

## Open-channel estimation of denitrification

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### Abstract

Estimates of denitrification based on standard methods often cannot easily be extrapolated to entire ecosystems because of high spatial heterogeneity in rates of denitrification. Diel changes in the concentration of dinitrogen ( $N_2$ ) in running waters provide the basis for estimating denitrification by an open-channel technique in much the same way that ecosystem metabolism can be estimated from diel changes in the concentration of dissolved oxygen. The open-channel  $N_2$  method was field tested at a site on the South Platte River downstream from Denver, CO; concentrations of  $N_2$  were measured by membrane inlet mass spectrometry. For a date in November 1998, the rate of denitrification was estimated to be  $0.19 \text{ mol } N_2 \text{ m}^{-2} \text{ d}^{-1}$  and was similar to rates reported in previous studies based on mass-balance analysis. The open-channel  $N_2$  method is the first method to provide direct, whole-system estimates of denitrification in flowing waters and may help to expand our understanding of nitrogen cycling in running waters.

Human-induced changes to the nitrogen cycle have led to a growing interest in processes that remove nitrogen from water. Denitrification, which is the microbial reduction of nitrate to gaseous nitrogen (mainly  $N_2$  plus small amounts of  $N_2O$ ), can remove a large fraction of the fixed nitrogen that reaches a body of water, but rates of denitrification are highly variable in space and time (Sjodin et al. 1998; Hill et al. 2000; Saunders and Kalff 2001).

In aquatic systems, rates of denitrification usually have been estimated in cores or chambers from rates of change in the concentration of  $N_2$  or  $N_2O$  (Seitzinger et al. 1993; Garcia-Ruiz et al. 1998). Most commonly, cores have been incubated in the laboratory, and denitrification has been estimated by the acetylene inhibition (block) technique (Sørensen 1978). The acetylene inhibition technique, however, tends to underestimate denitrification because acetylene inhibits nitrification and does not completely block the reduction of  $N_2O$  to  $N_2$  (Seitzinger et al. 1993). Although estimates also have been based on flux of  $N_2$

from sediment cores and chambers (Devol 1991; Seitzinger et al. 1993; Saunders and Kalff 2001), rates obtained by use of cores or chambers cannot easily be extrapolated to entire ecosystems where spatial heterogeneity in rates of denitrification is high.

Like denitrification, ecosystem metabolism (photosynthesis and respiration) has been estimated in chambers from rates of change in the concentration of dissolved  $O_2$  (e.g., Fellows et al. 2001). Integrated estimates of oxygen metabolism are possible by use of an open-channel method involving mass balance of  $O_2$  (Odum 1956; Marzolf et al. 1994; Ortiz-Zayas 1998; Fellows et al. 2001). Similarly, estimation of denitrification by an open-channel method is feasible in concept but would require measurement of small changes in the concentration of  $N_2$ . Rates of change in the concentration of  $N_2O$  can be measured with high precision relative to ambient concentrations, but open-channel estimates of denitrification based on  $N_2O$  would depend on assumptions about the ratio of  $N_2:N_2O$  produced by denitrification. In many aquatic systems, most of the  $N_2O$  produced during denitrification is subsequently reduced to  $N_2$ , but the ratio of  $N_2:N_2O$  is variable (Lindau et al. 1991; Mosier and Schimel 1993). Thus, given that  $N_2O$  presents apparently intractable problems of interpretation, open-channel estimation of denitrification from  $N_2$  flux would be ideal. Recently, Laursen and Seitzinger (2002) described an open-channel method based on measurement of  $N_2$  by membrane-inlet mass spectrometry (MIMS) (Kana et al. 1994; Kana et al. 1998; Cornwell 1999; Eyre et al. 2002). Alternatively, open-channel estimates of denitrification could be based on measurements of  $N_2$  by high-precision gas chromatography (Devol 1991; An and Joye 1997).

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MIMS allows rapid and precise measurement of  $N_2$  and is gaining widespread use in studies of sediment cores (Eyre et al. 2002). Typically, determination of  $N_2$  concentration by MIMS has been based on measurements of the  $N_2$ :Ar ratio and assumptions about the concentration of Ar. If Ar concentration in water is always at equilibrium with the atmospheric mixing ratio for ambient temperature and pressure (i.e., at saturation),  $N_2$  concentration can be computed from the  $N_2$ :Ar ratio. In natural aquatic systems, however, the assumption that Ar is at saturation rarely is valid. Even though Ar is biologically inert, the concentration of Ar changes on a diel basis in response to changes in temperature and pressure; diel variations in temperature and pressure result in continuous variation of the saturation concentration. The ambient concentration for a gas dissolved in water is driven toward saturation through exchange with the atmosphere (reaeration), but there is always a time lag between changes in saturation and changes in gas concentration. Because it cannot be assumed that Ar remains at saturation in natural systems, especially where the diel amplitude of temperature is large and the reaeration rate coefficient is low, application of the open-channel method to estimation of denitrification is most feasible through measurements of  $N_2$  concentration that do not depend on assumptions about Ar concentrations. Laursen and Seitzinger (2002) estimated concentrations of  $N_2$  from direct measurements of Ar concentration and the  $N_2$ :Ar ratio. Direct measurements of  $N_2$  concentration are possible with MIMS but require greater care than measurements of the  $N_2$ :Ar ratio.

The purpose of this paper is to describe an open-channel method for the estimation of denitrification in running waters. The technique described here depends on measurement of the concentration of dissolved  $N_2$  independently of other gas concentrations by MIMS. Hourly measurements were made over a 24-h period at a single station and estimation of denitrification was based on calculations of  $N_2$  flux, after correction for reaeration flux and groundwater flux. As shown here for a site on the South Platte River in Colorado, gas concentrations can be measured independently and precisely with MIMS, but only after careful calibration and correction for drift. Estimates presented here do not include losses of  $N_2O$  because  $N_2$  accounts for nearly all (>99%) of the gaseous losses associated with denitrification in the South Platte River (McMahon and Dennehy 1999).

### Materials and procedures

*Site description*—Diel changes in dissolved  $N_2$  were measured on 14 November 1998–15 November 1998 at a site on the South Platte River. The South Platte drains an area of almost 63,000 km<sup>2</sup> in Colorado, Wyoming, and Nebraska. Wastewater from the city of Denver is a major source of fixed nitrogen to the South Platte, as is agriculture. Previous studies of the South Platte have shown that denitrification is an important component of the mass balance for nitrogen (McMahon and Bohlke 1996; Sjodin et al. 1998). This study

was conducted at a site near Ft. Lupton, 55 km downstream of Denver (40°14'12"N, 105°38'3"W). At low flow, which characterized the period of sampling, the channel averages 40- to 50-m wide and is 30- to 70-cm deep.

*Sampling and field measurements*—Water samples for analysis of gas concentrations were collected hourly at three locations spaced evenly along a transect across the river (i.e., perpendicular to the channel).  $N_2$  samples were collected in 40-mL glass vials with 3.2-mm Teflon/silicone septa (I-Chem part nr. SB46-0040). Vials were filled with a sampler that flushed the vials with approximately 5 volumes of water, thus minimizing exchange of gases with the atmosphere (Kilpatrick et al. 1989). Vials were immersed in cold water (ca. 0°C) immediately after filling and were held there until analysis (within 12 to 24 h); no preservative was added to samples. Shallow groundwater also was collected from piezometers adjacent to the river, as necessary to correct for changes in  $N_2$  concentration caused by seepage (McCutchan et al. 2002).

Water temperature and barometric pressure were recorded at 10-min intervals; temperature was measured with a Hydrolab Datasonde III, and pressure was measured with a field barometer (Oregon Scientific). Discharge was estimated from measurements of stream depth and cross-sectional velocity profiles, as measured with a Marsh-McBirney flow meter, and the rate of groundwater seepage ( $m^3 s^{-1} d^{-1}$ ) was estimated from paired measurements of discharge (i.e., at the sampling location and at another point upstream). Samples for analysis of nitrate and ammonium concentrations were collected hourly.

Propane was used as a volatile tracer to estimate the reaeration rate (Kilpatrick et al. 1989). The rate coefficient for nitrogen ( $k_{nitrogen}$ ) was derived from the rate coefficient for propane using the indexing method of Gulliver et al. (1990). The reaeration rate was corrected for temperature using the following equation (Thomann and Mueller 1987):

$$k_{nitrogen,T} = k_{nitrogen,20} 1.024^{(T-20)} \quad (1)$$

where  $k_{nitrogen,20}$  is the reaeration rate for nitrogen at 20°C.

*Measurement of gas concentrations*—Concentrations of dissolved  $N_2$  were measured with a Balzers Prisma quadrupole mass spectrometer with a membrane inlet (Kana et al. 1994). Precise and independent measurement of  $N_2$  concentration depended on careful preparation of standards for calibration, slight modifications to the inlet line, and correction for machine drift. Analytical precision was further improved by correcting measured concentrations for diffusion during storage. Samples suspected of having high gas concentrations were diluted prior to analysis to avoid the formation of bubbles in the inlet line.

For this study, the membrane inlet of the MIMS was partially immersed in a water bath at constant temperature ( $5.0 \pm 0.05^\circ\text{C}$ ), and samples were drawn through the inlet line with a peristaltic pump. The water bath was covered with Styrofoam insulation and a submersible aquarium pump was used to irrigate the upper portion of the inlet. Maintenance of the

membrane inlet at a low and constant temperature eliminated bubbling in the line and, because the peristaltic pump was placed downstream of the inlet, cavitation in the pump did not disrupt flow through the inlet. Analytical standards were prepared by equilibrating deionized water with a calibrated gas mixture (77.97% N<sub>2</sub>, 0.998% Ar, balance O<sub>2</sub>) in jacketed vessels maintained at 6.0 ± 0.05°C and 20.0 ± 0.05°C. Temperature in the vessels and barometric pressure were measured frequently. The saturation concentration for N<sub>2</sub> was determined from relationships given by Colt (1984). Water in each vessel was circulated with a submersible aquarium pump, and analytical standards were siphoned from each vessel into narrow test tubes. The test tubes were filled from the bottom and overflowed 5 times; standards were analyzed immediately upon withdrawal of water from the vessels. Two standards from each calibration vessel were run after every five field samples.

Gas concentrations for samples were determined from detector currents after correction for background and drift. Concentrations of gases calculated for each calibration vessel remained constant over time except for slight variations caused by changes in temperature and barometric pressure. Corrections for the background detector current and drift were made as follows:

$$C_{sample} = (I_{sample} - I_{background})a_t + b_t \quad (2)$$

where  $C_{sample}$  is the gas concentration for a given sample analyzed at time  $t$ ;  $I_{sample}$  is the detector current for the sample;  $I_{background}$  is the background detector current; and  $a_t$  and  $b_t$  are the slope and intercept, respectively, for the line representing the relationship between the concentrations and detector currents for standards at time  $t$ . Changes over time in the slope and intercept for the calibration were estimated by cubic spline interpolation from measured detector currents and gas concentrations of standards.

Diffusion of dissolved gases across the interface between the septum and the vial during storage can affect estimates of gas concentrations, especially when samples with low concentrations are stored in cold water. Concentrations were corrected for diffusion during storage by application of a laboratory-derived diffusion coefficient (see Assessment section) as follows:

$$C_{collection} = C_{storage} - (C_{storage} - C_{analyzed})e^{k_{septum}t_{storage}} \quad (3)$$

where  $C_{collection}$  is the concentration for a sample at the time of collection;  $C_{storage}$  is the concentration in the storage water;  $C_{analyzed}$  is the concentration in the sample vial at the time of analysis;  $k_{septum}$  is the laboratory-derived diffusion coefficient for the septa (d<sup>-1</sup>); and  $t_{storage}$  is the time elapsed between collection and analysis (d).

Prior to analysis, groundwater samples, which often had high concentrations of N<sub>2</sub> (>700 μM), were diluted with water of known gas concentration. Dilution was accomplished by injecting a measured volume of water from the 20°C calibration vessel through the septum; a second needle allowed for the



**Fig. 1.** Dilution of samples suspected of high N<sub>2</sub> concentration; 25% to 50% of the water in a vial is replaced by warm water of known gas concentration.

displacement of a nearly equal volume of sample water without the introduction of air bubbles (Fig. 1). The use of two needles, one longer than the other, and inversion of the vial during injection prevented mixing between the water from the warm calibration vessel (20°C) and colder water of the sample (ca. 0°C). Water from the calibration vessel was less dense than the water of the sample and thus remained above the sample water during injection. Diluted samples were shaken after injection and then were analyzed in the normal fashion. The volume of each sample vial and the volume of water added with each dilution were determined gravimetrically; the concentrations for the samples prior to dilution were determined as follows:

$$C_{sample} = \frac{C_{diluted}V_{vial} - C_{syringe}V_{syringe}}{V_{vial} - V_{syringe}} \quad (4)$$

where  $C_{sample}$  is the concentration of N<sub>2</sub> in the undiluted sample;  $C_{diluted}$  is the concentration measured after dilution;  $V_{vial}$  is the volume of the vial;  $C_{syringe}$  is the concentration of N<sub>2</sub> in the 20°C calibration vessel; and  $V_{syringe}$  is the volume of 20°C water added to the sample.

*Estimation of denitrification*—After the concentrations of N<sub>2</sub> were established for each sample, rates of denitrification were estimated from equations developed for the estimation of ecosystem metabolism (Odum 1956; McCutchan et al. 2002). For a well-mixed stream with constant velocity and flux of groundwater, estimates of denitrification are based on a mass balance for N<sub>2</sub> over each interval between measurements, as follows:

$$\frac{dm}{dt} = C_g Q_g + P_{nitrogen} A + k_{nitrogen,T}(Sv_t - m_t) \quad (5)$$

where  $m_t$  is the mass of N<sub>2</sub> (mol N<sub>2</sub>) in a parcel (an arbitrary volume) of water at time  $t$ ;  $dm/dt$  is the rate of change in mass

with respect to time ( $\text{mol N}_2 \text{ d}^{-1}$ );  $C_g$  is the concentration of  $\text{N}_2$  in groundwater ( $\text{mol N}_2 \text{ m}^{-3}$ ); and  $Q_g$  is rate of groundwater flux to the parcel ( $\text{m}^3 \text{ d}^{-1}$ ).  $P_{\text{nitrogen}}$  is the rate of production of  $\text{N}_2$  (i.e., denitrification:  $\text{mol N}_2 \text{ m}^{-2} \text{ d}^{-1}$ );  $A$  is the area of the channel covered by the parcel ( $\text{m}^2$ );  $k_{\text{nitrogen},T}$  is the reaeration rate coefficient for  $\text{N}_2$  at temperature  $T$  ( $\text{d}^{-1}$ );  $S$  is the saturation concentration for  $\text{N}_2$ ;  $v_t$  is the volume of the parcel at time  $t$  ( $\text{m}^3$ ); and the rate of change in volume per unit time is equal to  $Q_g$ . It was assumed that barometric pressure,  $T$ ,  $C_g$ ,  $Q_g$ ,  $P$ ,  $A$ , and  $k_{\text{nitrogen},T}$  were constant over each interval. Estimates here are based on measurements at a single station over a diel period, although estimates obtained by the same method also could be based on the rates of change in concentration between two stations (cf. one-station vs. two-station estimates for metabolism based on  $\text{O}_2$  flux (Bott 1996; Ortiz-Zayas 1998). The method presented in Laursen and Seitzinger (2002) is a two-station approach but is not based on diel sampling.

The mean concentration of  $\text{N}_2$  was estimated at 10-min intervals by cubic spline interpolation from measured (hourly) concentrations. The rate of denitrification for each 10-min interval was estimated as the total flux of  $\text{N}_2$  minus the sum of groundwater flux and reaeration flux as follows:

$$P_{\text{nitrogen}} = \frac{C_t - C_0 Z}{\Delta t} - (C_g - C_t) \frac{Q_g}{A} - k_{\text{nitrogen},T} DZ \quad (6)$$

where  $C_0$  is the initial concentration for the interval and  $C_t$  is the final concentration;  $Z$  is the mean depth for the channel;  $Q_g/A$  is the flux of groundwater per unit area (i.e., the piston-velocity for net flux of groundwater); and  $D$  is the saturation deficit for  $\text{N}_2$ . The rate of groundwater seepage (estimated from paired measurements of discharge) was divided by channel width to obtain  $Q_g/A$ .

### Assessment

Precision for open-channel estimates of denitrification depends on the amount of analytical error in measurements of  $\text{N}_2$  concentration, but other factors also are important. An assessment of uncertainty in estimates of denitrification must incorporate uncertainty in all of the variables that affect the mass balance for  $\text{N}_2$  in the channel. Steps were taken to ensure that storage of samples between the times of collection and analysis did not bias estimates of concentration. The added effect of dilution (for groundwater samples) on precision for measurements of concentration was quantified, as was uncertainty in each of the factors that affect the mass balance for  $\text{N}_2$  in the channel. A Monte Carlo approach then was used to estimate uncertainty in the final estimate of denitrification.

**Handling of samples**—When concentrations of dissolved gases are not measured immediately after the collection of samples, it is important to quantify diffusion associated with sample storage and to ensure that biological or chemical processes during storage do not affect estimates of gas concentration. If gas concentrations in a sample are above saturation relative to

**Table 1.** Estimates of uncertainty for each variable used in the open-channel estimation of denitrification<sup>a</sup>

Variable	SD	Resolution
$\text{N}_2$ concentration <sup>b</sup>		
Channel, $\mu\text{M}$	4.7	0.1
Groundwater, $\mu\text{M}$	50.7	0.1
Depth <sup>b</sup> , m	0.007	0.01
Groundwater flux <sup>c</sup> , $\text{m d}^{-1}$	10% of value	0.01
Reaeration-rate coefficient <sup>b</sup> , $\text{d}^{-1}$	0.64	0.01
Temperature <sup>d</sup> , $^{\circ}\text{C}$	0.05	0.01
Barometric pressure <sup>d</sup> , Atm	0.0008	0.0013

<sup>a</sup>Except for groundwater flux, standard deviations (SD) were determined empirically or from published values.

<sup>b</sup>Determined empirically.

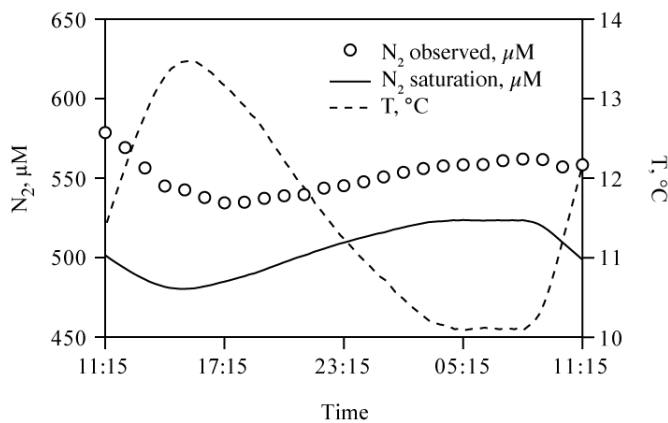
<sup>c</sup>See text.

<sup>d</sup>McCutchan et al. 1998.

the storage temperature, formation of bubbles in the vial can result in large errors in estimates of gas concentration. Storage of samples in water at  $0^{\circ}\text{C}$  helps prevent the formation of bubbles in sample vials. However, because cold water has high saturation concentrations for dissolved gases, diffusion could lead to an increase in concentration during storage and therefore could bias estimates of concentration. To estimate rates of diffusion during storage, vials were filled with water of known gas concentration (i.e., from the  $20^{\circ}\text{C}$  calibration vessel) and were kept submerged in ice water. Vials were removed periodically, and concentrations were measured as described above. For nitrogen, diffusion-rate coefficients for vials with new septa were less than  $0.025 \text{ d}^{-1}$  at  $0^{\circ}\text{C}$ . Because diffusion-rate coefficients for vials with old septa ( $> 4 \text{ y}$ ) were nearly twice as high as those for vials with new septa, only new septa were used in this study. For samples analyzed within 24 h of collection, corrections for diffusion generally were less than 1% of the concentration at the time of sampling. After correction for diffusion, the time between collection and analysis did not affect estimates of concentration for replicate field-collected samples; thus, there was no measurable production of  $\text{N}_2$  in vials between the times of collection and analysis.

Precision for measurements of concentration was high and decreased only slightly for diluted samples. The CV for replicate standards run on the same date was  $<0.9\%$  and typically was 0.1% to 0.4%; when standards from the  $6^{\circ}\text{C}$  vessel were diluted with an equal volume of water from the  $20^{\circ}\text{C}$  vessel, the CV for replicates was 2.1%.

**Error analysis**—In a manner similar to that described by McCutchan et al. (1998), a Monte Carlo approach was used to estimate uncertainty in the open-channel estimate presented here. Except for  $Q_g/A$ , standard deviations (SD) for each variable in Eq. 6 were determined empirically or were taken from published studies; the coefficient of variation for  $Q_g/A$  was set to 10% of the measured value (Table 1). Uncertainty in measurements of  $\text{N}_2$  concentration was calculated from the error distributions associated with calibration, dilution (for groundwater



**Fig. 2.** Diel changes in measured and saturation concentrations of N<sub>2</sub> on 14 November–15 November 1998, South Platte River; diel changes in temperature are shown for reference.

samples), and field replicates. The SD for measurement of the saturation deficit for N<sub>2</sub> depends on the SD for measurement of N<sub>2</sub> concentration and the SD for measurement of the saturation concentration. The SD for estimates of average depth and reaeration rate coefficient were determined from replicate measurements made in the field.

Commercially available software (@Risk, Pallisade Corporation) was used to sample randomly and repeatedly from the distributions that characterize each variable in Eq. 6. PetroPlot (available from the Lamont-Doherty Earth Observatory of Columbia University) was used to interpolate N<sub>2</sub> concentrations from hourly measurements for each iteration of the Monte Carlo simulation. For each random sampling from the error distributions, the rate of denitrification was estimated by Eq. 6; the mean and 95% confidence limits (CL) for the estimate of denitrification were calculated from the output distribution for the Monte Carlo simulations. The difference between total flux and reaeration flux (i.e., the estimated rate of denitrification assuming no net flux of groundwater) also was estimated for each resampling and the mean and 95% CL were calculated similarly from the output distribution.

**Results**—N<sub>2</sub> concentrations in the channel were well above saturation (107% to 116% of saturation; Fig. 2). Mean N<sub>2</sub> concentration for the channel was lowest in late afternoon, just after maximum stream temperature, and was highest near sunrise. Variation in concentrations for samples collected at the same time was small; the coefficient of variation (CV) for sets of samples collected from the channel at the same time averaged 0.9% (range 0.1% to 1.8%). The average concentration of N<sub>2</sub> for samples collected on the west side of the channel was significantly higher (6.7 µM; Tukey Kramer Honestly Significant Difference, *P* < 0.05) than the average for samples collected on the east side; the mean concentration for samples collected mid-channel did not differ significantly from the mean for either of the other sets of samples. The piston-

**Table 2.** Mean temperature, discharge (*Q*), depth (*Z*), concentration of N<sub>2</sub> in groundwater (*C<sub>g</sub>*), piston-velocity for groundwater flux (*Q<sub>g</sub>/A*), concentrations of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, reaeration rate coefficient for nitrogen (*k<sub>nitrogen,20°</sub>*), difference between total flux of N<sub>2</sub> and reaeration flux, and estimated rate of denitrification (*P<sub>nitrogen</sub>*) on 14 November 1998–15 November 1998, South Platte River<sup>a</sup>

<i>T</i> , °C		11.5 ± 1.19
<i>Q</i> , m <sup>3</sup> s <sup>-1</sup>		12.3 ± 1.13
<i>Z</i> , m		0.51 ± 0.007
<i>C<sub>g</sub></i> , µM		690 ± 26.6
<i>Q<sub>g</sub>/A</i> , m d <sup>-1</sup>		0.20 ± 0.02
NO <sub>3</sub> <sup>-</sup> , µM		436 ± 18.8
NH <sub>4</sub> <sup>+</sup> , µM		52 ± 22.9
<i>k<sub>nitrogen,20°</sub></i> , d <sup>-1</sup>		12.1 ± 0.64
Total flux – reaeration flux, mol N <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup>	0.23	One date; 95% CL = 0.19 to 0.25 Five dates; 95% CL = 0.21 to 0.23
<i>P<sub>nitrogen</sub></i> , mol N <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup>	0.094	One date; 95% CL = 0.16 to 0.22 Five dates; 95% CL = 0.18 to 0.21

<sup>a</sup>Values are mean ± SD, except for the difference between total flux and reaeration flux and *P<sub>nitrogen</sub>*, which are shown as mean (95% CL from simulations; expected 95% CL for the mean of 5 dates are also given).

velocity for groundwater flux (*Q<sub>g</sub>/A*) above the study site was 0.20 m d<sup>-1</sup>. The average concentration of N<sub>2</sub> for wells located adjacent to the channel was 690 ± 26.6 µM (mean ± SD).

Both the difference between total N<sub>2</sub> flux and reaeration flux and the estimated rate of denitrification were high (Table 2). As estimated from the Monte Carlo simulations, the 95% CL for the estimated difference between total flux and reaeration flux were within 13% of the mean; the 95% CL for the estimate of denitrification were within ~16% of the mean (Table 2). If uncertainty was similar across dates and estimates were made for five consecutive dates, rather than for a single date, the expected 95% CL would be ~6% for the difference between total flux and reaeration flux and ~7% for the estimate of denitrification (Table 2). Over the 24-h period, the rate of denitrification did not vary significantly with temperature.

### Discussion

In a manner similar to the open-channel method for estimation of oxygen metabolism, the open-channel N<sub>2</sub> method can provide integrated, whole-system estimates of denitrification in flowing waters. Depending on the characteristics of a particular stream and the questions of interest, this method can be employed as a single-station approach, as presented here, or as a two-station approach, as in Laursen and Seitzinger (2002). Whole-system estimates of denitrification are presently not possible with any other method except mass-balance analysis, which involves many more potential sources of error and more analytical effort than the open-channel N<sub>2</sub> method.

Our estimate with the open-channel method was high compared with most published estimates of denitrification

but was within the range of other estimates for the South Platte River based on residuals from mass-balance studies (Sjodin et al. 1998; Pribyl 2002). Assuming that rates are constant downstream, our estimate corresponds to complete removal of nitrate from the channel in 1.2 d of travel (instantaneous removal rate of 86% d<sup>-1</sup>). Laursen and Seitzinger (2002) also found high rates of denitrification compared with estimates based on the acetylene inhibition method. The acetylene inhibition method can underestimate rates of denitrification in cores (Seitzinger et al. 1993), and high spatial variability of denitrification can lead to underestimation of mean rates for whole systems if areas with the highest rates are not sampled. Thus, the open-channel N<sub>2</sub> method may contribute to an improved understanding of nitrogen retention in running waters.

The open-channel N<sub>2</sub> method provides measurements of net production of N<sub>2</sub>. Therefore, in systems where rates of N-fixation are high, the open-channel method will underestimate denitrification (Eyre et al. 2002). High concentrations of fixed nitrogen, as occur in the South Platte River, inhibit the activity of nitrogenase, the enzyme responsible for biological N-fixation (Postgate 1998). Although N-fixation may occur locally where high rates of uptake reduce the concentrations of nitrate and ammonium, ecosystem-level rates of N-fixation in the South Platte River probably are quite low and thus did not affect the estimation of denitrification.

In streams where the concentration of dissolved N<sub>2</sub> is well above saturation, precision for estimates of the difference between total flux and reaeration flux on a single date can be very high (95% CL within <15% of the estimated value for this study) using the methods described here. Where flux of groundwater to the channel is negligible, precision for estimates of denitrification also will be very high. However, where flux of groundwater contributes substantially to the mass balance of N<sub>2</sub> for the channel, relative uncertainty in estimates of denitrification will depend heavily on the precision for estimates of the concentration of N<sub>2</sub> in groundwater ( $C_g$ ) and the net flux of groundwater to the channel ( $Q_g/A$ ). In the South Platte River, neither  $C_g$  nor  $Q_g/A$  was a major source of uncertainty in the estimation of denitrification. Thus, precision for the estimated rate of denitrification (95% CL within ~16% of the estimated value for a single date) was only slightly below that for the difference between total flux and reaeration flux.

If open-channel estimates of denitrification are averaged over time (i.e., estimates are based on sampling across multiple dates), uncertainty will be lower than for a single date. Precision for individual estimates of denitrification based on chamber methods may be high under some circumstances, but spatial variability in rates of denitrification reduces overall precision when estimates from chambers are scaled to entire reaches. Although spatial heterogeneity in  $P_{\text{nitrogen}}$ ,  $C_g$ ,  $Q_g/A$ , or  $k_{\text{nitrogen},T}$  can affect the precision of open-channel estimates of denitrification, the effects of spatial heterogeneity on preci-

sion are smaller with open-channel studies than with chamber studies. The extent to which precision in open-channel studies is affected by spatial heterogeneity has not been quantified, but no other method for the estimation of denitrification in running waters can approach the level of precision presented here given a comparable level of effort.

There was not a significant relationship between temperature and the rate of denitrification in this study. However, the diel range of temperature in the channel at the time of this study was less than 3.4°C, and the diel range of temperature in the sediments was less than 1°C at a depth of 20 cm. Thus, it may not have been possible to detect a relationship between temperature and denitrification rate because of the small diel range of temperature.

Rates of denitrification in situ are poorly documented. The open-channel N<sub>2</sub> method for estimating rates of denitrification may lead to a great expansion in field estimates of denitrification. This method may therefore contribute substantially to our understanding of the nitrogen cycle and to efforts to control eutrophication locally and regionally.

### Comments and recommendations

The technique described here allows precise and accurate measurement of absolute concentration by MIMS without which estimation of denitrification from open-channel concentrations of N<sub>2</sub> would not be possible. The accuracy and precision of N<sub>2</sub> measurements can be affected substantially by the concentration of oxygen in samples and standards (Eyre et al. 2002). High precision and accuracy in measurements of concentration also depend on maintenance of the inlet line at low and constant temperature, careful handling of samples and preparation of standards, and correction for machine drift.

Oxygen present in samples and standards can react with N<sub>2</sub> in the ion source for the MIMS; reaction of O<sub>2</sub> with N<sub>2</sub> to form NO<sup>+</sup> reduces the detector currents for both N<sub>2</sub> and O<sub>2</sub> (Eyre et al. 2002). Based on information presented by Eyre et al. (2002), formation of NO<sup>+</sup> had only a slight effect (0.2% to 0.4%) on measurements of N<sub>2</sub> in the South Platte River because the O<sub>2</sub> concentration was similar in samples and standards. Nonetheless, oxygen can interfere with the accurate measurement of N<sub>2</sub> concentration by MIMS, particularly when samples and standards differ in oxygen concentration. Complete removal of O<sub>2</sub> can be accomplished, however, by placing a copper reduction column heated to 600°C between the inlet line of the MIMS and the ion source (Eyre et al. 2002). With such modifications, N<sub>2</sub> can be measured accurately by MIMS without regard for differences in O<sub>2</sub> between samples and standards. Additionally, Eyre et al. (2002) report improved precision for measurements of N<sub>2</sub> when the copper reduction column is used; thus, addition of the copper reduction column to the MIMS could extend the use of the open-channel N<sub>2</sub> method to streams with much lower rates of denitrification than are reported here.

Screw-top vials with flat septa are easily capped inside the flushing samplers used in this study. Although plug-type

septa provide a longer diffusion path than flat septa and thus are preferable if samples cannot be analyzed soon after collection, they are more difficult to use with the flushing samplers. Also, removal of plug septa produces a negative pressure in the sample vial and can affect measured gas concentrations; plug septa can be used effectively if a double-needle technique is used to withdraw water through the septa (as with the dilution technique).

One of the difficulties with MIMS in studies of denitrification is that samples with high gas concentrations (e.g., groundwater) often form bubbles in the inlet line. We were able to analyze samples with gas concentrations well above saturation by diluting these samples with water of known gas concentration (i.e., water from one of the calibration vessels). The dilution technique resulted in a slight loss of precision but greatly extended the range of samples that could be analyzed by MIMS. Even if rates of denitrification are low during winter months, samples collected at low temperature may require dilution because of high saturation concentrations. Because atmospheric pressure affects saturation concentrations as well, dilution also may be required if samples are collected at low elevation (e.g., near sea level) and analyzed at a substantially higher elevation (e.g., the University of Colorado).

The open-channel method as described here is best suited to the estimation of denitrification in low-gradient streams where rates of denitrification are high (McCutchan et al. 1998). In high-gradient streams, especially where rates of denitrification are low, uncertainty in estimates of  $N_2$  production will be high because concentrations of dissolved  $N_2$  remain near saturation. In standing waters, sampling at different depths can improve estimates of denitrification where vertical gradients in concentration exist, but the effects of spatial heterogeneity on the estimation of denitrification will be greater in standing waters than in well-mixed systems. Thus, the open-channel  $N_2$  method, without further modification of technique, probably is not well suited for use in cascading mountain streams or in standing waters. Although the reaeration rate coefficient in the South Platte River is relatively low for running waters, uncertainty associated with the flux of  $N_2$  in groundwater reduced the overall precision for estimation of denitrification in this study. It should be possible to achieve reasonable precision for estimates of denitrification in many streams with high rates of groundwater discharge, but only if  $C_g$  and  $Q_g/A$  can be estimated with high precision.

The method presented here is focused on measurement of dissolved  $N_2$  concentrations, but other processes (e.g., whole-system oxygen metabolism) also can be estimated by MIMS. If the methods presented here are applied to the measurement of dissolved  $O_2$ , additional care is necessary for precise and accurate estimates of concentration. Although  $O_2$  can be measured easily by other techniques and  $O_2$  can interfere with the measurement of  $N_2$ , it is possible to accurately measure  $O_2$  and  $N_2$  simultaneously by MIMS, but only if the oxygen concentration is similar between samples and standards; other-

wise, estimates of  $N_2$  concentration will be biased (Eyre et al. 2002). Unlike  $N_2$ ,  $O_2$  often changes in concentration considerably during storage in sample vials. When sample vials are stored at low temperature, diffusion can lead to substantial changes in concentration when anoxic or hypoxic samples are stored for long periods of time; metabolism during storage also can affect oxygen concentrations. Thus, immediate analysis or preservation followed by correction for diffusion during storage are critical for the accurate determination of dissolved  $O_2$  by MIMS, especially when concentrations are low.

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