Ammonium and nitrate uptake lengths in a small forested stream determined by \textsuperscript{15}N tracer and short-term nutrient enrichment experiments

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Introduction

Nutrient cycling is an important characteristic of all ecosystems, including streams. Nutrients often limit the growth rates of stream algae and heterotrophic microbes and the decomposition rate of allochthonous organic matter (ELWOOD et al. 1981, ROSEMOND et al. 1993, SUBERKROPP & CHAUVET 1995). Nutrient uptake length ($S_w$), defined as the mean distance traveled by a nutrient atom dissolved in stream water before uptake by biota (NEWBOLD et al. 1981, ELWOOD et al. 1983), is often used as an index of nutrient cycling in streams.

It is often overlooked, however, that $S_w$ is not a measure of nutrient uptake rate per se, but rather a measure of the efficiency with which a stream utilizes the available nutrient supply (i.e., it is the inverse of the fractional nutrient uptake rate per unit of stream length). NEWBOLD et al. (1982) showed that nutrient uptake rate ($U$, units of mass per unit time per unit area of stream) is related to nutrient uptake length as follows:

$$U = \frac{F_w}{S_w \times w}$$

(1)

where $F_w$ is the downstream nutrient flux in water (nutrient concentration * discharge, units of mass time$^{-1}$) and $w$ is average stream width. Thus, nutrient uptake length and uptake rate are closely related and one can be computed from the other if nutrient flux and stream width are known.

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preclude use of nutrient radiotracers (e.g., $^{32,33}$P) in field studies and methodological difficulties and high analytical costs have previously hindered the use of stable isotope nutrient tracers (e.g., $^{15}$N). Short-term nutrient enrichments are an alternative to nutrient tracer additions for measuring $S_w$ (STREAM SOLUTE WORKSHOP 1990). Several studies have used the short-term nutrient enrichment method in comparisons of $S_w$ within and across streams (e.g., MUNN & MEYER 1990, MARTI & SABATER 1996). In a previous study, however, MULHOLLAND et al. (1990) showed that use of the nutrient enrichment method can considerably overestimate $S_w$ for phosphate in streams. In the present study, we compare the short-term nutrient enrichment and tracer methods for determining $S_w$ of ammonium and nitrate in the same stream used for the previous phosphate study.

Keywords
stream, nutrient uptake length, nutrient cycling, ammonium uptake, nitrate uptake, nitrogen.

Materials and methods
Short-term (2-h) additions of ammonium chloride and potassium nitrate to stream water were conducted to determine $S_w$ of ammonium and nitrate using the nutrient enrichment approach in the West Fork of Walker Branch, a first-order forested stream in eastern Tennessee, USA. Addition of $^{15}$N-enriched ammonium chloride (10 atom % enrichment of $^{15}$N) to stream water was also used to determine $S_w$ of ammonium and nitrate in this stream (nutrient tracer approach). The 125-m study segment used for these experiments was the same segment used for previous measurements of $S_w$ of phosphate (NEWBOLD et al. 1981, 1983, MULHOLLAND et al. 1985, 1990). The nitrate enrichment experiment was performed on 27 March 1997, the ammonium
enrichment experiment on 31 March 1997, and the $^{15}$N addition experiment on 1 April 1997.

Stream discharge and weather conditions were similar for all experiments and each was conducted during the hours of 1000 and 1200.

In the nutrient enrichment experiments a concentrated solution of ammonium chloride or potassium nitrate was pumped at a constant rate into a well-mixed section of stream at a rate designed to increased the ammonium concentration by about 20 $\mu$g N l$^{-1}$ and the nitrate concentration by about 50 $\mu$g N l$^{-1}$ over background concentrations of about 2 and 20 $\mu$g N l$^{-1}$ for ammonium and nitrate, respectively. A conservative tracer (NaCl) was simultaneously added during each experiment to determine discharge rate at each station. Water samples were collected at several locations downstream from the addition just prior to the nutrient addition and again after steady state was achieved throughout the 125-m reach based on conservative tracer concentration (specific conductance) measurements (< 2 h). Water samples were filtered immediately (0.45 $\mu$m membrane filters), returned to the laboratory within 2 h, and refrigerated until analysis (within 5 d). Ammonium was analyzed by automated phenate colorimetry and nitrate plus nitrite by Cu-Cd reduction followed by azo dye colorimetry using a Bran Lubbe TRAACS 800 autoanalyzer (AMERICAN PUBLIC HEALTH ASSOCIATION 1992). Hereafter we refer to nitrate plus nitrite as nitrate because previous work has shown nitrite concentrations to be negligible in this stream. Nutrient uptake lengths were calculated from the regression of the rate of decline at steady state in the flux of added nutrient with distance downstream from the addition point ($x$) using the equation:

$$\ln(F_N) = \ln(F_{N0}) - kx,$$

where $F_{N0}$, $F_N$ are the steady state nutrient fluxes, corrected for background concentration, at the most upstream site where complete mixing of added nutrient was achieved and at any downstream
For the $^{15}\text{N}$ addition experiment, a solution of $^{15}\text{N}$-enriched ammonium chloride was pumped at a constant rate designed to increase the $^{15}\text{N}:^{14}\text{N}$ ratio in stream water ammonium by about 50% (see below). This resulted in a negligible increase in the ammonium concentration in stream water ($<0.1\mu g\text{ N l}^{-1}$). A conservative tracer (NaCl) also was simultaneously added during this experiment to account for the increase in discharge due to groundwater inflow in the reach. The $^{15}\text{N}$ addition was continued for six weeks as part of a larger intersite comparison study of nitrogen cycling in stream ecosystems (Lotic Intersite Nitrogen Experiment, LINX).

Approximately 6 hours after the initiation of $^{15}\text{N}$ addition, two large samples of stream water (4 liters) were collected upstream and at six locations downstream from the $^{15}\text{N}$ addition. Water samples were returned to the laboratory and filtered (0.45 μm) within 1 h of collection. Concentrations of ammonium and nitrate were determined on subsamples from each location.

An ammonium diffusion procedure (HOLMES et al. 1998) was used to determine $^{15}\text{N}$-ammonium in one of the water samples from each location. Briefly, this method involves diffusion of ammonia under basic conditions (by adding MgO) into the headspace of a sealed container and sorption of the headspace ammonia onto pre-combusted, acidified glass fiber filters (Whatman GF/D) sandwiched between two Teflon filters and floating on the surface of the water. Following 14 days of diffusion at 40°C while shaking, the filter packets were removed from the sample bottles, dried in a desiccator, and the $^{15}\text{N}:^{14}\text{N}$ ratio of ammonium absorbed on each filter was determined by mass spectrometry (Finnigan Delta S, Ecosystems Center, Marine Biological Laboratory).

The second water sample from each location was used for determination of $^{15}\text{N}$-nitrate.
Following removal of $^{15}$N-ammonium using the alkaline diffusion procedure (with the sample open to the atmosphere), DeVarda’s alloy was added to the sample to reduce nitrate to ammonium and the ammonium diffused into a closed headspace and trapped and analyzed for $^{15}$N as described above.

The $^{15}$N values are expressed as $\delta^{15}$N (units of $^{0}/_{\infty}$) according to the following equation

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

(3) where $R$ denotes the $^{15}$N:$^{14}$N ratio of the sample or standard, with the standard being air ($R_{\text{standard}} = 0.003663$). To determine the amount of tracer $^{15}$N in the ammonium and nitrate pools, $\delta^{15}$N values for the sample collected upstream from the $^{15}$N addition were subtracted from all $\delta^{15}$N values downstream (background-corrected). The flux of tracer $^{15}$N-ammonium or nitrate at each downstream location $i$ ($^{15}$N flux$_i$) was calculated according to the following equation:

$$^{15}\text{N flux}_i = \left(\frac{\delta^{15}\text{N}_i}{1000}\right) \times 0.003663 \times Q_i \times [N]_i$$

(4) where $\delta^{15}\text{N}_i$ is the background-corrected $\delta^{15}$N value, $[N]_i$ is the ammonium or nitrate concentration (determined either from the measurements on water samples or from the rates of total N recovery reported for the mass spectrometer analysis of each sample when concentrations were below the limit of detection for the water analysis), and $Q_i$ is the stream discharge at location $i$, and the term 0.003663 is the fractional abundance of $^{15}$N in air. Ammonium uptake length was then calculated using equation 2, except that tracer $^{15}$N-ammonium flux values were used rather than added nutrient flux values as for the enrichment experiment.

Determination of nitrate uptake length required fitting a 2-compartment model (ammonium and nitrate in stream water) to the longitudinal profile of tracer $^{15}$N-nitrate flux to account for the simultaneous production (nitrification) and removal (uptake) of streamwater $^{15}$N-nitrate. The model describes the change in $^{15}$N-nitrate flux ($N_x$) over distance $x$ as a function of
the flux of $^{15}$N-ammonium at the point of $^{15}$N addition ($A_0$) and the rate of decline in $^{15}$N-ammonium flux over distance ($k_1$, which is the inverse of the ammonium uptake length), the nitrification rate ($r$), and the nitrate uptake rate per unit distance ($k_2$) as follows:

$$\frac{d(N_x)}{dx} = rA_0 e^{-k_1x} - k_2N_x$$  \hspace{1cm} (5)$$

Solving for $N(x)$ yields:

$$N(x) = \left[ \frac{rA_0}{k_2-k_1} * (e^{k_1x} - e^{-k_2x}) \right] + N_0 e^{-k_2x}$$  \hspace{1cm} (6)$$

where $N_0$ is the flux of $^{15}$N-nitrate at the addition point ($x=0$). The values of $r$ (nitrification rate, in units of $\text{m}^{-1}$) and $k_2$ (nitrate uptake rate, in units of $\text{m}^{-1}$) were estimated by fitting the model to the longitudinal $^{15}$N-nitrate flux data using a spreadsheet least squares fitting procedure (Excel tool “Solver”). Nitrate uptake length was then calculated as the inverse of $k_2$.

**Results**

The first-order kinetics model (equation 2) used to compute the longitudinal uptake rate of added nutrient fit the nutrient enrichment data reasonably well (Fig. 1). For the ammonium experiments, the uptake rate of added NH$_4$ (enrichment) was considerably smaller than the uptake rate of the $^{15}$NH$_4$ tracer (Fig. 1A). Considering nutrient enrichment experiments only, the longitudinal uptake rate of added NO$_3$ (Fig. 1B) was considerably smaller than that for NH$_4$ (Fig. 1A).

The best fit of the 2-compartment nitrification/nitrate uptake model to the tracer $^{15}$NO$_3$ data gave a longitudinal uptake rate for nitrate of 0.0099 m$^{-1}$ (Fig. 2), about 4 times higher than
that for the nitrate enrichment experiment 5 days earlier (Fig. 1B). The effect of nitrate uptake on the longitudinal profile of tracer $^{15}$N-nitrate was clearly evident from a model simulation that considered nitrification only (dashed line in Fig. 2).

Summarizing the results of the nutrient enrichment and tracer experiments, mean uptake lengths were 2-fold (ammonium) to 4-fold (nitrate) shorter using the tracer approach than using the enrichment approach (Table 1). Discharge rates, water temperatures, and ambient nutrient concentrations were similar among all experiments.

Additionally, we can use the data from the nutrient enrichment experiments and divide the experimental stream segment into a longitudinal series of subsegments with declining nutrient concentrations to evaluate the relationship between enrichment level and uptake length. We calculated nutrient uptake rates and lengths for each subsegment using the nutrient concentrations, average discharge, and average stream width for each set of adjacent sampling locations. The uptake rate of ammonium increased with increasing ammonium concentration as expected (Fig. 3A); however, the increases in uptake rate were considerably less than the increases in concentration (i.e., values fell below 1:1 line). Consequently, ammonium uptake length declined from 45 to 65 m in the two most upstream subsegments with average ammonium enrichments of 12 to 17 $\mu$g N l$^{-1}$ to 30 to 40 m in the two most downstream subsegments with average ammonium enrichments of 2 to 4 $\mu$g N l$^{-1}$. In contrast, there was no apparent relationship between nitrate uptake rate and concentration in the nitrate enrichment experiment (Fig. 3B).

**Discussion and Conclusions**

Based on nutrient spiraling theory, the uptake length of a nutrient is directly related to its flux in available form in stream water (NEWBOLD et al. 1981, 1982, ELWOOD et al. 1983). Thus,
nutrient uptake lengths measured using short-term nutrient enrichment experiments are expected to be equivalent to uptake lengths under ambient (unenriched) nutrient conditions only if nutrient uptake rates increase in direct (1:1) proportion to the increase in nutrient flux with enrichment. This response in nutrient uptake rate with enrichment may be reasonable for low level enrichments of a nutrient in which stream biota are highly deficient (i.e., strongly limiting nutrient), but is unlikely for higher level enrichments or for non-limiting nutrients.

Our results show that ammonium and nitrate enrichments of about 20 $\mu$g N l$^{-1}$ and 50 $\mu$g N l$^{-1}$, respectively (increases of about 10 times and 3 times ambient concentrations), result in considerable overestimation of ambient uptake lengths for these nutrients in Walker Branch (as determined from $^{15}$N tracer additions). Although the uptake rate of ammonium increased with increasing concentration, the increase in uptake rate was not as great as the increase in ammonium flux and consequently uptake length increased with enrichment. These results are consistent with those of a previous study in Walker Branch showing that the enrichment approach overestimated phosphate uptake length by at least 50% even for relatively low level enrichments ($< 10 \mu$g P l$^{-1}$) (MULHOLLAND et al. 1990). The earlier study indicated that even at phosphate concentrations low enough to limit biological uptake rates ($< 5 \mu$g P l$^{-1}$), the increase in phosphate uptake with increasing concentration was less than the 1:1 ratio necessary to maintain a constant uptake length.

The overestimation of uptake length using enrichments was greater for nitrate than for ammonium, despite the fact that the fractional nitrate enrichment (3 times ambient levels) was smaller than the fractional ammonium enrichment (about 10 times ambient levels). This likely reflects the very low ambient ammonium concentrations and stronger biological demand for ammonium compared with nitrate, the latter requiring chemical reduction before it can be used as
a source of N in biosynthesis. Under ambient nutrient concentrations uptake rates of ammonium and nitrate (in terms of N), calculated from the tracer $^{15}$N data using equation 1, were approximately equal (Fig. 3). From the enrichment experiment data, it is evident that ammonium uptake rates responded positively to an increase in ammonium concentration, whereas nitrate uptake rates showed no consistent response to an increase in nitrate concentration, perhaps due to near-saturation of biological uptake at ambient concentrations.

Our results indicate that ideally nutrient uptake lengths should be determined using additions of nutrient tracers that do not result in an increase in nutrient concentrations. Uptake lengths determined using the short-term nutrient enrichment approach should be interpreted with caution. Nonetheless, enrichment-derived nutrient uptake lengths can be useful for comparisons within or between streams if the level of nutrient enrichment is low relative to ambient concentrations and is approximately the same among experiments. The overestimation of ambient nutrient uptake length when using the enrichment approach should be least for nutrients that are present in the lowest supply relative to their demands (most limiting).

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1. Regressions of the natural logarithm of the fractional flux of added nutrient or tracer (flux at station $i$ divided by flux at the most upstream station below the addition) versus distance downstream from the addition for (A) ammonium enrichment and $^{15}$NH$_4$ tracer addition experiments, and (B) nitrate enrichment experiment. The slope of the regression is the longitudinal uptake rate (units of m$^{-1}$) and the inverse of the slope is the uptake length. Fractional flux rather than total flux was used in these regressions for uptake length in order to more clearly show differences between the enrichment and tracer experiment results.

2. Best fit of a two-compartment model for $^{15}$N-nitrate flux to measured $^{15}$N-nitrate flux values during the $^{15}$N tracer experiment.

3. Uptake rates as a function of nutrient concentration determined from calculations of uptake length and concentration for adjacent sampling locations during the (A) ammonium and (B) nitrate enrichment experiments.
Table 1. Summary of conditions and results of nutrient enrichment and $^{15}$N tracer addition experiments for determining uptake lengths of ammonium and nitrate in Walker Branch.

Discharge, water temperature, and nutrient concentration data are for the upper station (5-10 m downstream from nutrient/isotope addition).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NH$_4$/NO$_3$ Enrichment</th>
<th>$^{15}$NH$_4$ Tracer Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NH$_4$ experiments:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>31 March 1997</td>
<td>1 April 1997</td>
</tr>
<tr>
<td>Discharge (L/s)</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Water temperature ($^\circ$C)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>NH$_4$ concentration ($\mu$gN/L)</td>
<td>&lt; 2 (ambient)</td>
<td>2.6</td>
</tr>
<tr>
<td>Mean NH$_4$ uptake length (m)</td>
<td>43.3</td>
<td>19.8</td>
</tr>
<tr>
<td>95% CI for uptake length (m)</td>
<td>37.2 - 51.8</td>
<td>15.3 - 28.2</td>
</tr>
<tr>
<td><strong>NO$_3$ experiments:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>27 March 1997</td>
<td>1 April 1997</td>
</tr>
<tr>
<td>Discharge (L/s)</td>
<td>9.4</td>
<td>9.2</td>
</tr>
<tr>
<td>Water temperature ($^\circ$C)</td>
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<td>14</td>
</tr>
<tr>
<td>NO$_3$ concentration ($\mu$gN/L)</td>
<td>22 (ambient)</td>
<td>17</td>
</tr>
<tr>
<td>Mean NO$_3$ uptake length (m)</td>
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<td>101</td>
</tr>
<tr>
<td>95% CI for uptake length (m)</td>
<td>254 - 1023</td>
<td></td>
</tr>
</tbody>
</table>
(A) $\text{NH}_4$ enrichment:

$\ln$ (fractional flux)

$Y = 0.987$

$slope = -0.050 \text{ m}^{-1}$

(B) $\text{NO}_3$ enrichment:

$\ln$ (fractional flux)

$Y = 0.917$

$slope = -0.0025 \text{ m}^{-1}$
8 measured

nitrification rate ($N_1$) = 0.0084 m$^{-1}$

nitrate uptake rate ($k2$) = 0.0099 m$^{-1}$
(A) NH$_4$ uptake

NH$_4$ uptake rate ($\mu$gN m$^{-2}$ s$^{-1}$)

NH$_4$ concentration (mgN/L)

1:1 line

- NH$_4$ enrichment
- $^{15}$N tracer

(B) NO$_3$ uptake

NO$_3$ uptake rate ($\mu$gN m$^{-2}$ s$^{-1}$)

NO$_3$ concentration (mgN/L)

1:1 line

- NO$_3$ enrichment
- $^{15}$N tracer