



Measurement of denitrification in rivers: an integrated, whole reach approach

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Abstract

Rivers are believed to play an important role in nitrogen removal via denitrification. Unfortunately, there are few data quantifying these processes *in situ*, primarily due to methodological constraints. We have developed a new approach for estimating denitrification in rivers at the whole reach scale and have applied this approach to three small rivers, the Millstone River in central New Jersey, and the Iroquois River and Sugar Creek in northwest Indiana–northeast Illinois (USA). The approach is based on measuring the change in dissolved N₂ concentration as a parcel of water moves downstream. Two volatile, non-reactive tracers (propane and isobutane) were co-injected, and the rate of change in the ratio of these gases was used to calculate a first-order transfer rate of N₂ (K_{N_2}) to correct for loss of the gas to the atmosphere. Nitrogen removal via denitrification ranged between 0.27 ± 1.21 mmol N m⁻² h⁻¹ in Sugar Creek during May 2000 and 15.81 ± 2.51 mmol N m⁻² h⁻¹ in the Millstone River during March 2001. This approach could permit testing of factors that are believed to control denitrification at the reach scale, such as nitrate concentration, discharge, temperature, and water residence time, and could provide a clearer picture of nitrogen transformations in rivers.

Introduction

Nitrogen inputs to watersheds have dramatically increased as a result of human activities, (Vitousek et al., 1997). A portion of this N enters river networks, degrading river water quality. In much of the Midwestern United States nitrogen derived from fertilizer application has substantially contributed to water quality degradation (Beaulac and Reckhow, 1982; David et al., 1997). Rivers export large quantities of nitrogen from watersheds to estuaries contributing to eutrophication of coastal marine ecosystems (National Academy of Sciences, 2000). However, not all nitrogen loaded into rivers is ultimately exported to estuaries. Processes such as denitrification, organic matter burial in sediments, sediment sorption, and plant and microbial uptake can remove N from the

river, and thus affect the amount of N that is transported by rivers to coastal ecosystems (Billen et al., 1991). Of these processes, only denitrification represents permanent nitrogen removal, as the endproducts (N₂ and N₂O), are not biologically available to most organisms. Denitrification has the potential to improve water quality in rivers and to limit nitrogen export to estuaries, thereby buffering the effects of nitrogen pollution in these ecosystems.

Several approaches have been used for measuring denitrification in aquatic systems, including stable N isotope tracer methods (Nishio et al., 1982; Binnerup et al., 1992; Nielsen, 1992; Risgaard-Petersen et al., 1999), and measurement of N₂ production in a gas-tight chamber (Seitzinger et al., 1980). These approaches have been adapted for *in situ* denitrification measurements in aquatic systems using benthic

chambers (e.g., Devol, 1991; Devol & Christensen, 1993; Devol et al., 1997; Christensen et al., 2000; Laursen & Seitzinger, 2002). However, adaptation of these methods for *in situ* denitrification measurements in rivers presents a unique challenge as physical and chemical gradients in sediments vary with current velocity (Huettel & Gust, 1992; Laursen unpublished data). The use of benthic chambers in lotic systems may alter these gradients and, consequently, alter rates of benthic processes such as denitrification. Chambers also encompass a small surface area and extrapolating denitrification rates to a larger area can introduce considerable error, particularly if there is substantial small-scale variability. Other approaches that integrate this small-scale variability may be more appropriate for measuring denitrification in rivers. These approaches include the use of natural abundance stable N isotope ratios in nitrate and dissolved N_2 (McMahon & Böhlke, 1996), nitrogen mass balance in which denitrification is estimated as the net loss in total nitrogen or change in nitrate concentration (Swank & Caskey, 1982; Hill, 1983; Billen et al., 1985; Jacobs & Gilliam, 1985; Chesterikoff et al., 1992; Mulholland, 1992; Jansson et al., 1994; Sjödin et al., 1997), or some combination of these approaches (Kellman & Hillaire, 1998). Natural abundance stable isotope and mass balance approaches can be applied to large rivers providing spatially integrated measures of river denitrification. However, both approaches are indirect measures of denitrification (i.e., they do not measure production of an endproduct), and both have substantial limitations. Measurement of natural abundance N isotopes can be costly and the isotopic fractionation by various other nitrogen cycling processes can make results difficult to interpret. Temporary storage of nitrogen in a reach by autotrophic uptake or physical retention can affect estimates of denitrification using a mass balance approach, and a complete budget of nitrogen inputs and outflows can be difficult to obtain. For these reasons a more simple and direct method of measuring denitrification in rivers is desirable.

Recent advances in membrane inlet mass spectrometry have made it possible to measure very small changes in concentrations of dissolved gases easily and rapidly (Kana et al., 1994). Denitrification can be measured directly as the increase in dissolved N_2 . We have developed a novel approach for quantifying denitrification in rivers based on measuring dissolved N_2 concentrations in river water, and correcting for atmospheric exchange. In the current study we have applied this approach to measure denitrification in two Mid-

western rivers and one New Jersey river. This technique provides a direct measure of denitrification (i.e., measurement of the endproduct) and integrates small-scale spatial variability in sediment processes. Further, this method measures denitrification at spatial and temporal scales appropriate to assess its importance to nitrogen removal from rivers.

Materials and methods

Study sites

The Millstone River is part of the Raritan River basin in central New Jersey, draining an area of mixed suburban and agricultural land use. The study site was a 6-km reach of the Millstone River near Griggstown, NJ. This reach has a relatively uniform channel morphology, no major lateral inputs of surface water, and no point sources of nitrogen along the reach. Annual average discharge is $\sim 11\text{--}14\text{ m}^3\text{ s}^{-1}$. The Millstone River flows north through a region of unglaciated piedmont, minimizing groundwater influence in the study reach.

The Iroquois River and Sugar Creek are part of the Iroquois River Basin, a major sub-system of the Illinois River Drainage Basin. The Iroquois River Basin is dominated by agricultural land use, primarily corn and soybean cultivation. Water quality in the Iroquois River Basin, including nitrogen concentrations, is being studied as part of the U.S. Geological Survey National Research Program and the USGS National Water Quality Assessment Program. The study sites included an 18-km reach on the Iroquois River near Foresman, IN, and a 24-km reach on Sugar Creek near Milford, IL. Annual average discharge is $\sim 7\text{--}10\text{ m}^3\text{ s}^{-1}$ in the Iroquois River and $\sim 1\text{--}2\text{ m}^3\text{ s}^{-1}$ in Sugar Creek.

Sample collection and analysis

A Lagrangian sampling scheme was used to collect water samples for dissolved gas analyses (N_2 :Ar) in the study reaches. In the Millstone River the same parcel of water was sampled at two different access points as the parcel moved downstream. Access points were located near bridges along the reach. In the Iroquois River the same parcel of water was sampled at seven different access points, and in Sugar Creek a parcel of water was sampled at six different access points along the reach. Mean current velocity at each sampling loc-

ation was used to estimate the time of travel for the parcel from one sampling location to the next downstream location. Samples were collected at mean depth in the area of greatest discharge (thalweg) using a peristaltic pump, or in shallow reaches using a bucket and carefully avoiding the creation of bubbles. Samples for N₂:Ar analysis were collected by gravity-filling 60-ml BOD bottles from the bucket using a teflon tube. The tube was inserted to fill BOD bottles from the bottom. During bottle filling, several volumes were allowed to overflow, preventing contamination of water samples by atmospheric N₂. Samples were preserved by pipetting a small volume of saturated HgCl₂ solution into each bottle (final concentration 0.25%, v/v) immediately before inserting the ground glass stopper. Prior to analysis, samples were stored under water 1–2 °C below *in situ* temperature.

Dissolved N₂:Ar was measured using a Membrane Inlet Mass Spectrometry (MIMS) system (Kana et al., 1994). Water samples were pushed through a stainless steel capillary using a peristaltic pump with Viton™ tubing. The capillary passed coaxially through a T-shaped glass inlet tube under high vacuum (~10⁻⁶ mmHg). Within the inlet tube, the water sample was pushed past a gas-permeable silicon membrane. The vacuum degassed the water sample as it passed the membrane, introducing the gases into a quadrupole mass spectrometer (Balzers Prisma™). A liquid nitrogen trap was used to freeze out water vapor and carbon dioxide, preventing interference of carbon dioxide with N₂ measurements. Peaks were measured for N₂ and Ar (*m/z*: 28, 40). Replicate samples generally have a lower percent standard deviation among N₂:Ar measurements (<0.05%) than among N₂ measurements (<0.10%). Therefore, increases in N₂ were calculated from the change in N₂:Ar through time. Argon concentration was assumed to behave conservatively, varying as a function of a temperature corrected first-order gas transfer coefficient (*K*_{Ar}) (detailed below).

The MIMS system was calibrated using a series of four standards, each with a different salinity (0–100 ppt). Standards were prepared by equilibrating water with the atmosphere at a constant temperature. Standards were stirred for a minimum of 48 h, and the stirring rate was slow to prevent entrainment of gas bubbles associated with turbulent mixing. The dissolved gas concentrations in standards (N₂ and Ar) were calculated using the solubility equations of Weiss (1970) for the appropriate temperature and salinity. Standards were interspersed among samples during

analysis (one standard after every three samples) to ascertain there was no instrument drift. We experimentally manipulated dissolved O₂ concentrations in standards using sodium hydrosulfite, and found that N₂:Ar measurements made using our MIMS system are not affected within the range of O₂ concentrations measured in our samples.

Channel measurements

In the Iroquois River and Sugar Creek, U.S. Geological Survey personnel measured wetted channel width, mean depth, and mean velocity to calculate discharge (*Q*) at each sampling location. In the Millstone River, wetted channel width and depth were measured at the upstream and downstream locations on each sampling date. Depth was measured at a minimum of seven vertical transects equally spaced across the width of the channel. Discharge at the downstream location was obtained from the U.S. Geological Survey on each sampling date (station 01402000, Blackwells Mills, NJ). The cross-sectional surface area and discharge were used to calculate mean velocity in the Millstone for each sampling date.

Denitrification

Denitrification can increase the N₂:Ar of river water over time in the absence of atmospheric exchange. The primary endproduct of denitrification, N₂, is lost from water through re-equilibration with the atmosphere. The change through time of N₂ concentration is, therefore, a function of production and atmospheric exchange. The dissolved N₂ concentration in river water can be described as:

$$[N_2] = f(N_2 \text{ production, atmospheric exchange}) \quad (1)$$

We have developed a model that predicts the N₂ concentration in river water as a function of the denitrification rate, atmospheric exchange of N₂, and channel morphology (i.e., mean width and depth). Given the dissolved N₂ concentration, atmospheric exchange rate, and channel morphology, it is possible to determine denitrification rates using the model.

Determination of atmospheric exchange rates

The atmospheric exchange of N₂ and Ar were determined by measuring the gas transfer rates of volatile,

non-reactive tracers. A similar approach was used to determine the gas transfer rate of CO₂ in the Hudson River (Clark et al., 1994). Because these tracers are non-reactive the primary mechanism of removal from water is atmospheric exchange. The transfer rates of the tracers were used to determine the loss rates of N₂ and Ar by relating gas transfer rates to molecular diffusion coefficients for the gases.

The atmospheric exchange of a gas across the air–water interface (F) is defined in terms of the first-order gas transfer rate (K), mean water column depth (h), and the difference between the mean gas concentration in the water column (C_{mean}) and the atmospheric equilibrium concentration (C_{equil}) according to:

$$F = hK(C_{\text{mean}} - C_{\text{equil}}) \quad (2)$$

assuming that the water column is well-mixed (homogeneous). Depth profiles of conductivity, temperature, and dissolved oxygen at several points across the channel suggested that the water column was well mixed in the Iroquois River and Sugar Creek at all sampling locations (data not shown). We assume similar homogeneity in the Millstone River, supported by several depth profiles of conductivity across the channel.

The first-order gas transfer rates of N₂ and Ar were determined in the Iroquois River on 27 June 1999 and 8 May 2000, in Sugar Creek on 7 May 2000, and in the Millstone River on 15 October 1999 and 12 March 2001. Propane and isobutane were used as volatile tracers. These gases were co-injected at the head of a river reach from a tank of mixed gases (5% propane, 5% isobutane, 90% N₂). The gases were bubbled into the river water using a perforated garden hose staked to the riverbed, transecting the channel perpendicularly.

Concentrations of propane and isobutane were measured at three locations along the study reaches. For example, in the Iroquois River water samples were collected for propane and isobutane analysis at 50 m below the point of injection, approximately 0.6 km below the point of injection, and 2.2 km below the point of injection. At each location, river water was sampled repeatedly, permitting us to measure the change in propane and isobutane concentrations through time. Water samples were collected from mean depth in the thalweg in 60-ml serum bottles. Samples were preserved by adding one crystal of KOH (approximately 20 mg) to the bottle. Bottles were stoppered (butyl rubber) underwater to prevent formation of a headspace. A headspace was created in each sample by replacing 3 ml of water with 3 ml of N₂. Propane and isobutane were measured following headspace equi-

libration at 25°C. Gas samples were injected into a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 1.8 m × 0.318 cm o.d. stainless steel column packed with HayeSep T, 80/100 mesh (N₂ carrier gas flow 30 ml min⁻¹). The total amounts of propane and isobutane dissolved in the water and gas phases of the samples were calculated using the Ostwald coefficients of Hayduk (1986) for the appropriate temperature. A propane and isobutane standard series used for instrument calibration was prepared from a 1.01 ppm propane, 0.99 ppm isobutane standard (Scott Specialty Gases). Standards were interspersed among samples during analysis (one standard after every five samples) to ascertain there was no instrument drift.

Propane and isobutane have different molecular diffusion coefficients and, therefore, different loss rates from water. The decrease in the absolute concentration of each gas through time was governed by mixing (dilution) and loss to the atmosphere. The change through time in the concentration of one gas relative to the other was governed only by loss to the atmosphere (Fig. 1). Measuring the rate of change in the concentration of one gas relative to the other allowed calculation of the first-order gas transfer rates for each tracer (K_{prop} and K_{isobut}) (Jähne et al., 1987), assuming transfer rates were related according to:

$$K_{\text{isobut}}/K_{\text{prop}} = (S_{\text{Cisobut}}/S_{\text{Cprop}})^{-n}, \quad (3)$$

where S_{Cisobut} and S_{Cprop} are the Schmidt numbers for isobutane and propane, respectively, and n is the Schmidt number coefficient. Schmidt numbers were calculated as the kinematic viscosity of water divided by the molecular diffusion coefficients in water of isobutane and propane, respectively. The molecular diffusion coefficient of propane in water was obtained from Witherspoon & Saraf (1965). The molecular diffusion coefficient of isobutane was calculated from the molecular diffusion coefficient of propane according to Graham's Law. Laboratory experiments have determined the Schmidt number coefficient to be $\sim 2/3$ for surfaces without waves, decreasing sharply to $\sim 1/2$ for wavy surfaces not broken by white caps (Jähne et al., 1987). Equation (3) can be rearranged to express K_{prop} in terms of the measured change in propane and isobutane concentrations and the Schmidt numbers for propane and isobutane (Appendix A). The first-order transfer rate of propane is expressed as:

$$K_{\text{prop}} = d\{\ln([\text{prop}]/[\text{isobut}]) / (1 - (S_{\text{Ciso}}/S_{\text{Cprop}})^{-n})\} / dt. \quad (4)$$

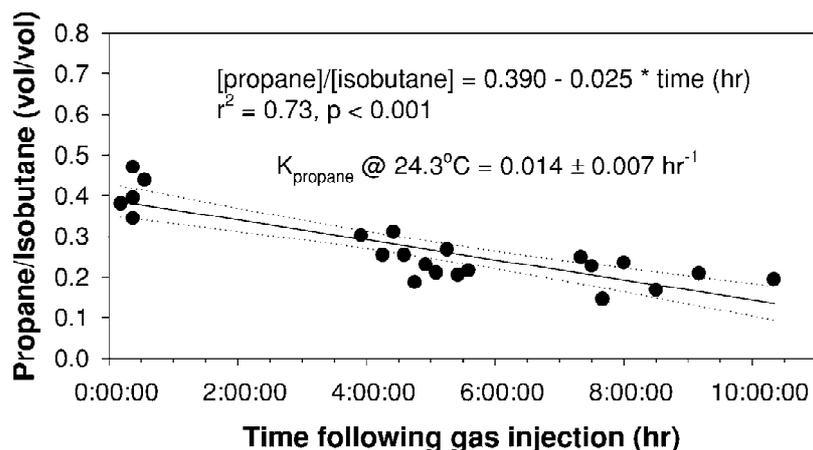


Figure 1. In (propane/isobutane) measured in Iroquois River following tracer injection on 27 June 1999. The first-order transfer rate of propane was calculated from the change in this ratio and used to derive K_{N_2} and K_{Ar} as described in the text.

The first-order gas transfer rate of N_2 is determined according to Equation (3), substituting K_{N_2} for K_{isobut} and Sc_{N_2} for Sc_{isobut} . Similarly the first-order transfer rate of Ar is calculated by substituting K_{Ar} for K_{isobut} and Sc_{Ar} for Sc_{isobut} in Equation (3). Atmospheric exchange of N_2 and Ar were then calculated from first-order transfer rates according to Equation (2).

First-order transfer rates measured in this manner are temperature specific. As the temperature of a parcel of water changes during downstream transport, transfer rates for N_2 were corrected according to:

$$K_{N_2^*}/K_{N_2} = (Sc_{N_2^*}/Sc_{N_2})^{-n}, \quad (5)$$

where $K_{N_2^*}$ is the unknown first-order transfer rate for N_2 at some new water temperature, K_{N_2} is the measured first-order transfer rate at the previous water temperature, $Sc_{N_2^*}$ is the Schmidt number for N_2 at the new temperature, and Sc_{N_2} is the Schmidt number for N_2 at the previous temperature. The temperature-specific first-order transfer rates for Ar were calculated in a similar fashion. There was no attempt to correct for differences in first-order transfer rates as a function of wind velocity. However, wind velocity did not vary much during the experimental period (period including measurement of transfer rate and Lagrangian sampling).

Determining N_2 concentrations from $N_2:Ar$

Argon standard curves were constructed by plotting the signal for $m/z = 40$ versus the theoretical equilibrium Ar concentration for each standard, and fitting

a least-squares linear regression line. These standard curves were then used to directly calculate the *in situ* Ar concentrations of samples. Using the measured *in situ* Ar concentration of the furthest upstream site as a starting point, we modeled the expected change in Ar concentration as the parcel of water moved downstream in one minute time steps, assuming that Ar concentration changed as a function of K_{Ar} (Table 1). The model allowed for changes in K_{Ar} due to temperature changes by recalculating Sc_{Ar} at each time step. The theoretical equilibrium Ar concentration was also allowed to change at each time step as temperature changed. Measured Ar concentrations at each sampling site, calculated directly from Ar standard curves, were plotted along with modeled Ar to determine how effectively the model described *in situ* concentrations of Ar (Fig. 2). There was relatively good agreement between modeled and measured Ar in most cases, giving us confidence in our model. It should be noted that while modeled and measured Ar were generally similar, they did not always match the theoretical equilibrium Ar concentration. In the Iroquois River and Sugar Creek during May 2000 K_{Ar} was high and Ar concentrations rapidly re-equilibrated as water temperature changed. Consequently, modeled and measured Ar concentrations were similar to the theoretical equilibrium Ar concentrations. Similarly, in the Millstone River during October 1999 re-equilibration was sufficient to move measured and modeled Ar concentrations toward the theoretical equilibrium Ar concentration at the downstream site. In the Iroquois River during June 1999

Table 1. Components and calculation procedures of model used to predict Ar concentrations at downstream locations

<i>Model inputs</i>	
<i>Measured</i>	<i>Derived</i>
Channel depth (m)	d Equilibrium Ar concentration ($\text{mmol m}^{-3} \text{ h}^{-1}$)
Channel width (m)	dt
Velocity (m min^{-1})	
Δ Time (h)	d Temperature ($^{\circ}\text{C h}^{-1}$)
Initial Ar concentration (mmol m^{-3})	dt
Initial equilibrium Ar concentration (mmol m^{-3})	
Initial temperature (upstream) ($^{\circ}\text{C}$)	
Final temperature (downstream) ($^{\circ}\text{C}$)	
Initial K_{Ar} (h^{-1})	
 <i>Calculations</i>	
Total Ar in the parcel of water (mmol) = Ar concentration (mmol m^{-3}) \times width (m) \times distance traveled in 1 min (m)	
At each time step, total Ar is recalculated as $\text{Ar} = \text{Ar}' - \text{Ar loss}$	
where:	
Ar' = total Ar in parcel at previous time step (mmol)	
Ar loss (mmol) = Ar flux ($\text{mmol m}^{-2} \text{ min}^{-1}$) \times 1 min \times width (m) \times distance (m) traveled in 1 min	
Ar flux ($\text{mmol m}^{-2} \text{ min}^{-1}$) = depth (m) \times (1h / 60 min) \times K_{Ar}' (h^{-1}) \times [$\text{Ar}_{\text{measured}}$ (mmol m^{-3}) - $\text{Ar}_{\text{equil}}'$ (mmol m^{-3})]	
K_{Ar}' (h^{-1}) = First-order transfer rate of Ar given the current temperature (K_{Ar}' varies through time as the water temperature changes)	
$\text{Ar}_{\text{equil}}'$ = equilibrium Ar concentration in the parcel at current time step given the current temperature ($\text{Ar}_{\text{equil}}'$ varies through time as the water temperature changes)	
Dissolved Ar (mmol m^{-3}) is then recalculated from total Ar after each 1-min time step	

and in the Millstone River during March 2001 K_{Ar} was lower and the change in water temperature was more rapid than the re-equilibration of Ar. As a result, modeled and measured Ar concentrations were not similar to theoretical Ar concentrations.

In situ N_2 concentrations could also be directly calculated by constructing a standard curve, plotting the signal for $m/z = 28$ versus the theoretical equilibrium N_2 concentration for each standard. However, N_2 in replicate samples calculated directly from standard curves generally have a higher percent standard deviation than N_2 :Ar measurements. Therefore, *in situ* N_2 concentrations were calculated from measured *in situ* N_2 :Ar rather than directly from N_2 standard curves. The error associated with modeling Ar was generally less than the error associated with directly calculating Ar in replicate samples (Fig. 2). Therefore, we used the model predicted Ar values (\pm error) at each site to calculate *in situ* N_2 from measured *in situ* N_2 :Ar. This approach allowed us to test the strength of our model which would later be adapted to estimate denitrification, and it allowed us to control for analytical error in estimating N_2 concentrations by permitting the use of N_2 :Ar rather than directly measured N_2 .

Modeling denitrification

Denitrification was determined by the change in the dissolved N_2 concentration of a parcel of water as it moved downstream. The model determined the rate of N_2 production required to achieve the observed change in N_2 , given K_{N_2} . Changes in N_2 were modeled using 1-min time steps (Table 2). The model allowed for changes in K_{N_2} due to temperature changes by recalculating Sc_{N_2} at each time step. The equilibrium N_2 concentration was also allowed to change at each time step as temperature changed. Conceptually, denitrification was determined by excess N_2 (i.e., an increase in N_2 not accounted for by atmospheric exchange). Measured concentrations of dissolved N_2 were compared with equilibrium N_2 concentrations in all study reaches. Water samples were supersaturated with respect to N_2 , although *in situ* concentrations generally tracked equilibrium concentrations, increasing as temperature decreased. Excess N_2 was determined by modeling the expected change in N_2 concentration if no denitrification occurred (i.e., if the change in N_2 were solely due to atmospheric exchange) and comparing this estimated N_2 concentration with the

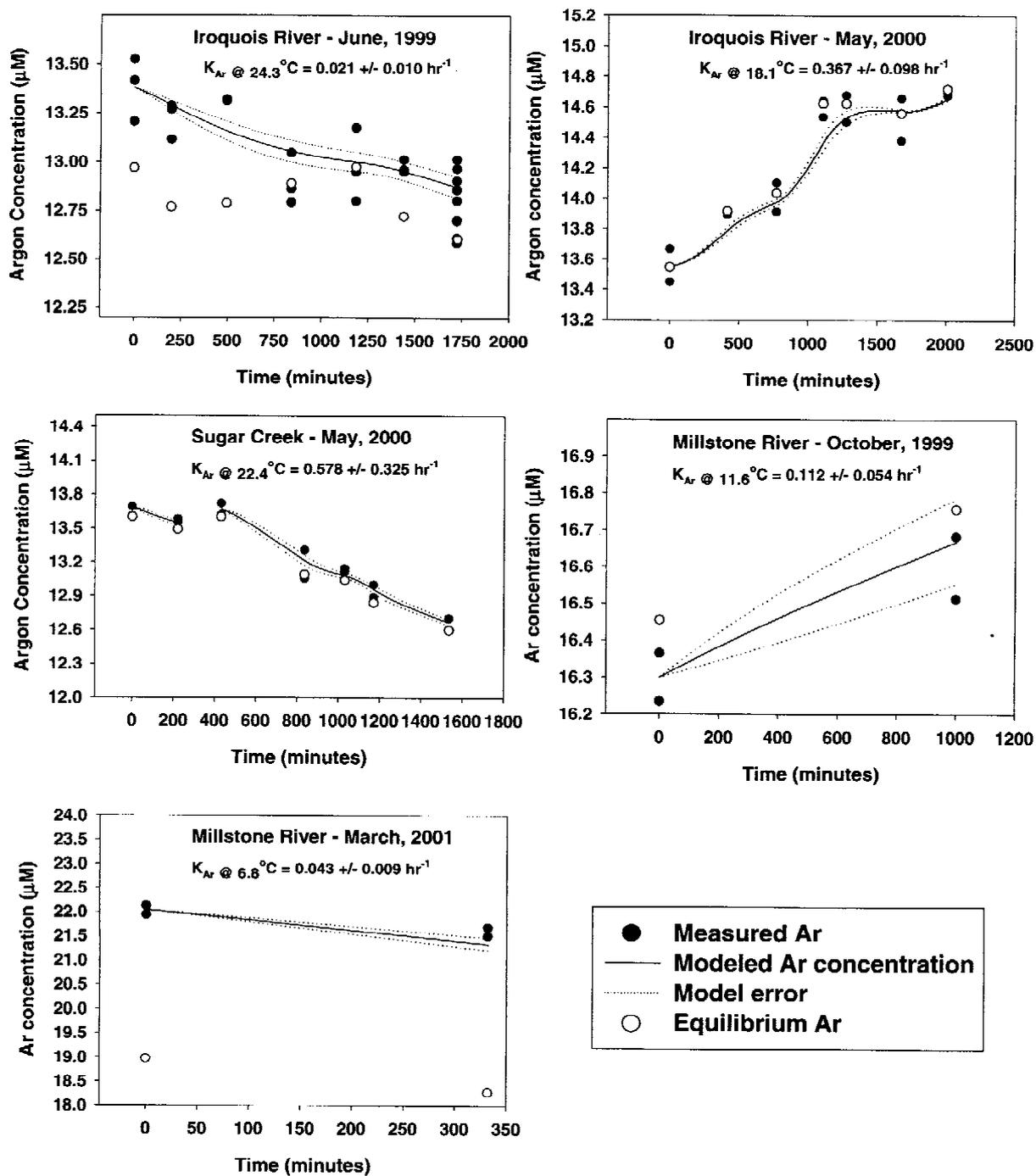


Figure 2. Dissolved Ar concentrations in study reaches. Black circles represent measured dissolved Ar at each sampling site. Solid lines represent the model predicted Ar concentrations based on first-order transfer rates and changes in temperature as the parcel of water moves downstream. Dotted lines represent the error in model predicted Ar concentration. White circles represent theoretical Ar concentration based on water temperature.

Table 2. Components and calculation procedures of model used to quantify denitrification from dissolved N₂ concentrations

<i>Model inputs</i>	
<i>Measured</i>	<i>Derived</i>
Channel depth (m)	d Equilibrium N ₂ concentration (mmol N ₂ m ⁻³ h ⁻¹)
Channel width (m)	dt
Velocity (m min ⁻¹)	
Δ Time (h)	d Temperature (°C h ⁻¹)
Initial N ₂ concentration (mmol N ₂ m ⁻³)	dt
Initial equilibrium N ₂ concentration (mmol N ₂ m ⁻³)	
Initial temperature (upstream) (°C)	
Final temperature (downstream) (°C)	
Initial K _{N2} (h ⁻¹)	
Denitrification rate (mmol N ₂ m ⁻³ min ⁻¹)	
<i>Calculations</i>	
Total N ₂ in the parcel of water (mmol N ₂) = N ₂ concentration (mmol N ₂ m ⁻³) × width (m) × distance traveled in 1 min (m)	
At each time step, total N ₂ is recalculated as N ₂ = (N ₂ ' + N ₂ prod) – N ₂ loss	
where:	
N ₂ ' = total N ₂ in parcel at previous time step (mmol N ₂)	
N ₂ prod is the total produced (mmol N ₂) within the parcel during the 1-min time step, calculated as denitrification rate (mmol N ₂ m ⁻³ min ⁻¹) × 1 min × width (m) × depth (m) × distance (m) traveled in 1 min	
N ₂ loss (mmol N ₂) = N ₂ flux (mmol N ₂ m ⁻² min ⁻¹) × 1 min × width (m) × distance (m) traveled in 1 min	
N ₂ flux (mmol N ₂ m ⁻² min ⁻¹) = depth (m) × (1 h / 60 min) × K _{N2} ' (h ⁻¹) × [N ₂ measured (mmol N ₂ m ⁻³) – N ₂ equil' (mmol N ₂ m ⁻³)]	
K _{N2} ' (h ⁻¹) = First-order transfer rate of N ₂ given the current temperature (K _{N2} ' varies through time as the water temperature changes)	
N ₂ equil' (mmol N ₂ m ⁻³) = equilibrium N ₂ concentration in the parcel at current time step given the current temperature (N ₂ equil' varies through time as the water temperature changes)	
Dissolved N ₂ (mmol N ₂ m ⁻³) is then recalculated from total N ₂ after each 1-min time step	

measured concentration. Denitrification was calculated based on the production required to explain this excess N₂ for each site.

Error terms

While the natural log of the molar ratio of propane to isobutane decreased linearly with time, there was some uncertainty in the rate at which this ratio decreased (Fig. 1). The standard deviation of the regression was used to determine the error in K_{prop} associated with this uncertainty. This was done by calculating K_{prop} assuming maximum and minimum error (± 1 standard deviation) in d(ln(prop/isobut)/dt). In addition, the maximum error in K_{prop} associated with uncertainty in the Schmidt number coefficient (*n*) was determined by calculating K_{prop} with *n* = 1/2 and with *n* = 2/3. The errors in K_{prop} associated with uncertainty in d(ln(prop/isobut)/dt and with the Schmidt number coefficient were assumed to be additive. Max-

imum and minimum values of K_{prop} were substituted into Equation (3), and K_{N2} and K_{Ar} were recalculated as before to determine the errors in these first-order transfer rates. Error in K_{N2}* was calculated by substituting maximum and minimum values of K_{N2} into Equation (5) and recalculating K_{N2}* for each sample temperature. Error in K_{Ar}* was similarly calculated. The analytical errors associated with dissolved N₂ measurements were determined from replicate standards. The error associated with N₂ measurements was ± 0.7 μM. Along the study reach, the Millstone River channel is fairly consistent with respect to width and depth. Cross-sectional areas were measured at 18 transects spaced equidistantly along the reach. The standard deviation as a percentage of the mean cross-sectional area was ~10%. Therefore, denitrification was modeled assuming that the average cross-sectional area of the stream reach was ± 10% of the measured cross-sectional area. The channels of the Iroquois River and Sugar Creek vary more with

respect to width and depth along their lengths. Denitrification in the Iroquois River and Sugar Creek was modeled assuming that the average cross-sectional areas of the stream reaches were $\pm 20\%$ of measured cross-sectional areas. All of these sources of error were assumed to be additive in modeling denitrification. Therefore, the error terms associated with denitrification rates reflect the maximum total error given the uncertainty in model input parameters and the analytical uncertainty in measuring N_2 . For the Iroquois River and Sugar Creek, reported denitrification rates are the average values for all individual reaches and the reported errors are calculated from the average error rates of individual reaches.

Results and discussion

Hydrological parameters

Denitrification measurements were conducted in study reaches spanning a range of hydrologic and physical conditions. Discharge in study reaches ranged from $1.62 \text{ m}^3 \text{ s}^{-1}$ in Sugar Creek during May 2000 to $18.76 \text{ m}^3 \text{ s}^{-1}$ in the Iroquois River in June 1999 (Table 3). Within the Iroquois and Millstone Rivers current velocity and discharge were inversely related to transit time. Mean temperature within study reaches ranged from 6.8 to 24.3°C .

First-order transfer rates

First-order transfer rates for N_2 and Ar varied as a function of mean wind velocity (Table 4) and to a lesser extent, water temperature. Transfer rates were particularly high in Sugar Creek and the Iroquois River during May 2000. These high transfer rates account for the similarity between measured and equilibrium Ar concentrations in these study reaches during May (Fig. 2), and for the large error in modeling N_2 concentrations in Sugar Creek (see below). First-order transfer rates were expressed as K_{600} to allow comparison between dates (600 is the Schmidt number of CO_2 at 20°C).

Excess N_2

Denitrification produces N_2 in excess of that expected based on re-equilibration with the atmosphere. The model was used to predict changes in dissolved N_2 as a parcel of water moved downstream. At each location,

measured N_2 was used as a starting point to predict the N_2 concentration at the next downstream location (based on first-order transfer rates and changes in water temperature, and assuming no *in situ* production). Excess N_2 at each location was then calculated as the difference between measured N_2 and the model predicted N_2 for that location. Excess N_2 was found in all study reaches and at all sites (Fig. 3, black bar exceeds gray bar for all locations), although this excess N_2 was not statistically significant at Iroquois River site 7 during June 1999, or at any site in Sugar Creek during May 2000. In the Iroquois River, excess N_2 averaged $1.61 \mu\text{M}$ (range 0.59 – $2.63 \mu\text{M}$) in June 1999. In June, the Iroquois River was treated as two study reaches (sites 3–4 and sites 5–7) because we measured a decrease in discharge between sites 4 and 5 that could not be explained. Therefore no estimate was made of excess N_2 at site 5. Excess N_2 was higher in the Iroquois River during May 2000, averaging $7.05 \mu\text{M}$ N_2 (range 2.5 – $10.4 \mu\text{M}$), suggesting higher rates of denitrification than in June 1999. Excess N_2 in Sugar Creek averaged $0.81 \mu\text{M}$ (range 0.28 – $1.32 \mu\text{M}$) in May 2000. Sugar Creek was functionally divided into two reaches (sites 4–5 and sites 7–10), as tributaries entered Sugar Creek between sites 5 and 7, potentially diluting or concentrating N_2 . Therefore no estimate was made of excess N_2 at site 7. The Millstone River had the greatest concentrations of excess N_2 . Excess N_2 in the Millstone was $8.33 \mu\text{M}$ in October 1999. In March 2001 excess N_2 was $39.91 \mu\text{M}$, suggesting very high rates of denitrification.

Importance of various sources of error in estimating denitrification

No one source of error consistently accounted for the majority of total error in estimating denitrification. Uncertainty in cross-sectional area accounted for 4% (Sugar Creek, May 2000) to 52% (Iroquois River, June 1999) of total error, on average accounting for 35% of total error. Analytical uncertainty in measuring dissolved N_2 accounted for 7% (Millstone River, March, 2001) to 64% (Sugar Creek, May 2000) of total error, on average accounting for 29%. Uncertainty in first-order transfer rates accounted for 25% (Iroquois River, May 2000) to 55% (Millstone River, March 2001) of total error, on average accounting for 36%. Of the error associated with uncertainty in first-order transfer rates, most was related to the measurement of $d(\ln(\text{prop}/\text{isobut})/dt$, and not uncertainty in the Schmidt number coefficient.

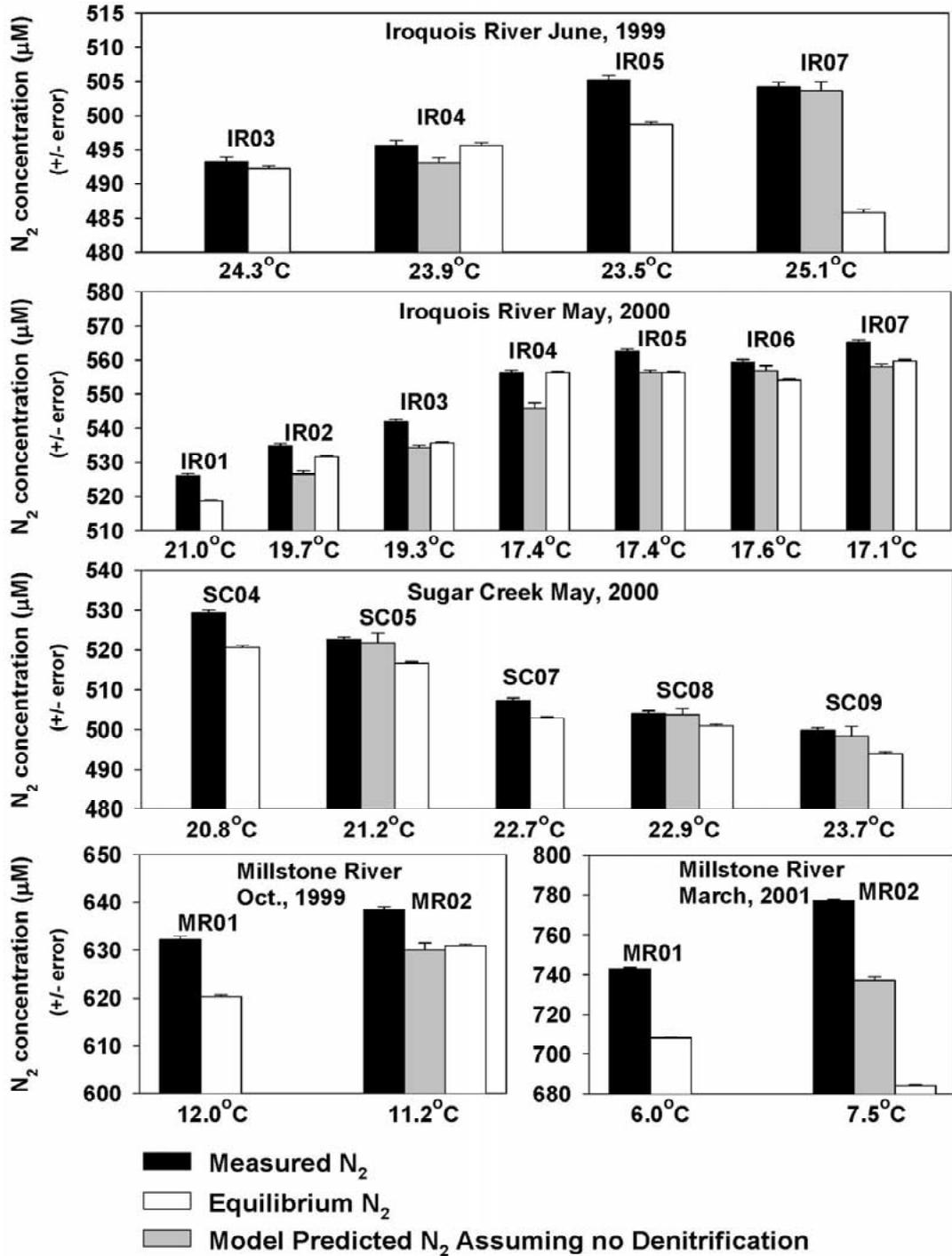


Figure 3. Dissolved N₂ in study reaches. Black bars represent measured N₂ at each sampling site. White bars represent the theoretical equilibrium dissolved N₂ concentration (based on water temperature) at each sampling site. Gray bars represent the model predicted dissolved N₂ assuming no *in situ* production of N₂ (based on first-order transfer rates and change in temperature as the parcel of water moves downstream). The difference between the black bar and the gray bar represents the excess N₂ at each sampling site as a result of denitrification. Sampling sites designated by number, for example IR01 = Iroquois River site 1, SC04 = Sugar Creek site 4, MR01 = Millstone River site 1, etc.

Table 3. Physical characteristics of rivers on sampling dates

Site, date	Q ($\text{m}^3 \text{s}^{-1}$)	Mean velocity (m s^{-1})	Transit time (h)	Mean depth (m)	Mean width (m)	Mean temp ($^{\circ}\text{C}$)
Iroquois River,						
25 – 26 June 1999 [†]	7.55	0.28	20.6	1.55	26.0	24.3
9 – 10 May 2000	18.76	0.34	15.7	2.08	29.2	18.1
Sugar Creek,						
8 – 9 May 2000	1.62	0.33	25.6	0.43	12.7	22.4
Millstone River,						
14 – 15 Oct. 1999	3.25	0.09	16.7	1.22	29.2	11.6
12 March 2001	10.33	0.33	5.3	1.02	30.8	6.8

[†] For reaches IR03–IR07.

Table 4. First-order gas transfer rates and wind velocities in study reaches

Site, Date	K_{Ar} (h^{-1})	K_{N_2} (h^{-1})	K_{600} (h^{-1})	Mean wind velocity (km h^{-1})
Iroquois River,				
25 – 26 June 1999	0.021 ± 0.010	0.011 ± 0.005	0.017 ± 0.008	5.0
9 – 10 May 2000	0.367 ± 0.098	0.205 ± 0.055	0.315 ± 0.084	32
Sugar Creek,				
8 – 9 May 2000	0.578 ± 0.325	0.315 ± 0.177	0.482 ± 0.271	35
Millstone River,				
14 – 15 Oct. 1999	0.112 ± 0.054	0.065 ± 0.031	0.100 ± 0.048	9.2
12 March 2001	0.043 ± 0.009	0.026 ± 0.005	0.040 ± 0.008	8.1

Table 5. Denitrification in study reaches

Site, Date	$\text{mmol N m}^{-2} \text{h}^{-1}$	mmol N km^{-1}
Iroquois River,		
25 – 26 June 1999 [†]	3.40 ± 1.32	93 ± 30
9 – 10 May 2000	8.47 ± 4.16	4015 ± 1971
Sugar Creek,		
8 – 9 May 2000	0.27 ± 1.21	55 ± 248
Millstone River,		
14 – 15 Oct. 1999	1.90 ± 0.85	925 ± 414
12 March 2001	15.81 ± 2.51	8111 ± 1309

Values are mean \pm error (calculated as described in text). Values for Iroquois River and Sugar Creek are averaged over all reaches.

[†] For reaches IR03–IR07.

Denitrification

Using the parameters listed in Tables 3 and 4, we estimated denitrification in the Iroquois River, Sugar Creek, and the Millstone River. In the Iroquois River, average denitrification rates in the sampled parcel were higher in May 2000 than in June 1999 (Table 5). Consequently loss of nitrogen as N_2 as the parcel of water moved downstream (km^{-1}) was more than a factor of 4 greater in May 2000 than in June 1999. The highest rates of denitrification and nitrogen loss during transport were in the Millstone River during March 2001. Denitrification and nitrogen loss were much lower in the Millstone River during October 1999. Denitrification rates and nitrogen loss during transport were lowest in Sugar Creek during May 2000. The measured denitrification rate in Sugar Creek was not significantly different than zero. Sugar Creek is a shal-

low stream (0.43 m), and at the time denitrification measurements were attempted, mean wind velocity was high (35 km h^{-1}) resulting in high exchange rates of dissolved gases. Under these conditions (shallow stream, high wind), this method for measuring denitrification is not very effective.

Significant groundwater inputs into a reach could influence our estimates of denitrification, particularly if the dissolved N_2 concentration of groundwater is greatly different than that of river water. In the Iroquois River during June 1999 discharge increased by $\sim 4\%$ between sites 3 and 4. Over this reach, excess N_2 was estimated as $2.63 \mu\text{M}$. For this to be an artifact of groundwater input, groundwater would have contained $\sim 65 \mu\text{M}$ excess N_2 , suggesting that we actually measured denitrification in this reach. Between sites 5 and 7, discharge in the Iroquois River decreased by $\sim 5\%$. Therefore, the excess N_2 estimated for this reach could not be attributed to groundwater influences. Similarly, in the Iroquois River during May 2000 there was no clear change in discharge over the study reach, suggesting that excess N_2 measured along the reach was the result of denitrification. Previous measurements of discharge in the Millstone River indicate that discharge also does not increase along this reach, thus excess N_2 must be the product of denitrification. Sugar Creek, however, does gain groundwater as indicated by increased discharge along the reach. Dissolved N_2 in groundwater could plausibly account for the excess N_2 measured in surface water along the reach, as excess N_2 concentrations in groundwater would not have had to exceed $10 \mu\text{M}$. These results suggest that this method is most appropriate for reaches that do not gain a significant amount of groundwater. However, sampling groundwater along a reach with the use of piezometers may permit estimates of groundwater contributions to excess N_2 measured in surface waters, allowing adaptation of this method for reaches that do gain groundwater.

Denitrification in river sediments has been studied previously using a variety of techniques. Most commonly, acetylene has been used to inhibit nitrous oxide reduction in sediment cores (Cooper & Cooke, 1984; Duff et al., 1984; Cooke & White, 1988; Christensen et al., 1989; Nielsen et al., 1990; Jansson et al., 1994; García-Ruiz et al., 1998a,b; Pattinson et al., 1998), or in benthic chambers (Torre et al., 1992). Riverine denitrification has also been estimated from NO_3^- flux data using sediment cores (Robinson et al., 1979) or benthic chambers (Chesterikoff et al., 1992), from NO_3^- microelectrode data (Laursen & Carlton,

1999), from direct N_2 flux data (Seitzinger, 1987, 1988), and using an isotope pairing technique (Pind et al., 1997). Denitrification rates reported in these studies were generally lower (median 0.61 , range $0\text{--}3.29 \text{ mmol N m}^{-2} \text{ h}^{-1}$) than those estimated for the Millstone and Iroquois Rivers. We might expect acetylene inhibition techniques to underestimate riverine denitrification, as nitrification and denitrification are often tightly coupled in aquatic systems (Jenkins & Kemp, 1984; Blackburn et al., 1994; Risgaard-Petersen et al., 1994), and as acetylene also inhibits nitrification (Berg et al., 1982; Bédard & Knowles, 1989). However, the Iroquois and Millstone Rivers are both low gradient rivers with fine, organically rich sediments and high spring nitrate concentrations ($> 300 \mu\text{M NO}_3^-$ in previously measured samples), and we might expect high rates of denitrification in these rivers.

A few studies have estimated area-specific rates of nitrogen removal in agriculturally impacted rivers based on nitrogen or nitrate mass balance. These approaches are perhaps more comparable to ours. In the South Platte River (Colorado, USA), nitrogen loss estimated over an annual cycle ranged between 0.14 and $7.14 \text{ mmol N m}^{-2} \text{ h}^{-1}$, averaging $2.00 \text{ mmol N m}^{-2} \text{ h}^{-1}$ (Sjodin et al., 1997). Nitrogen loss estimated from nitrate deficits in Duffin Creek (Ontario, Canada) averaged $0.48 \text{ mmol N m}^{-2} \text{ h}^{-1}$ (Hill, 1983) and $0.73 \text{ mmol N m}^{-2} \text{ h}^{-1}$ (Hill, 1988). Loss of nitrogen in the Nottawasaga River (Ontario, Canada) averaged $0.10 \text{ mmol N m}^{-2} \text{ h}^{-1}$ (Hill, 1983). While our estimates of denitrification in the Iroquois and Millstone Rivers exceed estimates of nitrogen loss from the Canadian rivers, they are similar to the nitrogen loss rates for the South Platte River and may be representative of low gradient, agriculturally impacted rivers.

Factors potentially regulating denitrification in rivers

A number of studies have examined factors that can control nitrogen retention and/or removal via denitrification in rivers, including nitrate concentration, organic carbon availability, sediment properties, benthic oxygen metabolism, flow rate, and land use (e.g., Robinson et al., 1979; Cooke & White, 1987; Christensen & Sorensen, 1988; Christensen et al., 1990; Jansson et al., 1994; Howarth et al., 1996). Several recent studies have examined physical characteristics of rivers (i.e., discharge, depth/water residence time) that can be related to efficiency of nitrogen removal in rivers via burial and/or denitrification (e.g., Howarth et al., 1996; Smith et al., 1997;

Alexander et al., 2000, Seitzinger et al., 2002). In previous studies, denitrification has not explicitly been measured at the reach-scale, as mass balance modeling approaches do not distinguish between denitrification and other processes removing nitrogen (i.e., sorption of nitrogen to sediments, nitrogen burial, and nitrogen uptake.), although annual mean estimates of nitrogen removal may approximate annual mean denitrification (e.g., Sjodin et al., 1997). The method described here explicitly measures N_2 production at the reach scale and is appropriate for testing what factors control denitrification within a stream reach. We are continuing to measure denitrification rates in these rivers and we are evaluating the importance of various factors (i.e., hydrological characteristics, nitrate concentrations, temperature, dissolved oxygen concentrations, land use, and temporal patterns (diel and seasonal)) in regulating denitrification both within and among rivers. With our collaborators (see acknowledgements) we are also generating data that will permit comparison of our measured rates with denitrification measured in core incubations, with denitrification measured using whole-reach ^{15}N tracer methods, and with nitrogen removal estimated by mass balance. Additionally, we are generating a data set that will permit calculation of annual average nitrogen removal efficiency (explicitly by denitrification), allowing comparison with previous studies relating hydrology to nitrogen loss in rivers (e.g., Howarth et al., 1996; Smith et al., 1997; Alexander et al., 2000; Seitzinger et al., 2002).

The effort to reduce nitrogen loading to coastal waters will require more detailed understanding of factors that regulate denitrification *in situ* and at the reach scale. The method described here is integral to this process as part of an emerging program to quantify de-

nitrification in rivers. This method has the advantages of being relatively simple and inexpensive, applicable to rivers of various sizes, and more direct than various other approaches incorporating nitrogen mass balance or stable isotope techniques, as the endproduct of denitrification (N_2) is directly measured. Further, the approach can be adapted to estimate *in situ* production of various other biogenic gases simultaneously, such as nitrous oxide and methane. As this technique evolves, we hope to apply it to longer reaches with greater structural complexity, and to scale it up from individual reaches to whole river systems.

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Appendix A: derivation of Equation (4)

$$\text{Step 1. } K_{\text{prop}} = d(\ln [\text{prop}])/dt, K_{\text{isobut}} = d(\ln [\text{isobut}])/dt$$

$$\text{Step 2. } K_{\text{prop}} - K_{\text{isobut}} = d(\ln [\text{prop}])/dt - d(\ln [\text{isobut}])/dt = d(\ln [\text{prop}]/[\text{isobut}])/dt$$

$$\text{Step 3. } K_{\text{prop}} - K_{\text{prop}} \times (K_{\text{isobut}}/K_{\text{prop}}) = d(\ln [\text{prop}]/[\text{isobut}])/dt$$

$$\text{Step 4. } K_{\text{prop}} \times (1 - (K_{\text{isobut}}/K_{\text{prop}})) = d(\ln [\text{prop}]/[\text{isobut}])/dt$$

$$\text{Step 5. } K_{\text{prop}} = d(\ln [\text{prop}]/[\text{isobut}])/(1 - (K_{\text{isobut}}/K_{\text{prop}}))/dt$$

$$\text{Step 6. } K_{\text{isobut}}/K_{\text{prop}} = (Sc_{\text{isobut}}/Sc_{\text{prop}})^{-n} \quad (\text{Equation (3) in text})$$

$$\text{Step 7. } K_{\text{prop}} = d\{\ln ([\text{prop}]/[\text{isobut}])/(1 - (Sc_{\text{iso}}/Sc_{\text{prop}})^{-n})\}/dt \quad (\text{Equation (4) in text})$$

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