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DENITRIFICATION IN NITRATE-RICH STREAMS: APPLICATION OF N_2 :Ar AND 15 N-TRACER METHODS IN INTACT CORES

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Abstract. Rates of benthic denitrification were measured using two techniques, membrane inlet mass spectrometry (MIMS) and isotope ratio mass spectrometry (IRMS), applied to sediment cores from two NO₃⁻-rich streams draining agricultural land in the upper Mississippi River Basin. Denitrification was estimated simultaneously from measurements of N₂:Ar (MIMS) and $^{15}N[N_2]$ (IRMS) after the addition of low-level $^{15}NO_3^-$ tracer ($^{15}N:N=0.03-$ 0.08) in stream water overlying intact sediment cores. Denitrification rates ranged from about 0 to 4400 µmol N m⁻² h⁻¹ in Sugar Creek and from 0 to 1300 µmol N m⁻² h⁻¹ in Iroquois River, the latter of which possesses greater streamflow discharge and a more homogeneous streambed and water column. Within the uncertainties of the two techniques, there is good agreement between the MIMS and IRMS results, which indicates that the production of N₂ by the coupled process of nitrification/denitrification was relatively unimportant and surfacewater NO₃⁻ was the dominant source of NO₃⁻ for benthic denitrification in these streams. Variation in stream NO₃⁻ concentration (from about 20 μmol/L during low discharge to 1000 μmol/L during high discharge) was a significant control of benthic denitrification rates, judging from the more abundant MIMS data. The interpretation that NO₃⁻ concentration directly affects denitrification rate was corroborated by increased rates of denitrification in cores amended with NO₃[−]. Denitrification in Sugar Creek removed ≤11% per day of the instream NO₃⁻ in late spring and removed roughly 15–20% in late summer. The fraction of NO₃ removed in Iroquois River was less than that of Sugar Creek. Although benthic denitrification rates were relatively high during periods of high stream flow, when NO₃ concentrations were also high, the increase in benthic denitrification could not compensate for the much larger increase in stream NO₃⁻ fluxes during high flow. Consequently, fractional NO₃⁻ losses were relatively low during high flow.

Key words: ¹⁵N; agriculture; denitrification; isotope ratio mass spectrometry; isotope tracer; membrane inlet mass spectrometry; Mississippi River Basin; sediment oxygen demand; streams.

Introduction

Nitrate (NO₃⁻) contamination of surface water and ground water is common in watersheds dominated by agricultural activities (Vitousek et al. 1997, Townsend et al. 2003). Substantial quantities of dissolved inorganic nitrogen, particularly NO₃⁻, are exported through loworder streams (Alexander et al. 2000, Donner et al. 2004). Denitrification, the microbial reduction of NO₃⁻ to the gaseous products nitric oxide (NO), nitrous oxide (N₂O), or dinitrogen (N₂), is an important mechanism for NO₃⁻ removal in a variety of suboxic environments (Seitzinger et al. 2006). Streams provide an opportunity for large quantities of NO₃⁻ to come into contact with oxygen-depleted sediments, thus creating an ideal environment for nitrogen transformations at or below

sheds (Hill 1983, Alexander et al. 2000), thus mitigating the effects of excess N on aquatic ecosystems and public health downstream. Despite the importance of denitrification, direct measurements of denitrification remain difficult, and results of different methods are not always in agreement (Groffman et al. 2006).

Methods for estimating benthic denitrification rates include measurements of NO₃⁻ disappearance (Andersen 1977), N₂ efflux (Seitzinger et al. 1980), N₂O

the sediment—water interface (Duff and Triska 2000). Denitrification in stream corridors is believed to account

for substantial losses of N leaving agricultural water-

Methods for estimating benthic denitrification rates include measurements of NO₃⁻ disappearance (Andersen 1977), N₂ efflux (Seitzinger et al. 1980), N₂O accumulation after acetylene inhibition of N₂O reduction (with and without the addition of chloramphenicol to prevent de novo synthesis of additional denitrifying proteins [Sørensen 1978]), ¹⁵N[N₂] efflux after addition of ¹⁵N-labeled tracers measured by isotope ratio mass spectrometry (IRMS) (Nishio et al. 1983), ¹⁵N dilution (Koike and Hattori 1978), ¹⁵N-¹⁵N isotope-pairing (Nielsen 1992), and N₂:Ar measured by membrane inlet mass spectrometry (MIMS) (Kana et al. 1994). The most

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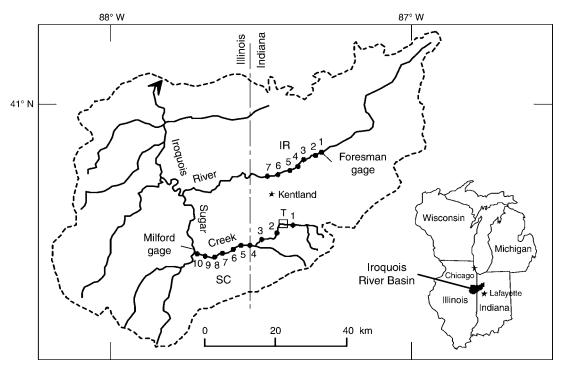


Fig. 1. Map showing locations of core sites. Foresman and Milford are USGS stream gage stations. Abbreviations are: IR, Iroquois River; SC, Sugar Creek.

commonly used techniques are based on laboratory incubations of sediment cores or sediment slurries. However, several techniques have been applied *in situ* to estimate denitrification in surface water, including direct assays of N₂ production or nitrogen isotope pairing using benthic landers (Devol 1991, Nielsen and Glud 1996), porewater profiling of nitrogen species (Vanderborght et al. 1977), and whole-reach or water column estimates from mass-balance approaches (Hill 1983), MIMS (Laursen and Seitzinger 2002, McCutchan et al. 2003) and in-stream ¹⁵N isotope tracer (Böhlke et al. 2004, Mulholland et al. 2004).

All of these methods have advantages and disadvantages and many studies comparing techniques have been done (Seitzinger 1988, Seitzinger et al. 1993, Raymond et al. 1992, Bernot et al. 2003, Steingruber et al. 2001, Groffman et al. 2006). However, there are relatively few direct measurements of denitrification in NO₃⁻-rich streams and no reported comparisons of MIMS and IRMS techniques applied to surface water overlying intact core samples. These two techniques have several advantages. For example, MIMS and IRMS are relatively benign methods with little modification to the biological systems because excess substrate additions or metabolic inhibition are avoided. In addition, MIMS measures total N₂ production and IRMS measures N₂ production from ¹⁵N-labeled NO₃⁻. Therefore, a direct comparison of simultaneous results of these methods also can provide information on the relative importance

of coupled nitrification/denitrification vs. NO_3^- supplied from overlying water.

As part of a larger U.S. Geological Survey (USGS) study on the fate of nitrogen during transport through agriculturally impacted rivers (Böhlke et al. 2004, Antweiler et al. 2005), we measured denitrification rates in two streams located in the upper Mississippi River Basin. The objectives of this study were to (1) compare two techniques to quantify denitrification rates in benthic sediments, and (2) provide much needed data on denitrification rates in streams receiving agricultural discharge. The study streams have high concentrations of NO₃⁻ that vary seasonally (Antweiler et al. 2005), and denitrification rates were measured during high and low periods of discharge to obtain data during periods of high and low NO₃ concentrations, respectively.

METHODS

Study site characteristics

The sites for this study are on the Iroquois River, a headwater drainage basin in the Mississippi River watershed, and Sugar Creek, a tributary to the Iroquois River (Fig. 1). The watersheds are located within the Upper Illinois River Basin, with drainage areas of approximately 1600 and 400 km² in the Iroquois River and Sugar Creek reaches, respectively. Nearly the entire area drained by both streams is devoted to agriculture, mostly corn and soybeans, with over 90% of the available land being farmed (R. B. Alexander, *personal*

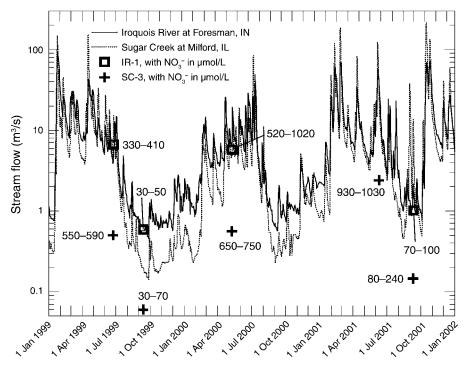


Fig. 2. Core sampling times compared to variations of daily streamflow. Continuous stream-flow records are from USGS gages at Foresman, Indiana, and Milford, Illinois (data available at \(\http://waterdata.usgs.gov/nwis \)). Symbols indicate coring times and corresponding flows near the upstream ends of the cored reaches. IR-1 is the same as the Foresman gage site. SC-3 is in the headwaters of Sugar Creek upstream from Milford (Fig. 1). Nitrate concentrations are reported as the range measured at all sites within the sample reach during a Lagrangian study that was conducted concurrent with each core collection trip (Antweiler et al. 2004).

communication). This study was done simultaneously with a Lagrangian mass transport study (Antweiler et al. 2005), in which discharge and water chemistry were measured at seven locations along a 22-km reach of Iroquois River (IR-1 to IR-7) downstream from the USGS stream gage (05524500) near Foresman, Indiana (40°52′ N, 87°18′ W) and at 11 locations along a 38-km reach of Sugar Creek (SC-T, SC-1 to SC-10) upstream from the USGS gage (05525500) near Milford, Illinois (40°37′ N, 87°43′ W; see Fig. 1). The sampling times of both studies are shown in relation to streamflow records in Fig. 2. Within the study area, Iroquois River is a fourth-order channel and Sugar Creek begins as a firstorder channel that becomes a second- and then a thirdorder channel before the downstream end of the study reach.

The average flow in each of these reaches is variable, depending on the season. The season of minimum discharge occurs in late summer/early fall, at which time the discharge in the Iroquois River study reach was around 0.6 m³/s (Fig. 2). During the same time period, flow in the Sugar Creek study reach ranged from 0.04 to 0.2 m³/s. Higher flows occur in spring with typical high discharge rates of approximately 10 m³/s in the Iroquois River and 0.4 to 2.5 m³/s in the Sugar Creek study reach. Stream depth in the Iroquois River study reach averaged 60 cm during late summer/early fall discharge and 180

cm during spring discharge. Stream depth in Sugar Creek averaged 15 cm during late summer and 40 cm during spring discharge. The streambed substrate in both study reaches is primarily sand, with fine sand and silt predominant in the Iroquois River study reach (median grain size 0.2 mm) and coarse sand and gravel predominant in the Sugar Creek study reach (range, 0.065–40 mm; median grain size, 1.4 mm) (Voytek et al. 2001). The organic carbon content of the streambed sediment is low (below a few percent) in both study reaches (Voytek et al. 2001). The Iroquois River has a higher suspended sediment load during most times of the year (Sugar Creek, <5–120 mg/L, mean 18; Iroquois, 17–80 mg/L, mean 44) composed mostly of silt (Antweiler et al. 2005).

Collection of sediment cores

Sediment cores and surface water samples were collected in June and September 1999, May 2000, and June and September 2001 in order to measure rates of denitrification and sediment oxygen demand at several high and low discharge periods, excluding storm flow peaks (Fig. 2). The core barrels consisted of Lexan tubing (inner diameter, 7.9 cm; length, 30.6 cm). These were hand driven into the sediments to depths ranging from about 10 to 20 cm (depth dependent upon substrate resistance) and then sealed along with over-

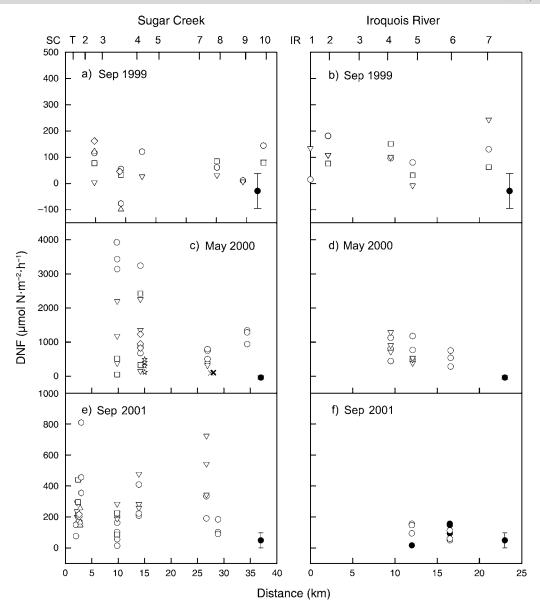


Fig. 3. Individual denitrification rates (DNF) measured by membrane inlet mass spectrometry (MIMS) in cores from Sugar Creek and Iroquois River. Sampling location names are shown across the top; distances along the streams are shown on the bottom axis. Solid circles with error bars show the mean \pm sp of denitrification rates measured in all blank cores evaluated during a given sampling trip. Panels (a) and (b) show data from September 1999. At this time, individual cores were collected from each sediment type present at the time of sampling, as indicated by the different symbols at each sampling location. Panels (c) and (d) show data from May 2000, with a few additional data from high-flow periods in June 1999 (×) and June 2001 (stars). In May 2000 and June 2001, each set of three cores collected at a sampling station was collected from the same sediment type, as indicated by similar symbols. Panels (e) and (f) show data from September 2001. Symbols are as in panels (c) and (d).

lying surface water by securing black rubber caps at both ends with hose clamps. Cores were collected in sets of three along with a 20-L carboy of stream water, which served as feed water during the sediment core incubations.

In an effort to characterize the range of denitrification rates within the Iroquois River Basin, sediment cores were collected from numerous locations and a variety of bottom types in Sugar Creek and Iroquois River (Figs. 1 and 3). In 1999, each individual core was collected to represent an area of stream bottom with distinctive properties such as grain size, organic material, periphyton, and stream velocity. In May 2000 and September 2001, each set of three cores represented a single bottom type, and multiple sets of cores were collected to represent different bottom types at a given location (Fig. 3). In June 2001, the sampling was designed mainly as a preliminary comparison of the MIMS and IRMS

techniques. All cores for this test were collected near site SC-4 in a relatively homogeneous environment around the edges of an emergent bar and back-pool area (approximately 18×2 m), where calm water and some vegetation caused settling of fine detrital organic-rich ooze in a thin layer on the sediment surface following a major flood event that scoured the stream bed several days earlier.

Laboratory incubations

All sediment cores were transported to the laboratory and set-up within 24 hours of collection, and incubations were run for 24–48 hours. In 1999 and 2000, sediment cores were flown to Colorado and incubated in a walk-in incubator at the University of Colorado, which was maintained at a temperature similar to the stream water temperatures at the time of sampling (June 1999, 22°C; September 1999, 21°C; May 2000, 22°C; June 2001, 23°C), and the cores remained in the dark until water samples were collected for dissolved gas analysis. In September 2001, sediment cores were driven to Purdue University and incubated in a laboratory that was maintained at 22°C with fluctuations in light levels reflecting shaded outdoor conditions.

Each set of three sediment cores, along with a "blank" core containing only stream water from the same core site, was maintained within a flow-through system in which stream water circulated above the intact sedimentwater interface with no headspace (Smith et al. 2000). The cores were continually supplied with stream water, which was collected from the core site in a 20-L carboy of water and was recirculated through the system. The surface water in each core barrel was stirred gently by a magnetic stir bar rotating at about 22 rotations per minute. Air was pumped into each carboy through an airstone to maintain oxygenated conditions in the stream water entering the cores (about 240–260 µmol/L O₂ at room temperature). Flow through the cores was provided by a multichannel Rainin peristaltic pump (Rainin Instruments, Woborn, Massachusetts, USA) set at a flow rate of about 0.2 L/h. For each individual core barrel, the inlet flow rate was measured volumetrically and the surface-water residence time was calculated from the flow rate and the volume of water above the sedimentwater interface. Typical residence times were between about two and six hours (longest for the "blank" cores with stream water only).

In June and September 2001, NO_3^- enriched in ^{15}N ($^{15}N = 99\%$) was added to the carboys to serve as an isotopic tracer of the source of NO_3^- undergoing denitrification to N_2 . The target value of isotopic enrichment for the total NO_3^- in the incubation water was of the order of 3-8% ^{15}N , which permitted the tracer study to be done without altering substantially the total NO_3^- concentration. The introduction of the tracer into the cores was accelerated by siphoning out the non-labeled stream water overlying the sediment and replacing it gently (without disturbing the sediment

surface) with tracer-labeled carboy water before the circulation system was turned on.

Nutrient addition experiments

Two nutrient addition experiments were conducted in September 2001 to evaluate the effect of elevated NO₃ and dissolved organic carbon (DOC) concentrations on denitrification and sediment oxygen demand. After completion of the routine denitrification and sediment oxygen demand measurements, cores from three representative sites in Sugar Creek and two sites in Iroquois River were redistributed randomly among the circulation systems and treated as follows. Three cores from Sugar Creek and three cores from Iroquois River were incubated without additions to serve as controls. Feed water for six cores from Sugar Creek and three cores from Iroquois River received NO₃⁻ additions including isotope tracer to increase the concentrations by about an order of magnitude while maintaining a similar level of ¹⁵N enrichment before and after the NO₃⁻ addition. The amounts of NO₃⁻ added to the core incubations were chosen to give changes in NO₃⁻ concentrations that were similar to the difference in ambient NO₃⁻ concentrations observed in the field between May 2000 and September 2001. Feed water for three cores from Sugar Creek received sodium acetate additions resulting in a surfacewater acetate concentration of 1600 umol/L as C, which was about three times as high as the average ambient DOC concentration of Iroquois River (550 µmol/L) and six times as high as that of Sugar Creek (270 µmol/L) (Antweiler et al. 2005).

Membrane inlet mass spectrometry

Denitrification rates in the sediment cores were measured using MIMS, which quantifies changes in dissolved N₂:Ar and O₂:Ar ratios within the water overlying the sediments (Kana et al. 1994). Triplicate water samples were collected from the inlet and outlet of each sediment core and blank core by overfilling 5-mL glass test tubes fitted with ground-glass stoppers. The sealed water samples, which were stored underwater at the same temperature as the walk-in incubator or laboratory, were analyzed within 4 hours of collection for dissolved N₂:Ar and O₂:Ar using a MIMS system with a Balzers Prisma quadrupole mass spectrometer (Pfeiffer Vacuum GmbH, Asslar, Germany).

In 1999 and 2000, the MIMS system was calibrated using water in equilibrium with two gas-tank standards (one with 21% O₂, 1% Ar, and 78% N₂ and one with 16% O₂, 1% Ar, and 83% N₂), which were humidified in Erlenmeyer flasks containing a small quantity of deionized water (DIW). The water-saturated gas standards were fed into the headspace of sealed containers partially filled with DIW, and the DIW was circulated with a submersible pump to accelerate the rate of equilibration between the DIW and the gas. This reservoir was maintained at the same temperature as the cores. In 2001, a single air-saturated water standard

was prepared by allowing room air to equilibrate with DIW in a similar manner. The equations of Colt (1984) were used to calculate the dissolved N_2 , Ar, and O_2 concentrations of the standards.

Isotopic analysis of nitrogen in nitrate

Samples for N isotopic analysis of NO₃ were filtered $(0.45 \mu m)$, frozen or preserved with KOH (pH > 11), and returned to the USGS Reston Stable Isotope Laboratory for analysis. For the June 2001 samples, in which NO₃⁻ concentrations were high (950–1120 μmol/ L), and for the September 2001 samples, in which NO₃ concentrations ranged from about 30-300 µmol/L, aliquots containing approximately 20 µmol of NO₃⁻ were freeze-dried, then loaded into quartz glass tubes with $Cu + Cu_2O$ and CaO. The tubes were evacuated, sealed, and combusted at 850°C to produce N2 gas from the NO₃⁻, cooled slowly to remove C, H, O, and S gases, then opened under vacuum in a tube cracker attached to the inlet of a Finnigan MAT 251 mass spectrometer (ThermoElectron, Waltham, Massachusetts, USA) and analyzed in dual-inlet mode (DI-IRMS; modified from Kendall and Grimm 1990, Böhlke and Denver 1995). In this case, the measured δ¹⁵N values represent NO₃⁻ plus NO₂⁻ and a fraction of the DON, but not NH₄⁺. For the September 2001 samples, additional aliquots containing approximately 20 nmol of NO₃⁻ were incubated with the denitrifier Pseudomonas aureofaciens to produce N₂O, which was purged with He, trapped with liquid N_2 , and released in a He carrier stream for analysis by continuous-flow mass spectrometry at m:z 44, 45, and 46 (Sigman et al. 2001, Casciotti et al. 2002). In this case, the measured δ^{15} N values represent NO₃⁻ plus NO₂⁻ only.

Measurements were calibrated by analyses of solutions prepared with NO₃⁻ isotopic reference materials with $\delta^{15}N$ normalized to values of +0.4% and +4730%for the international isotope reference materials IAEA-N1 and IAEA-311, respectively. Average reproducibilities (1 sigma) of normalized data range from approximately $\pm 0.2\%$ for $\delta^{15}N$ near 0% (non-tracer samples) to $\pm 250\%$ for δ^{15} N near 10 000% (tracer sample values ranged from about 7000 to 21000‰). For NO₃⁻ concentrations lower than about 80 µmol/L, freezedried and combusted aliquots of tracer samples returned systematically lower $\delta^{15}N$ values than aliquots analyzed by bacterial reduction owing to isotope dilution caused by minor amounts of non-tracer DON in the freezedried samples, which did not have a measurable effect at higher NO₃⁻ concentrations. Therefore, the NO₃⁻ isotope data used in this paper are from the bacterial N₂O method for September 2001 and the combustion N₂ (DI-IRMS) method for June 2001.

Isotopic analysis of dissolved nitrogen gas

Samples for isotopic analysis of dissolved N_2 were collected in He-flushed Wheaton 30-mL serum bottles fitted with 12 mm thick Bellco butyl rubber stoppers and

aluminum crimp seals. These bottles were prepared in the laboratory before sampling by degassing the stoppers under vacuum overnight, injecting approximately 100 µL of 12 mol/L NaOH into the bottles, inserting and crimping the stoppers, and then flushing the bottles with ultrapure He through inlet and outlet syringe needles for approximately 15 minutes at a flow rate of 600 mL/min (approximately 300 bottle volumes). Test analyses indicate that properly flushed bottles containing He at 1 atm pressure contained negligible amounts of air. To collect a sample, water was pumped from the inlet or outlet of a core incubation unit through a syringe needle into the bottle until the bottle was approximately half full, yielding a He headspace with about 2 atm total pressure and about 15 mL of sample water. The isotopic analyses were done by two different methods involving off-line combustion with DI-IRMS and on-line gas chromatography with continuous-flow mass spectrometry (GC-CFIRMS). For DI-IRMS, the high-pressure He-rich headspace in each of the 30-mL serum bottles was expanded in a high-vacuum extraction line into a pair of 20 cm long quartz glass tubes (9 mm outer diameter) containing 1.2 g of Cu₂O + Cu and 0.2 g of CaO. The tubes were flame sealed, combusted at 850°C, and analyzed by dual-inlet mass spectrometry, as were the high-concentration NO₃⁻ isotope samples. For GC-CFIRMS, the He-rich headspace was pressurized and released through a Nafion water trap (Perma Pure, Toms River, New Jersey, USA) into a closed loop, then injected into a 5A mol-sieve gas chromatograph attached to the inlet of a Finnigan Delta Plus XL mass spectrometer (ThermoElectron, Waltham, Massachusetts, USA) (modified from Revesz et al. 1999, Böhlke et al. 2004). The N_2 (m:z = 28, 29) and $O_2 + Ar$ (m:z = 32, 29) 34, 40) peaks were monitored by the mass spectrometer and converted to O_2 :Ar, N_2 :Ar, and $\delta^{15}N$ values by comparison with small aliquots of air that were injected into 30-mL, He-flushed, serum bottles and analyzed in the same way as the samples. The dissolved N2 results also were tested against aliquots of laboratory-equilibrated air-saturated water samples ($\delta^{15}N[N_2] = +0.7\%$) that were collected, prepared, and analyzed the same way as the core incubation samples. He-flushed serum bottles with and without water samples typically exhibit evidence of minor N2 addition as a function of storage time over periods of months to years, which is attributed to gradual leakage or degassing of the stoppers. The rate of change in the amount and isotopic composition of this added component were quantified by monitoring He-blank samples and reference-water samples after varying storage times, and all analyses were adjusted to remove this component from the total N₂ measured in each sample (maximum adjustments were of the order of 10-20% of the amount of N₂ in the sample). Corrected δ¹⁵N values had similar reproducibilities and were in agreement for replicate samples analyzed by DI-IRMS and GC-CFIRMS. The uncertainties (2 sigma) of the average $\delta^{15}N[N_2]$ values after blank adjustments are

larger than the analytical uncertainties and are estimated to range from approximately $\pm 1\%$ for $\delta^{15}N$ values near 0%, to $\pm 10\%$ for $\delta^{15}N$ near 100%, to $\pm 50\%$ for $\delta^{15}N$ near +500% (outlet values for core incubations ranged from 1 to 700%).

Data reduction

Rates of benthic denitrification and sediment oxygen demand were derived from measurements at the inlet and outlet of the stream water overlying sediment in each core barrel and expressed as equivalent benthic fluxes. Denitrification rates were calculated independently from changes in N₂ concentrations (MIMS) and from changes in the mole fraction of ¹⁵N in the N₂ when ¹⁵N-enriched NO₃⁻ was present (IRMS). From MIMS data, the total production rate of N₂-N (denitrification) or consumption rate of O₂ (sediment oxygen demand) in a core (PR_{tot}, in µmol/h) is given by

$$PR_{tot} = (C_o - C_i)V/\tau \tag{1}$$

where $C_{\rm o}$ and $C_{\rm i}$ are the concentrations of N₂-N or O₂ in the water samples collected from the outlet and inlet of each core, respectively (µmol N/L or µmol O₂/L), V is the volume of water overlying the sediment (L), and τ is the water residence time (h). To account for potential effects of water-column processes and artifacts such as residual bubble dissolution (collectively referred to as the core "blank" effect), it was assumed that the blank contribution to the total N₂ flux was proportional to the volume of surface water or the surface area of core barrel exposed directly to the water. Thus, the amount of the total production rate that is attributable to the benthic flux is given by

$$PR_{ben} = PR_{tot} - PR_{bl}(V/V_{bl})$$
 (2)

where the subscript "bl" refers to measurements in the core barrels with water only (no sediment). The benthic flux at the sediment–water interface (FL_{ben}, in $\mu mol~N \cdot m^{-2} \cdot h^{-1}$) is then given by

$$FL_{ben} = PR_{ben}/A \tag{3}$$

where A is the area of the sediment-water interface (set equal to the cross-section area of the core barrel, 0.0049 m²). Calculated N₂ production rates in the blank cores from each sampling trip were variable, but they had roughly normal distributions with averages near zero, indicating little or no water-column denitrification. Therefore, the average PR_{bl} value for each trip was used to adjust the sediment core data for the same trip, and the variability of the blank values was assumed to indicate the uncertainty of the method. The uncertainties of the MIMS-derived denitrification rates based on the variability of the blank data were (1 sigma, in µmol $N \cdot m^{-2} \cdot h^{-1}$) ±66 in September 1999, ±55 in May 2000, ± 138 in June 2001, and ± 49 in September 2001. O₂ consumption rates in the blank (water-only) core barrels were substantial in most cases, indicating that the surface water was an important O_2 sink. Therefore, the data for individual blank core barrels were used to adjust for benthic O_2 demand for each set of three sediment cores from a site.

Most of the MIMS sampling was done between about 18 and 28 hours after the circulation systems were started. In some cases, additional samples were taken another day later and, in a few cases, four times over a period of four days (nutrient addition experiments). Variability of results between samples taken on different days could be caused by analytical errors, physical changes in the cores, changes in geochemical conditions, or changes in the biological communities and/or their function, all of which contribute to the overall uncertainty with which the measurements reflect processes at a given site. With the exception of the NO₃ and acetate addition experiments, the average difference between the denitrification rates measured in the same cores on two different days was 22 ± 68 (n = 15). Because this comparison does not indicate a consistent trend in the time series, and to avoid misleading replication in the regional surveys, the reported rates are averages when multiple samples were taken from the same core.

From the N isotope data (IRMS), the total production rate of N₂ from isotopically labeled NO₃⁻ was calculated from isotope mass balance:

$$(N_{2,o})(x^{15}N_{2,o}) = (N_{2,i})(x^{15}N_{2,i}) + (N_{2,p})(x^{15}N_{2,p})$$
 (4)

where (N_2) is the concentration of dissolved N_2 (in μ mol N/L), $(x^{15}N_2)$ is the mole fraction of ^{15}N in the N_2 , and subscripts o, i, and p stand for nitrogen sampled at the outlet (o) or inlet (i), or produced by denitrification (p), respectively. This equation was rearranged to give

$$(N_{2,p}) = (N_{2,i}) \frac{(x^{15}N_{2,0}) - (x^{15}N_{2,i})}{(x^{15}N_{0,3}) - (x^{15}N_{2,0})}$$
(5)

in which ($x^{15}NO_3^-$) was substituted for ($x^{15}N_{2,p}$) and the MIMS concentration of N_2 was used for ($N_{2,i}$). The isotopically derived value of ($N_{2,p}$) is equivalent to $C_0 - C_i$ and can be inserted into Eq. 1 to obtain PR_{tot}, followed by Eqs. 2 and 3 to obtain the isotopically derived value of FL_{ben}. There was no isotopic evidence for denirtification (no appreciable enrichment in $N[N_2]$) in any of the blank (water-only) cores except for a few high-temperature experiments from June 2001 (not reported here) in which N_2 fell below about 30 $N[N_2]$ (about 1 mg/L). In September 2001, the IRMS data for the blank cores yielded an average denitrification rate of $N[N_2]$ (1 sigma), which gives an indication of the uncertainty at low rates.

RESULTS

Nitrate concentrations, denitrification rates, and sediment oxygen demand

Nitrate concentrations were relatively low during the September sampling trips, when streams were at low flow. For example, NO_3^- concentrations were lowest in September 1999, ranging from 30 to 70 μ mol/L (Antweiler et al. 2005). Nitrate was higher during times of greater discharge with NO_3^- ranging from 330 to 1030 μ mol/L during the May and June sampling trips. The highest NO_3^- concentrations were about 1000 μ mol/L in June 2001.

Denitrification rates determined by MIMS were highest in May 2000 in both Sugar Creek and Iroquois River, when streamflows and NO₃⁻ concentrations were high (Fig. 3). Denitrification rates in both streams were consistently low in September 1999 and 2001 during low flow, when NO_3^- concentrations were <100 μ mol/L. Although the flow was high and in-stream NO₃⁻ concentrations were of similar magnitude in May 1999 and 2000 and in June 2001 (Fig. 2), limited denitrification rates measured in June 1999 and June 2001 in Sugar Creek (stars and x's, Fig. 3c) were at the low end of the range measured in May 2000. Variability within and among sampling locations was greatest in Sugar Creek. For example, denitrification rates at several coring sites at SC-3 ranged from 50 to 3936 μmol N·m⁻²·h⁻¹, which encompassed the full spectrum of rates measured during 2000 in both streams. Denitrification rates ranged from 76 to 809 μmol N·m⁻²·h⁻¹ at several sites in the SC-T location (Fig. 3e), and the highest rates were observed at a site that had a thick growth of submerged aquatic vegetation (hexagons, Fig. 3e). Regardless of hydrologic regime, denitrification rates were highest at sites with thick periphyton or plant growth (e.g., circles representing SC-3 and SC-4, Fig. 3c) or in stagnant pool sites that had a thick upper layer of fine organic matter (e.g. upside-down triangles representing SC-7 in September 2001, Fig. 3e). Overall, denitrification rates were positively correlated with NO₃ concentration (slope = 0.77 mm/h, $r^2 = 0.26$, P = 0.0011 for Sugar Creek and slope = 0.68 mm/h, $r^2 = 0.85$, P < 0.0001 for Iroquois River; Fig. 4a).

Oxygen concentrations in the outlets of individual core incubations varied between about 40 and 260 μ mol/L. Sediment oxygen demand ranged from 50 to 6000 μ mol $O_2 \cdot m^{-2} \cdot h^{-1}$ with most of the rates falling below 4000 μ mol $O_2 \cdot m^{-2} \cdot h^{-1}$. No correlation was evident between sediment oxygen demand and NO_3^- in either stream (Sugar Creek, n = 38, $r^2 = 0.02$, P = 0.41; Iroquois River, n = 12, $r^2 = 0.35$, P = 0.41; Fig. 4b). Sediment oxygen demand rates and denitrification rates were weakly correlated (Sugar Creek, n = 38, $r^2 = 0.21$, P = 0.0042; Iroquois River, n = 12, $r^2 = 0.44$, P = 0.019; Fig. 4c).

Simultaneous denitrification rates measured by MIMS and IRMS

Comparisons were made between denitrification rates derived from total N_2 production rates by MIMS and those derived from ^{15}N -labeled N_2 production rates by IRMS in cores collected in June 2001 and September 2001. A total of 70 comparisons were made over a

variety of sites within and between streams, as well as over a wide range of denitrification rates. Comparisons were also made during nutrient addition experiments conducted in September 2001. The two sets of data provide a highly correlated fit near the one-to-one line with an r^2 of 0.92 and P value of <0.0001 (Fig. 5).

Although the two methods yield similar rates of denitrification, the relative magnitudes of the errors associated with each method vary over the range of measured denitrification rates. The majority of the analytical uncertainty in the MIMS method is attributed to variability of the denitrification rates measured in the blanks. Thus, the magnitude in the error for the MIMS method is relatively large at the lower range of denitrification rates but does not increase systematically with the rate. The uncertainties of the IRMS data are mainly related to the combined uncertainties of the δ^{15} N values of the N₂, and they are roughly proportional to the denitrification rates. Consequently, the IRMS method has a lower detection limit than the MIMS, but both methods have similar uncertainties at the high end of the range in denitrification rates (Fig. 5).

Nutrient addition experiments

The five sampling locations chosen for the nutrient addition experiments showed a positive response of denitrification to increased NO₃⁻ and acetate concentrations in comparison to the controls (Fig. 6a). Water samples for the IRMS method were collected at two time points over the four-day incubation period, whereas the MIMS samples were collected at four time points (compare Fig. 6a, b). Fluxes determined from MIMS data and IRMS data were in good agreement. The largest increase in denitrification rates occurred in the NO₃-amended cores collected from SC-T4, T-5, T-6. The response to increased NO₃⁻ concentrations in the cores from IR5 and IR6 was about half that of the Sugar Creek sediment cores and was similar in response to acetate amendments of cores collected from SC-8.

The response of sediment oxygen demand to the nutrient additions was more variable than the denitrification rate response. Sediment oxygen demand in the control cores did not appear to change over time (Fig. 6c), whereas sediment oxygen demand rates appeared to decrease slightly over time in cores that received NO₃⁻. However, variability was large between the two sets of treatment cores, indicating that the response of sediment oxygen demand to NO₃⁻ additions was not uniform. The Sugar Creek cores that received acetate appeared to increase slightly in their sediment oxygen demand rates over the incubation period.

DISCUSSION

Denitrification rates in streams receiving agricultural discharge

A wide range of rates for sediment denitrification has been reported for a variety of aquatic systems (e.g.,

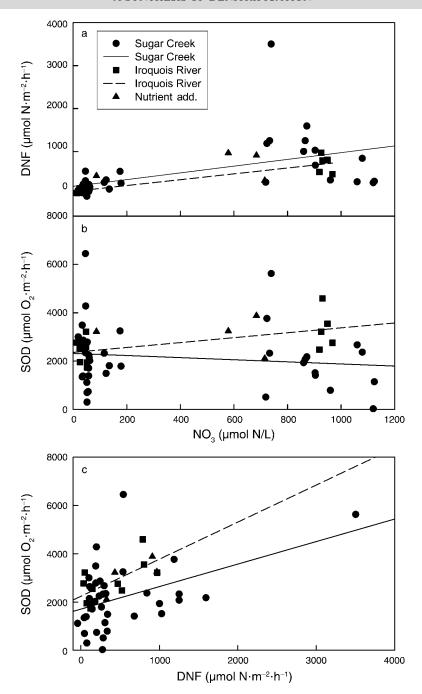


Fig. 4. The relationship between (a) denitrification (DNF) and NO_3^- concentration and (b) sediment oxygen demand (SOD) and NO_3^- concentration. Each data point represents the mean value for three cores collected at a sampling site. The relationship between sediment oxygen demand and denitrification is illustrated in (c). The lines show linear regressions for the Sugar Creek data (solid line) and the Iroquois River data (dashed line). Regressions do not include data from the nutrient addition experiments (nutrient add.).

Seitzinger 1988, Tuominen et al. 1998). The highest rates reported were associated with systems receiving large loading of anthropogenic nutrients similar to our study area. In Iroquois River and Sugar Creek we measured rates ranging from 0 to 3900 µmol·m⁻²·h⁻¹. Our highest rates were comparable to rates reported in other studies

of agriculturally impacted systems. For example, denitrification rates have been reported of greater than 1000 μ mol·m⁻²·h⁻¹ by Royer et al. (2004) in agricultural streams in Illinois, up to 2100 μ mol·m⁻²·h⁻¹ by Cooper and Cooke (1984) in a pasture stream in New Zealand, up to 2200 μ mol·m⁻²·h⁻¹ by Cooke and White (1987) in

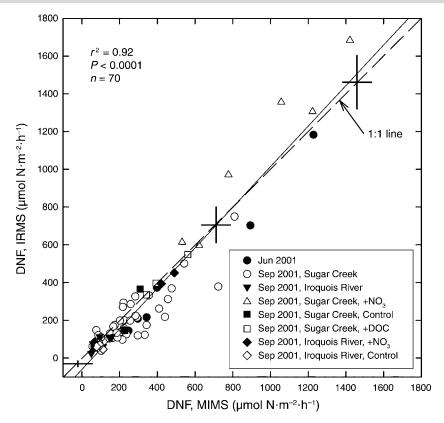


Fig. 5. Comparison of denitrification rates (DNF) determined by measurement of total N_2 gas production within sediment cores (membrane inlet mass spectrometry, MIMS) and by measurement of $^{15}N_2$. Production following low-level enrichment of $^{15}NO_3$ (isotope ratio mass spectrometry, IRMS). The solid line indicates the regression line, and the dashed line illustrates the 1:1 fit of the data. The three crosses indicate the estimated uncertainties (± 2 sigma) associated with each method at different rates.

the River Dorn, England, up to 2600 μmol·m⁻²·h⁻¹ by Garcia-Ruiz et al. (1998c) in the River Wiske, northeast England, up to 5700 μmol·m⁻²·h⁻¹ by Yan et al. (2004) in the Changjiang River, China and up to 8000 μmol·m⁻²·h⁻¹ by Jansson et al. (1994) in the River Raan, Sweden. As in our study, a large variability in rates was observed in these studies, which employed a variety of techniques to make measurements in various seasons and in rivers high in NO₃⁻ but that vary in other environmental conditions. Denitrification rates are controlled by complex interactions between geological, hydrological, biological and chemical factors, and the relative importance of these environmental factors is likely to differ within and between various types of environments and may vary seasonally.

Evaluation of methods

For this study, we chose to use intact core incubations in the laboratory and to measure N_2 , the final product of denitrification, by two techniques (IRMS and MIMS). Incubations done with intact cores in the laboratory maintain the physical integrity of the sediment (unlike slurry incubations) and permit the control of many of the important environmental controls on denitrification. However, they convert a system that in the field may

have been affected by hyporheic flow to a system in the laboratory where solute transport between surface water and sediment pore water is controlled mainly by diffusion. Even with steep gradients at the sedimentwater interface, diffusion-driven fluxes may be substantially lower than advection-driven fluxes. Thus, under these conditions the potential nitrogen removal for the stream might be underestimated. In order to obtain steady-state measurements with MIMS and IRMS on intact cores, incubation periods must be long enough to equilibrate temperatures and gas concentrations in the cores, plus sufficient time is needed for isotopically labeled NO₃⁻ to reach reaction sites in the sediments and for the labeled N2 gas to efflux to the overlying water. Total incubation time can vary from several hours to several days during which time there is the potential of changing the microbial community thus influencing denitrification rates (Bernot et al. 2003).

Despite a number of potential limitations, intact core incubations combined with analysis of water samples with MIMS and IRMS have some advantages over other techniques for measuring denitrification in steams. For example, techniques such as nitrogen mass balance measurements in streams do not measure denitrification directly. Denitrification is only one of several processes

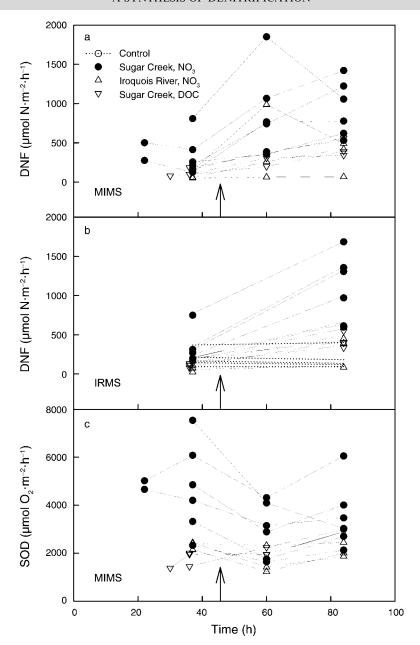


Fig. 6. Response of denitrification rates (DNF) to nutrient additions in sediment cores as measured by (a) membrane inlet mass spectrometry (MIMS) and (b) isotope ratio mass spectrometry (IRMS). (c) Response of sediment oxygen demand (SOD) to nutrient additions is illustrated in panel (c). Time zero indicates when the core incubations began, and sampling times are indicated as symbols. The arrows indicate the time of the nutrient additions.

in the nitrogen cycle that can affect the concentrations of nitrogen oxides and nitrogenous gases and therefore, estimates based on mass balance can be confounded by transformations of substrates and products by competing processes. MIMS and IRMS measure the product N_2 directly without the necessity of modifying the natural system by altering NO_3^- concentrations. This measurement technique is in contrast to the nitrogen isotope pairing technique, which typically is done with a substantial increase in the concentration of NO_3^- and

can stimulate denitrification and may overestimate in situ rates. Nor do IRMS and MIMS interfere with microbial processes by addition of inhibitors such as acetylene. The acetylene inhibition technique is widely used but has various potential limitations (Knowles 1990). An underestimate of denitrification will be observed if the inhibitory effect of acetylene on denitrifiers is incomplete (Oremland et al. 1984), or if the inhibition of nitrifying enzymes by acetylene (Sloth et al. 1992) reduces the flow of NO₃⁻ ions to denitrifiers

(Lohse et al. 1996). Conversely, under prolonged anaerobic incubation, acetylene may be used as a carbon source by denitrifiers and stimulate activity to give an overestimate of denitrification (Mosier and Heinemeyer 1985).

Whereas MIMS measures total N₂ production irrespective of the source of NO₃⁻, IRMS may underestimate total denitrification because only denitrification of isotopically labeled NO₃⁻ is measured, and NO₃⁻ resulting from nitrification within the sediments may not be accounted for. In our study, this contrast was used to address the importance of coupled nitrification/denitrification with moderate isotope enrichment. Both IRMS and MIMS methods may underestimate denitrification if reduction stops at an intermediate step (i.e., nitrous oxide) rather than continuing to completion. Nitrous oxide production via denitrification may be greater than N₂ production in some cases (Bergsma et al. 2001) but is typically much less. In our study, limited measurements of nitrous oxide concentrations in cores and streams indicate that it was a minor product in comparison to N_2 (Böhlke et al. 2004; R. L. Smith, unpublished data).

Coupled nitrification/denitrification

A direct comparison was possible between denitrification rates derived from the MIMS and IRMS methods for samples collected in June and September 2001. The MIMS method measures total N2 production rates, whereas the IRMS method measures only N2 production from isotopically labeled NO₃⁻ from surface water. In principle, if these rates were the same, then it could be concluded that surface-water NO₃ was the only source of NO₃⁻ for denitrification. If the IRMS denitrification rate was lower than the MIMS denitrification rate, then the results could be interpreted to indicate that nitrification (production of non-tracer NO₃⁻) was a significant source of NO₃ for denitrification. Within the uncertainties of the analyses, the denitrification rates derived from the two independent methods are in good agreement (Fig. 5), which means that the 15N concentration of NO₃ was not diluted substantially at the site of denitrification in the sediments by inputs of NO₃ derived from nitrification. Therefore, coupled nitrification/denitrification was not a major source of N₂ production. Although we cannot rule out a contribution when denitrification rates were lowest (precision of this comparison is relatively poor at low rates of denitrification), in no case was it the dominant source of N₂

Another line of evidence indicating little or no coupled nitrification/denitrification in this system is the measured sediment oxygen demand. Whereas sediment oxygen demand provides little direct information on the relative importance of aerobic and anaerobic processes of detrital mineralization, the relationship between sediment oxygen demand and denitrification can be used to infer the relative importance of the coupled process of nitrification/denitrification. Sediment oxygen

demand is a measure of total community metabolism and includes aerobic respiration of organic matter, as well as iron oxidation and the production of NO₃⁻ and sulfate by nitrification and sulfur oxidation. A linear relationship between sediment oxygen demand and denitrification has been reported in natural systems strongly affected by coupled nitrification/denitrification such as North Atlantic continental shelf, estuarine and some freshwater sediments (Seitzinger 1990, Seitzinger and Giblin 1996). In contrast, in NO₃⁻-rich systems, the sediment oxygen demand and denitrification are not correlated. In our study, sediment oxygen demand does not appear to be strongly correlated with denitrification rates in either stream (Fig. 4c).

Previous studies have indicated that coupled nitrification/denitrification may be a major source of N₂, especially in estuarine systems with low surface-water NO₃⁻ concentrations (Jenkins and Kemp 1984, Seitzinger 1988, Cornwell et al. 1999). In contrast, where surface waters have high NO₃⁻ concentrations, the relative importance of coupled nitrification/denitrification may be less. For example, Nishio et al. (1983) reported that the amounts of nitrogenous oxides produced by ammonium oxidation accounted for only 6-9% of the N₂ produced by denitrification in Tama Estuary. Kana et al. (1998) reported a positive correlation between denitrification rate and NO₃ concentration and interpreted this as evidence for a decreasing proportion of coupled nitrification/denitrification with increasing NO₃⁻ concentration (assuming nitrification was relatively constant). Our data for small mid-continent streams are consistent with these observations in estuaries with high NO₃⁻ surface waters.

Importance of nitrate and DOC concentrations

Denitrification rates in both Sugar Creek and Iroquois River were relatively high in May 2000, when stream flows and NO₃⁻ concentrations were high (Fig. 3) and low in September 1999 and 2001. Denitrification rates measured elsewhere in both freshwater and marine aquatic systems vary with both nutrient availability and time of year. Seasonal variations have been observed, which mainly depended on the temperature of the sediment and/or the concentration of dissolved oxygen in the overlying water (e.g., Kaplan et al. 1977, Kim et al. 1997, Richardson et al. 2004). In other studies of coastal sediments, maximum rates appear to be related to NO₃⁻ concentrations and are measured in the late winter and spring, and the lowest rates are measured in the summer (Jorgensen 1989, Rysgaard et al. 1995, Sundback and Miles 2000). In the current study, the timing of sample collection (May to September) included seasonal variations in streamflow and NO₃⁻ concentrations but largely avoided the effects of major temperature differences. Furthermore, all of the incubation experiments were done under oxic conditions (inlet oxygen concentrations were between 220 and 260 umol/L), and there were no systematic differences in

Table 1. Average nitrate concentrations measured during Lagrangian sampling ("Stream"; Antweiler et al. 2004) and in core incubations ("Cores"), denitrification (DNF), and sediment oxygen demand (SOD) rates, as measured by membrane inlet mass spectrometry (MIMS).

Date	NO_3 (µmol N/L)		DNF (μ mol N·m ⁻² ·h ⁻¹)			N	SOD (μ mol O ₂ ·m ⁻² ·h ⁻¹)		
	Stream	Cores	Mean ± sp	Range	n	$(\% \cdot \mathbf{d}^{-1})$	Mean ± sp	Range	n
Sugar Creek									
Sep 1999	43 ± 1	46 ± 13	76 ± 78	-99-202	23	14	1270 ± 775	71-3386	22
May 2000	717 ± 5	828 ± 90	1277 ± 1201	50-4371	32	11	2344 ± 1483	63-6277	35
Jun 2001		1096 ± 31	290 ± 151	114-493	6	2†	585 ± 622	10-1286	6
Sep 2001	135 ± 11	84 ± 51	327 ± 300	51-1851	52	19	2740 ± 1274	704-7528	65
Iroquois River	-								
Sep 1999	42 ± 1	33 ± 16	100 ± 67	-8-243	14	7	2589 ± 1149	180-5416	15
May 2000	840 ± 12	938 ± 20	711 ± 305	281-1288	15	2	3317 ± 1206	1299-6719	15
Jun 2001									
Sep 2001	79 ± 6	50 ± 3	181 ± 261	50-988	12	17	2123 ± 614	1127-2934	14

Notes: The mean \pm sp, range, and sample size are shown for the rates during each sampling trip. N refers to the mean percentage of water-column nitrate (stream) removed by denitrification, unless noted otherwise. Empty cells indicate that no data were available for that date.

sediment oxygen demand that were related to the denitrification rates. Although many different local features of the core samples may have caused variability in the measured denitrification rates, such as the structure and abundance of microbial communities, periphyton and SAV coverage and physiology, carbon availability, etc., there is evidence that the average rates were affected predominately by the NO_3^- concentrations.

The concentration of NO₃⁻ in overlying water has been identified as one of the main factors controlling denitrification in sediments (King and Nedwell 1988, Seitzinger 1988, Kemp and Dodds 2002). Studies involving NO₃⁻ additions and utilizing a variety of measurement techniques have demonstrated that denitrification rates in freshwater and estuarine sediments respond strongly to NO₃⁻ addition (Andersen 1977, Koike et al. 1978, Oremland et al. 1984, Oren and Blackburn 1979, Garcia-Ruiz et al. 1998a). Garcia-Ruiz et al. (1998a) measured denitrification rates along a river continuum including a highly polluted tributary. Rates increased from upland to lowland reaches and were positively correlated with NO₃⁻ concentration. Similar results were observed in an estuary and stream receiving water from a sewage-treatment facility (Warwick and McDonnel 1981, Ogilivie et al. 1997). These studies indicate that NO₃⁻ concentration is a major factor controlling rates of denitrification while other environmental factors may be responsible for much of the local variability observed. In our study, denitrification rates were positively correlated with NO₃ concentration (Fig. 4a; slope = 0.77 and 0.68 mm/h for Sugar Creek and Iroquois River, respectively), but there was appreciable scatter in the relationship particularly at higher concentrations in Sugar Creek ($r^2 = 0.26$, n = 41). The weaker relationship between denitrification and NO₃⁻ observed in the Sugar Creek data may reflect the higher degree in variability of the benthos, or may be a result of the larger sample size that captured the full array of denitrification rates within the sampling reach.

The September 2001 denitrification rates responded positively to manipulated increases in both NO₃⁻ and DOC concentrations. The final NO₃⁻ concentrations in the amended September 2001 cores were similar to the ambient stream concentrations in May 2000, and denitrification rates in the cores increased to rates similar to those measured in May 2000, from about 400 μ mol $N \cdot m^{-2} \cdot h^{-1}$ to 1100 μ mol $N \cdot m^{-2} \cdot h^{-1}$ (compare with rates in Table 1). Furthermore, the response to increased NO₃⁻ concentrations in the cores from Iroquois River was about half the response of the Sugar Creek sediment cores, and this result is similar to the seasonal differences in denitrification between the two streams. The direct correspondence between results of seasonal evaluations (high to low NO₃⁻ concentrations, low flow conditions) and the September NO₃⁻ addition experiments supports the conclusion that NO₃⁻ concentrations in the overlying water are a major control of denitrification in these streams.

The June 2001 results offer a notable exception to the relationship between NO₃⁻ concentrations and denitrification rates. Despite the high NO₃ concentrations, the denitrification rates measured in June 2001 were low compared to most of the rates measured in May 2000 and were similar to the rates measured in September 1999 and September 2001, when NO₃⁻ concentrations were much lower. The June 2001 cores were collected shortly after a major flood event in Sugar Creek that left debris well above the level of the floodplain and caused major scouring and sediment redistribution on the stream bottom, leaving almost no benthic plants or deposits of fine organic matter. Clearly, an important consideration in the evaluation of differences in denitrification rates during seasonally high and low discharge is that the bottom characteristics in shallow

 $[\]dagger$ NO₃ removal (%) based on nitrate concentrations measured by ion chromatography in water samples collected from carboys at the end of the experiments.

streams may change dramatically and affect denitrification rates at shorter time scales.

Inputs of DOC from aquatic biota, ground-water discharge, and runoff from fields can contribute substantial amounts of DOC to streams. Denitrification rates have been stimulated by C loading in sediment studies with both high and low NO₃⁻ concentrations in the overlying water (e.g., Sloth et al. 1995, Kelso et al. 1999). However, in natural systems where coupled nitrification/denitrification is important, organic loading can also inhibit denitrification rates as bacterial respiration outcompetes nitrification for O₂ (Blackburn 1990, Blackburn and Blackburn 1992, Sloth et al. 1995). In the current study, DOC in the form of acetate was added to select cores at concentrations from three to six times greater than stream concentrations of total DOC. These additions caused an increase in denitrification rates and stimulated total respiration, as indicated by increased rates of sediment oxygen demand. Kelso et al. (1999) added a range of organic C compounds to intact sediment cores collected from the Upper Bann River, Ireland, which drains an agricultural catchment, and measured denitrification by IRMS. They observed changes in the rates and shifts in the products of NO₃ reduction depending on the substrate. In contrast, Royer et al. (2004) did not record any effect on denitrification rates with the addition of DOC as glucose to acetylene-amended sediment slurries from agricultural streams in central Illinois.

Local variability

Spatial heterogeneity is a common feature of shallow aquatic sediment environments. The cores used in our study were collected from a wide variety of stream settings in order to capture a range in environmental factors that could affect benthic rates. In addition to temperature and the concentrations of NO₃⁻, O₂, and DOC in the stream, benthic denitrification rates depend on many other environmental variables including abundance of periphyton or submerged aquatic vegetation (SAV), quantity and quality of organic matter in bottom sediments, and the means of delivery of substrates to reaction sites either by advection (hyporheic flow) or by diffusion (for a general discussion of environmental controls of denitrification, see Tiedje 1988).

Greater variability was observed in denitrification rates measured within and among sampling locations in Sugar Creek than in Iroquois River. The sediment bottom in the Iroquois River is relatively homogeneous, whereas the sediments in Sugar Creek are more of a mosaic of different bottom types. The stream bottom in the shallower of the two reaches, Sugar Creek, is more susceptible to sediment resorting as a result of storms, flooding and other erosional events that produce variation in stream bed geomorphology (e.g., riffles, pools, etc.). In addition, light penetration is higher in Sugar Creek because of lower turbidity and average

stream depths that are less than 40 cm (Antweiler et al. 2005), and this shallow depth enhances the growth and development of aquatic plants, benthic algae and fresh reactive organic matter. Sediment type may be important in controlling microbial metabolism. Studies in other streams have documented variability in denitrification rates and have attributed it to differences in sediment characteristics such as grain size or carbon content (Hill and Sanmugadas 1985, Steinhart et al. 2000, Martin et al. 2001). Denitrification potential commonly is higher in fine-textured sediments because fine sediments commonly have higher organic carbon contents and a higher probability of anaerobic conditions (Groffman and Tiedje 1989, Garcia-Ruiz et al. 1998b, Steinhart et al. 2000). In contrast, depositional areas characterized by more coarsely textured substrates are likely to be more oxic and have lower potential for denitrification (Rysgaard et al. 1994). On the other hand, coarser sediments may permit larger fluxes of NO₃⁻ to reaction sites by diffusion or hyporheic flow.

In Sugar Creek, denitrification rates were highest in sampling locations with thick periphyton or plant growth (i.e., circles at SC-3 and SC-4; Fig. 3c). While plants and algal mats as substrates may not support higher rates of denitrification (Kemp and Dodds 2002, Schaller et al. 2004), macrophytes can increase the organic content of the sediment by trapping detritus and/or excreting organic carbon to the rhizosphere (Christensen and Sørensen 1988). High denitrification rates have been reported in Chesapeake Bay sediments colonized by Zostera marina (Caffrey and Kemp 1990). Although benthic algal communities can be a source of fresh carbon to the sediments, microphytobenthic activity can inhibit denitrification particularly if the NO₃⁻ concentrations are low (Sundback and Miles 2000). The benthic algal community can act as a filter controlling the flux of dissolved nutrients by assimilating NO₃⁻ from the overlying water, and it can control the timing and maximum rates of denitrification by changing the redox conditions in the sediments below via photosynthetic production of oxygen (e.g., Rysgaard et al. 1995). Shading will modulate temperature and light that can affect denitrification rates directly or indirectly by its effect on algal and plant growth. Light limitation in Iroquois River caused by high turbidity is likely to inhibit photosynthetic activity, which could affect denitrification rates.

Average denitrification rates in Sugar Creek and Iroquois River were similar in September 1999, but they were higher in Sugar Creek than in Iroquois River in May 2000 and September 2001 (Table 1). The two stream systems are appreciably different, but it is not clear what environmental factors explain this observation. Interestingly, denitrification rates measured at sites SC-8 and SC-9 were similar to rates measures at all Iroquois River sites. As Sugar Creek flows towards the Iroquois River, it incorporates three main tributaries, increasing discharge and sediment load, including fine

sand and silt. At locations SC-8 and SC-9, Sugar Creek is about 15 km from where it joins the Iroquois River, and these sites are qualitatively more like the Iroquois River than the upper portion of the Sugar Creek reach. The parts of Sugar Creek that yield relatively high denitrification rates tend to have a combination of permeable bed sediments that permit hyporheic flow and high primary production that generates new organic matter on a daily basis. Iroquois River and the parts of Sugar Creek that yield relatively low denitrification rates typically contain less permeable bed sediments, less light penetration to the bottom and presumably a higher fraction of less reactive material in the organic fraction (Voytek et al. 2001).

Impact on nitrogen load

Although results are highly variable, a few studies have shown that benthic sediments in NO₃⁻-rich streams draining agricultural watersheds are capable of high rates of denitrification (Garcia-Ruiz et al. 1998c, Royer et al. 2004, this study). We measured relatively high denitrification rates in cores from both Sugar Creek and Iroquois River in May, when streamflows and NO₃ concentrations were high, and relatively low rates in September, when streamflows and NO₃⁻ concentrations were much lower. We also observed an increase in denitrification rates in response to an increase in NO₃ concentrations in experiments with September samples from both streams. Nevertheless, because periods of high NO₃⁻ concentrations are also periods of high flow, the fractional rate of NO₃⁻ removal from the streams during those times is actually relatively low. For example, denitrification in Sugar Creek, the stream with lower discharge, removed ≤11% of the in-stream NO₃⁻ in May and removed roughly 15-20% in September. Iroquois River with its higher discharge removed an even smaller fraction of in-stream NO₃⁻ during comparable periods (Table 1). Royer et al. (2004) reached a similar general conclusion from some Illinois streams based on sediment slurry experiments with the acetylene inhibition method. A higher ratio of NO₃-rich water to sediment surface (including the subsurface) results in a decrease in the effective NO₃⁻ removal from the total NO₃⁻ load even with increased benthic denitrification rates. That is, the increase in denitrification rates cannot compensate for the decrease in relative exposure (i.e., effective residence time) of NO₃⁻ in reactive sites. Several other studies complementary to this one have estimated NO₃⁻ removal rates from Sugar Creek and Iroquois River (Laursen and Seitzinger 2002, Böhlke et al. 2004, Antweiler et al. 2005). Most times of the year, regardless of the approach used (N_2 :Ar, in-stream ^{15}N , NO₃⁻ tracer, or Lagrangian mass balance), denitrification does not appear to remove a major fraction of the NO₃⁻ load in this river basin on a daily basis. When NO₃ concentrations are highest, removal is particularly inefficient and can only account for less than a few percent removal, and when flow and NO₃⁻ concentrations are lower, measured rates can account for as much as 20–30% removal of NO₃⁻ per day (Böhlke et al 2004, Antweiler 2005, this study). These in-stream denitrification rates are lower than typical rates of fractional N loss estimated by large-scale regional modeling (Alexander et al. 2000), even in agricultural watersheds (David et al. 1997, David and Gentry 2000).

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