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Evaluating the source of streamwater nitrate using $\delta^{15}N$ and $\delta^{18}O$ in nitrate in two watersheds in New Hampshire, USA

Linda H. Pardo,[1]* Carol Kendall,[2] Jennifer Pett-Ridge[3] and Cecily C. Y. Chang[2]

[1] *USDA Forest Service, PO Box 968, Burlington, VT 05402, USA*
[2] *USGS, 345 Middlefield Road, MS 434, Menlo Park, CA 94025, USA*
[3] *Department of Environmental Science, Policy and Management, 151 Hilgard Hall, UC Berkeley, Berkeley, CA 94720-3110, USA*

Abstract:

The natural abundance of nitrogen and oxygen isotopes in nitrate can be a powerful tool for identifying the source of nitrate in streamwater in forested watersheds, because the two main sources of nitrate, atmospheric deposition and microbial nitrification, have distinct $\delta^{18}O$ values. Using a simple mixing model, we estimated the relative fractions in streamwater derived from these sources for two forested watersheds with markedly different streamwater nitrate outputs. In this study, we monitored $\delta^{15}N$ and $\delta^{18}O$ of nitrate biweekly in atmospheric deposition and in streamwater for 20 months at the Hubbard Brook Experimental Forest, New Hampshire, USA (moderate nitrogen export), and monthly in streamwater at the Bowl Research Natural Area, New Hampshire, USA (high nitrogen export). For rain, $\delta^{18}O$ values ranged from +47 to +77‰ (mean: +58‰) and $\delta^{15}N$ from −5 to +1‰ (mean: −3‰); for snow, $\delta^{18}O$ values ranged from +52 to +75‰ (mean: +67‰) and $\delta^{15}N$ from −3 to +2‰ (mean: −1‰). Streamwater nitrate, in contrast to deposition, had $\delta^{18}O$ values between +12 and +33‰ (mean: +18‰) and $\delta^{15}N$ between −3 and +6‰ (mean: 0‰). Since nitrate produced by nitrification typically has $\delta^{18}O$ values ranging from −5 to +15‰, our field data suggest that most of the nitrate lost from the watersheds in streamflow was nitrified within the catchment. Our results confirm the importance of microbial nitrogen transformations in regulating nitrogen losses from forested ecosystems and suggest that hydrologic storage may be a factor in controlling catchment nitrate losses. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS ^{15}N; ^{18}O; nitrate; nitrogen saturation

INTRODUCTION

Human activities continue to have an increasing impact on forest ecosystems globally (Vitousek *et al.*, 1997; Birdsey *et al.*, 2000). Of particular concern in the northeastern USA are impacts on the nitrogen cycle due to elevated nitrogen deposition (Aber *et al.*, 1998) and other alterations in global nutrient cycles caused by land-use change and elevated CO_2 emissions (Galloway, 1995; Vitousek *et al.*, 1997). Excess nitrogen in nitrogen-limited forested ecosystems can cause detrimental effects (including plant nutrient imbalances, soil and surface water acidification and increased aluminium mobility), decreased water, and increases in 'greenhouse' gas emissions (Aber *et al.*, 1989; Fenn *et al.*, 1998). There is tremendous variability in the response of forested catchments to elevated nitrogen deposition (Hornbeck *et al.*, 1997; Dise *et al.*, 1998; Goodale *et al.*, 2000; Lovett *et al.*, 2000). Hauhs *et al.* (1989) described a simple temporal relationship between increasing nitrogen deposition and increasing nitrogen losses in surface water. This relationship included a strong seasonal pattern of hydrologically driven nitrogen losses initially, followed by aseasonal elevated nitrogen losses (Stoddard, 1994). However, ecosystem response to elevated nitrogen deposition in the northeastern USA has been found to be considerably more complex.

* Correspondence to: Linda H. Pardo, USDA Forest Service, PO Box 968, Burlington, VT 05402, USA. E-mail: lpardo@fs.fed.us

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Factors that may be important in regulating ecosystem nitrogen loss include: nitrogen deposition (McNulty *et al.*, 1991), species composition (Aber *et al.*, 1995; Lovett and Rueth, 1999), land-use history (Goodale *et al.*, 2000; Goodale and Aber, 2001), disturbance history (Pardo *et al.*, 1995; Groffman *et al.*, 2001), soil C : N and microbial nitrogen cycling rates (Gundersen *et al.*, 1998; Goodale *et al.*, 2000; Ollinger *et al.*, 2002) soil type, and stand age (Fenn *et al.*, 1998). In spite of extensive research in the last decade (Aber *et al.*, 1998; Fenn *et al.*, 1998), understanding of disturbance and recovery in the nitrogen cycle of forested catchments is incomplete and cannot facilitate realistic predictions of ecosystem susceptibility to nitrogen saturation. At the high nitrogen deposition levels reported in Europe (over 60 kg ha^{-1} $year^{-1}$; Vitousek *et al.*, 1997), nitrogen saturation has been observed and may occur quickly, i.e. in decades (Dise *et al.*, 1998; Emmett *et al.*, 1998). In evaluating ecosystem response to the moderate levels of nitrogen deposition observed in the northeastern USA (3–13 kg ha^{-1} $year^{-1}$; Ollinger *et al.*, 1993), however, the critical need is to be able to understand the pathway and timing of an ecosystem moving toward nitrogen saturation. Elucidation of the pathways of nitrogen movement through an ecosystem will enhance our understanding of the factors that regulate nitrogen losses. In addition to addressing a basic scientific question about ecosystem nitrogen cycling, such knowledge is critical to the ability to predict ecosystem susceptibility to nitrogen saturation and, therefore, is an essential step in sustainable management of forest resources in the northeastern USA.

Stable isotopes of nitrogen and oxygen in nitrate can be used to determine the source of nitrate in streamwater from forested catchments, because of the distinct isotopic signatures of the two main sources of nitrate, i.e. atmospheric deposition and nitrification. This dual isotope approach has been used successfully at several sites (Böttcher *et al.*, 1990; Durka *et al.*, 1994; Kendall *et al.*, 1996; Williard *et al.*, 2001; Burns and Kendall, 2002; Campbell *et al.*, 2002). The wide separation in $\delta^{18}O$ in nitrate ($\delta^{18}O_{(NO_3^-)}$) between precipitation nitrate and microbially produced nitrate permits the use of a simple mixing model to evaluate the relative importance of the two sources (Kendall, 1998). Research in Europe demonstrated that declining forest stands had significant increases in the fraction of precipitation nitrate entering streamwater compared to healthy stands (Durka *et al.*, 1994). In a study in the USA that included the Rocky Mountains (CO), the Green Mountains (VT), and the Catskill Mountains (NY), Kendall *et al.* (1996) reported that the contribution of precipitation nitrate was negligible even during snowmelt. Burns and Kendall (2002) reported that nearly all streamwater nitrate in two Catskill catchments was microbially produced, except during a 10 year flood event. The range of precipitation $\delta^{18}O_{(NO_3^-)}$ values reported for US studies (+15 to +75‰; Kendall *et al.*, 1996; Kendall 1998; Williard *et al.*, 2001; Burns and Kendall, 2002; Campbell *et al.*, 2002) was much broader than that in European studies (+55 to +75‰; Durka *et al.*, 1994).

The isotopic signatures of precipitation and microbially produced nitrate are regulated by very different processes. Factors that are believed to affect $\delta^{18}O_{(NO_3^-)}$ in precipitation include isotopic fractionation during nitrate formation caused by lightning, the isotopic signatures of the reactive oxygen in the atmosphere that combines with NO_x to form NO_3, and any isotopic fractionations during reactions in the atmosphere (Kendall, 1998). The $\delta^{15}N$ in nitrate ($\delta^{15}N_{(NO_3^-)}$) in precipitation is a function of the source of the oxidized nitrogen, the temperature of and completeness of combustion of fossil fuels in power plants and vehicle exhaust, and any additional isotopic fractionation during nitrogen transformations in the atmosphere; hence, the $\delta^{15}N_{(NO_3^-)}$ of precipitation varies widely (Heaton, 1990; Freyer, 1991).

The $\delta^{18}O_{(NO_3^-)}$ of microbially produced nitrate is determined by the $\delta^{18}O$ of water and of the gaseous oxygen (O_2). The conventional theory is that two of the oxygen atoms in microbially produced nitrate are derived from water and one oxygen atom is from gaseous oxygen (Andersson and Hooper, 1983; Kumar *et al.*, 1983; Hollocher, 1984; Voerkelius, 1990; Durka *et al.*, 1994; Kendall, 1998). For soil solution that has $\delta^{18}O_{(H_2O)}$ in the normal range of −25 to +4‰, and $\delta^{18}O$ of soil O_2 equivalent to atmospheric O_2 (+23·5‰), the $\delta^{18}O_{(NO_3^-)}$ of microbially produced nitrate theoretically should range from −10 to +10‰. There are four assumptions inherent in this calculation: (1) there is no isotopic fractionation during incorporation of oxygen from water or O_2, (2) the proportion of oxygen from water to that from O_2 is the same in soils as is observed in laboratory cultures, (3) $\delta^{18}O_{(H_2O)}$ of water used by microbes is the same as that of bulk soil water, and (4) $\delta^{18}O$ of soil O_2 used by microbes is equivalent to $\delta^{18}O$ of atmospheric O_2. Campbell *et al.* (2002) noted

that several field studies have reported $\delta^{18}O_{(NO_3^-)}$ values of microbially produced nitrate that are as much as 5‰ higher than the theoretical maximum of +10‰ (Amberger and Schmidt, 1987; Aravena *et al.*, 1993; Kendall *et al.*, 1995; Wassenaar, 1995; Kendall, 1998; Seiler, 1999; Mayer *et al.*, 2001; Burns and Kendall, 2002). Based on an evaluation of many studies, Kendall (1998) concluded that +15‰ is a more appropriate upper value for $\delta^{18}O_{(NO_3^-)}$ of microbial nitrate. Kendall (1998) suggested that $\delta^{18}O$ of soil O_2 may become higher than that of atmospheric O_2 because soil air is not well mixed with the atmosphere, and the $\delta^{18}O$ of soil O_2 could increase as a result of oxygen isotope fractionation during respiration. In addition, Mayer *et al.* (2001) reported that, when ammonium is limiting, more oxygen may be derived from atmospheric O_2 than from water, resulting in $\delta^{18}O_{(NO_3^-)}$ of microbially produced nitrate up to +14‰.

The $\delta^{15}N_{(NO_3^-)}$ of microbially produced nitrate is a function of the isotopic fractionation during nitrification and the $\delta^{15}N$ of the ammonium that is nitrified. Koba *et al.* (1998) observed a range in $\delta^{15}N_{(NH_4^+)}$ from +2 to +16‰ in forest soils at Mt Ryuou, Japan.

The objective of this study was to evaluate the source of streamwater nitrate at two sites with different levels of nitrate loss in the White Mountains of New Hampshire. We hypothesized that the fraction of precipitation nitrate in streamwater at the site with higher nitrogen losses would be higher than that at the site with lower nitrogen losses (i.e. tighter nitrogen cycling).

SITE DESCRIPTIONS

This study was conducted in two watersheds in the White Mountains of New Hampshire, the Hubbard Brook Experimental Forest Watershed 6 (HBEF W6) and the Bowl Research Natural Area (Bowl RNA; Figure 1). These sites were selected because they span much of the range of streamwater nitrate concentration observed in northern New England (0–50 μeq l^{-1}; Hornbeck *et al.*, 1997). The Bowl RNA has high nitrogen losses (total inorganic nitrogen), and the HBEF W6 has moderate nitrogen losses (Table I). Streamwater chemistry has been measured at HBEF W6 continuously since 1963, and at the Bowl RNA for 18 months during 1973–74 and continuously since 1994. The sites are similar in soils, hardwood species present, nitrogen deposition, and climate (Table I). They differ in streamwater nitrate loss, species composition, size, slope, and land-use history (Table I). The Bowl RNA is a much larger watershed, being dominated by hardwoods in the lower, flat portion of the watershed and by conifers in the steeper, upper elevations, above 900 m (Martin and Bailey, 1999). The HBEF W6 is dominated by hardwoods, and in upper elevations has few areas dominated by conifers. Soils at low elevations at the Bowl RNA are deeper (>1 m) than at the HBEF (60 cm; Johnson, 1991). The Bowl RNA has been subject to natural disturbances only and is considered an old-growth forest; the HBEF W6 was logged between 1900 and 1910. The sites are described in detail elsewhere (Martin, 1979; Likens and Bormann, 1995; Martin and Bailey, 1999; Martin *et al.*, 2000).

METHODS

Precipitation and streamwater sample collection

Precipitation and streamwater samples were analysed for ammonium, nitrate, sulphate, chloride, (USDA Forest Service, Durham, NH; auto-analyser), dissolved organic carbon (DOC; Syracuse University, Syracuse, NY; Dohrmann TOC analyser), and $\delta^{15}N_{(NO_3^-)}$ and $\delta^{18}O_{(NO_3^-)}$ (USGS, Menlo Park, CA). Samples were collected at both watersheds between May 1996 and February 1998. At the HBEF, 20–40 l streamwater samples were collected biweekly above the weir at W6, scheduled to coincide with long-term monitoring chemistry measurements. At the Bowl RNA, 20 l streamwater samples were collected from the West Branch of the Wonalancet River once per month. In September and October 1996, samples were collected from the Main Branch of the Wonalancet River rather than the West Branch (Figure 1). Samples from the Main Branch were collected about 2 m below the point where the West Branch and the East Branch join, and about 4 m

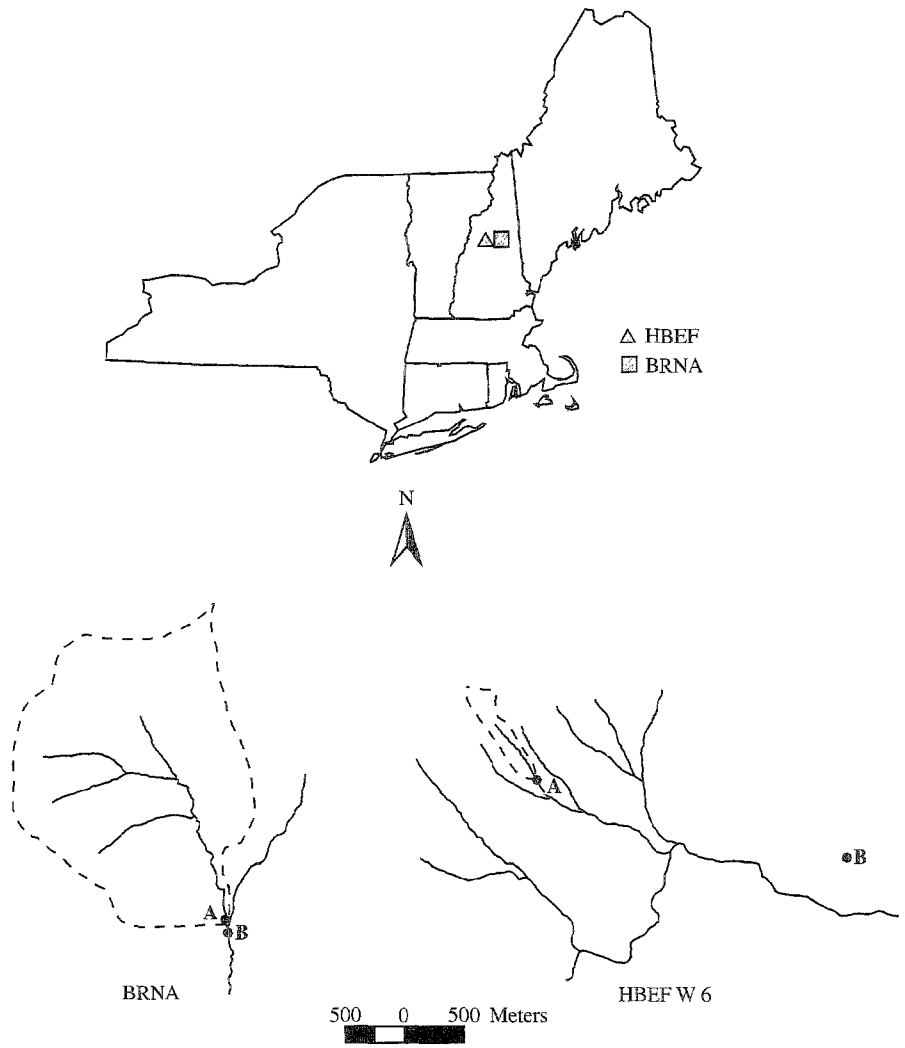

Figure 1. Site maps for the Bowl Research Natural Area (BRNA) and the Hubbard Brook Experimental Forest (HBEF). At the Bowl RNA, streamwater samples were collected at the West Branch (point A); the Main Branch collection point is shown (point B). At the HBEF, streamwater samples were collected at point A and precipitation samples were collected at point B

below the West Branch sampling location. In order to determine whether there was a difference in isotopic signature between the Main Branch and the West Branch, for the period September–December 1997, samples were collected from both the Main and the West branches. Samples were compared using a paired *t*-test, and there was no statistical difference between $\delta^{15}N_{(NO_3^-)}$ and $\delta^{18}O_{(NO_3^-)}$ in the Main and West Branches ($p < 0{\cdot}05$). Therefore, samples from the Main Branch were used on dates that no West Branch collection had been made.

Precipitation samples were collected over a period of 1 week every other week at the HBEF. Samples were collected in a large clearing used for NADP sample collection, which is maintained in order to minimize forest edge effects on the precipitation (Figure 1). Snow was collected in four buckets (50 cm in diameter) anchored to a platform 1·2 m above ground. Samples from the individual buckets were melted and combined. Bulk rain samples were collected using a 1·5 m × 1·7 m V-shaped collector, sized to ensure sufficient collection during

Table I. Characteristics of study sites

Characteristic	HBEF W6[a]	Bowl RNA[b]
Location	43°56′N, 71°45′W	43°56′N, 71°23′W
Size (ha)	13	206
Soil type	Coarse-loamy, mixed, frigid Aquic, Lithic, and Typic Haplorthods	Coarse-loamy, mixed, frigid Aquic, Lithic, and Typic Haplorthods
Bedrock	Schist, quartzite and granulite	Syenite and granite
Forest type	Northern hardwood	Mixed conifer and hardwood
Species composition	*Acer saccharum* 31%[c] *Fagus grandifolia* 36% *Betula alleghaniensis* 18%	*Acer saccharum* 15%[d] *Betula* 33% *Fagus grandifolia* 17% *Picea rubens* 14% *Abies balsamea* 8%
Land-use history	Last logged 1910–17	Natural disturbance only
Stand age (years)	~80	old growth, 35–200
Mean nitrogen deposition (mol ha^{-1} $year^{-1}$)	492[e]	~492[f]
Streamwater inorganic nitrogen outflux (mol ha^{-1} $year^{-1}$)	41[c]	~152[g]
Streamwater nitrate concentration (μmol l^{-1})	3.6[e]	15.4[h]

[a] Data for HBEF are from Likens and Bormann (1995), except where noted otherwise.
[b] Data for the Bowl RNA are from Martin (1979), except where noted otherwise.
[c] Data provided by G.E. Likens for the water years 1994–97.
[d] Martin and Bailey (1999).
[e] Data from G.E. Likens, Hubbard Brook Web-page (www.hubbardbrook.org) for the period 1965–92.
[f] Deposition was assumed to be similar to that at Hubbard Brook based on previous comparisons (Martin, 1979).
[g] Flux was estimated based on current concentrations at the Bowl RNA (Martin *et al.*, 2000), and flow estimates from Hubbard Brook for the period 1994–97 (J. Hornbeck, www.hubbardbrook.org).
[h] Data are from Martin *et al.* (2000) for the period June 1994–June 1997.

low rainfall periods. The samples were fed through a funnel with a polyester wool filter to eliminate debris, into tubing that fed through into a large plastic overflow container and into a 20 l collapsible carboy. On weeks when the carboy overflowed into the plastic container, the carboy was poured into the container, mixed, and a 20 l subsample collected in the collapsible carboy. The precipitation collector was covered with polyethylene sheeting each week; the sheeting and all other parts that contacted precipitation were acid-washed with HCl and rinsed with deionized water before being placed in the field.

Solution sample preparation and isotope analysis

All samples for isotope analysis were filtered immediately after collection with 0·45 μm pore size Gelman filter capsules. For nitrate isotope analysis, samples were dripped through two 20 ml columns each filled with 5 ml of ion-exchange resin. A cation-exchange column (AG50W-X8, 100–200 mesh, Biorad) was used in series with an anion-exchange column (AG2-X8, 100–200 mesh, Biorad) according to the method of Chang *et al.* (1999). Chang *et al.* (1999) report that this method minimizes the transfer of DOC to the anion column. DOC can confound the oxygen isotope analysis. Samples were kept in a cooler with ice-packs while they dripped through the ion-exchange columns overnight. A final 60 ml chemistry sample was collected from the last eluent to pass through the column and analysed for nitrate, sulphate, chloride, and ammonium, to ensure that all the nitrate in the sample had been retained by the column (i.e. to verify that there was no bleed-through of nitrate). Samples with insufficient nitrate for analysis or with detectable nitrate in the final chemistry sample were discarded. Samples for DOC analysis were filtered using a glass-fibre (Poretics GF-75,

0·7 μm) and 0·2 μm silver membrane filter (Poretics), in order to prevent microbial contamination. DOC was measured in order to identify samples with high DOC values that might confound oxygen isotope analysis (Chang *et al.*, 1999).

Ion-exchange columns were stored at 4 °C prior to analysis for $\delta^{15}N_{(NO_3^-)}$ and $\delta^{18}O_{(NO_3^-)}$ at the USGS laboratory in Menlo Park, CA. Nitrate was eluted from the anion column and processed according to the method of Silva *et al.* (2000).

Isotopic analyses were performed using a Micromass Optima isotope ratio mass spectrometer at the USGS in Menlo Park, CA. Nitrogen isotope values ($\delta^{15}N_{(NO_3^-)}$) are reported in per mil relative to atmospheric air, which is defined as 0‰; oxygen isotope values ($\delta^{18}O_{(NO_3^-)}$) are reported relative to the Vienna standard mean ocean water also defined as 0‰. For example:

$$\delta^{15}N = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \quad (1)$$

where R_{sample} represents the sample isotope ratio ($^{15}N/^{14}N$), and $R_{standard}$ is $^{15}N/^{14}N$ for atmospheric N_2, or 0·003 676 5. The precision for analysis of laboratory standards for $\delta^{15}N$ of KNO_3 was ±0·05‰ (standard deviation, SD) and for $\delta^{18}O$ of $AgNO_3$ was ±0·2‰.

Statistical analysis

Comparisons of the $\delta^{18}O_{(NO_3^-)}$ and $\delta^{15}N_{(NO_3^-)}$ in precipitation and streamwater were made using *t*-tests, by season for precipitation and for streamwater, by season within each site, using an α level adjusted for multiple comparisons (Table II). All statistical analyses were conducted using SAS (SAS Institute, 1988).

RESULTS

There was a wide separation in mean $\delta^{18}O_{(NO_3^-)}$ between precipitation (+62·2‰; SD = 8·5; $n = 26$) and streamwater (+17·9‰; SD = 4·9; $n = 32$) that was significant ($p < 0·05$; Figure 2, Table II). The separation in mean $\delta^{15}N_{(NO_3^-)}$ between precipitation (−2·3‰; SD = 1·7; $n = 26$) and streamwater (+0·2‰; SD = 1·3; $n = 32$) was smaller, but also significant ($p < 0·05$; Figure 2). Precipitation $\delta^{18}O_{(NO_3^-)}$ ranged from +46 to +75‰; precipitation $\delta^{15}N_{(NO_3^-)}$ ranged from −5 to +2‰ (Figure 3). Seasonal patterns in precipitation were significant (Figure 3). Mean snow $\delta^{18}O_{(NO_3^-)}$ (+67·4‰; SD = 7·2; $n = 11$) and $\delta^{15}N_{(NO_3^-)}$ (−1·2‰; SD = 1·4; $n = 11$) were significantly higher ($p < 0·05$; Figure 4) than mean rain $\delta^{18}O_{(NO_3^-)}$ (+58·5‰; SD = 7·5; $n = 14$) and $\delta^{15}N_{(NO_3^-)}$ (−3·1‰; SD = 1·5).

Table II. *t*-tests for $\delta^{15}N$ and $\delta^{18}O$ in precipitation and streamwater nitrate in the HBEF and the Bowl RNA, NA

Group	Variable	Degrees of freedom	*t*	$p > t^a$
Precipitation vs stream	$\delta^{15}N$	57	−6·22	<0·0001
	$\delta^{18}O^b$	34·5	22·72	<0·0001
Precipitation summer, vs winter	$\delta^{15}N$	22	−3·13	0·0049
	$\delta^{18}O$	22	−2·90	0·0083
HBEF stream, summer vs winter	$\delta^{15}N$	15	1·02	0·3241
	$\delta^{18}O^b$	8·52	−1·63	0·1386
BNRA stream, summer vs winter	$\delta^{15}N$	16	0·29	0·7741
	$\delta^{18}O$	15	−0·80	0·4380

[a] Significant differences occur at the 0.0125 level to account for multiple tests (Hayes, 1988)
[b] Satterthwaite (1996) method used for calculating degrees of freedom due to unequal variances.

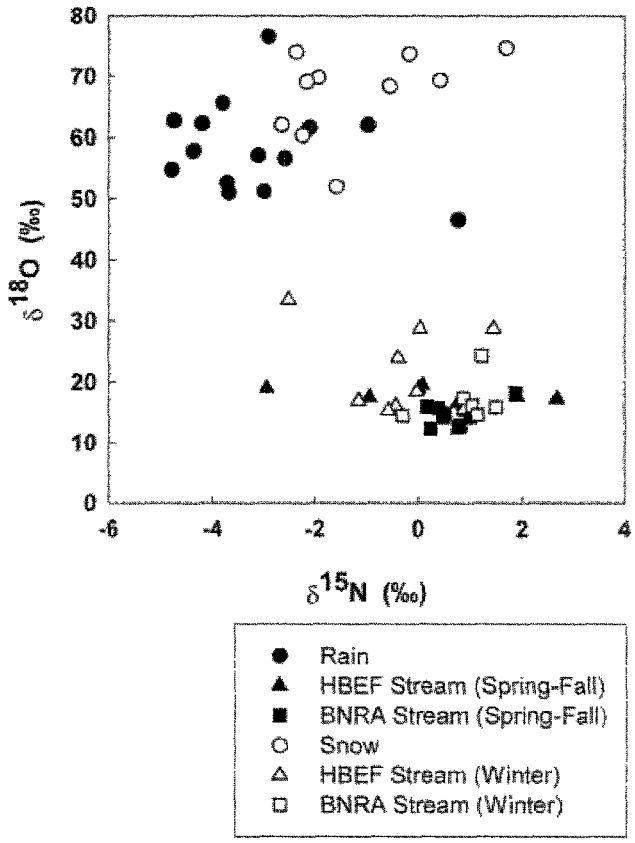

Figure 2. $\delta^{15}N$ and $\delta^{18}O$ in nitrate in precipitation and streamwater at the Bowl RNA and the HBEF

Differences in isotope values of streamwater nitrate between winter (December–April) and non-winter (May–November) periods were not significant at either site (Figure 4). The highest streamwater $\delta^{18}O_{(NO_3^-)}$ values occurred during the winter period (Figure 5).

DISCUSSION

The wide separation between precipitation and streamwater nitrate $\delta^{18}O_{(NO_3^-)}$ facilitates the use of a simple mixing model to determine the source of the streamwater nitrate. The $\delta^{18}O_{(NO_3^-)}$ expected in microbially produced nitrate should range from −5 to +15‰ (Durka *et al.*, 1994; Kendall, 1998; Mayer *et al.*, 2001; Williard *et al.*, 2001). Streamwater $\delta^{18}O_{(NO_3^-)}$ values at the HBEF and the Bowl RNA ranged from +12 to +33‰, suggesting that most of the nitrate exported in streamwater was produced via nitrification within the ecosystem. Using a simple mixing model for the range of $\delta^{18}O_{(NO_3^-)}$ values observed for precipitation (+46 to +75‰), the mean for streamwater (+18‰) in these watersheds, and the literature range for $\delta^{18}O_{(NO_3^-)}$ (−5 to +15‰) for microbially produced nitrate, approximately 55–100% of streamwater nitrate could have been produced within these catchments.

Streamwater $\delta^{15}N_{(NO_3^-)}$ was about 3‰ higher than precipitation $\delta^{15}N_{(NO_3^-)}$, perhaps due to microbial cycling of nitrogen. Streamwater $\delta^{15}N_{(NO_3^-)}$ was closer to the range of $\delta^{15}N$ measured in forest floor at these sites (Pardo, 1999; Pardo *et al.*, 2001, 2002). We note that the difference in precipitation and streamwater observed

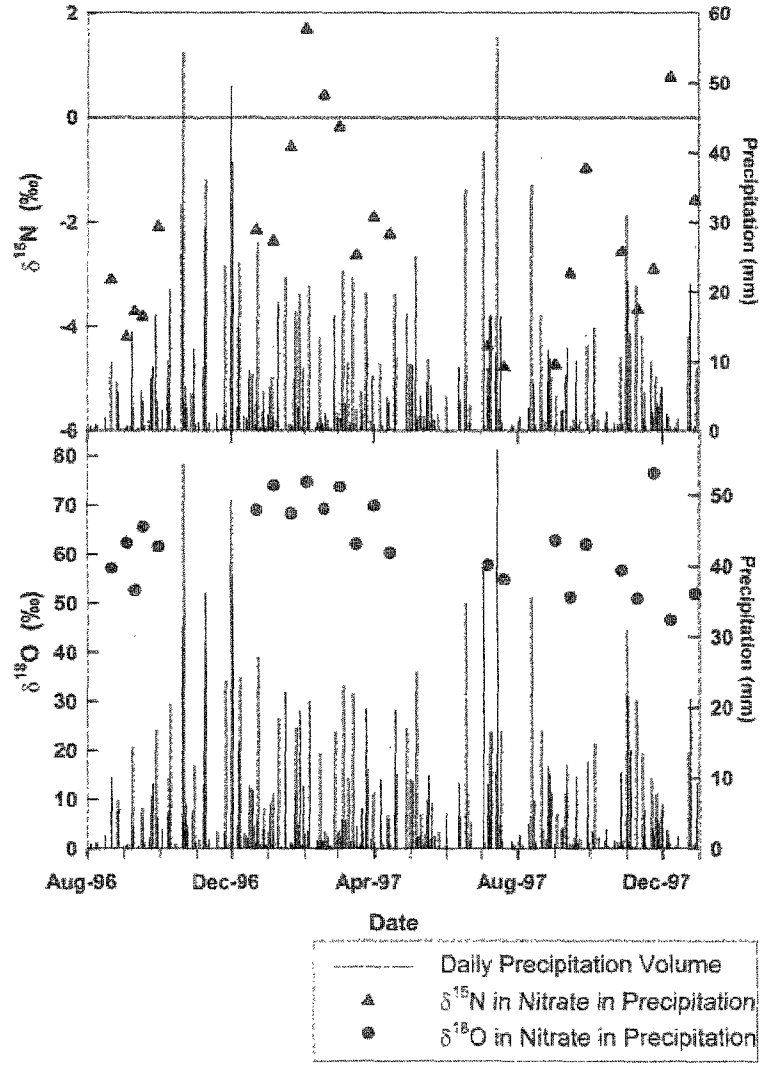

Figure 3. Precipitation volume and (a) $\delta^{15}N$ and (b) $\delta^{18}O$ in nitrate at the Isotope chemistry data are connected to precipitation volume data by dotted drop-lines

by Durka *et al.* (1994) followed the opposite pattern: streamwater $\delta^{15}N_{(NO_3^-)}$ was lower than precipitation $\delta^{15}N_{(NO_3^-)}$.

Mean $\delta^{18}O_{(NO_3^-)}$ in snow was 9‰ higher than in rain, and mean $\delta^{15}N_{(NO_3^-)}$ in snow was 2‰ higher than in rain. Similar seasonal differences have been observed in Germany and in West Virginia (Voerkelius, 1990; Durka *et al.*, 1994; Williard *et al.*, 2001). Most previous work in the USA on evaluating $\delta^{18}O_{(NO_3^-)}$ has not shown such a clear distinction (Kendall *et al.*, 1996; Burns and Kendall, 2002; Campbell *et al.*, 2002); however, several of those studies noted that snowpack or precipitation $\delta^{18}O_{(NO_3^-)}$ was significantly higher in winter than in summer. There are several possible explanations for the difference between snow and rain $\delta^{18}O_{(NO_3^-)}$: (1) The predominant source of NO_x in the atmosphere may vary with season. Power plant emissions may have higher $\delta^{18}O$ in NO_x than do automobile emissions (Kendall, 1998). Hence, if the relative proportion of power plant to automobile emissions increases in the winter in the eastern USA, then this could cause the $\delta^{18}O_{(NO_3^-)}$

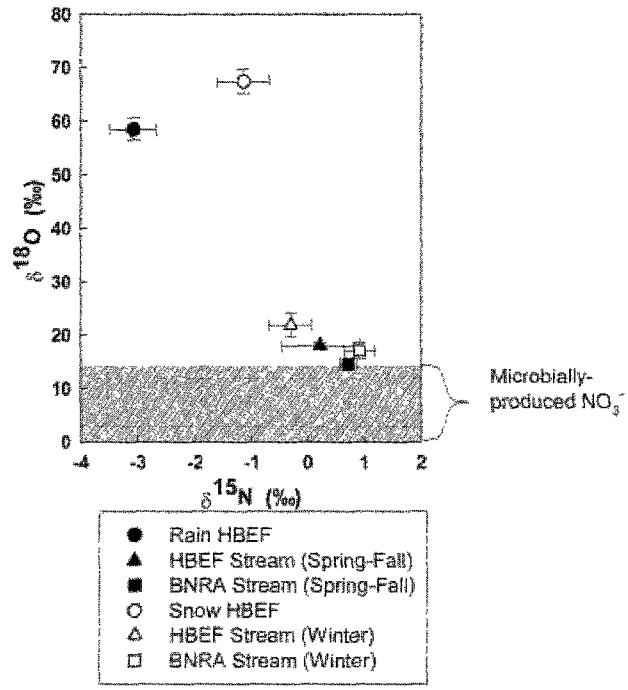

Figure 4. Mean $\delta^{15}N$ and $\delta^{18}O$ in nitrate in precipitation, streamwater and microbially produced nitrate. The mean $\delta^{15}N$ and $\delta^{18}O$ in nitrate in rain and snow at the HBEF and the streamwater separated by season for the Bowl RNA and the HBEF are shown with standard error bars. $\delta^{18}O$ in microbially produced nitrate is shown as the range reported in the literature

in snow nitrate to be higher than that of rain. (2) The form of the precipitation itself may affect the isotopic signature, although this seems unlikely. (3) Seasonal differences in atmospheric chemistry may sufficiently alter the relative abundance of reactive oxygen, such that the photochemically reactive hydroxyl radical with a low $\delta^{18}O$ value is more likely to react with NO_x to form nitrate in the summer than in the winter, compared with the less reactive ozone with a higher $\delta^{18}O$ that would dominate the reactions in the winter (Dentener and Crutzen, 1993; Johnston *et al.*, 1995; Krankowsky *et al.*, 1995). This explanation would account for processes that occur at various sites independent of particular seasonal variations in the proportion of power plant to automobile emissions. However, sites in Colorado, New York, and West Virginia offer contradictory evidence, since the range of precipitation $\delta^{18}O_{(NO_3^-)}$ is broad and includes many low winter snow $\delta^{18}O_{(NO_3^-)}$ values at some sites (Kendall *et al.*, 1996; Williard *et al.*, 2001; Campbell *et al.*, 2002). If power plant and automobile exhaust $\delta^{18}O_{(NO_3^-)}$ signatures are consistently distinct, then the storm track (the path that the precipitation travels before being deposited at the HBEF) may strongly influence the $\delta^{18}O_{(NO_3^-)}$ of precipitation, as it does for many solutes at the HBEF (Munn *et al.*, 1984).

Similar seasonal patterns in $\delta^{15}N_{(NO_3^-)}$ have been observed in the Catskill and Rocky Mountains based on snowmelt and snowpack rather than precipitation (snow fall). Burns and Kendall (2002) reported that $\delta^{15}N_{(NO_3^-)}$ in snowmelt was significantly higher than in wet deposition; Campbell *et al.* (2002) reported that $\delta^{15}N_{(NO_3^-)}$ in snowmelt and snowpack were significantly higher than bulk precipitation (rain). The difference in mean $\delta^{15}N_{(NO_3^-)}$ in snow compared to rain may suggest similar seasonal variation in the $\delta^{15}N$ values of nitrogen emissions or in the sources of nitrogen emissions.

We expected to see an increase in $\delta^{18}O_{(NO_3^-)}$ in nitrate in streamwater during snowmelt periods in the winter, assuming that during snowmelt the precipitation-derived snowpack nitrate would be a more significant contributor to streamwater nitrate. Although the mean streamwater $\delta^{18}O_{(NO_3^-)}$ at the HBEF was 6‰ higher

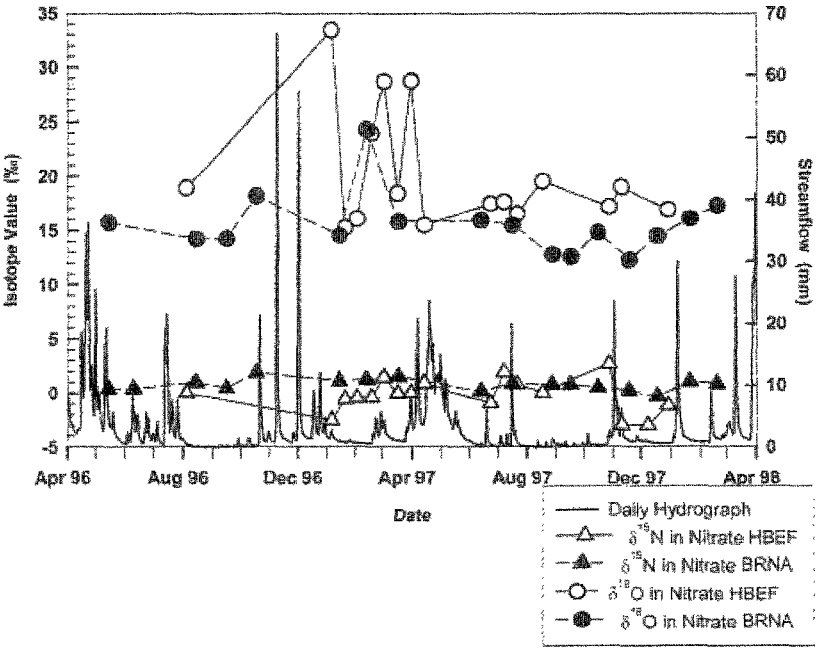

Figure 5. $\delta^{15}N$ and $\delta^{18}O$ in nitrate in streamwater at the Bowl RNA and the HBEF and streamwater hydrograph. The daily stream hydrograph from the reference watershed (W6) at the HBEF is shown. Streamflow data are not available from the Bowl RNA

during the winter than during the non-winter period, this difference was not statistically significant. There were four $\delta^{18}O_{(NO_3^-)}$ values that ranged from +23 to +33‰, while five other snowmelt values ranged from +15 to +18‰. It might have been easier to detect a difference, if one existed, had streamwater sampling been by melt event instead of biweekly; stream chemistry at HBEF W6 returns to antecedent concentrations relatively quickly (<24 h) even after large hydrologic events (Buso *et al.*, 2000).

The study was motivated by the desire to understand why some forested watersheds lose more nitrate than others at a given nitrogen deposition level. We expected that the Bowl RNA would have a higher fraction of nitrate from precipitation (relative to microbially produced nitrate) compared with the HBEF, where the nitrogen flux is lower. However, we did not observe this pattern. The absence of a significant difference in streamwater $\delta^{18}O_{(NO_3^-)}$ at the Bowl RNA between winter and non-winter samples is consistent with several pieces of evidence that suggest hydrology may play a significant role in regulating streamwater nitrate at this site. First, the Bowl RNA is steep at the upper elevations (up to 75% slope, with a watershed mean of 32%; Martin *et al.*, 2000), and is fairly flat at the low elevations. Because soils are absent (bare ledge) or are shallow to bedrock on the steep slopes, and because the colluvial, gravelly soils in the flat area are relatively deep (>3 m) and porous (J. Homer, NRCS, unpublished data, 1996), water may drain more quickly into deeper soils at the base of the watershed. Thus, precipitation-derived soil solution may enter into a relatively well-mixed soil or groundwater storage pool, before being discharged into streamwater, hence dampening any potential seasonal changes in relative proportions of sources. The importance of topography in controlling nitrate concentration and export was reported by Schiff *et al.* (2002): compared with a watershed with shallower slopes, in an adjacent watershed with steeper slopes, they observed a deeper water table that reduced the potential for plant uptake of nitrogen and led to the elevated nitrate concentrations they observed.

A second factor that suggests hydrology may play a significant role in regulating streamwater nitrate at this site is that streamwater nitrate concentrations at the Bowl RNA do not have the seasonal pattern characteristic of many watersheds in the White Mountains, with increased nitrate concentration and flux at snowmelt

(Martin, 1979; Martin *et al.*, 2000). The aseasonal elevated nitrate loss pattern is considered an indicator that an ecosystem is moving towards nitrogen saturation (*sensu* Stoddard, 1994). However, the aseasonal pattern at the Bowl RNA may be the result of a time lag between nitrate leaching below the rooting zone and being exported via streamwater.

Finally, a recent study evaluating significant damage from the January 1998 ice storm at the Bowl RNA found a moderate aseasonal increase (approximately double the baseline) in streamwater nitrate concentration in spite of removal of 53% of the canopy, compared with seasonal increases of 1–2 orders of magnitude at the HBEF (Houlton *et al.*, 2003). These findings, in contrast to large seasonal increases in streamwater nitrate concentration at other sites in the region (Houlton *et al.*, 2003), support the hypothesis that significant subsurface storage of water may moderate any pulses of nitrate loss.

The Bowl RNA catchment is more than an order of magnitude larger than HBEF W6 (Table I; Figure 1). Although size may certainly affect nitrogen cycling dynamics, it is insufficient alone to explain the aseasonal pattern observed at the Bowl RNA. For example, the main Hubbard Brook catchment, which is significantly larger, exhibits the same seasonal patterns for sulphate and calcium as HBEF W6 (Likens *et al.*, 1998; 2002) suggesting that simply increasing size without significantly altering topography or other factors is not sufficient to alter the seasonal pattern in nitrate concentration.

If streamwater nitrate concentration is regulated largely by the hydrology of the catchment, then this could alter our understanding of the stages of nitrogen saturation as proposed by Stoddard (1994). The aseasonal elevated streamwater nitrate concentration does, indeed, suggest that plants are not utilizing all available nitrate (at this site, available nitrogen exceeds plant and microbial demand). However, the inability of biota to utilize the nitrate could be influenced by the rate at which water moves within the catchment and out of the rooting zone, rather than simply by the capacity of plants and microbes to take up nitrogen. Collecting samples by snowmelt event would have enabled us to compare the seasonal patterns in streamwater $\delta^{18}O_{(NO_3^-)}$ by evaluating baseflow and event samples at the Bowl RNA with greater certainty. However, the lack of seasonal pattern in the streamwater $\delta^{18}O_{(NO_3^-)}$ at the Bowl RNA supports the other aseasonal observations described above that suggest hydrology may be a significant factor in regulating temporal nitrate loss patterns.

CONCLUSIONS

The oxygen isotope ratios of streamwater nitrate suggest that most nitrate that is exported in streamwater has been cycled through microbes before leaving the ecosystem. These results are significant because they confirm the importance of microbial transformations in regulating nitrogen loss from ecosystems. We did not find that the site with higher nitrogen losses had a higher fraction of precipitation-derived nitrate in the streamwater. The research also highlights the potential importance of hydrological storage in controlling nitrate export from forested catchments, and the need for interdisciplinary research in evaluating catchment-level controls on nutrient cycling.

Sampling during snowmelt events would clarify the relationship between snowmelt and streamwater nitrate. Event sampling of precipitation would also be useful, in order to evaluate the relationship between storm track and precipitation isotopic signatures.

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REFERENCES

Aber J, Nadelhoffer K, Steudler PA, Melillo JM. 1989. Nitrogen saturation in northern forest ecosystems—hypothesis and implications. *BioScience* **39**: 378–386.

Aber J, Magill A, McNulty SG, Boone RD, Nadelhoffer KJ, Downs M, Hallett R. 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water, Air, and Soil Pollution* **85**: 1665–1670.

Aber J, McDowell W, Nadelhoffer KJ, Magill A, Berntson G, Kamakea M, McNulty SG, Currie W, Rustad L, Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems. *BioScience* **48**: 921–934.

Amberger A, Schmidt HL. 1987. Natürliche Isotopengehalte von Nitrat als Indikatoren für dessen Herkunft. *Geochimica et Cosmochimica Acta* **51**: 2699–2705.

Andersson KK, Hooper AB. 1983. O_2 and H_2O and each the source of one O in NO_2 produced from NH_3 by *Nitrosomonas*: ^{15}N evidence. *FEBS Letters* **164**: 236–240.

Aravena R, Evans ML, Cherry JA. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Ground Water* **31**: 180–186.

Birdsey RA, Mickler RA, Hom J, Heath LS. 2000. Summary of prospective global change impacts on northern U.S. forest ecosystems. In *Responses of Northern U.S. forests to Environmental Change*, Mickler RA, Birdsey RA, Hom J (eds). Ecological Studies 139, Springer-Verlag: New York; 543–568.

Böttcher J, Strebel O, Voerkelius S, Schmidt HL. 1990. Using isotopic fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. *Journal of Hydrology* **114**: 413–424.

Burns DA, Kendall C. 2002. Analysis of sources of ^{15}N and ^{18}O to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. *Water Resources Research* **38**: DOI: 10·1029/2001WR000292.

Buso DC, Likens GE, Eaton JS. 2000. *Chemistry of precipitation, stream water and lake water from the Hubbard Brook Ecosystem Study: a record of sampling protocols and analytical procedures*. USDA Forest Service, Northeastern Research Station, General Technical Report NE-275, Newtown Square, PA.

Campbell DH, Kendall C, Chang CCY, Silva SR, Tonnessen KA. 2002. Pathways for nitrate release from an alpine watershed: determination using $\delta^{18}O$ and $\delta^{15}N$. *Water Resources Research* **38**: DOI: 10·1029/2001WR000294.

Chang CCY, Langston J, Riggs M, Campbell DH, Silva SR, Kendall C. 1999. A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. *Canadian Journal of Fisheries and Aquatic Science* **56**: 1856–1864.

Dentener FJ, Crutzen PJ. 1993. Reaction of N_2O_5 on tropospheric aerosols: impact on the global distributions of NO_x, O_3, and OH. *Journal of Geophysical Research* **98**(D4): 7149–7163.

Dise NB, Matzner E, Gundersen P. 1998. Synthesis of nitrogen pools and fluxes from European forest ecosystems. *Water, Air, and Soil Pollution* **105**: 143–154.

Durka W, Schulze ED, Gebauer G, Voerkelius S. 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. *Nature* **372**: 765–767.

Emmett BA, Kjønaas OJ, Gundersen P, Koopmans CJ, Tietema A, Sleep D. 1998. Natural abundance of ^{15}N in forests across a nitrogen deposition gradient. *Forest Ecology and Management* **101**: 9–18.

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* **8**(3): 706–733.

Freyer HD. 1991. Seasonal variation of $^{15}N/^{14}N$ ratios in atmospheric nitrate species. *Tellus Series B: Chemical and Physical Meteorology* **43**: 30–44.

Galloway JN. 1995. Acid deposition: perspectives in time and space. *Water, Air, and Soil Pollution* **85**: 15–24.

Goodale CL, Aber JD. 2001. The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. *Ecological Applications* **11**: 253–267.

Goodale CL, Aber JD, McDowell WH. 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. *Ecosystems* **3**: 433–450.

Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL. 2001. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry* **56**(2): 191–213.

Gundersen P, Emmett BA, Kjønaas OJ, Koopmans CJ, Tietema A. 1998. Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. *Environmental Pollution* **102**: 403–407.

Hauhs M, Rost-Siebert K, Raben G, Paces T, Dillon PJ. 1989. Summary of European data. In *The Role of Nitrogen in the Acidification of Soils and Surface Waters*, Malanchuk JL, Nilsson J (eds.). Miljørapport.

Hayes WL. 1988. *Statistics*. Holt, Rinehart, Winston Inc.: New York.

Heaton THE. 1990. $^{15}N/^{14}N$ ratios of NO_x from vehicle engines and coal-fired power stations. *Tellus Series B: Chemical and Physical Meteorology* **42**: 304–307.

Hollocher TC. 1984. Source of the oxygen atoms of nitrate in the oxidation of nitrite by *Nitrobacter agilis*: evidence against a P–O–N anhydride mechanism in oxidative phosphorylation. *Archives of Biochemistry and Biophysics* **233**: 721–727.

Hornbeck JW, Bailey SW, Buso DC, Shanley JB. 1997. Streamwater chemistry and nutrient budgets for forested watersheds in New England: variability and management implications. *Forest Ecology and Management* **93**: 73–89.

Houlton BZ, Driscoll CT, Fahey TJ, Likens GE, Groffman PM, Bernhardt ES, Buso DC. Nitrogen dynamics in ice-storm damaged forest ec0-systems: implications for nitrogen limitation theory. *Ecosystems* **6**: 431–443.

Johnson CE. 1991. Soil nitrogen status 8 years after whole-tree clear-cutting. *Canadian Journal of Forest Research* **25**: 1346–1355.

Johnston JC, Cliff SS, Thiemens MH. 1995. Measurement of multioxygen isotopic (delta18O and delta17O) fractionation factors in the stratospheric sink reactions of nitrous oxide. *Journal of Geophysical Research* **100**(D8): 16 801–16 804.

Kendall C. 1998. Tracing nitrogen sources and cycling in catchments. In *Isotope Tracers in Catchment Hydrology*, Kendall C, McDonnell JJ (eds). Elsevier: Amsterdam; 519–576.

Kendall C, Campbell DH, Burns DA, Shanley JB, Silva SR, Chang CCY. 1995. Tracing sources of nitrate in snowmelt runoff using the oxygen and nitrogen isotopic compositions of nitrate. In *Biogeochemistry of Seasonally Snow-Covered Catchments*, Tonnessen K, Williams M, Tranter M (eds). IAHS Publication No. 228. IAHS Press: Wallingford; 339–347.

Kendall C, Silva SR, Chang CCY, Burns DA, Campbell DH, Shanley JB. 1996. Use of the Delta 18-O and Delta 15-N of nitrate to determine sources of nitrate in early spring runoff in forested catchments. In *Isotopes in Water Resources Management. International Atomic Energy Agency Symposium*, vol. 1; 167–176.

Koba K, Tokuchi N, Yoshioka T, Hobbie EA, Iwatsubo G. 1998. Natural abundance of nitrogen-15 in a forest soil. *Soil Science Society of American Journal* **62**: 778–781.

Krankowsky D, Bartecki F, Klees GG, Mauersberger K, Schellenbach K, Stehr J. 1995. Measurement of heavy isotope enrichment in tropospheric ozone. *Geophysical Research Letters* **22**: 1713–1716.

Kumar S, Nicholas DJD, Williams EH. 1983. Definitive ^{15}N NMR evidence that water serves as a source of 'O' during nitrite oxidation by *Nitrobacter agilis*. *FEBS Letters* **152**: 71–74.

Likens GE, Bormann FH. 1995. *Biogeochemistry of a Forested Ecosystem*, 2nd edn. Springer-Verlag: New York.

Likens GE, Driscoll CT, Buso DC, Siccama TG, Johnson CE, Lovett GM, Fahey TJ, Reiners WA, Ryan DA, Martin CW, Bailey SW. 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* **41**: 89–173.

Likens GE, Driscoll CT, Buso DC, Mitchell MJ, Lovett GM, Bailey SW, Siccama TG, Reiners WA, Alewell C. 2002. The biogeochemistry of sulfur at Hubbard Brook. *Biogeochemistry* **60**: 235–316.

Lovett GM, Rueth H. 1999. Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient. *Ecological Applications* **9**: 1330–1344.

Lovett GM, Weathers KC, Sobczak W. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. *Ecological Applications* **10**: 73–84.

Martin CW. 1979. Precipitation and streamwater chemistry in an undisturbed forested watershed in New Hampshire. *Ecology* **60**: 36–42.

Martin CW, Bailey AS. 1999. Twenty years of change in a northern hardwood forest. *Forest Ecology and Management* **123**: 253–260.

Martin CW, Driscoll CT, Fahey TJ. 2000. Changes in streamwater chemistry after 20 years from forested watersheds in New Hampshire, USA. *Canadian Journal of Forest Research* **30**: 1206–1213.

Mayer B, Bollwerk SM, Mansfeldt T, Hütter B, Veizer J. 2001. The oxygen isotope composition of nitrate generated by nitrification in acid forest soils. *Geochimica et Cosmochimica Acta* **65**: 2743–2756.

McNulty SG, Aber JD, Boone RD. 1991. Spatial changes in forest floor and foliar chemistry of spruce-fir forests across New England. *Biogeochemistry* **14**: 13–29.

Munn RE, Likens GE, Weisman B, Hornbeck JW, Martin CW, Bormann FH. 1984. A meteorological analysis of the precipitation chemistry event samples at Hubbard Brook, New Hampshire. *Atmospheric Environment* **18**: 2775–2779.

Ollinger SV, Aber JD, Lovett GM, Millham SE, Lathrop RG, Ellis JM. 1993. A spatial model of atmospheric deposition for the northeastern U.S. *Ecological Applications* **3**: 459–472.

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, Aber JD. 2002. Regional variation in foliar chemistry and nitrogen cycling among forests of diverse history and composition. *Ecology* **83**: 339–355.

Pardo LH. 1999. *Natural abundance of ^{15}N as a tool for assessing patterns of nitrogen loss from forest ecosystems*. PhD dissertation, Massachusetts Institute of Technology, Cambridge, MA.

Pardo LH, Driscoll CT, Likens GE. 1995. Patterns of nitrate loss from a chronosequence of clear-cut watersheds. *Water, Air, and Soil Pollution* **85**: 1659–1664.

Pardo LH, Hemond HF, Montoya JP, Siccama TG. 2001. Long-term patterns in forest floor nitrogen-15 natural abundance at Hubbard Brook, NH. *Soil Science Society of America Journal* **65**: 1279–1283.

Pardo LH, Hemond HF, Montoya JP, Fahey TJ, Siccama TG. 2002. Response of natural abundance of ^{15}N in soils and foliage to high nitrate loss following clear-cutting. *Canadian Journal of Forest Research* **32**: 1126–1136.

SAS Institute Inc. 1988. *SAS/STAT® User's Guide*, Release 6·03 Edition. SAS Institute Inc.: Cary, NC.

Satterthwaite FW. 1946. An approximate distribution of estimates of variance components. *Biometrics Bulletin* **2**: 110–114.

Schiff SL, Devito KJ, Elgood RJ, McCrindle PM, Spoelstra J, Dillon P. 2002. Two adjacent forested catchments: dramatically different NO_3^- export. *Water Resources Research* **38**: 1292. DOI: 10·1029/2000WR000170.

Seiler RL. 1999. *A chemical signature for ground water contaminated by domestic wastewater*. PhD dissertation, University of Nevada Reno, Reno, NV.

Silva SR, Kendall C, Wilkison DH, Ziegler AC, Chang CCY, Avanzino RJ. 2000. A new method for collection of nitrate from fresh water and analysis of the nitrogen and oxygen isotope ratios. *Journal of Hydrology* **228**: 22–36.

Stoddard JL. 1994. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In *Environmental Chemistry of Lakes and Reservoirs*, Baker A (ed). Advances in Chemistry Series No. 237 American Chemical Society: Washington, DC: 223–284.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman D. 1997. Human alteration of the global nitrogen cycle: causes and consequences. *Ecological Applications* **7**(3): 737–750.

Voerkelius S. 1990. *Isotopendiskriminierungen bei der Nitrifikation und Denitrifikation: Grundlagen und Anwendungen der Herkunfts-Zuordnung von Nitrat und Disickstoffmonoxid*. PhD dissertation, Technische Universität München.

Wassenaar L. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. *Applied Geochemistry* **10**: 391–405.
Williard KWJ, DeWalle DR, Edwards PJ, Sharpe WE. 2001. ^{18}O isotopic separation of stream nitrate sources in mid-Appalachian forested watersheds. *Journal of Hydrology* **252**: 174–188.

