Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested watersheds in the northeastern U.S.

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Abstract Nitrogen from atmospheric deposition serves as the dominant source of new nitrogen to forested ecosystems in the northeastern U.S. By combining isotopic data obtained using the denitrifier method, with chemical and hydrologic measurements we determined the relative importance of sources and control mechanisms on nitrate (NO_3^-) export from five forested watersheds in the Connecticut River watershed. Microbially produced NO_3^- was the dominant source (82–100%) of NO_3^- to the sampled streams as indicated by the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-. Seasonal variations in the $\delta^{18}O$–NO_3^- in streamwater are controlled by shifting hydrologic and temperature affects on biotic processing, resulting in a relative increase in unprocessed NO_3^- export during winter months. Mass balance estimates find that the unprocessed atmospherically derived NO_3^- stream flux represents less than 3% of the atmospherically delivered wet NO_3^- flux to the region. This suggests that despite chronically elevated nitrogen deposition these forests are not nitrogen saturated and are retaining, removing, and reprocessing the vast majority of NO_3^- delivered to them throughout the year. These results confirm previous work within Northeastern U.S. forests and extend observations to watersheds not dominated by a snow-melt driven hydrology. In contrast to previous work, unprocessed atmospherically derived NO_3^- export is associated with the period of high recharge and low biotic activity as opposed to spring snowmelt and other large runoff events.

Abbreviations

ANOVA	Analysis of variance
CASTNet	Clean Air Status and Trends Network
DIN	Dissolved inorganic nitrogen
IAEA	International Atomic Energy Agency
^{18}O	Oxygen-18
^{15}N	Nitrogen-15
NADP	National Atmospheric Deposition Program
NH_4^+	Ammonium
NO_3^-	Nitrate
NO_2^-	Nitrite
VSMOW	Vienna standard mean ocean water
δ	delta
‰	per mill

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Introduction

Increased reactive nitrogen in the biosphere has led to several environmental problems including alteration of forest processes (e.g. Aber et al. 1998), increased nitrate (NO_3^-) export (Stoddard 1994), and the degradation of coastal waters (NRC 2000). These problems occur despite the suggestion that the vast majority of nitrogen added to our landscape is not exported to the coastal ocean (Boyer et al. 2002; Schaefer and Alber 2007; Van Breemen et al. 2002). Atmospheric N deposition is one source of anthropogenic nitrogen loading affecting the northeastern U.S., with nitrate (NO_3^-) comprising the majority of inorganic nitrogen (66%) delivered via precipitation in Connecticut (Luo et al. 2003).

Forests in the northeastern US now receive 5- to 10-fold more nitrogen via atmospheric deposition relative to pre-industrial conditions (Galloway et al. 2004), and understanding how these ecosystems respond to an increase in a limiting nutrient remains a major research question (Aber et al. 2003). Uncovering the effects of increased atmospheric deposition to forest ecosystem processes can be difficult due to the number of factors shown to effect nitrogen cycling within forested ecosystems, including past land use and disturbance history (Aber and Driscoll 1997; Aber et al. 1997; Goodale et al. 2000), stand successional trends (Vitousek and Reiners 1975), climate change (Mitchell et al. 1996), geology (Holloway et al. 1998; Williard et al. 2005), elevation (Lawrence et al. 2000), and hydrology (Band et al. 2001).

In forested watersheds where stream NO_3^- fluxes have not increased despite elevated nitrogen deposition, excess nitrogen is retained within the ecosystem or removed via denitrification. Alternatively, an increase in NO_3^- export indicates possible nitrogen saturation (Stoddard 1994). However, due to the varied responses seen in watersheds the regional long-term impacts of chronic nitrogen deposition are still debated (Aber et al. 2003).

The isotopic composition of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) provides unique insights into the nitrogen dynamics in forested watersheds because the dominant sources of stream NO_3^-, microbial nitrification and atmospheric deposition, have distinctive $\delta^{18}O–NO_3^-$ values (e.g. Burns and Kendall 2002; Campbell et al. 2002; Durka et al. 1994) due to the highly enriched nature of the $\delta^{18}O–NO_3^-$ delivered via atmospheric deposition

(e.g. Kendall 1998). Greater export of ^{18}O enriched NO_3^- will occur if atmospheric deposition exceeds the biological demand for NO_3^-, enabling the use of a two end member mixing model to apportion sources. Currently the majority of U.S. studies using the dual isotopes of NO_3^- have occurred in a narrow range of ecosystems that receive relatively moderate rates of nitrogen deposition and have seasonal snow cover and therefore a large spring melt event. These studies indicate that microbial nitrification is the source of NO_3^- export from forests to streams except during snow melt and large storm events when a fraction of exported NO_3^- is derived directly from atmospheric deposition (Burns and Kendall 2002; Campbell et al. 2002; Ohte et al. 2004; Pardo et al. 2004). Furthermore, the majority of these studies used an offline combustion technique which has been shown to yield potentially biased $\delta^{18}O–NO_3^-$ values due to exchange between the quartz reaction tube and the CO_2 produced from the sample (Révész and Böhlke 2002).

Snow cover can be an important ecosystem variable with respect to biogeochemistry (Groffman et al. 2001), yet many U.S. forested systems are not in regions dominated by snow and this study was designed to extend these measurements into forested ecosystems without a snowmelt driven hydrology. We hypothesized that the lack of a snow melt driven hydrology would result in a dampened seasonal pattern in stream $\delta^{18}O–NO_3^-$ as compared to similar studies conducted in northern New England. To test this hypothesis, we measured the dual isotopic composition of NO_3^- in stream and rain water using the denitrifier method (Casciotti et al. 2002; Sigman et al. 2001), a relatively novel technique not utilized by the majority of previously conducted studies. We applied both mixing models and mass balance techniques to isotopic and NO_3^- concentration data and calculated the proportion of unprocessed atmospheric NO_3^- contributing to stream NO_3^- export and the annual amount of atmospherically deposited NO_3^- retained within the watershed.

Methods

Spatial analysis

Watersheds were delineated using ArcHydro tools in ArcMap 9.1 (ESRI, Redlands, CA) using NHDPlus

data (USGS and USEPA 2005). Land use and impervious cover (MRLC 2005), surficial materials (Stone et al. 1992) and bedrock geology (Rodgers 1985) datasets were obtained from both federal and state agency websites. These data were then analyzed using tools in ArcMap 9.1 to determine land use/land cover, surficial materials, and bedrock geology of each watershed.

Nitrogen deposition fluxes

Nitrogen deposition data were obtained from two sources: the Connecticut Nitrogen Deposition Monitoring Network (1997–2001) for Mohawk Mountain (73°17′47″ W, 41°49′17″ N) (Carley et al. 2001, P. Stacey unpublished data) and the National Atmospheric Deposition Program (NADP) and Clean Air Status and Trends Network (CASTNet) for their site in Abington, CT (1994–2006, 72°0′36.36″ W, 41°50′24″ N) (NADP 2007; USEPA 2007). Data from the Abington, CT site were used for deposition flux estimates for the watershed in north−central Connecticut (CB) for 2005 and 2006. Data for Mohawk Mountain were only available through 2001, therefore 2005 and 2006 atmospheric fluxes were calculated based on the relationships ($R^2 > 0.90$) between reported fluxes from the two sites for the years of data overlap (1997–2001). The annual estimated flux for Mohawk Mountain was used for the four watersheds in northwestern Connecticut and southwestern Massachusetts (HSR, RB, SB, WBFR).

Sample collection

Streamwater was collected bi-monthly from five first-order streams in the Connecticut River Watershed over a 14 month period (June 2005–August 2006). Stream flow was measured at each site at the time of sample collection using a Marsh-McBirney electromagnetic current meter. Stream water was collected in acid-washed HDPE bottles and filtered through 0.7 μm GF/F filters in the field and stored on ice until returning to lab. Water samples collected for nitrate isotopic analyses were brought to pH 11 using 6 M NaOH and frozen along with the samples reserved for [NO_3^-], [NO_2^-], and [NH_4^+] analyses.

Precipitation samples were collected on an event basis throughout northern and central Connecticut from June 2006 to March 2007 with the cooperation of

wastewater treatment plant operators in Manchester, Vernon, Winsted, Canton and Farmington Connecticut. Four liter glass beakers were placed in open areas preceding a rainstorm and were collected shortly after it ended to minimize evaporation and the collection of dry deposition; rainwater was immediately transferred to acid washed polycarbonate bottles and frozen until analyses.

Collection of soil samples occurred during July and October of 2006 at seven sites within the five watersheds. We sampled representative areas of each watershed based on results from the GIS analysis of land use and surficial material (5 forest-till, 1 wetland-till, and 1 wetland-swamp). Three soil cores (0.813″ × 8″) were taken at each location, combined, and air dried for approximately one week. A sub-sample of each soil was dried in a muffle furnace at 60°C for 24 hours and then homogenized with a Spex/CentriPrep 6750 freezer mill.

Sample analysis

Nitrogen ion analyses (NO_3^-, NO_2^-, and NH_4^+) were performed using an Astoria 2 Flow Analyzer with a detection limit of 0.36 μmol l^{-1}. Isotopic analyses were performed using the denitrifier method (Casciotti et al. 2002; Sigman et al. 2001) with *Pseudomonas aureofaciens*, by which NO_3^- and NO_2^- were quantitatively converted to N_2O. The $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the N_2O were then analyzed on a Finnigan Delta^{PLUS} XP IRMS. These analyses were standardized on AIR and VSMOW scales, respectively, by parallel analyses of NO_3^- reference materials USGS32, USGS34, and USGS35. Duplicate measurements were made on all samples, with standard deviations falling within the cited reproducibility of 0.3‰ and 0.5‰ (1 standard deviation) for $\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^-, respectively. For all samples where [NO_2^-] made up more than 1% of [$NO_3^- + NO_2^-$] samples were corrected following the method discussed previously (Casciotti et al. 2007; Casciotti and McIlvin 2007), whereby the isotopic composition of NO_2^- is measured by the azide method (McIlvin and Altabet 2005) and subtracted from NO_3^- and NO_2^- $\delta^{15}N$ and $\delta^{18}O$ to yield the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-.

The ^{15}N content of atmospheric NO_3^- determined from isotopic measurements of N_2O must also be corrected for the contribution of $^{14}N^{14}N^{17}O$ to the mass 45 peak. Using the average ratio of $\delta^{17}O/\delta^{18}O$

of rain samples collected in Princeton, NJ (Kaiser et al. 2007, Meredith G. Hastings, personal communication) the following relationship was assumed ($\delta^{17}O \approx 0.90 \times \delta^{18}O$) to correct the measured $\delta^{15}N$ of NO_3^- in rain for the ^{17}O contribution to the $^{15}N/^{14}N$ ratio (see work by Hastings et al. (2004) for a similar correction).

Daily flow estimation and hydrograph separation

Daily flow information for the sampled streams was estimated using the Maintenance of Variance-Extension, type 1(MOVE.1) method, a record-extension technique (Helsel and Hirsch 1992), utilizing both field measurements and daily discharge records from the USGS's National Water Inventory (USGS 2007). Field flow measurements were compared to at least three gauging station datasets (all data were log_{10} transformed) and the correlation coefficient (R^2) for each gauging station-field data pair was calculated. The gauging station with the highest R^2 ($R^2 > 0.93$) was chosen to estimate the mean daily flow for each stream using the MOVE.1 equation which results in estimates that are similarly statistically distributed to actual streamflow measurements (Helsel and Hirsch 1992). The estimated daily flow data were subsequently entered into a web-based hydrograph analysis tool (Lim et al. 2005) to determine the approximate flow conditions (i.e. percent of baseflow) at the time of sampling.

Statistical methods

Paired t-tests were used to determine if there were statistical seasonal differences between N concentrations, $\delta^{15}N–NO_3^-$, and $\delta^{18}O–NO_3^-$ in stream water at each sampling location. Comparisons between the isotopic composition of nitrate in stream and precipitation samples was done using Analysis of Variance (ANOVA). Finally, two-sample t-tests were used to examine the potential seasonality of the $\delta^{15}N–$ and $\delta^{18}O–NO_3^-$ in precipitation samples. All statistical analyses were conducted using Minitab (Minitab Inc.) and an α level of 0.05 was used to determine significance.

Site description

The sampled streams drain forested watersheds located in northern Connecticut and southwestern Massachusetts: headwaters of the West Branch of the Farmington River (WBFR), Riiska Brook (RB), headwaters of the Still River (HSR), Charter's Brook (CB), and Sandy Brook (SB). The watersheds are dominated by forests and wetlands (90–98%) (Table 1) with forest cover typical of southern New England, including both mixed deciduous and coniferous stands. The surficial and bedrock materials do not differ appreciably between watersheds, with glacial till overlying metamorphic and igneous bedrock in all of the watersheds (Rodgers 1985; Stone et al. 1992) (Table 1). The amount of open water is minimal in all of the watersheds except for WBFR, where a dam creates a large impoundment surrounded by wetlands (Table 1). It is important to note that while a portion of some of the watersheds (up to 10%) are classified as urban or agricultural land use, in all cases greater than 95% of this land is designated as open space or pasture.

The NW portion of the sampling region (watersheds WBFR, SB, RB, HSR) received more dissolved inorganic nitrogen (DIN) via atmospheric deposition (8.16 kg N ha^{-1} year^{-1} in 2005 and 8.35 kg N ha^{-1} year^{-1} in 2006) than the CB watershed (in north-central CT) which received 5.61 kg N ha^{-1} year^{-1} in 2005 and 5.68 kg N ha^{-1} year^{-1} in 2006. This gradient in deposition rates is in accordance with the pattern found by Luo et al. (2003) in their analysis of three years of deposition data taken at eight locations throughout CT. The southwest portion of CT had the greatest amount of nitrogen deposition (~ 19 kg ha^{-1} year^{-1}) with the northeast corner receiving approximately 7 kg ha^{-1} year^{-1} less (Luo et al. 2003). On average, NO_3^- and NH_4^+ in wet deposition contribute 41% and 20% to total atmospheric N fluxes. Dry deposition contributes an average of 33% of the total N deposition at these sites. Overall, NO_3^- makes up 69% and 59% of total (wet plus dry) deposition at Abington and Mohawk Mountain site, respectively, with NH_4^+ and dissolved organic nitrogen making up the remainder. Precipitation in this region is distributed almost evenly throughout the year with snow making up a minor component ($\sim 10\%$) of the average annual precipitation budget of 1140 mm (Miller et al. 2002).

Results

Streamwater NO_3^- concentrations were low throughout the year ($[NO_3^-] < 30$ μmol L^{-1}) (Fig. 1). The

Table 1 Watershed attributes and summary data for each of the five watersheds sampled

Stream	Sampling location	Area (km^2)	Land use/ land cover	Bedrock geology	Surficial material	Average water yield (m year^{-1})	Average flow weighted NO$_3^-$ flux (kg km^{-2} year^{-1})
WBFR	73°6′40.95 W 42°13′47.13 N	35.9	78.2% forest 7.8% wetland 3.9% water 1.6% ag 8.5% urban	58% sulfidic schists 41% granite	93% till 7% gravel & sand	1.4	44.8
RB	73°8′15.39 W 42°2′45.68 N	4.1	87.3% forest 7% wetland 1.2% water 1.2% ag 3.9% urban	48% quartzose metasandstone 37% mafic gneiss 14% granite	92% till 7% gravel	0.7	40.8
SB	73°9′39.15 W 42°4′44.01 N	11.2	87% forest 3.3% wetland 3.1% water 4.1% ag 2.5% urban	66% quartzose metasandstone 24% granite 6% mafic gneiss	99% till 1% gravel & sand	1.0	10.1
HSR	72°4′29.44 W 41°53′5.16 N	1.9	97.3% forest 0% wetland 0% water 1.4% ag 1.4 urban	59% pelitic schist 30% granite 11% quartzose metasandstone	93% till 7% gravel & sand	0.9	5.9
CB	72°24′42.47 W 41°55′35.45 N	7.5	75% forest 17.7% wetland 0% water 2.5% ag 4.7% urban	92% granite	98% till 2% swamp	1.3	16.0

Averages are based on all available measurements and weighted appropriately; average NO$_3^-$ flux is weighted by flow measured in the field, average isotopic values for NO$_3^-$ are flux weighted, and the average δ^{15}N for soil are weighted by area

highest NO$_3^-$ concentrations occurred during the lowest flow period (August 2005) and lowest concentrations coincide with high flow events (October 2005 and June 2006) (Fig. 1). The highest NO$_3^-$ fluxes generally occurred during the winter due to significantly greater discharge during these months ($p = 0.05$) (Fig. 1). It should be noted that in CB and HSR, NO$_3^-$ concentrations were higher during the summer than winter, with no measurable NO$_3^-$ export occurring in the winter (Fig. 1b, e).

Nitrate was the dominant form of dissolved inorganic nitrogen (DIN) in three of the five streams sampled, making up 72%, 63%, and 59% of the flow-weighted annual DIN export in CB, RB and SB, respectively, with NH$_4^+$ only being a significant contributor during high flow events (Fig. 1b, c, d). Ammonium made up a significant portion of DIN at WBFR throughout the sampling period (Fig. 1a) and at HSR in the winter and spring months (Fig. 1e).

The δ^{15}N–NO$_3^-$ and δ^{18}O–NO$_3^-$ in stream waters varied between 0.1‰ and 5.7‰ and −3.9‰ and 9.7‰, respectively (Fig. 2). Average streamwater δ^{18}O–NO$_3^-$ was significantly greater ($p = 0.002$) in the winter and spring (6.1‰) than the summer (−2.2‰) (Fig. 2). The δ^{15}N–NO$_3^-$ in rain averaged −2.3‰ (SD = 2.9‰, n = 29) and had δ^{18}O ranging
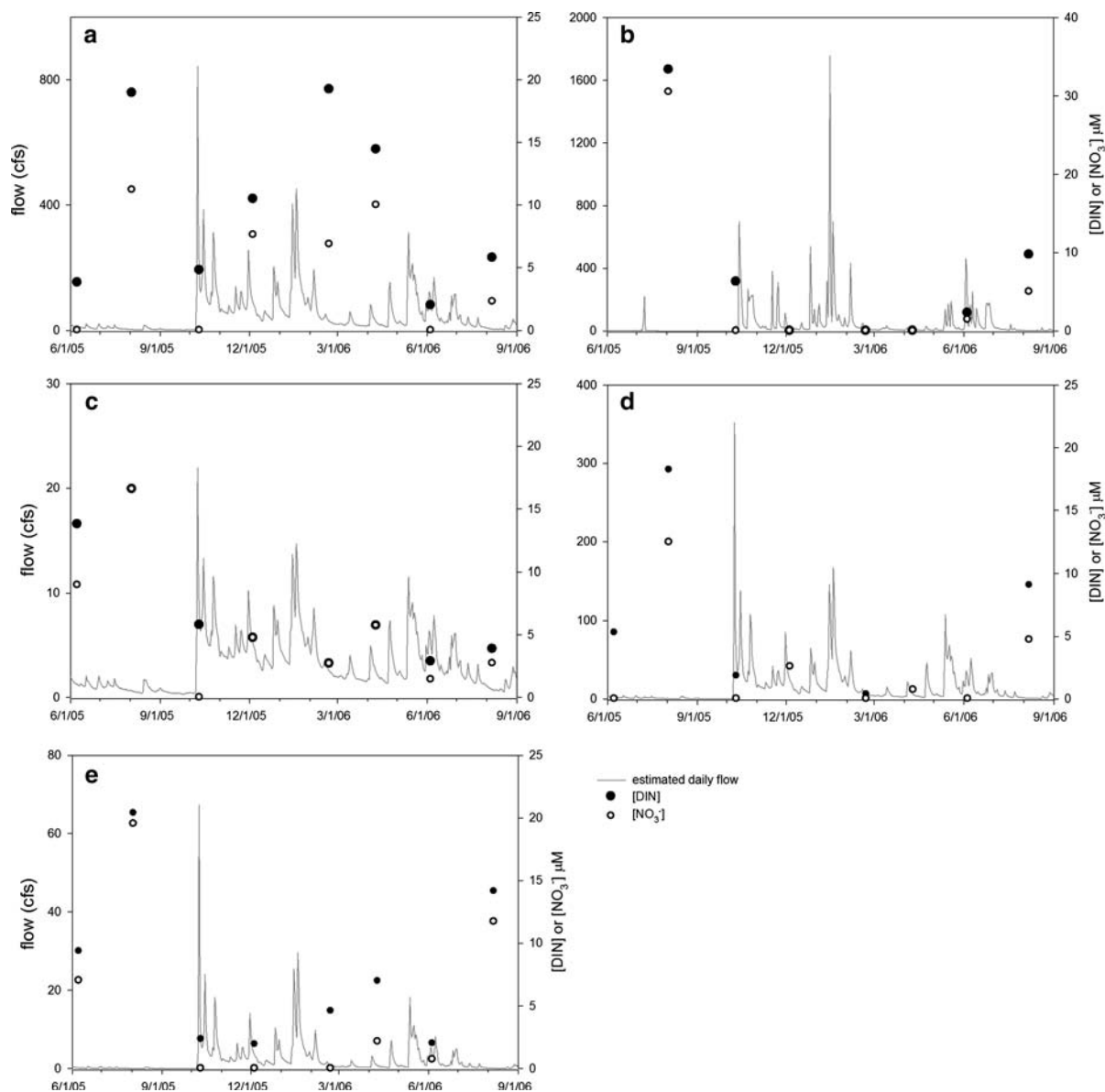
Fig. 1 Bimonthly concentrations of nitrate (NO_3^-), dissolved inorganic nitrogen (DIN), and estimated daily flow values (cfs) from June 2005 to August 2006 within each of the five sampled streams: (**a**) headwaters of the West Branch of the Farmington River, (**b**) Charter's Brook, (**c**) Riiska Brook, (**d**) Sandy Brook, and (**e**) headwaters of the Still River

from 50.4‰ to 83.5‰ (avg = 70.9‰) with no significant seasonal patterns (Fig. 2). The isotopic composition of stream NO_3^- was statistically different ($p < 0.001$) from atmospheric deposition for both $\delta^{15}N$ and $\delta^{18}O$, with the $\delta^{18}O$ of NO_3^- in rain averaging 70‰ higher than that in streamwater (Fig. 2).

Baseflow separation estimates indicate 15 times greater baseflow from October 2005 to April 2006 as compared to June 2005 through September 2005. Sampling events occurred at or near baseflow conditions (baseflow > 90%) except for October 2005 and June 2006. However, it is important to note that the December 2005 and April 2006 sampling events occurred directly after the receding limb of the hydrograph (Fig. 3). Given the estimated nature of our daily flow data it is therefore possible that runoff contributed to streamflow during those two sampling events.
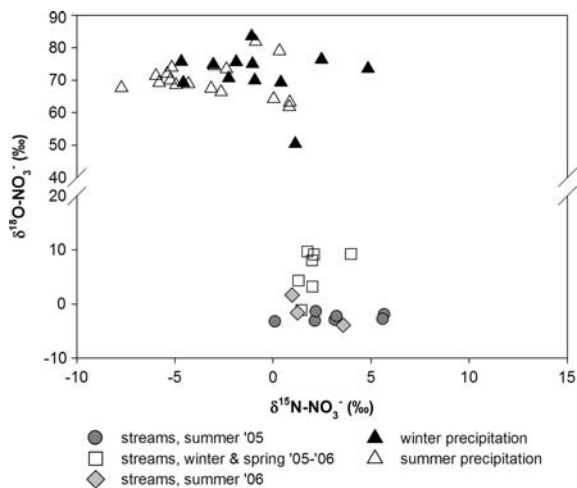
Fig. 2 The $\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^- of stream water and precipitation samples. Stream and precipitation samples are grouped by sampling date into summer and winter/spring subsets

Discussion

Seasonality of the isotopic composition of stream NO_3^-

Differences between $\delta^{15}N$– and $\delta^{18}O$–NO_3^- of precipitation and stream water strongly suggest that atmospherically derived NO_3^- is being processed in a stepwise fashion; NO_3^- is first taken up by biota, converted into organic nitrogen, mineralized to ammonium, and later oxidized back to NO_3^- during nitrification. These processes could lead to the enrichment of ^{15}N in the residual NO_3^- pool and may be responsible for the average 5‰ relative enrichment of streamwater $\delta^{15}N$–NO_3^- as compared to atmospheric deposition $\delta^{15}N$–NO_3^- (Fig. 2). The processing of NO_3^- within a watershed also removes the high $\delta^{18}O$ values of atmospheric NO_3^-, with the $\delta^{18}O$ essentially reset by assimilation and subsequent nitrification to reflect the oxygen used as substrates of this microbial reaction. The $\delta^{18}O$ of NO_3^- in soil and stream water can also be lowered relative to atmospheric deposition by isotope dilution through the microbial oxidation of atmospherically derived NH_4^+ or dissolved organic nitrogen.

In the three streams (WBFR, SB and RB) with measurable NO_3^- flux during the winter there was a clear seasonal pattern in the $\delta^{18}O$ of stream NO_3^-, with measurements falling into summer and winter/
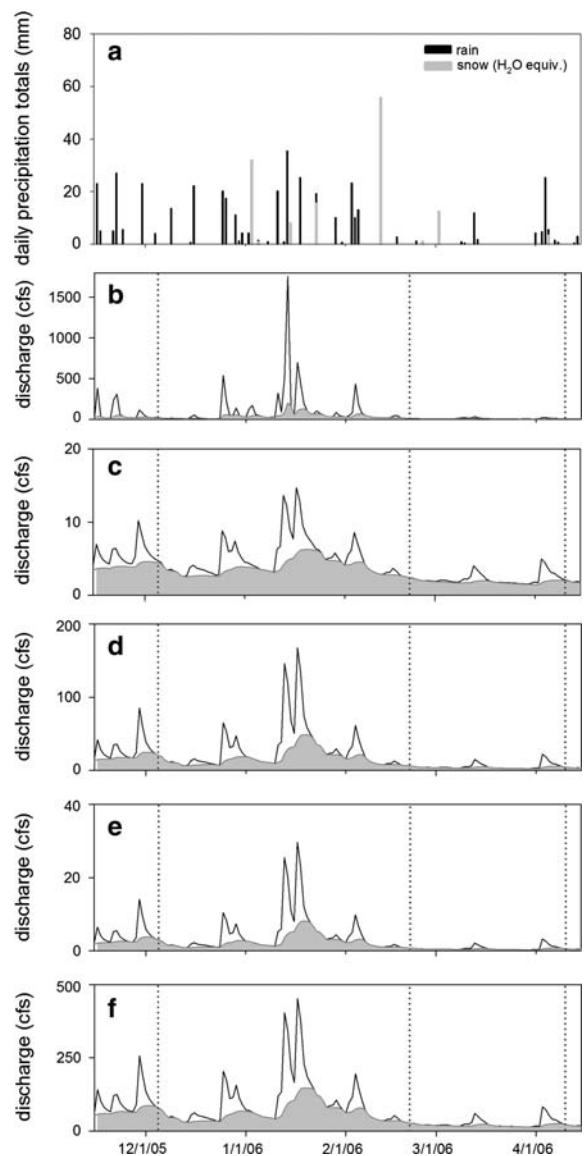

Fig. 3 Daily precipitation totals and estimated hydrographs for study sites for the winter and spring sampling period (11/15/05 through 4/15/06). Precipitation totals (**a**) are for Bradley International Airport and snow amounts are given in water equivalents (as estimated by the following relationship: water equivalent = snow total/10). Estimated hydrographs for each watershed are shown (**b**) Charter's Brook, (**c**) Riiska Brook, (**d**) Sandy Brook, (**e**) headwaters of the Still River, (**f**) headwaters of the West Branch of the Farmington River. The derivation of daily flow values and the baseflow separation calculations are discussed in the text. The dotted vertical lines denote sampling events

spring clusters (Fig. 2). While both clusters fall within the broad range of values given in the literature for microbial nitrification (Kendall 1998)

the statistical difference suggests that either the sources of NO_3^- to the stream or the extent of processing of NO_3^- shifts between seasons.

Seasonality in precipitation $\delta^{18}O–NO_3^-$ could explain seasonal stream patterns, yet our precipitation data showed no significant seasonal trend. It is important to note that this lack of seasonal variation in precipitation $\delta^{18}O–NO_3^-$ suggests that the minimal temporal overlap between rain and stream sample collection should not present a problem for our analyses. If the percentage of atmospherically derived NO_3^- undergoing processing within the watershed changes seasonally, the signal imparted by atmospheric deposition on the exported stream NO_3^- should vary. We tested this hypothesis by entering stream and atmospheric deposition isotopic values ($\delta^{18}O–NO_3^-$) into a simple two end-member mixing model (Eq. 1) to determine the fraction of riverine NO_3^- made up of unprocessed atmospheric NO_3^- (f_{atm}), versus NO_3^- that had been produced by nitrification within the watershed.

$$\frac{\delta^{18}O_{stream} - \delta^{18}O_{nitrification}}{\delta^{18}O_{atm} - \delta^{18}O_{nitrification}} = f_{atm} \qquad (1)$$

Errors associated with this model, due to choices of the end-member values and seasonal variation of end-member values and sources, are discussed below.

The $\delta^{18}O$ of the microbial nitrification source was not directly measured at our sites. Instead we calculated an end member value assuming that microbes incorporate oxygen in a two to one ratio from ambient H_2O and O_2, respectively (Andersson and Hooper 1983; Hollocher 1984; Kumar et al. 1983), using our precipitation $\delta^{18}O–H_2O$ values (−16.02‰ to −0.08‰, R. Barnes unpublished data) and a constant $\delta^{18}O–O_2$ (23.5‰). This calculation yields a range of values from −2.85‰ to 7.78‰ for $\delta^{18}O–NO_3^-$ produced via nitrification. The $\delta^{18}O$ values of streamwater NO_3^- observed in this study (−3.9‰ to +9.7‰), however, imply that for at least part of the year the nitrification end member is below the calculated range, which may reflect a greater influence of $\delta^{18}O–H_2O$ on the $\delta^{18}O–NO_3^-$ produced by nitrification than assumed in the 2:1 H_2O:O_2 ratio (Casciotti et al. 2002). Therefore, in the mixing model we used the lowest measured streamwater $\delta^{18}O–NO_3^-$ value at this site (−3.9‰) to represent the nitrification end member (Table 2). Field studies

examining the $\delta^{18}O–NO_3^-$ from microbial nitrification at other sites have not shown systematic seasonal variations in the $\delta^{18}O$ of NO_3^- produced by nitrification (Burns and Kendall 2002) and therefore we assumed this value did not change seasonally. To test the sensitivity of our interpretations to potential variations in precipitation $\delta^{18}O–NO_3^-$, ($\delta^{18}O_{atm}$ in Eq. 1) we applied the minimum, maximum and average $\delta^{18}O–NO_3^-$ values of the sampled rainwater (50.4‰, 83.5‰ and 70.9‰, respectively) as the atmospheric deposition end-member (Eq. 1, Table 2).

Our calculations suggest that on average 1–3% of the summer and 10–18% of the winter/spring exported stream NO_3^- is derived from direct atmospheric deposition (Table 2), which equals 11–12% of the annual flux-weighted exported stream NO_3^-. Therefore the majority of the NO_3^- exported from these forests is derived from within the catchment and that variation in the amount of processing of atmospherically derived NO_3^- within the watershed can account for the seasonal signal of $\delta^{18}O–NO_3^-$ observed in the streams. Interestingly, this annual average is similar to the estimate given for the snow dominated Catskill Mountains, NY (8%, Burns and Kendall 2002) and within the range (0–45%) presented by Pardo et al. (2004) for two streams in snow dominated New Hampshire.

The peak in $\delta^{18}O–NO_3^-$ for many of these streams occurs in the winter and early spring, opposed to during spring snowmelt or following large storm events as found in other studies (e.g. Burns and Kendall 2002; Campbell et al. 2002; Pardo et al. 2004; Williard et al. 2001). Unlike previous studies, the enrichment found in these non-snow dominated systems could not be attributed solely to runoff events. On average, the amount of processed NO_3^- entering streams in the winter and spring is less than NO_3^- entering the streams in summer and fall. This could be due to either changes in hydrology (e.g. flow paths, recharge rates), temperature affects on the microbial processing of NO_3^-, or both.

High baseflow percentages coincided with the peak $\delta^{18}O–NO_3^-$ of these systems (Fig. 3) and therefore it is unlikely that runoff contributed to the observed $\delta^{18}O–NO_3^-$ patterns. Both higher recharge rates and reduced water demand by plants during the winter favor shorter flow paths (Burns et al. 1998). We believe that the export of unprocessed

Table 2 Mixing model calculations determining the percentage of NO_3^- derived directly from atmospheric deposition (AD) using the minimum and maximum measured $\delta^{18}O-NO_3^-$ values (50.37 to 83.52‰) for AD and −3.9‰ (minimum stream measurement) and −2.85‰ (calculated minimum) for microbial nitrification (MN). *indicates the mixing model calculation yielded a negative percent

Stream	Date	Streamwater $\delta^{18}O-NO_3^-$ (‰)	% of stream NO_3^- from atmospheric deposition			
			AD: 50.37 MN: −3.9	AD: 83.52 MN: −3.9	AD: 50.37 MN: −2.85	AD: 83.52 MN: −2.85
RB	6/2005	−3.0	1.6	1.0	0.0	0.0
HSR	6/2005	−3.3	1.1	0.7	*	*
WBFR	8/2005	−3.2	1.3	0.8	*	0.0
CB	8/2005	−2.0	3.5	2.2	1.6	1.0
RB	8/2005	−2.8	2.0	1.2	0.0	0.0
SB	8/2005	−2.3	2.9	1.8	1.0	0.6
HSR	8/2005	−1.4	4.5	2.8	2.6	1.6
WBFR	12/2005	9.2	24.1	14.9	22.6	13.9
RB	12/2005	4.3	15.2	9.4	13.5	8.3
SB	12/2005	8.1	22.1	13.7	20.5	12.6
WBFR	2/2006	9.2	24.2	15.0	22.7	14.0
RB	2/2006	3.2	13.1	8.1	11.4	7.0
WBFR	4/2006	9.7	25.1	15.6	23.6	14.5
RB	4/2006	−1.1	5.1	3.2	3.2	2.0
WBFR	8/2006	−3.9	0.0	0.0	*	*
CB	8/2006	−1.6	4.2	2.6	2.3	1.4
HSR	8/2006	1.6	10.2	6.3	8.4	5.2

atmospherically derived NO_3^- is due in large part to these shorter flow paths, which reduce the opportunity for NO_3^- processing.

Net nitrification potential measurements and modeling results also indicate that microbial processes responsible for DIN export are strongly influenced by soil temperature and moisture (Christ et al. 2002; Hong et al. 2006). Therefore, lowered rates of microbial nitrification may also contribute to the higher stream $\delta^{18}O-NO_3^-$ values in winter and spring (Fig. 4). The lack of a similar relationship between temperature and stream $\delta^{15}N-NO_3^-$ (Fig. 4) could be due to the different effects of microbial processing on $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$. Complete turnover of the NO_3^- pool may result in little observed $\delta^{15}N-NO_3^-$ change, while $\delta^{18}O-NO_3^-$ is lowered from high atmospheric $\delta^{18}O-NO_3^-$ values to those of microbial nitrification. These processes could result in the observed disconnect between the seasonal trends in stream $\delta^{15}N$- and $\delta^{18}O-NO_3^-$ with relatively higher $\delta^{18}O-NO_3^-$ observed during the winter. However, the significant positive relationship between discharge and $\delta^{18}O-NO_3^-$ ($p < 0.0001$)

could indicate that the relationship between $\delta^{18}O-NO_s^-$ and temperature may only be due to simultaneous changes in hydrology.

Comparison of $\delta^{18}O-NO_3^-$ values across studies

The $\delta^{18}O-NO_3^-$ values in streams sampled in this study (−3.9‰ to 9.7‰) are generally lower than those in other northeastern U.S. studies ($\sim$10‰ to 32‰, Burns and Kendall 2002; Pardo et al. 2004), despite similar estimates of unprocessed atmospherically derived NO_3^- export. One possible explanation is true variation in the $\delta^{18}O$ of the substrates (H_2O and O_2) used during nitrification at the different sites. Isotopic maps of $\delta^{18}O-H_2O$ (Kendall and Coplen 2001) in river water suggests that the $\delta^{18}O-H_2O$ in the Catskills (−10 to −8‰) and White Mountains (−12 to −10‰) is similar or slightly depleted in ^{18}O relative to our sites, providing no explanation for the observed difference. Although micro-scale influences (e.g. respiration, exchange with fine particulate organic matter, denitrification) on these substrates are possible, it is also possible that the discrepancy is methodological. The
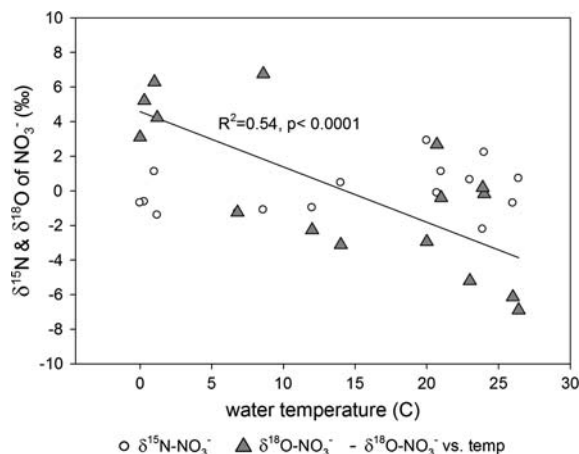
Fig. 4 Water temperature (°C) at time of sampling versus measured $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-. The regression line represents the significant inverse relationship between water temperature and $\delta^{18}O$–NO_3^-, $R^2 = 0.54$ ($p < 0.0001$)

studies mentioned within this paper, with the exception of Ohte et al. (2004), used the method described by Silva, Chang and colleagues (Chang et al. 2002; Silva et al. 2000) and not the denitrifier method used here (Casciotti et al. 2002; Sigman et al. 2001). It should be noted that the study conducted by Ohte et al. (2004), reported a range of $\delta^{18}O$–NO_3^- values (−7.7‰ to 18.3‰) in stream water which encompass our values. The off-line combustion procedure used in the other studies has been shown to yield biased $\delta^{18}O$–NO_3^- values as compared to samples using on-line combustion due to isotopic exchange between the sample derived CO_2 and the quartz combustion tube (Révész and Böhlke 2002). Furthermore, until recently there were not a range of $\delta^{18}O$ NO_3^- standards that allowed for more than a one-point calibration (Böhlke et al. 2003), it was therefore difficult to detect the presence or magnitude of the problem. More recent studies using off-line combustion, such as Hales et al. (2007) used a range of standards to calibrate their NO_3^- isotopic measurements and therefore it is far less likely that their $\delta^{18}O$–NO_3^- values are biased.

Retention of atmospheric NO_3^-

Unprocessed atmospherically derived NO_3^- accounts for up to 25% of the stream NO_3^- flux during the winter and early spring months (Table 2), however the stream NO_3^- flux represents a small fraction of the total wet atmospheric NO_3^- flux; implying that

the remainder is retained or removed within the ecosystem. We estimated the fraction of atmospherically deposited NO_3^- that leaves the catchment unprocessed using equation 2, where F_{atm} is the unprocessed atmospheric NO_3^- deposition flux, FN_s is the estimated stream NO_3^- flux, and f_{atm} the fraction of stream NO_3^- from unprocessed atmospheric deposition (Eq. 1).

$$\frac{f_{atm} \times FN_s}{FN_{atm}} = f_{unprocessed} \qquad (2)$$

Calculations indicate that no more than 2% of NO_3^- entering the watersheds via precipitation goes unprocessed annually. It is important to note that these calculations are based on wet deposition NO_3^- fluxes only and therefore represent an upper bound on $f_{unprocessed}$.

Overestimation of NO_3^- retention (1-$f_{unprocessed}$) is possible due to undetectable levels of NO_3^- in 38% of our samples. In order to account for potential NO_3^- export associated with these samples we repeated the calculations with estimated NO_3^- fluxes using the following assumptions: (1) all samples with NO_3^- concentrations below the detection limit had [NO_3^-–N] of 0.18 μM, (2) during base flow conditions the proportion of unprocessed atmospheric NO_3^- (f_{atm}) was set equal to the monthly average f_{atm} as calculated from the other streams, and (3) during high flow conditions (October 2005, June 2006) f_{atm} was set to 100%. Application of these assumptions did not change the estimates by more than 1% except in CB where retention estimates decreased from 100% to 61%.

It is our understanding that estimates of atmospheric deposition retention within a watershed have never been made based on isotopic mixing model calculations and we acknowledge that our estimates are based on a limited number of observations. Therefore, as a check, we applied the same method to results presented by Pardo et al. (2004) and calculated N retention estimates ranging from 96 to 99% and 86 to 97% in Hubbard Brook Experimental Forest and the Bowl Research Natural Area, respectively. These estimates are in line with studies documenting high N retention in Hubbard Brook (e.g. Bernhardt et al. 2005), including during the non-growing season when Groffman et al. (2001) calculated N retention ranging from 84.1 to 99.9%.

These retention estimates provide evidence that the vast majority of atmospherically derived NO_3^- is

retained or removed within the watershed despite chronically elevated levels of N deposition, suggesting these forests have not reached nitrogen saturation. Furthermore, even without a large above-ground biological demand, watersheds are capable of retaining NO_3^- during the winter via biotic (e.g. microbial immobilization (Brooks et al. 1999)) and abiotic mechanisms such as the reduction of iron (II) in organic soils followed by the conversion of NO_2^- to dissolved organic nitrogen via reactions with dissolved organic material (Davidson et al. 2003).

Conclusions

By using measurements of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation and streamwater in conjunction with estimates of the isotopic composition of microbially produced NO_3^- we distinguished sources of exported NO_3^- across forested watersheds in southern New England. We found that throughout the year soil N processes are the dominant source of exported NO_3^- to streams, confirming the results of similar studies conducted in snowmelt dominated watersheds (e.g. Burns and Kendall 2002; Hales et al. 2007; Pardo et al. 2004). However, in contrast to previous studies, we found that the enrichment of ^{18}O in streamwater NO_3^- during the winter and spring months not associated with large runoff events. Instead it is likely associated with reduced biotic uptake and reprocessing due to shorter flow paths associated with the period of groundwater recharge. Finally, retention estimates illustrate that despite increases in NO_3^- export during the winter and spring months, the watersheds are retaining, removing, or reprocessing 98% of annual atmospheric NO_3^- wet deposition.

Understanding how anthropogenic inputs of nitrogen affect the processing and export of nitrogen from forests to streams is important, as elevated rates of N deposition will continue. In particular, we need a better understanding of soil nitrification and how the isotopic signatures of NO_3^- produced by nitrification vary spatially and temporally. Comparing studies across the Northeastern U.S. points to the importance of seasonal changes in hydrology on soil nitrogen processing and the need of more research that examines how watershed hydrology controls nitrogen export and cycling.

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