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^{18}O isotopic separation of stream nitrate sources in mid-Appalachian forested watersheds

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Abstract

The $\delta^{18}O$ values of atmospheric nitrate deposition, microbe-produced nitrate, and stream nitrate were measured to determine the dominant source of stream nitrate in 27 mid-Appalachian headwater forested watersheds (12–771 ha) with varying bedrock geologies, land disturbance histories, and stand ages. The 12 monthly composite nitrate $\delta^{18}O$ values of wet deposition and throughfall exhibited similar pronounced seasonal trends, with relatively depleted $\delta^{18}O$ values during the summer. Wet deposition and throughfall nitrate $\delta^{18}O$ values were not significantly different between northern (Leading Ridge, PA) and southern (Fernow, WV) regional sampling sites, indicating that $\delta^{18}O$ values were spatially similar across the study area. Atmospheric nitrate $\delta^{18}O$ values were significantly greater than microbe-produced nitrate $\delta^{18}O$ values, allowing the two sources of stream nitrate to be separated. During four baseflow and three stormflow sampling periods, microbe-produced nitrate was the dominant (>70%) source of nitrate in the study streams. This result does not mean atmospheric nitrogen deposition should be discounted as a source of forested stream nitrate, because atmospheric deposition is the primary external contributor to the long-term soil nitrogen pool that ultimately drives soil nitrate production rates. Stream nitrate $\delta^{18}O$ values were greater during stormflow periods compared to baseflow periods, indicating greater contributions of atmospheric nitrate during storm events. Neither microbe-produced nitrate $\delta^{18}O$ values from incubated forest soil samples nor stream nitrate $\delta^{18}O$ values showed strong relationships with land disturbance history or stand age. However, watersheds dominated by Pottsville/Allegheny bedrock and associated extremely acid soils had greater summer stream nitrate $\delta^{18}O$ values than watersheds containing predominantly Catskill/Chemung/Pocono and Mauch Chunk/Greenbrier bedrock. Inhibited microbial nitrate production by low soil pH could account for the greater proportions of atmospheric nitrate deposition in streams draining Pottsville/Allegheny bedrock.

Keywords: Oxygen-18; Isotope; Nitrate; Atmospheric deposition; Nitrification; Forested watershed

1. Introduction

Traditionally, northern temperate forests were considered nitrogen limited, but this assumption has been challenged by researchers who discovered substantial export of nitrate in streams draining relatively undisturbed forests (Aber et al., 1989; Driscoll et al., 1989; Edwards and Helvey, 1991; Johnson and Lindberg, 1992; Murdoch and Stoddard, 1992; Peterjohn et al., 1996). High rates of atmospheric nitrate deposition in the region could be directly responsible for high nitrate export, because atmospheric deposition

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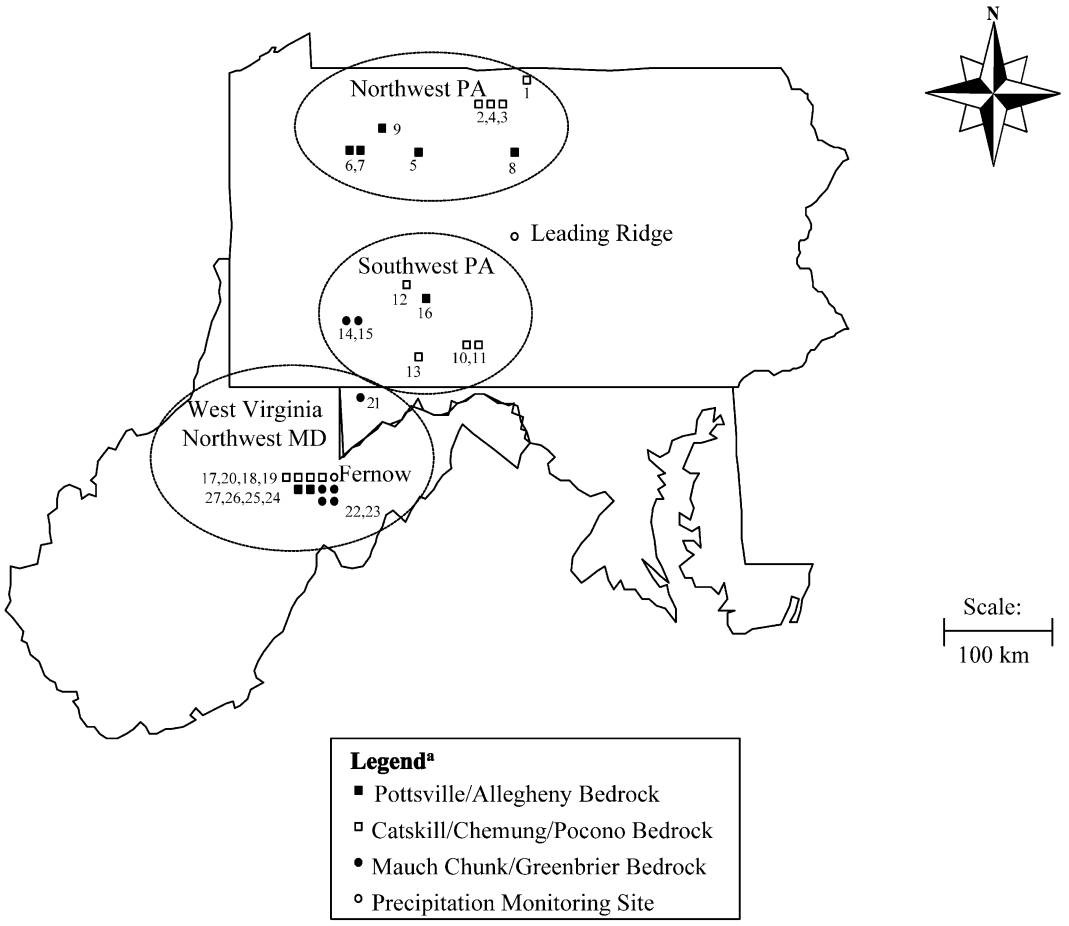
is a significant contributor of nitrogen to forested ecosystems. The mid-Appalachian region receives some of the highest rates of atmospheric nitrate deposition in the United States (Adams et al., 1993; Lynch et al., 1989–1994). High rates of atmospheric nitrate deposition have been correlated strongly to stream nitrate loads in broad surveys of European forested catchments (Dise and Wright, 1995), while the most extensive survey of forested watersheds in North America, the Integrated Forest Study, did not show a strong correlation between atmospheric nitrate deposition and stream nitrate (Johnson and Lindberg, 1992). Williard et al. (1997) also found that stream nitrate export from nine forested watersheds in the mid-Appalachians was not correlated with atmospheric deposition rates. This lack of correlation may be due, in part, to a lack of direct hydrologic connection between precipitation and streamflow in forested watersheds. For example, Burns et al. (1998) found that a deep bedrock flow system containing high nitrate waters recharged in the early spring and late fall was responsible for high summer nitrate concentrations in Catskill Mountain streams. In the summer, this deep flow system was disconnected from the shallow flow system, which contained relatively low nitrate concentrations. Thus, it is important to consider hydrologic flow paths and residence times (Maloszewski et al., 1983) when trying to relate atmospheric deposition rates to stream nitrate loads. Also, a correlation may exist between atmospheric deposition and stream nitrate loads that is not demonstrated by rate-based analyses alone. Through isotopic separation, it is now possible to determine the percentage of stream nitrate that is of atmospheric origin (Durka et al., 1994).

^{15}N and ^{18}O isotopes of nitrate have both been used to estimate the probable source and origin of nitrate in surface waters (Amberger and Schmidt, 1987; Durka et al., 1994; Kendall et al., 1996). Durka et al. (1994) focused on separating the two primary sources of forested stream nitrate (atmospheric deposition and microbe-produced nitrate). They found ^{18}O isotopes were more useful than the ^{15}N isotopes, because the ^{18}O signatures of atmospheric nitrate (52.5–60.9‰) and microbe-produced soil nitrate (0.8–5.8‰) differed significantly, while the ^{15}N signature ranges for the two sources overlapped (Durka et al., 1994).

Theoretically, microbe-produced nitrate should have lower ^{18}O signatures than atmospheric nitrate deposition. *Nitrosomonas* and *Nitrobacter*, the bacteria responsible for nitrification, derive two of the oxygen atoms in nitrate from soil water and one from atmospheric O_2 (Hollocher et al., 1981; Andersson and Hooper, 1983; Kumar et al., 1983; Hollocher, 1984). Atmospheric O_2 has an $\delta^{18}O$ value of approximately 23.5‰ (Kroopnick and Craig, 1972), while soil water exhibits a range of ^{18}O values (−25 to +4‰) (Kendall, 1998). The significant range in soil water ^{18}O exists because of seasonal variation in the ^{18}O signatures of precipitation (Clark and Fritz, 1997). Based on this range, the calculated, theoretical $\delta^{18}O$ range of microbe-produced nitrate is −2.2 to +3.2‰. These calculations assume no oxygen exchange occurs between nitrate and water, which was first demonstrated in laboratory investigations by Bunton et al. (1952).

Kendall (1998) found a wide range of nitrate deposition $\delta^{18}O$ values (14–75‰) in a data compilation ($n = 232$ samples) of North American and German studies. She suggested that the observed variation could be due to fractionation associated with thunderstorms, incomplete combustion of fossil fuels in power plants and automobile exhaust, and photochemical reactions in the atmosphere. The compiled data set did not contain any temporal trend analysis, and Kendall (1998) stated that almost nothing is known about seasonal differences in atmospheric nitrate $\delta^{18}O$.

In this study, Durka et al.'s (1994) general approach was applied to mid-Appalachian watersheds of differing bedrock, land disturbance history, and stand age to determine if differing percentages of atmospheric nitrate deposition in stream nitrate account for some of the significant regional variation in nitrate export (DeWalle and Pionke, 1994). The study was designed to meet four objectives. Objective 1 was to determine the seasonal variability of $\delta^{18}O$ in atmospheric nitrate deposition. Monthly precipitation sampling allows the calculation of a more representative nitrate deposition $\delta^{18}O$ value than less frequent sampling, yielding more effective separation between atmospheric nitrate and microbe-produced nitrate. Objective 2 was to evaluate the effects of transformations within the forest canopy on the atmospheric nitrate isotopic signature. Durka et al. (1994) measured nitrate ^{18}O in throughfall but



[a]See Table 1 for watershed numbers and names.

Fig. 1. Locations of the 27 study watersheds and two precipitation monitoring sites in the mid-Appalachian region of the United States.

not in open precipitation. Therefore, the effects of in-canopy transformations on the atmospheric nitrate isotopic signature could not be assessed in Durka et al.'s (1994) study. Objective 3 was to determine the variability of microbe-produced nitrate $\delta^{18}O$ among the study watersheds. Since soil water ^{18}O signatures likely vary among the study watersheds, $\delta^{18}O$ values of microbe-produced nitrate also were expected to vary among the study watersheds. Objective 4 was to determine the fractional contribution of atmospheric nitrate to stream nitrate in mid-Appalachian forested watersheds. Percentages of atmospheric nitrate in stream nitrate were compared seasonally, at baseflow and stormflow levels, and across different geologic and watershed types to test if this factor can explain any of the significant variation in nitrate export that exists in the mid-Appalachian region.

2. Study sites

Twenty-seven forested watersheds were sampled for stream nitrate ^{18}O signatures in three regions of the mid-Appalachians: northwestern Pennsylvania, southwestern Pennsylvania, and West Virginia and northwestern Maryland (Fig. 1, Table 1). Six watersheds in West Virginia were chosen for more intensive monthly stream sampling [(18) Fernow 4, (19) Fernow 10, (23) Freeland Run, (24) Karly Run, (25) West Three Spring, and (26) Otter Run] (Fig. 1, Table 1; the

Table 1
Geographic region, geology, and watershed type for the 27 study watersheds

	Watershed	Region	Geology category[a]	Watershed type
1	E. Branch Pine Creek	NW PA	CCP	Farmed
2	Jones Run	NW PA	CCP	Farmed
3	Baker Hollow	NW PA	CCP	Logged
4	Jacob Run	NW PA	CCP	Logged
5	Red Mill Run	NW PA	PVA	Burned
6	West Branch	NW PA	PVA	Logged
7	The Branch	NW PA	PVA	Logged
8	Beech Bottom Run	NW PA	PVA	Old-growth
9	East Fork Run	NW PA	PVA	Old-growth
10	Bowl Run	SW PA	CCP	Logged
11	Betsy Gap Run	SW PA	CCP	Logged
12	Lick Run	SW PA	CCP	Old-growth
13	Sweet Root Gap	SW PA	CCP	Old-growth
14	Nedrow Run	SW PA	MCG	Farmed
15	Roaring Run	SW PA	MCG	Farmed
16	Deadlift Run	SW PA	PVA	Logged
17	Clover Run	WV MD	CCP	Farmed
18	Fernow 4	WV MD	CCP	Logged
19	Fernow 10	WV MD	CCP	Logged
20	Bear Run	WV MD	CCP	Old-growth
21	Little Bear Run	WV MD	MCG	Farmed
22	Freeland Run	WV MD	MCG	Logged
23	Salamander Run	WV MD	MCG	Logged
24	Karly Run	WV MD	MCG	Logged
25	West Three Spring	WV MD	MCG	Old-growth
26	Otter Run	WV MD	PVA	Logged
27	Condon Run	WV MD	PVA	Logged

[a] Geology Categories. CCP = Catskill formation, Chemung formation, and Pocono group. PVA = Pottsville group and Allegheny group. MCG = Mauch Chunk formation and Greenbrier member.

number in parentheses preceding the watershed name refers to its location identifier on Fig. 1). All study watersheds were 100% forested with no disturbances in the past 60 years. Most of the watersheds contained first or second order streams and were relatively small (<2 km^2). Prevalent overstory species across the 3 regions were sugar maple (*Acer saccharum* Marsh.), black cherry (*Prunus serotina* L.), and eastern hemlock (*Tsuga canadensis* L.).

Study watersheds were grouped into three geologic categories: Pottsville/Allegheny sandstone (PVA), Catskill/Chemung/Pocono shale and sandstone (CCP), and Mauch Chunk/Greenbrier shale/limestone (MCG) (Table 1). The bedrock geology types were determined from quadrangle geology maps (Reger, 1923; Reger, 1931; Maryland Geological Survey, 1953; Berg and Dodge, 1981) and the geologic categories were constructed based on relative differences in published groundwater pH values (Ponce et al., 1979; Taylor et al., 1982, 1983; DeWalle et al., 1987), with PVA, CCP, and MCG exhibiting low, mid, and high pH values, respectively.

The watersheds were further stratified according to their land use or disturbance history (farmed, logged, burned, and old growth) (Table 1). Farmed watersheds were cultivated 60–90 years ago and left to revert back to forestland. Historical aerial photographs, as well as present day evidence (personal communication, old fence posts, rock piles, foundations), were used to document the presence of past farming. The logged watersheds were cut between 60 and 90 years ago and received no significant harvesting since that date. Burned watersheds had severe fires 60 to 90 years ago and were left to re-establish forest cover. Old-growth stands were defined as stands greater than 150 years old. Historical fire records, maps, and stand

inventories were examined at the Allegheny National Forest, PA, Monongahela National Forest, WV, and Buchanan State Forest, PA headquarters to locate the burned, logged, and old-growth watersheds.

3. Methods

3.1. Open precipitation and throughfall ^{18}O analysis

Open precipitation and throughfall were sampled at a northern and southern regional site within the mid-Appalachians. The northern site was Leading Ridge Experimental watershed 1 in Huntingdon County, Pennsylvania (40°40′25″ N, 77°56′15″ W); the southern site was Fernow watershed 4 on the Fernow Experimental Forest near Parsons, WV (39°03′15″ N, 79°41′20″ W). Both sampling sites contained mature, deciduous forest stands. Open precipitation and throughfall were sampled for one year (December 1997–November 1998). The samples were collected bimonthly and composited monthly. Bulk precipitation and throughfall were sampled in open polyethylene tubs during the winter and in polyethylene funnel/bottle collection devices during the spring and summer. The vast majority of nitrate collected by the bulk containers was wet and dry atmospheric nitrate deposition, however, it is possible that some of the sampled nitrate could have originated from microbial mineralization and/or nitrification of wet and dry dissolved organic nitrogen (DON) and ammonium in the forest canopy.

$HgCl_2$ was added to collection bottles at the beginning of each biweekly sampling period during spring and summer to inhibit any microbe-mediated nitrogen transformations in the samples. Bulk precipitation and throughfall sampled in tubs during the winter were preserved immediately with $HgCl_2$ upon returning to The Pennsylvania State University.

Approximately 4 l of monthly bulk precipitation and throughfall samples were collected to obtain the necessary 2 mg of nitrate-N for ^{18}O analysis. Each sample was vacuum filtered through 0.45 μm Millipore nitrocellulose filter paper and refrigerated until shipped on ice to the University of Waterloo Isotope Laboratory in Ontario, Canada for ^{18}O analysis of nitrate.

At the University of Waterloo Isotope Laboratory, nitrate was isolated using anion exchange columns (12.7 cm long polypropylene Evergreen Scientific columns containing integral 90–130 micrometer filters and Bio Rad AG 1-X8 Cl^- 100–200 mesh resin). Nitrate was eluted with 3M HCl and converted to silver nitrate by the addition of silver oxide. After freeze drying, the silver nitrate was combusted with graphite in a quartz breakseal at 800°C, producing CO_2 gas that was analyzed for ^{18}O and ^{16}O with a VG Prism Mass Spectrophotometer (Micromass, Inc). The $^{18}O/^{16}O$ isotope ratios were reported in delta notation ($\delta^{18}O$ in per mil units) relative to Standard Mean Ocean Water. Duplicate sample analyses indicated analytical error of the nitrate $\delta^{18}O$ measurements was 1.0‰. Monthly bulk precipitation and throughfall samples at the two regional sites also were analyzed for nitrate concentrations at the University of Waterloo Isotope Laboratory with a Dionex Ion Chromatograph.

3.2. Soil leachate ^{18}O analysis

Three soil cores were taken in August 1998 from each of the 27 watersheds to determine the $\delta^{18}O$ values of microbe-produced nitrate. The three cores were taken along an elevation gradient in each watershed. The low elevation cores were taken at each watershed mouth near the stream water sampling point in well-drained soils, which were not gleyed. The mid-slope cores were taken near the middle of each watershed. The upslope cores were taken on the ridge top in the headwaters of each watershed. Soil cores were sampled in 15.2 cm diameter PVC tubes to a depth of 10 cm and were transported to the laboratory where they were flushed with 2.5 l of deionized water to remove existing atmospheric and microbe-produced nitrate. Cores then were incubated at room temperature (15–29°C) in the laboratory for five weeks. During incubation, the cores were left free to drain and covered with paper towels to prevent particulate contamination. At 2.5 weeks into incubation, 200 ml of deionized water was added to each core to maintain soil moisture levels in a favorable range for microbial nitrification. After the five week incubation period, cores were flushed again with 2.5 l of deionized water; the resulting nitrate in solution was assumed to be microbe-produced nitrate. Leachate from the three incubated cores from each watershed

was composited, preserved with $HgCl_2$, and sent to the University of Waterloo for ^{18}O analysis of nitrate.

3.3. Stream water ^{18}O analysis

The same 27 headwater streams were sampled during winter (March 1998) and summer (August 1998) baseflow periods for stream nitrate ^{18}O analysis. Grab samples were preserved with $HgCl_2$ at streamside and put on ice during transit to The Pennsylvania State University. There samples were filtered and shipped on ice to the University of Waterloo Isotope Laboratory for ^{18}O analysis of nitrate.

Six streams in WV (Fernow 4, Fernow 10, Otter Run, Salamander Run, West Three Spring, and Karly Run) were chosen for more intensive monthly sampling, which began in March 1998. Four baseflow samples and three high flow samples were taken from each stream for nitrate ^{18}O analysis. Single storm event sampling on Fernow 4 also was conducted on May 18, 1999. Stream samples were taken prior to the event, during the rising limb, near the peak, and during the falling limb of the hydrograph. Storm sewer outflow for a major residential area in State College, PA (Walnut Springs) was sampled near peakflow during an April event to verify that the isotopic integrity of direct contributions of atmospheric nitrate in overland flow were maintained as a test of the ^{18}O separation method.

3.4. Calculation of the percent contribution of atmospheric nitrate to stream nitrate

$\delta^{18}O$ values from atmospheric, microbe-produced, and stream nitrate samples were used to calculate the percentage of atmospheric nitrate contributing directly to stream nitrate with the following equation:

$$\%\ \text{atomospheric nitrate} = \frac{\delta^{18}O_{NO_3\ \text{stream}} - \delta^{18}O_{NO_3\ \text{nitrifcation}}}{\delta^{18}O_{NO_3\ \text{atmosphere}} - \delta^{18}O_{NO_3\ \text{nitrifcation}}} \times 100 \quad (1)$$

The equation is based on the derivation of the two-component model in hydrology and assumes that atmospheric nitrate deposition and nitrate originating from soil nitrification are the only two sources of stream nitrate in forested watersheds.

4. Results and discussion

4.1. Open precipitation and throughfall nitrate $\delta^{18}O$

Open precipitation and throughfall nitrate $\delta^{18}O$ values exhibited significant seasonal variation at the Fernow and Leading Ridge sampling sites (Figs. 2 and 3). Open precipitation and throughfall were relatively depleted of ^{18}O during the summer months (July, August, and September). The range of open precipitation and throughfall $\delta^{18}O$ values in this study (17–76‰) was similar to the range of values (14–75‰) cited by Kendall (1998) from precipitation in North America and Germany. However, Kendall's (1998) data set was not presented temporally, so no seasonal variation inferences can be made. Kendall (1998) did state that little is known about the temporal variability of nitrate $\delta^{18}O$. Durka et al.'s (1994) and Voerkelius' (1990) results hint of seasonal trends in precipitation $\delta^{18}O$ values. Fall and spring precipitation had a relatively tight range of $\delta^{18}O$ values (60.3–73.4‰) with a mean of 64.5 ± 4.8‰ (Durka et al., 1994). Voerkelius (1990) found nitrate ^{18}O in summer precipitation was slightly depleted (mean $\delta^{18}O$ value 57.5 ± 2.9‰) compared to Durka et al.'s (1994) fall and spring results.

Throughfall and open precipitation were measured monthly at Leading Ridge and Fernow to determine if $\delta^{18}O$ values in precipitation were altered by interactions within the canopy. Throughfall and open precipitation essentially tracked each other throughout the year at Leading Ridge and Fernow (Figs. 2 and 3) and were found to be not significantly different ($\alpha = 0.05$) at both sites according to paired t-tests. Thus, canopy effects on nitrate $\delta^{18}O$ values were minimal, and open precipitation nitrate $\delta^{18}O$ can be used as the input to the forest floor.

4.2. Microbe-produced nitrate $\delta^{18}O$

Soil incubation experiments were conducted to obtain microbe-produced nitrate $\delta^{18}O$ values for the 27 study watersheds (Table 2). Deionized water, with a depleted $\delta^{18}O$ value of -13‰, served as the soil water source for the microbes in the incubation experiment. Nitrifying bacteria derive 2/3 of the oxygen atoms in nitrate from soil water (-13‰) (Hollocher et al., 1981; Kumar et al., 1983), and 1/3

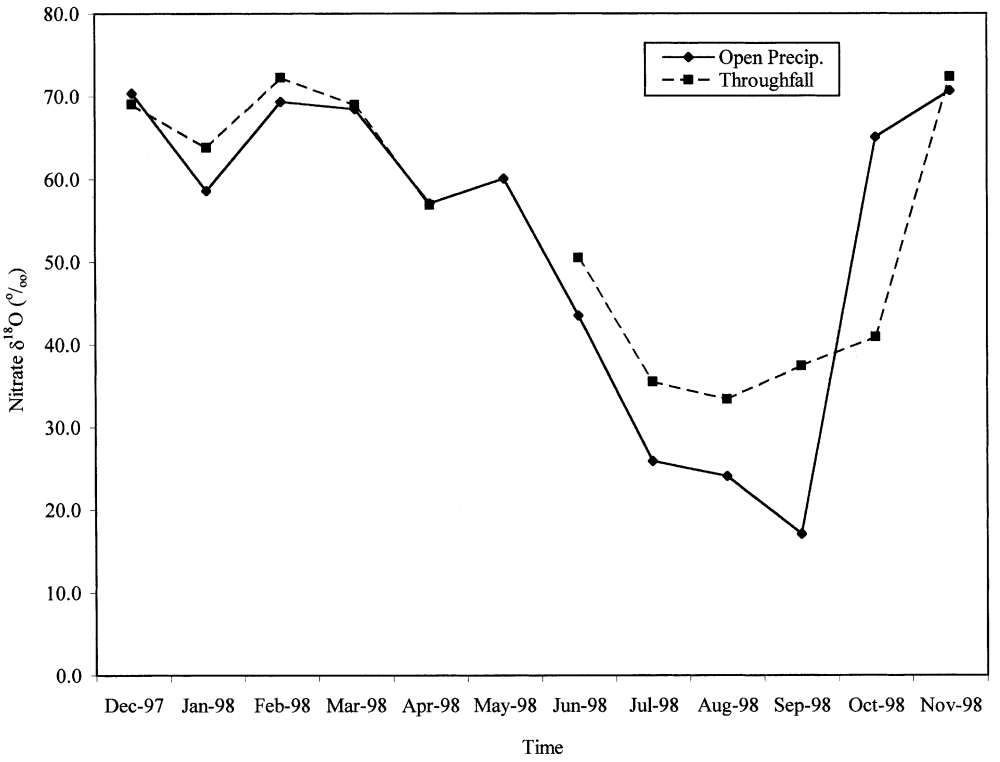


Fig. 2. Seasonal variation in nitrate $\delta^{18}O$ values for open precipitation and throughfall at Fernow watershed 4, WV.

from atmospheric oxygen (23.5‰) (Kroopnick and Craig, 1972). Therefore, the calculated, theoretical $\delta^{18}O$ value of microbe-produced nitrate in the study was −0.8‰. Since neither source likely varied in their $\delta^{18}O$ values, differences in microbe-produced nitrate $\delta^{18}O$ were not expected among the study watersheds.

Microbe-produced nitrate $\delta^{18}O$ values varied from 0.2 to 13.7‰ among 20 watersheds (Table 2). The remaining 7 watersheds did not yield enough microbe-produced nitrate in the soil cores for isotopic analysis. Microbe-produced nitrate $\delta^{18}O$ values were within the range (0.8 to 5.8‰), (3.3‰), and (16‰) measured by Amberger and Schmidt (1987), Voerkelius (1990), and Burns and Kendall (in press) , respectively. However, the majority (13) of the study watersheds had microbe-produced nitrate $\delta^{18}O$ values at the upper end (8.1‰–13.7‰) of the observed range. These elevated $\delta^{18}O$ values could be due to denitrification effects, nitrification by different types of soil microbes (fungi), and/or analytical interference by organic anions.

Denitrification increases $\delta^{15}N$ and $\delta^{18}O$ in the residual nitrate at a $\delta^{15}N/\delta^{18}O$ ratio of 2:1 (Amberger and Schmidt, 1987; Voerkelius, 1990; Bottcher et al., 1990; Kendall, 1998). To assess denitrification effects it also is necessary to measure nitrate ^{15}N, which was not possible within the project budget. Significant denitrification rates in forest soils are generally limited to broad, floodplain soils where anoxic conditions are prevalent (Pinay and Decamps, 1988; Cooper et al., 1990; Schipper et al., 1993). The soil cores did not show any evidence of anoxic conditions such as gleying or mottling. Also, given the relatively narrow valley segments of the headwater streams in this study, denitrification rates probably were not significant.

In forest soils, low pH can inhibit the nitrifying potential of autotrophic bacteria (Alexander, 1977; Paul and Clark, 1996). Fungi can serve as important agents of nitrate production in acidic soils. It is possible that fungi utilize different oxygen sources than autotrophic bacteria, resulting in different nitrate

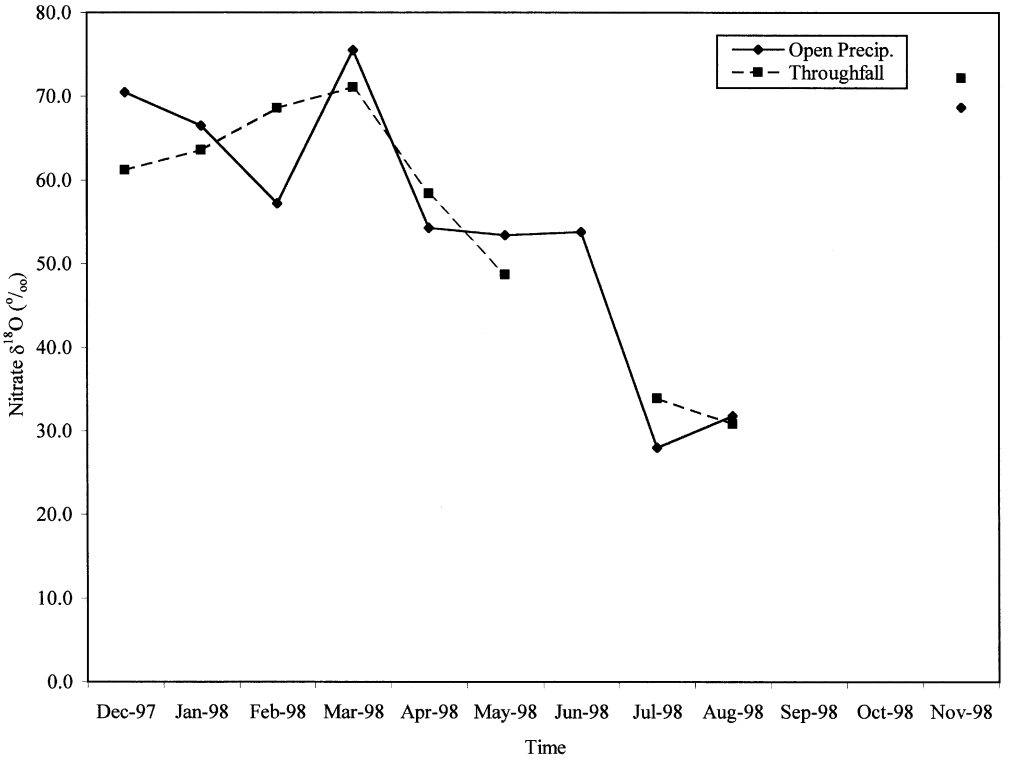


Fig. 3. Seasonal variation in nitrate $\delta^{18}O$ values for open precipitation and throughfall at Leading Ridge, PA.

$\delta^{18}O$ values. Oxygen sources in fungal nitrification have not been assessed, based on the literature. If fungal nitrification did result in different $\delta^{18}O$ values, one would expect those differences to be more pronounced in German forest soils, which generally are more acidic. However, incubations of German forest soils (Amberger and Schmidt, 1987; Voerkelius, 1990) yielded nitrate $\delta^{18}O$ values closest to the theoretical range of -2.2 to $+3.2$‰

Lastly, the elevated soil nitrate $\delta^{18}O$ values could be due to analytical interference by organic anions. To perform nitrate ^{18}O analysis, the nitrate must first be isolated on ion exchange columns and then eluted. High levels of dissolved organic anions can overload the exchange columns, which can potentially contaminate the nitrate elution step. Samples contaminated with organic anions show enriched $\delta^{18}O$ values. However, stream dissolved organic nitrogen (DON) concentration, an index to organic anion levels, was not correlated with $\delta^{18}O$ values of microbe-produced nitrate in either summer or winter. Therefore, it is difficult to establish a cause for the elevated soil nitrate $\delta^{18}O$ values observed in this study.

Soil nitrate $\delta^{18}O$ values were not significantly different among geology categories or land disturbance types. There was also no relationship between the dominant overstory vegetation types and soil nitrate $\delta^{18}O$. Therefore, the mean soil nitrate $\delta^{18}O$ value of 8.4 ± 3.8‰ was assigned to the 7 watersheds without soil nitrate $\delta^{18}O$ data to calculate the percent of atmospheric deposition in stream nitrate.

4.3. Stream nitrate $\delta^{18}O$

Stream nitrate $\delta^{18}O$ values were more similar to soil nitrate $\delta^{18}O$ values (Table 2) than to precipitation $\delta^{18}O$ values, indicating that most of the stream nitrate originated from microbe-produced nitrate. Source separation calculations yielded relatively low mean percentages of atmospheric deposition in March ($1.8 \pm 1.5\%$) and August ($8.2 \pm 3.9\%$) baseflow stream nitrate (Table 2).

Table 2
Soil and stream nitrate $\delta^{18}O$ values and the percentage of atmospheric deposition in stream nitrate for the 27 study watersheds. - Denotes that there was not enough nitrate for ^{18}O analysis

	Watershed	Soil Nitrate $\delta^{18}O$ (‰) August 98	Stream Nitrate $\delta^{18}O$ (‰) March 98	Stream Nitrate $\delta^{18}O$ (‰) August 98	% Atmos.[ab] Dep. in stream Nitrate — March 98	% Atmos. Dep. in stream Nitrate — August 98
1	E. Branch Pine Crk.	-	7.4	9.5	0.0	2.3
2	Jones Run	9.1	-	4.8	-	0.0
3	Baker Hollow	-	9.5	4.9	0.9	0.0
4	Jacob Run	12.1	5.7	0.6	0.0	0.0
5	Red Mill Run	8.3	8.7	14.2	0.8	12.5
6	West Branch	-	4.9	15.7	0.0	15.5
7	The Branch	-	7.8	-	0.0	-
8	Beech Bottom Run	3.5	-	14.0	-	20.2
9	East Fork Run	9.2	10.9	21.9	14.2	27.4
10	Bowl Run	0.2	4.2	15.3	0.0	27.3
11	Betsy Gap Run	-	4.5	13.1	7.8	10.0
12	Lick Run	-	10.9	21.0	5.3	26.8
13	Sweet Root Gap	-	6.8	12.0	0.0	7.6
14	Nedrow Run	4.5	10.9	11.4	1.8	13.5
15	Roaring Run	11.6	9.6	9.1	10.0	0.0
16	Deadlift Run	5.1	7.1	-	0.0	-
17	Clover Run	6.1	4.3	3.0	0.0	0.0
18	Fernow 4	10.4	4.2	3.8	0.0	0.0
19	Fernow 10	6.6	4.9	14.0	0.0	15.1
20	Bear Run	10.1	0.0	1.4	0.0	0.0
21	Little Bear Run	8.2	8.4	9.8	0.0	3.4
22	Freeland Run	12.5	8.8	9.3	1.3	0.0
23	Salamander Run	11.9	9.9	9.9	0.0	0.0
24	Karly Run	8.1	1.9	-	0.0	-
25	West Three Spring	3.1	3.4	10.3	0.6	13.7
26	Otter Run	13.7	-	14.6	-	2.2
27	Condon Run	13.6	9.2	11.2	0.0	0.0
				Mean =	1.8 ± 1.5	8.2 ± 3.9

[a] Individual watershed soil nitrate $\delta^{18}O$ values and the mean annual $\delta^{18}O$ value of throughfall (55.5‰) were used in the separation calculations.

[b] The mean soil nitrate $\delta^{18}O$ value of 8.4‰ was assigned to the seven watersheds without soil nitrate ^{18}O data.

Separation calculations utilized the individual watershed soil nitrate $\delta^{18}O$ values and the mean annual throughfall nitrate $\delta^{18}O$ value (55.5‰). Given the seasonal variation in throughfall $\delta^{18}O$, ideally growing and dormant season mean $\delta^{18}O$ values should be used. However, one would need to determine the residence time of precipitation in each watershed to correctly assign either a growing or dormant season $\delta^{18}O$ mean to each separation calculation. Residence time estimates for forested headwater streams of similar size within our study region range from 1 to >5 years (DeWalle et al., 1997; McGuire, 1999). Given this range, it would be ideal to measure the hydraulic residence time of each study watershed. Since measuring the residence time of precipitation in each watershed was not in the scope of this study, the mean annual value was utilized for all separations.

Fifteen of the watersheds sampled in March and ten of the watersheds sampled in August exhibited negative percentages of atmospheric deposition in stream nitrate (Table 2), because stream nitrate was more depleted in ^{18}O than soil nitrate. These negative percentages are denoted as zeros in Table 2.

High percentages of microbe-produced nitrate in stream baseflow nitrate can be explained by differences in the relative magnitude of atmospheric and microbial nitrate pools in forest ecosystems. On a

Table 3
Mean $\delta^{18}O$ values of soil and stream nitrate and percent atmospheric deposition in stream nitrate for the three geology categories

Parameter	PVA	CCP	MCG
Soil Nitrate $\delta^{18}O$ (‰)	8.9 a[a]	7.8 a	8.6 a
Winter Stream Nitrate $\delta^{18}O$ (‰)	8.1 a	5.7 a	7.6 a
Summer Stream Nitrate $\delta^{18}O$ (‰)	15.3 a	8.6 b	10.0 ab
Winter % Atmos. Dep. in Stream Nitrate	2.5 a	1.3 a	1.9 a
Summer % Atmos. Dep. in Stream Nitrate	13.0 a	7.4 a	5.1 a

[a] Parameter means with different letters within a row are significantly different at $\alpha = 0.05$ using Tukey's HSD mean separation procedure.

load basis, microbe-produced nitrate within mid-Appalachian forest ecosystems is much greater than atmospheric nitrate deposition. The mid-Appalachian region receives on average 7.70 kg ha^{-1} yr^{-1} of wet and dry nitrate-N deposition (Lynch et al., 1989–1994; U.S. Environmental Protection Agency 1995, and P. Edwards for Fernow-written comm.), while annual soil nitrate-N production rates were estimated to be 70 kg ha^{-1} yr^{-1} at Fernow 4 (Gilliam et al., 1996). Even though Fernow 4 has relatively high soil nitrification rates compared to other watersheds (Williard, 1999), the soil nitrate production rates in most watersheds should be substantially higher than atmospheric nitrate deposition rates. Given the great difference in nitrate loads between the two sources, the atmospheric nitrate ^{18}O signal could be overwhelmed by the ^{18}O signature of microbial nitrate.

Stream nitrate $\delta^{18}O$ values ranged from 0.0 to 10.9‰ in winter and 0.6–21.9‰ in summer. The variation in winter stream nitrate $\delta^{18}O$ was not related to geology, land disturbance history, or stand age. However, the variation in summer stream nitrate $\delta^{18}O$ was related to differences in geology.

In summer, PVA geologic category had a greater mean stream nitrate $\delta^{18}O$ value (15.3‰) than MCG (10.0‰) and CCP geologic categories (8.6‰*) (* denotes significant difference at $\alpha = 0.05$) (Table 3). This resulted in a greater calculated, mean percentage of atmospheric deposition in summer stream nitrate for the PVA geologic category compared to the two other geologic categories (Table 3). Higher percentages of atmospheric deposition in stream nitrate in the PVA geology watersheds may be due to lower soil N mineralization rates in the relatively acidic soils (Williard, 1999). Greater N mineralization rates in the soils overlying CCP and MCG could be overwhelming the atmospheric nitrate $\delta^{18}O$ signal in these watersheds, as discussed earlier. Greater percentages of atmospheric deposition in stream nitrate from PVA watersheds also may be due to reduced uptake by slower growing vegetation on the more acidic soils of PVA watersheds. It also could be a function of more shallow flow paths on these watersheds, providing more rapid delivery of atmospheric nitrate to the streams.

4.4. Intensive watershed study of stream nitrate $\delta^{18}O$

4.4.1. Baseflow vs. stormflow nitrate $\delta^{18}O$

Six watersheds in West Virginia were chosen for more intensive sampling of stream nitrate $\delta^{18}O$ at baseflow and stormflow conditions. All six watersheds exhibited greater mean nitrate $\delta^{18}O$ values during stormflow periods than baseflow, however, flow regime $\delta^{18}O$ differences were significant in only two of the watersheds (Table 4).

The mean annual throughfall nitrate $\delta^{18}O$ value (55.5‰) and Voerkelius (1990) mean soil nitrate $\delta^{18}O$ value (3.3‰) were used in the separation of baseflow nitrate sources (Table 5). Monthly throughfall nitrate $\delta^{18}O$ values and Voerkelius (1990) mean soil nitrate $\delta^{18}O$ value were used in the separation of stormflow nitrate sources (Table 5). Voerkelius (1990) mean soil nitrate $\delta^{18}O$ value was used in the separation calculations instead of our measured values to better depict the relative difference between baseflow and stormflow periods. Our measured values would have resulted in zero percent contributions for all baseflow and stormflow sampling dates in three of the watersheds (Fernow 4, Salamander Run, and Karly Run).

Even though atmospheric nitrate deposition contributions were not statistically different between stormflow and baseflow, the mean values were greater during stormflow (Table 5). The greater importance of atmospheric nitrate deposition during stormflow was expected, given the relatively direct contributions of nitrate deposition by channel interception and

Table 4
Stream nitrate $\delta^{18}O$ values (‰) during baseflow and stormflow on six West Virginia forested watersheds

Sampling Dates	Fernow 4	Fernow 10	Otter Run	Salamander Run	W. Three Spring	Karly Run
Baseflow						
March 12–13, 1998	4.2	4.9	–	9.9	3.4	1.9
May 18–19, 1998	4.1	1.7	4.6	7.1	3.9	1.0
June 1–3, 1998	3.6	4.2	2.1	5.9	4.6	3.4
August 3–5, 1998	3.8	14.0	14.6	9.9	10.3	–
Mean	3.9 A[a]	6.2 A	7.1 **A**	8.2 A	5.6 A	2.1 A
Stormflow						
April 9–10, 1998	3.3	2.6	11.3	9.8	6.1	3.4
June 29–30, 1998	8.6	11.7	14.3	11.9	11.2	4.8
May 14, 1999	4.8	7.1	9.6	11.7	8.6	6.4
Mean	5.6 A	7.1 A	11.7 A	11.1 B	8.6 A	4.9 B

[a] Baseflow and stormflow means for each stream with different letters were significantly different at $\alpha = 0.05$ using a two-sample *t*-test.

shallow interflow mechanisms during stormflow. However, seasonal variations of atmospheric nitrate $\delta^{18}O$ values in baseflow may be confounding the results somewhat.

Mean percentage contributions of atmospheric nitrate to stream nitrate during stormflow periods ranged from 3.4 to 17.4% (Table 5). These percentages could be explained by channel precipitation inputs alone. Crayosky et al. (1999) found direct channel precipitation constituted an average of 19% of streamflow during six events in a mid-Appalachian headwater catchment.

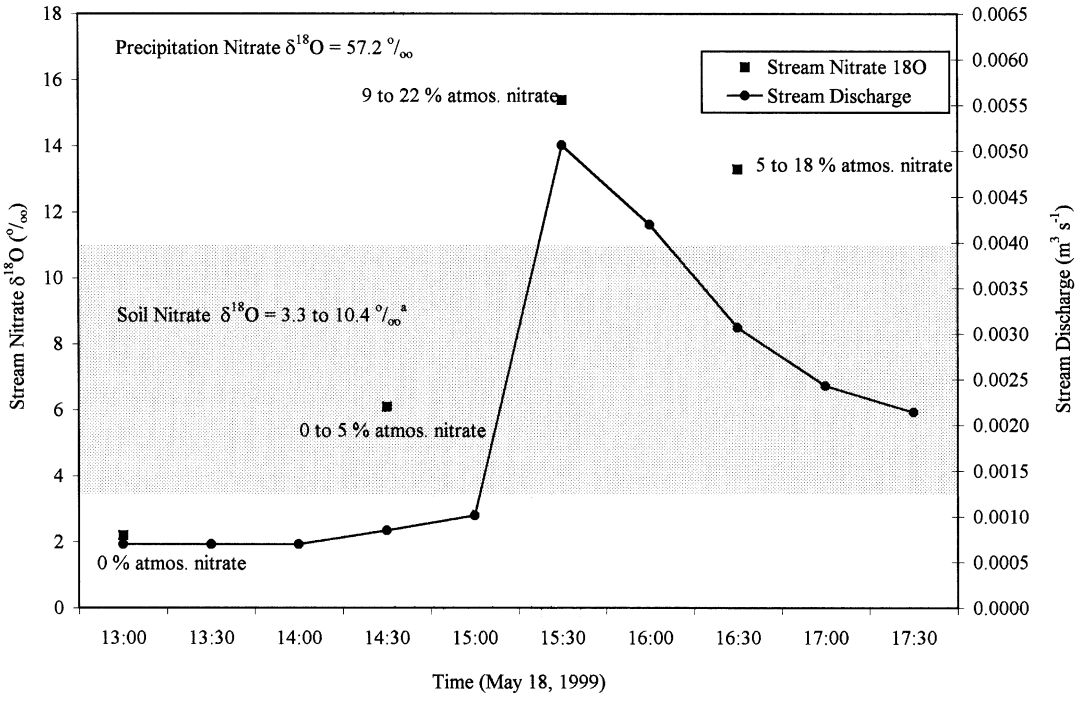
4.4.2. $\delta^{18}O$ variations within a storm event

On Fernow watershed 4, stream samples were collected on the rising limb, peak, and falling limb of the hydrograph resulting from a 2.06 cm rain event. Nitrate $\delta^{18}O$ essentially tracked the stormflow hydrograph with the peak sample exhibiting the highest $\delta^{18}O$ value (Fig. 4), indicating stream water was carrying the greatest percentage of dissolved atmospheric nitrate (9–22%) during the highest flow. This agrees with the N flushing hypothesis proposed by Hornberger et al. (1994) and supported by Boyer et al. (1995) and Creed et al. (1996), where accumulated

Table 5
Percentage contribution of atmospheric deposition to stream nitrate from six West Virginia forested watersheds. (The mean annual throughfall nitrate $\delta^{18}O$ value (55.5‰) and Voerkelius (1990) mean soil nitrate $\delta^{18}O$ value (3.3‰) were used in the separation of baseflow nitrate sources; Monthly throughfall nitrate $\delta^{18}O$ values and Voerkelius (1990) mean soil nitrate $\delta^{18}O$ value were used in the separation of stormflow nitrate sources)

Sampling dates	Fernow 4	Fernow 10	Otter run	Salamander run	W. Three spring	Karly run
Baseflow						
March 12–13, 1998	1.7	3.1	–	12.6	0.2	0.0
May 18–19, 1998	1.5	0.0	2.5	7.3	1.1	0.0
June 1–3, 1998	0.6	1.7	0.0	5.0	2.5	0.2
August 3–5, 1998	1.0	20.5	21.6	12.6	13.4	–
Mean	1.2 A[a]	6.3 A	8.0 A	9.4 A	4.3 A	0.1 A
Stormflow						
April 9–10, 1998	0.0	0.0	14.9	12.1	5.2	0.2
June 29–30, 1998	11.2	17.8	23.3	18.2	16.7	3.2
May 14, 1999	3.3	8.4	13.9	18.5	11.7	6.8
Mean	4.8 A	8.7 **A**	17.4 A	16.3 A	11.2 A	3.4 A

[a] Baseflow and stormflow means were compared for each watershed independently. Means with different letters within a watershed were significantly different at $\alpha = 0.05$ using a two-sample *t*-test.



[a]3.3 °/oo and 10.4 °/oo correspond to Voerkelius (1990) and our measured soil nitrate ^{18}O values, respectively.

Fig. 4. Stream nitrate $\delta^{18}O$ values during a spring storm event on Fernow watershed 4.

nitrate in the upper soil horizons, including atmospheric nitrate, is flushed to the stream by a rising water table during a storm event. Elevated atmospheric nitrate during peak stream discharge may also be supported by rapid macropore flow of precipitation through the soil matrix, provided that atmospheric nitrate is not immobilized by microbes in the organic and upper mineral horizons (Hill et al., 1999).

Separation calculations based on Voerkelius (1990) soil nitrate $\delta^{18}O$ value better represent expected atmospheric contributions during the rising limb of the hydrograph. During initial storm response, atmospheric nitrate contributions should be detectable due to direct channel precipitation inputs. However, microbe-produced nitrate remained the dominant source of stream nitrate over the entire duration of the storm hydrograph using either our or Voerkelius (1990) soil nitrate $\delta^{18}O$ value (Fig. 4).

The stream nitrate $\delta^{18}O$ value from Walnut Springs storm sewer (51.4‰) was similar to the atmospheric nitrate $\delta^{18}O$ value (49.7‰). This finding supports the conservation assumption underlying the separation method; that is, little fractionation occurs with minimal nitrate contributions from other sources. However, most urban stormwater does not pass through soil so this check does not completely validate the methodology.

5. Conclusions

In addressing study objective 1, seasonal trends in precipitation (throughfall and open precipitation) nitrate $\delta^{18}O$ values were found at both Leading Ridge, PA and Fernow, WV. More enriched $\delta^{18}O$ values occurred in winter than summer. In satisfying study objective 2, throughfall nitrate $\delta^{18}O$ values were found to be not significantly different from open precipitation, suggesting that canopy wash-off of dry deposition or biological exchange on plant surfaces does not significantly

alter the nitrate ^{18}O signature. Neither throughfall nor open precipitation $\delta^{18}O$ values differed significantly between Leading Ridge, PA and Fernow, WV, suggesting that throughfall and open precipitation $\delta^{18}O$ values were relatively uniform across the mid-Appalachian region.

Stream nitrate $\delta^{18}O$ values were not strongly related to any differences in land disturbance history or stand age. However, variation in summer stream nitrate $\delta^{18}O$ values was related to differences in bedrock geology. Pottsville/Allegheny geology watersheds had significantly higher stream nitrate $\delta^{18}O$ values than the other two geology types, likely due to inhibition of soil N mineralization rates and reduced plant uptake of N in the associated acidic soils. Stream nitrate $\delta^{18}O$ values were generally higher during storm flow than baseflow, indicating that atmospheric nitrate was a greater contributor to stream nitrate during storm events.

The ability to use nitrate ^{18}O to precisely separate sources of stream nitrate is questionable due to the variability of $\delta^{18}O$ values in both microbe-produced nitrate (study objective 3) and atmospheric nitrate. Variability in microbe-produced nitrate $\delta^{18}O$ values could be caused by differences in soil water $\delta^{18}O$ values and/or different proportions of nitrifying organisms (bacteria/fungi) among watersheds. It is not possible to apply the observed seasonal variability in atmospheric $\delta^{18}O$ to baseflow source separation calculations without determining the hydraulic residence time of groundwater in a watershed.

Even with the aforementioned uncertainty, $>70\%$ of nitrate in these mid-Appalachian forested streams was microbial in origin (study objective 4). High rates of microbial nitrate production help explain these significant contributions. However, atmospheric nitrogen deposition remains an important factor in nitrate leaching to forested streams, because it is the most significant external input of nitrogen to forest soils, and thereby, helps control microbial nitrate production. Stream nitrate responses to changes in atmospheric deposition will likely be delayed rather than direct, because the majority of atmospheric nitrogen deposition will first be assimilated into the forest nitrogen cycle before it contributes to stream nitrate.

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