

# Whole-system Estimates of Nitrification and Nitrate Uptake in Streams of the Hubbard Brook Experimental Forest

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## ABSTRACT

Although they drain remarkably similar forest types, streams of the Hubbard Brook Experimental Forest (HBEF) vary widely in their  $\text{NO}_3^-$  concentrations during the growing season. This variation may be caused by differences in the terrestrial systems they drain (for example, varying forest age or composition, hydrology, soil organic matter content, and so on) and/or by differences between the streams themselves (for example, contrasting geomorphology, biotic nitrogen [N] demand, rates of instream nitrogen transformations). We examined interstream variation in N processing by measuring  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake and estimating nitrification rates for 13 stream reaches in the HBEF during the summers of 1998 and 1999. We modeled nitrification rates using a best-fit model of the downstream change in  $\text{NO}_3^-$  concentrations following short-term  $\text{NH}_4^+$  enrichments. Among the surveyed streams, the fraction of  $\text{NH}_4^+$  uptake that was subsequently nitrified varied, and this variation was positively correlated with ambient streamwater  $\text{NO}_3^-$  concentrations. We examined whether this

variation in instream nitrification rates contributed significantly to the observed variation in  $\text{NO}_3^-$  concentrations across streams. In some cases, instream nitrification provided a substantial portion of instream  $\text{NO}_3^-$  demand. However, because there was also substantial instream  $\text{NO}_3^-$  uptake, the net effect of instream processing was to reduce rather than supplement the total amount of  $\text{NO}_3^-$  exported from a watershed. Thus, instream rates of nitrification in conjunction with instream  $\text{NO}_3^-$  uptake were too low to account for the wide range of streamwater  $\text{NO}_3^-$ . The relationship between streamwater  $\text{NO}_3^-$  concentration and rates of instream nitrification may instead be due to a shift in the competitive balance between heterotrophic N uptake and nitrification when external inputs of  $\text{NO}_3^-$  are relatively high.

**Key words:** ammonium; nitrate; nitrification; streams; Hubbard Brook Experimental Forest; nutrient cycling; nutrient uptake.

## INTRODUCTION

Nitrogen (N) cycling in forests has received increased attention during the past several decades as

scientists and managers recognize the impacts of environmental pollution on the water quality of downstream ecosystems as well as the health of the terrestrial forest (Aber and others 1989, 1998; Vitousek and others 1997). Much of this research has focused on understanding mechanisms that lead to losses of  $\text{NO}_3^-$  from forests due to disturbance (Bormann and others 1974; Likens and Bormann 1974).

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Mass balances of  $\text{NO}_3^-$  are calculated by comparing streamwater output to precipitation inputs to determine whether the terrestrial system is retaining or losing N (Bormann and Likens 1967). Studies are then done of forest vegetation and forest soils to understand what factors are causing  $\text{NO}_3^-$  leaching.

Typically, instream processing is ignored as a potentially important component in determining watershed outputs. Instream conversion, uptake, and release of N could significantly affect interpretations about terrestrial processes and mass balances for watershed ecosystems made from samples taken at gauging weirs. There is still much to be learned about the mechanisms by which N is retained during downstream transport. However, recent studies have shown that despite their relatively small proportion of watershed surface area, streams can be important sites for transformations and retention of nutrients (Fisher and others 1998; Alexander and others 2000; Peterson and others 2001). In streams of the Hubbard Brook Experimental Forest (HBEF), this function has been demonstrated for phosphorus (P) (Meyer 1979; Meyer and Likens 1979), carbon (Fisher and Likens 1972; McDowell 1985), N (Richey and others 1985; Steinhart and others 2001),  $\text{H}^+$  (Hall and others 1987; Hedin and others 1990), and calcium (Ca) (Hall and others 2001).

Streams of the HBEF vary widely in concentrations of  $\text{NO}_3^-$ . As in most temperate streams, there is substantial seasonal variation in  $\text{NO}_3^-$  concentrations within individual streams (Likens and Bormann 1995). There is also considerable spatial variation in  $\text{NO}_3^-$  concentration, both within and among streams draining remarkably similar watersheds (Johnson and others 1969; Sloane 1979; Likens and Bormann 1995). This variation in  $\text{NO}_3^-$  concentration among watersheds is probably due to a number of factors, including differences in (a) land-use history (Vitousek and Reiners 1975; Aber and others 1989; Reynolds and others 1994), (b) forest composition (Lewis and Likens 2000; Lovett and others 2000), (c) watershed geology (Holloway and others 1998), or (d) the hydrology of the watersheds (Burns and others 1998). This variation in streamwater nutrient concentrations could also be due to differences between the streams themselves, such as (e) stream geomorphology (Valett and others 1996), or (f) instream N sorption or transformations (Triska and others 1994; Jones and Holmes 1996; Fisher and others 1998). Instream N transformations, processes by which N is converted among its various inorganic oxidation states, or between organic and inorganic, dissolved or particulate forms are important in many stream ecosystems (Richey and others 1985; Grimm 1987; Steinhart

and others 2001; Triska and others 1989; Jones and others 1995; Holmes and others 1996).

Nitrification is a critical transformation in the nitrogen cycle of upland soils in that it is a prerequisite for downstream losses of  $\text{NO}_3^-$  in solution, especially following disturbance to forest vegetation (Likens and others 1969; Rosswall 1982). Bohlen and others (2001) found an elevational gradient in soil nitrification that they suggested could account for elevational variation in  $\text{NO}_3^-$  export by streams. Nitrifiers fix carbon dioxide ( $\text{CO}_2$ ) from the energy gained by oxidizing the easily bound cation  $\text{NH}_4^+$  to the mobile anion  $\text{NO}_3^-$ . This transformation can reduce the capacity of a system to retain N within forest soils or stream sediments. The production of  $\text{NO}_3^-$  is also important in cases where nitrification and denitrification are closely linked (Focht and Verstraete 1977; Cooke and White 1987). Especially when inputs of  $\text{NO}_3^-$  to stream ecosystems are low (that is, growing-season levels at the HBEF) (Likens and Bormann 1995), nitrification within stream sediments can potentially become the dominant source of  $\text{NO}_3^-$  to denitrifiers (Duff and Triska 2000). We currently have a poor understanding of the factors controlling nitrification in freshwater ecosystems.

Previous work at HBEF measured high nitrification potential in one study where  $\text{NH}_4^+$  was added to an entire stream and in several laboratory studies (Sloane 1979; Richey and others 1985). We measured rates of whole-stream  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake and nitrification in 13 stream reaches within the HBEF and the surrounding White Mountains National Forest during June and July of 1998 and 1999. The goal of this study was to examine the variation in instream  $\text{NO}_3^-$  concentrations and  $\text{NO}_3^-$  production and consumption among and within streams, in an effort to quantify to what degree instream nitrification can control stream transport and watershed exports of inorganic N.

## METHODS

### Study Sites

We measured nitrification in streams throughout the Hubbard Brook valley and nearby White Mountain National Forest. The HBEF is located in the White Mountains of New Hampshire, USA (43°56'N, 75°45'W). The climate is humid continental with about one-third of the annual precipitation as snow. The basin is forested primarily by American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*), with red spruce (*Picea rubens*), balsam fir

(*Abies balsamea*), and white birch (*Betula papyrifera*) at higher elevations (Likens and Bormann 1995). Within the HBEF, all six of the south-facing experimental watersheds were included in this survey, as were Hubbard Brook and three of its larger tributaries: Bear, Paradise, and Cascade brooks. We performed repeated measurements of  $\text{NO}_3^-$  uptake and nitrification in the streams draining watersheds (W) 3 and 6 (seven dates in the W3 stream, 11 dates in the W6 stream). Outside the HBEF, we also sampled the West Inlet to adjacent Mirror Lake and the outlet of nearby Cone Pond. All streams used for this survey have an extensive biogeochemical record at HBEF. We used the same stream reaches as those described in Hall and others (2002). They are all oligotrophic, heavily shaded, saturated with dissolved oxygen (O), and with a pH of 6.5 or less (Likens and Bormann 1995).

### Field Methods

From June 1998 to November 1999, we estimated nitrification by measuring  $\text{NO}_3^-$  produced during short-term injections of  $\text{NH}_4\text{Cl}$  into each stream. Ammonium chloride was added to increase stream  $\text{NH}_4^+$  concentrations by 15–50  $\mu\text{g/L}$  above background  $\text{NH}_4^+$  concentrations (approximately 1–5  $\mu\text{g N/L}$ ). This level of enrichment was chosen as the minimum level of enrichment at which we could reliably detect any downstream differences in  $\text{NO}_3^-$  concentrations.

Within each stream, we delineated study reaches that would allow at least 1 h of travel time between the upstream and downstream sampling points. We did not include culverts or gauging weirs within any of these reaches. The upstream addition site was chosen to facilitate quick mixing of our solution into the stream. In small streams, we selected waterfalls that accounted for more than 75% of the total streamflow; in large streams, we added solution to constrained points in the streambed. Average stream-wetted width was measured at 10 sites within each reach.

In each stream, discharge was measured either from a gauging weir immediately upstream or downstream of the study reach, or by mass balance of short-term  $\text{NaCl}$  solution releases. Before initiating the addition, three to five background samples were collected throughout the study reach to determine ambient concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , Cl, and Br. Nutrient releases were done slightly differently in 1999 than in 1998. In 1998, we added a solution of both  $\text{NaNO}_3$  and  $\text{NH}_4\text{Cl}$ , along with an  $\text{NaCl}$  hydrologic tracer (target concentration increases in streamwater were 50  $\mu\text{g NO}_3\text{-N}$ , 15  $\mu\text{g NH}_4\text{-N}$ , 5–7  $\text{mg/L Cl}$  above background levels). In

1999, we did two separate releases. First, we released  $\text{NH}_4\text{Cl}$  and  $\text{NaCl}$ ; this release was immediately followed by a second release of  $\text{NaNO}_3$  along with  $\text{NaBr}$  (target concentration increase in streamwater for Br was 50  $\mu\text{g/L}$ ). We changed methods because we had difficulty calculating  $\text{NO}_3^-$  uptake in streams where nitrification rates were high when the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  releases were done simultaneously. This problem led us to underestimate  $\text{NO}_3^-$  uptake in many of the 1998 releases. In both years, solutions were added to the stream using a Watson-Marlow peristaltic pump that allows fine-scale adjustment of the addition rate. The downstream change in conductivity due to the added  $\text{NaCl}$  was monitored until conductivity reached a plateau, (Stream Solute Workshop 1990). At plateau eight to 10 samples were taken throughout the entire reach at measured intervals. In 1999, after sampling the first  $\text{NH}_4^+$  and Cl release, we initiated the  $\text{NO}_3^-$  and Br release. A second set of samples was then collected after the same time to plateau calculated for the  $\text{NH}_4^+$  and Cl release. All samples were maintained at 4°C and analyzed within 24 h for  $\text{NH}_4^+$  or within 1 week for  $\text{NO}_3^-$ , Cl, and Br.

We surveyed 10 streams in June/July 1998 and seven streams in June 1999. We also performed repeated measurements of nutrient uptake and nitrification in two streams (W3 and W6) on eight occasions throughout 1998 and 1999.

### Laboratory Methods

Unfiltered  $\text{NH}_4^+$  samples were analyzed by hand using the phenol-hypochlorite technique with a 10-cm cell (Solorzano 1969). We measured concentrations of  $\text{NO}_3\text{-N}$ , Cl, and Br for each sample using a DIONEX 500 Ion Chromatograph with an AS4A anion column. These samples were filtered in the field using Gelman A/E glass fiber filters. The lowest standards detectable with these analytical techniques were typically 2  $\mu\text{g/L NH}_4\text{-N}$ , 2  $\mu\text{g/L NO}_3\text{-N}$ , and 5  $\mu\text{g/L Cl}$  and Br.

### Data Analysis

Ammonium and  $\text{NO}_3^-$  uptake lengths were calculated for each release by calculating the slope of the line relating  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations to downstream distance using the linear form of the following exponential uptake equations:

$$\ln A_x = \ln A_0 - k_A X \quad (1)$$

$$\ln N_x = \ln N_0 - k_{\text{NO}_3} X \quad (2)$$

where  $A_x$  and  $N_x$  are  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations ( $x$ ) meters downstream from the addition site

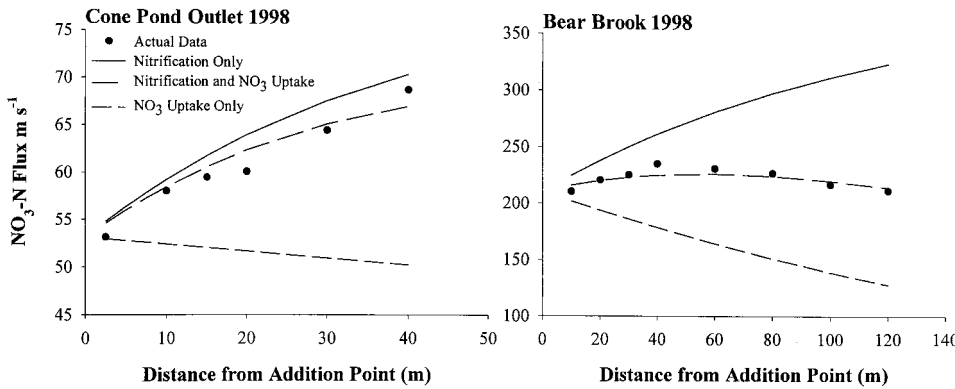


Figure 1. Two examples of the best-fit two-compartment model for NO<sub>3</sub><sup>-</sup> flux for estimating nitrification rates in each stream reach. The actual data are shown as (●), with lines representing the estimated fluxes determined by the model with NO<sub>3</sub><sup>-</sup> uptake only, nitrification only, and with both NO<sub>3</sub><sup>-</sup> uptake and nitrification.

(0 m), A<sub>0</sub>/N<sub>0</sub> are NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> concentrations at the addition site, and k<sub>A</sub>, k<sub>NO</sub> is the per meter uptake rate of NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> (Newbold and others 1981). The uptake length of NH<sub>4</sub><sup>+</sup> (S<sub>NH4</sub>) or NO<sub>3</sub><sup>-</sup> (S<sub>NO3</sub>) is calculated as k<sup>-1</sup> and is equivalent to the average downstream distance traveled by an N atom prior to removal from the water column. To correct for groundwater dilution, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were divided by the concentration of Cl or Br, our conservative hydrologic tracers (Webster and Ehrman 1996).

Per square meter uptake rates (U) of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (μg N m<sup>-2</sup> s<sup>-1</sup>) were calculated using the following equations:

$$U_{NH4} = (Q \cdot A_{bkg}) / S_{NH4} \cdot w \quad (3)$$

$$U_{NO3} = (Q \cdot N_{bkg}) / (S_{NO3} \cdot w) \quad (4)$$

where Q is stream discharge (m<sup>3</sup>/s), A<sub>bkg</sub> and N<sub>bkg</sub> are background NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations in μg/m<sup>3</sup>, and w is stream width (Newbold and others 1981).

To compare uptake among streams, we calculated the uptake velocity of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. The uptake velocity (V<sub>f</sub>) (m/s), also called the “mass transfer coefficient” (Stream Solute Workshop 1990), is calculated as follows:

$$V_{INH4} = (Q/w) / S_{NH4} \quad (5)$$

$$V_{INO3} = (Q/w) / S_{NO3} \quad (6)$$

Uptake velocities allow the comparison of uptake among streams that differ in depth and velocity, which are important determinants of solute uptake length in HBEF streams (Hall and others 2002).

The fraction of NH<sub>4</sub><sup>+</sup> that was immediately nitrified was determined by fitting a two-compartment model (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> flux) to the longitudinal profile of NO<sub>3</sub><sup>-</sup> concentration, which is both in-

creasing through nitrification and decreasing due to NO<sub>3</sub><sup>-</sup> uptake (Mulholland and others 2000). The model describes the change in NO<sub>3</sub><sup>-</sup> flux (N<sub>x</sub>·Q μg/s) over distance x as:

$$d(N_x)/dx = k_{NIT}(Q \cdot A_0)e^{(-k_A)x} - k_N N_{Ox} \quad (7)$$

where k<sub>NIT</sub> is the nitrification rate and k<sub>N</sub> is the NO<sub>3</sub><sup>-</sup> uptake rate per unit distance, or the inverse of the NO<sub>3</sub><sup>-</sup> uptake length (1/S<sub>NO3</sub> m<sup>-1</sup>). Solving for N<sub>x</sub> yields:

$$N_x = [(k_{NIT}(Q \cdot A_0)/k_N - k_A) \cdot (e^{-k_A x} - e^{-k_N x})] + (Q \cdot N_0)e^{-k_N x} \quad (8)$$

The values of k<sub>NIT</sub> (nitrification rate, in units of m<sup>-1</sup>) were estimated by fitting the model to the longitudinal NO<sub>3</sub><sup>-</sup> flux. K<sub>n</sub> and K<sub>NIT</sub> were systematically varied to minimize the sums of squares between model and data using a least-squares fitting procedure (MS Excel Solver) (Figure 1). The NO<sub>3</sub><sup>-</sup> flux value was corrected for background concentration and the concentration of the tracer so that the model only considered change in NO<sub>3</sub><sup>-</sup> concentration over background and corrected for dilution. In situations where our enrichment-estimated NO<sub>3</sub><sup>-</sup> uptake lengths were extremely long (more than 10× reach length) and the model fits were extremely poor, we allowed the model to estimate k<sub>N</sub> as well as k<sub>NIT</sub>. For a detailed description of this model, see Mulholland and others (2000). Our approach differs somewhat from that of Mulholland and others (2000) in that they are modeling the flux of <sup>15</sup>N, added as NH<sub>4</sub><sup>+</sup>, through the NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> pools. Because our approach involved multiple streams within the same valley and repeated measurements of the same streams, the use of an isotopic tracer was impractical.

The output from this model calculated the fraction of the added NH<sub>4</sub><sup>+</sup> that was nitrified. To cal-

**Table 1.** Fraction of  $\text{NH}_4^+$  Uptake Nitrified, Modeled Nitrification Rates, Ambient  $\text{NO}_3\text{-N}$  Concentrations, and  $\text{NO}_3^-$  Uptake Velocities for the HBEF Stream Survey

Stream Reach	Release Date	$k_{\text{NIT}}/k_{\text{A}}$	$\text{NO}_3\text{-N}$ ( $\mu\text{g/L}$ )	$V_{\text{NO}_3}$ ( $\text{mm min}^{-1}$ )
Watershed 6-1	6/29/98	0.02	3	0.08
Watershed 3	6/30/98	0.547	7.2	0.04
Bear Brook	7/10/98	0.716	18.4	1.29
Watershed 2	7/14/98	0.46	4	0.21
Paradise Brook	7/16/98	1	58.5	0.02
Cone Pond Outlet	7/17/98	1	46	0.11
Watershed 5	7/21/98	0.05	2	0.91
Hubbard Brook	7/22/98	0.651	40	0.44
Watershed 4	7/28/98	1	305	1.63
West Inlet	7/31/98	0.514	2	0.85
Paradise Brook	6/14/99	1	315	1.21
West Inlet	6/16/99	0.051	8	n.d.
Watershed 3	6/17/99	1	76	1.40
Watershed 1	6/18/99	0.144	14	n.d.
Watershed 6-1	6/21/99	0.587	26.6	0.58
Watershed 6-2	6/21/99	1	38.1	0.30
Bear Brook	6/22/99	0.439	61.7	0.21
Cascade Brook	6/23/99	0.118	4.1	0.33
Hubbard Brook	6/25/99	0.441	115.4	0.02

*n.d., no data*

$\text{NH}_4^+$  uptake velocities can be found in Hall and others (2002). Because streamwater  $\text{NH}_4^+$  concentrations were at or near our detection limit, we do not report those data here, although they are used in calculating our nitrification estimates.

culate a nitrification rate ( $\mu\text{g m}^{-2} \text{h}^{-1}$ ), we used the following equation:

$$\text{Nitrification rate} = (k_{\text{NIT}}/k_{\text{A}}) \cdot U_{\text{NH}_4} \quad (9)$$

To estimate how much  $\text{NO}_3^-$  could be formed or consumed in each of the south-facing watersheds, we multiplied the nitrification rate and the  $\text{NO}_3^-$  uptake rate by the area of stream channel upstream of the weir. This allowed us to compare instream production of  $\text{NO}_3^-$  to instream  $\text{NO}_3^-$  uptake and also to compare these rates to the amount of  $\text{NO}_3^-$  lost over the weir over the same time period ( $Q_{\text{weir}} \cdot [\text{NO}_3\text{-N}]_{\text{weir}}$ ).

Relationships between  $\text{NO}_3^-$  concentrations and  $k_{\text{NIT}}/k_{\text{A}}$  (% of  $\text{NH}_4^+$  uptake nitrified) and  $\text{NH}_4^+$  uptake rates were estimated with correlation and regression analyses using the statistical package SYSTAT 4.1. We ran each nitrification model with  $k_{\text{NIT}}$  set at 0%, 25%, 50%, 75%, and 100% of  $k_{\text{A}}$  ( $\text{NH}_4^+$  uptake) as a comparison against the Solver-derived estimate. In all cases, the sums of squares at the estimated  $k_{\text{n}}$  were less than half those determined for the  $k_{\text{n}}$  at the closest quartile, giving us confidence that our % nitrification estimates were within 25% of the actual proportion of  $\text{NH}_4^+$  nitrified.

## RESULTS

### Ammonium, Nitrate, and Nitrification Rates

Ammonium concentrations in HBEF streams were always low ( $<5 \mu\text{g/L}$   $\text{NH}_4\text{-N}$ ). Ammonium uptake lengths varied between 1.4 and 270 m, and uptake velocities varied between 0.83 and 10.8  $\text{mm min}^{-1}$ . Ammonium uptake rate estimates varied between 0.32 and 716  $\text{mg N m}^{-2} \text{d}^{-1}$ . These data are presented in Hall and others (2002).

Nitrate concentrations varied much more widely than  $\text{NH}_4^+$  concentrations, ranging between less than 2 and approximately 300  $\mu\text{g/L}$   $\text{NO}_3\text{-N}$  across all streams sampled (Table 1). Nitrate uptake velocities were lower and less variable than  $\text{NH}_4^+$  uptake velocities, falling between 0.02 and 1.7  $\text{mm min}^{-1}$  (Table 1). Nitrate uptake rates varied between 0.3 and 716  $\text{mg N m}^{-2} \text{d}^{-1}$  (Table 2).

Between 0 and 100% of the  $\text{NH}_4^+$  added during our short-term enrichments was immediately nitrified, showing that some streams nitrified all additional  $\text{NH}_4^+$  whereas others nitrified none (Table 1). This high variability in  $k_{\text{NIT}}/k_{\text{A}}$  (% of  $\text{NH}_4^+$  uptake nitrified) caused high variability in modeled nitrification rates that varied between 0 and 18  $\text{mg N m}^{-2} \text{d}^{-1}$  across all streams surveyed (Table 2).

**Table 2.** A Direct Comparison of Instream Areal  $\text{NO}_3^-$  Uptake Rates and Nitrate Production Rates for all HBEF Survey Streams

Stream Reach	Release Date	$\text{NO}_3^-$ Uptake ( $\text{mg m}^{-2} \text{d}^{-1}$ )		Nitrification ( $\text{mg m}^{-2} \text{d}^{-1}$ )
Watershed 6	6/29/98	0.32	<	1.13
Watershed 3	6/30/98	0.40	<	0.67
Bear Brook	7/10/98	34.30	>	11.95
Watershed 2	7/14/98	1.19	<	8.85
Paradise Brook	7/16/98	1.54	<	4.55
Cone Pond Outlet	7/17/98	7.40	<	10.14
Watershed 5	7/21/98	2.63	>	0.23
Hubbard Brook	7/22/98	25.54	>	17.76
Watershed 4	7/28/98	715.97	>	13.69
West Inlet	7/31/98	2.45	>	0.00
Paradise Brook	6/14/99	268.9	>	2.35
West Inlet <sup>a</sup>	6/16/99	n.d.		2.18
Watershed 3	6/17/99	153.70	>	5.16
Watershed 1 <sup>a</sup>	6/18/99	n.d.		1.29
Watershed 6-1	6/21/99	22.26	>	7.85
Watershed 6-2	6/21/99	16.52	>	2.20
Bear Brook	6/22/99	18.48	>	4.19
Cascade Brook	6/23/99	1.96	>	1.26
Hubbard Brook	6/25/99	3.19	>	1.29

*n.d.*, no data

<sup>a</sup> $\text{NO}_3^-$  uptake was not measured for the West Inlet and watershed 1 in June 1999.

**Table 3.** Budgets for South-facing Watersheds of HBEF Comparing Instream Production and Consumption to  $\text{NO}_3^-$  Flux over the Weir

Watershed	Year	Estimated Nitrification in 750 m <sup>2</sup> ( $\text{mg d}^{-1}$ )	Estimated $\text{NO}_3^-$ Uptake in 750 m <sup>2</sup> ( $\text{mg d}^{-1}$ )	Net Change in $\text{NO}_3\text{-N}$ ( $\text{mg d}^{-1}$ )	Is	$\text{NO}_3\text{-N}$ Flux at weir ( $\text{mg d}^{-1}$ )
Watershed 6	1998	848	244	608	<	1477
Watershed 3	1998	503	297	206	<	9456
Watershed 2 <sup>a</sup>	1998	6638	896	5741	>	294
Watershed 5	1998	173	1974	-1801	<	275
Watershed 4	1998	10,268	536,974	-526,707	<	16,865
Watershed 3	1999	3870	115,276	-111,406	<	53,385
Watershed 6	1999	5888	16,698	-10,811	<	3264

We assumed a stream reach area of 750 m<sup>2</sup>.

<sup>a</sup>In only one case was the net effect of instream processing estimated to increase  $\text{NO}_3^-$  export over the weir.

### Effects of Stream N Processing

In only five of the 17 cases where we had same-day measurements of both  $\text{NO}_3^-$  production and  $\text{NO}_3^-$  consumption was instream production of  $\text{NO}_3^-$  from nitrification able to meet or exceed the measured uptake of  $\text{NO}_3^-$  from the water column (Table 2). When these nitrification and  $\text{NO}_3^-$  uptake rates were scaled to whole watersheds, our data indicated that in six of seven cases instream processing led to reduced losses of  $\text{NO}_3^-$  over the weirs

(Table 3). In several cases, more  $\text{NO}_3^-$  was removed from streamwater than was exported from the watershed (Table 3).

### Controls on Nitrification and $\text{NH}_4^+$ Uptake

Across all streams for both years, differences in  $\text{NO}_3^-$  concentration explained 58% of the variation in the fraction of added  $\text{NH}_4^+$  that was nitrified ( $P < 0.001$ ) (Figure 2). This relationship was consistent between years; for 1998 data alone,  $\text{NO}_3^-$

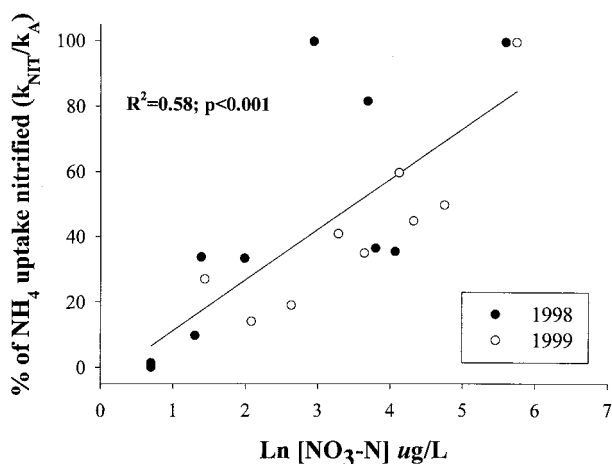


Figure 2. Nitrification (as the fraction of  $\text{NH}_4^+$  nitrified) versus log-transformed  $\text{NO}_3^-$  concentration for streams surveyed in 1998 and 1999.

concentration explained 59% of the variation in the  $k_{\text{NIT}}/k_{\text{A}}$  ( $P = 0.009$ ); (Figure 2). The trend for 1999 data alone was similar, with  $\text{NO}_3^-$  concentration explaining 75% of the variation in  $k_{\text{NIT}}/k_{\text{A}}$  ( $P = 0.002$ ) (Figure 2). Our repeated measurements of nitrification in the W3 stream showed no significant relationship between  $\text{NO}_3^-$  concentration and  $k_{\text{NIT}}/k_{\text{A}}$  (Figure 3a). In W6, however, there was a significant positive relationship, with  $\text{NO}_3^-$  concentration explaining 68% of the variation in  $k_{\text{NIT}}/k_{\text{A}}$  ( $P = 0.012$ ) (Figure 3b).

For the entire 1998 and 1999 data set (including survey and repeated measures of W3 and W6), streamwater  $\text{NO}_3^-$  concentrations accounted for 21% of the variation in  $\text{NH}_4^+$  uptake velocity ( $V_{\text{f}}$ ) ( $P = 0.01$ ) (Figure 4). This pattern was especially clear for the 1998 survey data alone, where  $\text{NO}_3^-$  concentrations accounted for 44% of the variation in  $\text{NH}_4^+$  uptake velocity ( $P = 0.02$ ) (Figure 4). Data from 1999 alone did not follow this trend (Figure 4).

## DISCUSSION

Our survey of HBEF stream nitrogen cycling found a great deal of variability in nitrogen uptake, the relative demand for  $\text{NH}_4^+$  versus  $\text{NO}_3^-$ , and the potential for nitrification within stream sediments. A recent cross-biome study of stream dissolved inorganic nitrogen (DIN) dynamics found substantial variation in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake and nitrification (Peterson and others 2001). It is notable that our survey of multiple streams within only one of the areas included in their larger survey found a similarly wide range of variation in streamwater

$\text{NO}_3^-$  concentrations,  $\text{NO}_3^-$  uptake, and nitrification.

Our understanding of the fate of DIN in streams is increasing, but there are still many unknowns. Ammonium uptake can occur through sorption onto stream sediments, as well as assimilation by autotrophic and heterotrophic stream organisms. Some proportion of this  $\text{NH}_4^+$  uptake in stream sediments will be nitrified. Nitrate uptake can be caused by biological assimilation or through uptake by denitrifying bacteria. Although we can measure DIN removal from the water column, it has been difficult to apportion that uptake into the various uptake compartments.

Ammonium uptake velocities in HBEF streams were high—and within the range found for other streams (Hall and others 2002). Nitrate uptake rates were twice as variable as those measured for  $\text{NH}_4^+$ , in part because  $\text{NO}_3^-$  concentrations varied much more than  $\text{NH}_4^+$  concentrations between streams. Although a much smaller proportion of the  $\text{NO}_3^-$  flux is taken up by stream sediments,  $\text{NO}_3^-$  uptake typically exceeds  $\text{NH}_4^+$  uptake because the supply of  $\text{NO}_3^-$  is typically 10–1000 times greater. Hydraulic parameters, such as transient storage, can exert some control on  $\text{NH}_4^+$  uptake (Hall and others 2002), but we have not found a relationship between transient storage and  $\text{NO}_3^-$  uptake velocities (R. O. Hall unpublished). Aside from these physical controls on DIN uptake, the availability of high-quality organic C strongly influences both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  demand in these heterotrophic streams (Bernhardt and Likens forthcoming; Bernhardt forthcoming).

Differences in stream hydraulic features and organic carbon availability may account for some of the interannual variation in our survey patterns. In addition to these differences, an ice storm in January of 1997 caused severe forest canopy damage in several of the experimental watersheds. As had been found during previous clear-cutting experiments, the ice storm damage resulted in increased losses of  $\text{NO}_3^-$  from forest soils (B. Z. Houlton personal communication). These losses led to very high streamwater  $\text{NO}_3^-$  concentrations during the summer of 1999 in some of our surveyed streams (B. Z. Houlton personal communication). W3 was severely impacted, but  $\text{NO}_3^-$  concentrations in W6 changed very little because of this disturbance (G. E. Likens unpublished). The difference in the extent of ice storm damage and associated  $\text{NO}_3^-$  inputs may explain in part why we see a relationship between  $\text{NO}_3^-$  and  $k_{\text{NIT}}/k_{\text{A}}$  for our 1998 and 1999 data in W6 but not in W3. Of course, more mechanistic examinations of nitrification in streams

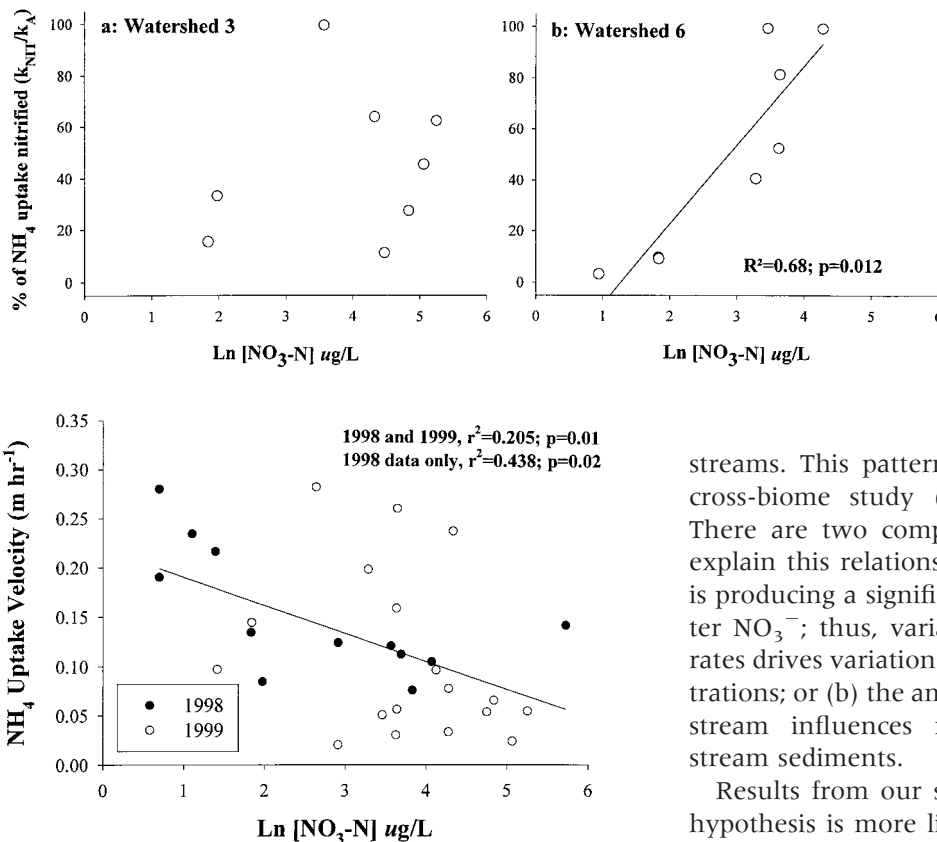


Figure 3. Nitrification (as the fraction of  $\text{NH}_4^+$  nitrified) versus log-transformed  $\text{NO}_3^-$  concentration for repeated releases (a) in the watershed 3 stream, and (b) in the watershed 6 stream.

Figure 4. Ammonium uptake velocity versus  $\text{NO}_3^-$  concentration for streams surveyed in 1998 and 1999. This graph includes both repeated measures of the W3 and W6 streams, as well as data from the whole stream survey.

are necessary before we can fully explain why there is so much variation in nitrification within stream sediments.

However, despite the high variability in inorganic N demand and turnover, the net effect of instream processing is typically a reduction in downstream DIN concentrations. In HBEF streams, at least during the growing season, instream uptake of  $\text{NO}_3^-$  commonly exceeds rates of  $\text{NO}_3^-$  export over the weir (B. Z. Houlton personal communication). Although we cannot equate DIN uptake with N retention in stream sediments, it is clear that streams are transforming or even retaining a significant proportion of the N that enters them from the surrounding forest (Alexander and others 2000; Peterson and others 2001).

The extreme variation in  $k_{\text{NIT}}/k_A$  within these similar streams was surprising, with anywhere from 2% to 100% of the  $\text{NH}_4^+$  added during short-term enrichments being converted to  $\text{NO}_3^-$ . We found that variation in  $\text{NO}_3^-$  concentration explains over half of the variation in  $k_{\text{NIT}}/k_A$  across our surveyed

streams. This pattern has been noted in another cross-biome study (Peterson and others 2001). There are two competing hypotheses that might explain this relationship: (a) Instream nitrification is producing a significant proportion of stream water  $\text{NO}_3^-$ ; thus, variation in instream nitrification rates drives variation in streamwater  $\text{NO}_3^-$  concentrations; or (b) the amount of  $\text{NO}_3^-$  imported to the stream influences rates of nitrification within stream sediments.

Results from our study suggest that the second hypothesis is more likely. Because instream  $\text{NO}_3^-$  uptake in most cases exceeds instream  $\text{NO}_3^-$  production, it appears unlikely that instream nitrification could be producing most streamwater  $\text{NO}_3^-$ . Although instream nitrification may contribute a large portion of the  $\text{NO}_3^-$  used within the stream itself, not enough  $\text{NO}_3^-$  is produced within the stream to account for the wide range of  $\text{NO}_3^-$  concentrations among the streams we surveyed. This conclusion is more strongly supported when we consider that the differences between  $\text{NO}_3^-$  production and consumption reported here are likely to be conservative. Nutrient uptake is often underestimated using enrichment techniques (Mulholland and others 1990), while our measurements of  $\text{NO}_3^-$  production during  $\text{NH}_4^+$  releases were likely to be maximum nitrification estimates due to our enrichment method. In addition, the simultaneous additions of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  during our 1998 releases probably caused even greater underestimates of  $\text{NO}_3^-$  uptake than in 1999, and all of the cases in which we found nitrification to exceed  $\text{NO}_3^-$  uptake were in the first summer.

Results from other studies at the HBEF also lead us to reject the first hypothesis. If indeed instream nitrification was the driving variable explaining variation in streamwater  $\text{NO}_3^-$  concentration, we would expect to see  $\text{NO}_3^-$  concentrations increasing longitudinally along stream courses. Instead,



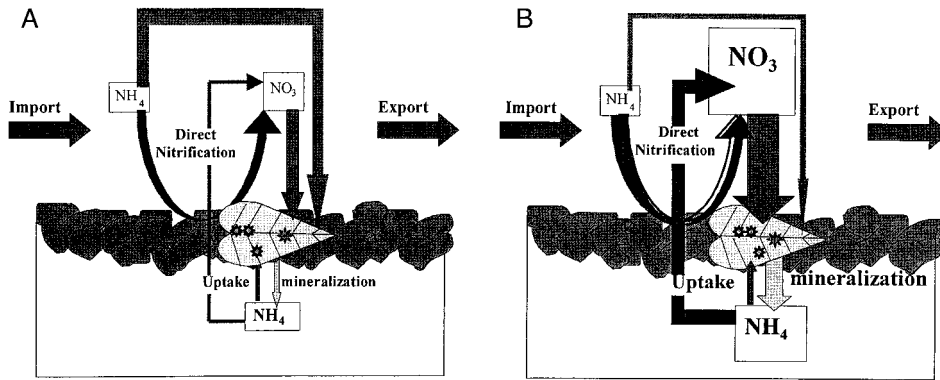


Figure 5. A conceptual diagram of the potential effect of increased nitrate concentrations on instream nitrification. In Panel (A), the nitrogen cycle is shown with the pools of streamwater  $\text{NO}_3^-$  and  $\text{NH}_4^+$  being equal. In (B), higher  $\text{NO}_3^-$  availability might lead to reduced demand (smaller arrows) for water column  $\text{NH}_4^+$  by heterotrophs and/or to increased mineralization (larger arrows)—increasing the size of the  $\text{NH}_4^+$  pool. For clarity,  $\text{NH}_4^+$  is shown here as two separate pools. The streamwater  $\text{NH}_4^+$  pool represents inputs of  $\text{NH}_4^+$  from the surrounding forest, whereas the  $\text{NH}_4^+$  pool pictured here in the sediments represents  $\text{NH}_4^+$  derived from mineralization of organic matter. In reality,  $\text{NH}_4^+$  from both sources makes up one pool.

surveys of HBEF streams have found the opposite pattern (Lawrence and others 1987; E. S. Bernhardt unpublished; C. T. Driscoll unpublished; G. E. Likens unpublished):  $\text{NO}_3^-$  concentrations were often found to be highest in the headwaters and lowest at their confluence with Hubbard Brook. Recent work by Bohlen and others (2001) found that soil N mineralization and nitrification rates increase with elevation. Bohlen and others (2001) have suggested that the declines in streamwater  $\text{NO}_3^-$  concentrations with decreasing elevation may be a consequence of this pattern, with reduced N inputs from forest to stream at lower elevations. Preliminary information on the sources of  $\text{NO}_3^-$  in streamwater, based on stable isotope analysis ( $^{18}\text{O}$ ), suggests that most streamwater  $\text{NO}_3^-$  is derived from microbially produced soil  $\text{NO}_3^-$  (L. Pardo unpublished), which makes this scenario plausible.

If inputs of  $\text{NO}_3^-$  to stream ecosystems are largely driven by soil microbial processing, as HBEF researchers have suggested (Bohlen and others 2001; L. Pardo unpublished), then the correlation between streamwater  $\text{NO}_3^-$  and instream nitrification becomes more puzzling. We speculate that as the amount of  $\text{NO}_3^-$  entering the stream increases, competitive demand for  $\text{NH}_4^+$  within stream sediments is reduced, and a greater fraction of the  $\text{NH}_4^+$  is thereby available to nitrifiers. To some extent, our observation that increased  $\text{NO}_3^-$  availability is associated with reduced  $\text{NH}_4^+$  uptake velocities supports this hypothesis. Nitrate inputs could influence competition for  $\text{NH}_4^+$  indirectly, with higher streamwater DIN stimulating more rapid cycling of N and thus greater rates of N mineralization (Jones

and others 1995) (Figure 5). Alternatively, high  $\text{NO}_3^-$  concentrations in streamwater might directly satisfy more of the heterotrophic N demand, lowering competition and thus demand for vanishingly small concentrations of  $\text{NH}_4^+$ . This reduced competition may allow nitrifying bacteria to take up more  $\text{NH}_4^+$  from streamwater (Figure 5).

We hypothesize that  $\text{NO}_3^-$  availability thus mediates competition for  $\text{NH}_4^+$  between heterotrophs and nitrifiers. Rates of nitrification are typically limited by the supply of  $\text{NH}_4^+$  (Roberston 1982; Triska and others 1990, 1993; Verhagen and Laanbroek 1991) and ammonium-oxidizing bacteria are considered poor competitors for  $\text{NH}_4^+$  relative to heterotrophic bacteria and other autotrophs (Vitousek and others 1982; Gerards and others 1998; Carnol 1999). Thus, reducing the competitive demand for  $\text{NH}_4^+$  should stimulate nitrification rates. Verhagen and others (1991, 1992) found that nitrification within soils is inhibited at high C:N ratios when the competitive demand for nitrogen by heterotrophic bacteria is higher. In a survey of forest soils across a gradient of N deposition, Lovett and Rueth (1999) found rates of soil nitrification to be significantly positively related to levels of atmospheric N deposition. Increased  $\text{NO}_3^-$  availability led to reduction in soil organic matter C:N ratios and increased rates of N mineralization and thus higher nitrification rates (Lovett and Rueth 1999).

Two recent studies in streams suggest that the addition of labile C increases N limitation of heterotrophic processes and thus reduces  $\text{NH}_4^+$  availability to competing nitrifiers. In a microcosm experiment, Strauss and Lamberti (2000) found that by increasing

streamwater C:N ratios, total microbial activity was increased while nitrification was significantly reduced. In addition, they found that higher-quality glucose inhibited nitrification more strongly than lower-quality leaf leachate (Strauss and Lamberti 2000). In a whole-system experiment, Bernhardt and Likens (forthcoming) found that the addition of potassium acetate to a stream at HBEF led to an increase in heterotrophic respiration but a reduction in the fraction of  $\text{NH}_4^+$  uptake that was nitrified. Both studies suggest that stream nitrifiers are poor competitors for limiting levels of  $\text{NH}_4^+$ .

Determining which is the controlling variable and which is the dependent variable in this relationship between nitrification and streamwater  $\text{NO}_3^-$  concentration is critical to our understanding of watershed  $\text{NO}_3^-$  losses. Current interpretations of watershed-level N dynamics would have to be reevaluated if a significant fraction of the  $\text{NO}_3^-$  in streamwater resulted from instream nitrification. Management strategies for eutrophication of downstream ecosystems will be quite different depending on whether streamwater  $\text{NO}_3^-$  concentrations are driven in large part by within-stream nitrification, or if the availability of  $\text{NO}_3^-$  to stream heterotrophs were to influence nitrification rates in stream sediments by lessening the competitive demand for  $\text{NH}_4^+$ . We suspect that—at least for HBEF and probably for most regions where stream  $\text{NH}_4^+$  concentrations are low relative to  $\text{NO}_3^-$  concentrations—instream nitrification is not sufficient to account for a significant proportion of the variation in streamwater  $\text{NO}_3^-$  concentrations. In this study, the net effect of instream processes appears to be the reduction of  $\text{NO}_3^-$  concentrations in streamwater. This result is consistent with other recently published findings in which headwater streams were found to retain large amounts of  $\text{NO}_3^-$  (Alexander and others 2000; Mulholland and others 2000; Peterson and others 2001).

## CONCLUSIONS

Within streams of the Hubbard Brook Valley, instream nitrification was insufficient to explain the variation among streams in streamwater  $\text{NO}_3^-$ . Although there was a relationship between  $\text{NO}_3^-$  concentration and the fraction of  $\text{NH}_4^+$  uptake nitrified ( $k_{\text{NIT}}/k_{\text{A}}$ ), we conclude that at the low  $\text{NH}_4^+$  concentration that characterizes HBEF streams, nitrification cannot produce enough  $\text{NO}_3^-$  to dramatically alter streamwater  $\text{NO}_3^-$  concentrations. Instead, our results suggest that  $\text{NO}_3^-$  concentration may indirectly influence nitrification rates by mediating the competitive demand for  $\text{NH}_4^+$  between

heterotrophs and nitrifiers. This model suggests the potential for a positive feedback between upland soils and stream sediments, with increases in  $\text{NO}_3^-$  inputs from the terrestrial watershed stimulating nitrification in receiving streams.

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