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Isotopic signature of nitrate in two contrasting watersheds of Brush Brook, Vermont, USA

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Abstract We used the dual isotope method to study differences in nitrate export in two subwatersheds in Vermont, USA. Precipitation, soil water and streamwater samples were collected from two watersheds in Camels Hump State Forest, located within the Green Mountains of Vermont. These samples were analyzed for the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-. The range of $\delta^{15}N$–NO_3^- values overlapped, with precipitation –4.5‰ to +2.0‰ ($n = 14$), soil solution –10.3‰ to +6.2‰ ($n = 12$) and streamwater +0.3‰ to +3.1‰ ($n = 69$). The $\delta^{18}O$ of precipitation NO_3^- (mean 46.8 ± 11.5‰) was significantly different ($P < 0.001$) from that of the stream (mean 13.2 ± 4.3‰) and soil waters (mean 14.5 ± 4.2‰) even during snowmelt periods. Extracted soil solution and streamwater $\delta^{18}O$ of NO_3^- were similar and within the established range of microbially produced NO_3^-, demonstrating that NO_3^- was formed by microbial processes. The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- suggests that although the two tributaries have different seasonal NO_3^- concentrations, they have a similar NO_3^- source.

Keywords $\delta^{15}N \cdot \delta^{18}O \cdot$ Nitrate · Nitrogen isotopes · Oxygen isotopes · Vermont

Abbreviations

ANOVA	Analysis of variance
C/N	Ratio of carbon to nitrogen
δ	Delta
DI	Deionized distilled
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
IAEA	International Atomic Energy Agency
N	Nitrogen
^{15}N	Nitrogen-15
NADP	National Atmospheric Deposition Program
NH_4^+	Ammonium ion
NO_3^-	Nitrate ion
^{18}O	Oxygen-18
^{18}Ow	Oxygen-18 of H_2O
‰	Per mil
spp	Species
USGS	United States Geological Survey
V-SMOW	Vienna Standard Mean Ocean Water
VT	Vermont

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Introduction

The source of streamwater NO_3^- in forested watersheds may vary spatially and temporally. In the northeastern US, NO_3^- export patterns from forested watersheds vary seasonally, with the highest leaching losses found in the spring, during snowmelt periods. However in some watersheds, NO_3^- leaching losses have been documented throughout the growing season, indicating that N inputs and availability may exceed N demand (Ågren and Bossatta 1988; Aber 1992; Burns et al. 1998; Pregitzer et al. 2004; Gundersen et al. 2006). Across a range of sites and deposition levels, a general correlation between N deposition and N leaching rates has been established in Europe (Gundersen et al. 1998b; Vesely et al. 2002). However, in most instances there does not appear to be a direct correlation between N deposition and N leaching rates, and N leaching rates may be controlled by other factors, such as plant uptake and soil microbial transformations as well as land use history (Goodale and Aber 2001; Jussy et al. 2004; Pregitzer et al. 2004), C/N (Gundersen et al. 1998a) and species composition (Lovett et al. 2002). Alternatively, Burns et al. (1998) attributed elevated NO_3^- concentrations in streamwater during the growing season to groundwater seeps that recharge streams with water from previous dormant seasons. In this case, hydrological control supersedes biochemical processes.

Nitrate leaching losses from forested ecosystems have been associated with several detrimental effects including soil base cation leaching, soil acidification, nutrient imbalances and eventual forest decline (Aber 1992; Aber et al. 1998; Emmett et al. 1998). Therefore, it is important to understand the factors that regulate N losses in order to predict how an ecosystem may respond to increased levels of nitrogen deposition.

Streamwater NO_3^- may be derived either from atmospheric deposition, which passes directly into the stream without alteration, or NO_3^- that is microbially transformed prior to (or after) entering the stream. The natural abundance of ^{15}N and ^{18}O of NO_3^- in precipitation, soil water and streamwater has been used to trace and distinguish sources of NO_3^- in forested ecosystems (Durka et al. 1994; Kendall et al. 1995; Kendall 1998; Spoelstra et al. 2001). The $\delta^{15}N$ of precipitation NO_3^- varies by location and season with values ranging from –4‰ to +9‰, and these values are similar to those reported for microbially produced NO_3^- (Kendall 1998). The $\delta^{18}O$ of precipitation NO_3^- is often distinct from microbially produced NO_3^-, with values typically ranging from +20‰ to +60‰, and reported values as high as +90‰ (Kendall 1998; Spoelstra et al. 2001; Ohte et al. 2004). From field studies, the $\delta^{18}O$ of microbially produced NO_3^- is reported to range from –10‰ to +16‰ (Kendall 1998; Mayer et al. 2001; Burns and Kendall 2002). Due to the wide separation between the $\delta^{18}O$ of atmospherically and microbially produced NO_3^-, the natural abundance of ^{18}O in NO_3^- can be a powerful tool to identify NO_3^- sources.

We investigated N movement and transformations in two contrasting watersheds with elevated N deposition levels in the Green Mountains of Vermont, USA. Two tributaries of Brush Brook, with different average pH values (4.8 vs. 6.8) were found to have dramatically different NO_3^- concentrations throughout the year, despite draining areas containing soils with similar chemistry (Ross et al. 1994). Flow paths varied between the two watersheds. The watershed draining the higher pH and NO_3^- export stream is underlain by fractured bedrock, suggesting a deeper groundwater source, while the watershed draining the acidic tributary contains a layer of dense basal till (see Ross et al. (1994) for a comprehensive description of the site). Because the two watersheds had different streamwater NO_3^- concentrations and flow paths, the watersheds may also have differed in their ability to utilize soil NO_3^-, resulting in increased leaching of atmospheric NO_3^-. One objective of our study was to determine if the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation, extracted soil solution and streamwater differentiated between NO_3^- sources in these two adjacent tributaries of Brush Brook located in two distinct watersheds. The values for the isotope signature of microbially produced NO_3^- have usually been derived from theoretical calculations or incubated soil samples. Sampling disturbance has been found to stimulate net nitrification (Ross and Hales 2003) and likely alter the $\delta^{15}N$ (Hales and

Ross, submitted). A second objective was to measure the isotope signature of microbially produced NO_3^- in soils processed immediately after sampling to minimize disturbance effects.

Methods

Site description

All experiments were conducted in Camels Hump State Park, located in the Green Mountains of VT, in the towns of Huntington and Duxbury (44°19′ N lat, 72°53′ W long). Both watersheds are completely forested. Vegetation below 820 m is composed of yellow birch (*Betula alleghaniensis*) and sugar maple (*Acer saccharum*) with some red spruce (*Picean rubens*) and balsam fir (*Abies balsamea*) and soils are Spodosols classified as a Houghtonville–Houghtonville Variant complex (Typic Haplorthods) (Ross et al. 1994; US Department of Agriculture, unpublished mapping 1985). Above 820 m, vegetation is dominated by red spruce (*Picean rubens*), balsam fir (*Abies balsamea*) and paper birch (*Betula papyrifa*) and soils are classified as a Stratton-Glebe complex (Thixotropic Humic Cryorthods) (Ross et al. 1994; US Department of Agriculture, unpublished mapping 1985). Annual wet N deposition (NO_3^- and NH_4^+) reported at nearby Underhill, VT (44.53′ N lat, 72.87′ W long; 399 m elevation) during the study period averaged 5.67 kg N ha^{-1} $year^{-1}$ (NADP). Precipitation amounts were relatively constant throughout the year, with most of the precipitation from December through March falling as snow (NOAA).

Sample collection

Precipitation

Precipitation samples were collected intermittently, during all seasons, from 1/13/1998 to 3/7/2000. Rain samples were collected on an event basis from a 122 cm × 122 cm wooden platform covered with a clean polyethylene sheet draining into a collection container lined with plastic. Snow samples were collected at 2–week intervals from an 80-l container lined with plastic. In the laboratory, snow samples were stored at room temperature until melted. All precipitation was filtered through a 0.45 μm filter (Aquaprep 600 Groundwater Capsules, Pall Gelman Sciences, East Hills, NY) in the laboratory and stored at 4°C for analysis. This study did not attempt to establish a comprehensive record of atmospheric deposition chemistry at this site, but rather to confirm that the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation corresponded to samples collected in similar regions (e.g., Kendall 1998).

Streamwater

Streamwater sample sites 1, 13, 17, 18 and 19 were selected from sites previously researched by Ross et al. (1994) (Fig. 1). Sampling site 1 was at the base of a stream draining the southern slope of Camels Hump into Brush Brook. Steam sites 13, 17, 18 and 19 were located along a tributary draining the north-facing slope of Mount Ethan Allen and in an area containing fractured bedrock and numerous groundwater seeps. Site 18 was within a groundwater seep (with higher NO_3^- and pH) that drained into the stream directly above site 17. Site 19 was located above the inflow from the seep and site 13 at the base of the stream before draining into Brush Brook. Sites 13, 17 and 18 contained areas of nettles (*Laportia* spp.).

Streamwater samples were collected in 125-ml polyethylene pre-rinsed bottles on a biweekly basis, as conditions permitted, from September 1996 to May 1999. Samples for NO_3^- isotopic analysis were collected and stored in pre-rinsed 4-l polyethylene cubitainers and 4-l polyethylene wide-mouthed bottles and passed through a 0.45 μm filter (Aquaprep 600 Groundwater Capsules, Pall Gelman Sciences, East Hills, NY). The sample volume collected at each site ranged from 4 l to 20 l and was dependent on the estimated NO_3^- concentration (100 μmol NO_3^- target). Samples for DOC analysis were collected in 250 ml amber glass pre-rinsed bottles and filtered through a GF-F filter in the laboratory. All streamwater samples were stored at 4°C until analysis.

Snowmelt periods were determined by examining hydrographs from two nearby streams,

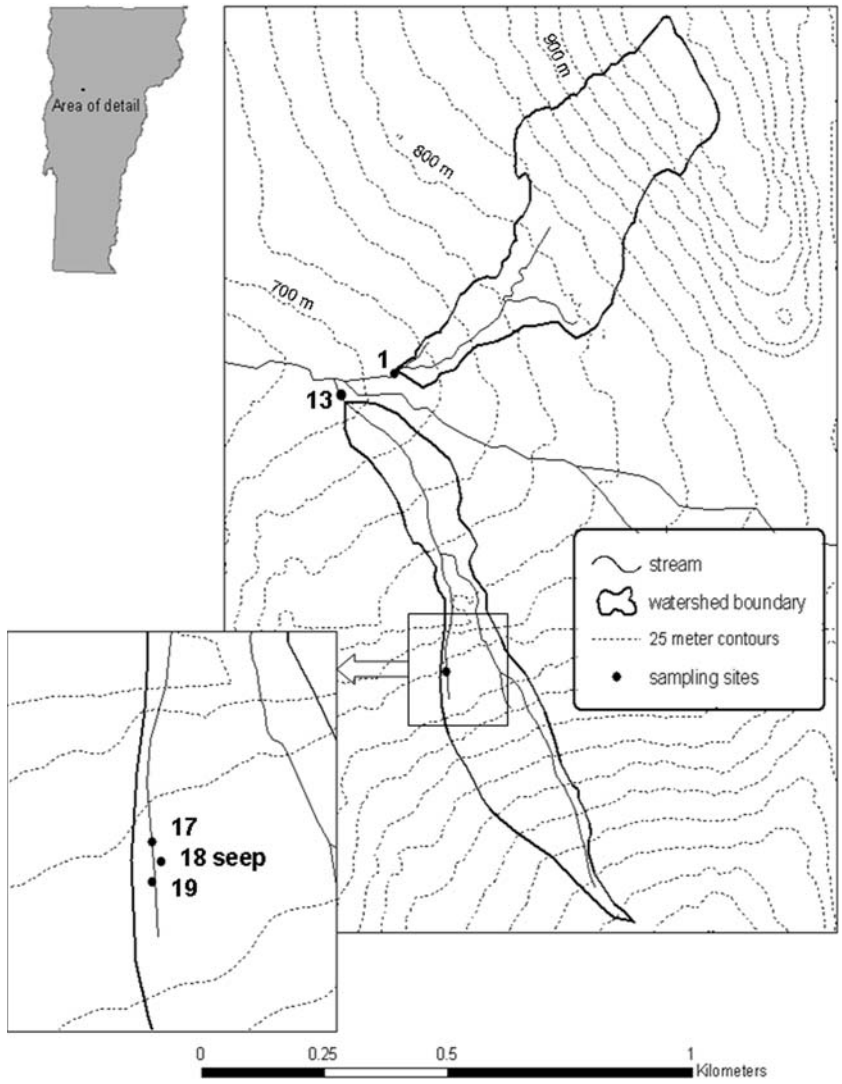

Fig. 1 Location of the study area and streamwater sampling sites. The study area was located within the Green Mountains of VT

Nettle Brook in Underhill, VT and the New Haven River in Bristol, VT (Shanley personal communication; USGS 2006). Thin-plate 90° V-notch weirs were installed at sampling sites 1 and 13 in 2002 and 2001 respectively. Stream stage was recorded at 15 min intervals using a pressure transducer at site 1 and a shaft encoder (stage potentiometer) at site 13.

Soil and extracted soil solution

Oa or A horizons were collected from soils within the two watersheds. Soil sampling sites were selected randomly with a focus on methodology for soil characterization, rather than attempting to relate soil and streamwater chemistry. Sampling sites were also selected to avoid any possible previous disturbance. Samples were collected and mixed (large debris removed) on a clean polyethylene sheet and then either extracted in the field or placed in polyethylene bags and put on ice for return to the laboratory.

Soils were extracted for isotope analysis by two different methods ($n = 12$). Soil solution for initial samples ($n = 3$) was extracted by miscible displacement (Adams 1974). But miscible displacement could not be performed in the field, so all subsequent work was done on water extracts

obtained in the field as soon as feasible after sampling to avoid disturbance-induced increases in nitrification (Ross and Hales 2003). All field extractions were done in duplicate ($n = 9$).

In the field, after the soil was mixed by hand, soil NO_3^- for $\delta^{15}N$ and $\delta^{18}O$ analysis was extracted with distilled-deionized (DI) water (2 l soil:2 l DI water in a polyethylene 4-l bottle), shaken for 5 min, and then filtered through a sparging bag (mesh size 0.34 mm) to remove large particulates. Soil removed from the solution was discarded, and the filtrate kept on ice and returned to the lab, within 2–3 h, for centrifugation at 12,000 × *g* for 15 min, followed by immediate filtration through a pre-rinsed polystyrene 0.45 μm filter (AquaPrep 600 Groundwater Capsules, Pall Gelman Sciences, East Hills, NY). All filtered extracted soil solution was stored at 3°C. Because the disturbance caused by sampling has been found to increase net nitrification in similar soils (Ross and Hales 2003), we extracted and filtered the extracted solution as quickly as possible to minimize the disturbance effect.

Extracted soil solution samples 97-5, 97-7 and 97-9 were collected by miscible displacement in a cold room (10°C) in the laboratory, begun within 2–3 h of initial soil sampling. Miscible displacement (Adams 1974) forces soil solution out of a soil column by simple piston flow through slowly adding another solute (usually water) to the top. Approximately 1 l of soil was packed into a 9 cm diameter plastic pipe with DI H_2O dripped through the top of the column via a 0.5 mm capillary tube. Samples were collected in a series of 125 ml plastic bottles (approximately 500–750 ml collected), filtered through a 0.45 μm filter (Millipore, Billerica, MA) and stored at 3°C.

Laboratory analyses

Precipitation samples ($n = 14$) were analyzed for pH, Cl^- ($n = 13$), NO_3^- ($n = 13$), SO_4^{2-} ($n = 13$), DOC ($n = 4$), $\delta^{18}O$ in water ($n = 8$), $\delta^{15}N$ ($n = 7$) and $\delta^{18}O$ ($n = 8$) of NO_3^-. Streamwater samples ($n = 69$) were analyzed for pH, Cl^-, NO_3^-, SO_4^{2-}, $\delta^{18}O$ in water, DOC, $\delta^{15}N$ and $\delta\ ^{18}O$ of NO_3^-. Additional streamwater samples were collected ($n = 176$) and analyzed for pH, Cl^-, NO_3^- and SO_4^{2-}. Anion concentrations were determined by ion chromatography (Dionex 2000 series, Dionex Corp., Sunnyvale, CA). DOC analysis was conducted at the USGS laboratory, Troy NY (Dohrmann Total Carbon Analyzer, Mason OH). Soils were analyzed for pH, C/N, $\delta^{15}N$ and $\delta^{18}O$ of soil NO_3^- ratios, and total soil ^{15}N. Soil pH was measured with field-moist soils in a 2:1 v:v in distilled water. Soil C and N analyses were conducted on dried, ground and sieved (0.125 mm) soil samples by CHN elemental analyzer (CE 440, Exeter Analytical Inc., North Chelmsford, MA). Samples were run in duplicate with a data quality coefficient of less than 5%.

Isotope procedure

The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation, streamwater and extracted soil solution samples were determined using an adaptation of the method described by Silva et al. (2000) and Chang et al. (1999). The samples were dripped, in series, through 4 ml of pre-rinsed Bio-Rad cation exchange resin (AG 50W-X8, 100–200 mesh) to reduce potential clogging of the anion exchange column by DOC and to reduce potential contamination of the sample (Chang et al. 1999), and then passed through pre-packaged, pre-rinsed Bio-Rad anion exchange resin (AG1-X8, 200–400 mesh) at a flow rate of less than 1 l per hour to retain NO_3^-. For each sample, we loaded approximately 100 μmol of NO_3^- onto the anion exchange resin.

NO_3^- was eluted from the column with 3 M HCl and neutralized with Ag_2O to form $AgNO_3$. The solution was then divided into two aliquots, one for $\delta^{15}N$ analysis and the other for $\delta^{18}O$. For $\delta^{15}N$ analysis the sample was freeze-dried, combustion ingredients added, the sample combusted to produce N_2 gas (Kendall and Grim 1990) and cracked on the mass spectrometer. For $\delta^{18}O$ analysis, $BaCl_2$ was added to precipitate barium sulfate (and possibly barium phosphate), and the solution was passed through a cation exchange resin, freeze dried, and spectrographic graphite added. The sample was then combusted at 850°C for one minute, carbon dioxide extracted cryogenically on the vacuum line and transferred into a sample tube, and analyzed for $\delta^{18}O$ of carbon

dioxide on the mass spectrometer. The $\delta^{15}N$ value of NO_3^- is expressed relative to the standard, atmospheric N_2 (AIR) and the $\delta^{18}O$ value of NO_3^- is expressed relative to the standard, Vienna Mean Ocean Water (V-SMOW). Samples were analyzed using a VG SIRA Series II stable isotope mass spectrometer (VG Isotech, Wythenshawe, Manchester, UK) at the University of Vermont, Stable Isotope Laboratory.

Samples analyzed for total soil $\delta^{15}N$ ratios were taken from bulked samples. Soils were dried (55°C), ground with a mortar and pestle and prepared for ^{15}N analysis according to the method of Kendall and Grim (1990). The $\delta^{18}O$ in water was measured by equilibration with CO_2 and extracted cryogenically on a vacuum line (Socki et al. 1992). Results are reported in per mil deviations relative to a standard, V-SMOW.

Quality control

To ensure accurate results throughout the isotope procedure, we investigated potential interferences and analyzed a number of NO_3^- standards.

(1) We tested the potential effect of Cl^- and dissolved organic matter (DOM) concentration on NO_3^- sorption onto the anion exchange column. Extracted soil solution was collected from an Oa horizon and extracted (DI Water) on March 1, 2000. The extracted soil solution was divided into 0.5, 1 and 2 l aliquots, in duplicate. One ml of 0.09 mol l^{-1} KNO_3 (US Department of Commerce NBS Standard 193) was added to each aliquot, (the extracted soil solution NO_3^- concentration was 12 μmol l^{-1}), aiming for a final NO_3^- composition of 100 μM. Each sample was loaded onto anion exchange resins as described above. We also prepared an additional 1 l aliquot of extracted soil solution for analysis using a Sep-Pak C18 cartridge (Waters Corp, Milford MA). DOC ranged from 4.3 mg l^{-1} to 17.0 mg l^{-1} and Cl^- from 22.9 μmol l^{-1} to 91.6 μmol l^{-1} in the extracted soil solutions (Table 1). The added KNO_3 had a $\delta^{15}N$ value of +0.8‰. DOC and Cl^- concentrations had no discernable effect on either the $\delta^{15}N$ or $\delta^{18}O$ of NO_3^- in these samples ($P < 0.05$). However, the $\delta^{18}O$ of DOM has been reported to be approximately +26‰ (Chang et al. 1999), and we may not have conclusively demonstrated a lack of ^{18}O contribution from DOM because the $\delta^{18}O$ of our added KNO_3 was +22‰.

(2) The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in standard KNO_3 salts (USGS and IAEA standards) were analyzed in duplicate and compared to published values when available (Table 2). For $\delta^{15}N$ of NO_3^-, we also used an in-house KNO_3 sample from the US Department of Commerce (NBS Standard 193) for repeated method validation. We established a method precision for $\delta^{15}N$ of ±0.1 and $\delta^{18}O$ of ±0.3‰. The $\delta^{18}O$ of NO_3^- in precipitation may be biased because we did not run a standard that was within the reported range (Böhlke et al. 2003). However, we did demonstrate adequate accuracy and precision for $\delta^{18}O$ of NO_3^- in the range of values reported for stream and soil samples. Also, the method used to analyze the $\delta^{18}O$ of nitrate has been hypothesized to be biased (0.3–0.7 times higher) than samples

Table 1 The effect of dissolved organic carbon (DOC) concentration on the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- at different extraction volumes

Sample volume (l)	NO_3^- isotope values		Cl^- (μmol l^{-1})	SO_4^{2-} (μmol l^{-1})	DOC (mg l^{-1})
	$\delta^{15}N$ (‰)	$\delta^{15}O$ (‰)			
0.5	1.2	22.3	22.9	8.5	4.3
1.0	1.0	22.2	45.8	16.9	8.5
1.0 (c18*)	0.8	23.4	45.8	16.9	nd
2.0	1.0	22.0	91.6	33.8	17.0
Mean	1.0	22.5	51.5	19.0	9.9
1 SD	0.2	0.6	28.8	10.6	6.5

The extracted solution contained 12 μmol l^{-1} NO_3^- and each volume was spiked with 90 μmol of a standard KNO_3

* Samples were pre-eluted through Waters Sep-Pak C18 column (Milford, MA) to remove DOC

nd: Not determined, but solution color was removed

Table 2 $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in several standards

Sample	Laboratory values (this study)		Reported values	
	NO_3^- isotope values		NO_3^- isotope values	
	$\delta^{15}N$ (‰)	$\delta^{15}O$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
KNO3 Salt 1 (in-house)	0.8		0.7*	
	0.9		0.8*	
	0.9[a]			
	0.9[a]			
IAEA NO-3		22.9		22.7
		22.7		
		22.7		
USGS-32		22.4		22.7
		22.6		
KNO_3 Salt 2 (in-house)	3.7	25.0	3.6	
	3.6	25.4		
IAEA N-2 $(NH_4)_2SO_4$	19.9		20.3	
	20.0			

One liter of 100 μmol l^{-1} (unless otherwise noted) of standard NO_3^- was prepared and put through the sample isotope procedure

[a] 2 l of 100 μmol l^{-1}

* Value determined by combustion of pure KNO_3 salt

IAEA NO-3, values reported by Révész et al. (1997)

USGS-32, values reported by Révész et al. (1997)

analyzed by the on-line combustion technique due to potential isotope exchange between the CO_2 generated from the sample and the quartz combustion tube (Révész and Böhlke 2002). Although, based on our results (Table 2) it does not appear that our samples were biased.

Statistical methods

We used an analysis of variance (ANOVA) to determine if there were any differences in the $\delta^{15}N$ or $\delta^{18}O$ of streamwater NO_3^- or the $\delta^{18}O$ in water among sampling sites 1, 13, 18 and 19. The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- and $\delta^{18}O$ in water were compared using a Dunnett test. The means of the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation, extracted soil solution and streamwater were compared by using a Kruskal–Wallis test. Pair-wise tests were done with a Mann–Whitney test. The $\delta^{15}N$ and $\delta^{18}O$ of streamwater samples collected during snow-melt and non-melt periods were compared by using *t*-tests. All statistical analyses were conducted using SAS (SAS Institute), except for paired *t*-tests (EXCEL). An α level of 0.05 was used to determine significance.

Results

Precipitation chemistry

Precipitation type and chemistry varied throughout the sampling period (Table 3). Precipitation samples collected for this study ($n = 14$), predominantly in 1998, had a mean NO_3^- concentration of 31.4 (±18) μmol l^{-1}. The $\delta^{15}N$ of NO_3^- ranged from –4.5‰ to +2.0‰ (mean –1.1) and the $\delta^{18}O$ from 25.7‰ to 57.3‰ (mean 46.8). The arithmetic mean of $\delta^{18}O$ in water in precipitation was –12.4‰ ($n = 8$). The $\delta^{18}O$ in water of rain averaged (arithmetic mean) –8.4‰ (±3.0; $n = 4$), which was significantly different than snowfall (mean –16.5‰ ±2.8; $n = 4$) ($P < 0.01$, *t*-test).

At nearby Underhill, VT (NADP site VT99) precipitation collected during the study period had a mean pH of 4.5 and NO_3^- concentration of 19.6 μM. Differences between samples collected during this research project and the NADP data may be due to the fewer number of samples we collected, the timing of sample collection, or possible biological activity within the snow-collector (Rascher et al. 1987; Spoelstra et al. 2001).

Table 3 Chemistry of precipitation samples

Date	pH	Cl^- (μmol l^{-1})	NO_3^- (μmol l^{-1})	SO_4^{2-} (μmol l^{-1})	DOC (mg l^{-1})	NO_3^- isotope values			Form
						$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\delta^{18}Ow$ (‰)	
01/13/98	6.1	31.2	43.1	22.5	nd	2.0	33.3	–12.3	Snow
02/02/98	6.2	10.8	23.3	8.4	nd	0.2	57.3	–18.2	Snow
03/11/98	nd	23.7	28.2	10.7	nd	0.7	53.0	nd	Snow
03/25/98	6.6	nd	nd	nd	nd	–4.5	45.6	–17.2	Snow
04/22/98	4.8	6.4	25.7	20.3	nd	nd	nd	nd	Rain
05/05/98	4.3	4.9	44.9	33.1	2.1	–2.3	50.1	–8.9	Rain
05/18/98	4.7	61.5	64.2	77.0	3.0	nd	25.7	–5.0	Rain
05/21/98	4.8	13.6	55.5	39.2	nd	nd	nd	nd	Rain
05/29/98	4.5	9.4	41.4	49.5	nd	nd	nd	nd	Rain
06/10/98	5.3	4.4	10.9	6.1	nd	nd	nd	nd	Rain
06/17/98	4.8	1.8	8.6	6.7	nd	nd	nd	–12.1	Rain
07/02/98	5.2	4.2	4.3	3.1	nd	nd	nd	nd	Rain
07/17/98	4.8	3.4	26.0	32.5	1.2	–2.3	55.3	–7.6	Rain
03/07/00	5.0	13.3	31.8	13.4	3.9	–1.1	54.6	–18.2	Snow
Mean	5.2	14.5	31.4	24.8	2.6	–1.1	46.8	–12.4	
SD	0.7	16.5	18.0	21.3	1.1	2.2	11.5	5.1	
Median	4.8	9.4	28.2	20.3	2.6	–1.1	51.6	–12.2	

nd: Not determined

Streamwater chemistry

Nitrate concentrations varied among stream sampling sites (Fig. 2a and Tables 4, 5). Sites 1 and 13, each at the base of the contrasting watersheds, had differences in how they varied in NO_3^- seasonal concentration (Fig. 2a). Hydrograph data from sites 1 and 13 collected during the spring of 2003 demonstrate the differences in streamflow (Fig. 2b). The pattern shown in Fig. 2a continued through the 2003 time period. Site 18, the groundwater seep draining to site 13, demonstrated less seasonal variation than sites 1, 13 and 19, and had higher NO_3^- concentrations. Streamwater NO_3^- concentrations from sites 1 ($n = 43$) and 13 ($n = 46$) were significantly different ($P < 0.001$, t-test), with site 13 having higher NO_3^- concentrations. Stream sites 1 ($n = 41$) and 13 ($n = 44$) also had significantly different pH ($P < 0.001$, t-test). The $\delta^{15}N$ of streamwater NO_3^- from site 1 was significantly different than that of sites 13, 18 and 19 (Fig. 3; $P < 0.03$). There were no significant differences in the $\delta^{18}O$ of NO_3^- or $\delta^{18}O$ in water ($P = 0.06$, ANOVA) among stream sampling sites. When streamwater sites were paired by date, there were no significant differences in the $\delta^{15}N$ among sites. The difference found when all samples were analyzed was likely the result of seasonality. Streamwater NO_3^- concentrations were too low during much of the growing season to make sampling possible at site 1. Consequently, samples from site 1 were primarily collected during periods of higher flow and NO_3^- concentration, and this may have lead to the observed separation. For all streamwater samples, the mean $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- was +1.9‰ (±0.6) and +13.2‰ (±4.3), respectively. The mean $\delta^{18}O$ in water was –12.5‰ (±0.9). The average streamwater NO_3^- concentration (20.1 ± 13.9 μmol l^{-1}) was similar to the range found in precipitation (31.4 ± 18.0 μmol l^{-1}).

Soil nitrate

Soils had C/N ratios ranging from 15 to 25 (mean 18.9) and pH from 3.3 to 4.2. The $\delta^{15}N$ of total soil ranged from 0.3‰ to 5.3‰ (mean 3.2‰). Of the 12 soils studied, 11 were Oa horizons (%C 28.8–48.1), with one A horizon (%C 17.2). The $\delta^{15}N$ value of extracted soil solution NO_3^- ranged from –10.3‰ to +6.2‰ (mean –2.6, ±4.9), while the $\delta^{18}O$ ranged from +9.1‰ to +21.6‰ (mean 14.5, ±4.2) (Table 6).

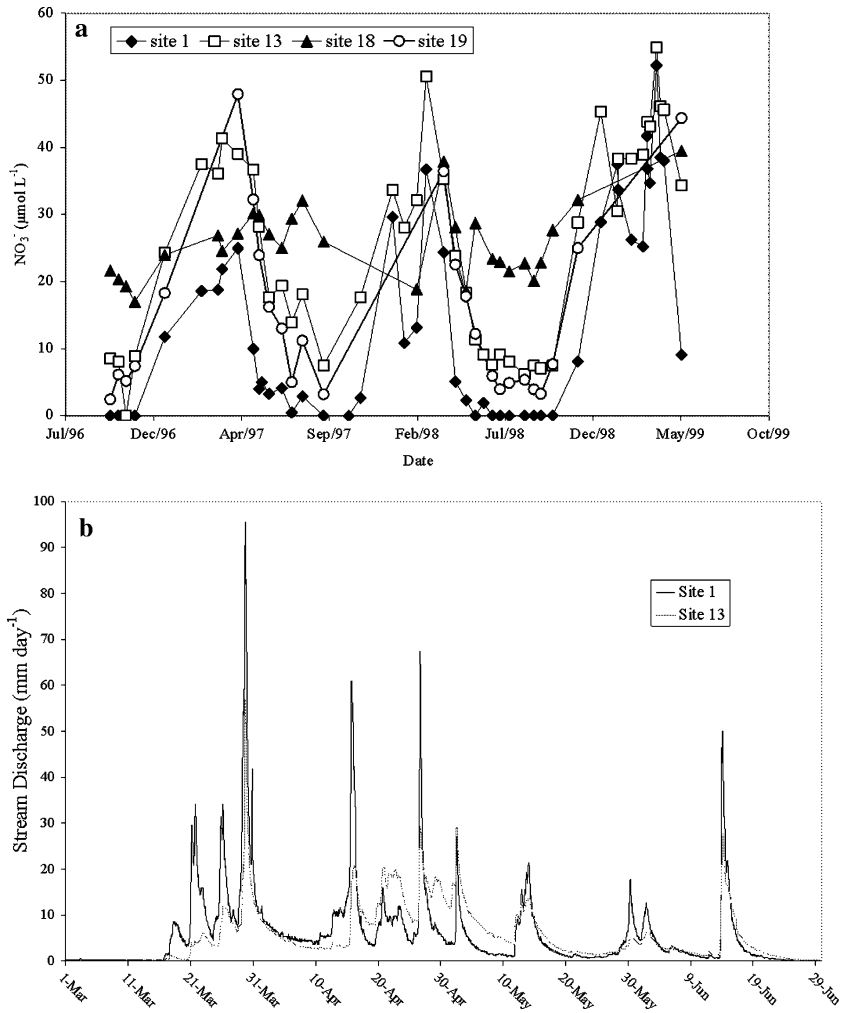

Fig. 2 (**a**) Streamwater NO_3^- concentrations collected between September 1996 and May 1999 from sites 1, 13, 18 and 19. See Fig. 1 for location of sampling sites. (Site 18 is the seep.) (**b**) Spring 2003 discharge for the two subwatersheds. The site 1 subwatershed showed more rapid response and earlier snowmelt, reflecting the shallow soil depth and south-facing aspect

Discussion

Streamwater nitrate dynamics

The different NO_3^- concentrations and patterns found at stream sampling sites 1 and 13 suggest that different biogeochemical processes or flowpaths are occurring within the two subwatersheds. Nitrate concentrations from site 1 varied seasonally, with the highest concentrations during the dormant and snowmelt periods, while NO_3^- concentrations from site 13 (located in a different subwatershed) were less variable, with elevated NO_3^- concentrations during the growing season. The groundwater seep (site 18) contributed a relatively constant concentration of NO_3^- to the tributary draining to site 13, and may have damped the seasonal pattern of nitrogen export within that subwatershed.

Ross et al. (1994) studied flow paths within the Brush Brook research area and identified a layer of dense basal till in the subwatershed draining to site 1, and no dense basal till within the subwatershed draining to site 13. There were no differences in B horizon soil pH between the two subwatersheds. They hypothesized that the tributary draining to site 1 was supplied by soil water that flowed only as deep as the B horizon, whereas in the tributary draining to site 13, the source of stream flow was from below the soil profile. The presence of fractured bedrock and groundwater seeps within the watershed draining to site 13 also suggested a deeper groundwater source. Nitrate export patterns in this watershed

Table 4 Streamwater chemistry statistics for all samples collected

Site	*n*	pH		NO_3^-		Cl^-		SO_4^{2-}		Ca^{2+}		Mg^{2+}		K^+	
		Mean	SD	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})
1	48	5.0	0.3	13.8	15.2	6.6	2.7	52.7	8.2	32.9	14.7	17.8	8.4	4.1	3.3
13	45	6.6	0.2	25.5	15.1	5.1	3.3	67.4	9.0	69.6	18.7	40.0	11.6	9.5	6.0
17	28	6.6	0.3	21.5	10.3	5.5	1.9	73.4	11.2	70.3	19.9	38.8	11.7	9.8	4.3
18	29	7.0	0.2	25.7	5.5	6.4	2.3	88.2	13.5	101.7	16.6	57.8	9.4	15.4	5.1
19	26	5.3	0.3	14.8	13.2	5.0	2.7	62.3	13.4	43.5	7.1	22.3	4.3	3.4	4.1

Table 5 Streamwater chemistry of samples analyzed for the isotopic composition of NO_3^-

Site	*n*	pH		NO_3^-		Cl^-		SO_4^{2-}		Ca^{2+}		Mg^{2+}		K^+		DOC		$\delta^{15}N$		$\delta^{18}O$		$\delta^{18}Ow$	
		Mean	SD	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (μmol l^{-1})	SD (μmol l^{-1})	Mean (mg l^{-1})	SD (mg l^{-1})	Mean (‰)	SD (‰)	Mean (‰)	SD (‰)	Mean (‰)	SD (‰)
1	7	5.2	0.5	30.7	16.9	7.1	2.7	45.6	3.4	27.1	3.3	14.1	2.7	2.7	2.4	2.2	0.9	2.7	0.4	16.5	4.9	–12.4	0.6
13	22	6.6	0.2	27.8	21.9	4.9	3.8	68.4	15.9	65.3	13.7	37.5	8.6	6.5	5.0	1.5	0.6	1.8	0.7	13.0	5.0	–12.4	0.8
17	9	6.6	0.5	21.0	6.2	5.0	1.4	77.7	13.1	69.5	19.9	38.3	11.8	7.1	3.8	2.0	0.7	1.8	0.2	12.3	3.3	–12.9	0.7
18	16	6.9	0.1	27.5	4.9	6.6	2.8	89.8	14.2	91.1	9.6	51.9	6.0	12.3	4.3	1.1	0.4	1.9	0.5	12.6	2.7	–12.8	0.5
19	15	5.3	0.3	13.4	9.2	3.8	2.0	64.4	17.7	46.0	14.0	23.7	8.0	2.5	4.9	3.1	0.5	1.6	0.6	13.2	4.4	–12.1	1.3

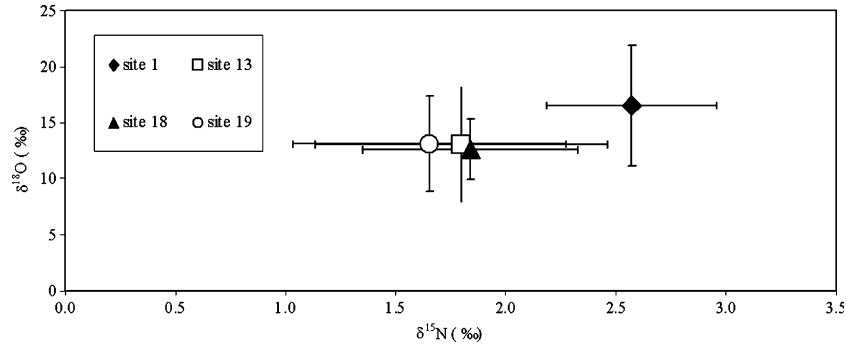

Fig. 3 The mean $\delta^{15}N$ and $\delta^{18}O$ in streamwater NO_3^- collected from sampling sites 1, 13, 18 and 19 with 1 SD. Samples were collected from May 1997 to May 1999. The $\delta^{15}N$ of site 1 is significantly different than sites 13, 18 and 19 ($P < 0.02$). There are no significant differences among sampling sites when samples are date-paired

Table 6 The natural abundance of ^{15}N and ^{18}O of NO_3^- in extracted soil solution

Date	Soil sample	NO_3^- isotope values	
		$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
7/28/1997	97-5	–10.3	nd
10/8/1997	97-7	–6.8	nd
12/18/1997	97-9	–2.4	9.1
5/20/1998	98-3	–0.2	14
6/8/1998	98-4	–0.9	nd
7/10/1998	98-5	10.2	13.9
9/28/1998	98-6	2.9	20.2
10/7/1998	98-7	–3.5	13.1
5/14/1999	99-8	–3.1	11.8
7/20/1999	99-10	0.2	16.8
7/27/1999	99-11	6.2	21.6
9/27/1999	99-13	–3.5	10.4

nd: Sample destroyed during analysis

may depend on flow paths and appear to vary depending on the subwatershed (Ross et al. 1994).

Burns et al. (1998) also attributed increased NO_3^- concentrations in streamwater during the growing season to groundwater seeps that recharge streams with water from previous dormant seasons. Similarly, at Hubbard Brook, Pardo et al. (2004) hypothesized that hydrology may have a significant impact on masking any seasonal patterns of streamwater NO_3^-. Others have suggested that when the capacity of plants and soils to retain N is exceeded, due to increased N inputs, rates of N leaching increase, indicating that N inputs exceed demand (i.e., nitrogen saturation) (Ågren and Bossatta 1988; Aber 1992; Pregitzer et al. 2004). The hydrological control of seasonal NO_3^- export patterns contrasts with N saturation theory. This juxtaposition and the presence of similar vegetation in our two study sub-watersheds suggests that N saturation does not explain the differences in streamwater NO_3^- concentrations identified during this study, as increased leaching and increased export are not necessarily the same. Several factors must be considered when determining the source of streamwater NO_3^-, and the potential importance of hydraulic flow paths must be considered when studying N movement through an ecosystem.

Streamwater nitrate sources

The major groundwater seep (site 18) appeared to have a strong influence on the streamwater NO_3^- concentrations of the tributary draining to site 13. By using the wide separation between the $\delta^{18}O$ of precipitation and microbially produced NO_3^-, documented by other researchers, we attempted to distinguish NO_3^- sources (Durka et al. 1994; Kendall et al. 1995; Kendall 1998; Williard et al. 2001). Despite the differences in NO_3^- export, the $\delta^{18}O$ of NO_3^- indicates that both tributaries of Brush Brook have a similar NO_3^- source. Nitrate in samples from site 19, above the seep, where the stream is more acidic and intermittent, also did not have a different isotopic signature, suggesting that NO_3^- with a longer residence time was not isotopically distinguishable from that from presumably shallower flow. Because there were no differences in date-paired samples (site 1 vs. site

13), we assume that the overall higher $\delta^{15}N$ of NO_3^- at site 1 (Fig. 3) was related to the preponderance of samples from high flow events. Therefore, neither the $\delta^{15}N$, the $\delta^{18}O$ was able to separate the NO_3^- in these two tributaries.

Soil nitrate

Nitrate that is microbially produced in the soil is likely a significant source of streamwater NO_3^-. We analyzed the NO_3^- from twelve surface soil horizons to investigate the potential range of isotope ratios. The soils were extracted in the field immediately after sampling because soils collected from this study area have been found to undergo rapid changes in net nitrification after disturbance (Ross and Hales 2003; Ross et al. 2004). The $\delta^{18}O$ of extracted soil solution NO_3^- ranged from +9.1 to +21.6 (mean 14.5‰) and while reported values of the isotopic composition of soil NO_3^- are limited, our data fell within the reported range of microbially produced NO_3^- (+2‰ to +20‰) (Kendall 1998; Mayer et al. 2001). During soil nitrification, 2/3 of O_2 utilized is from soil water (global range –25‰ to +4‰) and 1/3 from the soil atmosphere (+23.5‰) (Böttcher et al. 1990; Kendall 1998; Durka et al. 1994). Therefore, the $\delta^{18}O$ of biologically produced soil NO_3^- would be expected to range from –10‰ to +15‰ (Durka et al. 1994; Kendall 1998; Spoelstra et al. 2001). In this study, the calculated theoretical value of $\delta^{18}O$ of microbially produced NO_3^- is –0.5‰, using the average $\delta^{18}O$ in water of precipitation. The $\delta^{18}O$ of extracted soil solution NO_3^- from soils is approximately 10‰ to 20‰ higher than calculated theoretical values (Kendall 1998). This deviation from calculated values may be due to several factors that include differences in the proportion of oxygen sources and fractionations resulting from the incorporation of oxygen from water or O_2 (Kendall 1998). An additional explanation for this deviation may be that the $\delta^{18}O$ of oxygen may not remain +23.5‰ throughout our extraction procedure. Reasons for the wide variation in the $\delta^{15}N$ of soil NO_3^- (Fig. 4a, b) are not readily apparent. However, there was even more variation in the $\delta^{15}N$ of NO_3^- from further extractions of theses same samples after 2–3 days of incubation (Hales and Ross submitted). The factors that may affect the $\delta^{15}N$ of soil NO_3^- include the isotopic composition of the reactant pool (i.e., NH_4^+), the rate of reaction and/or denitrification. Rapid changes in net nitrification apparently affect the isotopic composition. Further work is warranted to determine the most suitable technique for extracting soil nitrate for isotopic analysis.

Comparison among precipitation, streamwater and soil solution

The $\delta^{15}N$ and $\delta^{18}O$ of precipitation, soil and streamwater NO_3^- were consistent with other observations in the literature (Kendall 1998). Studies in the US and Europe have demonstrated that the $\delta^{18}O$ value of atmospheric NO_3^- and microbially produced NO_3^- differ significantly, while the $\delta^{15}N$ ranges for the two overlap (Durka et al. 1994; Kendall 1998). The $\delta^{18}O$ of precipitation NO_3^- is expected to range from +20‰ to +80‰ (Durka et al. 1994; Kendall et al. 1996; Kendall 1998; Williard et al. 2001; Ohte et al. 2004; Pardo et al. 2004). These reported values are similar to those obtained during this study, with a mean $\delta^{15}N$ of –1.1 and $\delta^{18}O$ of 46.8‰ (Table 3).

The range of $\delta^{15}N$ values of precipitation, extracted soil solution and streamwater NO_3^- overlapped. The $\delta^{18}O$ of precipitation NO_3^- was significantly higher from that of the streamwaters and extracted soil solutions (Fig. 4a, b; $P < 0.001$, Kruskal–Wallis test). Extracted soil solution and streamwater $\delta^{18}O$ of NO_3^- were similar and within the established range of microbially produced NO_3^-, suggesting that NO_3^- was formed by microbial processes prior to entering the stream (Fig. 4a, b; Kendall 1998). Pardo et al. (2004) found similar results in streamwater samples collected from Hubbard Brook where the mean $\delta^{18}O$ was +18‰, indicating that most of the NO_3^- entering the stream was nitrified within the catchment.

Snow melt

In Europe, declining forest stands displayed significant increases in the amount of precipitation NO_3^- entering streams compared to healthy

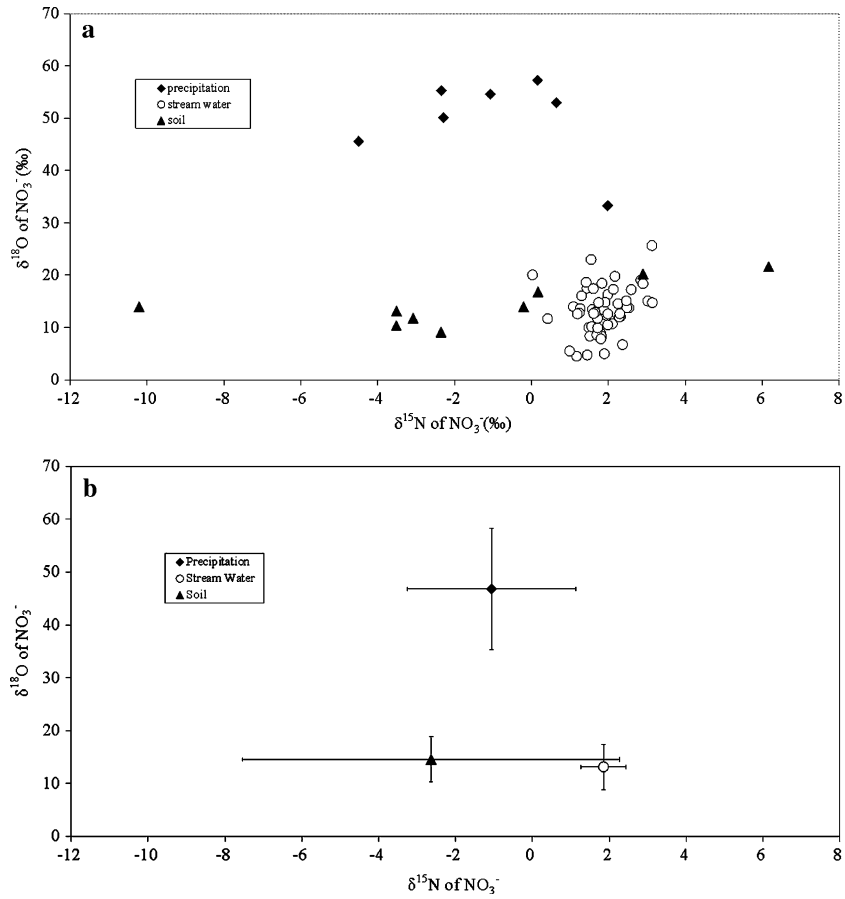

Fig. 4 (**a**) The natural abundance of ^{15}N and ^{18}O of NO_3^- in precipitation, streamwater and extracted soil solution. (**b**) The natural abundance of ^{15}N and ^{18}O of NO_3^- in precipitation, streamwater and extracted soil solution represented by means with 1 SD

stands (Durka et al. 1994). However, in the US, the contribution of precipitation NO_3^- to streamwaters has been determined to be very small, even during snowmelt periods (Kendall et al. 1995, 1996; Pardo et al. 2004). Recent studies have demonstrated that atmospherically derived NO_3^- may have a larger influence on streamwater NO_3^- than previously thought, primarily during the initial stage of snowmelt (Ohte et al. 2004). Our results (Fig. 5) demonstrated that in samples taken during snowmelt (i.e., high flow) periods the $\delta^{15}N$ and $\delta^{18}O$ of streamwater NO_3^- was primarily derived from a microbial source. However, our sampling and analytical techniques limited our ability to collect snowmelt samples on a time-scale as fine as Ohte et al. (2004), and we may not have collected streamwater samples from the initial melt period. One limitation of our analysis of streamwater during melt periods was that we did not collect flow-related samples and may not have collected samples during peak discharge NO_3^- concentrations. However, all samples were collected during the afternoon when diurnal melt cycles were peaking. During the snowmelt (i.e., high flow) period the $\delta^{15}N$ of streamwater NO_3^- ranged from 1.5‰ to 3.2‰ (mean 2.3‰) and the $\delta^{18}O$ from 5 to 25‰ (mean 15.9‰) (Fig. 5). Also during melt periods, there was an apparent simultaneous increase in $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- (Fig. 5). The $\delta^{15}N$ of streamwater NO_3^- collected during snowmelt periods was significantly different than samples collected during non-melt periods ($P < 0.05$, t-test). However, the $\delta^{18}O$ of streamwater NO_3^- was not significantly different between melt and non-melt samples ($P = 0.07$, t-test), although the highest $\delta^{18}O$ values of streamwater NO_3^- were collected during spring snow melt ($\delta^{18}O$ of 25.7, site 1). Flow-

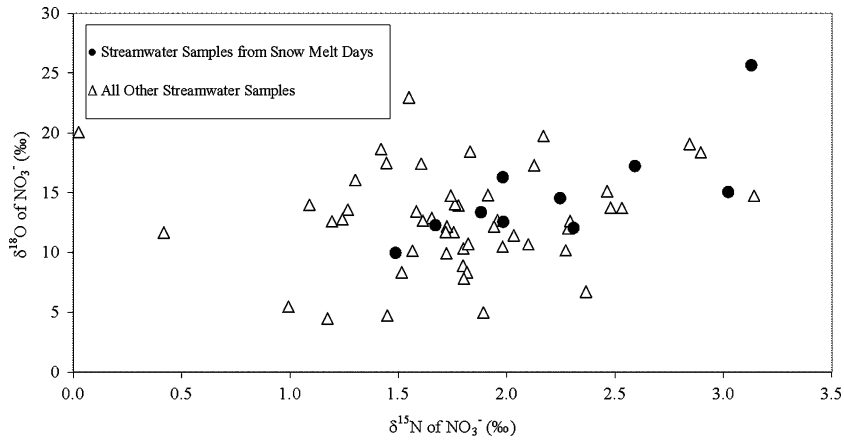

Fig. 5 The natural abundance of ^{15}N and ^{18}O in streamwater NO_3^- comparing samples collected during snowmelt and non-melt periods

paced sampling on a finer time scale is needed to further clarify the relative contribution of the nitrate sources.

Conclusions

We used the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation, streamwater and extracted soil solution to distinguish between atmospherically derived and microbially produced NO_3^-, with the separation primarily due to $\delta^{18}O$. Despite the differences in streamwater chemistry between sites 1 and 13, the source of NO_3^- was found to be similar and the $\delta^{18}O$ of NO_3^- was within the accepted range of microbially produced NO_3^-, even during snowmelt periods. This demonstrates that soil N processes are an important source of N to streamwater and confirms the results from several other studies (Kendall et al. 1996; Burns et al. 1998; Pardo et al. 2004). Also, the presence of numerous groundwater seeps in the watershed draining to site 13 suggests that hydraulic flow paths are an important control of NO_3^- to these streams, particularly as a source during the growing season. The different seasonal NO_3^- concentrations between the two tributaries are apparently the result of differences in hydrology and not differences in the degree of N saturation.

Due to the continued inputs of N to northeastern forest soils, it is important to understand how these inputs may affect soil N cycling and export. The relationship between N deposition and N export can only be understood if we gain a better understanding of soil nitrification processes. Nitrate from deposition appears to be cycled through the microbial pool prior to export and a greater understanding of watershed N dynamics is needed to predict how increased N inputs may affect soil processes and regulate N losses.

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