Stream denitrification and total nitrate uptake rates measured using a field \textsuperscript{15}N tracer addition approach

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Abstract

We measured denitrification and total nitrate uptake rates in a small stream (East Fork of Walker Branch in eastern Tennessee) using a new field \textsuperscript{15}N tracer addition and modeling approach that quantifies these rates for entire stream reaches. The field experiment consisted of an 8-h addition of 99 atom\% K\textsuperscript{15}NO\textsubscript{3} and a conservative solute tracer. Two \textsuperscript{15}N tracer addition experiments were performed on consecutive days, the first under ambient NO\textsubscript{3} concentrations (23 \mu g N L\textsuperscript{-1}) and the second with a NO\textsubscript{3} addition of approximately 500 \mu g N L\textsuperscript{-1}. We fit first-order NO\textsubscript{3} uptake and two-box denitrification models to the longitudinal measurements of tracer \textsuperscript{15}N in dissolved NO\textsubscript{3}, N\textsubscript{2}, and N\textsubscript{2}O in stream water to determine rates. Total NO\textsubscript{3} uptake rates were 0.028 m\textsuperscript{-2} (0.32 \mu g N m\textsuperscript{-2} s\textsuperscript{-1}) and 0.01 m\textsuperscript{-2} (1.6 \mu g N m\textsuperscript{-2} s\textsuperscript{-1}) under ambient NO\textsubscript{3} and with NO\textsubscript{3} addition, respectively. Denitrification rates were 0.0046 m\textsuperscript{-2} (uncertainty range of 0.002 to 0.008 m\textsuperscript{-2}) and 9 \times 10\textsuperscript{-4} m\textsuperscript{-2} (uncertainty range of 3 \times 10\textsuperscript{-5} to 21 \times 10\textsuperscript{-5} m\textsuperscript{-2}) under ambient NO\textsubscript{3} and with NO\textsubscript{3} addition, respectively. Denitrification resulted almost exclusively in N\textsubscript{2} production (\textgreek{N}2 production) and comprised about 16\% (\textpm{}10\%) of total NO\textsubscript{3} uptake rate under ambient NO\textsubscript{3} concentrations and about 1\% (\textpm{}1\%) of total NO\textsubscript{3} uptake rate with NO\textsubscript{3} addition. Denitrification rate expressed on a mass flux basis was about 12 \mu mol m\textsuperscript{-2} h\textsuperscript{-1} under ambient NO\textsubscript{3} concentrations, a value within the range reported for other streams with low NO\textsubscript{3} concentrations.

Humans have greatly altered the nitrogen (N) cycle in recent decades, more than doubling the inputs of fixed N to the biosphere (Vitousek et al. 1997). The increased inputs have led to increased hydrologic export of N from landscapes and consequent large increases in the inputs of N, primarily as nitrate–nitrogen (NO\textsubscript{3}–N), via rivers to estuaries and coastal oceans (Howarth et al. 1996; Jordan and Weller 1996). The increases in N loading to streams and rivers have accelerated rates of eutrophication and the development of extensive areas of anoxia and may be linked to harmful algal blooms in a number of coastal ecosystems (Turner and Rabalais 1994; Nixon et al. 1996; Glasgow and Burkholder 2000).

Regional budgets have shown that only 20\% to 30\% of the N added to the land by humans is exported to the ocean (Howarth et al. 1996; Boyer et al. 2002), indicating that substantial N sinks exist between the land where N is applied and the oceans receiving N loads. In a recent study of the Mississippi River drainage basin, Alexander et al. (2000) showed that rivers were substantial sinks for N originating from terrestrial runoff. Alexander and colleagues found that N retention was inversely related to river size, with retention rates declining exponentially with increasing river depth. Seitzinger et al. (2002) reported that 37\% to 78\% of the N
inputs to rivers were removed during transport through river networks based on application of a regression model to 16 rivers in the northeastern United States. In contrast to the findings of Alexander et al. (2000), however, Seitzinger et al. (2002) pointed to the importance of larger rivers, suggesting that about one-half of basin-scale N retention occurred in higher order (i.e., ≥fifth) systems.

In an experimental study using $^{15}$NH$_4$ additions to 12 streams representing multiple biomes, Peterson et al. (2001) reported that the average distance traveled by an ammonium ion before being removed from stream water (defined as the uptake length, $S_w$) was strongly related to stream discharge, with longer values of $S_w$ (i.e., lower uptake efficiencies) in larger streams. Using a model based on their experimental results, Peterson and colleagues showed that N uptake could reduce inorganic N concentrations by about two-thirds over a 1-km reach of headwater stream with the model most sensitive to nitrate uptake rate.

Although there are a number of processes that remove inorganic N from water, including assimilation by plants and microbes, sorption to sediments, deposition of particulate organic N, and denitrification, it is primarily denitrification that results in permanent loss because the other processes represent primarily internal processes of transformation or relocation. Rates of denitrification in streams and rivers have been measured almost exclusively using the acetylene block technique on sediment cores or slurries returned to the laboratory or in cores or chambers incubated in situ. Studies using this technique have generally shown that denitrification rates are highly variable in space and time. Variation in denitrification rates has been shown to be related primarily to redox status and secondarily to nitrate concentrations and/or the availability of labile organic carbon (Holmes et al. 1996; Duff et al. 1996; Kemp and Dodds 2002). However, the acetylene block technique involves substantial handling of sediments if performed in the laboratory or modification of hydraulic conditions if conducted in field chambers. Thus, this technique may not provide accurate measures of denitrification rates for entire stream ecosystems.

In the past decade, $^{15}$N techniques have been developed for determining denitrification rates in aquatic sediments (Nielsen 1992; Rysgaard et al. 1993). These techniques avoid the artifacts associated with the use of acetylene, but past applications still involved use of chambers or cores to isolate sediments and overlying water. Chambers are often problematic to install in streams with coarse or mixed substrata. In addition, there is considerable work on stream biogeochemistry emphasizing the importance of hydrologic exchange between surface and shallow subsurface water (Boulton et al. 1998; Jones and Mulholland 2000), and chamber installation may alter the flow of water through streambed sediments and change the environmental conditions to which denitrification is highly sensitive. Given the recognized potential for stream ecosystems to act as points of substantial N retention along the continuum from land to the ocean, it is important to accurately quantify denitrification rates in streams.

We have developed a field $^{15}$N tracer addition approach to quantify denitrification and total nitrate uptake rates for entire stream reaches without physical or chemical perturba-

### Materials and methods

**Study site**—The study was conducted in the East Fork of Walker Branch, a first-order stream draining a 59.1-ha deciduous forest catchment in the Ridge and Valley region of eastern Tennessee (35°58’N, 84°17’W). Mean annual precipitation is about 140 cm, and mean annual temperature is about 14.5°C. The catchment is underlain by several layers of siliceous dolomite, and stream water is slightly basic. The stream originates in a headwater spring approximately 100 m above the study reach, and the stream bottom is comprised primarily of cobble, gravel, and fine-grained organic-rich sediments. The average gradient of this stream is quite low (approximately 0.02 m m$^{-1}$).

The experiments were conducted on 2–3 October 2002 when discharge was low and stable. Although nutrient concentrations are low year-round (<0.1 mg N L$^{-1}$ and <0.01 mg P L$^{-1}$ of inorganic N and P), they are highest at this time of year just prior to substantial leaf-fall inputs, which peak in early November. Previous studies during summer and autumn have indicated that the hydrologic transient storage zone (presumed to be primarily the hyporheic zone) was 0.5 to 1.0 times the size of the surface zone (i.e., $A_t$:A ratio of 0.5–1.0). Previous measurements of denitrification using the acetylene block technique on sediments returned to the laboratory indicated low to moderate activity (mean rate of 78 ng N$_2$O g ash-free dry mass$^{-1}$ h$^{-1}$) limited primarily by nitrate concentrations (Martin et al. 2001).

**Experimental procedures**—We conducted 8- to 9-h continuous additions of 99% $^{15}$N-enriched KNO$_3$ to the stream on successive days, with the first beginning about 1000 h on 2 October 2002 (day 1) and the second about 0830 h on 3 October 2002 (day 2). The day 1 experiment was conducted under ambient stream NO$_3^-$ concentrations, whereas the day 2 experiment involved addition of unlabeled KNO$_3$ intended to increase streamwater NO$_3^-$ by about 0.5 mg N L$^{-1}$. Addition of K$^{15}$NO$_3$ increased the $^{15}$N:$^{14}$N ratio of streamwater NO$_3^-$ by about 40 times relative to the ambient ratio in both experiments. The injection solution consisted of K$^{15}$NO$_3$ (0.9 g and 6.2 g 99% $^{15}$N-enriched KNO$_3$ on day 1 and 2, respectively) added to 15 liters of deionized water together with NaCl (200 g on each date) to provide a conservative solute tracer for each experiment. On day 2, 2.5 g of unlabeled KNO$_3$ were also added to the 15-liter injection solution. The K$^{15}$NO$_3$ addition resulted in a small increase in the streamwater NO$_3^-$ concentration—approximately 15% above the ambient concentration (23 μg N L$^{-1}$) on day 1 and 15% above the target enrichment on day 2.

The injection solution was pumped using a fluid metering
pump (FMI Inc.) into the stream at approximately 25 ml min\(^{-1}\) over the duration of each experiment. The injection site was immediately above a constricted, turbulent section of stream that afforded complete mixing of the injected solution prior to the first sampling station 7 m downstream. Samples of water for isotopic and chemical analysis were collected at an upstream station (5 m upstream from the injection site) and at four to seven stations downstream from the injection location 7 to 8 h after the injection began.

Water temperature and samples for \(^{15}\)NO\(_1\), chloride (Cl\(^-\)), NO\(_3\), and ammonium (NH\(_4\)) were collected from the upstream and four downstream stations ranging in distance from 7 to 87 m from the \(^{15}\)N injection location. Single samples were collected from the second and fourth stations and replicate samples from the first and third stations downstream from the \(^{15}\)N injection. All samples were returned to the laboratory and filtered through Whatman GFF glass-fiber filters (nominal pore size = 0.7 \(\mu\)m) within 2 h of collection. Spikes of unlabeled KNO\(_3\) of approximately 200 \(\mu\)g N L\(^{-1}\) (day 1) and 5 mg N L\(^{-1}\) (day 2) were added to 1-liter samples for \(^{15}\)N-NO\(_3\) analysis to reduce \(^{15}\)N:14N ratios to the ideal working range for mass-spectrometric measurement. Identical spikes were also added to 1-liter samples of deionized water to calculate N recovery and determine the \(^{15}\)N:14N ratio of the NO\(_3\) spike. Cl was determined by ion chromatography, NO\(_3\) by automated Cu-Cd reduction followed by azo dye colorimetry, and NH\(_4\) by automated phenate colorimetry, the latter two analyses on a Bran Luebbe auto analyzer 3. The NO\(_3\) measurement is actually NO\(_3\) + NO\(_2\) but NO\(_2\) is assumed to be negligible in this well-oxygenated stream (Mulholland 1992).

Processing of samples for \(^{15}\)N-NO\(_3\) analysis was modified from the method of Sigman et al. (1997). Samples ranging in volume from 0.05 to 1 liter (depending on NO\(_3\) concentration) were added to glass flasks together with 5 g of NaCl and 3 g of MgO. For small samples (high NO\(_3\) concentrations), deionized water was added to bring the initial sample volume to 200 ml. The samples were then brought to a gentle boil on a hot plate until the volume was reduced to about 100 ml, thereby concentrating \(^{15}\)NO\(_3\) and removing NH\(_4\) produced from NH\(_3\) under alkaline conditions. The concentrated samples were then cooled, transferred to 250-ml high density polyethylene bottles, and refrigerated until further processing. The \(^{15}\)NO\(_3\) in the concentrated samples was captured using a reduction/diffusion/sorption procedure as follows. An additional 0.5 g of MgO and 3 g of Devarda’s alloy was added to each sample to reduce all NO\(_3\) to NH\(_4\). A filter packet consisting of a precombusted 1-cm glass-fiber filter (Whatman GFD) to which 25 \(\mu\)l of 2.5 mol L\(^{-1}\) KHSO\(_4\) was added to absorb NH\(_3\), was sealed between two Teflon filters (Millipore white nitex LCWP 25-mm diameter, 10-\(\mu\)m pore size). The filter packet was then immediately placed on the surface of the concentrated sample. Parafilm® placed over the bottle mouth, and the bottle tightly capped. Samples were then heated to 60°C for 2 d and shaken at room temperature for an additional 7 d to allow reduction of NO\(_3\) to NH\(_4\). conversion of NH\(_3\) to NH\(_4\), diffusion of NH\(_3\) into the sample headspace, and absorption of NH\(_3\) onto the GFD filter. The filter packets were then removed from the sample bottles and dried in a desiccator for 2 d, after which the Teflon filter packet was opened and the GFD filter removed. The GFD filters with absorbed NH\(_3\) were encapsulated in tins, placed in a 96-well titer plate with each well capped, and sent to the stable isotope laboratory at the University of California at Davis (http://stableisotopefacility.ucdavis.edu) for \(^{15}\)N:14N ratio analysis by mass spectrometry using a Europa Integra continuous flow isotope mass spectrometer (IRMS) coupled to an in-line elemental analyzer for automated sample combustion.

Samples for \(^{15}\)N analysis of dissolved N\(_2\) and N\(_2O\) were collected from the upstream station and 7 m downstream from the \(^{15}\)N injection site. At each station, 50 ml of stream water was collected in 60-ml plastic syringes (Becton-Dickinson 60-ml disposable syringes) and all visible air bubbles were expelled. The samples were then kept submerged in stream water after collection until the headspace equilibration was performed (within 30 min). Duplicate samples were collected from the first four stations downstream from the \(^{15}\)N injection, while single samples were collected from the other stations. After all samples were collected, needles were affixed to the syringes, 5 ml of water expelled (leaving a sample volume of 45 ml), and 15 ml of high purity helium was added to each syringe from a gas bag submerged in stream water to minimize air contamination. Samples were then shaken gently for 15 min while submerged in stream water to allow for equilibration of dissolved gases between water and the He headspace. Approximately 13 ml of headspace gas was then injected into preevacuated 12-ml exetainers (Labco, evacuated/labeled type 3 screw-cap with septa) while the syringe and exetainer were submerged in a large bucket filled with stream water. A bead of silicone sealant was placed over the exetainer septa and the samples shipped to the stable isotope laboratory at the University of California, Davis, for \(^{15}\)N:14N ratio analysis by mass spectrometry using a Europa Hydra Model 20/20 continuous flow IRMS. These analyses were performed within 3 weeks of sample collection.

Six additional samples for \(^{15}\)N analysis of N\(_2\) were collected during the NO\(_3\) addition experiment on day 2 (three samples from the upstream station and three samples from a station 28 m downstream from the \(^{15}\)NO\(_3\) injection). Water samples were collected in evacuated 150-ml glass bulbs until approximately one-half full by submerging the bulb in the stream and opening the stopcock. The glass bulbs contained 1 ml of a saturated HgCl solution to prevent the microbial transformation of N after sample collection. On a vacuum line, the headspace gas was cryogenically separated to remove water vapor and CO\(_2\), then collected in a Pyrex tube containing silica gel and copper oxide. Once the tube was sealed, it was then combusted to remove oxygen and analyzed on a Finnigan MAT Delta S mass spectrometer for \(^{15}\)N:14N ratios at the Marine Biological Laboratory, Woods Hole, Massachusetts. Nitrogen:argon ratios were also recorded from the mass-spectrometer analysis and used to check for atmospheric N\(_2\) contamination of the samples. Samples were analyzed within 2 months of collection.

Measurements of \(^{15}\)N:14N ratio are expressed as \(\delta^{15}\)N values (units of \(\%\)) according to the following equation:
\[ \delta^{15}N = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000 \]  

(1)

where \( R_{\text{sample}} \) is the \(^{15}\text{N}:^{14}\text{N} \) ratio in the sample and \( R_{\text{standard}} \) is the \(^{15}\text{N}:^{14}\text{N} \) ratio in atmospheric \( \text{N}_2 \) (\( R_{\text{standard}} = 0.0036765 \)).

We measured air–water gas exchange rates using a propane/conservative tracer injection method (sensu Marzolf et al. 1994) the day following the \(^{15}\text{NO}_3^-\) additions (day 3) under similar discharge rates. Propane and a conservative tracer (100 g L\(^{-1}\) NaCl solution) were injected simultaneously at constant rates. Propane was injected using a large bubble diffuser and NaCl using the same pump used for the \(^{15}\text{NO}_3^-\) injection. Water samples for dissolved propane were collected at two stations (7 and 43 m) downstream from the injection site approximately 3 h after the injection began. Six 45-mL water samples were collected in 60-mL polyethylene syringes at each station, and 10 mL of headspace air was added to each syringe. Samples were immediately returned to the laboratory, shaken gently for 2 h, and a subsample of the headspace was removed and analyzed for propane by gas chromatography using a flame ionization detector. Propane readings were normalized to the increase in conservative tracer concentration at each station determined as the increase in specific conductance using a YSI Model 30 field conductivity meter (Yellow Springs Instrument Co.) to account for dilution due to groundwater input.

The gas exchange rate for propane (\( k_p \), m\(^{-1}\)) was calculated as the slope of a regression of the natural log of conservative tracer-normalized propane readings versus distance.

Gas exchange rates of \( \text{N}_2 \) and \( \text{N}_2 \text{O} \) were calculated from the measured values of \( k_p \) using the relative values of their Schmidt numbers (Sc). Gas transfer rates of two gases, denoted as \( k_\alpha \) and \( k_p \), can be related through the Schmidt number \( \text{Sc} \), defined as the kinematic viscosity of the water divided by the diffusion coefficient of the gas (MacIntyre et al. 1995):

\[ k_\alpha = k_p \left( \frac{\text{Sc}_\alpha}{\text{Sc}_p} \right)^x \]  

(2)

where \( x \) is the Schmidt number dependence that ranges between \(-2/3\) for smooth water surfaces and \(-1/2\) for rough surfaces (Jähne et al. 1987). For streams, the value of \( x \) is likely to be about \(-1/2\) (Wanninkhof et al. 1990; MacIntyre et al. 1995). Because at a given temperature the kinematic viscosity of water is the same in the numerator and denominator of Eq. 2, we can substitute the diffusion coefficients \( D_\alpha \) and \( D_p \) for the Schmidt numbers. Using Eq. 2 and data from table A1 in Wanninkhof (1992) based on \( \text{N}_2 \) and \( \text{N}_2 \text{O} \) diffusion coefficients determined by Jähne et al. (1987) and a propane diffusion coefficient determined by Wise and Houghton (1966), we calculated that the air–water exchange rates of \( \text{N}_2 \) and \( \text{N}_2 \text{O} \) were 0.98 and 0.96 times the measured values of \( k_p \). These relationships appear to be essentially independent of temperature over the range experienced by temperate streams (Rainwater and Holley 1984).

**Calculations of tracer \(^{15}\text{N} \) flux**—Tracer \(^{15}\text{N} \) flux was calculated from the measured \( \delta^{15} \)N values by first converting all \( \delta^{15} \)N values to \(^{15}\text{N}/(^{15}\text{N} + ^{14}\text{N}) \) ratios using the following equation:

\[ \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} = \left( \frac{\delta^{15} \text{N}}{1,000} + 1 \right) \times 0.0036765 \]  

(3)

Hereafter we will call \(^{15}\text{N}/(^{15}\text{N} + ^{14}\text{N}) \) the isotopic mole fraction of \(^{15}\text{N} \) (MF).

We then corrected \(^{15}\text{NO}_3^-\) MF values for the added nitrate spike using the following equation:

\[ \text{MF}_i = \left( \left( [\text{NO}_3^- - N_i] + [\text{NO}_3^- - N_{sp}] \right) \text{MF}_{iu} \right) - [\text{NO}_3^- - N_{sp})] / [\text{NO}_3^- - N_i] \]  

(4)

where \([\text{NO}_3^- - N_i] \) is the measured nitrate N concentration at station \( i \) (\( \mu g \text{ N L}^{-1} \)), \([\text{NO}_3^- - N_{sp}] \) is the nitrate N concentration increase resulting from the nitrate spike (\( \mu g \text{ N L}^{-1} \), same for all stations), \( \text{MF}_{iu} \) is the MF at station \( i \) calculated from the measured \( \delta^{15} \)N values on spiked samples from station \( i \) using Eq. 3, \( \text{MF}_{sp} \) is the MF of the nitrate spike calculated from the measured \( \delta^{15} \)N values of nitrate in the deionized water samples that also received the nitrate spike, and \( \text{MF}_i \) is the true MF of nitrate at station \( i \).

We then computed total \(^{15}\text{NO}_3^-\) mass flux at each station \( i \) (\(^{15}\text{N}_{\text{flux}, i}\), units of \( \mu g \text{ s}^{-1} \)) by multiplying \( \text{MF}_i \) by the streamwater nitrate concentration ([\( \text{NO}_3^- \) - \( N_i \)]) and stream discharge \( (Q_i) \) at each station \( i \) as follows:

\[ ^{15}\text{N}_{\text{flux}, i} = \text{MF}_i \times [\text{NO}_3^- - N_i] \times Q_i \]  

(5)

Stream discharge at each station \( (Q_i) \) was determined from the increase in streamwater \( \text{Cl}^- \) concentration during the injection as follows:

\[ Q_i = ([\text{Cl}_i] - Q_{\text{pump}})/([\text{Cl}_i] - [\text{Cl}_b]) \]  

(6)

where the \( \text{Cl}^- \) injection rate (\( \mu g \text{ s}^{-1} \)) was determined as the product of the \( \text{Cl}^- \) concentration in the injection solution ([\( \text{Cl}_i \)]) and the solution injection rate \( \left( Q_{\text{pump}} \right) \), and the increase in \( \text{Cl}^- \) concentration at each station \( i \) is the difference between [\( \text{Cl}_i \)] during the injection ([\( \text{Cl}_i \)]) and the measured \( \text{Cl}^- \) concentration just prior to the \(^{15}\text{N} \) injection (i.e., background concentration, [\( \text{Cl}_b \)]).

Finally, we computed tracer \(^{15}\text{NO}_3^-\) mass flux at each station \( i \) by subtracting background \(^{15}\text{NO}_3^-\) mass flux from the total \(^{15}\text{NO}_3^-\) mass flux. Background \(^{15}\text{NO}_3^-\) mass flux at each station \( i \) was calculated using Eq. 5 except that the MF determined for the station upstream from the \(^{15}\text{N} \) addition was used instead of the measured value of \( \text{MF}_i \).

For \(^{15}\text{N}_i \) and \(^{15}\text{NO}_3^-\) we first corrected the measured headspace \( \delta^{15} \)N values for equilibrium isotopic fractionation during the headspace equilibration using the estimated masses of \( N \) in the gas and liquid phases of the equilibration system and the following equation:

\[ \delta^{15}N_{i.e} = \delta^{15}N_{i} - \varepsilon \]  

(7)

where \( \delta^{15}N_{i.e} \) is the fractionation-corrected \( \delta^{15} \)N value, \( \delta^{15}N_{i} \) is the measured \( \delta^{15} \)N value, and \( \varepsilon \) is the isotopic enrichment factor for \( \text{N}_2 \) (\(-0.85\%_\varepsilon\); Klots and Benson 1963) and \( \text{N}_2 \text{O} \).
(−0.75% of Inoue and Mook 1994), respectively. The concentrations of N₂ and N₂O were calculated from N mass values determined in the headspace as part of the mass-spectrometric analysis and corrected for incomplete gas transfer into the headspace using the relative volumes of water and headspace and the Bunsen coefficients for N₂ and N₂O at the temperature and pressure at which the headspace equilibration was performed. MF, values for each gas were then calculated using Eq. 3 except that δ¹⁵N values were used instead of the measured δ¹⁴N values. The fluxes of total ¹⁵N in N₂ and N₂O at each station i were calculated from the MF, values, the N₂ and N₂O concentrations in stream water, and stream discharge using Eq. 5. The stream discharge at each station was determined as described above (Eq. 6) and interpolated for stations at which ¹⁵N₂ and ¹⁵N₂O samples but no ¹⁵NO samples were collected. Finally, fluxes of tracer ¹⁵N in N₂ and N₂O at each station i were determined by subtracting the background ¹⁵N flux at that station (calculated by Eq. 5 but using the MF determined for the station upstream from the ¹⁵N addition rather than MF) from the total ¹⁵N flux.

Calculation of NO₃⁻ nitrate uptake rate and length—The total uptake rate of NO₃⁻, expressed as a fractional uptake rate per unit distance (k₂), was calculated from the regression of ln (tracer ¹⁵NO₃⁻ flux) versus distance from the ¹⁵N injection for each injection. The inverse of the slope of these regressions is the uptake length of NO₃⁻ (S_W; Newbold et al. 1981; Stream Solute Workshop 1990). The total NO₃⁻ uptake rate was also calculated as a mass removal rate from water per unit area (U) using the following equation:

\[
U = \frac{F \times k_{\text{tot}}}{w}
\]

where \( F \) is the average flux of NO₃⁻·N in streamwater in the experimental reach (determined as the product of average NO₃⁻·N concentration and average discharge) and \( w \) is the average stream wetted width (Newbold et al. 1981). Total NO₃⁻ uptake rate was also calculated as a mass transfer velocity (V_f) using the following equation (Stream Solute Workshop 1990):

\[
V_f = \frac{U}{C}
\]

where \( C \) is the average stream NO₃⁻ concentration.

Determination of N₂ and N₂O production rates via denitrification—Production rates of N₂ and N₂O (considered separately) were estimated by fitting a model of N gas production to the longitudinal pattern in the fluxes of tracer ¹⁵N as N₂ and N₂O over the study reach. The model simulates N₂ and N₂O production from NO₃⁻ (k_{den}), air–water exchange (k₂) of N₂ and N₂O, and the assimilative uptake of NO₃⁻ (k_U) in a 1-m reach of stream (Fig. 1). Change in tracer ¹⁵N fluxes with distance x are expressed as follows:

\[
\frac{\partial^{15}\text{NO}_3}{\partial x} = -(k_{\text{den}} + k_U)^{15}\text{NO}_3
\]

\[
\frac{\partial^{15}\text{N}_{\text{gas}}}{\partial x} = k_{\text{den}}^{15}\text{NO}_3 - k_2^{15}\text{N}_{\text{gas}}
\]

where \(^{15}\text{NO}_3\) is the tracer ¹⁵N in NO₃⁻ and \(^{15}\text{N}_{\text{gas}}\) is the tracer ¹⁵N in N₂ or N₂O. The fractional total uptake rate of NO₃⁻ (k_{tot}) is the sum of denitrification and assimilatory uptake (i.e., k_{den} + k_U). The steady state solutions for \(^{15}\text{NO}_3\) and \(^{15}\text{N}_{\text{gas}}\) are

\[
^{15}\text{NO}_3 = (^{15}\text{NO}_3)_0 \times e^{-(k_{\text{den}} + k_U)x}
\]

\[
^{15}\text{N}_{\text{gas}} = \frac{k_{\text{den}}^{15}\text{NO}_3}_0}{k_2 - k_{\text{den}} - k_U} \times (e^{-(k_{\text{den}} + k_U)x} - e^{-k_2x})
\]

where \((^{15}\text{NO}_3)_0\) is the tracer ¹⁵NO₃ flux at the injection site (x = 0).

We used a least-squares fitting procedure in conjunction with a spreadsheet model of Eq. 13 (using Microsoft Excel optimization tool “Solver”; Microsoft) to estimate values of k_{den} from the tracer ¹⁵N mass flux data for N₂ and N₂O separately (¹⁵N_{gas}). Values of k_{tot} were determined from the regression of ln (tracer ¹⁵NO₃ flux) versus distance as described above. Values of k₂ were determined from the measured rates of propane gas exchange converted to N₂ and N₂O exchange rates using Eq. 2. Denitrification rates were also calculated as NO₃⁻ mass removal rates per unit area (DN) for N₂ and N₂O production separately using Eq. 8 with k_{den} substituted for k_{tot}.

Figure 2 presents model simulations of tracer ¹⁸N_{gas} flux for a stream with a NO₃⁻ uptake length of 50 m, equivalent to a fractional total uptake rate (k_{tot} = k_U + k_{den}) of 0.02 m⁻¹, and three different combinations of the gas exchange rate (k₂) and denitrification rate (k_{den}). The tracer ¹⁵N_{gas} flux curve is hump shaped because of the longitudinal decline in labeled substrate (i.e., tracer ¹⁵NO₃) available for denitrification. The maximum tracer ¹⁵N_{gas} flux is primarily dependent on the value of k_{den} relative to k₂ (higher for high k_{den} relative to k₂). The distance at which maximum tracer ¹⁵N_{gas} flux occurs is dependent on the value of k₂ relative to k_{tot} (e.g., shorter for high k₂ relative to k_{tot}).
as a mass transfer velocity ($V_f$) declined about threefold when NO$_3^-$ concentrations were increased compared with ambient conditions (Fig. 3C,D; Table 2). This resulted in a threefold increase in NO$_3^-$ uptake length ($S_{\text{ct}}$) with NO$_3^-$ addition. However, the total mass removal rate of NO$_3^-$ from water per unit area ($U$) increased more than fivefold with NO$_3^-$ addition (Table 2), indicating that total NO$_3^-$ uptake from stream water was stimulated by NO$_3^-$ addition.

There was evidence that the $^{15}$N$_2$ samples were contaminated with atmospheric N$_2$. The headspace N$_2$ mass determined for samples collected during the ambient experiment ranged from 100 to 160 µmol, with the exception of one sample with 220 µmol. The N$_2$ mass determined for samples during the NO$_3^-$ addition experiment ranged from 180 to 220 µmol, with the exception of two samples with 290 and 320 µmol. The N$_2$ mass expected in our headspace samples for water in equilibrium with the atmosphere is about 20 µmol; thus, our samples contained 5 to 16 times more N$_2$ than expected. Because it is unlikely that the stream water was more than a few percent supersaturated with N$_2$, the high N$_2$ mass values measured were probably the result of inadvertent contamination by atmospheric N$_2$, possibly introduced during sampling, sample equilibration, or storage of the etainers. The excess N$_2$ would dilute the tracer $^{15}$N in the sample and lead to smaller background-corrected MF values. The three samples with the highest levels of air contamination relative to the others for that experiment were not included in subsequent analyses. Although important for N$_2$, these levels of air contamination are relatively unimportant for N$_2$O, which exists at only trace levels in air.

Values of $^{15}$N MF for N$_2$ and N$_2$O generally exhibited hump-shaped distributions with distance (Fig. 4A,B). $^{15}$N MF values for N$_2$O were considerably higher and the spatial distribution of the data more consistent than for N$_2$, a result of the much lower mass of N$_2$O (ranging from 0.13 to 0.20 nmol) that permitted detection of a much larger tracer $^{15}$N signal in our samples. There was little difference in the $^{15}$N MF values for N$_2$ between experiments; however, the MF values for N$_2$O were considerably higher during the ambient NO$_3^-$ addition than during the ambient NO$_3^-$ experiment.

Measurements of $^{15}$N-N$_2$ in the evacuated glass bulb samples collected during the NO$_3^-$ addition experiment at the upstream and 28-m stations indicated substantial enrichment

Table 1. Conditions during the $^{15}$N tracer addition experiments in the East Fork of Walker Branch. Values for discharge, water temperature, and N and Cl concentrations are averages for the study reach (mean of values measured at 7, 28, 57, and 87 m) during the experiments (range is given in parentheses). Average wetted width is the mean of measurements made at 1-m intervals along the study reach (range in parentheses). The values for NO$_3^-$ concentration include the effect of a small increase in concentration due to the $^{15}$NO$_3^-$ addition (about 15%). The ambient experiment values given below are typical for this stream during this time of year.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ambient NO$_3^-$ experiment (2 October 2002)</th>
<th>NO$_3^-$ addition experiment (3 October 2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge (L s$^{-1}$)</td>
<td>0.4 (0.3–0.7)</td>
<td>0.4 (0.3–0.6)</td>
</tr>
<tr>
<td>Average wetted width (m)</td>
<td>0.92 (0.6–2.0)</td>
<td>0.92 (0.6–2.0)</td>
</tr>
<tr>
<td>Average water velocity (cm s$^{-1}$)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>20.0 (19.5–20.5)</td>
<td>20.1 (19.5–20.7)</td>
</tr>
<tr>
<td>NO$_3^-$ concentration (µg N L$^{-1}$)</td>
<td>26 (19.6–39.8)</td>
<td>380 (166–580)</td>
</tr>
<tr>
<td>NH$_4^+$ concentration (µg N L$^{-1}$)</td>
<td>4 (1.8–8)</td>
<td>5 (3.8–7.8)</td>
</tr>
<tr>
<td>Cl$^-$ concentration (mg L$^{-1}$)</td>
<td>1.1 (1.09–1.15)</td>
<td>1.1 (1.09–1.15)</td>
</tr>
</tbody>
</table>
Stream denitrification and nitrate uptake

Table 2. Total NO\textsubscript{3} uptake rates and denitrification rates (N\textsubscript{2} and N\textsubscript{2}O production separately and in total) presented as fractional uptake rates ($k$), mass flux rates per unit area ($U$ and DN), and mass transfer velocities ($V_f$). Uptake lengths for NO\textsubscript{3} ($S_W$) based on total NO\textsubscript{3} uptake and denitrification (sum of N\textsubscript{2} and N\textsubscript{2}O production rates) are also given.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ambient NO\textsubscript{3} experiment</th>
<th>NO\textsubscript{3} addition experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{tot}}$ (m\textsuperscript{-1})</td>
<td>0.028</td>
<td>0.010</td>
</tr>
<tr>
<td>$S_W$ (m)</td>
<td>35.7</td>
<td>100</td>
</tr>
<tr>
<td>$U$ (\mu g N m\textsuperscript{-2} s\textsuperscript{-1})</td>
<td>0.32</td>
<td>1.65</td>
</tr>
<tr>
<td>$V_f$ (m h\textsuperscript{-1})</td>
<td>0.044</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Fig. 3. \textsuperscript{15}N-NO\textsubscript{3} MF values for the upstream station (−5 m, triangles) and four stations downstream (circles) from the \textsuperscript{15}N addition location for the (A) ambient NO\textsubscript{3} and (B) NO\textsubscript{3} addition experiments. MF values of replicate samples at 7 and 57 m were within 0.0002 of each other and appear as one point on these plots. Also shown are plots of ln (tracer \textsuperscript{15}NO\textsubscript{3} flux) versus distance downstream from the \textsuperscript{15}NO\textsubscript{3} addition for the (C) ambient and (D) NO\textsubscript{3} addition experiments. The slopes of the regression lines are the total fractional NO\textsubscript{3} uptake rates ($k_{\text{tot}}$) and the inverse of the slopes are the uptake lengths for NO\textsubscript{3} ($S_W$). Fluxes calculated from replicate samples collected from the 7- and 57-m stations during both experiments differed by <3%, and the mean values at these stations were used in the regressions.

The air–water exchange rate of propane was 0.056 m\textsuperscript{-1} (95% CI: 0.048 to 0.064 m\textsuperscript{-1}). This measurement was for the reach from 7 to 43 m and was similar to a propane exchange rate of 0.051 m\textsuperscript{-1} measured for the reach from 7 to 25 m downstream from the \textsuperscript{15}N addition. The mean \textsuperscript{15}N-N\textsubscript{2} MF at the 28-m station was 3.678 × 10\textsuperscript{-3} (SD = 0.002 × 10\textsuperscript{-3}, n = 3), significantly higher than the mean \textsuperscript{15}N-N\textsubscript{2} MF at the upstream station (3.666 × 10\textsuperscript{-3}, SD = 0.001 × 10\textsuperscript{-3}, n = 3, p = 0.004). In addition, the mean \textsuperscript{15}N-N\textsubscript{2} MF for the bulb samples at 28 m was about 0.006 higher than the MF determined using the headspace equilibration and exetainer gas storage method at the same station and time. Based on N\textsubscript{2}/Ar ratios measured in the bulb samples, there appeared to be little if any contamination of these samples by atmospheric N\textsubscript{2} (Suzanne Thomas, Marine Biological Laboratory, unpubl. data). Thus, the higher \textsuperscript{15}N-N\textsubscript{2} MF for the bulb sample compared with the \textsuperscript{15}N-N\textsubscript{2} MF for the exetainer sample appears to be the result of dilution of tracer \textsuperscript{15}N due to atmospheric N\textsubscript{2} contamination in the latter. The air–water exchange rate of propane was 0.056 m\textsuperscript{-1} (95% CI: 0.048 to 0.064 m\textsuperscript{-1}). This measurement was for the reach from 7 to 43 m and was similar to a propane exchange rate of 0.051 m\textsuperscript{-1} measured for the reach from 7 to 25 m downstream from the \textsuperscript{15}N addition. The mean \textsuperscript{15}N-N\textsubscript{2} MF at the 28-m station was 3.678 × 10\textsuperscript{-3} (SD = 0.002 × 10\textsuperscript{-3}, n = 3), significantly higher than the mean \textsuperscript{15}N-N\textsubscript{2} MF at the upstream station (3.666 × 10\textsuperscript{-3}, SD = 0.001 × 10\textsuperscript{-3}, n = 3, p = 0.004). In addition, the mean \textsuperscript{15}N-N\textsubscript{2} MF for the bulb samples at 28 m was about 0.006 higher than the MF determined using the headspace equilibration and exetainer gas storage method at the same station and time. Based on N\textsubscript{2}/Ar ratios measured in the bulb samples, there appeared to be little if any contamination of these samples by atmospheric N\textsubscript{2} (Suzanne Thomas, Marine Biological Laboratory, unpubl. data). Thus, the higher \textsuperscript{15}N-N\textsubscript{2} MF for the bulb sample compared with the \textsuperscript{15}N-N\textsubscript{2} MF for the exetainer sample appears to be the result of dilution of tracer \textsuperscript{15}N due to atmospheric N\textsubscript{2} contamination in the latter. The air–water exchange rate of propane was 0.056 m\textsuperscript{-1} (95% CI: 0.048 to 0.064 m\textsuperscript{-1}). This measurement was for the reach from 7 to 43 m and was similar to a propane exchange rate of 0.051 m\textsuperscript{-1} measured for the reach from 7 to 25 m downstream from the \textsuperscript{15}N addition.
Denitrification rates \( (k_{\text{den}}) \) were determined by fitting the denitrification model to the tracer \(^{15}\text{N}_2\) flux data for \( \text{N}_2 \) and \( \text{N}_2\text{O} \) production separately. The best-fit \( N_2 \), \( k_{\text{den}} \) was 0.0046 m\(^{-1}\) under ambient \( \text{NO}_3^- \) concentrations, representing about 16% of \( k_{\text{den}} \) (Fig. 5A; Table 2). \( N_2 \) \( k_{\text{den}} \) declined to \( 8.8 \times 10^{-5} \) m\(^{-1}\), or about 1% of \( k_{\text{den}} \) when \( \text{NO}_3^- \) concentration was increased on day 2 (Fig. 5B; Table 2). The mass flux rate of \( \text{N}_2 \) production per unit area (DN-\( \text{N}_2\)) was nearly threefold higher under ambient \( \text{NO}_3^- \) concentrations than under \( \text{NO}_3^- \) addition (Table 2). \( \text{N}_2\text{O} \) \( k_{\text{den}} \) values were considerably lower than \( N_2 \) \( k_{\text{den}} \) values, accounting for about 0.02% of \( k_{\text{den}} \) under ambient \( \text{NO}_3^- \) concentrations (Fig. 6A; Table 2) and about 0.03% of \( k_{\text{den}} \) when \( \text{NO}_3^- \) concentration was increased (Fig. 6B; Table 2). The mass flux rate of \( \text{N}_2\text{O} \) production per unit area (DN-\( \text{N}_2\text{O}\)) was about sixfold higher under \( \text{NO}_3^- \) than under ambient \( \text{NO}_3^- \) concentration (Table 2).

To provide an estimate of the impact of gas exchange rates on the uncertainty in the rates of \( k_{\text{den}} \) for \( \text{N}_2 \) and \( \text{N}_2\text{O} \) production, the denitrification model was fit to the data using values of \( k_{\text{den}} \) (\( \text{N}_2 \) gas exchange rate) one-half and two times the values of \( k_{\text{den}} \) determined from the air–water exchange rates of propane. As a further analysis of uncertainty, we determined the values of \( k_{\text{den}} \) required to bound the majority of tracer \(^{15}\text{N}_2\) and \(^{15}\text{N}_2\text{O}\) flux data points for each experiment. These simulations are shown in Figs. 5 and 6, and the \( k_{\text{den}} \) values are summarized in Table 3. Based on this uncertainty analysis, \( k_{\text{den}} \) for \( \text{N}_2 \) production is constrained to range from 0.002 to 0.008 m\(^{-1}\) for the ambient \( \text{NO}_3^- \) experiment and from 3 to 21 \( \times 10^{-5} \) m\(^{-1}\) for the \( \text{NO}_3^- \) addition experiment. Similarly, \( k_{\text{den}} \) for \( \text{N}_2\text{O} \) production is constrained...
Table 3. Results of uncertainty analysis of denitrification rate ($k_{\text{out}}$) for N$_2$ and N$_2$O production for the ambient and NO$_3^-$ addition experiments. Results are shown for the least-squares fit of the denitrification model to the tracer $^{15}$N flux data using the air–water gas exchange rates ($k_g$) calculated from the mean gas exchange rate of propane. Results are also shown for two other scenarios to assess uncertainty: (1) the least-squares fit of the denitrification model to the tracer $^{15}$N flux data using values of $k_g$ approximately one-half and 2 times the measured $k_g$ value and (2) the minimum and maximum $k_{\text{out}}$ values necessary to bound the majority of the data points (see Figs. 5 and 6). The second maximum $k_{\text{out}}$ value listed for N$_2$ production for the NO$_3^-$ addition experiment is the model fit to the bulb sample (open data point in Fig. 5B).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ambient</th>
<th>NO$_3^-$ addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_2$ production:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best model fit ($k_g = 0.055$ m$^{-1}$)</td>
<td>0.0046</td>
<td>8.8x10^{-6}</td>
</tr>
<tr>
<td>Uncertainty scenario 1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 $k_g$ (0.03 m$^{-1}$)</td>
<td>0.0031</td>
<td>5.5x10^{-6}</td>
</tr>
<tr>
<td>2 $k_g$ (0.11 m$^{-1}$)</td>
<td>0.0075</td>
<td>15.7x10^{-6}</td>
</tr>
<tr>
<td>Uncertainty scenario 2:</td>
<td></td>
<td></td>
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<tr>
<td>Minimum</td>
<td>0.0017</td>
<td>3x10^{-6}</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.0080</td>
<td>16x10^{-6}</td>
</tr>
<tr>
<td>Maximum (bulb sample)</td>
<td>—</td>
<td>21x10^{-6}</td>
</tr>
<tr>
<td>N$_2$O production:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best model fit ($k_g = 0.054$ m$^{-1}$)</td>
<td>6.8x10^{-6}</td>
<td>3.0x10^{-6}</td>
</tr>
<tr>
<td>Uncertainty scenario 1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 $k_g$ (0.03 m$^{-1}$)</td>
<td>4.6x10^{-6}</td>
<td>1.9x10^{-6}</td>
</tr>
<tr>
<td>2 $k_g$ (0.11 m$^{-1}$)</td>
<td>10.8x10^{-6}</td>
<td>5.3x10^{-6}</td>
</tr>
<tr>
<td>Uncertainty scenario 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>3.5x10^{-6}</td>
<td>2.0x10^{-6}</td>
</tr>
<tr>
<td>Maximum</td>
<td>11.0x10^{-6}</td>
<td>3.5x10^{-6}</td>
</tr>
</tbody>
</table>

1$^5$N tracer addition approach (Peterson et al. 2001; Webster et al. 2003). The high $k_{\text{out}}$ and short $S_w$ were primarily due to the low discharge, low average water velocity, and low water depth in the East Fork, which together enhance the contact time of stream water with sediments and biofilms where N uptake takes place. Total NO$_3^-$ mass removal rate per unit area ($U$) and mass transfer velocity ($V_t$) in the East Fork under ambient NO$_3^-$ concentrations were in the lower portion of the range reported for the streams in the Peterson et al. (2001) study, in part reflecting the low concentrations of NO$_3^-$ characteristic of this stream.

Short-term (several hours) NO$_3^-$ addition experiments also have been used to estimate NO$_3^-$ uptake lengths and rates in streams (e.g., Munn and Meyer 1990; Valett et al. 1996; Martí et al. 1997). Our value of $S_w$ for the East Fork of Walker Branch under ambient NO$_3^-$ was considerably shorter than values reported for other streams using the NO$_3^-$ solute addition approach (generally 100 to >1,000 m). Although the relatively low discharge of the East Fork certainly accounts for some of these differences, methodological differences are also important. Mulholland et al. (2002) have shown that the nutrient addition approach results in overestimates of nutrient uptake length, with the magnitude of the overestimate a function of the degree of nutrient limitation and the magnitude of the nutrient addition. It may be possible to use a graphical extrapolation technique that involves extrapolation of uptake length–nutrient addition level relationships determined from nutrient addition experiments to obtain accurate estimates of uptake length, although this approach involves either multiple addition experiments under similar conditions or multiple sampling locations and the assumption of longitudinal homogeneity in stream conditions affecting nutrient uptake (Rob Payn and Jack Webster pers. comm.). Where feasible, however, the tracer addition approach such as used here with $^{15}$NO$_3^-$ is the most accurate and straightforward method for determining nutrient uptake length and uptake rate under ambient conditions in streams.

**Denitrification rates**—Our reach-scale, field $^{15}$N addition and modeling approach indicated that the denitrification rate (expressed as a fractional NO$_3^-$ removal rate, $k_{\text{out}}$) in the East Fork of Walker Branch under ambient NO$_3^-$ concentrations was 0.0046 m$^{-1}$, with an uncertainty of about ±0.003 m$^{-1}$. Thus, denitrification represented about 16% of the total NO$_3^-$ removal rate from stream water under ambient conditions, with an uncertainty of about ±10%. On a mass flux per unit area basis, the denitrification rate was 0.045 µg N m$^{-2}$ s$^{-1}$, with an uncertainty of about ±0.03 µg N m$^{-2}$ s$^{-1}$ under ambient NO$_3^-$ concentrations. Denitrification consisted almost entirely of N$_2$ production (>99%), with very little N$_2$O production occurring.

Our uncertainty analysis included varying the air–water gas exchange rate ($k_g$) by a factor of 2 and determining the denitrification rates ($k_{\text{out}}$) necessary to bound most of the data points (Figs. 5 and 6). We believe that this analysis provides reasonable outer bounds on the uncertainty in the denitrification rate. The atmospheric N$_2$ contamination of our samples likely was the source of a considerable amount of the relatively high variability in the tracer $^{15}$N$_2$ flux data compared with the longitudinal pattern expected (Fig. 5). Sub-

to range from 3.5 to 11 × 10$^{-6}$ m$^{-1}$ for the ambient NO$_3^-$ experiment and from 1.9 to 5.3 × 10$^{-6}$ m$^{-1}$ for the NO$_3^-$ addition experiment. For the ambient and NO$_3^-$ addition experiments, respectively, the constrained ranges in N$_2$ production were 6.1% to 28.6% and 0.3% to 1.6% of $k_{\text{out}}$. Similarly, N$_2$O production was 0.01% to 0.04% and 0.02% to 0.05% of $k_{\text{out}}$ for the ambient and NO$_3^-$ addition experiments, respectively.

The uptake length of NO$_3^-$ resulting from the total denitrification rate (sum of N$_2$ and N$_2$O production rates) was 217 m under ambient NO$_3^-$ concentrations but increased to nearly 11 km when NO$_3^-$ concentration was increased (Table 2). Based on the uncertainty analysis, the total denitrification-based NO$_3^-$ uptake length is constrained to range from about 125 to 600 m for the ambient NO$_3^-$ experiment and from about 5 to 32 km with NO$_3^-$ addition.

**Discussion**

**Total NO$_3^-$ uptake rates**—Total NO$_3^-$ uptake rate per unit distance ($k_{\text{out}}$) measured under ambient conditions in the East Fork of Walker Branch was among the highest and the NO$_3^-$ uptake length ($S_w$) among the shortest values of these parameters reported for a number of small streams using the
sequent testing has implicated background N\textsubscript{i} in the extainers prior to use as the likely source of most of the contamination (S. Hamilton unpubl. data). This might be avoided by reevacuating the extainers and storing them under water prior to use. Nonetheless, our longitudinal \textsuperscript{15}N data clearly indicate the presence of tracer \textsuperscript{15}N in the dissolved N\textsubscript{i} and N\textsubscript{2}O pools in stream water, and the longitudinal pattern showing a hump-shaped distribution of tracer \textsuperscript{15}N flux in these pools is consistent with theoretical simulations using the denitrification model (Fig. 2).

The nearly sixfold increase in NO\textsubscript{3} mass removal rate from water per unit area (U) with the approximately tenfold increase in NO\textsubscript{3} concentration during the NO\textsubscript{3} addition experiment appeared to be almost entirely the result of stimulation of assimilatory NO\textsubscript{3} uptake. Denitrification rate expressed as a mass flux per unit area (DN) unexpectedly declined during the NO\textsubscript{3} addition experiment relative to the ambient NO\textsubscript{3} experiment (Table 2). A previous study using the C\textsubscript{2}H\textsubscript{2} inhibition technique on Walker Branch sediments incubated in the laboratory suggested that denitrification was NO\textsubscript{3} limited (Martin et al. 2001). Consequently, we had expected to observe an increase in the mass flux denitrification rate with NO\textsubscript{3} addition. The upper bound estimate of fractional denitrification rate for the NO\textsubscript{3} addition experiment (21 $\times$ 10^-3 m\textsuperscript{-1}; Table 3) translates to a mass flux denitrification rate that is about 70\% of the ambient mass flux rate, so it is possible that the mass flux denitrification rate did not change much with NO\textsubscript{3} addition. Regardless, our results suggest that denitrifiers were not capable of responding rapidly (within hours) to increases in streamwater NO\textsubscript{3} in the field, as might occur during storms or other transient events, possibly due to carbon limitation or some other constraint. Carbon limitation may have been particularly strong at the time of this study because of low streamwater dissolved organic carbon concentrations (approximately 0.5 mg L\textsuperscript{-1}), low algal production rates (due to low light levels below the dense forest canopy), and low standing stocks of leaf detritus prior to the onset of autumn leaf fall.

The very low N\textsubscript{2}O production rate relative to N\textsubscript{2} production rate in the East Fork of Walker Branch (N\textsubscript{2}O/N\textsubscript{2} production ratios of 0.0015 and 0.032 for the ambient and NO\textsubscript{3} addition experiments, respectively) are consistent with many previous studies of aquatic sediments. For example, in a survey of N\textsubscript{2}O/N\textsubscript{2} production ratios reported for river, lake, and coastal marine sediments, Seitzinger (1988) reported that N\textsubscript{2}O/N\textsubscript{2} production ratios were generally <0.05 and often <0.01. The ratios reported by Seitzinger also may be high when considering only denitrification because N\textsubscript{2}O is also produced during nitrification and may account for some of the N\textsubscript{2}O production in these studies. The relative proportions of N\textsubscript{2}O and N\textsubscript{2} produced via denitrification are related to pH, oxygen, and H\textsubscript{2}S concentrations, with higher N\textsubscript{2}O production under more acid conditions or higher dissolved oxygen and H\textsubscript{2}S concentrations (Seitzinger 1988). Recent work on soils suggests that the percentage water-filled pore space, which is proportional to the extent of anoxic conditions within the soil matrix, is a predictor of the N\textsubscript{2}O/N\textsubscript{2} production ratio (Davidson et al. 2000). The pH of the East Fork of Walker Branch is approximately 7.5, and although dissolved oxygen concentrations of surface water are relatively high (approximately 8 mg L\textsuperscript{-1}), sediments and biofilms likely present great heterogeneity in redox conditions. Thus, in contrast to the relatively thorough anoxia typical of lake or ocean sediments, small streams could present a range of redox conditions, yet our results suggest that stream denitrification efficiently consumes nearly all of its N\textsubscript{2}O intermediaries.

The denitrification rate in the East Fork of Walker Branch (12 $\mu$mol N m\textsuperscript{-2} h\textsuperscript{-1}) was generally within the range of denitrification rates in other streams with NO\textsubscript{3} concentrations <0.1 mg N L\textsuperscript{-1} (Table 4). Denitrification rates reported for streams and rivers with high NO\textsubscript{3} concentrations (>1 mg N L\textsuperscript{-1}) are considerably greater (generally >100 $\mu$mol m\textsuperscript{-2} h\textsuperscript{-1}). Comparisons between the denitrification rate for the East Fork of Walker Branch and those reported for other streams and rivers are problematic, however, because of methodological differences and limitations. The rates reported using the C\textsubscript{2}H\textsubscript{2} inhibition technique suffer from artifacts related to difficulties of adding C\textsubscript{2}H\textsubscript{2} uniformly within sediments. The NO\textsubscript{3} flux methods do not distinguish between denitrification and assimilatory NO\textsubscript{3} uptake and may not account for nitrification. The C\textsubscript{2}H\textsubscript{2}, NO\textsubscript{3} flux, and N\textsubscript{2} flux methods all require use of chambers or cores, which may reduce the exchange of surface water into and out of sediments. In addition, it can be problematic to extrapolate measurements made using chambers or cores to the entire stream ecosystem due to the complex spatial heterogeneity characteristic of most lotic ecosystems.

The field \textsuperscript{15}N addition and modeling approach presented here does not suffer from the limitations described above and provides a reach-scale measure of denitrification in streams. However, this method does not include denitrification resulting from tightly coupled mineralization/nitrification/denitrification occurring entirely within sediments. Because we added tracer \textsuperscript{15}NO\textsubscript{3} only to the surface water and only for a short period, our approach includes only denitrification of NO\textsubscript{3} that was originally in surface water or that exchanges rapidly with surface water NO\textsubscript{3} pools. Thus, our method may underestimate total denitrification rate in stream ecosystems.

Seitzinger (1988), in her review of denitrification rates in aquatic ecosystems, reported that coupled mineralization/nitrification/denitrification in sediments comprised >75\% of total denitrification in the Potomac and Delaware rivers, based on observations of high denitrification rates and low net NO\textsubscript{3} flux into sediments. However, denitrification of streamwater NO\textsubscript{3} may be more important in small streams with coarser sediments and more extensive and rapid mixing between surface and subsurface waters than in larger rivers characterized by fine-grained sediments with lower hydraulic conductivity. In a study of a small nitrate-rich stream in Denmark, Christensen et al. (1990) reported that the NO\textsubscript{3} source for denitrification was primarily surface water NO\textsubscript{3}, with minimal contribution from mineralization/nitrification in the sediments. In a study of denitrification in a small desert stream, Holmes et al. (1996) determined that denitrification rates were highest at downwelling areas where input of surface-derived organic matter and streamwater NO\textsubscript{3} provided the substrates for denitrification in the sediments. Clearly, further research focusing on the coupling of mineralization,
Stream denitrification and nitrate uptake

Table 4. Denitrification rates measured in streams and rivers.

<table>
<thead>
<tr>
<th>Stream/river, Location</th>
<th>Watershed type</th>
<th>Denitrification rate (μmol m⁻² h⁻¹)</th>
<th>NO₃⁻ concentration (mg N L⁻¹)</th>
<th>Method*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salto River, Costa Rica</td>
<td>Lowland swamp forest</td>
<td>5.25-25</td>
<td>0.2</td>
<td>1</td>
<td>Duff et al. (1996)</td>
</tr>
<tr>
<td>Duffin Creek, Ontario</td>
<td>Forest</td>
<td>6.5-475</td>
<td>0.5</td>
<td>2</td>
<td>Hill (1979)</td>
</tr>
<tr>
<td>Sycamore Creek, Arizona</td>
<td>Desert shrub</td>
<td>10-123</td>
<td>0.3</td>
<td>3</td>
<td>Duff et al. (1996)</td>
</tr>
<tr>
<td>Kings Creek, Kansas</td>
<td>Prairie</td>
<td>1-3</td>
<td>0.03</td>
<td>4</td>
<td>Holmes et al. (2002)</td>
</tr>
<tr>
<td>Little Lost Man Creek, California</td>
<td>Forest</td>
<td>2</td>
<td>0.6±0.1</td>
<td>5</td>
<td>Duff et al. (1994)</td>
</tr>
<tr>
<td>Delaware River, New Jersey</td>
<td>Lowland agriculture</td>
<td>40-460</td>
<td>4±13</td>
<td>6</td>
<td>Christensen et al. (1990)</td>
</tr>
<tr>
<td>Potomac River, Maryland</td>
<td>Mixed</td>
<td>210-324</td>
<td>0.03±0.03</td>
<td>7</td>
<td>Christensen et al. (1998)</td>
</tr>
<tr>
<td>East Fork Walker Branch, Tennessee</td>
<td>Forest</td>
<td>160-232</td>
<td>0.014</td>
<td>8</td>
<td>Seitzinger (1988)</td>
</tr>
<tr>
<td>Sugar Creek, Indiana</td>
<td>Agriculture</td>
<td>250-450</td>
<td>1.8</td>
<td>9</td>
<td>Seitzinger (1994)</td>
</tr>
<tr>
<td>Tennessee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Böhle et al. 2004</td>
</tr>
</tbody>
</table>

The field ¹⁵N addition approach has also been used to determine denitrification rates within an experimentally generated groundwater NO₃⁻ plume in a salt marsh (Tobias et al. 2001). The authors of this study estimated denitrification rates of about 1,000 μmol N m⁻² h⁻¹, although they were unable to determine ¹⁵N, and ¹⁵NO₂⁻ evasion rates because a conservative volatile tracer was not co-injected in the experiment.

A field ¹⁵N addition approach quite similar to ours was recently used by Böhle et al. (2004) to determine the denitrification rate in Sugar Creek, an agricultural stream in the upper Mississippi basin. The authors report a denitrification rate about an order of magnitude greater than that measured in Walker Branch, reflecting the 30-fold higher NO₃⁻ concentration in Sugar Creek (Table 4). Our study and that by Böhle et al. demonstrate the usefulness of the field ¹⁵N tracer addition approach for determining denitrification rates at the scale of entire stream reaches.

The field ¹⁵N tracer addition approach may be impractical in streams and rivers with high discharge rates or high NO₃⁻ concentrations due to the cost of adding enough ¹⁵N to achieve a sufficiently high ¹⁵N enrichment of streamwater NO₃⁻. We used a ¹⁵N-NO₃⁻ enrichment of about 40,000‰, although considerably lower enrichment levels should be sufficient if denitrification rates are relatively high. For example, Böhle et al. (2004) used a ¹⁵N-NO₃⁻ enrichment about tenfold lower than we used in our study. The field ¹⁵N tracer addition approach may also be problematic in high gradient streams with very high air–water gas exchange rates. Nonetheless, for many streams, our approach should be tractable and provides a reach-scale measure of the denitrification rate of NO₃⁻ in stream water as well as total NO₃⁻ uptake rate and length.

References


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