Mobility of Nitrogen-15-Labeled Nitrate and Sulfur-34-Labeled Sulfate during Snowmelt

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The objective of this study was to investigate the winter dynamics of $SO_4^{2−}$ and $NO_3^{−}$ in a forested soil to better understand controls on these acidifying anions during snowmelt. In February 2004, a stable isotopic tracer solution with 93 atom% $^{34}S$ as $H_2^{34}SO_4$ and 99 atom% $^{15}N$ as $NH_4^{15}NO_3$ was applied to the snowpack at the Hubbard Brook Experimental Forest in New Hampshire. The chemical and isotopic compositions of throughfall, snow, snowmelt, and forest floor leachates were monitored for 10 mo following the addition of the tracers. The $^{34}SO_4^{2−}$ and $^{15}NO_3^{−}$ tracer amounts in forest floor leachates were highest in the first fractions of meltwater and declined exponentially until returning to ambient levels in mid-May. Isotopic mass balances indicated that $SO_4^{2−}$ and $NO_3^{−}$ were conservative in the snowpack, with tracer recoveries near 100%. In contrast, only 54 to 62% of the $^{34}SO_4^{2−}$ and 49 to 58% of the $^{15}NO_3^{−}$ were recovered in forest floor leachates, suggesting that much of the $SO_4^{2−}$ and $NO_3^{−}$ that infiltrated the forest floor during snowmelt was retained or transformed. Microbial biomass $^{15}N$ values in the forest floor remained low during snowmelt and the natural abundance values of $δ^{18}O–NO_3^{−}$ in forest floor leachates were indicative of an atmospheric rather than a microbial source. These results suggest that, in this study, microbial immobilization and subsequent mineralization and nitrification of snowpack $NO_3^{−}$ was insignificant in the forest floor during snowmelt.

Abbreviations: DNRA, dissimilatory nitrate reduction to ammonia; HBEF, Hubbard Brook Experimental Forest; PVC, polyvinyl chloride.

There has been much recent interest in understanding biogeochemical cycling in forest soils during winter. This interest stems from relatively recent findings that have shown that considerable microbial activity can occur in the forest floor and mineral soil beneath the snowpack at temperatures near freezing (Brooks et al., 1996; Monson et al., 2006; Schmidt and Lipson, 2004). While winter was once considered a period of dormancy, it is increasingly recognized as an important time for biogeochemical processes (Campbell et al., 2005). Despite the growing awareness of winter’s critical role in nutrient cycling, many questions remain unanswered. In particular, there is much uncertainty about the extent and means by which nutrients are retained or transformed in forest soils during cold weather.

Sulfur and N cycling have been studied extensively in North America and Europe, largely because atmospheric deposition of these elements has been elevated as a result of emissions due to the combustion of fossil fuels as well as other human activities. Stricter emissions regulations have led to a reduction of S deposition since 1975 in the United States, yet N deposition remains high (Driscoll et al., 2001; Kahl et al., 2004). In forests of the northeastern United States, $SO_4^{2−}$ and $NO_3^{−}$ are, respectively, the dominant forms of S and N in precipitation, throughfall, soil water, and surface waters. Atmospheric $SO_4^{2−}$ and $NO_3^{−}$ inputs accumulate in the snowpack during the winter and are transferred in a relatively short period during snowmelt. For example, half of the water exported annually at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire occurs during the months of March through May, which includes snowmelt (Bailey et al., 2003). During this same time period, approximately 52% of annual $SO_4^{2−}$ and 68% of annual $NO_3^{−}$ are exported in stream water (Likens and Bormann, 1995). The pulsed release of $SO_4^{2−}$ and $NO_3^{−}$ during snowmelt commonly causes episodic acidification in poorly buffered ecosystems, resulting in short-term decreases in acid-neutralizing capacity and pH (Wigington et al., 1996).

Both $SO_4^{2−}$ and $NO_3^{−}$ are thought to be conservative during winter when biological assimilation is minimal. Microbial activity has been detected in soils at temperatures near and below 0°C (Schadt et al., 2003; Sommerfeld et al., 1993), however, and there is increasing evidence that indicates strong biotic retention of $SO_4^{2−}$ and $NO_3^{−}$ in forest soil beneath the snowpack (Houle et al., 2004; Judd et al., 2007; Preston et al., 1990). Evidence of subniveal heterotrophic activity is further corroborated by studies that have used the
natural abundance of stable isotopes to identify sources of stream water SO$_4^{2-}$ and NO$_3^-$ The isotopic changes in N and S solutes that occur in the forest floor and upper mineral soil, even during winter, indicate that a large proportion of SO$_4^{2-}$ and NO$_3^-$ passes through the microbial pool before being exported to drainage waters (Campbell et al., 2006; Kendall et al., 1996; Mayer et al., 1995; Pardo et al., 2004; Patek et al., 2005; Shanley et al., 2005).

In addition to biological controls on SO$_4^{2-}$ and NO$_3^-$, abiotic processes may also regulate the export of these anions. Abiotic retention of SO$_4^{2-}$ has long been recognized as an important process; some reactions, such as adsorption-desorption, have been studied in detail while other reactions are chemically complex and are still not fully understood (see Mitchell et al., 1992). The two abiotic SO$_4^{2-}$ retention mechanisms that have been studied most extensively are SO$_4^{2-}$ mineral formation and especially SO$_4^{2-}$ adsorption. The former has been suggested to occur when pH is low and Al concentrations are high, resulting in precipitation of aluminum sulfate minerals. The latter occurs by a variety of mechanisms including when SO$_4^{2-}$ displaces OH$^-$ on Al and Fe hydroxides. Sulfate adsorption can be important in forest soils and can explain spatial and temporal patterns of SO$_4^{2-}$ retention (e.g., Johnson and Mitchell, 1998). Far less is known about abiotic retention of NO$_3^-$; however, several studies have suggested that this may be an important process affecting ecosystem N retention (Dail et al., 2001; Davidson et al., 2003; Fitzhugh et al., 2003).

Few studies have used isotopic labeling techniques to gain an understanding of winter processing of NO$_3^-$ and SO$_4^{2-}$ in forested ecosystems and none have simultaneously applied isotopically enriched NO$_3^-$ and SO$_4^{2-}$ tracers. There have been no previous snowpack $^{34}$SO$_4^{2-}$ isotope tracer additions, mainly because the limited availability and exorbitant cost of highly enriched $^{34}$SO$_4^{2-}$ has precluded its use in field applications. In one experiment, radiogenic $^{35}$SO$_4^{2-}$ was applied to the snowpack in a boreal coniferous forest in Quebec, Canada (Houle et al., 2004). Results indicated strong retention and transformation of SO$_4^{2-}$ in soils during winter. Snowpack additions of isotopically labeled NO$_3^-$ are also rare, and past studies have generally used isotopic tracers combined with N fertilization. Williams et al. (1996) applied $^{15}$NO$_3^-$ and $^{15}$NH$_4^+$ to snow at an alpine site in Colorado and found no transformation of N within the snowpack. In an N fertilizer experiment in British Columbia, Canada, Preston et al. (1990) applied 100 kg N ha$^{-1}$ as $^{15}$NO$_3^-$ to the snowpack of an 11-yr-old lodgepole pine (Pinus contorta Dougl. var. latifolia Engelm.) forest. After one growing season, 40% of the labeled N was recovered in soils, indicating that some of the NO$_3^-$ was retained.

The purpose of our study was to examine the mobility of NO$_3^-$ and SO$_4^{2-}$ deposited in snow in a northern hardwood forest ecosystem exposed to levels of N and S deposition that are considered high (5.3 kg N ha$^{-1}$ yr$^{-1}$ and 5.6 kg S ha$^{-1}$ yr$^{-1}$) for North America. Isotopically labeled N and S were applied simultaneously to the snowpack in February at the HBEF to investigate retention and transformation of NO$_3^-$ and SO$_4^{2-}$ during spring snowmelt, and LiBr was applied as a biologically inert hydrologic tracer. We designed our study as a tracer experiment, whereby the addition of NH$_4$NO$_3$-N and H$_2$SO$_4$-$S$ was negligible (1.7 g N ha$^{-1}$ yr$^{-1}$ and 1.8 g S ha$^{-1}$ yr$^{-1}$) compared with annual ambient N and S deposition. The use of highly enriched stable isotopic tracers (99 atom% $^{15}$N and 93 atom% $^{34}$S) enabled us to apply extremely small quantities of N and S, comparable to the amount deposited in a dusting of natural snowfall (~0.2-mm water equivalent). Thus, background concentrations of NO$_3^-$, NH$_4^+$, H$^+$, and SO$_4^{2-}$ were minimally altered as a result of the tracer addition. Tracers were confined to the area above the lysimeters and water fluxes were measured, rather than modeled, resulting in more accurate and sensitive measurements of tracer recovery. The goals of this research were to: (i) determine the extent to which atmospherically deposited NO$_3^-$ and SO$_4^{2-}$ are transformed in the snowpack; (ii) quantify how much snowpack NO$_3^-$ and SO$_4^{2-}$ is retained or transformed in the forest floor during snowmelt; and (iii) determine the importance of microbial immobilization of snowpack NO$_3^-$ that infiltrates the forest floor.

To accomplish these goals, we quantified concentrations, fluxes, and isotopic compositions of NO$_3^-$ and SO$_4^{2-}$ in throughfall, snow, snowmelt, and forest floor leachates for 10 mo following the addition of the isotopic tracers. Additionally, we assessed the importance of microbial NO$_3^-$ immobilization by tracing the labeled $^{15}$NO$_3^-$ into the forest floor microbial biomass N pool.

**MATERIALS AND METHODS**

**Site Description**

This research was conducted at the HBEF in the White Mountain National Forest, New Hampshire. The study site is on a south-facing slope east of Norris Brook (43°56′52″ N, 71°42′14″ W) at an elevation of 270 m. The climate is humid continental, with average maximum daily air temperatures ranging from 26°C in July to −3°C in January (Bailey et al., 2003). Twenty-seven-year average annual precipitation at the study site is 1210 mm, with 30% falling as snow. The winter snowpack is typically continuous, with a mean maximum depth of 490 mm (12-yr mean). The long-term (12-yr) mean maximum frost depth is 73 mm, and soil frost is present during winter approximately 3 out of 4 yr, depending on the depth and timing of snowfall. The forest is classified as northern hardwood and was cut heavily from 1910 to 1917. In the 1950s, selective cutting resulted in an uneven-aged forest, dominated by American beech (Fagus grandifolia Ehrh.), sugar maple (Acer saccharum Marsh.), and yellow birch (Betula alleghaniensis Britt.). Soils are acidic Spodosols (Haploorthods) with a thick (10-cm), organic forest floor layer at the surface. The soils have a sandy loamy texture and are well drained and shallow, with bedrock occurring at a depth of 1 m.

**Field Equipment and Sampling**

A total of eight 5- by 5-m plots were selected randomly at the study site. Forest floor and snow lysimeters were installed in the center of four of the plots, and the remaining four plots were designated for destructive snow and soil sampling. Forest floor lysimeters were installed 8 mo before the isotope addition to ensure that there was sufficient time for the soil to equilibrate after installation disturbance. The lysimeters were constructed of heavy-duty (6-mm) polyvinyl chloride (PVC), and were large enough (1.064 m$^3$) to allow frequent sampling and also to provide adequate water volume for isotopic analyses (Fig. 1). The forest floor was excavated to the top of the mineral soil, and this intact excavated layer was placed in the forest floor lysimeter. The lysimeter was installed in the soil at a depth of 10 cm, which was consistent with the depth of the forest floor it contained. Snow lysimeters were placed directly on the surface of the forest floor to capture meltwater from the bottom of the snowpack. Snow and forest floor lysimeters were angled slightly so that leachates drained by gravity through a PVC pipe to an underground storage container. The storage container was accessible through the surface of the snow and was insulated to prevent the drainage water from freezing. Three of the storage containers housed 20-L
polyethylene jugs for collecting water samples. A fourth storage container housed tipping buckets for measuring water volume. Tipping buckets were connected to a datalogger that measured snowfall and forest floor leachate volumes at 15-min intervals during the study. Water samples were collected on an individual snowmelt event basis and were stored in the dark underground at cool temperatures. Water samples from each of the snow and forest floor lysimeters were transferred from the 20-L collection containers to 250-mL bottles for ionic analyses (i.e., concentrations of SO$_4^{2-}$, NO$_3^-$, and NH$_4^+$). Samples for isotopic analyses consisted of a composite of equal amounts of water from each of the three snow and forest floor lysimeters.

Destructive sampling of snow and soil was conducted at separate dates so that the lysimeters remained undisturbed. Sampling areas, identical to the lysimeters in size and shape, were marked by vertical stakes in the autumn of 2003 before the snowpack developed. Snow samples for isotopic analyses were collected on four occasions by removing all the snow with an acid-washed, high-density polyethylene shovel in the 1-m$^2$ areas that received the tracer application. Snow samples for measuring concentrations of NO$_3^-$, SO$_4^{2-}$, and NH$_4^+$ in the snowpack were collected more frequently (2-wk intervals) using an acid-washed, beveled PVC tube. A sample consisted of multiple vertical cores, depending on the depth of snow, of the entire snowpack. All snow samples were placed in polyethylene bags and were transported to the laboratory on the same day of collection.

Soil samples were collected on three occasions: before the isotope addition (18 Feb. 2004), during peak snowmelt (4 Mar. 2004), and during the summer after the isotope addition (12 July 2004). In February and March 2004, soil samples were collected from the same location where the snow sample was removed. On each sampling date, three cores of the forest floor were collected using a 6-cm-diameter stainless steel soil corer. The three samples were placed in separate plastic bags and were immediately transported to a laboratory at the HBEF.

Throughfall was collected biweekly during the snow-covered period (6 Dec. 2003–3 Mar. 2004) at six randomly selected plots. Throughfall snow collectors for water volume and ionic analyses consisted of 15-cm-diameter by 50-cm-long PVC pipes lined with polyethylene collection bags. The collectors were mounted on vertical stakes 1.5 m above the ground surface. Since the isotopic analyses required a large sample volume, six 40-L polyethylene containers were co-located with throughfall collectors. Throughfall samples for isotopic analyses consisted of a composite sample from these collectors. Throughfall samples were transported immediately to the laboratory in collection bags for further processing.

Snow depth and snow water equivalence were measured adjacent to the site on a weekly basis as part of the long-term sampling program at HBEF. At Mt. Rose snow tube was used to core and weigh the snow at 10 sampling points along a transect. Soil frost was measured biweekly at the six throughfall collector locations using soil frost gauges (Ricard et al., 1976). Soil temperature thermistors were installed at six depths (1, 3, 7, 15, 30, and 50 cm) in two locations. Soil temperature probes were connected to a datalogger and recorded temperatures at 15-min intervals. Air temperature also was measured at 15-min intervals at one location.

**Tracer Application**

A tracer solution was applied to the surface of the snowpack above each lysimeter on 18 Feb. 2004, when snowpack depth and water equivalence were near maximum values for the winter (285 and 36 mm, respectively). The tracer consisted of 93 atom% $^{34}$S applied as H$_2^{34}$SO$_4$ and 99 atom% $^{15}$N applied as NH$_4^{15}$NO$_3$. The quantity of the isotopic tracers applied to the snowpack (91 $\mu$g $^{15}$N m$^{-2}$ and 182 $\mu$g $^{34}$S m$^{-2}$) was calculated to increase the isotopic composition of the snowpack significantly above natural abundance levels without exceeding the analytical constraints of the laboratory (1000‰ for $\delta^{15}$N and 200‰ for $\delta^{34}$S). In addition to isotopic tracers, 152 mg Br$^{-}$ m$^{-2}$ was added (as LiBr) as a conservative chemical tracer. The dilute solution (mixed with 500 mL of distilled, deionized water) was applied uniformly to the snowpack above each lysimeter using a hand-pump sprayer. The tracer solution also was applied to areas designated for destructive snow and soil sampling. Since lateral water movement through snow and soil can hamper tracer recovery (e.g., Houle et al., 2004; Williams et al., 1996), an effort was made to contain the water and tracers within the application area. Vertical stakes and plastic sheeting were used to create a boundary around the perimeters of the lysimeters and soil sampling areas. Sheetimg was added and removed as snow depth changed to maintain a snow depth within the application area that was consistent with the surrounding area.

**Laboratory Procedures for Aqueous Samples**

All aqueous samples were transported to the analytical laboratory at the USDA Forest Service, Northern Research Station, Durham, NH, on the day they were collected. Throughfall and snowpack samples were melted in polyethylene containers at room temperature immediately after returning to the laboratory. Throughfall water volumes were measured and samples for ionic analyses were transferred into 250-mL bottles and stored in the dark at 2°C until analysis. Measurements of SO$_4^{2-}$, NO$_3^-$, NH$_4^+$, and Br$^-$ concentrations were made using ion chromatography with an in-line cellulose membrane filter (0.15-μm pore size), usually within 2 d of collection. Samples for isotopic analyses were filtered through 0.45-μm high-capacity filter capsules and were acidified to pH 3 with dilute HCl to remove HCO$_3^-$ and Cl$^-$ in the samples. Appropriate sample volumes then were passed through anion exchange resin columns (Bio-Rad AG 1-X8) to retain and store the amount of NO$_3^-$, and SO$_4^{2-}$—necessary for analysis (1 mg NO$_3^-$ and 1 mg SO$_4^{2-}$). Resin columns were shipped immediately to the Isotope Science Laboratory at the University of Calgary, AB, Canada.

The procedures for the determination of N and O isotopy ratios of NO$_3^-$ and S isotopy ratios of SO$_4^{2-}$ are based on methods described by Silva et al. (2000) and Giesemann et al. (1994), respectively. In short, NO$_3^-$ and SO$_4^{2-}$ were eluted from anion exchange resins with 15 mL of 3 mol L$^{-1}$ HCl solution. One mL of 0.2 mol L$^{-1}$ BaCl$_2$ solution was added to the eluant to precipitate SO$_4^{2-}$ as BaSO$_4$, which subsequently was isolated on a 0.45-μm membrane filter. After removing excess Ba$^{2+}$ in a cation exchange column (Bio-Rad AG 50W-X8, Bio-Rad Laboratories, Hercules, CA), the remaining eluant solution containing HNO$_3$ and HCl was neutralized by adding Ag$_2$O. The reaction causes AgCl to precipitate, leaving Ag$^+$ and NO$_3^-$ in solution after removal of AgCl by filtration. The solution was freeze-dried to yield pure AgNO$_3$. In addition to these isotopic analyses, a small subset (five samples) of forest floor leachate samples also was analyzed for $^{15}$N/$^{14}$N of NH$_4^+$ at the University of Calgary. The purpose...
Laboratory Procedures for Soil Samples

In the laboratory at the HBEF, sticks, roots, and stones were removed from soil samples by hand. A subsample of soil was used for determination of potential N mineralization and nitrification following the procedure described by Robertson et al. (1999). Samples were placed in airtight glass jars and were incubated in the dark at 25°C for 10 d. Ammonium and NO₃⁻ were extracted from initial and incubated soil samples using 2 mol L⁻¹ KCl. The extract solutions were transported to the laboratory at the Institute of Ecosystem Studies, Millbrook, NY, where they were analyzed for NH₄⁺ and NO₃⁻ concentrations using a continuous flow colorimeter (salicylate and Cd reduction methods, respectively). Net N mineralization was calculated as the accumulation of total inorganic N (NH₄⁺ and NO₃⁻) during the incubation. Net nitrification was calculated as the accumulation of NO₃⁻ only. Potential net N mineralization and nitrification values are reported in milligrams N per kilogram dry soil per day.

Microbial biomass N contents were measured using the chloroform-fumigation incubation method (Paul et al., 1999). Soil subsamples were fumigated with chloroform to kill and lyse any living cells. The fumigated samples subsequently were inoculated with fresh soil and sealed in an airtight jar for 10 d at 25°C. Microorganisms from the inoculated soil grow vigorously, using the dead cells as a substrate. The inorganic N produced during the incubation was assumed to be proportional to the amount of N in the microbial biomass of the original samples. Inorganic N was determined by KCl extraction followed by measurement on a continuous flow colorimeter, as described above.

A N diffusion technique (modified from Brooks et al., 1989) was used to determine soil KCl-extractable ¹⁵N/¹⁴N and microbial biomass ¹⁵N/¹⁴N ratios. The procedure is similar to that described above for δ¹⁵N in the microbial pool, atom% X applied, and atom% X pool

\[ \delta(‰) = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} \right] - 1 \times 10^3 \]  

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the ³⁴S/³²S, ¹⁵N/¹⁴N, or ¹⁸O/¹⁶O ratio of the sample and the standard, respectively. The internationally accepted standards are: Vienna-Canyon Diablo troilite for S isotopes, AIR for N isotopes, and Vienna standard mean ocean water for O isotopes. The analytical uncertainty, including extraction, gas preparation, and isotope measurement, was ±0.5‰ for δ³⁴S–SO₄²⁻, δ¹⁵N–NO₃⁻, and δ¹⁸O–NO₃⁻ measurements.

Statistical Analyses

An analysis of variance was conducted to determine if there were significant differences (α = 0.05) among throughfall, snowmelt, and forest floor leachate concentrations for each ion investigated (SO₄²⁻, NH₄⁺, Br⁻, and NO₃⁻). Where significant variations existed, pairwise comparisons for post hoc determination of significant differences between means were made using a Tukey-Kramer test. Snowpack samples consisted of a composite sample collected at each sampling interval. Therefore, replicate snowpack samples were not collected, and we could not determine if the snowpack concentrations were significantly lower than other concentrations. Analysis of variance was applied also to flux data to determine if there were significant differences among throughfall, snowmelt, and forest floor leachate fluxes for SO₄²⁻, NH₄⁺, and NO₃⁻.

RESULTS

Water and Nutrient Fluxes

There was continuous snow cover from 6 December through 22 March during the winter of 2003–2004. Approximately 270 mm of precipitation fell during the snow-covered period, which was 20% of the annual precipitation of the 2003–2004 water year (based on a 1 June–31 May water year). Due to unusually low snowfall, the snowpack during the study winter had the lowest maximum snow water equivalent (36 mm) measured within the long-term record from 1993 to 2005, about 30% of the 13-yr mean maximum snow water equivalent (123 mm). No measurable snowmelt occurred for 10 d following the tracer application because of cold air temperatures (Fig. 2). The major spring snowmelt event began on 29 Feb. 2004 and ended on 22 Mar. 2004. A rain-on-snow event occurred on 4 Mar. 2004, which caused significant melting and infiltration, resulting in a marked increase in snow and forest floor leachate water volume shortly thereafter.
Soil Characteristics and Processes

The low snow accumulation during the study winter resulted in low soil temperatures and deeper-than-average soil frost (Fig. 4). Temperatures at the 7-cm depth or less were <0°C for 52 days during the winter, whereas temperatures at depths of 15 cm and greater consistently remained >0°C. There was a slight increase in Br− concentrations in snowmelt and forest floor leachates, the isotopic tracer declined more precipitously in snowmelt than in forest floor leachates. The isotopic tracer (δ34S and δ15N) values followed an exponential decline (where x is the day fraction following the initial sample collection):

\[ \delta^{15}N_{\text{snowmelt}} = 7.4 \exp[13.7/(x + 2.9)] \]
\[ R^2 = 0.999, P = 0.037 \]

\[ \delta^{34}S_{\text{snowmelt}} = 1.1 \exp[18.1/(x + 3.7)] \]
\[ R^2 = 0.999, P = 0.022 \]

\[ \delta^{15}N_{\text{forest floor leachates}} = 6.7 \exp[36.0/(x + 7.9)], \]
\[ R^2 = 0.998, P < 0.001 \]

\[ \delta^{34}S_{\text{forest floor leachates}} = 6.0 \exp[9.5/(x + 3.8)], \]
\[ R^2 = 0.997, P = 0.002 \]

The isotopic tracer declined more precipitously in snowmelt than in forest floor leachates. The temporal trend for the Br− tracer was generally similar to that of δ34S−SO4^2− and δ15N−NO3^− values, but there was a slight increase in Br− concentrations in forest floor leachates during sampling dates in mid-April and mid-May (Fig. 5d). We continued to track the tracer in forest floor leachates for 10 mo following the addition. The isotopic values returned to pretreatment levels by 11 May 2004 (53 d after the

### Table 1. Water flux and mean concentrations of SO4^2−, NH4^+−N, and NO3^−−N in throughfall, snowpack, snowmelt, and forest floor leachates, during the snow-covered period (6 Dec. 2003–22 Mar. 2004).

<table>
<thead>
<tr>
<th>Pool</th>
<th>Water flux</th>
<th>SO4^2−</th>
<th>NH4^+−N</th>
<th>NO3^−−N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughfall</td>
<td>217</td>
<td>0.73 (0.07) a</td>
<td>0.27 (0.06) a</td>
<td>0.69 (0.06) a</td>
</tr>
<tr>
<td>Snowpack</td>
<td>–</td>
<td>0.14</td>
<td>0.07</td>
<td>0.25</td>
</tr>
<tr>
<td>Snowmelt</td>
<td>136</td>
<td>1.03 (0.10) ab</td>
<td>0.32 (0.08) a</td>
<td>0.88 (0.09) a</td>
</tr>
<tr>
<td>Forest floor leachates</td>
<td>131</td>
<td>1.20 (0.10) b</td>
<td>1.35 (0.08) b</td>
<td>0.54 (0.09) a</td>
</tr>
</tbody>
</table>

† For each ion, different lowercase letters indicate significant differences among throughfall, snowmelt, and forest floor leachate concentrations (α = 0.05). Numbers in parentheses are standard errors of the group means. Water flux and snowpack samples were omitted from the ANOVA because replicate measurements were not made.
traced into NH4 extractable NO3

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sampling dates. Either the pretreatment or March

also significantly higher in July than the pretreatment sampling
date. Microbial biomass 15N was also significantly higher in July than either the pretreatment or March sampling dates.

Table 2. Summary of selected soil properties measured in the forest floor. Values are means (n = 3) taken on three sampling dates: before the tracer addition (18 Feb. 2004), during snowmelt (4 Mar. 2004), and midsummer (12 July 2004). Numbers in parentheses are standard errors of the means.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Microbial N, mg N kg⁻¹ soil</td>
<td>400 (17)</td>
<td>354 (19)</td>
<td>389 (127)</td>
</tr>
<tr>
<td>Potential net N mineralization, mg N kg⁻¹ dry soil d⁻¹</td>
<td>4.4 (0.4)</td>
<td>1.0 (0.4)</td>
<td>3.3 (1.1)</td>
</tr>
<tr>
<td>Potential net nitrification, mg N kg⁻¹ dry soil d⁻¹</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>&lt;0.01 (&lt;0.01)</td>
</tr>
<tr>
<td>Soil NH4⁺–N, mg N kg⁻¹ dry soil</td>
<td>19.6 (3)</td>
<td>23.1 (2)</td>
<td>120.0 (65)</td>
</tr>
<tr>
<td>Soil NO3⁻–N, mg N kg⁻¹ dry soil</td>
<td>1.0 (0.07)</td>
<td>0.8 (0.02)</td>
<td>0.5 (0.07)</td>
</tr>
</tbody>
</table>

Fig. 4. Mean daily soil temperature (primary y axis) at six depths (1, 3, 7, 15, 30, and 50 cm) and soil frost depth (secondary y axis) measured from January to April 2004. Error bars for soil frost indicate standard errors of the means.

DISCUSSION

By the time the snowpack fully melted, nearly all of the applied 34SO4⁻² and 15NO3⁻ had been recovered in meltwater, indicating that SO4⁻² and NO3⁻ in the snowpack were conservative and were not transformed to other forms of N and S. Biological reactions can contribute to transformations of snowpack NO3⁻, and some studies in the northeastern United States have indicated N cycling within the snowpack before melting (Burns and Kendall, 2002; Schaefer and Driscoll, 1993). Similarly, studies in polar regions have shown that NO3⁻ within the snowpack can be depleted by photolysis (Blunier et al., 2005; Hastings et al., 2004). Our results for NO3⁻ are similar to the findings of Williams et al. (1996), who reported no significant transformations of NO3⁻ in snow following a K15NO3⁻ addition to an alpine snowpack at Niwot Ridge, CO. Compared to NO3⁻, far less is known about SO4⁻² transformations within the snowpack. While there are no similar isotopic tracer studies of snowpack SO4⁻², isotopic natural abundance isotope ratios indicate little transformation of SO4⁻² within the snowpack (Campbell et al., 2006).

In contrast to our snowpack results, recovery of labeled 34SO4⁻² and 15NO3⁻ in forest floor leachates was much lower. Only about 54 to 62% of the 34SO4⁻² and 49 to 58% of the 15NO3⁻ applied to the snow surface was recovered in the forest floor lysimeters. Based on isotopic mass balances, these data suggest that some of the SO4⁻² and NO3⁻ that infiltrated the forest floor during snowmelt

Fig. 3. Fluxes of SO4⁻²–S, NH4⁺–N, and NO3⁻–N in throughfall, snowmelt, and forest floor (FF) leachates for the snow-covered period (6 Dec. 2003–22 Mar. 2004). For each ion, different lowercase letters indicate significant differences among throughfall, snowmelt, and forest floor leachate fluxes (α = 0.05). Error bars indicate the standard error of the group means.
Canada. Despite extremely high experimental N application rates part of a snowpack fertilization experiment in British Columbia, balance approach to measure soil NO$_3^-$ value given by Houle et al. (2004).

In the forest floor was similar (38–46%), but slightly higher than the indicated that 33% of the 35SO$_4^{2-}$ retention during snowmelt in a boreal coniferous forest in Quebec, using a radioactive isotopic tracer, 35SO$_4^{2-}$, was retained or transformed. Houle et al. (2004) measured SO$_4^{2-}$ throughfall, snowpack, snowmelt, and forest floor (FF) leachates from 31 Dec. 2003 to 8 June 2004. The dashed line indicates the date of the tracer application (18 Feb. 2004), which consisted of enriched 34SO$_4^{2-}$, 15N–NO$_3^-$, and Br$^-$. There were no significant differences between throughfall and snowmelt for concentrations and fluxes of all ions (Table 1, Fig. 3), supporting the hypothesis that there are no major sinks for these ions in the snowpack. Concentrations of ions within the snowpack were lower than concentrations in throughfall and snowmelt. This finding is well documented and is attributed to ion exclusion and export that occurs during partial melts in winter (Trantor and Jones, 2001). As snow freezes and thaws, ions become more concentrated on snow grain surfaces and are quickly released through the snowpack and organic soil during the initial spring snowmelt. This finding is well documented and is attributed to ion exclusion and export that occurs during partial melts in winter (Trantor and Jones, 2001). As snow freezes and thaws, ions become more concentrated on snow grain surfaces and are quickly released through the snowpack and organic soil during the initial spring snowmelt event that began on 29 Feb. 2004.

Concentrations of SO$_4^{2-}$ in forest floor leachates were significantly higher than throughfall concentrations (Table 1); however, there were no marked differences in the fluxes of SO$_4^{2-}$ among throughfall, snowmelt, and forest floor leachates (Fig. 3). Concentrations and fluxes of SO$_4^{2-}$-entering and leaving the forest floor were similar (Table 1, Fig. 3). Hence, SO$_4^{2-}$-flux data without isotopic tracers provide no indication of retention in the forest floor, and exemplify how isotopic tracers can provide unique

(100 kg N ha$^{-1}$), recovery of 15N in organic soil was nevertheless 31% of the total 15NO$_3^-$ added to the snowpack, which is only slightly less than the forest floor NO$_3^-$ retention and transformation values we calculated in our study using the mass balance approach (42–51%) under ambient N input levels. Lower N recovery in soil might be expected with high N additions, as the system is pushed toward N saturation. Even under high NO$_3^-$-amendment rates during the winter, however, when there is a marked reduction in N uptake by forest vegetation, the capacity of the forest soil to retain or transform NO$_3^-$ is remarkable.

Bromide recovery in the snow lysimeters was comparable to 34SO$_4^{2-}$–S and 15NO$_3^-$–N recoveries, whereas Br$^-$ recovery in the forest floor lysimeters far exceeded 34SO$_4^{2-}$–S and 15NO$_3^-$–N recoveries. Even though plastic sheeting confined the area above the lysimeters, there was still a possibility that a portion of the tracers could have leaked through the seams of the barriers. The high recovery rates of Br$^-$ provide evidence that 34SO$_4^{2-}$–S and 15NO$_3^-$–N were retained or transformed in the forest floor, and were not lost by leakage. Despite relatively high Br$^-$ recovery, it was <100% in snow and forest floor lysimeters (89 and 83%, respectively). Although Br$^-$ is used widely as a tracer, there is some evidence that suggests Br$^-$ is not entirely conservative and may be affected by geochemical and biogeochemical processes, particularly in organic soils (Gerrite and George, 1988). Hence, some of the Br$^-$ may have been retained in the forest floor. Another possible explanation for the incomplete recovery of Br$^-$ is that samples for chemical analyses and water volume were collected from separate lysimeters because shallow bedrock prevented the installation of water sample collection vessels directly beneath the tipping buckets. Consequently, differences in the timing of snowmelt between lysimeters used to measure water volume and those used to collect samples for chemical analyses could have affected the precise quantification of Br$^-$ recovery.

Chemical concentrations and fluxes of SO$_4^{2-}$, NO$_3^-$, and NH$_4^+$ in throughfall and snowmelt were consistent with isotopic results. There were no significant differences between throughfall and snowmelt for concentrations and fluxes of all ions (Table 1, Fig. 3), supporting the hypothesis that there are no major sinks for these ions in the snowpack. Concentrations of ions within the snowpack were lower than concentrations in throughfall and snowmelt. This finding is well documented and is attributed to ion exclusion and export that occurs during partial melts in winter (Trantor and Jones, 2001). As snow freezes and thaws, ions become more concentrated on snow grain surfaces and are quickly released in an ionic pulse at the initiation of spring snowmelt (Bales et al., 1989). Our tracer results provided additional evidence of the rapid elution of ions from the snowpack. Enhanced melting at the snowpack surface mobilized these ions, producing high concentrations in the first fractions of meltwater. Even though the tracers were applied to the surface of the snow, they quickly passed through the snowpack and organic soil during the initial spring snowmelt event that began on 29 Feb. 2004.
Table 3. Recovery of Br$^-$, $^{34}$SO$_4^{2-}$, and $^{15}$NO$_3^-$ in snowmelt and forest floor leachates during the 10 mo following tracer application. Recovery values were calculated using Eq. [2] and are expressed as the percentage recovered of the total added to the snowpack surface.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Br$^-$ recovery</th>
<th>$^{34}$S recovery</th>
<th>$^{15}$N recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snowmelt</td>
<td>89</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Forest floor leachates</td>
<td>83</td>
<td>54−62</td>
<td>49−58</td>
</tr>
</tbody>
</table>

Nitrification rates have been found to be highly variable in time and space at the HBEF (Bohlen et al., 2000; Groffman et al., 2001; Melillo, 1977; Venterena et al., 2003), and low net nitrification rates indicate either limited production or high consumption of NO$_3^-$ by immobilization, denitrification, or dissimilatory nitrate reduction to ammonia (DNRA).

The results of our study are inconsistent with the expectation of high NO$_3^-$ immobilization by microbes during spring snowmelt. Many studies that have examined the natural abundance O isotope ratios of NO$_3^-$ ($\delta^{18}$O–NO$_3^-$) in ecosystems during spring snowmelt have shown that a large fraction of the snowpack NO$_3^-$ flux is assimilated and subsequently mineralized and nitrified in soils before being released to streams (e.g., Campbell et al., 2006; Kendall et al., 1996; Pardo et al., 2004; Platek et al., 2005). Microbial immobilization is driven by high C availability (Hart et al., 1994), which is common in HBEF soils (Fahey et al., 2005). Since snow is a thermal buffer between the soil and cold winter air, below-average snowfall during the study period in our study resulted in deeper than average soil frost. A portion of the forest floor remained unfrozen, however, indicating that soil temperatures and free water were adequate for microbial activity (Fig. 4). Despite the potential for microbial N retention during winter, it did not appear important during the snowmelt period. There was no significant difference in microbial biomass $\delta^{15}$N between the preapplication (18 Feb. 2004) and post-application spring (4 Mar. 2004) sampling dates (Fig. 6). These data suggest that the NO$_3^-$ released from melting snow was
not incorporated into microbial biomass during spring snowmelt even though 87% of the total amount of $^{15}$NO$_3$ recovered in leachates had passed through the forest floor by the second sampling date (4 Mar. 2004). Differences in soil extractable NO$_3$ between the pretreatment and spring sampling dates were also insignificant, suggesting that the bulk of the tracer was not simply held on soil exchange sites during this period. Conclusive evidence for a lack of microbial immobilization during snowmelt would require repeated measurements of microbial biomass $\delta^{15}$N to account for the possibility of turnover. The lack of change in microbial biomass $\delta^{15}$N indicates that there was no net immobilization; however, this does not eliminate the possibility that there was microbial immobilization followed by turnover.

Interestingly, microbial biomass N pools were significantly enriched in $^{15}$N on the summer sampling date (12 July 2004) relative to both the pretreatment and snowmelt (4 Mar. 2004) sampling dates (Fig. 6). It is not possible to determine, however, whether the increase in microbial biomass $\delta^{15}$N was due to immobilization of the $^{15}$N tracer or to natural variability in microbial biomass $\delta^{15}$N values. Mean microbial $\delta^{15}$N values were only slightly above the range measured at Hubbard Brook in a similar forest type (–3.8 to 4.1‰; L.M. Christenson, unpublished data, 2007) and these background samples were collected earlier in the year (May) at a different location.

Nitrogen isotope fractionation is typically small in N-limited forest ecosystems (e.g., Campbell et al., 2006). Therefore it is unlikely that fractionation processes markedly influenced N isotope ratios in snowmelt and forest floor leachates. Because $\delta^{15}$N–NO$_3^-$ values of various sources often are indistinct, N isotope ratios are often not useful in determining sources of NO$_3^-$. The O isotope ratio of NO$_3^-$ ($\delta^{18}$O) is typically more useful than $\delta^{15}$N because $\delta^{18}$O values of atmospheric NO$_3^-$ are higher than those of soil microbially produced NO$_3^-$ (Durka et al., 1994). In our study, $\delta^{18}$O–NO$_3^-$ values (Fig. 5e) suggest that the NO$_3^-$ that infiltrated the forest floor during snowmelt was not immobilized by microbes, followed by mineralization and nitrification. From January through March, $\delta^{18}$O–NO$_3^-$ values in forest floor leachates were similar to values of atmospherically derived NO$_3^-$ characterized by high $\delta^{18}$O–NO$_3^-$ (Fig. 5e; Elliott et al., 2005, Kendall, 1998). After spring snowmelt, $\delta^{18}$O–NO$_3^-$ values in the forest floor leachates declined sharply by 45%. The range in $\delta^{18}$O–NO$_3^-$ values in the forest floor leachates during the snow-free period (3.3–25.3‰) was similar to the range in microbially produced NO$_3^-$ reported in the literature (Ambberger and Schmidt, 1987; Burns and Kendall, 2002; Mayer et al., 2001; Williard et al., 2001). During nitrification, one of the O atoms used to produce NO$_3^-$ is derived from atmospheric O$_2$ and two O atoms are taken from ambient soil water with negative $\delta^{18}$O values (Andersson and Hooper, 1983; Hollocher, 1984; Hollocher et al., 1981; Kumar et al., 1983). Therefore, the low $\delta^{18}$O–NO$_3^-$ values after spring snowmelt indicate that NO$_3^-$ in the leachates was produced by microbial nitrification. The comparatively high $\delta^{18}$O–NO$_3^-$ values in forest floor leachates during the period of snow cover indicate that the NO$_3^-$ did not cycle through the biomass and came directly from the atmosphere unaltered.

There are several possible explanations in addition to microbial immobilization that could explain how NO$_3^-$ is retained or transformed in the forest floor during winter. Vegetation can substantially lower soil NO$_3^-$ concentrations during the growing season; however, the forest floor material in the lysimeters was isolated from tree roots and there were very few seedlings and herbaceous plants growing in the lysimeters. Furthermore, roots are not typically active at the HBEF until after snowmelt in late spring (Tierney et al., 2003). Another possible explanation is DNRA, which can be an important sink for NO$_3^-$ in forest soils (Silver et al., 2001). Our analysis of $^{15}$N–NH$_4^+$ on selected soil water samples suggested that the labeled $^{15}$N in NO$_3^-$ was not transformed into NH$_4^+$ by DNRA (Fig. 5c). It is possible, however, that DNRA occurred and the produced NH$_4^+$ did not leach out of the forest floor. A third possible process for overwinter NO$_3^-$ loss is denitrification, which could have lowered the availability of NO$_3^-$ during snowmelt in poorly aerated, water-saturated soils (Groffman et al., 1993; Nyborg et al., 1997). Intermittent measurements of denitrification indicate that rates are low at Hubbard Brook during winter (Groffman et al., 2001); however, denitrification may be highly variable, particularly during the snowmelt period. On all sampling dates, the forest floor had a high moisture content (61–72%); therefore, any NO$_3^-$ produced could have been denitrified, resulting in low residual soil NO$_3^-$.

Lastly, the NO$_3^-$ could have been retained abiotically. While this process is poorly understood and its relative importance is unclear, evidence suggests that abiotic NO$_3^-$ retention has the potential to rapidly retain large quantities of N in soil (Davidson et al., 2003).

**CONCLUSIONS**

Our results indicate that there were no significant transformations of SO$_4^{2-}$ and NO$_3^-$ in the snowpack, but that retention and transformation in the forest floor were significant. Most of the labeled $^{34}$S–SO$_4^{2-}$ and $^{15}$N–NO$_3^-$ added to the snowpack infiltrated the forest floor during spring snowmelt, where 38 to 50% of the SO$_4^{2-}$ and 42 to 51% of the NO$_3^-$ subsequently were retained or transformed. Mass balances of SO$_4^{2-}$ indicated that even though a significant amount of SO$_4^{2-}$ was retained or transformed in the forest floor, a nearly equal amount was released, resulting in no significant difference between forest floor inputs and outputs of SO$_4^{2-}$. A significant amount of NH$_4^+$ was produced in the forest floor, indicating that N mineralization can be important, even when soil temperatures are near freezing. By contrast, net nitrification rates were very low and the forest floor was a sink for NO$_3^-$. Low $^{15}$N–NO$_3^-$ tracer recovery in microbial biomass and high $\delta^{18}$O–NO$_3^-$ values in forest floor leachates suggest that microbes did not immobilize and subsequently mineralize and nitrify snowpack NO$_3^-$. Other processes such as plant uptake, DNRA, denitrification, and abiotic NO$_3^-$ retention were probably more important in retaining or transforming NO$_3^-$ during snowmelt. Based on our results, we conclude that SO$_4^{2-}$ and NO$_3^-$ are not conservative in organic soil horizons underneath the snowpack, and both biotic and abiotic processes may control the retention and release of these anions during winter. These controls not only influence N and S dynamics during the snowmelt period, but also have effects that are manifested later during the growing season. Hence, understanding N and S cycling during winter is critical to our understanding of longer term N and S trends and responses to ecosystem disturbances such as climate change.

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