Biogeochemistry (2005) 73: 439–455
DOI 10.1007/s10533-004-0565-4

© Springer 2005

Whole-system estimation of denitrification in a plains river: a comparison of two methods

ALENA L. PRIBYL, JAMES H. MCCUTCHAN Jr.*, WILLIAM M. LEWIS Jr., and JAMES F. SAUNDERS III

*Center for Limnology, Cooperative Institute for Research in Environmental Sciences and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309-0216, USA; *Author for correspondence (e-mail: mccutchj@cires.colorado.edu; phone: 303-492-5191; fax: 303-492-0928)*

Received 26 January 2004; accepted in revised form 8 June 2004

Key words: Denitrification, Mass balance, Membrane-inlet mass spectrometry, Nitrogen, Open-channel N_2 method, South Platte River

Abstract. Whole-system denitrification in the South Platte River was measured over a 13-month period using an open-channel N_2 method and mass-balance measurements. Concentrations of dissolved N_2 were measured with high precision by membrane-inlet mass spectrometry and estimates of denitrification were based on the mass flux of N_2, after correction for reaeration and groundwater flux. Open-channel estimates of denitrification ranged from 0 to 3.08 g N m^{-2} d^{-1} and the mean annual rate was 1.62 g N m^{-2} d^{-1}, which corresponds to removal of approximately 34% of the nitrate transported by the river over a distance of 18.5 km. Over the same period of time, estimates of denitrification based on mass-balance measurements ranged from 0.29 to 5.25 g N m^{-2} d^{-1} and the mean annual rate was 2.11 g N m^{-2} d^{-1}. The two methods revealed similar seasonal patterns of denitrification the highest rates were measured from late April to August and the lowest rates were in winter. Both methods provide whole-system estimates of denitrification in running waters; where reaeration rate coefficients are low and flux of groundwater is well quantified, the open-channel method has fewer sources of uncertainty and is easier to implement.

Introduction

Anthropogenic sources now dominate the supply of fixed nitrogen to many aquatic ecosystems, and concentrations of dissolved forms of fixed nitrogen are increasing in both surface waters (Howarth et al. 1996; Vitousek et al. 1997; Lovett et al. 2000) and groundwaters (Nolan et al. 1997). Because nitrogen can limit primary production in both freshwater and marine systems (Morris and Lewis 1988; Bergmann et al. 2002; Tank and Dodds 2003) and high concentrations of nitrate in drinking water pose health risks to humans (Nolan et al. 1997), it has become critically important to understand the biogeochemical processes that comprise the nitrogen cycle.

Mass-balance studies of the Mississippi River drainage (Alexander et al. 2000) have shown that small streams are generally more retentive of nitrogen than are large streams. Additionally, ^{15}N-tracer studies have shown that rates of uptake and transformation for N tend to be highest in small streams

(Peterson et al. 2001). Mechanisms that remove inorganic nitrogen from streams include storage (through sorption or conversion to organic matter) as well as gaseous losses (e.g., denitrification). The process of denitrification is of particular interest because it leads to the loss of fixed nitrogen from aquatic ecosystems through the reduction of nitrate to inert, gaseous forms (primarily N_2, but also small amounts of N_2O). Rates of denitrification have been difficult to estimate for entire ecosystems, however, because denitrification occurs only under specific biogeochemical conditions that are difficult to sample or replicate in a laboratory.

There are two common ways of estimating denitrification in aquatic systems. The first involves laboratory incubation of sediment cores (Seitzinger et al. 1993; Pfenning and McMahon 1996; Garcia-Ruiz et al. 1998; Kana et al. 1998; Bernot et al. 2003). Typically, estimates from cores have been based on the acetylene inhibition (block) technique (Sørensen 1978); acetylene blocks the reduction of N_2O to N_2 and rates of denitrification are calculated from the production of N_2O. Denitrification rates within cores also can be estimated from the rate of disappearance of NO_3^- or the rate of production of N_2. It is difficult to extrapolate results from cores to whole systems, however, because conditions of incubation may differ from those of the natural setting and because spatial heterogeneity in rates can be high (Hill et al. 2000; Saunders and Kalff 2001). A second approach involves mass-balance estimates based on transport and transformation rates (Hill 1981; Hill 1983; Sjodin et al. 1997). The mass-balance approach provides system-level estimates of denitrification, but requires measurements of multiple forms of nitrogen and estimates of their rates of transformation. Thus, the accumulation of measurement errors can lead to substantial errors in estimates of denitrification (Cornwell et al. 1999). Although the mass-balance approach has been used successfully where rates of denitrification are high and transport is well quantified, a method that does not depend on estimates of rates for multiple transformations could achieve higher precision than is possible with estimates based on mass-balance measurements.

Recently, Laursen and Seitzinger (2002) and McCutchan et al. (2003) described a new method for estimating denitrification at the ecosystem level on the basis of N_2 flux in open channels. The open-channel N_2 method has the advantage of directly measuring the end product of denitrification (i.e., N_2) rather than calculating rates from the residual of other measurements. Thus, open-channel estimates are subject to fewer sources of error than estimates based on concentration and transport of multiple nitrogen species, and open-channel estimates of denitrification are not subject to many of the difficulties associated with incubation of cores. Open-channel methods have been used to estimate oxygen metabolism in running waters for some time (Odum 1956), but nitrogen gas had not been used previously to measure system-level rates of denitrification because of the difficulty of precisely measuring changes in N_2 concentrations and of differentiating N_2 produced by denitrification from atmospheric N_2 flux. Recent developments in membrane-inlet mass spectrometry have made it possible to measure concentrations of N_2 dissolved in water rapidly and accurately (Kana et al. 1994). Also, the use of volatile tracers to estimate rates of reaeration (e.g., Kilpatrick et al. 1989; Laursen and Seitzinger 2002; McCutchan et al. 2003) has improved estimates of gas flux in open channels. For many streams and rivers, the open-channel N_2 method could provide the most accurate estimates of denitrification rates presently available because it directly measures denitrification *in situ*, instead of indirectly, and could be used where denitrification rates are too low to be quantified by mass balance. The present study compares the open-channel and mass-balance approaches for estimating denitrification in a plains reach of the South Platte River in Colorado.

Site description

The study area is an 18.5-km reach of the South Platte River near Fort Lupton, Colorado (Figure 1). Two of the sampling sites are located on the South Platte River and one is at the mouth of Big Dry Creek, a tributary that joins the South Platte within the sampling reach. The sampling reach increases in width and decreases in depth in the downstream direction. At the upstream site,

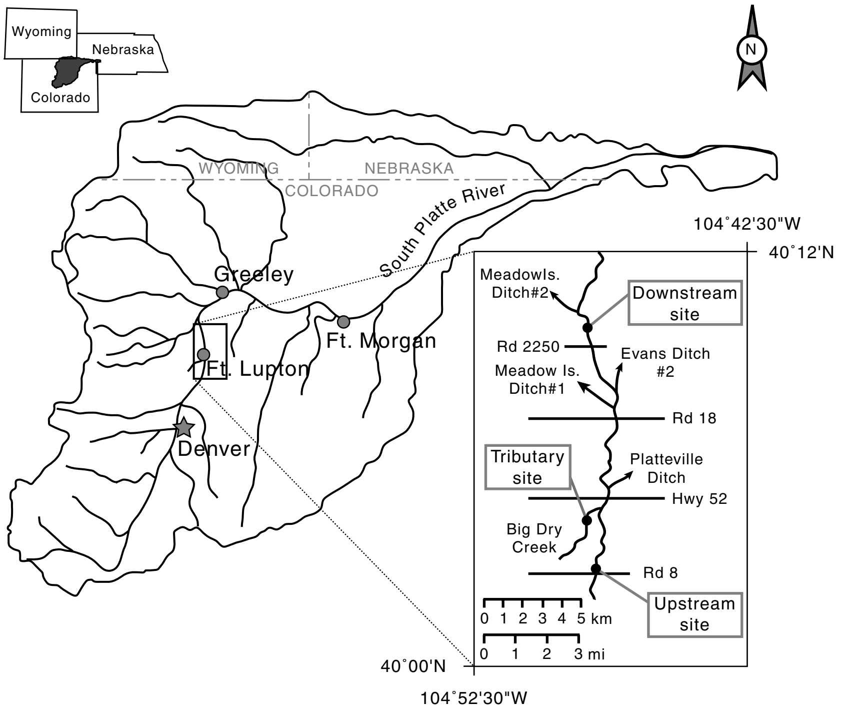

Figure 1. Map of the study area.

width averaged 23.5 m and depth averaged 0.66 m; at the downstream site, width averaged 48 m and depth averaged 0.31 m. Mean annual temperature for the study reach is approximately 13 °C; from November to March, daily mean temperatures generally are < 11 °C and daily mean temperature is near 24 °C in mid summer (G. Cronin, unpublished data).

The South Platte River, which originates in the Colorado Rockies, is fed by snowmelt and transmountain diversions. Much of the water of the South Platte is removed for urban and agricultural use within 200 km of Denver. Below Denver, the return of water to the river occurs primarily by wastewater discharges and return of irrigation water, either overland or by seepage. The hydrology of the South Platte River is characterized by a strong seasonal peak associated with snowmelt, a period of lower base flow extending from fall to early spring, and brief spates associated primarily with summer storms. The natural flow regime has been altered by impoundments, withdrawals, and other human interventions. The primary effects of water management on the South Platte main stem below Denver have been to increase the seasonal base flows and to suppress the spring flood flows as compared with the natural hydrograph (Saunders and Lewis 2003).

Like many rivers and streams of the Great Plains, the study reach has a bed of sand and fine gravel; silt and clay account for less than 5% of the total dry mass of bed sediments in this section of the South Platte River (Bradley et al. 1995). At the downstream site, total organic carbon in the upper 10 cm of sediments ranged from 20 to 120 μg C g^{-1}; oxygen is present in the upper sediments (ca. 3 cm) and the lower sediments are anoxic (G. Cronin and S. Klemm, unpublished data). The easily mobilized bed creates an unstable substrate for algal growth. During spring and summer, high flows associated with snowmelt and storm-related episodes remove algal biomass from the sediment surface; at flows > 10 m^3 s^{-1}, rates of photosynthesis are near zero (G. Cronin and J. McCutchan, unpublished data). The effects of high flows on rates of denitrification in the South Platte River are not known.

The South Platte River below Denver is an ideal setting in which to compare the open-channel method for estimating denitrification with the more traditional mass-balance approach. Rates of denitrification in the South Platte River are high, presumably because concentrations of nitrate and organic carbon are high and the substrate of coarse sand facilitates the exchange of water between the channel and the sediments (McMahon and Bohlke 1996; Sjodin et al. 1997; McCutchan et al. 2003). Nitrogen gas comprises over 99% of the gaseous products of denitrification in the South Platte below Denver (McMahon and Dennehy 1999). Thus, estimates of denitrification based on flux of N_2 should provide good estimates of the total amount of fixed N removed by denitrification. Previous studies have shown that denitrification rates are quite high in the reach below Denver (McMahon and Bohlke 1996; Pfenning and McMahon 1996; Sjodin et al. 1997). Additionally, seasonal variation in temperature, discharge, and concentrations of nitrate and organic matter should produce a wide range of denitrification rates over which to test the methods.

Methods

Hydrologic measurements

Between May 2000 and August 2001, discharge was estimated for the upstream and downstream sites from records of flow at a gaging station (USGS 068020500) located at Henderson, 18 km upstream of the study reach. Water removed by ditch diversions below the gage was subtracted and entry of seepage and tributary water was added. Estimated flows were checked periodically against depth-velocity profiles at the upstream and downstream sites.

Seepage to the reach was estimated from a relationship developed from the historical record of flow residuals between the Henderson gage and a gage at Ft. Lupton (USGS 06721000). For each date, the rate of groundwater seepage (S, $m^3\ s^{-1}\ d^{-1}$) was estimated from measurements of discharge at the Henderson gage as follows:

$$\begin{aligned} &S = 0.486 - 0.450(\ln Q_t/\ln Q_{t-1}) - 0.369(\ln Q_t/\ln Q_{t-1})\ln Q; \\ &r^2 = 0.45,\ \ p < 0.0001 \end{aligned} \tag{1}$$

where Q_t is daily mean discharge at the Henderson gage and Q_{t-1} is daily mean discharge at the Henderson gage on the previous day.

Open-channel method

Water samples at a given station were collected at three to five points spaced evenly along a transect across the river. Samples were collected at the downstream station hourly for 24-h periods during the summer, fall, and spring of 2000–2001 and for one 12-h period during the summer of 2001. On five additional dates between July 2000 and June 2001, samples for N_2 analysis were collected once during the day. Samples of groundwater were collected from piezometers adjacent to the stream channel. Samples for N_2 analysis were collected with a flushing sampler (Kilpatrick et al. 1989) in 40-ml vials and were capped with teflon-silicone septa (I-Chem #288-0022) prior to removal from the sampler. Vials were kept submerged in cold (ca. 0 °C) water and samples were analyzed within 30 h of collection.

On dates when samples were collected for N_2 analysis and on some of the dates when nutrient samples were collected for mass-balance measurements (see below), stream temperature was measured at 10-min intervals at the downstream site with a Hydrolab Datasonde III. Stream temperature also was measured bi-weekly at the time of collection for nutrient samples. Barometric pressure was measured at 10-min intervals with a Honeywell Precision Barometer (Model HPB200).

Concentrations of dissolved N_2 in the channel and in groundwater were measured with a membrane-inlet mass spectrometer (MIMS; Kana et al. 1994), with modifications as described by McCutchan et al. (2003). Denitrification was estimated from the mass flux of N_2 as given by McCutchan et al. (2003):

$$P_{\mathrm{nitrogen}} = \frac{C_t - C_0}{\Delta t} Z - (C_{\mathrm{g}} - C_t)\frac{Q_{\mathrm{g}}}{A} - k_{\mathrm{nitrogen,T}} DZ \tag{2}$$

where P_{nitrogen} is the rate of denitrification (g N m^{-2} d^{-1}), C_0 is the initial concentration of N_2 (i.e., the average concentration for a set of samples taken across the channel) and C_t is the final concentration (g m^{-3} = mg l^{-1}), Z is the average depth (m), Δt is time interval between measurements (d), C_{g} is the concentration of N_2 in groundwater (g m^{-3}), Q_{g}/A is the flux of groundwater per unit area (m d^{-1}), k_{nitrogen} is the reaeration rate coefficient for N_2 (d^{-1}) and D is the saturation deficit for N_2. Rates of seepage (Eq. (1)) for each date were divided by the channel width (m) to obtain rates of groundwater flux per unit area (Q_{g}/A).

In November 1997, six estimates of the reaeration rate coefficient were made with propane according to the method described by Kilpatrick et al. (1989). Propane was injected upstream of the reach at a constant rate through eight diffuser plates spaced evenly across the channel and propane concentrations were measured at two stations downstream of the injection point. Rhodamine dye was injected concurrently (as a slug) with propane to estimate travel time between the stations where samples were collected for propane analysis. At each station, samples for propane analysis were collected at five points spaced evenly along a transect across the river; samples were collected by the same method used to collect N_2 samples, but a 10-ml headspace was created prior to sealing each vial. Within 24 h of collection, propane in the headspace was analyzed by gas chromatography using a flame ionization detector. A flow-through field fluorometer was located at each station where propane was measured and concentrations of rhodamine dye were measured continuously (5-s intervals); travel time between the stations was computed from the times of peak concentration. Reaeration rate coefficients for propane (k_{propane}) were calculated from the rate of loss of propane between the two stations (Kilpatrick et al. 1989); reaeration rate coefficients for nitrogen (k_{nitrogen}) were derived from the rate coefficients for propane using the indexing procedure described by Gulliver et al. (1990). Rate coefficients were adjusted to a common temperature as follows (Bowie et al. 1985; Thomann and Mueller 1987):

$$k_{\mathrm{nitrogen,T}} = k_{\mathrm{nitrogen}}, 20^{\circ}\theta^{(T-20)} \tag{3}$$

where k_{nitrogen}, 20° is the reaeration rate coefficient for nitrogen at 20 °C and θ is set to 1.024. After adjustment to a common temperature, the reaeration rate coefficient for N_2 did not vary significantly with discharge; thus, the mean rate at 20 °C (12.1 ± 0.64 d^{-1}) was applied to all dates. The mean rate then was adjusted for changes in temperature at 10-min intervals according to Eq. 3.

On five dates, temperature, atmospheric pressure, and depth were measured at hourly intervals but N_2 concentration was measured only at a single point in time. For dates with only one measurement of N_2 concentration, a simulation model similar to that described by McCutchan et al. (1998) was used to estimate rates of denitrification. From a set of initial conditions, the model predicts temporal changes in N_2 concentration from changes in temperature, atmospheric pressure, and depth and the assumption that the rate of denitrification adjusted to 20 °C (P_{nitrogen}, 20°) is constant over the 24-h period. For the 24-h period preceding the measured N_2 concentration, the initial concentration (i.e., the concentration 24 h before the time of collection) was set to the measured concentration. For each 10-min interval, k_{nitrogen}, 20° and P_{nitrogen}, 20° were adjusted for changes in temperature according to Equation 3; θ for denitrification was set to 1.045 (DiToro and Connolly 1980). P_{nitrogen}, 20° was adjusted to obtain the best fit between the measured and predicted concentrations and P_{nitrogen} was calculated as the average rate over the 24-h period. The reaeration rate was sufficiently high that estimation of the denitrification rate was insensitive to the initial concentration of N_2; variation of the initial concentration by 2 mg l^{-1} (70 μM N_2) had no measurable effect on the estimated rates.

Mass-balance method

Water samples for nutrient analyses were collected bi-weekly from the three sampling sites (Figure 1). Samples were collected in the middle of the channel in 1-l polyethylene bottles. Cross-sectional measurements of specific conductance showed that the river was well mixed at each site. Samples were filtered through glass-fiber (Whatman GF/C) filters within 6 h of collection; after filtration, samples were stored at −20 °C until they were analyzed.

Analyses of water samples for nutrient concentrations included tests for ammonium, nitrate, and total dissolved nitrogen; nitrite concentrations were negligible. Ammonium was measured colormetrically (Grasshoff 1976) and nitrate was analyzed with a Dionex DX-120 ion chromatograph. Total dissolved nitrogen was determined by ion chromatography after all dissolved nitrogen was converted to nitrate by persulfate digestion (Davi et al. 1993). Dissolved organic nitrogen was estimated as the difference between the concentration of total dissolved nitrogen and the sum of the inorganic nitrogen fractions.

Denitrification was estimated by mass balance from transport and transformation rates affecting nitrate within the study reach as follows:

$$O = I + S + N - D \tag{4}$$

where O is the mass flux of nitrate-N in surface outflow from the reach (g N d^{-1}), I is the flux of nitrate-N in surface inflow to the reach, S is flux of nitrate-N entering the reach in groundwater, N is nitrification conversion of ammonium to nitrate; (g N d^{-1}), and D is the rate of denitrification (g N d^{-1}). Because all variables except D can be estimated from field data, denitrification can be estimated as a residual. The same basic equation was used for estimating ammonification rates from the decline in mass of organic nitrogen and nitrification rates from the decline in mass of ammonium. The sequence of procedures for estimates was as follows: (1) estimate ammonification from mass-balance of dissolved organic nitrogen; (2) estimate nitrification from the rate of ammonification and mass-balance of ammonium; (3) estimate denitrification from the rate of nitrification and mass-balance of nitrate. Mass fluxes then were divided by reach area to obtain areal rates ammonification, nitrification, and denitrification (g N m^{-2} d^{-1}).

Rates were adjusted for changes in temperature over the reach by use of Eq. (3). Temperature coefficients (θ) were as follows: ammonification, 1.02 (Bowie et al. 1985), nitrification, 1.08 (DiToro and Connolly 1980), denitrification, 1.045 (DiToro and Connolly 1980). On dates when temperature was measured at a single time rather than at 10-min intervals, mean daily temperature was estimated from the measured temperature and time of day using a model of diel temperature oscillation (McCutchan et al. 1998); times of minimum and maximum temperature were estimated from day of the year with a relationship developed from long-term records of temperature at the upstream and downstream sites (G. Cronin and J. Saunders, unpublished data). Typically, estimates of mean daily temperature based on the model of diel temperature oscillation were within 1 °C of the measured values (i.e., means calculated from measurements made at 10-min intervals).

Results

Open-channel estimates

In the channel, concentrations of dissolved N_2 over the 24-h sampling periods typically were above saturation and fluctuated approximately in parallel with changes in saturation (Figure 2). Concentrations of dissolved N_2 were highest in the cool months (Nov–Mar; 15–16 mg l^{-1}; 535–570 μM N_2), when saturation concentrations were highest, and were lowest in the warm months (Apr–Oct; 10.5–14 mg l^{-1}; 375–500 μM N_2), when saturation concentrations were lowest. Variation in N_2 concentration among samples collected at the same time was small; on a given date, the average coefficient of variation for samples collected from the channel at the same time was generally $<0.5\%$ (range, 0.03–0.97%).

The mean ($\pm$SD) concentration of N_2 in groundwater was 19.2 $\pm$ 1.4 mg l^{-1} (682 ± 51 μM N_2). Concentrations in groundwater were slightly higher during the cool months, but not significantly so.

Open-channel estimates of denitrification varied from 0 to 3.08 g N m^{-2} d^{-1} (Figure 3). The highest rate was recorded in late July and the lowest value (0 g N m^{-2} d^{-1}) was recorded in February. The mean rate ($\pm$SE) for the

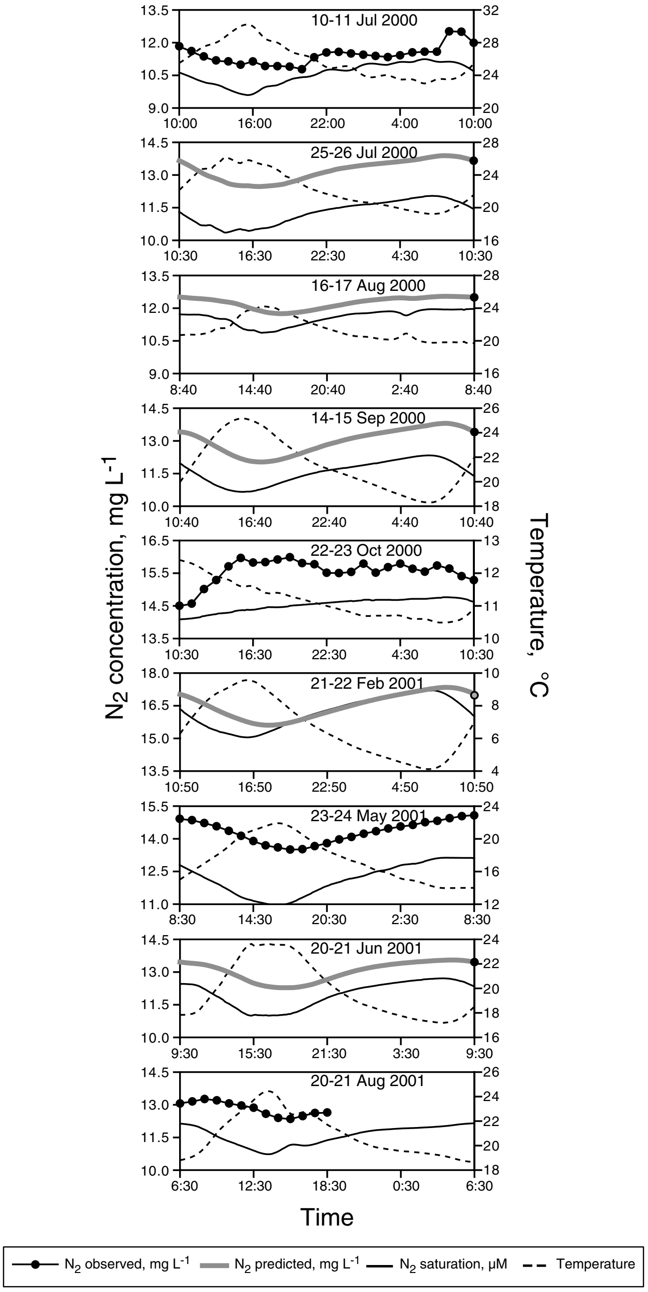

Figure 2. Open-channel concentrations of dissolved N_2. The saturation concentration and temperature are shown for reference. On dates when only one measurement of concentration was made, the predicted diel changes in N_2 concentration are shown (see text for further details).

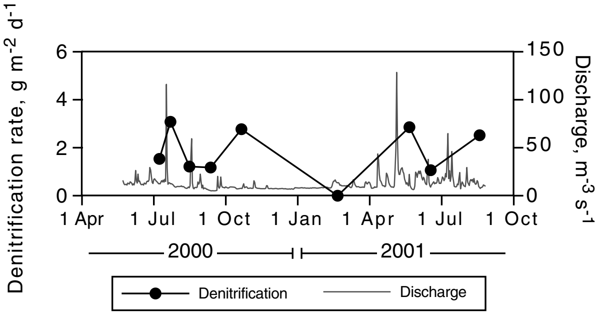

Figure 3. Open-channel estimates of denitrification. Discharge at the downstream site is shown for reference.

warm season (Apr–Oct) was 2.03 ± 0.30 g N m^{-2} d^{-1} ; only one measurement was made during the cool season (Nov–Mar).

Estimates from mass-balance

In the absence of new sources, DON declines as organic N is converted to ammonium though ammonification (Figure 4). The mass flux of ammonium declined over the reach, indicating that nitrification proceeded faster than ammonification. Nitrate accumulated, but at a rate lower than would be expected if denitrification were not occurring.

Areal rates of ammonification, nitrification, and denitrification from mass balance all showed slight seasonal trends (Figure 5). Rates of ammonification were higher during the cool months than in the warm months (Student's t test, $t = 3.71$, $p = 0.0012$); rates of nitrification also were higher during the cool months, but not significantly so (Student's t test, $t = 1.19$, $p = 0.245$). Rates of denitrification were higher during the warm months, but not significantly so (Student's t test, $t = 1.77$, $p = 0.089$).

Comparison of methods

Mean rates of denitrification based on linear interpolation across nine sampling dates (Figure 6) were 1.62 g N m^{-2} d^{-1} for the open-channel method and 2.04 g N m^{-2} d^{-1} for the mass- balance method. Over the 18.5-km reach, the mean rates of denitrification corresponded to a loss of 1450 kg d^{-1} (open-channel) and 1870 kg d^{-1} (mass-balance), which were equivalent to the removal of 34–45% of the nitrate that entered the reach. The two methods showed similar seasonal patterns over the study period, particularly in 2001 (Figure 6) and, for the nine dates, temperature-corrected rates of denitrifica-

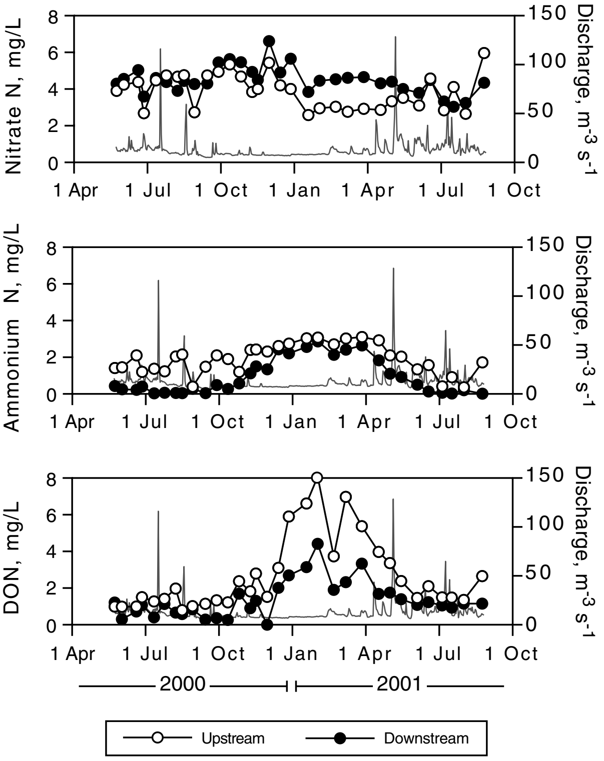

Figure 4. Concentrations of dissolved and particulate forms of nitrogen at the upstream and downstream sites. Discharge at the downstream site is shown for reference.

tion were not significantly different between the two methods (Wilcoxon signed-rank test, $p = 0.30$).

Discussion

Whole-system estimates of denitrification have been possible with mass-balance measurements of multiple nitrogen species, but the open-channel N_2 method offers an alternative that may produce superior estimates of denitrification under many conditions. Although the methods compared here are subject to different sources of uncertainty and may not produce equivalent estimates of denitrification under some conditions, the two did produce similar estimates of denitrification for the South Platte River. Open-channel estimates were lower than estimates from mass balance, although not significantly so.

As presented here, the open-channel N_2 method is best suited for use in well-mixed streams where (1) concentrations of N_2 do not vary laterally or with depth, and (2) processes affecting the mass balance for N_2 are spatially

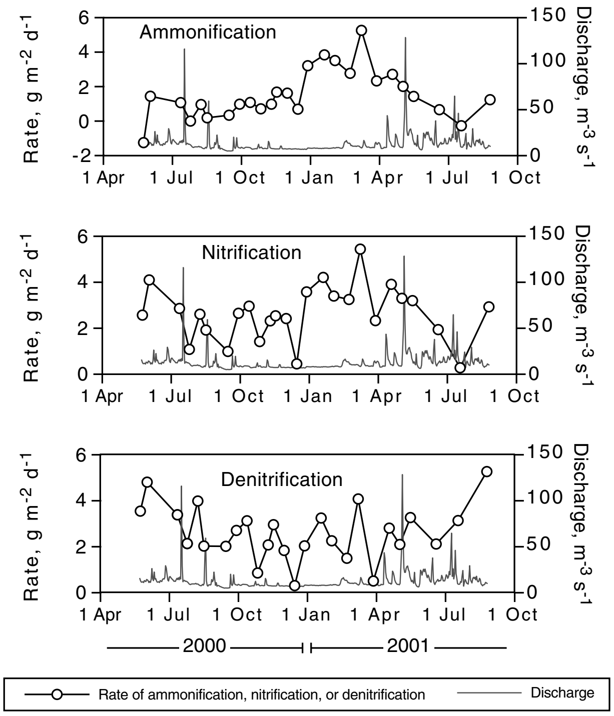

Figure 5. Estimates of ammonification, nitrification, and denitrification from mass-balance measurements. Discharge at the downstream site is shown for reference.

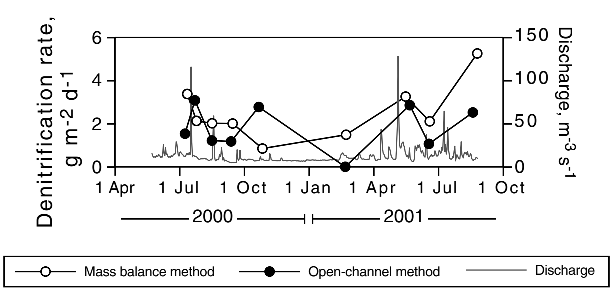

Figure 6. Comparison of open-channel estimates of denitrification with those based on mass-balance measurements. Discharge at the downstream site is shown for reference.

homogeneous. Application of the open-channel method to poorly-mixed systems (e.g., wetlands or streams with abundant emergent vegetation) should be possible, but would require modification of the method to account for spatial heterogeneity in N_2 concentrations. Although high spatial variability in the processes affecting the mass balance for N_2 (e.g., variation in reaeration rates between cascades and pools or high rates of denitrification associated with biogeochemical ‘hot spots’) will affect the precision of the method, the extent to which spatial variability affects open-channel estimates has not been explored.

Precision with the open-channel method will be the highest where rates of denitrification are high and rates of reaeration are low (McCutchan et al. 1998, 2003), as occurs in the South Platte River. In this study, the saturation concentration for N_2 varied in response to changes in temperature, but concentrations remained well above the saturation concentration except during winter (Figure 2). Although some of the excess N_2 was derived from N_2 dissolved in groundwater, N_2 supersaturation in the South Platte River is largely the result of high rates of denitrification in combination with low rates of reaeration. As long as groundwater flux and the N_2 concentration for groundwater are well quantified, the open-channel N_2 method can provide precise estimates even where rates of groundwater flux are high (McCutchan et al. 2003). Where precision will be low with the open-channel method (e.g., where rates of denitrification are very low or rates of reaeration are high) or where information on spatial heterogeneity in rates is required, other methods (e.g., chamber techniques) may be more suitable.

Other potential sources of error with the open-channel method include ebullition (bubbling), nitrogen fixation, and losses of N_2O. N_2O was not measured in this study but accounts for $< 1\%$ of the nitrogen lost through denitrification in the South Platte River (McMahon and Dennehy 1999). Ebullitive losses associated with high rates of gas production can be captured with chamber methods (e.g., Smith et al. 2000), but such losses may not be measured by open-channel methods, especially in shallow-water systems. Ebullition has been observed on some dates in the South Platte River, particularly in association with algal mats that form along the river margin after extended periods of low discharge (J. McCutchan and J. Saunders, personal observation). Gas collected by inverting vials over streams of bubbles in the water column were analyzed by MIMS and concentrations of N_2 in the bubbles typically were higher than concentrations in the channel or in groundwater (> 18 mg l^{-1}; J. McCutchan, unpublished data). Although ebullition probably did not result in large errors in estimates of denitrification, the potential for ebullitive losses should be recognized in open-channel studies of denitrification. The contribution of nitrogen fixation (microbial conversion of N_2 to ammonium) to the mass-balance for N_2 was not considered here, but rates of N-fixation in the South Platte probably are very low due to the high concentrations of fixed nitrogen. Although the actual rates of denitrification for the South Platte River may have been higher than the rates reported here, uncertainty associated with ebullition, nitrogen fixation, and losses of N_2O probably is quite small.

With the mass-balance approach, errors in estimates of ammonification or nitrification affect the estimation of denitrification. Precision with the mass-balance approach is not affected by reaeration rates, ebullition, or losses of N_2O, but other factors can be important. Uncertainty in estimates of groundwater flux and concentrations of nitrogen species in groundwater also affect this method.

Uptake of nitrate or ammonium by algae is not considered in Equation 4. At the downstream site, rates of photosynthesis estimated by the open-channel oxygen method (McCutchan et al. 2002) range from near zero in winter to 3.1 g C m^{-2} d^{-1} during summer at low discharge (G. Cronin, unpublished data). If the molar C:N ratio for algal biomass is assumed to be 7, uptake of N by algae would be 0–0.44 g N m^{-2} d^{-1}. Thus, the potential effects of algal uptake on estimates of denitrification would be no more than about 10%; because nitrogen turns over more slowly in algal biomass than does carbon, errors related to algal uptake probably are even lower than this estimate would indicate.

The estimates presented here are high compared to estimates for other rivers (Hill 1983; Seitzinger 1988), but are similar to those reported in previous studies of downstream reaches of the South Platte River. McMahon and Bohlke (1996), working with cores, estimated rates between 1.26 and 2.50 g N m^{-2} d^{-1} near Greeley, about 40 km downstream from the study reach; Sjodin et al. (1997), using mass balance, estimated an average rate of 0.84 g N m^{-2} d^{-1} about 55 km downstream from the study reach (Figure 1).

Rates of denitrification in the South Platte River were the highest in summer and lowest in winter. The supply of nitrate can limit rates of denitrification in some systems (Bradley et al. 1995; Garcia-Ruiz et al. 1998; Pattinson et al. 1998), but concentrations of nitrate in the South Platte River remained high year-round (Figure 4). The concentration of nitrate was more variable at the upstream site than at the downstream site, but there was not a significant linear relationship between the concentration of nitrate at the upstream site and the rate of denitrification ($r^2 = 0.01, p = 0.55$). Thus, the seasonal pattern in rates of denitrification probably was not caused by seasonal variation in nitrate concentration. Temperature may have regulated rates of denitrification in the South Platte River. Although there was a weak linear relationship between temperature and the rate of denitrification ($r^2 = 0.17$, $p = 0.03$), the mechanisms by which temperature affected denitrification are not clear. At the downstream site, the daily mean concentration of oxygen averaged 8.2 mg l^{-1} in January and 5.7 mg l^{-1} in June (G. Cronin, unpublished data). The seasonal pattern of denitrification may have been caused by direct effects of temperature on microbial processes or indirectly by the effects of temperature and community respiration on the concentration of dissolved oxygen in the sediments.

Conclusions

The open-channel N_2 method provides an alternative to estimation of denitrification from mass-balance measurements of transport and transformation. Analytical requirements associated with mass-balance measurements are simple and estimation of denitrification is unaffected by reaeration, but estimates based on mass-balance measurements are calculated as residuals (i.e., estimates are indirect) and are generally feasible only where rates of denitrification are high. Although specialized analytical equipment is necessary for the open-channel N_2 method, it provides direct estimates of denitrification and is capable of high precision where rates of reaeration are low and the flux of groundwater is well quantified. Use of the open-channel N_2 method is particularly attractive where the open-channel oxygen method is already in use for the estimation of ecosystem metabolism because both methods depend on measurements of reaeration rates. Although the open-channel N_2 method in its present form is best suited to low-gradient streams where rates of reaeration are low, it is probably the most reliable means of making whole-system estimates of denitrification in many streams and rivers.

Acknowledgments

This work was supported by the Regional Integrated Sciences and Assessments (RISA) Program of the National Oceanic and Atmospheric Administration (NOAA; award NA17RJ1229), by the Department of Environmental, Population, and Organismal Biology, University of Colorado, by the Edna Bailey Sussman Foundation, and by the Denver Metro Wastewater Reclamation District. We thank Greg Cronin and Stephan Klemm (University of Colorado, Denver), who provided data for total organic carbon, oxygen, and temperature. Craig Wilks helped with sampling, as did Sonja Chavez de Baca, and Sujay Kaushal; Lesley Smith provided assistance in the laboratory. In addition, we thank Bob Stahl (Water Resources Division of the Colorado Department of Natural Resources), who provided flow records for irrigation ditches.

References

Alexander R.B., Smith R.A. and Schwartz G.E. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403: 758–761.

Bergmann T., Richardson T.L., Paerl H.W., Pinckney J.L. and Schofield O. 2002. Synergy of light and nutrients on the photosynthetic efficiency of phytoplankton populations from the Neuse River Estuary, North Carolina. J. Plankton Res. 24: 923–933.

Bernot M.J., Dodds W.K., Gardner W.S., McCarthy M.J., Sobolev D. and Tank J.L. 2003. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. Appl. Environ. Microbiol. 69: 5950–5956.

Bowie G.L., Mills W.B., Porcella D.B., Campbell C.L., Pagenkopf J.R., Rupp G.L., Johnson K.M., Chan P.H., Gherini S. and Chamberlain C.E. 1985. Rates, Constants, and Kinetics Formulations in Surface Water Quality Modeling. Report No: EPA/600/3-85/040. Environmental Research Laboratory, Athens GA.

Bradley P.M., McMahon P.B. and Chapelle F.H. 1995. Effects of carbon and nitrate on denitrification in bottom sediments of an effluent-dominated river. Water Resour. Res. 31: 1063–1068.

Cornwell J.C., Kemp W.M. and Kana T.M. 1999. Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. Aquat. Ecol. 33: 41–54.

Davi M.L., Bignami S., Milan C., Liboni M. and Malfatto M.G. 1993. Determination of nitrate in surface waters by ion-exchange chromatography after oxidation of total organic nitrogen to nitrate. J. Chromatogr. 644: 345–348.

DiToro D.M. and Connolly J.P. 1980. Mathematical Models of Water Quality in Large Lakes. Part 1: Lake Huron and Saginaw Bay. US Environmental Protection Agency, Ecological Research Series.

Garcia-Ruiz R., Pattinson S.N. and Whitton B.A. 1998. Denitrification in river sediments: relationships between process rate and properties of water. Freshwater Biol. 39: 467–476.

Grasshoff K. 1976. Methods of Seawater Analysis. Weinham, Verlag Chemie, New York, 317 pp.

Gulliver J.S., Thene J.R. and Rindels A.J. 1990. Indexing gas transfer in self-aerated flows. J. Environ. Eng. 116: 503–523.

Hill A.R. 1981. Nitrate-nitrogen flux and utilization in a stream ecosystem during low summer flows. Can. Geogr. 25: 225–239.

Hill A.R. 1983. Nitrate-nitrogen mass balances for two Ontario rivers. In: Fontaine T.D. and Bartell S.M. (eds), Dynamics of Lotic Ecosystems. Ann Arbor Science, Ann Arbor.pp 457–477.

Hill A.R., DeVito K.J., Campagnolo S. and Sanmugadas K. 2000. Subsurface denitrification in a forest riparian zone: interactions between hydrology and supplies of nitrate and organic carbon. Biogeochemistry 51: 193–223.

Howarth R.W., Billen G., Swaney D., Townsend A., Jaworski N., Lajtha K., Downing J.A., Elmgren R., Caraco N., Jordan T., Berendse F., Freney J., Kudeyarov V., Murdoch P. and Zhu-Liang Z. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochemistry 35(1): 75–139.

Kana T.M., C. Darkangelo C., Hunt M.D., Oldham J.B., Bennett G.E. and Cornwell J.C. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2 and Ar in environmental water samples. Anal. Chem. 66(23): 4166–4170.

Kana T.M., Sullivan M.B., Cornwell J.C. and Groszkowski K. 1998. Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. Limnol. Oceanogr. 43: 334–339.

Kilpatrick F.A., Rathbun R.E., Yotsukura N., Parker G.W. and DeLong L.L. 1989. Determination of stream reaeration coefficients by use of tracers. Techniques of Water-Resources Investigations of the U.S. Geological Survey 3-A18.

Laursen A.E. and Seitzinger S.P. 2002. Measurement of denitrification in rivers: an integrated, whole-reach approach. Hydrobiologia 485: 67–81.

Lewis W.M. Jr. 2002. Yield of nitrogen from minimally disturbed watersheds of the United States. Biogeochemistry 57(58): 375–385.

Lovett G.M., Weathers K.C. and Sobczak W.V. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. Ecol. Appl. 10: 73–84.

McCutchan J.H. Jr., Lewis W.M. Jr. and Saunders J.F. III 1998. Uncertainty in the estimation of stream metabolism from open-channel oxygen concentrations. J. N. Am. Benthol. Soc. 17: 155–164.

McCutchan J.H. Jr., Saunders J.F. III, Lewis W.M. Jr. and Hayden M.G. 2002. Effects of groundwater flux on open-channel estimates of stream metabolism. Limnol. Oceanogr. 47: 321–324.

McCutchan J.H. Jr., Saunders J.F. III, Pribyl A.L. and Lewis W.M. Jr. 2003. Open-channel estimation of denitrification. Limnol. Oceanogr.: Meth. 1: 74–81.

McMahon P.B. and Bolke J.K. 1996. Denitrification and mixing in a stream-aquifer system: effects on nitrate loading to surface waters. J. Hydrol. 186: 105–128.

McMahon P.B. and Dennehy K.F. 1999. N_2O emissions from a nitrogen-enriched river. Environ. Sci. Technol. 33: 21–25.

Morris D.P. and Lewis W.M. Jr. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. Freshwater Biol. 20: 315–327.

Nolan B.T., Ruddy B.C., Hitt K.J. and Helsel D.R. 1997. Risk of nitrate in groundwaters of the United States—a national perspective. Environ. Sci. Technol. 31: 2229–2236.

Odum H.T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 1: 102–117.

Pattinson S.N., Garcia-Ruiz R. and Whitton B.A. 1998. Denitrification and nitrous oxide production in sediments of the Wiske, a lowland eutrophic river. Sci. Tot. Environ. 210((211): 307–320.

Peterson B.J., Wollheim W.M., Mulholland P.J., Webster J.R., Meyer J.L., Tank J.L., Martí E., Bowden W.B., Valett H.M., Hershey A.E., McDowell W.H., Dodds W.K., Hamilton S.K., Gregory S. and Morrall D.D. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292: 86–90.

Pfenning K.S. and McMahon P.B. 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. J. Hydrol. 187: 283–295.

Saunders D.L. and Kalff J. 2001. Denitrification rates in the sediments of Lake Memphemagog, Canada-USA. Water Res. 35: 1879–1904.

Saunders J.F. III. and Lewis W.M. Jr. 2003. Implications of climate variability for regulatory low flows in the South Platte Basin, Colorado. J. Am. Water Resour. Assoc. 39: 33–45.

Seitzinger S.P. 1988. Denitrification in fresh water and coastal marine ecosystems: Ecological and geochemical significance. Limnol. Oceanogr. 33: 702–724.

Seitzinger S.P. 1993. Denitrification and nitrification rates in aquatic sediments. In: Kemp P.F. (ed), Handbook of Methods is Aquatic Microbial Ecology. Lewis Publishers, Boca Raton. 633pp.

Seitzinger S.P., Nielson L.P., Caffrey J. and Christensen P.B. 1993. Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry 23(3): 147–167.

Sjodin A.L., Lewis W.M. Jr. and Saunders J.F. III 1997. Denitrification as a component of the nitrogen budget for a large plains river. Biogeochemistry 39: 327–342.

Smith L.K., Lewis W.M. Jr., Chanton J.P., Cronin G. and Hamilton S.K. 2000. Methane emissions from the Orinoco River floodplain, Venezuela. Biogeochemistry 51: 113–140.

Sørensen J. 1978. Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl. Environ. Microbiol. 36: 139–143.

Tank J.L. and Dodds W.K. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biol. 48: 1031–1049.

Thomann R.V. and Mueller J.A. 1987. Principles of Surface Water Quality Modeling and Control. Harper & Row Publishers, Inc., pp. 297–301.

Vitousek P.M., Aber J., Bayley S.E., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.M. and Tilman G.D. 1997. Human alteration of the global nitrogen cycle: causes and consequences. Ecol. Issues 1: 1–15.