1   ***Running Head:*** Processing of Atmospheric N in NE Forests

2   ***Article Type:*** Original Paper

3   ***Title:*** Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested

4   watersheds in the northeastern U.S.

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1 ***Key words:*** atmospheric deposition, forests, nitrogen, stable isotopes

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3 ***Abstract:*** Nitrogen from atmospheric deposition serves as the dominant source of new nitrogen

4 to forested ecosystems in the northeastern U.S.. By combining isotopic data obtained using the

5 denitrifier method, with chemistry and hydrology measurements we determined the relative

6 importance of sources and control mechanisms on nitrate (NO_3^-) export from five forested

7 watersheds in the Connecticut River watershed. Microbially produced NO_3^- was the dominant

8 source (82-100%) of NO_3^- to the sampled streams as indicated by the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-.

9 Seasonal variations in the $\delta^{18}O$-NO_3^- in streamwater are controlled by shifting hydrology and

10 temperature affects on biotic processing, resulting in a relative increase in unprocessed NO_3^-

11 export during winter months. Mass balance estimates find that the unprocessed atmospherically

12 derived NO_3^- stream flux represents less than 3% of the atmospherically delivered wet NO_3^- flux

13 to the region. This suggests that despite chronically elevated nitrogen deposition these forests

14 are not nitrogen saturated and are retaining, removing, and reprocessing the vast majority of

15 NO_3^- delivered to them throughout the year. These results confirm previous work within

16 Northeastern U.S. forests and extend observations to watersheds not dominated by a snow-melt

17 driven hydrology. In contrast to previous work, unprocessed atmospherically derived NO_3^-

18 export is associated with the period of high recharge and low biotic activity as opposed to spring

19 snowmelt and other large runoff events.

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1   *Abbreviations:*

2   ANOVA   Analysis of Variance

3   CASTNet   Clean Air Status and Trends Network

4   DIN   dissolved inorganic nitrogen

5   IAEA   International Atomic Energy Agency

6   ^{18}O Oxygen-18

7   ^{15}N Nitrogen-15

8   NADP   National Atmospheric Deposition Program

9   NH_4^+ Ammonium

10   NO_3^- Nitrate

11   NO_2^- Nitrite

12   VSMOW   Vienna Standard Mean Ocean Water

13   δ   delta

14   ‰   per mill

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16   **Introduction**

17   Increased reactive nitrogen in the biosphere has led to several environmental problems

18   including alteration of forest processes (e.g. Aber et al. 1998), increased nitrate (NO_3^-) export

19   (Stoddard 1994), and the degradation of coastal waters (NRC 2000). These problems occur

20   despite the suggestion that the vast majority of nitrogen added to our landscape is not exported to

21   the coastal ocean (Boyer et al. 2002; Schaefer & Alber 2007; Van Breemen et al. 2002).

22   Atmospheric N deposition is one source of anthropogenic nitrogen loading affecting the

1   northeastern U.S., with nitrate (NO_3^-) comprising the majority of inorganic nitrogen (66%)

2   delivered via precipitation in Connecticut (Luo et al. 2003).

3   Forests in the northeastern US now receive 5 to 10 fold more nitrogen via atmospheric

4   deposition relative to pre-industrial conditions (Galloway et al. 2004), and understanding how

5   these ecosystems respond to an increase in a limiting nutrient remains a major research question

6   (Aber et al. 2003). Uncovering the effects of increased atmospheric deposition to forest

7   ecosystem processes can be difficult due to the number of factors shown to effect nitrogen

8   cycling within forested ecosystems, including past land use and disturbance history (Aber &

9   Driscoll 1997; Aber et al. 1997; Goodale et al. 2000), stand successional trends (Vitousek &

10   Reiners 1975), climate change (Mitchell et al. 1996), geology (Holloway et al. 1998; Williard et

11   al. 2005), elevation (Lawrence et al. 2000), and hydrology (Band et al. 2001).

12   In forested watersheds where stream NO_3^- fluxes have not increased despite elevated

13   nitrogen deposition, excess nitrogen is retained within the ecosystem or removed via

14   denitrification. Alternatively, an increase in NO_3^- export indicates possible nitrogen saturation

15   (Stoddard 1994). However, due to the varied responses seen in watersheds the regional long-

16   term impacts of chronic nitrogen deposition are still debated (Aber et al. 2003).

17   The isotopic composition of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) provides unique insights into the

18   nitrogen dynamics in forested watersheds because the dominant sources of stream NO_3^-,

19   microbial nitrification and atmospheric deposition, have distinctive $\delta^{18}O-NO_3^-$ values (e.g. Burns

20   & Kendall 2002; Campbell et al. 2002; Durka et al. 1994) due to the highly enriched nature of

21   the $\delta^{18}O-NO_3^-$ delivered via atmospheric deposition (e.g. Kendall 1998). Greater export of ^{18}O

22   enriched NO_3^- will occur if atmospheric deposition exceeds the biological demand for NO_3^-,

23   enabling the use of a two end member mixing model to apportion sources. Currently the majority

1   of U.S. studies using the dual isotopes of NO_3^- have occurred in a narrow range of ecosystems

2   that receive relatively moderate rates of nitrogen deposition and have seasonal snow cover and

3   therefore a large spring melt event. These studies indicate that microbial nitrification is the

4   source of NO_3^- export from forests to streams except during snow melt and large storm events

5   when a fraction of exported NO_3^- is derived directly from atmospheric deposition (Burns &

6   Kendall 2002; Campbell et al. 2002; Ohte et al. 2004; Pardo et al. 2004). Furthermore, the

7   majority of these studies used an offline combustion technique which has been shown to yield

8   potentially biased $\delta^{18}O$-NO_3^- values due to exchange between the quartz reaction tube and the

9   CO_2 produced from the sample (Révész & Böhlke 2002).

10   Snow cover can be an important ecosystem variable with respect to biogeochemistry

11   (Groffman et al. 2001), yet many U.S. forested systems are not in regions dominated by snow

12   and this study was designed to extend these measurements into forested ecosystems without a

13   snowmelt driven hydrology. We hypothesized that the lack of a snow melt driven hydrology

14   would result in a dampened seasonal pattern in stream $\delta^{18}O$-NO_3^- as compared to similar studies

15   conducted in northern New England. To test this hypothesis, we measured the dual isotopic

16   composition of NO_3^- in stream and rain water using the denitrifier method (Casciotti et al. 2002;

17   Sigman et al. 2001), a relatively novel technique not utilized by the majority of previously

18   conducted studies. We applied both mixing models and mass balance techniques to isotopic and

19   NO_3^- concentration data and calculated the proportion of unprocessed atmospheric NO_3^-

20   contributing to stream NO_3^- export and the annual amount of atmospherically deposited NO_3^-

21   retained within the watershed.

22

23   **Methods**

1 *Spatial Analysis*

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

3 using NHDPlus data (USGS & USEPA 2005). Land use and impervious cover (MRLC 2005),

4 surficial materials (Stone et al. 1992) and bedrock geology (Rodgers 1985) datasets were

5 obtained from both federal and state agency websites. These data were then analyzed using tools

6 in ArcMap 9.1 to determine land use/land cover, surficial materials, and bedrock geology of each

7 watershed.

8

9 *Nitrogen deposition fluxes*

10   Nitrogen deposition data were obtained from two sources: the Connecticut Nitrogen

11 Deposition Monitoring Network (1997-2001) for Mohawk Mountain (73°17'47" W, 41°49'17"

12 N) (Carley et al. 2001, P. Stacey unpublished data) and the National Atmospheric Deposition

13 Program (NADP) and Clean Air Status and Trends Network (CASTNet) for their site in

14 Abington, CT (1994-2006, 72°0'36.36" W, 41°50'24" N) (NADP 2007; USEPA 2007). Data

15 from the Abington, CT site were used for deposition flux estimates for the watershed in north-

16 central Connecticut (CB) for 2005 and 2006. Data for Mohawk Mountain were only available

17 through 2001, therefore 2005 and 2006 atmospheric fluxes were calculated based on the

18 relationships (R^2>0.90) between reported fluxes from the two sites for the years of data overlap

19 (1997-2001). The annual estimated flux for Mohawk Mountain was used for the four watersheds

20 in northwestern Connecticut and southwestern Massachusetts (HSR, RB, SB, WBFR).

21

22 *Sample collection*

1   Streamwater was collected bi-monthly from five first-order streams in the Connecticut

2   River Watershed over a 14 month period (June 2005 to August 2006). Stream flow was

3   measured at each site at the time of sample collection using a Marsh-McBirney electromagnetic

4   current meter. Stream water was collected in acid-washed HDPE bottles and filtered through 0.7

5   μm GF/F filters in the field and stored on ice until returning to lab. Water samples collected for

6   nitrate isotopic analyses were brought to pH 11 using 6M NaOH and frozen along with the

7   samples reserved for $[NO_3^-]$, $[NO_2^-]$, and $[NH_4^+]$ analyses.

8   Precipitation samples were collected on an event basis throughout northern and central

9   Connecticut from June 2006 to March 2007 with the cooperation of wastewater treatment plant

10   operators in Manchester, Vernon, Winsted, Canton and Farmington Connecticut. Four liter glass

11   beakers were placed in open areas preceding a rainstorm and were collected shortly after it ended

12   to minimize evaporation and the collection of dry deposition; rainwater was immediately

13   transferred to acid washed polycarbonate bottles and frozen until analyses.

14   Collection of soil samples occurred during July and October of 2006 at seven sites within

15   the five watersheds. We sampled representative areas of each watershed based on results from

16   the GIS analysis of land use and surficial material (5 forest-till, 1 wetland-till, and 1 wetland-

17   swamp). Three soil cores (0.813" x 8") were taken at each location, combined, and air dried for

18   approximately one week. A sub-sample of each soil was dried in a muffle furnace at 60°C for 24

19   hours and then homogenized with a Spex/CentriPrep 6750 freezer mill.

20

21   *Sample analysis*

22   Nitrogen ion analyses (NO_3^-, NO_2^-, and NH_4^+) were performed using an Astoria 2 Flow

23   Analyzer with a detection limit of 0.36 μmol L^{-1}. Isotopic analyses were performed using the

1   denitrifier method (Casciotti et al. 2002; Sigman et al. 2001) with *Pseudomonas aureofaciens*, by

2   which NO_3^- and NO_2^- were quantitatively converted to N_2O. The $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of

3   the N_2O were then analyzed on a Finnigan Delta^{PLUS} XP IRMS. These analyses were

4   standardized on AIR and VSMOW scales, respectively, by parallel analyses of NO_3^- reference

5   materials USGS32, USGS34, and USGS35. Duplicate measurements were made on all samples,

6   with standard deviations falling within the cited reproducibility of 0.3‰ and 0.5‰ (1 standard

7   deviation) for $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-, respectively. For all samples where $[NO_2^-]$ made up

8   more than 1% of $[NO_3^-+NO_2^-]$ samples were corrected following the method discussed

9   previously (Casciotti et al. 2007; Casciotti & McIlvin 2007), whereby the isotopic composition

10   of NO_2^- is measured by the azide method (McIlvin & Altabet 2005) and subtracted from NO_3^-

11   and NO_2^- $\delta^{15}N$ and $\delta^{18}O$ to yield the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-.

12   The ^{15}N content of atmospheric NO_3^- determined from isotopic measurements of N_2O

13   must also be corrected for the contribution of $^{14}N^{14}N^{17}O$ to the mass 45 peak. Using the average

14   ratio of $\delta^{17}O/\delta^{18}O$ of rain samples collected in Princeton, NJ (Kaiser et al. 2007, Meredith G.

15   Hastings, personal communication) the following relationship was assumed ($\delta^{17}O \approx 0.90 \times \delta^{18}O$)

16   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

17   work by Hastings and others (2004) for a similar correction).

18

19   *Daily Flow Estimation and Hydrograph Separation*

20   Daily flow information for the sampled streams was estimated using the Maintenance of

21   Variance-Extension, type 1(MOVE.1) method, a record-extension technique (Helsel & Hirsch

22   1992), utilizing both field measurements and daily discharge records from the USGS's National

23   Water Inventory (USGS 2007). Field flow measurements were compared to at least three

1   gauging station datasets (all data were $\log_{10}$ transformed) and the correlation coefficient (R^2) for

2   each gauging station-field data pair was calculated. The gauging station with the highest R^2 ($R^2 >$

3   0.93) was chosen to estimate the mean daily flow for each stream using the MOVE.1 equation

4   which results in estimates that are similarly statistically distributed to actual streamflow

5   measurements (Helsel & Hirsch 1992). The estimated daily flow data were subsequently entered

6   into a web-based hydrograph analysis tool (Lim et al. 2005) to determine the approximate flow

7   conditions (i.e. percent of baseflow) at the time of sampling.

8

9   *Statistical methods*

10   Paired *t*-tests were used to determine if there were statistical seasonal differences between

11   N concentrations, $\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ in stream water at each sampling location.

12   Comparisons between the isotopic composition of nitrate in stream and precipitation samples

13   was done using Analysis of Variance (ANOVA). Finally, two-sample t-tests were used to

14   examine the potential seasonality of the $\delta^{15}N\text{-}$ and $\delta^{18}O\text{-}NO_3^-$ in precipitation samples. All

15   statistical analyses were conducted using Minitab (Minitab Inc.) and an α level of 0.05 was used

16   to determine significance.

17

18   **Site description**

19   The sampled streams drain forested watersheds located in northern Connecticut and

20   southwestern Massachusetts: headwaters of the West Branch of the Farmington River (WBFR),

21   Riiska Brook (RB), headwaters of the Still River (HSR), Charter's Brook (CB), and Sandy

22   Brook (SB). The watersheds are dominated by forests and wetlands (90-98%) (Table 1) with

23   forest cover typical of southern New England, including both mixed deciduous and coniferous

1   stands. The surficial and bedrock materials do not differ appreciably between watersheds, with

2   glacial till overly metamorphic and igneous bedrock in all of the watersheds (Rodgers 1985;

3   Stone et al. 1992) (Table 1). The amount of open water is minimal in all of the watersheds

4   except for WBFR, where a dam, creates a large impoundment surrounded by wetlands (Table 1).

5   It is important to note that while a portion of some of the watersheds (up to 10%) are classified

6   as urban or agricultural land use, in all cases greater than 95% of this land is designated as open

7   space or pasture.

8   The NW portion of the sampling region (watersheds WBFR, SB, RB, HSR) received

9   more dissolved inorganic nitrogen (DIN) via atmospheric deposition (8.16 kg N ha^{-1}yr^{-1} in 2005

10   and 8.35 kg N ha^{-1}yr^{-1} in 2006) than the CB watershed (in north-central CT) which received 5.61

11   kg N ha^{-1}yr^{-1} in 2005 and 5.68 kg N ha^{-1}yr^{-1} in 2006. This gradient in deposition rates is in

12   accordance with the pattern found by Luo and others (2003) in their analysis of three years of

13   deposition data taken at eight locations throughout CT. The southwest portion of CT had the

14   greatest amount of nitrogen deposition (~19 kg ha^{-1} yr^{-1}) with the northeast corner receiving

15   approximately 7 kg ha^{-1} yr^{-1} less (Luo et al. 2003). Nitrate makes up a majority of nitrogen in

16   atmospheric deposition, 69% and 59% at Abington and Mohawk Mountain sites, respectively.

17   On average NO_3^- and NH_4^+ in wet deposition contribute 41% and 20% to total atmospheric N

18   fluxes, with dry deposition contributing an average of 33% of the total N deposition to these

19   sites. Precipitation in this region is distributed almost evenly throughout the year with snow

20   making up a minor component (~10%) of the average annual precipitation budget of 1140 mm

21   (Miller et al. 2002).

22

23   **Results**

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

2   (Figure 1). The highest NO_3^- concentrations occurred during the lowest flow period (August

3   2005) and lowest concentrations coincide with high flow events (October 2005 and June 2006)

4   (Figure 1). The highest NO_3^- fluxes generally occurred during the winter due to significantly

5   greater discharge during these months ($p = 0.05$) (Figure 1). It should be noted that in CB and

6   HSR, NO_3^- concentrations were higher during the summer than winter, with no measurable NO_3^-

7   export occurring in the winter (Figure 1b,e).

8   Nitrate was the dominant form of dissolved inorganic nitrogen (DIN) in three of the five

9   streams sampled, making up 72%, 63%, and 59% of the flow-weighted annual DIN export in

10   CB, RB and SB, respectively, with NH_4^+ only being a significant contributor during high flow

11   events (Figure 1b,c,d). Ammonium made up a significant portion of DIN at WBFR throughout

12   the sampling period (Figure 1a) and at HSR in the winter and spring months (Figure 1e).

13   The $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- in stream waters varied between 0.1‰ and 5.7‰ and -

14   3.9‰ and 9.7‰, respectively (Figure 2). Average streamwater $\delta^{18}O$-NO_3^- was significantly

15   greater ($p=0.002$) in the winter and spring (6.1‰) than the summer (-2.2‰) (Figure 2). The

16   $\delta^{15}N$-NO_3^- in rain averaged -2.3‰ (SD=2.9‰, n=29) and had $\delta^{18}O$ ranging from 50.4‰ to

17   83.5‰ (avg=70.9‰) with no significant seasonal patterns (Figure 2). The isotopic composition

18   of stream NO_3^- was statistically different ($p < 0.001$) from atmospheric deposition for both $\delta^{15}N$

19   and $\delta^{18}O$, with the $\delta^{18}O$ of NO_3^- in rain averaging 70‰ higher than that in streamwater (Figure

20   2).

21   Baseflow separation estimates indicate 15 times greater baseflow from October 2005 to

22   April 2006 as compared to June 2005 through September 2005. Sampling events occurred at or

23   near baseflow conditions (baseflow > 90%) except for October 2005 and June 2006. However, it

1   is important to note that the December 2005 and April 2006 sampling events occurred directly

2   after the receding limb of the hydrograph (Figure 3). Given the estimated nature of our daily

3   flow data it is therefore possible that runoff contributed to streamflow during those two sampling

4   events.

5

6   **Discussion**

7   *Seasonality of the isotopic composition of stream NO_3^-*

8   Differences between $\delta^{15}N$- and $\delta^{18}O$-NO_3^- of precipitation and stream water strongly

9   suggest that atmospherically derived NO_3^- is being processed in a stepwise fashion; NO_3^- is first

10   taken up by biota, converted into organic nitrogen, mineralized to ammonium, and later oxidized

11   back to NO_3^- during nitrification. These processes lead to the enrichment of ^{15}N in the residual

12   NO_3^- pool and may be responsible for the average 5‰ relative enrichment of streamwater $\delta^{15}N$-

13   NO_3^- as compared to atmospheric deposition $\delta^{15}N$-NO_3^- (Figure 2). The processing of NO_3^-

14   within a watershed removes the high $\delta^{18}O$ values of atmospheric NO_3^-, with the $\delta^{18}O$ essentially

15   reset by assimilation and subsequent nitrification to reflect the oxygen used as substrates of this

16   microbial reaction. The $\delta^{18}O$ of NO_3^- in soil and stream water can also be lowered relative to

17   atmospheric deposition by isotope dilution through the microbial oxidation of atmospherically

18   derived NH_4^+ or dissolved organic nitrogen.

19   In the three streams (WBFR, SB and RB) with measurable NO_3^- flux during the winter

20   there was a clear seasonal pattern in the $\delta^{18}O$ of stream NO_3^-, with measurements falling into

21   summer and winter/spring clusters (Figure 2). While both clusters fall within the broad range of

22   values given in the literature for microbial nitrification (Kendall 1998) the statistical difference

1   suggests that either the sources of NO_3^- to the stream or the extent of processing of NO_3^- shifts

2   between seasons.

3   Seasonality in precipitation $\delta^{18}O$-NO_3^- could explain seasonal stream patterns, yet our

4   precipitation data showed no significant seasonal trend. It is important to note that this lack of

5   seasonal variation in precipitation $\delta^{18}O$-NO_3^- suggests that the minimal temporal overlap

6   between rain and stream sample collection should not present a problem for our analyses. If the

7   percentage of atmospherically derived NO_3^- undergoing processing within the watershed changes

8   seasonally, the signal imparted by atmospheric deposition on the exported stream NO_3^- should

9   vary. We tested this hypothesis by entering stream and atmospheric deposition isotopic values

10   ($\delta^{18}O$-NO_3^-) into a simple two end-member mixing model (eqn 1) to determine the fraction of

11   riverine NO_3^- made up of unprocessed atmospheric NO_3^- (f_{atm}), versus NO_3^- that had been

12   produced by nitrification within the watershed.

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

14   Errors associated with this model, due to choices of the end-member values and seasonal

15   variation of end-member values and sources, are discussed below.

16   The $\delta^{18}O$ of the microbial nitrification source was not directly measured at our sites.

17   Instead we calculated an end member value assuming that microbes incorporate oxygen in a two

18   to one ratio from ambient H_2O and O_2, respectively (Andersson & Hooper 1983; Hollocher

19   1984; Kumar et al. 1983), using our precipitation $\delta^{18}O$-H_2O values (-16.02‰ to -0.08‰, R.

20   Barnes unpublished data) and a constant $\delta^{18}O$-O_2 (23.5‰). This calculation yields a range of

21   values from -2.85‰ to 7.78‰ for $\delta^{18}O$-NO_3^- produced via nitrification. The $\delta^{18}O$ values of

22   streamwater NO_3^- observed in this study (-3.9‰ to +9.7‰), however, imply that for at least part

23   of the year the nitrification end member is below the calculated range, which may reflect a

1 greater influence of δ^{18}O-H$_2$O on the δ^{18}O-NO$_3^-$ produced by nitrification than assumed in the

2 2:1 H$_2$O:O$_2$ ratio (Casciotti et al. 2002). Therefore, in the mixing model we used the lowest

3 measured streamwater δ^{18}O-NO$_3^-$ value at this site (-3.9‰) to represent the nitrification end

4 member (Table 2). Field studies examining the δ^{18}O-NO$_3^-$ from microbial nitrification at other

5 sites have not shown systematic seasonal variations (Burns & Kendall 2002) in the δ^{18}O of NO$_3^-$

6 produced by nitrification and therefore we assumed this value did not change seasonally. To test

7 the sensitivity of our interpretations to potential variations in precipitation δ^{18}O-NO$_3^-$, (δ^{18}O$_{atm}$ in

8 eqn. 1) we applied the minimum, maximum and average δ^{18}O-NO$_3^-$ values of the sampled

9 rainwater (50.4‰, 83.5‰ and 70.9‰, respectively) as the atmospheric deposition end-member

10 (eqn. 1, Table 2).

11   Our calculations suggest that on average 1-3% of the summer and 10-18% of the

12 winter/spring exported stream NO$_3^-$ is derived from direct atmospheric deposition (Table 2),

13 which equals 11-12% of the annual flux-weighted exported stream NO$_3^-$. Therefore the majority

14 of the NO$_3^-$ exported from these forests is derived from within the catchment and that variation in

15 the amount of processing of atmospherically derived NO$_3^-$ within the watershed can account for

16 the seasonal signal of δ^{18}O-NO$_3^-$ observed in the streams. Interestingly, this annual average is

17 similar to the estimate given for the snow dominated Catskill Mountains, NY (8%, Burns &

18 Kendall 2002) and within the range (0-45%) presented by Pardo and others (2004) for two

19 streams in snow dominated New Hampshire.

20   The peak in δ^{18}O-NO$_3^-$ for many of these streams occurs in the winter and early spring,

21 opposed to during spring snowmelt or following large storm events as found in other studies (e.g.

22 Burns & Kendall 2002; Campbell et al. 2002; Pardo et al. 2004; Williard et al. 2001). Unlike

23 previous studies, the enrichment found in these non-snow dominated systems could not be

1   attributed solely to runoff events. On average, the amount of processed NO_3^- entering streams in

2   the winter and spring is less than NO_3^- entering the streams in summer and fall. This could be

3   due to either changes in hydrology (e.g. flow paths, recharge rates), temperature affects on the

4   microbial processing of NO_3^-, or both.

5   High baseflow percentages coincided with the peak $\delta^{18}O\text{-}NO_3^-$ of these systems (Figure

6   3) and therefore it is unlikely that runoff contributed to the observed $\delta^{18}O\text{-}NO_3^-$ patterns. Both

7   higher recharge rates and reduced water demand by plants during the winter favor shorter flow

8   paths (Burns et al. 1998). We believe that the export of unprocessed atmospherically derived

9   NO_3^- is due in large part to these shorter flow paths, which reduce the opportunity for NO_3^-

10   processing.

11   Net nitrification potential measurements and modeling results also indicate that microbial

12   processes responsible for DIN export are strongly influenced by soil temperature and moisture

13   (Christ et al. 2002; Hong et al. 2006). Therefore, lowered rates of microbial nitrification may

14   also contribute to the higher stream $\delta^{18}O\text{-}NO_3^-$ values in winter and spring (Figure 4). The lack

15   of a similar relationship between temperature and stream $\delta^{15}N\text{-}NO_3^-$ (Figure 4) could be due to

16   the different effects of microbial processing on $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$. Complete turnover of

17   the NO_3^- pool could result in little observed $\delta^{15}N\text{-}NO_3^-$ change, while $\delta^{18}O\text{-}NO_3^-$ is lowered from

18   high atmospheric $\delta^{18}O\text{-}NO_3^-$ values to those of microbial nitrification. These processes could

19   result in the observed disconnect between the seasonal trends in stream $\delta^{15}N\text{-}$ and $\delta^{18}O\text{-}NO_3^-$

20   with relatively higher $\delta^{18}O\text{-}NO_3^-$ observed during the winter. However, the significant positive

21   relationship between discharge and $\delta^{18}O\text{-}NO_3^-$ (*p<0.0001*) could indicate that the relationship

22   between $\delta^{18}O\text{-}NO_s^-$ and temperature may only be due to simultaneous changes in hydrology.

23

15

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

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2   The $\delta^{18}O$-NO_3^- values in streams sampled in this study (-3.9‰ to 9.7‰) are generally

3   lower than those in other northeastern U.S. studies (~10‰ to 32‰, Burns & Kendall 2002;

4   Pardo et al. 2004), despite similar estimates of unprocessed atmospherically derived NO_3^- export.

5   One possible explanation is true variation in the $\delta^{18}O$ of the substrates (H_2O and O_2) used during

6   nitrification at the different sites. Isotopic maps of $\delta^{18}O$-H_2O (Kendall & Coplen 2001) in river

7   water suggests that the $\delta^{18}O$-H_2O in the Catskills (-10 to -8‰) and White Mountains (-12 to -

8   10‰) is similar or slightly depleted in ^{18}O relative to our sites, providing no explanation for the

9   observed difference. Although micro-scale influences (e.g. respiration, exchange with fine

10   particulate organic matter, denitrification) on these substrates are possible, it is also possible that

11   the discrepancy is methodological. The studies mentioned within this paper, with the exception

12   of Ohte and others (2004), used the method described by Silva, Chang and colleagues (Chang et

13   al. 2002; Silva et al. 2000) and not the denitrifier method used here (Casciotti et al. 2002; Sigman

14   et al. 2001). It should be noted that the study conducted by Ohte and others (2004), reported a

15   range $\delta^{18}O$-NO_3^- values (-7.7‰ to 18.3‰) in stream water which encompass our values. The off-

16   line combustion procedure used in the other studies has been shown to yield biased $\delta^{18}O$-NO_3^-

17   values as compared to samples using on-line combustion due to isotopic exchange between the

18   sample derived CO_2 and the quartz combustion tube (Révész & Böhlke 2002). Furthermore, until

19   recently there were not a range of $\delta^{18}O$ NO_3^- standards that allowed for more than a one-point

20   calibration (Böhlke et al. 2003), it was therefore difficult to detect the presence or magnitude of

21   the problem. More recent studies using off-line combustion, such as Hales and others (2007)

22   used a range of standards to calibrate their NO_3^- isotopic measurements and therefore it is far less

23   likely that their $\delta^{18}O$-NO_3^- values are biased.

1

2   *Retention of atmospheric NO_3^-*

3   Unprocessed atmospherically derived NO_3^- accounts for up to 25% of the stream NO_3^-

4   flux during the winter and early spring months (Table 2), however this estimate represents a

5   small fraction of the total wet atmospheric NO_3^- flux; implying that the remainder is retained or

6   removed within the ecosystem. We estimated the fraction of atmospherically deposited NO_3^- that

7   goes unprocessed by using atmospheric NO_3^- deposition estimates (FN_{atm}), calculated stream

8   NO_3^- flux measurements (FN_s), and the results of the isotopic mixing model, (eqn. 1, f_{atm}):

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

10   Calculations indicate that no more than 2% of NO_3^- entering the watersheds via precipitation

11   goes unprocessed annually. It is important to note that these calculations are based on wet

12   deposition NO_3^- fluxes and therefore represent a conservative estimate.

13   Overestimation of NO_3^- retention (1-$f_{uprocessed}$) is possible due to undetectable levels of

14   NO_3^- in 38% of our samples. In order to account for potential NO_3^- export associated with these

15   samples we repeated the calculations with estimated NO_3^- fluxes using the following

16   assumptions: (1) all samples with NO_3^- concentrations below the detection limit had [NO_3^--N] of

17   0.18 μM, (2) during base flow conditions the proportion of unprocessed atmospheric NO_3^- (f_{atm})

18   was set equal to the monthly average f_{atm} as calculated from the other streams, and (3) during

19   high flow conditions (October 2005, June 2006) f_{atm} was set to 100%. Application of these

20   assumptions did not change the estimates by more than 1% except in CB where retention

21   estimates decreased from 100% to 61%.

22   It is our understanding that estimates of atmospheric deposition retention within a

23   watershed have never been made based on isotopic mixing model calculations and we

1   acknowledge that our estimates are based on a limited number of observations. Therefore, as a

2   check, we applied the same method to results presented by Pardo and others (2004) and

3   calculated N retention estimates ranging from 96 to 99% and 86 to 97% in Hubbard Brook

4   Experimental Forest and the Bowl Research Natural Area, respectively. These estimates are in

5   line with studies documenting high N retention in Hubbard Brook (e.g. Bernhardt et al. 2005),

6   including during the non-growing season when Groffman and others (2001) calculated N

7   retention ranging from 84.1 to 99.9%.

8   These retention estimates provide evidence that the vast majority of atmospherically

9   derived NO_3^- is retained or removed within the watershed despite chronically elevated levels of

10   N deposition, suggesting these forests have not reached nitrogen saturation. Furthermore, even

11   without a large above-ground biological demand, watersheds are capable of retaining NO_3^-

12   during the winter via biotic (e.g. microbial immobilization (Brooks et al. 1999)) and abiotic

13   mechanisms such as the reduction of iron (II) in organic soils followed by the conversion of NO_2^-

14   to dissolved organic nitrogen via reactions with dissolved organic material (Davidson et al.

15   2003).

16

17   **Conclusions**

18   By using measurements of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in precipitation and streamwater in

19   conjunction with estimates of the isotopic composition of microbially produced NO_3^- we

20   distinguished sources of exported NO_3^- across forested watersheds in southern New England.

21   We found that throughout the year soil N processes are the dominant source of exported NO_3^- to

22   streams, confirming the results of similar studies conducted in snowmelt dominated watersheds

23   (e.g. Burns & 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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1 **Figure 1:** Bimonthly concentrations of nitrate (NO_3^-), dissolved inorganic nitrogen (DIN), and

2 estimated daily flow values (cfs) from June 2005 to August 2006 within each of the five sampled

3 streams: (a) headwaters of the West Branch of the Farmington River, (b) Charter's Brook, (c)

4 Riiska Brook, (d) Sandy Brook, and (e) headwaters of the Still River.

5

6 **Figure 2**: The $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- of stream water and precipitation samples. Stream and

7 precipitation samples are grouped by sampling date into summer and winter/spring subsets.

8

9 **Figure 3:** Daily precipitation totals and estimated hydrographs for study sites for the winter and

10 spring sampling period (11/15/05 through 4/15/06). Precipitation totals (a) are for Bradley

11 International Airport and snow amounts are given in water equivalents (as estimated by the

12 following relationship: water equivalent = snow total/10). Estimated hydrographs for each

13 watershed are shown (b) Charter's Brook, (c) Riiska Brook, (d) Sandy Brook, (e) headwaters of

14 the Still River, (f) headwaters of the West Branch of the Farmington River. The derivation of

15 daily flow values and the baseflow separation calculations are discussed in the text. The dotted

16 vertical lines denote sampling events.

17

18 **Figure 4:** Water temperature (°C) at time of sampling versus measured $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-.

19 The regression line represents the significant inverse relationship between water temperature and

20 $\delta^{18}O$-NO_3^-, R^2=0.54 (*p<0.0001*).

21

22

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

2   Averages are based on all available measurements and weighted appropriately; average NO_3^-

3   flux is weighted by flow measured in the field, average isotopic values for NO_3^- are flux

4   weighted, and the average $\delta^{15}N$ for soil are weighted by area.

5

6   **Table 2**: Mixing model calculations determining the percentage of NO_3^- derived directly from

7   atmospheric deposition (AD) using the minimum and maximum measured $\delta^{18}O\text{-}NO_3^-$ values
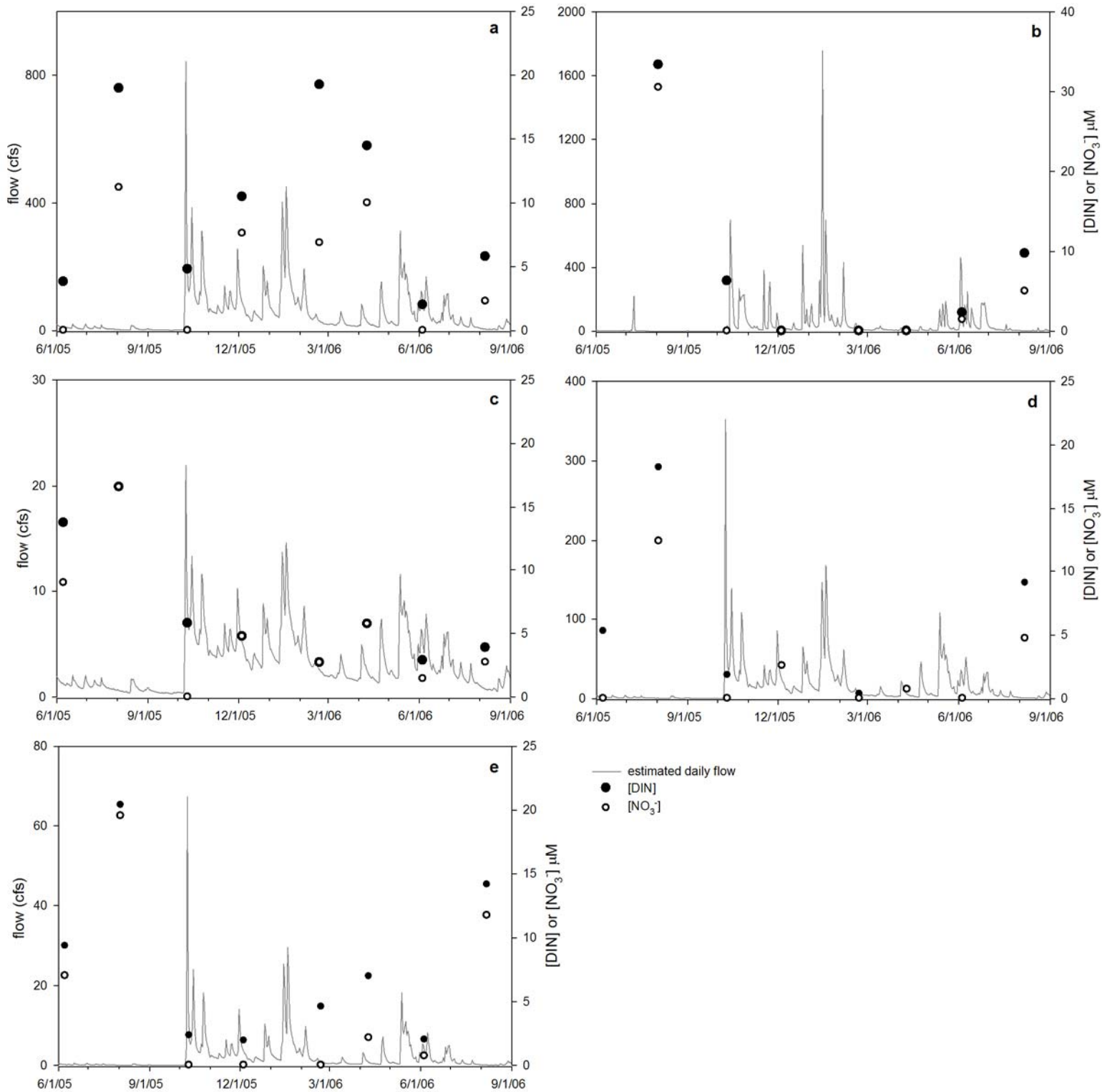
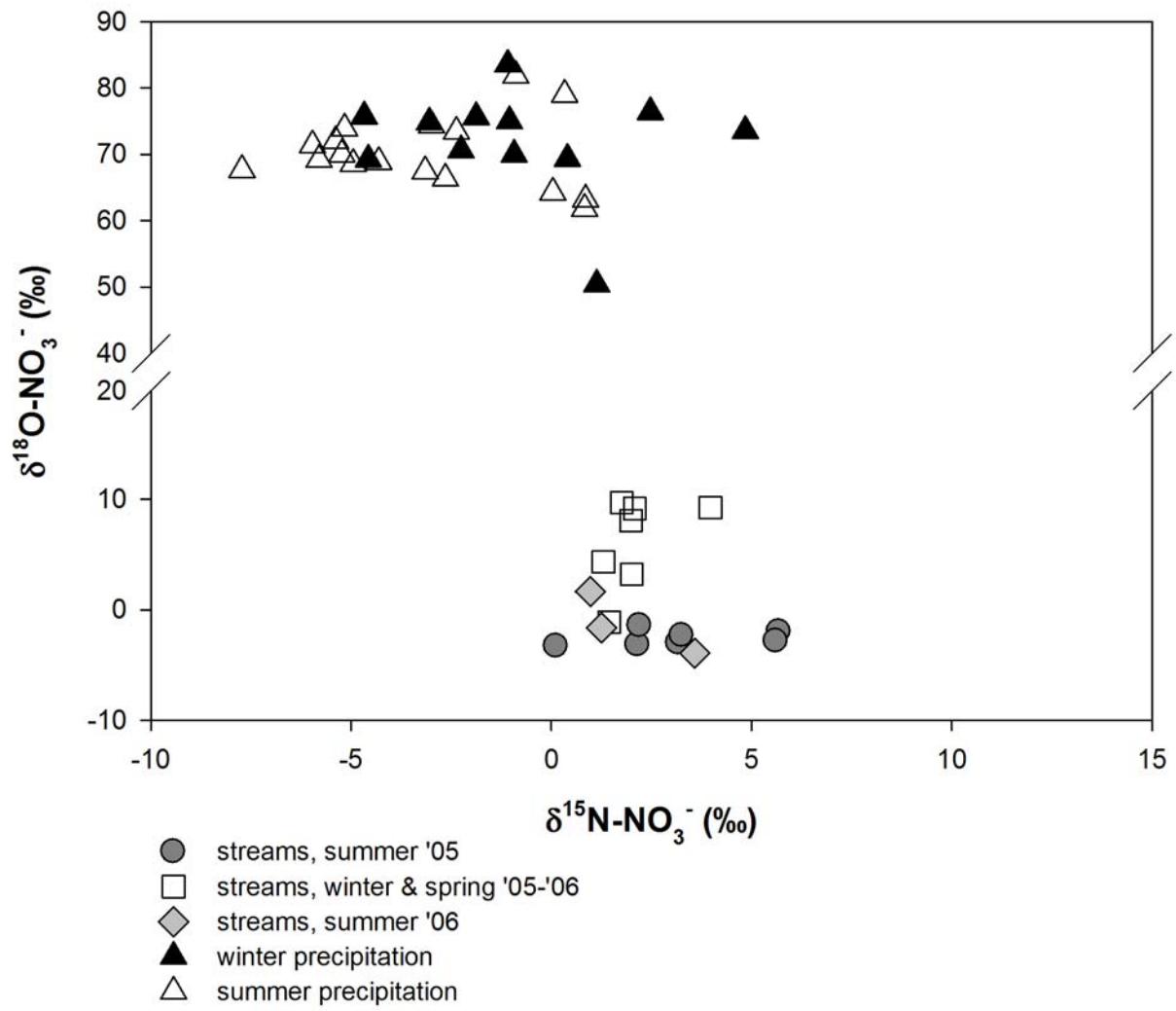8   (50.37 to 83.52‰) for AD and -3.9‰ (minimum stream measurement) and -2.85‰ (calculated

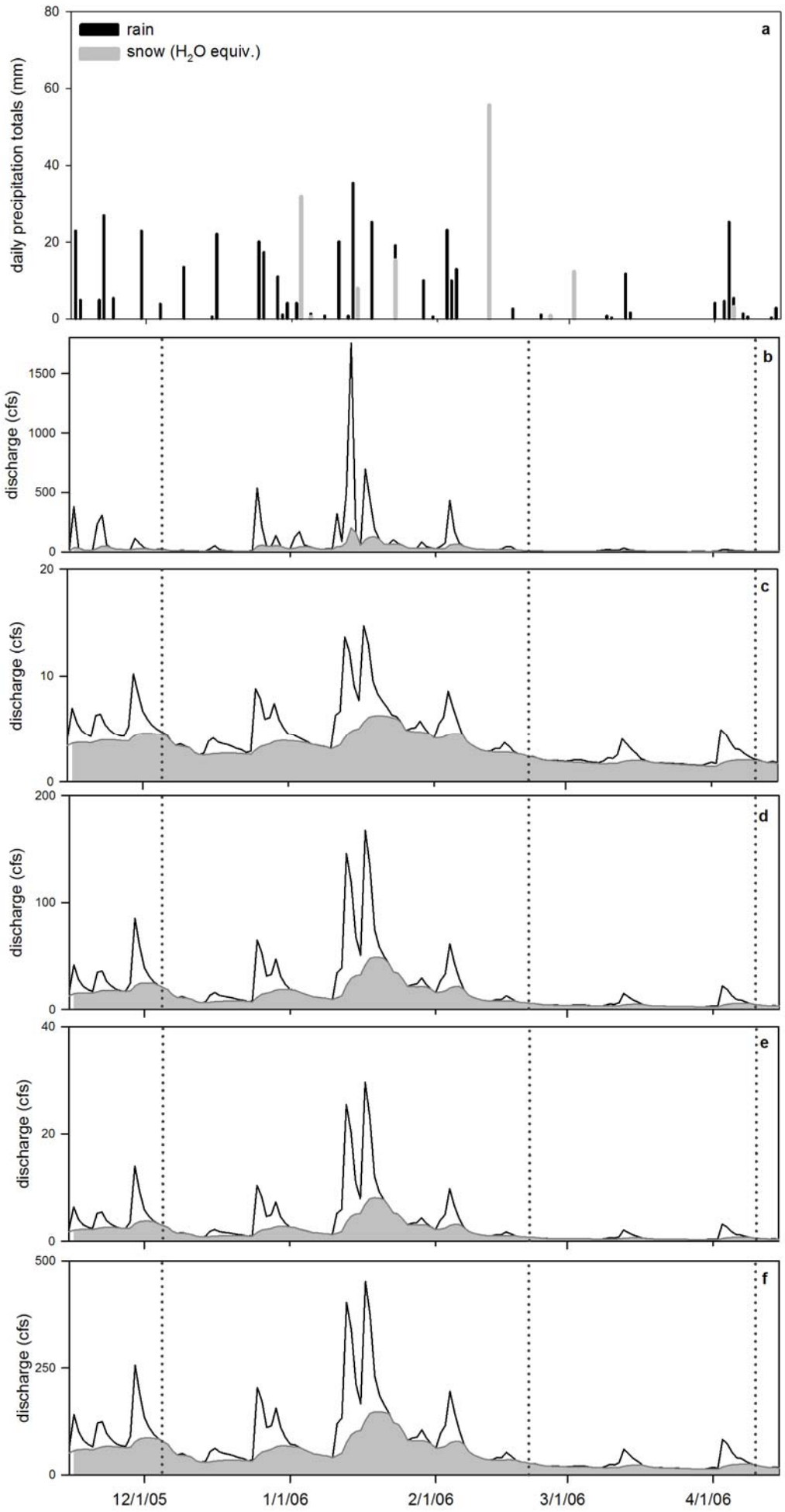9   minimum) for microbial nitrification (MN). * indicates the mixing model calculation yielded a
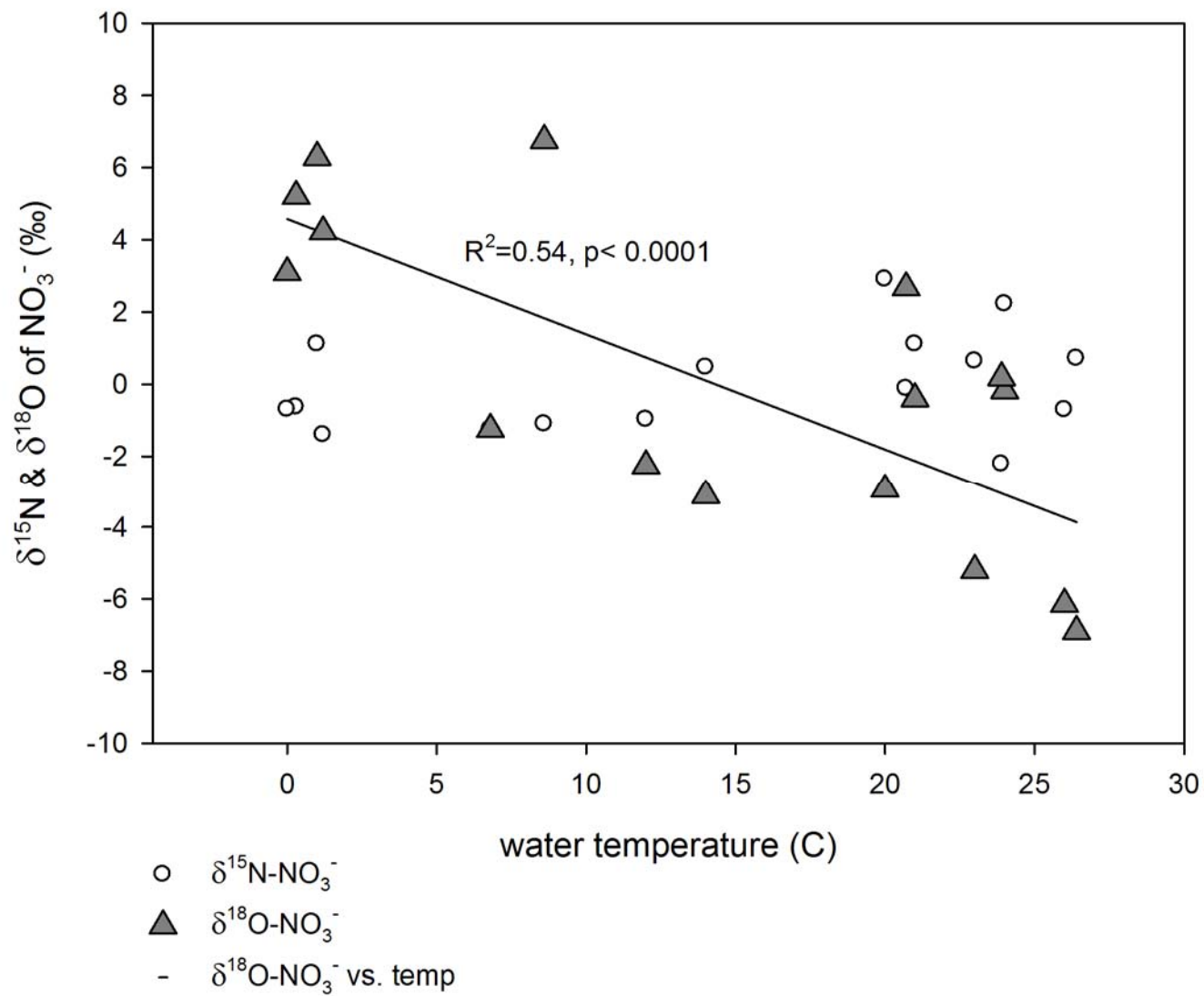
10   negative percent

11

12

Figure showing δ¹⁸O-NO₃⁻ (‰) versus δ¹⁵N-NO₃⁻ (‰).

Legend:
- streams, summer '05
- streams, winter & spring '05-'06
- streams, summer '06
- winter precipitation
- summer precipitation

R^2=0.54, p< 0.0001

water temperature (C)

$\delta^{15}N$ & $\delta^{18}O$ of NO$_3^-$ (‰)

○　$\delta^{15}N$-NO$_3^-$

▲　$\delta^{18}O$-NO$_3^-$

–　$\delta^{18}O$-NO$_3^-$ vs. temp

Table 1

stream	sampling location	area	land use/land cover	bedrock geology	surficial material	avg water yield	average flow weighted NO_3- flux
		km^2				$m\ yr^{-1}$	$kg\ km^{-2}\ yr^{-1}$
WBFR	73°6'40.95W 42°13'47.13N	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°8'15.39W 42°2'45.68N	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°9'39.15W 42°4'44.01N	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°4'29.44W 41°53'5.16N	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°24'42.47W 41°55'35.45N	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

Table 2

stream	date	streamwater $\delta^{18}O\text{-}NO_3^-$	AD: 50.37 MN: -3.9	AD: 83.52 MN: -3.9	AD: 50.37 MN: -2.85	AD: 83.52 MN: -2.85
		‰	\%\ of stream NO_3^- from atmospheric deposition			
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