positive correlation between $\delta^{15}N$ values and (a) concentrations of riverine nitrate and (b) N inputs with manure and sewage indicate that denitrification was not the major cause for the elevated $\delta^{15}N$ values observed in riverine nitrate at the outlet of some of the 16 watersheds.

Since most of the variability in the $\delta^{15}N$ values of riverine nitrate from the 16 different watersheds could be sufficiently explained by mixing of nitrate from various sources, the variability of the oxygen isotope ratios of riverine nitrate was also evaluated in terms of multiple source mixing. A significant linear relation ($r^2 = 0.76$; $p = 0.001$; $n = 16$) between mean $\delta^{18}O$ values of riverine nitrate and inverse nitrate concentrations (Figure 6) suggests two source mixing. One source of nitrate causing variable but high concentrations had a $\delta^{18}O$ value of +12‰ (= y intercept in Figure 6). Such oxygen isotope ratios are typical for nitrate derived from nitrification processes in manure, sewage, or soils (Figure 1). The second source responsible for low nitrate concentrations was apparently associated with higher $\delta^{18}O_{nitrate}$ values of more than +17‰. $\delta^{18}O_{nitrate}$ values higher than +17‰ can be caused by denitrification or by mixing of nitrification nitrate ($\delta^{18}O_{nitrate} < +14$‰) with fertilizer nitrate ($\delta^{18}O_{nitrate} \sim +22$‰) or atmospherically deposited nitrate ($\delta^{18}O_{nitrate} > +25$‰). To evaluate the influence of the latter two nitrate sources on the oxygen isotope ratios of riverine nitrate, mean $\delta^{18}O_{nitrate}$ values were plotted versus the N inputs with atmospheric nitrate deposition (Figure 7(a)) and synthetic fertilizers (Figure 7(b)) to the 16 watersheds expressed as percentage of total N input.

Mean $\delta^{18}O_{nitrate}$ values showed a weak positive correlation ($r^2 = 0.63$, $p = 0.01$; $n = 16$) with the percentage of atmospheric NO_3^--N deposition of the total N inputs (Figure 7(a)). A tendency to increasing $\delta^{18}O_{nitrate}$ values was particularly obvious when atmospheric NO_3^--N deposition represented more than 40% of the entire N inputs to the watersheds, as in the Kennebec, Penob-

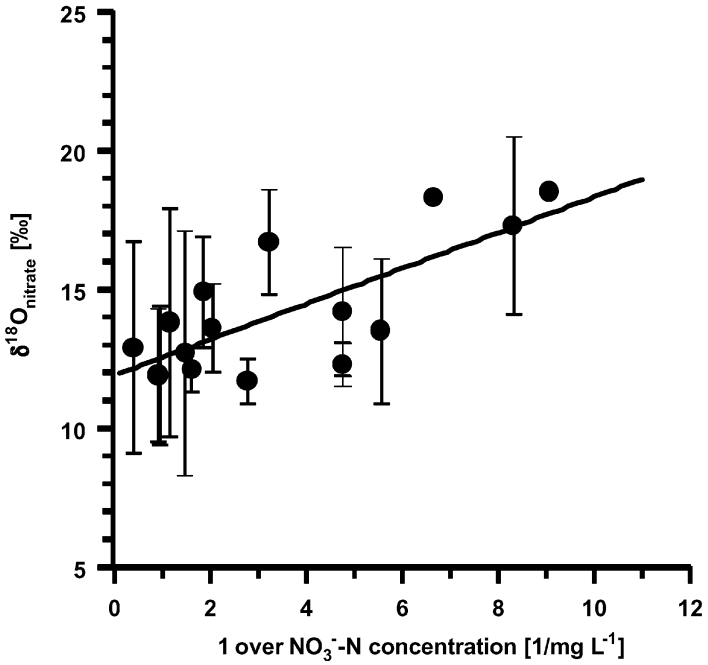

Figure 6. Mean $\delta^{18}O$ values of riverine nitrate versus inverse nitrate concentration ($r^2 = 0.76$, $p = 0.001$, $n = 16$).

scot, and Saco catchments, which yielded $\delta^{18}O_{nitrate}$ values between +16 and +19‰. The comparatively high $\delta^{18}O$ values suggest that not all nitrate in the surface runoff from these predominantly forested (>79%) watersheds was derived from nitrification ($\delta^{18}O_{nitrate} < +14‰$). Since $\delta^{18}O$ values of atmospheric nitrate vary typically between +25 and more than +70‰ (e.g. Durka et al. 1994; Kendall 1998), $\delta^{18}O$ values of up to +19‰ potentially indicate that a small proportion of riverine nitrate might have been derived from atmospheric NO_3^- deposition. This was only observable in predominantly forested watersheds with nitrate deposition as the dominant N input and at low concentrations of riverine nitrate. Oxygen isotope ratios of less than +15‰ in riverine nitrate in most other watersheds revealed no direct contribution of atmospheric nitrate to surface runoff. Apparently, nitrate from atmospheric deposition is intensively cycled through the organic N pool in all watersheds. Its origin is no longer isotopically recognizable after one immobilization/mineralization cycle, since nitrate derived from re-mineralization of the organic nitrogen compounds acquires $\delta^{18}O$ values of less than +15‰ (Mayer et al. 2001).

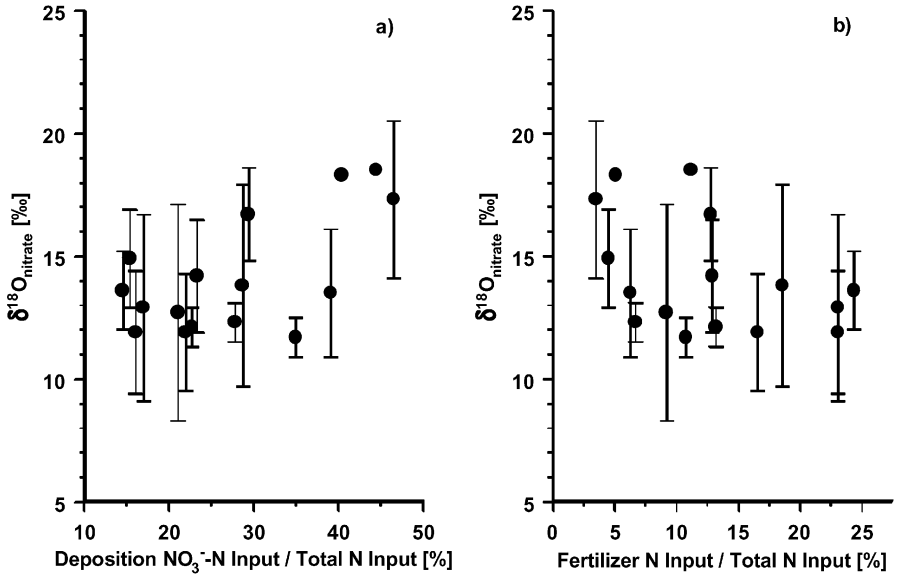

Figure 7. Mean $\delta^{18}O$ values of riverine nitrate versus N inputs with (a) atmospheric nitrate deposition and (b) with synthetic fertilizers to the 16 watersheds expressed as percentage of total N input.

$\delta^{18}O_{nitrate}$ values did not vary with the percentage of fertilizer N on the total N input (Figure 7(b)) indicating that nitrate-containing fertilizers ($\delta^{18}O \sim +22‰$) were not a major source of riverine nitrate. The lowest $\delta^{18}O$ values of riverine nitrate were observed in watersheds with the highest fertilizer N inputs. The absence of a trend of increasing $\delta^{18}O_{nitrate}$ values with increasing application of commercial fertilizers has in all likelihood two reasons. Uptake of fertilizer-nitrate by crops and microbes followed by re-mineralization and nitrification produces nitrate with $\delta^{18}O$ values below +15‰ (Mayer et al. 2001), thereby changing the original oxygen isotope ratio of the fertilizer-nitrate. Also, less than 50% of the fertilizers applied in agricultural areas of the northeastern U.S.A. contain nitrate (c.f. Boyer et al. 2002). Nitrogen from ammonium or urea-containing fertilizers must be nitrified before it can contribute to riverine nitrate. In analogy to what was described above, nitrate derived from nitrification of ammonium or urea-based fertilizers is typically characterized by $\delta^{18}O$ values of less than +15‰. This may explain the observed tendency of decreasing $\delta^{18}O$ values in riverine nitrate with increasing fertilizer loads (Figure 7(b)). Most ammonia and urea-containing fertilizers have $\delta^{15}N$ values around 0‰. Hence, the isotopic composition of nitrate derived from nitrification of these fertilizers can not be distinguished isotopically from that generated by nitrification of organic soil N.

$\delta^{18}O_{nitrate}$ data suggest that nitrate-containing synthetic fertilizers do not directly contribute to riverine nitrate, but may do so after cycling through the organic N pool of the watersheds.

Denitrification

Microbial denitrification, the reduction of nitrate to N_2O and N_2 when oxygen is limited and degradable organic carbon is available (Knowles 1982), constitutes another mechanism, which increases $\delta^{18}O_{nitrate}$ values while decreasing nitrate concentrations (Figure 6). Therefore, this process deserves attention even though most of the observed variability in the isotope composition of riverine nitrate can be explained by mixing of nitrate from various sources.

Denitrification is potentially important in various compartments of large watersheds. These include soils (e.g. Ostrom et al. 1998), aquifers (Fustec et al. 1991; Aravena & Robertson 1998), riperian zones (Warwick & Hill 1988; Lowrance et al. 1995; Hill 1996), hyporheic zones (Duff & Triska 1990), and stream sediments (Cooper 1990; Seitzinger et al. 2002). The extent of denitrification and its influence on N budgets is difficult to assess on a watershed scale, because of the spatial and temporal variability of this process.

Kinetic isotope effects during microbial denitrification progressively enrich the remaining nitrate in ^{15}N and ^{18}O as concentrations decrease. In closed systems, this isotopic enrichment obeys the kinetics of a Rayleigh process, with isotopic enrichment factors for nitrogen varying from less than 10‰ to more than 30‰ depending on environmental conditions (e.g. Mariotti et al. 1981; Mariotti et al. 1988; Fustec et al. 1991). Oxygen isotope fractionation during microbial denitrification varies typically between 8 and 15‰ (Böttcher et al. 1990; Aravena & Robertson 1998; Cey et al. 1999; Mengis et al. 1999). Closed system conditions are often prevalent in anaerobic soil compartments, along the groundwater flowpath in aquifers and riparian zones, and occasionally in river sediments (e.g. Kellman & Hillaire-Marcel 1998). Nitrate that has undergone partial denitrification in these watershed compartments should, therefore, have elevated $\delta^{15}N$ and $\delta^{18}O$ values and lowered concentrations compared to input values. Nitrate entering such compartments has usually undergone at least one immobilization/mineralization cycle resulting in $\delta^{18}O$ values of less than +15‰ (Mayer et al. 2001).

Of the N removal processes within rivers (uptake by biota, burial in sediments, leakage into underlying aquifers, microbial denitrification below the water-sediment interface), only denitrification is accompanied by significant isotope fractionation enriching the remaining nitrate in ^{15}N (Kellman & Hillaire-Marcel 1998). Therefore, changes in the concentration and the isotopic composition of riverine nitrate might be expected, if in-stream denitrification is occurring. Since our samples were taken at the outlet of the 16

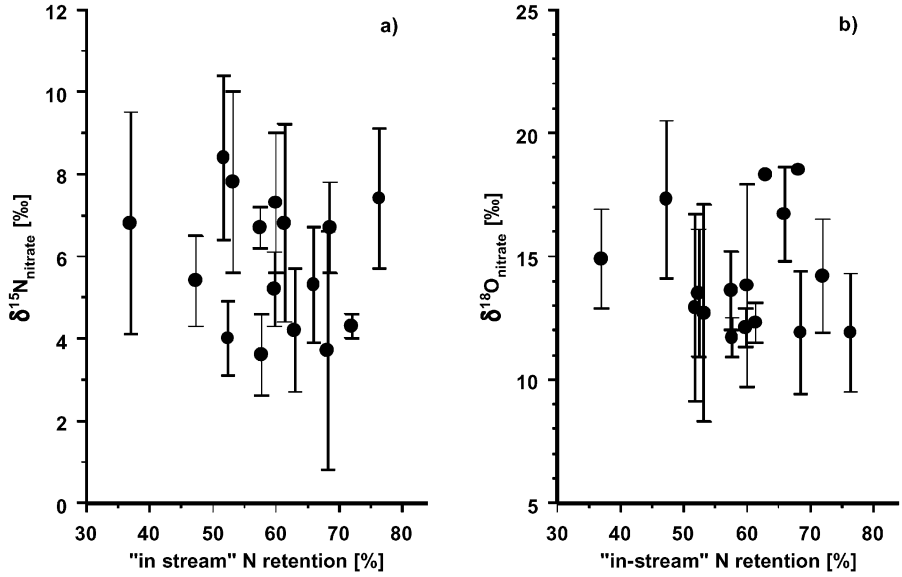

Figure 8. Mean δ^{15}N values (a) and δ^{18}O values (b) of riverine nitrate versus the extent of 'in-stream' N retention in the 16 rivers as determined by Seitzinger et al. (2002).

watersheds, and between 37% and 72% of the nitrogen entering the rivers was removed by in-stream processes (Seitzinger et al. 2002), in-stream denitrification was a potential candidate for influencing the isotopic composition of riverine nitrate.

Figures 8a and 8b show mean δ^{15}N and δ^{18}O values of riverine nitrate plotted versus in-stream N retention estimated by Seitzinger et al. (2002). Although these authors pointed to denitrification as the major in-stream N removal process, we found no statistically significant trend of increasing nitrogen or oxygen isotope ratios of riverine nitrate with increasing in-stream N retention. There are at least two possible explanations for this apparent contradiction. Microbial denitrification below the water-sediment interface may be limited by the diffusion of nitrate from the well-mixed aerobic water column to the anaerobic sediments, a process, which does not discriminate isotopically (Sebilo et al. forthcoming). This would result in little nitrogen and oxygen isotope fractionation during microbial denitrification in benthic sediments. Furthermore, while denitrification decreases nitrate concentrations, concomitant admixture of nitrate from other sources such as waste water and/or animal waste tends to increase riverine nitrate concentrations. Hence, even if denitrification would generate increasing δ^{15}N and δ^{18}O values in riverine nitrate, the simultaneous addition of nitrate from sewage or manure in watersheds with significant urban and agricultural land use would readily

mask any isotopic denitrification signal. This is true also for partially denitrified nitrate entering rivers from soils, aquifers, riparian and hyporheic zones. Because in-stream denitrification is not a single source closed system process, occasional analysis of riverine nitrate at the outlet of large watersheds is clearly inappropriate for determining the location and the extent of microbial denitrification in large watersheds. Detailed monitoring of the evolution of both the concentration and the isotopic composition of dissolved nitrate along a river holds more promise for a conclusive evaluation of the role of in-stream denitrification during riverine N removal (c.f. Kellman & Hillaire-Marcel 1998).

Conclusions

Detailed nitrogen budgets for 16 watersheds with significant variability of nitrogen inputs enabled us to evaluate the usefulness of the isotopic composition of riverine nitrate as a potential tracer for N sources and N transformations in large watersheds. Our data indicate that the isotopic composition of riverine nitrate collected at the outlet of a watershed provides mainly information about nitrate sources.

In predominantly forested watersheds, nitrate was mainly derived from nitrification processes in soils, resulting in comparatively low concentrations of riverine nitrate and $\delta^{15}N_{nitrate}$ values of less than +5‰. In watersheds with significant agricultural and urban land use, $\delta^{15}N$ of riverine nitrate increased to values between +6 and +9‰. Elevated $\delta^{15}N_{nitrate}$ values in some rivers were predominantly caused by admixture of sewage- and manure-derived nitrate. Directly introduced into rivers, waste water caused marked increases in $\delta^{15}N$ of riverine nitrate at fluxes as low as 150 kg N km^{-2} a^{-1}. By contrast, animal waste increased $\delta^{15}N_{nitrate}$ values only at inputs exceeding 1000 kg N km^{-2} a^{-1}. Since increasing $\delta^{15}N$ values were positively correlated with increasing nitrate concentrations, we conclude that sewage was the primary and manure a secondary source of nitrate responsible for increasing concentrations of riverine nitrate, and thus increasing N export from the watersheds. Hence, nitrogen isotope ratios of riverine nitrate appeared to reflect land use practices, with increasing percentages of urban or agricultural land causing increasing $\delta^{15}N$ values of riverine nitrate. Oxygen isotope analyses of riverine nitrate revealed that nitrate in atmospheric deposition and from commercial fertilizers was not a major source of riverine nitrate in the 16 watersheds. Despite significant in-stream denitrification (Seitzinger et al. 2002), this process was not revealed by the isotopic composition of riverine nitrate sampled at the outlet of the 16 watersheds. We suggest that comparatively low isotopic enrichment factors for nitrogen and oxygen

during diffusion-controlled denitrification in river sediments in concert with concomitant admixture of nitrate from waste water and manure is responsible for this finding.

We conclude that a combined evaluation of concentrations and isotope compositions of riverine nitrate can provide information about nitrate sources, whereas detection of N removal processes such as in-stream denitrification was not possible using isotope techniques and the here employed sampling protocol.

Acknowledgements

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