

IN-STREAM DENITRIFICATION: PILOT STUDIES AND SITE CHARACTERISTICS
ON A HEADWATER STREAM
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ABSTRACT

Increased loads of watershed nitrogen (N) are a major cause of water quality degradation, especially in coastal waters. According to regional budgets, only 20-30% of watershed N inputs reach coastal waters indicating there are N “sinks” across the landscape. Headwater streams can generate substantial N processing; however, several studies have observed minimal N retention within-streams. We assessed the reach-scale in-stream N removal and denitrification rates in one Rhode Island headwater stream. Using the constant rate injection method, we tracked the fate of isotopically enriched nitrate along a 500 meter stream reach. The objectives of this study were to assess the resolution of this method for use in Rhode Island, to investigate the sensitivity of the method to seasonal variation and to evaluate the extent of the intra-seasonal variation.

In-stream $\text{NO}_3\text{-N}$ removal was estimated using three approaches: NO_3^- disappearance using $\text{NO}_3^- : \text{Br}^-$ ratios, denitrification gas flux using the ^{15}N signature of the dissolved N_2 and N_2O gases, and mass balance of the $^{15}\text{NO}_3\text{-N}$ in the stream water. Removal was examined in summer and fall with three trials per season. During each trial the stream was dosed at a rate of $0.002 \text{ L second}^{-1}$ for 2.9 hours. The dosing solution contained ^{15}N -enriched potassium nitrate ($^{15}\text{N-KNO}_3$), a conservative solute tracer potassium bromide (KBr^-) and a conservative gas tracer sulfurhexafluoride (SF_6). Stream water samples were collected at six downstream stations and analyzed for nitrate, isotopic enrichment in the stream water, dissolved gases and concentration of conservative tracers. DOC, pH, and temperature were also assessed. A solute transport model, One Dimensional Transport with Inflow and Storage (OTIS) was used to estimate stream hydrologic parameters such as residence time and degree of entrainment. Uptake length, which is used to determine the average distance traveled

by a nitrate molecule before it is denitrified in a stream reach, was calculated directly from denitrification rates and by using the Spatially Referenced Regressions on Watershed Attributes (SPARROW) model.

Although flow rate, depth, temperature and DOC varied between the summer and fall trials, stream velocity and ambient $\text{NO}_3\text{-N}$ concentrations were very similar between seasons. Given the assumption that $\text{NO}_3\text{-N}$ transformation rates will follow first order kinetics (Mulholland et al., 2000), the lack of seasonal differences in these characteristics could explain the lack of difference in reach-scale $\text{NO}_3\text{-N}$ transformation between the summer and fall. In one trial of both the summer and fall experiments, the NO_3^- disappearance approach indicated significant N removal, but this method displayed high variability within the reach and within a season. The ^{15}N gas flux and the mass balance of $^{15}\text{NO}_3\text{-N}$ consistently generated results indicating little or no N removal for all trials in the summer and fall. The observed uptake length was greater than 170 km, which agreed with predictions generated using the SPARROW model. Results from the OTIS model indicate that the stream storage zone area and storage zone exchange were small in the summer and fall.

Seasonal variation in stream hydrologic and chemical conditions did not appear to affect the consistent, negligible removal rates found with the ^{15}N data. Similarly, the intra seasonal variations in-stream characteristics did not have a notable impact on the rates of N removal. The denitrification rates observed in this study are comparable to several studies; but a number of studies found markedly higher amounts of N disappearance. The minimal denitrification rates coupled with the low retention times associated with this headwater stream argue that it is a negligible sink for watershed N. The uncertainties surrounding in-stream N removal warrant further investigation, with consideration given to headwater streams with extended retention times and benthic interactions.

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PREFACE

This thesis is organized into manuscript format as described in the guidelines on thesis preparation of the URI Graduate School. The body of the text corresponds to the format of a journal article.

TABLE OF CONTENTS

Abstract.....	ii
Acknowledgements.....	iv
Preface.....	v
Table of Contents.....	vi
List of Tables.....	viii
List of Figures.....	ix
Introduction.....	1
Methodology	
Site description.....	4
Stream water dosing and sampling methods.....	8
Stream morphology field measurements.....	13
Analytical methods	
Sample Analysis.....	13
Analytical calculations and modeling.....	15
Estimates of in-stream NO ₃ -N removal.....	16
Spatial Analysis of Headwater Streams in Rhode Island.....	22
Statistical Analysis.....	23
Results	
Ambient characteristics.....	24
Analytical calculations and modeling	26
Estimates of in-stream NO ₃ -N removal.....	29
Spatial Analysis of Headwater Streams in Rhode Island.....	39
Discussion.....	43
Literature Cited.....	57

Bibliography.....66

LIST OF TABLES

1. Hydrologic characteristics of the study stream.....	7
2. Ambient and plateau $\text{NO}_3\text{-N}$ concentrations collected before and during the constant rate injection.....	12
3. Transport parameters determined from Br^- tracer data and OTIS simulation.....	27
4. Inter-quartile values of calculated reach-scale delivery fraction of N flux and calculated uptake rates.....	28
5. Stream NO_3^- disappearance estimated from Br^- and NO_3^- ratios.....	33
6. Estimates of stream reach denitrification gas flux and % stream $\text{NO}_3\text{-N}$ flux removed by denitrification.....	35
7. Summary of 1 st and 2 nd order streams in Rhode Island.....	41
8. Summary of three digital datasets of river and stream of Rhode Island.....	42
9. Stream characteristics, denitrification rates and uptake lengths for in-stream N removal studies.....	50
10. Uptake lengths of a Rhode Island headwater stream.....	52

LIST OF FIGURES

1. Map of study site and location of sampling stations.....	6
2. Quantile box plots of stream ambient $\text{NO}_3\text{-N}$, a) summer and b) fall...21	
3. Quantile box plots of stream ambient Br^- , a) summer and b) fall.....	25
4. Relative concentrations of $\text{NO}_3\text{-N}$ and Br^- collected at six downstream stations during a constant rate injection.....	30
5. $^{15}\text{NO}_3\text{-N}$ flux at five downstream stations during a constant rate injection.....	37
6. Daily hydrograph of the Chipuxet River for the dates surrounding the fall constant rate injection.....	47
7. In-stream rates of nutrient removal for the Waikato SPARROW models and other New Zealand watershed studies in relation to stream flow.....	53
8. In-stream rates of N removal for the Waikato SPARROW N model and other watershed studies in relation to stream channel depth.....	54

Introduction

Increased loads of nitrogen (N) from terrestrial ecosystems are a major cause of environmental degradation to coastal ecosystems. The increase in loads has been attributed to human activities, such as fertilizer application, N fixation by legume crops, human and animal waste, increased atmospheric deposition, and fossil fuel combustion (Vitousek et al., 1997). Nitrogen loading from streams and rivers to coastal waters has been linked to greater instances of eutrophication, leading to harmful algal blooms, loss of spawning habitat and anoxia that has resulted in fish kills (Howarth et al., 1996). Terrestrial nitrogen is transported via surface runoff and groundwater flow into a drainage network of streams, rivers and lakes. Currently, we are unable to fully account for the fate of anthropogenic N that is added to our land and waters. According to regional budgets, only 20-30% of this N reaches the ocean waters; therefore, most of the added N is assumed to be retained or removed by a mix of different settings and processes within terrestrial systems, groundwater or surface waters before reaching coastal water bodies (Howarth et al., 1996; Boyer et al., 2002). These locations, called N “sinks,” primarily transform inorganic N into organic N via plant and/or microbial uptake or into N gases by the microbially mediated anaerobic process of denitrification (Hill, 1996)

By identifying the locations and inherent processes responsible for watershed N sinks, we can restore and maximize the extent of these removal processes. River systems are a major pathway for moving watershed N into coastal water bodies and recent research suggests that some river systems function as important N sinks (Bohlke et al., 2004; Mulholland et al., 2004; Peterson et al., 2001). However, the extent of in-stream N removal and the range of stream conditions that foster N removal are poorly understood.

Recent studies of riverine N dynamics have identified lower order “headwater” streams as possible N sinks in the landscape (Alexander et al., 2000; Mulholland et al., 2004; Bohkle et al., 2004). Some research indicates that these headwater streams can generate substantially greater amounts of N loss than their larger, higher order streams (Alexander et al., 2000; Dodds et al., 2000; Seitzinger et al., 2002). Because lower order headwater streams generally comprise 70-85% of total stream length within a watershed (Rosenblatt et al., 2001; Peterson et al., 2001), there is considerable opportunity for headwater stream environments to transmit – and possibly transform – N generated by agriculture and other human activities.

Stream N removal has been examined through a variety of methods including spatially referenced statistical modeling (e.g., the SPARROW model; Smith et al., 1997; Alexander et al., 2000), sediment denitrification assays (Royer et al., 2004) and whole reach studies (Bohkle et al., 2004; Grimm et al., 2005; Mulholland et al., 2000; Tank et al., 2000). Stream reach studies integrate N dynamics over a range of settings, such as debris dams, microbial biofilms and hyporheic zones and can serve to validate ecosystem behavior predicted by site specific and process level studies. An increasing number of reach-scale studies have employed a constant rate injection method using stable N isotopes. This method involves adding isotopically enriched $\text{NO}_3\text{-N}$ along with conservative tracers to a stream and measuring the flux of different N forms as the $\text{NO}_3\text{-N}$ moves downstream. It has been used to assess the N loss potential in agricultural (Bohkle et al., 2004), urban (Grimm et al., 2005) and forested streams (Mulholland et al., 2004; Webster et al., 2003; Tank et al., 2000). Much is still unknown about the controlling factors and rate of N losses in these low order streams, and to date there have been very few reach-scale in-stream N removal studies in the New England region.

I assessed the efficacy of the constant rate injection method with isotopically enriched N for quantifying reach-scale in-stream N removal in a small, New England headwater stream. My research addressed the following questions:

1. Does the constant rate injection method have the resolution necessary to evaluate in-stream N removal rates in the settings and conditions found within lower-order streams in Rhode Island?
2. How sensitive is the method to seasonal variation?
3. What is the extent of the intra-seasonal variation?

The results of this work will contribute to the range of methods available for assessing N sinks and could help identify stream and riparian characteristics that can be expected to generate elevated N removal within stream ecosystems.

Methodology

Site Description

I studied a small, Rhode Island headwater stream to evaluate use of the constant rate injection method to investigate in-stream N removal. The study site is a 500 meter reach of a lower order tributary of the Chickasheen River in the Wood-Pawcatuck River Watershed; West Kingston, Rhode Island (Fig. 1). The stream was a first order stream for the first 122 meters of the reach, where an ephemeral tributary joined the study reach. During high flows in the fall and spring, the final 378 meters became a second order stream. The stream drained a small watershed of about 0.3 km², consisting of 29% forested wetland, 41% upland forest, 12% agriculture and 18% light residential (RIGIS, 2007). About 480 meters downstream from the last sampling station (station 6; 500 meters), the stream joined the Chickasheen Brook, a larger third-order tributary of the Wood-Pawcatuck River with a yearly average discharge of 122 L s⁻¹ (RIDEM, 2004).

The riparian area was forested and mostly wetland, with red maple (*Acer rubrum*), sweet pepperbush (*Clethra alnifolia*), cinnamon fern (*Osmunda cinnemomea*) and Massachusetts fern (*Thelypteris simulata*) as the dominant vegetation. The geology of the site was outwash consisting of glaciofluvial deposits. Using a USGS gravelometer (sensu Wolman, 1954), the stream bottom sediments ranged from cobble/ gravel in the upstream reaches to fine sands and silts in the downstream reaches. In the summer and fall of 2006, aquatic vegetation existed in the stream in small patches of burr reed (*Sparganium sp.*), skunk cabbage (*Symplocarpus foetidus*), smartweed (*Polygonum sp.*) and American waterweed (*Elodea canadensis*). In the fall, there was considerable leaf litter in the stream from the surrounding woody vegetation. The Rhode Island State Soil Survey mapped the

riparian zone soils within the study site as Scarboro mucky sandy loam (Rector, 1981). With a sinuosity of 1.55, a slope < 0.02 , and an average width to depth ratio of 22, the stream was classified as a Rosgen Level 1 Type C stream: *A low gradient, slightly entrenched riffle/pool stream with well defined meandering channels* (Rosgen, 1996).

Flow rates at the upper 10 m and lower 10 m sections of the stream reach ranged from 4-6 L s⁻¹ in the summer and 28-50 L s⁻¹ in the fall of 2006 (Table 1). An increase in downstream flow during both the summer and fall indicated groundwater inputs along the stream reach. These flow rates were comparable to those selected for other constant rate injection studies of stream biogeochemistry (Mulholland et al., 2002).

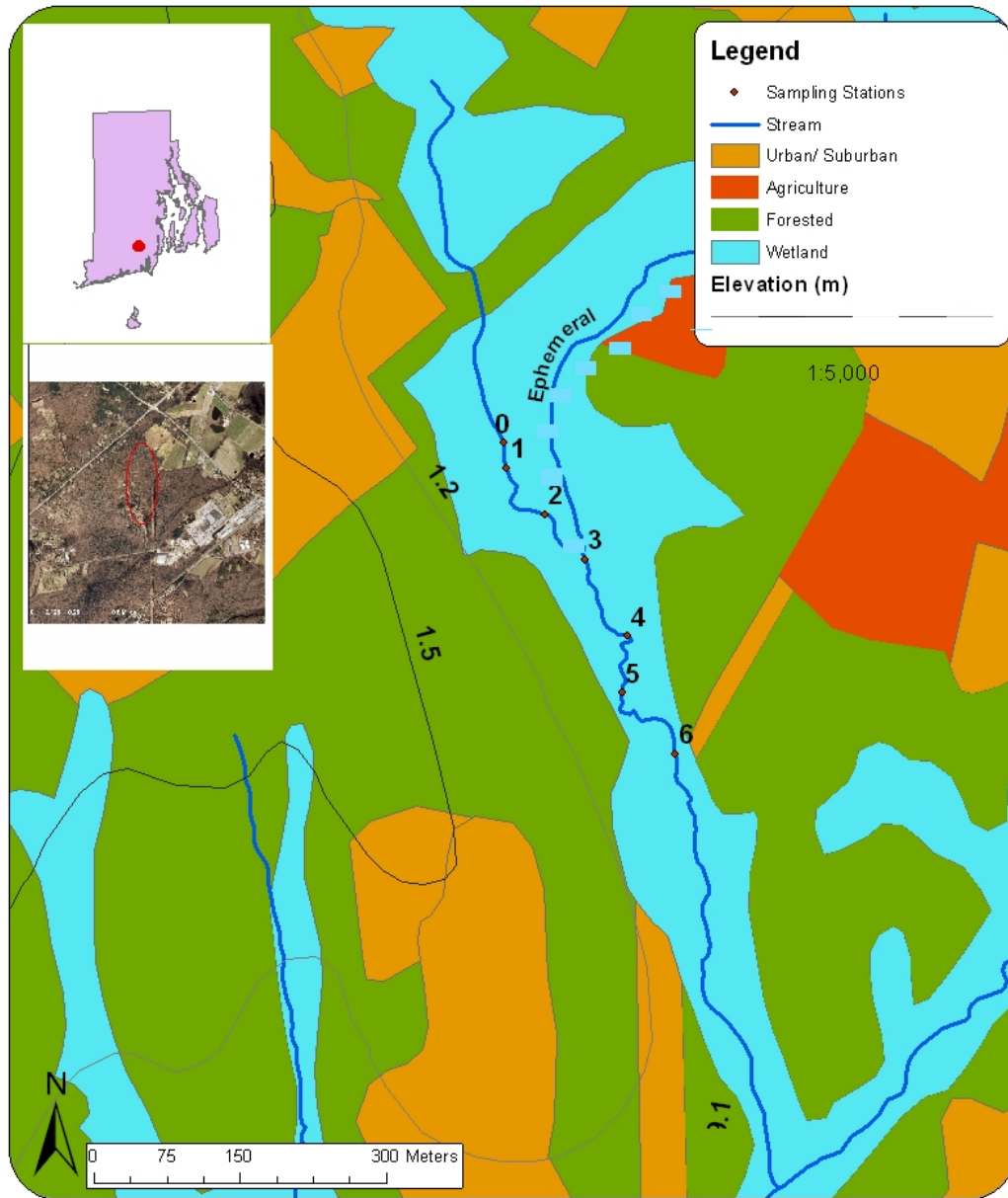


Fig. 1. Study site is a 500 meter reach of 1st/2nd order stream located in West Kingston, Rhode Island. Dosing station designated as 0, stations 1-6 are the downstream sampling locations.

Table 1. Hydrologic characteristics of a headwater stream in Rhode Island during summer 2006 (August) and fall 2006 (November). Upstream values were obtained at uppermost sampling station, and the downstream values were collected at the farthest downstream sampling location (500 meters). All values are mean (SE; n=3) unless otherwise indicated.

	Upstream Discharge † (L s ⁻¹)	Downstream Discharge † (L s ⁻¹)	Mean Stream Depth ‡ (m)	Time of Travel § (h)	Mean Reach Velocity (m s ⁻¹) ¶
Summer 2006	4.3 (0.37)	5.3 (0.48)	0.06 (0.42)	1.30 (0.01)	0.11 (0.01)
Fall 2006	28 (1.48)	49 (8.26)	0.14 (0.48)	1.24 (0.01)	0.11 (0.01)

† Discharge estimated from bromide (Br⁻) concentrations obtained during constant rate injection trials as described by Kilpatrick and Wilson, 1989

‡ Stream depth obtained from 15 channel cross-sectional measurements (10 measurements per cross section)

§ Time of travel estimated using Br⁻ data obtained during the constant rate injection trials as described by Kilpatrick and Wilson, 1989

¶ Mean velocity calculated as reach length divided by time of travel

Stream water dosing and sampling methods

The constant rate injection method, based on applications of isotopically enriched nitrate-N ($\text{NO}_3\text{-N}$), bromide (Br^- , a conservative solute tracer), and sulfur hexafluoride (SF_6), a gas tracer to the stream, was used to assess stream reach estimates of:

- 1) Nitrate disappearance using $\text{NO}_3 : \text{Br}^-$ ratios
- 2) Denitrification flux using isotopically enriched dinitrogen gas and nitrous oxide gas ($^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$)
- 3) Reach-scale mass balance of $^{15}\text{NO}_3\text{-N}$
- 4) Spiraling metrics using $^{15}\text{NO}_3\text{-N}$ and $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ gases.

A total of six constant rate injection trials were conducted. This method was used three times during low flow within a five day span in August 2006 and three times during high flow within a nine day span in November 2006.

The constant rate injection with an isotope tracer is a continuous application of amendments over a length of time resulting in a steady-state concentration (i.e. plateau) throughout the stream channel. When isotopically enriched N is added to a stream using the constant rate injection method, rates and lengths of both N uptake and denitrification can be measured using the downstream decrease in concentration of tracers and isotopically enriched $\text{NO}_3\text{-N}$ (Payn et al., 2005). I used this method to estimate reach-scale denitrification rates by examining the downstream concentrations of the amendments, $\text{NO}_3\text{-N}$, $^{15}\text{NO}_3\text{-N}$, Br^- , SF_6 , and by measuring the isotopically enriched N_2 and N_2O gases produced in the stream (Bohlke et al., 2004; Mulholland et al., 2004).

Before each of the seasonal amendment studies, I collected water samples in air-tight syringes and plastic nalgene bottle samples at each of the six downstream stations to obtain information on ambient ammonium (NH_4), bromide (Br^-), pH, and

SF₆, dissolved organic carbon (DOC), and ambient NO₃-N, ¹⁵N-NO₃ and ¹⁵N-N₂O and ¹⁵N-N₂ gases. Water samples to be analyzed for DOC were collected in brown glass vials, filtered and amended with 75 µl of phosphoric acid to achieve a pH of 2.0 or less. All samples were stored at 4 °C until analysis.

To apply the isotope tracer method successfully, three conditions must be satisfied : 1) The introduced ¹⁵N thoroughly mixes with the naturally occurring isotopic signature (Payn et al., 2005); 2) the addition of the ¹⁵N-NO₃ is small and does not significantly change the ambient NO₃-N concentration in the stream; thus providing an approximation of the ambient nitrate removal rate, which is presumed to be a first order kinetic process (Mulholland et al., 2000); and 3) the ambient groundwater inputs along the stream reach have the same isotopic signature as the stream's ambient conditions.

The location of the first sampling station was initially determined using mixing length calculations from Kilpatrick and Cobb (1985):

$$L_{\text{mix}} = K_{\text{mix}} (CX^2)/(g^{1/2}Y)$$

where:

C = Chezy's C

X = average reach width

Y = average reach depth

g = gravitational acceleration

Kmix = 0.500

For computational purposes, I used the following version of the above equation:

$$L_0 = 0.088 (vB^2)/(d^{3/2}d^{1/2}) \quad (1)$$

where:

L₀= distance required for optimum mixing, in feet

d= mean depth of the stream, in feet

s= the surface- water slope, in feet per foot

v = mean stream velocity

B= average stream width, in feet

Additional sampling stations were determined in the field using a combination of distance downstream and changes in stream features. To estimate the appropriate injection parameters (pumping rate and amendment concentration), validate the required mixing distance from the injection point to the first sampling station and to confirm the timing and duration of the steady state concentration (plateau) period at each of the six stations during the N amendment studies, I conducted a pre-assessment of the velocity and discharge of the stream along the entire study stream reach using constant rate injections of 0.64 g L⁻¹ of rhodamine dye (Kilpatrick and Wilson, 1989) in both the summer and fall. I estimated the arrival of the plateau at each sampling station using the pre-assessment rhodamine dose. Samples were analyzed for rhodamine dye concentration using a fluorometer (Shimadzu RF-1501 Spectrofluorophotometer, Kyoto, Japan). Discharge (Q) in L sec⁻¹ was calculated using the following equation:

$$Q = (qC)/c \quad (2)$$

where:

q = rate of injection (L sec⁻¹)

C = concentration of dye entering the stream (mg L⁻¹)

c = concentration of the dye in the downstream sample (mg L⁻¹)

For the constant rate amendment dosings, I used a peristaltic pump (Masterflex E/S 07571-00, Cole Parmer, IL) and injected the dosing solution at a location 30 m upstream of Station 1 to assure complete mixing before the amendments arrived at the study reach. The dosing solution was amended with potassium bromide (KBr), SF₆, and 25-50% ¹⁵N-enriched potassium nitrate (¹⁵N-KNO₃). Br⁻ dosing concentrations were 15,705 mg L⁻¹ and 98,603 mg L⁻¹, and NO₃-N dosing concentrations were 2,094 mg L⁻¹ and 3,433 mg L⁻¹ in the summer and fall seasons respectively. Pure SF₆ was bubbled into the dosing solution for five minutes

to achieve a concentration high enough to be detected at the final sampling station (500 m). The injection occurred at a continuous rate of 0.002 L sec^{-1} for 2.9 hours. In-stream concentrations of Br^- and SF_6 were increased substantially (e.g. > 10x) to ensure that they were detected in all downstream samples, while the addition of NO_3^- -N, was set to achieve a 20% increase above ambient NO_3^- -N concentrations observed at the downstream end of the study reach (Table 2).

At station 1 and 6, water samples were collected at 5-15 min intervals to observe the rising and falling limbs of the NO_3^- -N and Br^- tracer plateau and gain insight into flow characteristics. Sampling at Stations 1 and 6 occurred throughout the entire injection period and for an additional 2 hours after injection ceased. At all six stations we collected at least two 120-ml water samples in airtight syringes and two 1-liter water samples in nalgene bottles after the steady-state plateau of the injection was achieved. All sample bottles were kept cool in insulated coolers in the field and in a refrigerator at $4 \text{ }^\circ\text{C}$ in the lab until analysis or further processing. Water samples for analysis of denitrification gases and SF_6 were obtained using airtight syringes fitted with a stopcock, which was closed immediately after stream water sample collection; these syringes were stored in buckets under water to prevent atmospheric contamination of gases within the water sample. In addition, I gathered dissolved oxygen (DO) and temperature data at each sampling station using a YSI 550A DO meter (YSI, Yellow Springs, OH).

Table 2. NO₃⁻-N concentrations collected at the farthest downstream sampling station (500 meters from injection site) before and during the plateau phase of constant rate injection studies. Values represent mean concentrations (SE). The constant rate injection method was conducted three times during the summer and three times during the fall.

	n	Ambient NO ₃ ⁻ (µg N l ⁻¹)	n	Plateau NO ₃ ⁻ (µg N l ⁻¹)	Percent above ambient
Summer					
8/7/2006	5	1055 (14.2)	5	1325 (6.2)	20.3
8/9/2006	5	950 (16.1)	5	1219 (5.2)	22.1
8/11/2006	6	1123 (157)	2	1310 (3.0)	14.3
Fall					
11/7/2006	12	883 (5.1)	3	1472 (11.8)	40.0
11/10/2006	11	666 (10.6)	3	896 (7.9)	25.7
11/15/2006	11	715 (7.9)	3	879 (22.4)	18.6

Stream Morphology Field Measurements

I collected stream width and three depth measurements at 100 foot intervals along the study stream reach. Stream area measurements were calculated using these width and depth values. I assessed stream length and sinuosity using three techniques: 1) field measurements with a surveying tape; 2) computer estimates with data points collected using a Garmin Global Positioning System (GPS) map; and 3) computer estimates (Hernden, VA) using NHDplus digital stream maps acquired from Horizon Systems Inc. For the GPS measurements I collected 160 data points as I walked down the thalweg of the stream channel with a handheld unit. These data points were downloaded into ESRI ArcMap 9.1 as X and Y coordinates and then projected on top of other digital maps acquired from the Rhode Island Digital Imagery Server (RIGIS). The length of the stream reach was measured using ArcMap calculator for GPS and NHDplus digital stream points. Sinuosity, which is the ratio of channel length to valley length, was assessed using the Rosgen method (Rosgen 1996).

Analytical methods

Sample Analysis: Denitrification Gases

Upon return to the lab, we recorded the temperature of the stream water in buckets holding syringe samples, which were used to assess evasion rates, and added 20 ml of high-purity helium (He) gas into each syringe underwater, creating a 20 ml headspace. The syringes were then placed in a shaker and agitated for five minutes to allow the gas in the water to come into equilibrium with the headspace. We extracted the gas headspace using the equilibrium headspace extraction technique (Lemon, 1981; Davidson and Firestone, 1988) and collected replicate gas samples in two 12-ml evacuated glass vials (Labco International Inc., Houston, TX).

These gas vials were stored in 50-ml plastic centrifuge tubes (Labco International Inc., Houston, TX) filled with DI water at room temperature to prevent atmospheric contamination. One set of 12-ml gas samples was used to evaluate N_2O and SF_6 concentrations using a 5890A Hewlett Packard Gas Chromatograph with a 2 m Poropak Q column and electron capture detector at 350°C . The other set of 12-ml gas samples was analyzed for isotopic enrichment of $^{15}\text{N-N}_2\text{O}$ and $^{15}\text{N-N}_2$ on a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer coupled to a PDZ Europa TGII trace gas analyzer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, UC Davis, (Davis, CA).

Sample Analysis: ^{15}N Enriched $\text{NO}_3\text{-N}$

Stream water samples collected in the 1-liter bottles were filtered and amended with sulfuric acid to achieve a pH of 2.0 and stored at room temperature. These samples were then spiked with $\text{NO}_3\text{-N}$ to reach ^{15}N enrichment in the range required (< 2000 per mil) for $^{15}\text{N-NO}_3^-$ analysis on a GV Instruments IsoPrime isotope ratio mass spectrometer (Boston University Stable Isotope Laboratory, Boston University, Boston, MA). Samples were prepared for the isotope ratio mass spectrometer at Boston University using the diffusion method (Khan et al., 1998). Fall water samples collected for $^{15}\text{N-NO}_3^-$ analysis were not included in the results because of unidentified analytical problems encountered by the Boston University Laboratory.

Sample Analysis: Br^- , DOC, NH_4 and NO_3^-

I filtered the remaining stream water in the syringes into 125-ml nalgene bottles and stored them at 4°C in the refrigerator for later analysis of DOC, Br^- , NH_4 and NO_3^- . $\text{NO}_3\text{-N}$ was analyzed using the SM 4500 NO_3^- automated cadmium

reduction method (APHA, 1998), Br⁻ was analyzed colorimetrically using the Zitomer and Lambert (1963) and the Marti and Arozarena (1981) methods, and NH₄ was analyzed following the US EPA method 350.1 (US EPA, 1984) on an Astoria Pacific Auto Analyzer (Astoria-Pacific Inc., Clackamas, OR). DOC samples were analyzed via high-temperature catalytic oxidation (Sugimura and Suzuki, 1988; Deflandre and Gagne, 2001) on a Shimadzu TOC 5050 at the Institute of Ecosystem Studies (Millbrook, NY).

Analytical Calculations and Modeling

Stream Solute Transport Model

The OTIS stream solute transport model (Runkel, 1998) was used to obtain hydrologic parameters such as residence time and degree of entrainment of water in the hyporheic zone or in slow-moving pockets (Runkel, 1998; Battlin et al., 2003; Bohlke et al., 2004). The solute concentration curve of the constant rate injection trials, obtained from the 5 and 10 minute sampling of the rising limb of Br⁻ at stations 1 and 6, provided the primary input to the model. For each trial, the model generated values for the following characteristics for each stream reach section: dilution from groundwater and surface water inputs; transient-storage cross-sectional area (A_s ; composed of the hyporheic zone and small pockets of slow-moving water); the transient-storage zone exchange coefficient (α); and the dispersion coefficient (D) (Runkel, 1998; Battlin et al., 2003; Bohlke et al., 2004). The ratio A_s :Area indicates the degree to which stream water may be entrained in benthic sediments or slow-moving pools. The average storage residence time (T_s) was estimated for each sub-reach using the relation $\tau_s = A_s \text{Area}^{-1} \alpha^{-1}$ (Bohlke et al., 2004).

Spatially Referenced Regressions on Watershed Attributes (SPARROW) model

Uptake length is a common metric used to estimate the average distance traveled by a $\text{NO}_3\text{-N}$ molecule before it is denitrified in the stream reach. In this study, I used statistical approaches based on stream attributes and field measurements of $\text{NO}_3\text{-N}$ transformations to obtain estimates of uptake length. The SPARROW model estimates uptake length based on the reach-scale N delivery fraction that is derived from physical stream properties such as stream flow and depth (Stream Solute Workshop, 1990), using the following equation from Chapra (1997):

$$A (Z_i^s, A_i^R; \Theta_s, \Theta_R) = \exp (-\Theta_{s1} D_i^{\Theta_{s2}} T_i^s) \quad (3)$$

where

A = the fraction of N flux originating in and delivered to reach i that is transported to the reach's downstream node

T_i^s = mean water time of travel (day^{-1}) in reach i

D_i = mean water depth in reach i

Θ_{s1} ($\text{m}^{-1} \text{day}^{-1}$) = 0.0513

Θ_{s2} = -1.319

} estimated coefficients (Alexander et al., 2007)

Based on calculated A values, I obtained first order loss coefficients k_c , in terms of distance⁻¹ and time of travel⁻¹, based on the definition of $A = C_x/C_0$ and the first order expression (Gibson and Meyer, 2007):

$$\ln C_x = \ln C_0 - k_c x \quad (4)$$

where C_x = dilution-corrected nutrient concentration (x) kilometers from the top of the reach

C_0 = nutrient concentration at the top of the reach

Uptake length was then calculated from the reciprocal of the rate constants.

Estimates of uptake length based on equation 4 were obtained from $\text{NO}_3\text{-N}$ disappearance field trials and denitrification gas flux rates.

Estimates of In-Stream NO_3^- Removal

NO_3^- Disappearance Using $\text{NO}_3\text{-N}$: Br Ratios

I calculated reach-scale NO₃-N disappearance using the relative concentrations of NO₃⁻ and Br⁻ obtained during the constant rate injection method. The method uses changes in the relative concentration ratios (C/C₀) of NO₃-N and Br⁻ concentrations at station 1 (C₀) to concentrations at each downstream station (C) to infer NO₃-N loss from in-stream processes, without pinpointing the specific removal processes, such as denitrification, immobilization or plant uptake (Burns and Nguyen, 2002). The percentage of NO₃⁻ removed was calculated by combining stream flow (Q) with downstream observed NO₃-N concentrations and the “theoretical, conservative” downstream NO₃-N concentration estimate if the stream NO₃-N behaved in the same conservative fashion as the Br⁻ tracer concentration. Reductions observed in the Br⁻ C/C₀ from stations 2 through 6 reflect concentration changes due to physical (i.e., dilution) rather than biogeochemical processes. Biogeochemical cycling of NO₃-N is inferred if the decline in C/C₀ for NO₃-N is greater than noted for Br⁻.

To obtain percentage NO₃-N removal, mass flux of NO₃-N was calculated for each flow path distance from the tracer injection as follows:

$$\text{Theoretical, conservative daily mass flux of NO}_3^- = (\text{Br}^- \times T) \times Q \quad (5)$$

Where Q = discharge day⁻¹ and T = mass ratio of NO₃-N / Br⁻ in sample solution at the first sampling station.

The actual mass flux of NO₃-N was then subtracted from the theoretical, conservative mass flux of NO₃-N to calculate NO₃⁻ disappearance as:

$$\text{Mass of NO}_3\text{-N removed daily} = \text{Theoretical mass flux of NO}_3\text{-N} - \text{observed mass flux of NO}_3\text{-N} \quad (6)$$

The percentage of NO₃-N removal was then obtained from the ratio of the Mass of NO₃-N removed daily over the theoretical mass flux of NO₃-N. Because ambient NO₃-N concentrations increased from station 1 to station 6 (Figure 2), the ambient NO₃-N concentration observed at each station immediately prior to injection

was subtracted from the NO_3^- -N measurement obtained during the plateau sampling to estimate the concentration of the dosed NO_3^- -N at each station. To account for the considerable daily variability observed for ambient NO_3^- -N, I generated distinct values of disappearance using station 6 values for downstream ambient NO_3^- -N, and calculated the standard error of the results to compute ranges of N disappearance at the last sampling station (station 6, 500 m) for each of the three trials in the summer and the fall. I used these N disappearance ranges to calculate the mean and S.E. of the percentage of NO_3^- -N removed from the stream reach per day and the uptake length (equation 4.)

Denitrification Gas Flux

For each constant rate injection trial I calculated reach-scale cumulative denitrification rates. The masses of N_2O -N and N_2 gases (μg) in the water samples were calculated using the equations and constants provided by Tiedje (1982) and Mosier and Klemetsson (1994). The masses of N_2O -N and N_2 were converted to the masses of $^{15}\text{N}_2\text{O}$ -N and $^{15}\text{N}_2$ by multiplying by their respective ^{15}N sample enrichment proportion (ratio of plateau atom % of the dissolved N_2 and N_2O -N to dosed NO_3^- -N atom %, both corrected for ambient atom %). The gas tracer, SF_6 , was used with the appropriate Bunsen absorption coefficient to correct the estimates of the denitrification rates for gas evasion (i.e., gas flux from the water to the air) using the gas tracer and the measured concentrations of the individual denitrification gases (N_2O and N_2) in surface water and air (Cole and Caraco, 1998; Laursen and Seitzinger, 2002; McCutchan et al., 2003). The solute tracer, Br^- , was used to correct the estimates of denitrification rates for dilution and dispersion. The denitrification gas flux is expressed as:

N gas flux from stream (g d^{-1}) = concentration (g L^{-1}) of N_2O and N_2 generated from

the stream reach multiplied by the stream discharge ($L d^{-1}$) at the final sampling station (station 6).

Mass Balance $^{15}NO_3-N$ (15-N Mass Flux)

I also obtained estimates of NO_3-N removal from a mass balance based on the difference between the mass flux of $^{15}NO_3-N$ of the first station and each subsequent station for each constant rate injection trial. The mass flux of $^{15}NO_3-N$ at each station was computed by combining the measured molar fraction (Mf_t) and concentration of NO_3-N with the discharge using the following equation from the LINX II methodology (2004):

$$^{15}N_{flux\ i} = MF_t \times [NO_3 - N] \times Q_i \quad (7)$$

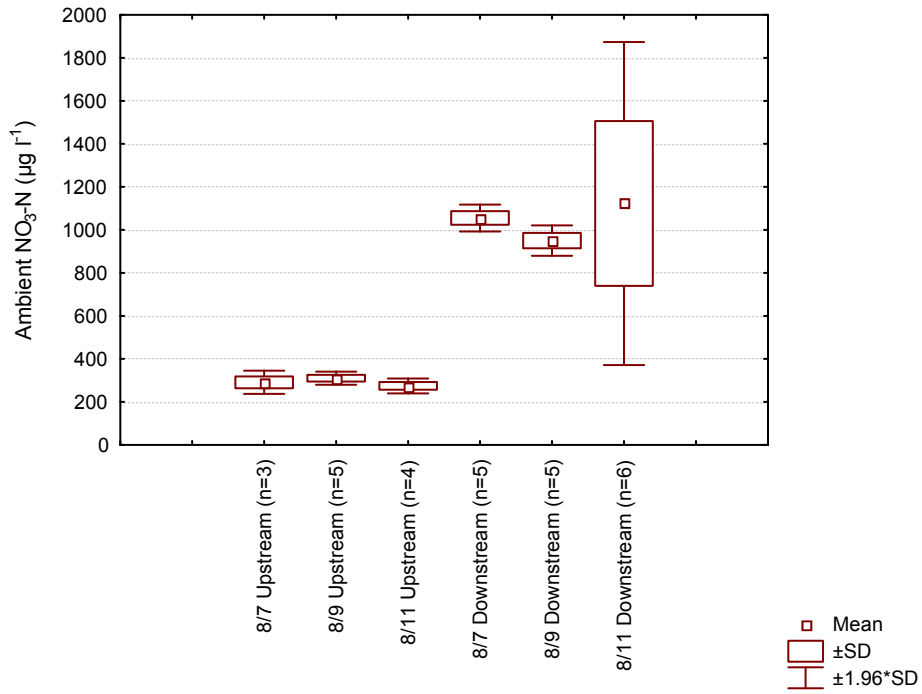
where: Q_i is the stream discharge, and Mf_t was calculated using the following equation:

$$\frac{^{15}N}{^{15}N + ^{14}N} = \frac{\left(\frac{\delta^{15}N}{1,000} + 1\right) \times 0.0036765}{1 + \left[\left(\frac{\delta^{15}N}{1,000} + 1\right) \times 0.0036765\right]} \quad (8)$$

Tracer $^{15}NO_3-N$ mass flux was then calculated by subtracting ambient $^{15}NO_3-N$ flux, obtained from the ambient stream water samples collected immediately prior to the dosing events, from the total $^{15}NO_3-N$ mass flux (Mulholland et al., 2004). The mass flux of NO_3-N is expressed as $g d^{-1}$, assuming that the discharge observed during the constant rate injection trials was comparable to the daily flow rate. A reach-scale mass balance was computed as the mass of ^{15}N at the first sampling station (30 m) minus the mass of ^{15}N at the downstream station (500 m). I calculated the standard

deviation of residuals of the $^{15}\text{NO}_3\text{-N}$ mass flux using 12 pairs of duplicate samples to provide insight into the extent of analytical error.

A. Summer



B. Fall

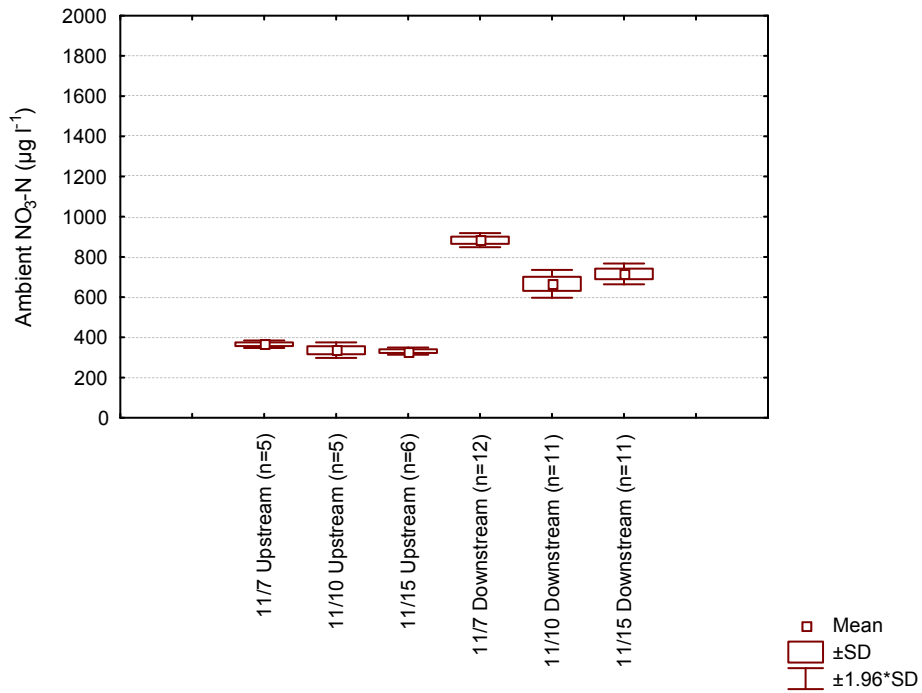


Fig 2. Quantile box plots of ambient $\text{NO}_3\text{-N}$ values collected from the first (33 m) and last (500 m) sampling station on a Rhode Island headwater stream. All samples were taken before constant rate injection trials performed three times in the summer and fall of 2006. Values are in $\mu\text{g l}^{-1}$

Spiraling Metrics

Total uptake rate of $\text{NO}_3\text{-N}$ (k_{tot}) was not calculated for each constant rate injection trial because the calculations require a negative slope of the downstream ^{15}N flux, indicating a decrease in downstream ^{15}N flux. These calculations can not be used when the ^{15}N flux is ≥ 0.0 in downgradient stations. Because the ^{15}N flux was never significantly > 0 ($p \leq 0.05$) in downgradient stations for all summer trials, the spiraling metrics were not calculated.

Spatial Analysis of Headwater Streams In Rhode Island

I used Geographic Information System (GIS) ArcMap 9.1 (Environmental Systems Research Institute, 1999) software, data available through the RIGIS Rhode Island GIS Data (RIGIS, 2007), and national hydrography data (NHDplus) to assess the proportion of first and second order streams in Rhode Island relative to their concurrent land use, surficial geology, slope, and soil type. This information was used to assess the range of settings and conditions found within headwater streams in Rhode Island and to determine the representativeness of the study site compared to other headwater streams within the state. Spatial attributes were obtained from the NHDplus dataset with 1:100,000 improved hydrography (Horizon Systems, 2007) along with 1995 Rhode Island state wide land use with 0.5 acre minimum resolution, the Rhode Island state wide glacial geology dataset derived from 1965 USGS classification and mapping (spatial resolution of plus or minus 40 feet), and the 1996 USDA/NRCS SSURGO soils dataset (1:24000) for Rhode Island (RIGIS, 2007). I generated a random sample of first and second order streams in Rhode Island using a random point generator (Beyer, 2004). Eleven random points were buffered with a 1 km radius, and all first and second order stream lengths that fell within the buffer area were measured. The random sample included 54 first order and 19 second

order RI streams. Percent impervious cover in the study stream watershed was determined using land use coverage and constants provided by the MANAGE model (Joubert et al., 1996).

Statistical Analysis

I used one-way analysis of variance (ANOVA) to analyze the variability and examine potential differences between N removal, stream hydrologic and chemical parameters during low-flow and high-flow conditions. Differences in chemical parameters collected once a season, such as DO and temperature, were assessed using a t-test. I also analyzed the variability of the aforementioned characteristics within the low-flow and the high-flow periods to assess the repeatability of these methods and to explore the inherent variability associated with the combination of natural variation and the type of methods used to assess in-stream denitrification. Within-season variability was addressed by using univariate techniques (i.e. box plots) to determine different measures of variation and central tendency. All statistical analyses were considered significant at the $p < 0.05$ level.

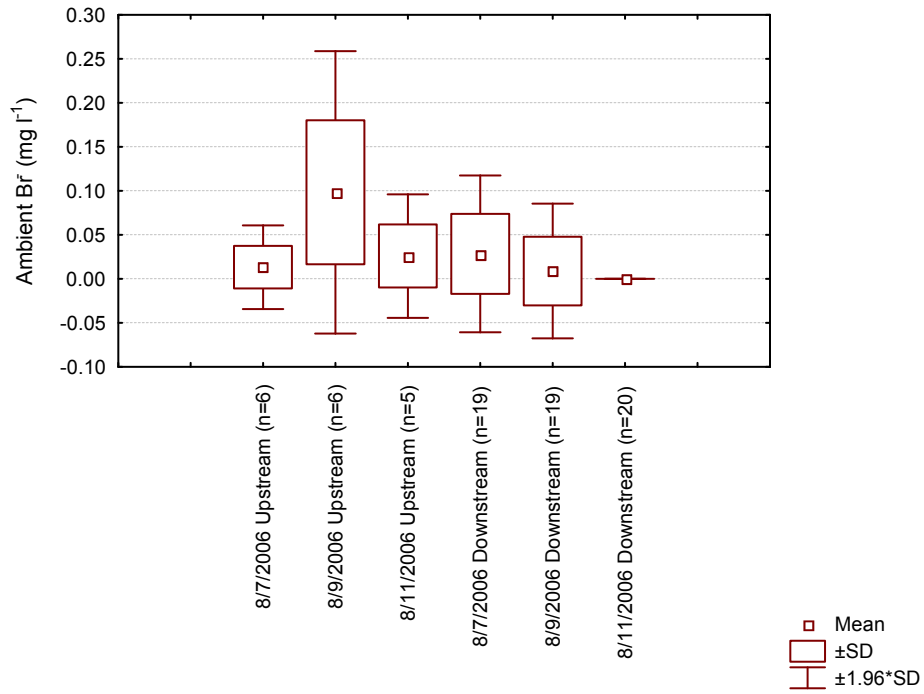
Results

Ambient Characteristics

Ambient concentrations of Br^- , SF_6 , NO_3^- , and $^{15}\text{NO}_3^-$ as well as pH, stream water temperature, DOC and DO were assessed during the summer and fall. SF_6 was absent from ambient stream water, and Br^- concentrations were consistently low, $< 0.3 \text{ mg L}^{-1}$ (Fig.3). There was no significant difference within season or between season difference in ambient SF_6 and Br^- levels. For all trials within both seasons, the ambient $\text{NO}_3\text{-N}$ levels were significantly higher ($p < 0.05$) downstream than upstream (Fig. 2). Fluctuating ambient $\text{NO}_3\text{-N}$ concentrations in the summer were observed downstream and contributed to the variability associated with N removal assessments, but NO_3^- concentrations were not significantly different between the same stations on different days within the same season (Fig. 2). The isotopic signature for all ambient samples at all stations remained relatively constant with an average (SE) mole fraction value of 0.0039 (0.00006).

Fluctuations in DOC were evident between seasons, with a significant increase ($p < 0.05$) in DOC in the fall, corresponding to visual differences in extent of leaf litter within the stream. Average (SE) summer and fall DOC were 4.1 (0.9) and 9.2 (0.9) mg l^{-1} respectively. The stream water temperature was significantly higher in the summer than the fall, with temperature ranges of 17.1-18.4 and 11-12 °C, respectively. There were no significant seasonal differences between DO or pH, with 8.0 mg/L average DO in the summer and 7.4 mg/L average DO in the fall. Upstream and downstream DO ranged between 7.5 and 8.0 mg l^{-1} during the summer and fall. Average (SE) pH in the summer and fall was 6.6 (0.04) and 6.4 (0.04) respectively.

A. Summer



B. Fall

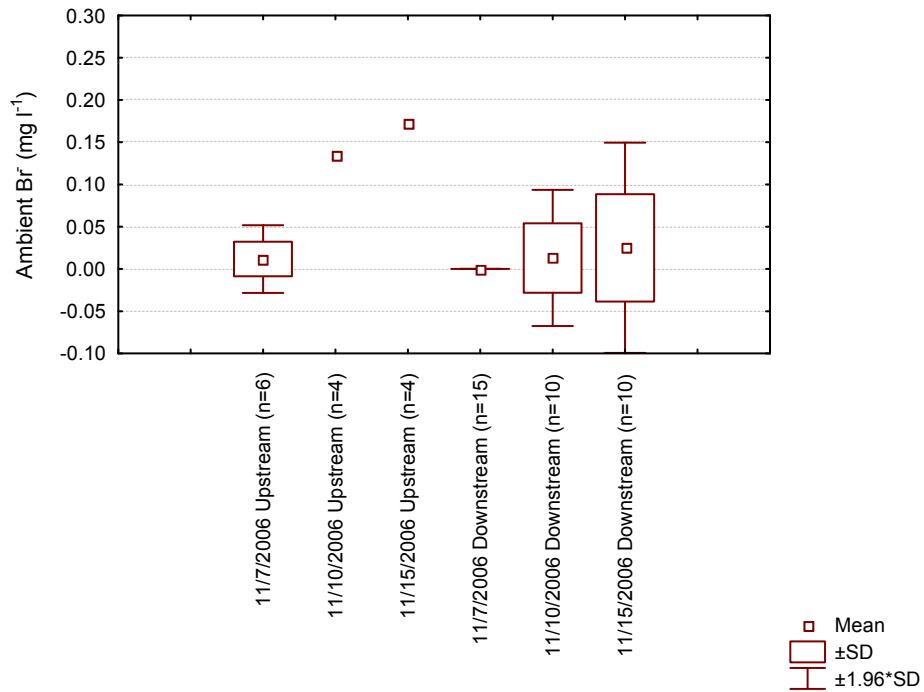


Fig 3. Quantile box plots of ambient Br⁻ values collected from the first (33 m) and last (500 m) sampling station on a Rhode Island headwater stream. All samples were taken before a constant rate injection performed three times in the summer and fall of 2006. Values are in mg L⁻¹.

Analytical Calculations and Modeling Results

Stream transport solute model

Based on the solutions derived from the OTIS model (Table 3), the storage zone area (A_s) and storage zone exchange (α) were predicted to be relatively minor for the summer and fall trials of the constant rate injection. Although stream cross-sectional area, hydrologic inputs, storage zone area, storage zone exchange and dispersion were greater in the fall than in the summer, the ratio of A_s :Area, which gives indication of entrainment of stream water in benthic sediments or slow moving pools was small and not significantly different between the seasons (Table 3). Average storage residence time varied from 0.04 to 0.08 hours in the summer and 0.02 to 0.16 hours in the fall (Table 3). These values predict that 4.7% (summer) and 5.5 % (fall) of the tracer travel time within the study reach occurred in either the hyporheic zone (benthic sediments) or trapped within slow moving pools.

Spatially Referenced Regressions on Watershed Attributes (SPARROW) model

Expected nitrogen loss-rate coefficients (A), which represent the reach-scale delivery of fraction N flux, were calculated using the stream depth inter-quartiles and the average time of travel (day^{-1}). All values indicate that minimal removal is expected to occur in this headwater stream, with even less chance for removal in the fall. Uptake rate, or aquatic loss (k_c) calculated using stream depth inter-quartiles, average time of travel (day^{-1}) and the length of stream reach (km) and can be found in Table 4.

Table 3. Transport parameters determined from Br⁻ tracer data and OTIS simulation on one day of each season.

	Stream section	Reach section length (m)	Groundwater Input (m ² s ⁻¹)	Stream dispersion (D) (m ² s ⁻¹)	Stream Area m ²	Storage Area (A _s) m ²	Storage exchange coefficient α (h ⁻¹)	Degree of entrainment (A _s : Area)	Average storage residence time (T _s) hours (A _s Area ⁻¹ α ⁻¹)
Summer 8/7/2006	0	20	0 (ns)	0.05	0.12	0.0001	0.001	8.3e-4	0.08
	1	61	7.3e ⁻⁷ (5.9e ⁻⁷)	0.1	0.12	0.0001	0.001	8.3e-4	0.08
	2	122	1.6 e ⁻⁶ (9.5e ⁻⁷)	0.1	0.12	0.0001	0.001	8.3e-4	0.08
	3	122	6.3e ⁻⁶ (1.4e ⁻⁶)	0.4	0.13	0.0001	0.001	7.7e-4	0.08
	4	76	6.2e ⁻⁶ (9.5e ⁻⁷)	0.4	0.14	0.0001	0.001	7.1e-4	0.07
	5	92	1.9e ⁻⁵ (3.9e ⁻⁶)	0.4	0.25	0.0001	0.001	4.0e-4	0.04
	6	500	0 (ns)	0.07	0.15	0.0001	0.001	6.7e-4	0.07
Fall 11/7/2006	0	20	0 (ns)	0.05	0.12	0.0001	0.004	8.3E-04	0.02
	1	61	1.2e ⁻⁵ (6.7e ⁻⁶)	0.2	0.13	0.0001	0.004	7.7E-04	0.02
	2	122	1.0e ⁻⁴ (4.3e ⁻⁵)	0.2	0.14	0.0001	0.004	7.1E-04	0.02
	3	122	2.0e ⁻⁵ (9.2e ⁻⁶)	0.2	0.14	0.0001	0.004	7.1E-04	0.02
	4	76	5.3e ⁻⁵ (3.2e ⁻⁶)	0.5	0.15	0.0001	0.004	6.7E-04	0.02
	5	92	8.1e ⁻⁵ (1.2e ⁻⁵)	3.0	0.33	0.0020	0.004	6.1E-03	0.15
	6	500	0 (ns)	1.0	0.31	0.0020	0.004	6.5E-03	0.16

ns = not significant

Table 4. Inter-quartile values of the calculated reach-scale delivery fraction of N flux (A) and calculated uptake rates (k_c). Values obtained from all combinations of the inter quartiles of observed stream depth, mean stream discharge and stream length data collected from a Rhode Island headwater stream in the summer and fall of 2006. Data can be used to assess nutrient delivery and is used with the SPARROW model (Smith et al., 1997)

<u>Date</u>	<u>A value</u> [†]	<u>k_c day⁻¹</u> [‡]	<u>k_c km⁻¹</u> [‡]
Summer			
8/7/2006	0.763- 0.919	1.56-5.01	0.17-0.54
8/9/2006	0.763- 0.919	1.56-5.01	0.17-0.54
8/11/2006	0.759- 0.918	1.56-5.01	0.17-0.54
Fall			
11/7/2006	0.945- 0.972	0.53-1.07	0.06-0.11
11/10/2006	0.947- 0.973	0.53-1.07	0.05-0.11
11/15/2006	0.947- 0.973	0.53-1.07	0.05-0.11

[†]Chapra (1997); constants from Alexander et al. (2007)

[‡]Alexander et al. (2002).

Estimates of in-stream NO₃⁻ Removal

Nitrate Disappearance Using NO₃⁻ / Br⁻ Ratios

Visual comparison of relative concentrations (C/C_o) indicate little difference in the fate of NO₃-N and Br⁻ within the stream for virtually all stations and all trials with the exception of the last sampling station on the first trial date (August 7, 2006) (Fig. 4). During the summer trials, the NO₃-N and Br⁻ relative concentrations tracked very closely throughout the first 5 stations and Station 6 was the only location where the mean C/C_o values of NO₃-N and Br⁻ diverged. Somewhat greater discrepancies in the relative ratios along the stream reach were observed during the fall, but there were few instances where the relative NO₃-N concentrations declined below the relative Br⁻ concentrations. These results suggest negligible in-stream NO₃-N disappearance. The variation in ambient NO₃-N concentrations observed at Station 6 generated substantial uncertainty in NO₃⁻ disappearance on several trial dates, minimizing the resolution of this method for providing evidence of low levels of NO₃⁻ disappearance. The NO₃⁻ disappearance method indicated no removal for three of the six trials, and only two trials were found to generate significant reach-scale NO₃⁻ disappearance (Table 5).

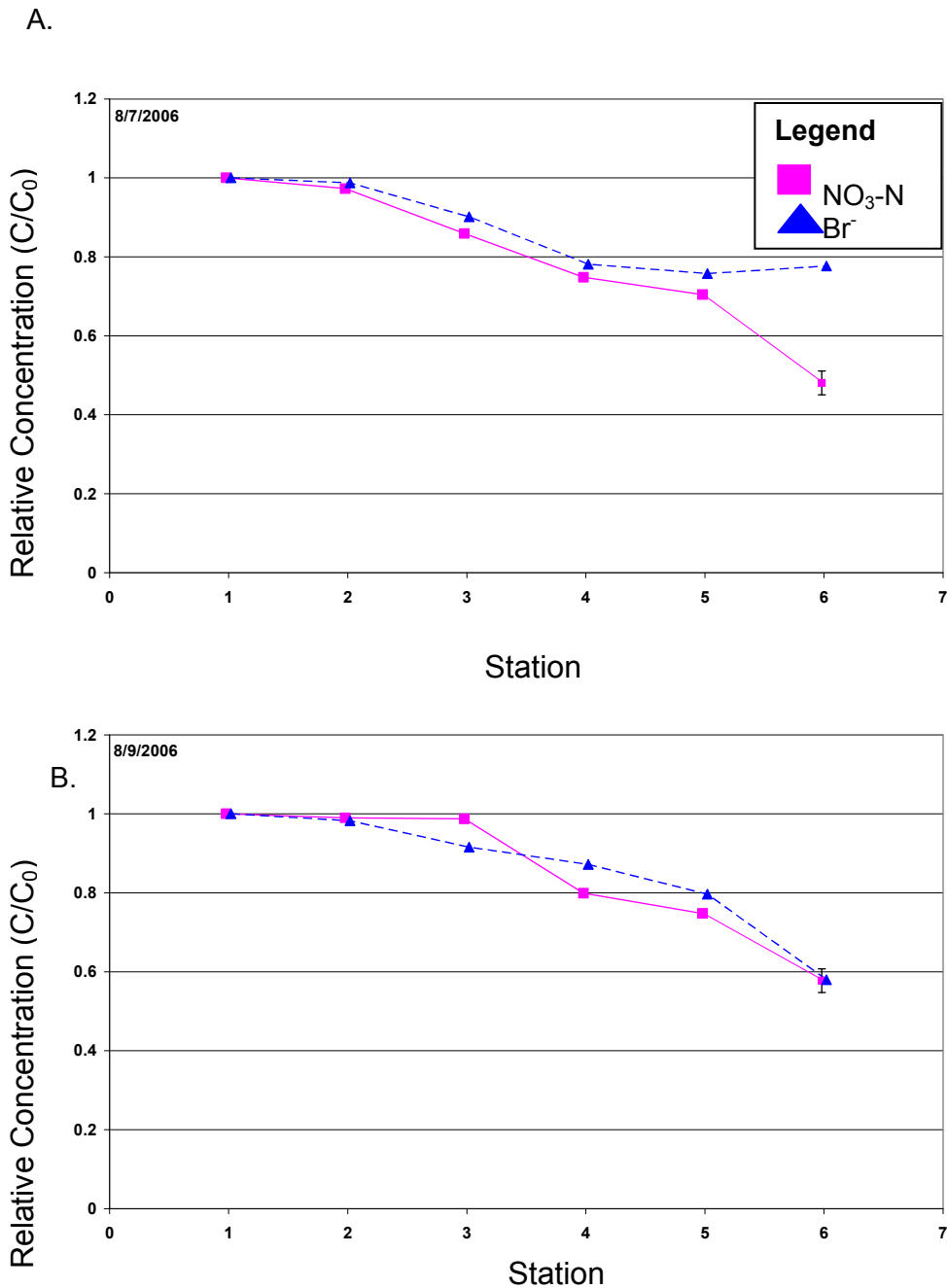
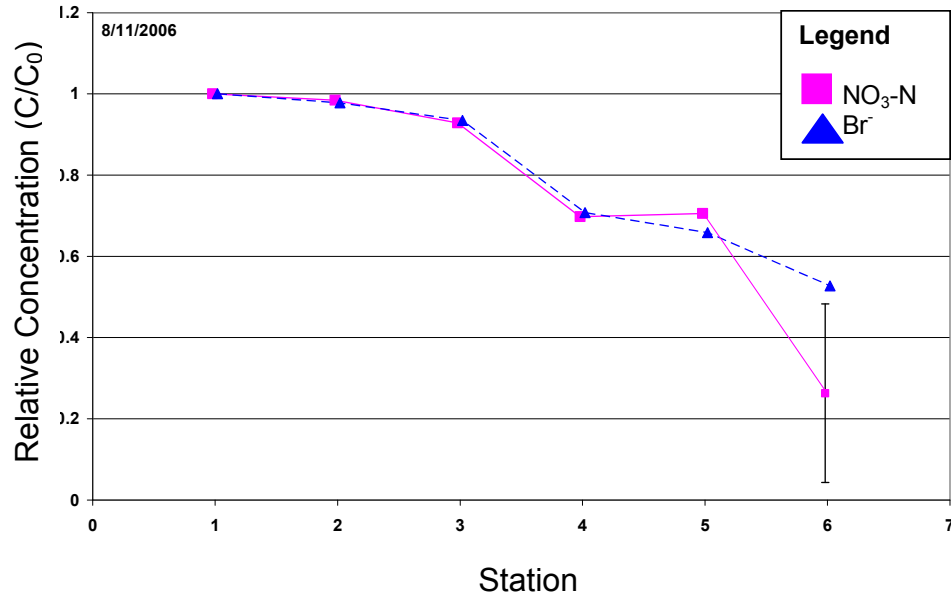


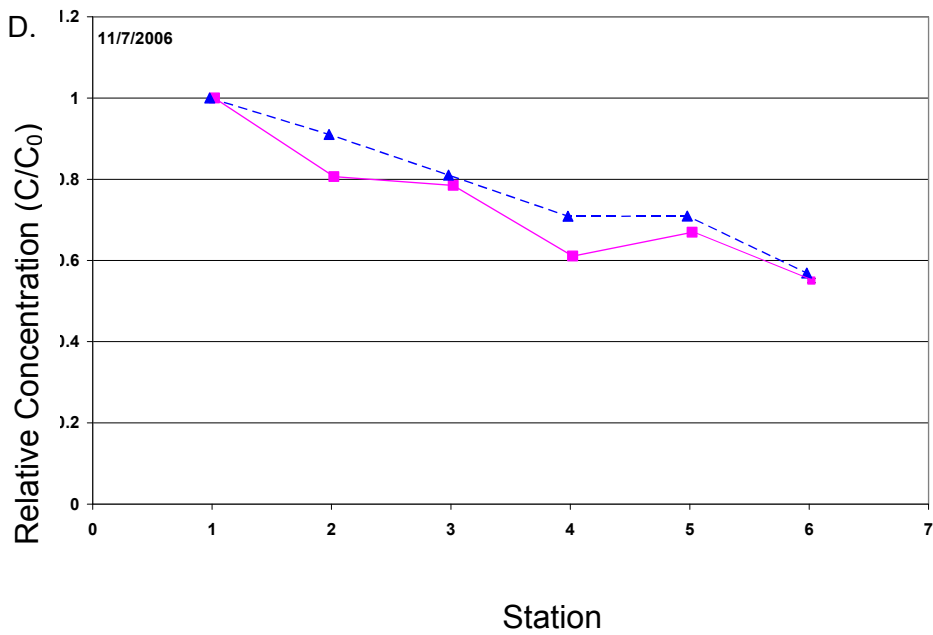
Fig 4. Relative concentrations (C/C_0) of $\text{NO}_3\text{-N}$ and Br^- from three replicate constant rate injections employed on a headwater Rhode Island stream in the summer (A, B, and C) and fall (D, E, and F) of 2006. Values represent ratios of mean Br^- and $\text{NO}_3\text{-N}$ plateau concentrations sampled at 6 downstream locations. Estimates of C_0 are derived from plateau measurements at Station 1. Pre-injection (ambient) $\text{NO}_3\text{-N}$ concentrations at stations 2-6 were subtracted from plateau values before relative concentration were computed. At Station 6, $\text{NO}_3\text{-N}$ relative concentrations include SE based on observed variability in pre-injection (ambient) concentrations.^a

^a Downstream ambient NO₃-N showed considerable within season and daily variability. Distinct values of disappearance using all values for downstream ambient NO₃-N were generated, and SE was calculated from these results.

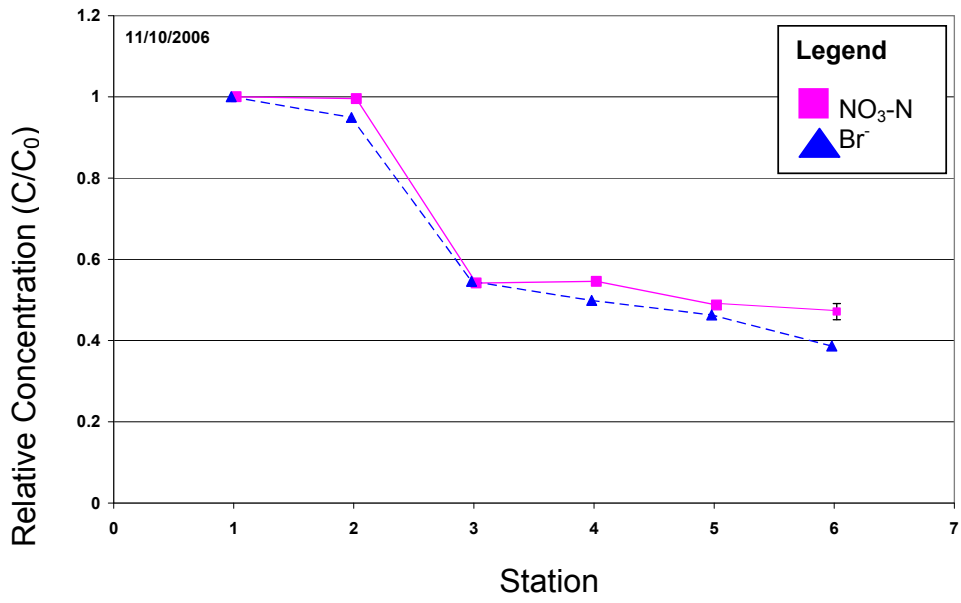
C.



D.



E.



F.

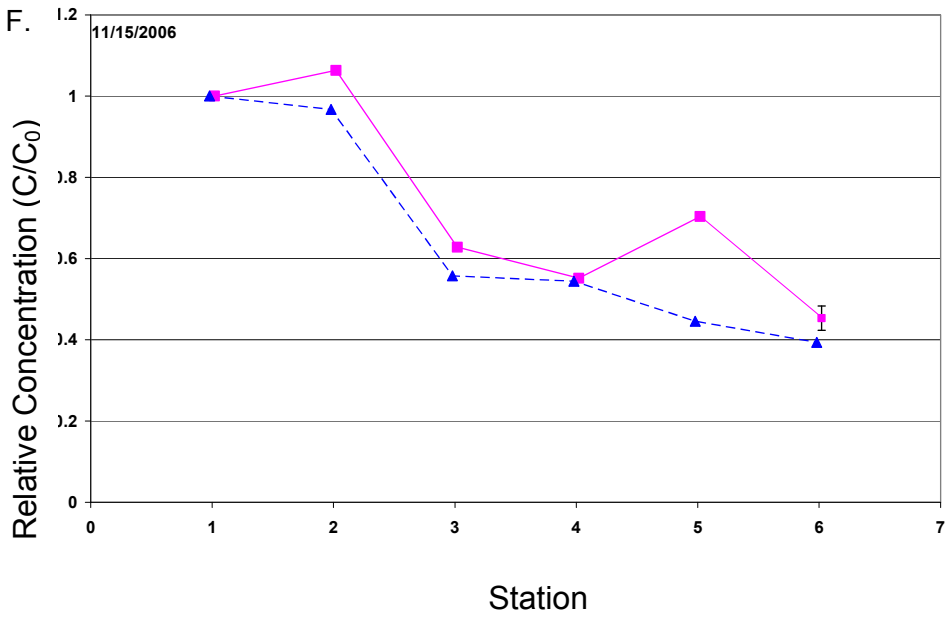


Table 5. Stream NO_3^- disappearance estimated from $\text{Br}^- : \text{NO}_3^-$ added during constant rate injections on three days in the summer and three days in the fall. Values represent mean Br^- and mean NO_3^- (SE) cumulative disappearance over a 500 meter reach of a Rhode Island headwater stream.

	Cumulative Removal Station 6 (500 m)		
	N removal [†] (g day ⁻¹)	% removal	Uptake length (km)
Summer			
8/7/2006	66.25 (6.21)*	36	1.1
8/9/2006	< 0.1 (9.30)	0	ns
8/11/2006	48.32 (32.20)	24	1.8
Fall			
11/7/2006	59.45 (14.25)*	4	12.2
11/10/2006	< 0.1 (45.79)	0	ns
11/15/2006	< 0.1 (37.90)	0	ns

[†] Standard error of the residuals calculated using distinct values of station 6 values for downstream ambient NO_3^- , thereby generating ranges of N disappearance from which SE was obtained (Fig 2).

* Significantly different from 0.0 ($p \leq 0.05$).

ns= not significant

Denitrification Flux Using $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ gases

The mass of ^{15}N gases ($^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ gases) generated during the constant rate injection method was negligible and accounted for less than 1% of the NO_3^- flux in the stream on each of the study dates (Table 6). Gas generation was consistently low at all stations and all trials. N_2 accounted for a greater proportion of N gases produced during the constant rate injection. Overall, the mass of $^{15}\text{N}_2$ gases were 10X greater in magnitude than $^{15}\text{N}_2\text{O}$ gases when present. Results of SF_6 gas monitoring did not demonstrate substantial gas evasion over the stream reach. More evasion occurred in the summer trials, with an average (SE) downstream value of 61% (3.8) remaining, than during the fall with an average (SE) downstream value of 79%(0.3) remaining.

Table 6. Estimates of stream reach denitrification gas (N₂O and N₂) flux and % stream NO₃⁻-N flux removed by denitrification. Values derived from constant rate injection ¹⁵N amendment studies. Values represent mean (SE) of three replicate samples per trial obtained during a 15 minute sampling period from Station 6, located at the end of a 500 reach of a headwater stream in Rhode Island. Mass removal detection rate for N₂O and N₂ is +/- 0.04 atom % and +/- 0.2 atom % respectively.

	Mass Removal		Percent Removal	Uptake lengths (km)
	g day ⁻¹	μmoles/m ² /hr		
Summer				
8/7/2006	0.12 (0.08)	0.7	< 0.1	2510
8/9/2006	0.03 (0.01)	0.2	< 0.1	9030
8/11/2006	1.82 (1.33) ^a	10.8	< 0.1	180
Fall				
11/7/2006	0.08 (0.01)	0.5	< 0.1	7340
11/10/2006	1.31 (0.91)	7.8	< 0.1	340
11/15/2006	1.41 (0.71)	8.4	< 0.1	340

^a n = 2

Mass Balance of $^{15}\text{NO}_3\text{-N}$

Mass balance analyses of $^{15}\text{NO}_3\text{-N}$ flux during the summer trials did not indicate $\text{NO}_3\text{-N}$ removal along the study stream reach during the constant rate injection method trials. Analytical error associated with the isotopically enriched water samples was low and accounted for less than a 2% change in all $^{15}\text{NO}_3\text{-N}$ δ^{15} values, where δ^{15} values above zero give indication of enrichment above natural abundance. The isotopic enrichment of the samples collected during the constant rate injection were substantially higher than the ambient levels observed at all sampling stations, with an average (SE) isotopic enrichment nearly an order of magnitude (8.8x) above ambient conditions for each sampling station. In several instances, the $^{15}\text{NO}_3\text{-N}$ flux at the last station (station 6) displayed a marked increase compared to all the other stations, indicating error associated with either sample contamination, analyses or the downstream flow estimates for that particular station. As a result, station 6 $^{15}\text{NO}_3\text{-N}$ flux values were not used in mass balance estimates and the results are given for the first 411 m portion of the stream reach. For all trials where $^{15}\text{N-NO}_3$ data were used (summer trials only) the ^{15}N flux did not decrease throughout the reach (Fig. 5), thereby negating the computation of other spiraling metrics using these data (Fig. 5).

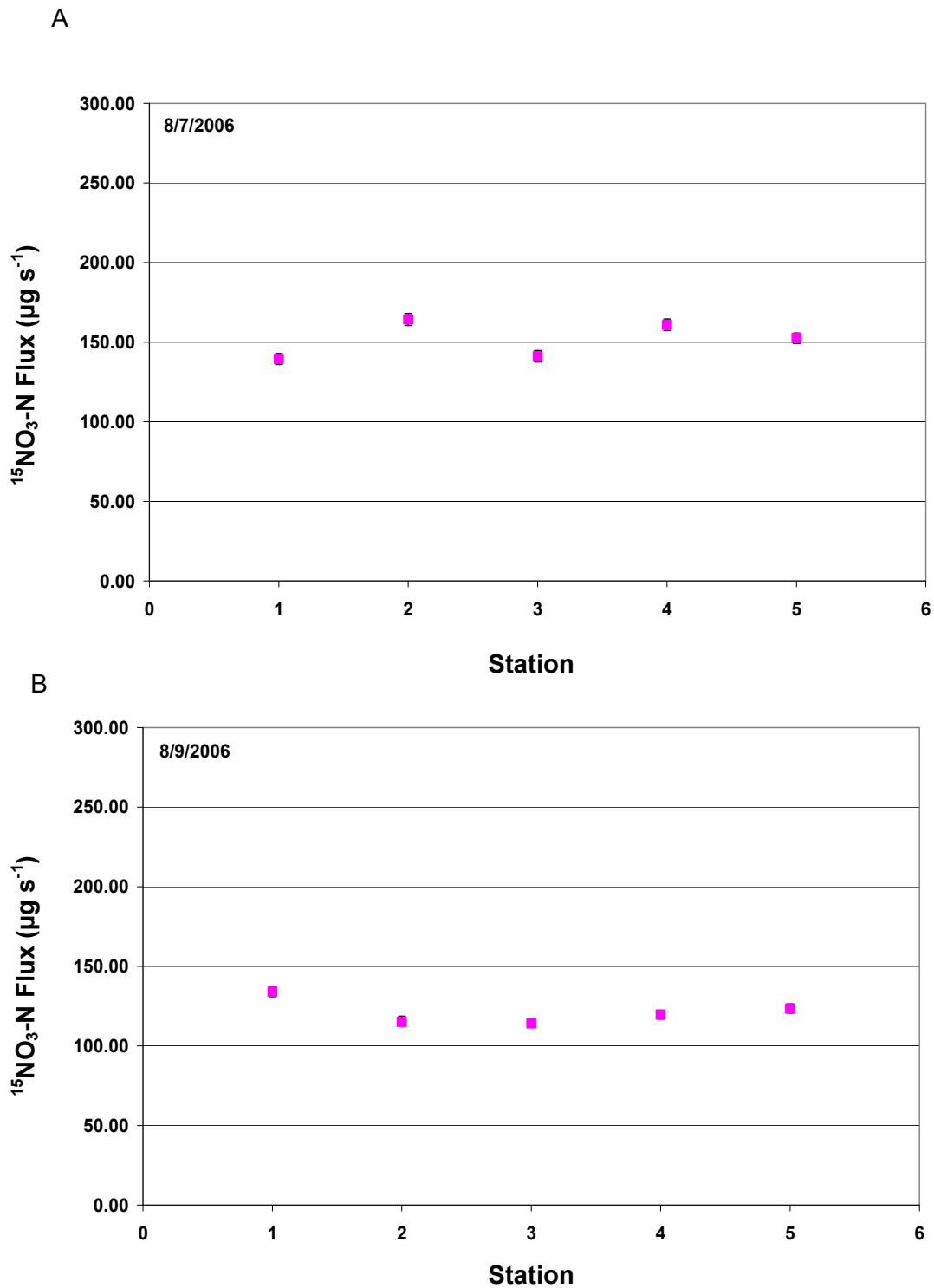
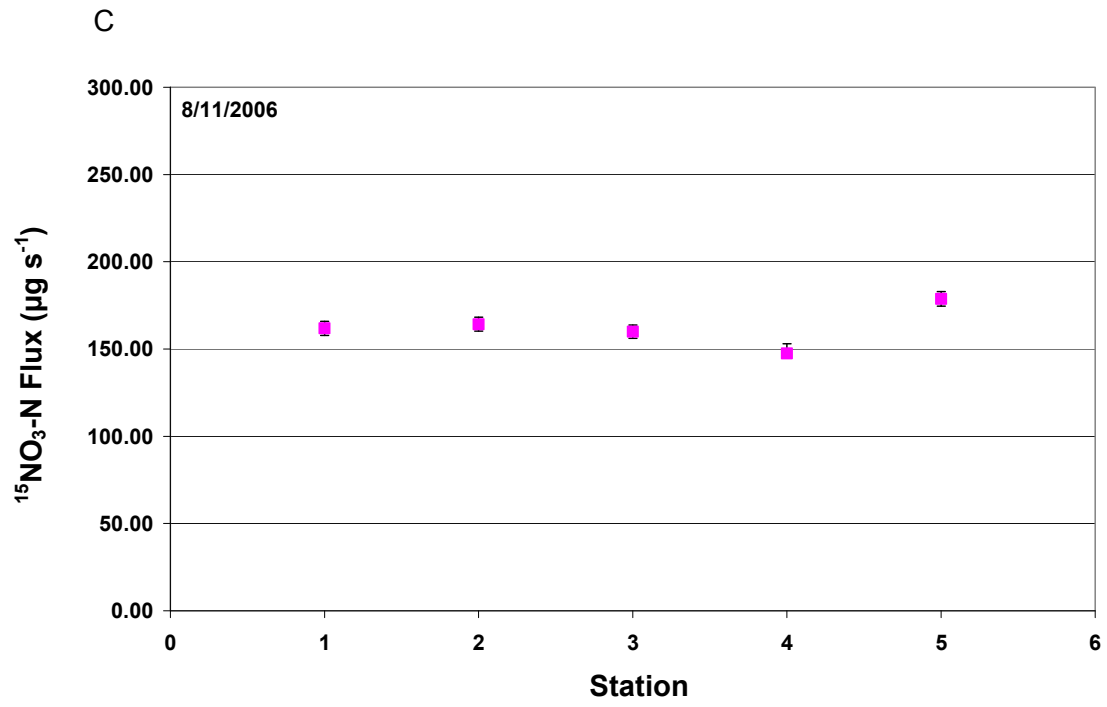


Fig 5. $^{15}\text{NO}_3\text{-N}$ Flux from three replicate constant rate injections in summer 2006 using isotopically enriched $\text{NO}_3\text{-N}$ on a Rhode Island headwater stream. Last sampling station is 411 m downstream. All values represent mean of two replicate samples collected 15 minutes apart.



Spatial Analysis of Headwater Streams in Rhode Island

Available digital river and stream datasets (1:100,000) show that the cumulative length of first order streams is considerably greater than the cumulative length of second order streams in the state of Rhode Island. Headwater streams, composed of all first and second order streams, account for 70% of the length of the drainage network Rhode Island (Table 7). Approximately 45% of the headwater stream length in RI was found to have comparable surficial geology, soils and land cover to the stream reach used in this study (e.g., outwash geomorphic setting, hydric soils with a forested riparian zone).

Although there are higher resolution spatial stream datasets for Rhode Island, (e.g., the 1:24,000 RIGIS hydroline dataset and the 1:5,000 RIGIS streams dataset), neither of these datasets include stream order designation. The study stream reach was not depicted on the 1:100,000 dataset, nor on the 1:24,000, suggesting that these databases may underestimate the length of headwater streams in RI. A comparison of the three river and stream datasets available for the state revealed dramatic differences in the number and total length of all rivers and streams between the three datasets (Table 8). The RIGIS streams 1:5000 dataset suggests that length of rivers and streams in the state is more than twice than contained in the other databases.

Based on the RIGIS streams 1:5000 dataset the study stream reach length was found to be 0.44 km. The study site stream length yielded similar estimates from three different methods. Based on field reconnaissance with a surveyor's tape, the study stream length from the upper sampling station to the farthest sampling station was found to be 0.50 km. With a field GPS reconnaissance combined with ArcMap 9.1 analyses, the stream length was estimated at 0.46 km.

Land use within a 3 km radius of the study stream is rural and consists of a mosaic of small parcels: 15% light residential, 19% agricultural, 37% forested and brushland, 4% water and 25% wetland. Based on impervious cover information provided by the MANAGE model (Joubert et al., 1996), the area surrounding the study reach is estimated to have less than 5% impervious cover – suggesting that the hydrology of the catchment is relatively undisturbed.

Table 7. Summary of first and second order streams in Rhode Island. Data obtained from the National Hydrography Dataset plus (NHDplus) improved hydrography digital dataset (1:100,000). Data analyzed with Geographic Information System (GIS) ArcMap 9.1 (Environmental Systems Research Institute 1999) software.

	Total length (km)	% of all rivers and streams	Number of streams	% of all rivers and streams
First order streams	1178	57	925	50
First order streams in similar landscape ^a	700	34	469	25
Second order streams	337	16	377	21
Second order streams in similar landscape ^a	250	12	213	11
Headwater (first and second)	1515	73	1302	70
Headwater streams in similar landscape ^a	920	45	682	36

^a site is in outwash with hydric soils and the riparian zone is forested.

Table 8. Summary of three digital datasets of river and streams of Rhode Island. Data resolution is given in parentheses.

	NHDplus (1:100K)	RIGIS Hydrolines (1:24000)	RIGIS streams (1:5000)
Total length of all rivers and streams (km)	2074	2240	4850
Total number of rivers and streams	1870	4470	31603

Discussion

Does the constant rate injection method have the resolution necessary to evaluate in-stream N removal rates in the settings and conditions found within lower-order streams in Rhode Island?

The use of isotopic tracers for in-stream N removal studies is a highly regarded approach because it accounts for groundwater dilution, groundwater N enrichment, fluctuating ambient $\text{NO}_3\text{-N}$ conditions, and provides reach-scale estimates of denitrification and nitrate transformations. In this study, isotope tracers were used to generate two individual assessments of nitrate removal: the denitrification flux using the $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ gases and the mass balance of $^{15}\text{NO}_3\text{-N}$. Bohlke et al. (2004) found that the estimation of denitrification rate from these methods is quite reliable. The in-stream denitrification method, which identifies denitrification as the specific removal process by enriching downstream pools of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ gases, consistently demonstrated negligible $\text{NO}_3\text{-N}$ removal occurring in the stream. The mass balance of $^{15}\text{NO}_3\text{-N}$ data exhibited unexplained fluctuations and analytical complications which caused it to be more ambiguous than the denitrification gas flux results.

The NO_3^- disappearance method, based on relative changes of $\text{NO}_3\text{-N}$ and Br^- through the study reach provided inconsistent results. Results varied within season and no significant removal was found in two of the three trials that occurred in both the summer and fall seasons. Within a given trial dramatic differences in NO_3^- disappearance were observed within the stream reach and in several trials the data suggested that $\text{NO}_3\text{-N}$ was generated within the stream reach. Resolution of the method was likely compromised by the dynamic spatial and temporal fluctuations of ambient $\text{NO}_3\text{-N}$ within the study reach during each trial. More specifically, the results emerging from the nitrate disappearance method were probably confounded by two

factors: 1) the stream $\text{NO}_3\text{-N}$ concentrations were only elevated slightly above ambient levels during the injection study to mimic the ambient nitrate removal rate, which is presumed to be a first order kinetic process (Mulholland et al., 2000) and 2) the ambient $\text{NO}_3\text{-N}$ fluctuated considerably during the course of the constant rate injection trials, which swamped the signal of the introduced $\text{NO}_3\text{-N}$. In the trials with significant nitrate disappearance, uptake lengths calculated using the NO_3^- disappearance deviated substantially from the uptake lengths estimated from in-stream denitrification (Table 5 & 6). Overall, the efficacy associated with the $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ denitrification gas flux was the highest. There was close agreement between the isotopically enriched gas and stream sample data, further arguing that the $\text{NO}_3^-:\text{Br}^-$ ratio method is less effective and reliable.

How sensitive is the method to seasonal variation?

Seasonal variation in a number of stream hydrologic and chemical conditions did not appear to affect the consistent, negligible removal rates found with the ^{15}N labeled denitrification gas flux method. In the fall, leaf litter was abundant in the stream, the DOC was elevated compared to the summer, the stream temperature was lower and the stream depth was much greater. Although stream discharge in the fall was 10 times greater than in the summer the velocities and retention time within the study reach were comparable. Ambient $\text{NO}_3\text{-N}$ concentrations within the study reach were comparable between seasons and did not change with discharge, in contrast to the findings of Fulweiler and Nixon (2005) who found $\text{NO}_3\text{-N}$ concentrations leaving the Wood-Pawcatuck watershed to be negatively associated with the river's discharge.

Other studies have found that the seasonal differences associated with select stream characteristics observed in this study can influence removal rates, but the

specific mix of seasonal changes and seasonal similarities can confound the results. Many studies equate the elevated DOC found in the fall trials with higher transformation rates (Groffman, 1994; Bachand and Horne, 2000; Bernhardt and Likens, 2002; McClain et al., 2003; Arango et al., 2007). However, transformation rates are expected to decrease with the lower temperatures observed in the fall trials (Kaplan et al., 1979; Bachand and Horne, 2000). In addition, as stream depth increases in the fall due to increased flow there is less interaction between the stream water and the bottom sediments and hyporheic zone, suggesting a lower potential for in-stream transformations.

Two important characteristics did not change substantially between seasons: the ambient $\text{NO}_3\text{-N}$ concentrations and stream reach retention times. Given the assumption that $\text{NO}_3\text{-N}$ transformation rates will follow first order kinetics (Mulholland et al., 2000), the lack of seasonal differences in these characteristics could explain the lack of difference in reach-scale $\text{NO}_3\text{-N}$ transformation between the summer and fall.

What is the extent of the intra seasonal variation?

In both the summer and fall trials stream velocity and time of travel were similar and displayed low variability; however discharge was highly variable within the fall trials (Table 1). These patterns of discharge are not unexpected. No precipitation occurred during the entire period surrounding the summer trials, but based on the rain gage at Kingston RI (5 km from the study site) 2.5 cm of precipitation occurred as a result of several precipitation events during the fall trials (Preliminary data from National Weather Service Cooperative Observer Station, 2006). The stable discharge observed during the summer is typical of vegetated watersheds located over permeable soils in RI. During the summer these areas do not typically generate

overland runoff or ground water recharge during summer conditions, because evapotranspiration (ET) exceeds precipitation and the soil moisture generally remains below field capacity (Gold et al., 1990). In contrast, during the fall when ET declines, recharge and runoff often respond to precipitation, creating more dynamic hydrographs. Over the 9 days of fall sampling, a United States Geological Survey (USGS) gauged local river, the Chipuxet River, which is within the Wood-Pawcatuck Watershed, also experienced extremely variable flows due to the combination of rain events and lack of ET (Fig. 6). The fall trials had greater variability in discharge (Table 1), but much less variation in the downstream ambient $\text{NO}_3\text{-N}$. Overall, the intra seasonal variations in-stream characteristics did not have a notable impact on the rates of N removal.

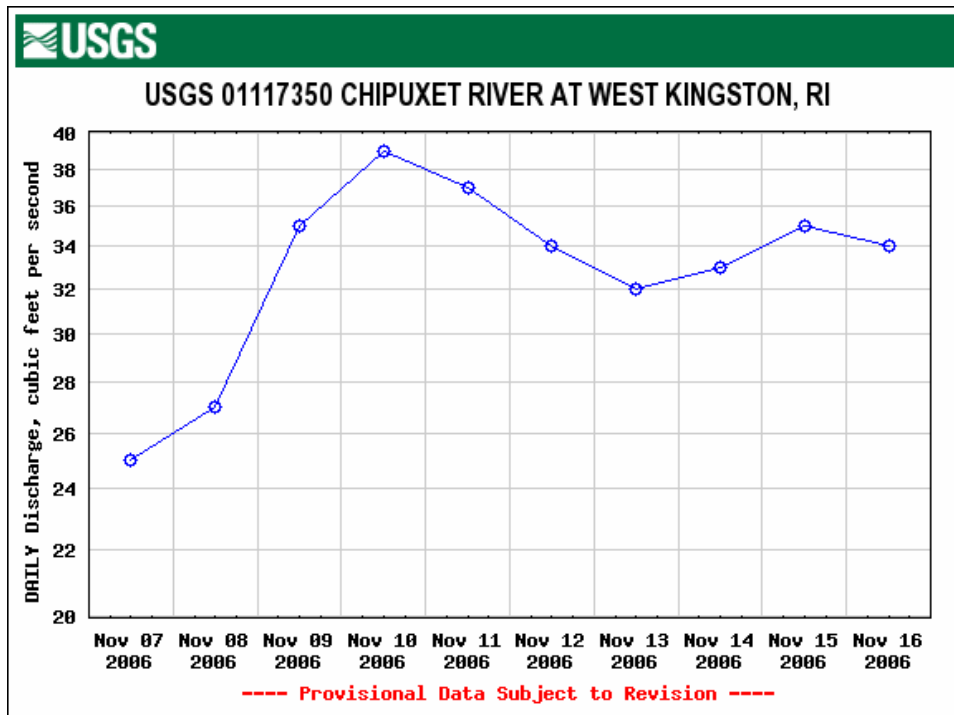


Fig 6. Daily hydrograph of the Chipuxet River for the dates surrounding the fall constant rate injection trials on November 7, 10 and 15, 2006. Values reflect real-time data acquired from USGS stream gauging station. (USGS, 2006)

How do my results compare to other studies?

This study assessed the constant rate injection method as a tool to study the potential for headwater streams to reduce watershed N loading. The rates of denitrification that I observed were similar to several studies; but a number of studies found markedly higher rates of N disappearance, through a combination of denitrification and other processes such as plant uptake or immobilization (Table 9). Based on the denitrification gas flux results in my study, the denitrification rates in the study reach ranged from 0.2 – 10.8 $\mu\text{mol}/\text{m}^2/\text{hour}$. The upper range of my observed denitrification rates approached those found in recent studies of a highly pristine stream (Mulholland et al., 2004) but were much lower than rates observed by others working on streams with both higher ambient nitrate concentrations (Royer et al., 2004) and comparable concentrations (Bohkle et al., 2004). Uptake length, the average downstream distance traveled by a nutrient molecule before it is removed by denitrification from the water column, was found to be very long, greater than 200 km, suggesting that in-stream denitrification is a negligible watershed N sink. Uptake lengths are a function of both the denitrification rate and the ambient nitrate concentrations. The uptake lengths in this study are comparable to those found by Royer et al. (2004), but those results occurred with higher denitrification rates in agricultural streams where the $\text{NO}_3\text{-N}$ concentrations were 10-20 times greater than the Rhode Island headwater stream, causing long uptake lengths and relatively minor removal. Where uptake lengths were found to occur over short distances (0.217 - 0.5 km), such as the pristine stream at Walker Branch (Mulholland et al., 2000; Mulholland et al., 2004), stream velocities and ambient nitrate levels were dramatically less than levels observed in the Rhode Island headwater stream. In addition the results of the OTIS simulations of transport parameters suggest that processing time within the hyporheic zone was comparatively minor in the headwater

stream examined in this study. The average residence time and degree of entrainment in the benthic sediments or slow moving pools determined from the bromide tracer data and the OTIS simulation model were more than an order of magnitude lower than those found by Bohlke et al. (2004)

Table 9. Stream characteristics, denitrification rates and uptake lengths for in-stream N removal studies. All numbers represent averages (unless a range is given).

Study Site	Mean Discharge (l s ⁻¹)	Mean Depth (m)	Mean Width (m)	Temp (°C)	Ambient NO ₃ ⁻ (mg l ⁻¹)	Velocity (m s ⁻¹)	Denitrification Rate (μmoles/m ² /hr)	Denitrification Uptake length (km)
*‡Sugar Creek Indiana (Bohkle et al., 2004)	45	0.19	4.5		0.99	0.43	117.5	17,950
†‡Walker Branch Tennessee (Mulholland et al., 2000)	9.6	0.05	3.1	12.4	0.02	0.07	123.3	0.5
*‡Walker Branch Tennessee (Mulholland et al., 2004)	0.4	0.03	0.92	20	0.03	0.02	12.0	0.217
§Illinois agricultural stream (Royer et al., 2004)	10-80	0.31			7.78	0.19	1.9	166 - >200
*‡Rhode Island stream (Milliman)	4.3-49	0.10	2.0	11- 18.4	0.90	0.11	0.2- 10.8	176- 9,030
§Ohio agricultural stream (Herrman et al., 2007 unpub. data)	9.8	0.16	0.92	8.8-19.3	9.7	0.05		210,000
§Ohio forested stream (Herrman et al., 2007 unpub. data)	8.4	0.10	1.85	8.8-19.3	10.7	0.03		80,000

* ¹⁵NO₃-N tracer experiment

† ¹⁵NH₄-N tracer experiment

§ C₂H₂ Inhibition method

‡ Cobble- gravel stream channel substrate

The importance of the stream velocity and ambient nitrate concentrations observed in the RI headwater stream on uptake length can be illustrated by recomputing uptake lengths for the RI headwater stream using the higher denitrification rates found by Mulholland et al (2000) rather than the rates found in my study. Those rates were 10 fold greater than I observed. Nevertheless, even with elevated denitrification rates, uptake length in the Rhode Island headwater stream would exceed 14 km, well in excess of the 1 km length of most headwater streams found within the state (Table 10).

The observed fraction of N denitrified in the summer and fall constant rate injection trials was very low and the export of ^{15}N enriched $\text{NO}_3\text{-N}$ was high, which suggests high values of expected nitrate export (low N removal) from the stream. The field results do not vary widely from estimates of N removal derived using the New England SPARROW model (Table 4; Smith et al. 1997) with the physical features of the study reach as model inputs (Moore et al. 2004). Alexander et al. (2002) calibrated the SPARROW model using data from 37 streams in New Zealand and compared their results with other in-stream N removal studies. By comparing calculated uptake rates (Table 4; k_c) for this study stream with rates of removal for the New Zealand SPARROW models and other watershed studies, it is evident that the uptake rates calculated for this stream reach are lower than other stream sites with comparable stream flow or depth. However, most of the relationships used in the New Zealand study are based on NO_3^- disappearance with few studies focused on denitrification (Figs. 7 and 8).

Table 10. Actual and hypothetical uptake lengths of a Rhode Island headwater stream. Actual values represent maximum denitrification rates observed during a constant rate injection method. Hypothetical values based on denitrification rates from another study on a stream in Tennessee (Mulholland et al., 2000)

	Ambient NO ₃ ⁻ N (mg l ⁻¹)	Velocity (m s ⁻¹)	Denitrification Rate (μmoles/m ² /hr)	Denitrification Uptake length (km)
Summer ^a				
*Actual Upper Rate	1.1	0.11	10.8	176
†Hypothetical	1.1	0.11	123.3	15
Fall ^b				
*Actual Upper Rate	0.7	0.11	8.4	338
†Hypothetical	0.7	0.11	123.3	23

^a Mean stream depth: 0.06 m, mean discharge: 4.8 liters second⁻¹, stream water temp: 18.4 °C.

^b Mean stream depth: 0.14 m, mean discharge: 38.5 liters second⁻¹, stream water temp: 11 °C.

* Maximum denitrification rates obtained from three replicate constant rate injection trials in the summer and fall of 2006

† Denitrification rate (123.3 μmoles/m²/hr) from Walker Branch, Tennessee (Mulholland et al., 2000)

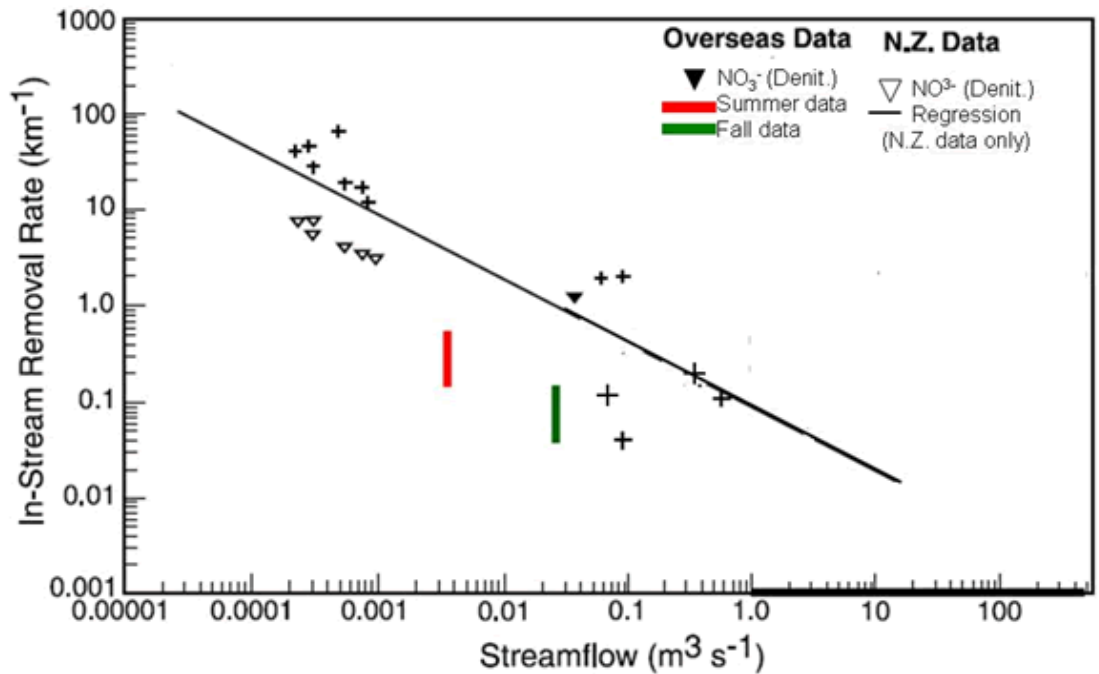


Fig 7. In-stream rates of nutrient removal for the New Zealand SPARROW models (Alexander et al. 2002) and other New Zealand watershed studies in relation to stream flow (modified from Rutherford et al. 1987). Nutrient forms include TN (total nitrogen), NH_4^+ (ammonium), NO_3^- , TDN (total dissolved nitrogen), TP (total phosphorus), DRP (dissolved reactive phosphorus), and TDP (total dissolved phosphorus). Results, given as a range, from the Rhode Island headwater stream study are calculated from inner quartiles of the observed stream depth and the mean stream flow in the summer (red) and fall (green) of the constant rate injection method.

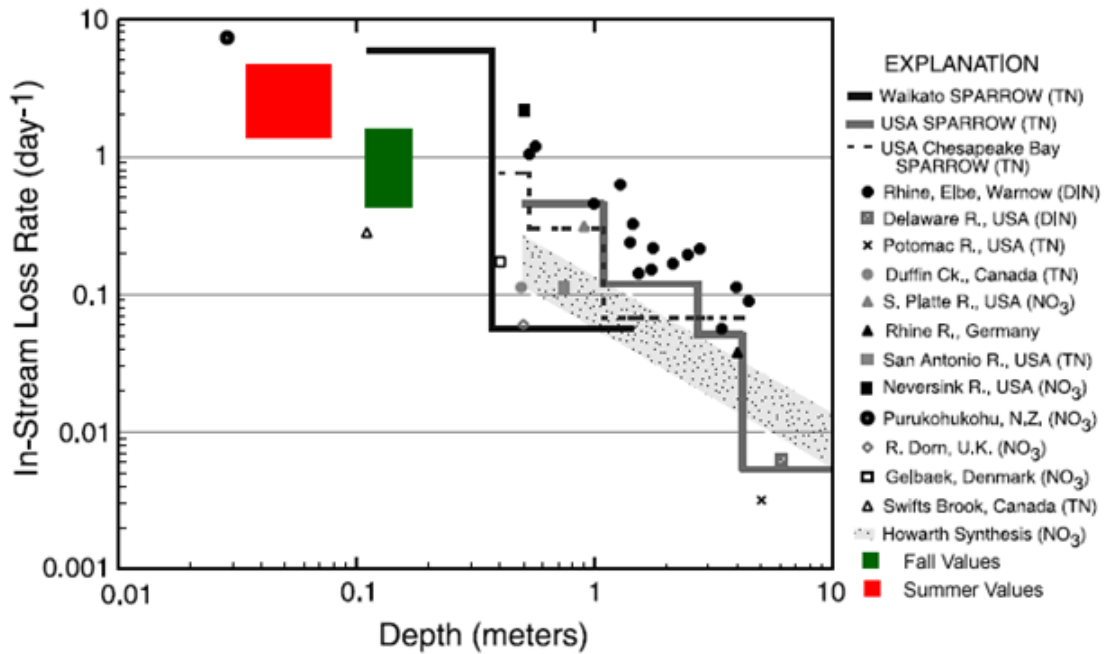


Fig 8. In-stream rates of N removal for the New Zealand SPARROW nitrogen model (Alexander et al. 2002) and other watershed studies in relation to stream channel depth (modified from Alexander et al. 2000 and Alexander et al. 2002); data for Purukohukohu from Cooper and Cooke (1984); data for R. Dorn, Gelbaek, and Swifts Brook from Seitzinger et al. (2002); other estimates from Alexander et al. (2000). Summer (red) and fall (green) estimates for the Rhode Island headwater stream study site are values calculated from inner quartiles of the observed stream depth in the summer and fall of the constant rate injection.

Applicability of the constant rate injection method for assessing the N sink potential of RI headwater streams

The constant rate injection method is a costly approach for assessing in-stream denitrification, warranting a careful assessment of the likely importance and variability of denitrification in RI headwater streams. In watersheds with agricultural and suburban land uses, RI streams often have NO₃-N concentrations in excess of the 1.1 mg l⁻¹ observed in the study stream (Da Silva, 2003). Assuming discharge rates and concentrations comparable to the ranges in the headwater stream examined in this study, a 3 hour constant rate study with ¹⁵N enrichment levels of 25 atom% in the dosing solution would require \$250-\$5000 of enriched ¹⁵N for a single trial – and this assumes that the cost of 99% ¹⁵N enriched NO₃-N remain in the current range of \$175-\$280 per gram. For streams with longer travel times, higher flow rates or higher ambient NO₃-N, the dosing cost per trial would increase.

Given the wide variation of in-stream reach N processing observed by many researchers, it would not be prudent to simply extend the negligible denitrification rates observed at a single site to headwater streams throughout RI. However, the physical attributes of the study stream and its watershed appear to represent many other headwater streams, suggesting that other types of watershed sinks may prove more effective at N retention. Based on the NHDplus dataset (Horizon Systems, 2007), 45% of headwater stream lengths in Rhode Island occur in watersheds with the same glacial geology, soil type and riparian zone land use (Table 7). Based on a random sampling of 54 first order and 19 second order Rhode Island streams, the average first order stream length in Rhode Island was found to be 0.51 km, and the average second order stream length was found to be only 0.59 km (RIGIS, 2007), comparable to the 0.5 km reach that was examined in this study.

It can be argued that this study stream has attributes that should not create unusually low in-stream N losses or unusually long uptake lengths in comparison with other RI headwater streams. The watershed is likely to generate substantial baseflow due to the permeable outwash soils, high forest cover and low extent of impervious cover. These watershed features in conjunction with a low stream gradient are likely to create stream retention times that are on the higher end of what may be expected within Rhode Island. For example, headwater catchments with till deposits or more urbanization are more likely to have flashy hydrographs, generating runoff with higher velocities and less retention times, characteristics unlikely to favor in-stream N removal..

The uncertainties surrounding in-stream N removal warrant further investigation of settings with extended retention times and benthic interactions. Higher order river systems should be secondary targets of study, since removal processes are reduced as flow increases and residence time decreases. Consideration of additional study should be given to headwater streams with retention features such as impoundments and streams that are connected to ponds and swamps and thus may increase chances of N removal. The constant rate injection method needs to be reviewed for these types of field studies, particularly in light of the costs associated with situations with long retention times.

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