

## Denitrification in a nitrogen-limited stream ecosystem

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**Abstract.** Denitrification was measured in hyporheic, parafluvial, and bank sediments of Sycamore Creek, Arizona, a nitrogen-limited Sonoran Desert stream. We used three variations of the acetylene block technique to estimate denitrification rates, and compared these estimates to rates of nitrate production through nitrification. Subsurface sediments of Sycamore Creek are typically well-oxygenated, relatively low in nitrate, and low in organic carbon, and therefore are seemingly unlikely sites of denitrification. However, we found that denitrification potential (C & N amended, anaerobic incubations) was substantial, and even by our conservative estimates (unamended, oxic incubations and field chamber nitrous oxide accumulation), denitrification consumed 5–40% of nitrate produced by nitrification. We expected that denitrification would increase along hyporheic and parafluvial flowpaths as dissolved oxygen declined and nitrate increased. To the contrary, we found that denitrification was generally highest at the upstream ends of subsurface flowpaths where surface water had just entered the subsurface zone. This suggests that denitrifiers may be dependent on the import of surface-derived organic matter, resulting in highest denitrification rate at locations of surface-subsurface hydrologic exchange. Laboratory experiments showed that denitrification in Sycamore Creek sediments was primarily nitrogen limited and secondarily carbon limited, and was temperature dependent. Overall, the quantity of nitrate removed from the Sycamore Creek ecosystem via denitrification is significant given the nitrogen-limited status of this stream.

## Introduction

Denitrification, the microbially-mediated reduction of nitrate to nitrous oxide or dinitrogen, is a sink for fixed nitrogen and thus influences nutrient availability in aquatic, terrestrial, and marine ecosystems (Knowles 1982; Seitzinger 1988). For example, Seitzinger (1988) estimates that “denitrification decreases by about 40% the amount of continentally derived, riverborne nitrogen transported to the oceans.” Most studies of denitrification in fluvial ecosystems have been performed in nitrate-enriched agricultural catchments and have focused on riparian zones as sites for nitrogen retention (Peterjohn & Correll 1984; Jacobs & Gilliam 1985; Lowrance et al. 1985; Pinay et al. 1993; Pinay et al. 1994). These studies have shown that riparian zones often intercept

>80% of nitrate-nitrogen in lateral groundwater before it is discharged into the surface stream.

Denitrification may also remove nitrate-nitrogen from water flowing downstream, either in benthic or subsurface sediments (Chatarpaul et al. 1979; Triska & Oremland 1981; Duff et al. 1984; Hill & Sanmugadas 1985; Sørensen et al. 1988; Duff & Triska 1990; Christensen et al. 1990, Jansson et al. 1994). In gravel-bed streams, surface water exchanges with subsurface regions, and consequently subsurface processes influence nutrient supply to the surface stream (Grimm & Fisher 1984; Triska et al. 1990; Triska et al. 1993; Holmes et al. 1994a). Denitrification improves surface-water quality in nitrate-contaminated systems, whereas in nitrogen-limited streams, loss of nitrate through denitrification may strongly influence primary productivity by exacerbating nitrogen limitation.

In this study, we examine denitrification in hyporheic, parafluvial, and bank sediments of Sycamore Creek, Arizona, a nitrogen-limited Sonoran Desert stream. We define the hyporheic zone as the region of saturated sediments directly below the surface stream, and the parafluvial zone as that part of the active channel without surface water (Holmes et al. 1994a). Bank sediments are located at the parafluvial-riparian interface. While other authors have considered the parafluvial zone to be part of the hyporheic zone (Stanford & Ward 1988; Triska et al. 1989), we distinguish between them based on their unique faunal communities (Boulton et al. 1992) and differing vegetation and disturbance regimes (Holmes et al. 1994b). Because primary productivity in Sycamore Creek is limited by nitrogen availability, any loss of nitrate through denitrification should have important implications for the functioning of the ecosystem. For example, nitrate availability is an important control on algal abundance and productivity (Grimm & Fisher 1986), which in turn affects processes such as secondary production and nutrient retention.

Previous research in Sycamore Creek has shown that nitrate increases and dissolved oxygen declines as water flows along hyporheic and parafluvial flowpaths, although sediments typically remain well-oxygenated (Grimm et al. 1991; Valett et al. 1994; Holmes et al. 1994a; Holmes et al. 1994b; Jones et al. 1995a). Denitrification is most prevalent under anoxic or hypoxic conditions and requires sufficient nitrate to act as an electron acceptor (Knowles 1982). Thus, we predicted that if denitrification occurs in Sycamore Creek sediments, its rate would increase along subsurface flowpaths.

The objectives of this study were 1) to determine if denitrification occurs in Sycamore Creek sediments, and if so, at what rates, 2) to investigate factors controlling denitrification rate and resulting spatial patterns, and 3) to assess the importance of denitrification to the functioning of this nitrogen-limited stream ecosystem.

## Study Site

Sycamore Creek is located ~35 km northeast of Phoenix, Arizona. Elevations reach 2164 m at the headwaters and drop to 427 m at its confluence with the Verde River. Catchment area is 505 km<sup>2</sup> and stream length is about 65 km. The geomorphology of Sycamore Creek is characteristic of Sonoran Desert streams and can be broadly divided into three sections (Wertz 1963). The channel in the upper section of the catchment is composed of boulders and exposed bedrock, and the middle section is characterized by coarse sand and gravel sediments. The channel is underfit in this region as the wetted perimeter typically occupies only part of the active channel (Graf 1988), creating extensive gravel bars that comprise the parafluvial zone (Holmes et al. 1994a). The downstream section of the stream channel has deep alluvial sediments that only rarely support surface flow.

The study site was a 100-m reach in the middle section of Sycamore Creek (elevation ~650 m). The surface stream is shallow (5 cm) and wide (20 m), and is bounded by a wide band of alluvial sediments (20 m, the parafluvial zone). The watertable is generally within 25 cm of the surface of parafluvial gravel bars and baseflow surface discharge is ~0.05 m<sup>3</sup>/s (discharge ranges from 0 to >400 m<sup>3</sup>/s).

## Methods

### *System configuration and hydrology*

The 100-m study reach was mapped in August 1993 to document system configuration (position of surface stream, parafluvial gravel bars, and riparian zone) and hydrologic linkage among subsystems. Watertable and sediment topography were surveyed at 5-m intervals along the 100-m study reach and at 1-m intervals across it. Elevations were referenced to an arbitrary benchmark. Watertable contours were generated using a geostatistical kriging program (GEOEAS). Hydrologic flowpaths were inferred from the piezometric surface, and corroborated by tracing the movement of fluorescein dye through parafluvial gravel bars over time. Interstitial flow rate was determined as the time required for parafluvially-injected dye to appear in the surface stream divided into travel distance. Upwelling zones (the downstream end of hyporheic flowpaths where hyporheic water enters the surface stream) and downwelling zones (the upstream end of hyporheic flowpaths where surface water enters the hyporheic zone) were identified by measuring vertical hydraulic gradient (VHG) between the surface stream and 25 cm below the benthic surface (Lee and Cherry 1978). Downwelling was indicated by a negative VHG and upwelling by a positive VHG.

*Physico-chemical characteristics of interstitial water*

Physico-chemical characteristics of interstitial water were assessed in three subsystems of Sycamore Creek (hyporheic, parafluvial, and bank sediments) on two dates (18 August and 23 October 1993). The hyporheic zone was sampled at upwelling and downwelling regions, and parafluvial gravel bars were sampled at inwelling zones (where surface water enters the parafluvial zone) and at 1.0 and 5.0 m along parafluvial flowpaths. Interstitial water was sampled with mini-piezometers ( $n = 3$  sampling points per subsystem; Holmes et al. 1994a). Piezometers were inserted to 25 cm below the water table in the bank and parafluvial zones, and 25 cm below the benthic surface in the hyporheic zone. Samples were collected using a battery-powered peristaltic pump fitted with an in-line 0.8- $\mu\text{m}$  cellulose-acetate filter (Geofilter<sup>®</sup>, 142 mm diameter). At each sampling point we first measured dissolved oxygen (Leeds & Northrup Instruments probe), temperature, and conductivity; then we collected triplicate water samples in acid-washed, 60-ml polyethylene bottles for nitrate and dissolved organic carbon (DOC) analyses. Separate triplicate 25-ml water samples were collected in glass vials and fixed in the field with phenol reagent before spectrophotometric ammonium analysis by the phenolhypochlorite method (Solorzano 1969). Nitrate samples were analyzed on a Bran and Luebbe TRAACS 800<sup>®</sup> autoanalyzer using a modified version of Technicon Industrial Method No. 818–87T. Dissolved organic carbon was analyzed by high temperature oxidation using a Shimadzu Model 5000 total organic carbon (TOC) analyzer. All samples were placed on ice immediately after collection and stored at 4°C until analyzed. Nitrate and ammonium samples were analyzed the day they were collected; DOC was analyzed within two days or frozen until later analysis.

*Denitrification in Sycamore Creek sediments*

Sediment denitrification rate was measured using three variations of the acetylene block technique (Yoshinari and Hynes 1976; Tiedje et al. 1989); 1) denitrification potential (anaerobic sediment incubations, C & N amended), 2) unamended denitrification (aerobic sediment incubations, unamended), and 3) field chamber nitrous oxide accumulation. Laboratory sediment incubations (1 & 2 above) were performed in 250-ml serum bottles using sediments collected from the six subsystems (hyporheic upwelling, hyporheic downwelling, stream-parafluvial interface, 1 & 5 m along parafluvial flowpaths, bank). Replication consisted of three samples per subsystem with three replicates per treatment for each sampling point. Approximately 150 g (wet mass) of sediment was added to each bottle. For the denitrification potential treatment, 40 ml of a solution containing 200 mg  $\text{NO}_3\text{-N/L}$  (as  $\text{KNO}_3$ ) and 3.3

g C/L (as  $C_6H_{12}O_6$ ) was added to each bottle; for the unamended treatment, 40-ml distilled water was added. Denitrification potential bottles were made anoxic by purging with  $N_2$  for 15 minutes. Acetylene (10 ml) was added to each incubation bottle and the bottles were vigorously shaken to speed equilibration of acetylene between the vapor and aqueous phases. After 15 minutes, pressure was equalized by briefly piercing the bottle septa with a large bore syringe needle. Laboratory incubations were performed at  $24^\circ C$  and lasted 4 h. Initial and final headspace gas samples were collected in 3-ml evacuated containers. Denitrification rate was calculated from the difference between final and initial headspace nitrous oxide content (corrected for nitrous oxide dissolved in the aqueous phase, Bunsen coefficient = 0.54) and was expressed per unit sediment dry mass. Denitrification potential is thought to correspond to maximum denitrification rate because all necessary substrates are present in excess, while unamended rate may underestimate actual denitrification rate because 1) acetylene blocks nitrification, a potentially important source of nitrate to denitrifiers (Hynes & Knowles 1978; Tiedje et al. 1989), 2) incubation with distilled water dilutes natural substrate concentrations, and 3) incubations may interrupt natural anoxic microsites.

In addition to laboratory sediment incubations, we measured nitrous oxide accumulation in the field using static chambers constructed of polyvinyl-chloride (PVC) and capped at one end (10 cm diameter, 30 cm high). Vials containing 3.5 g calcium carbide were placed on the sediment surface and injected with 2 ml water, resulting in a rapid acetylene-generating reaction. Field chambers were placed over the vials immediately after addition of water. Chambers were installed in all subsystems ( $n = 5$  chambers per subsystem per date) except the hyporheic zone where surface water would interfere with gas diffusion. Samples were collected through rubber septa in the chamber lids and stored in evacuated containers until analyzed. Denitrification rate was calculated for the upper 10 cm of the watertable (chambers were inserted to a depth of 10 cm below the watertable) as the difference between initial and final nitrous oxide concentrations in the chamber headspace (4 hour incubation period) expressed on an areal basis (area of chamber =  $78.5\text{ cm}^2$ ). This rate was extrapolated to the entire depth of bank, parafluvial, and hyporheic sediments. A previous study reported a mean sediment depth of 62 cm in this section of Sycamore Creek (Valett et al. 1990). However, substantial sediment deposition has occurred since that time, and consequently we assumed a mean sediment depth of 1 m for this study.

Nitrous oxide can be produced by nitrification as well as denitrification (Davidson & Swank 1986; Matson and Vitousek 1990); thus, nitrous oxide in our chambers could come from either of these sources. In order to determine if any nitrous oxide present in our chambers was produced by nitrification,

we installed additional chambers without acetylene. If nitrous oxide did not accumulate in these chambers, we could conclude that all nitrous oxide had been produced by denitrifiers, which in the absence of acetylene was further reduced to  $N_2$ .

### *Factors controlling denitrification rate*

Effects of temperature, nitrate concentration, organic carbon availability, and oxygen on denitrification rate were examined in three laboratory experiments. Initial and final headspace gas samples were taken during each experiment (incubation time  $\sim 24$  h). The first experiment was designed to determine what factor or factors limit denitrification in Sycamore Creek sediments (nitrate availability, organic carbon availability, or oxygen). Parafluvial sediments ( $\sim 150$  g wet mass) were incubated in 250-ml bottles with acetylene in all possible combinations of the following treatments:  $\pm$  C amendment (3.3 g C/L);  $\pm$  N amendment (200 mg  $NO_3$ -N/L); oxic/anoxic. All treatment combinations were run in triplicate.

The second experiment addressed the effect of temperature on denitrification rate. Sediments were amended with 40 ml of a solution containing carbon and nitrate (in the above concentrations), and incubated anaerobically at either 10, 24, or 35 °C.

The third experiment assessed the influence of nitrate concentration on denitrification rate. Sediments were amended with 40 ml solution containing 3.3 g C/L (as  $C_6H_{12}O_6$ ) and rendered anoxic by purging with  $N_2$  for 15 minutes, and then amended with nitrate to one of the following concentrations: 0, 0.05, 0.1, 0.5, 1, 5, 10, 100, 200 mg  $NO_3$ -N/L. All treatments were run in triplicate and initial and final headspace gas samples were collected.

All nitrous oxide samples were analyzed on a Varian<sup>®</sup> Model 3300 gas chromatograph equipped with an electron capture detector. Carrier gas flow was 30 ml/min, and injector, column, and detector temperatures were 100, 80, and 350 °C, respectively.

Statistical analyses were performed using SYSTAT (1992). Denitrification potential, unamended denitrification rate, and accumulation in field chambers were analyzed using two-way analysis of variance (ANOVA) with location and date as factors. Significant differences were further examined using Tukey's multiple comparisons.

### *Nitrification*

We measured sediment nitrification rate to compare the magnitude of nitrate loss through denitrification to the rate of nitrate production through nitrification. Net nitrification rate was measured by placing approximately 160 g (wet

mass) sediments in 250-ml Erlenmeyer flasks and incubating with 100-ml distilled water for 20–24 h at 24°C. Sediments from each sampling point were run in triplicate. After the end of the incubation, a 5-ml water sample was collected and analyzed for nitrate. Nitrification rate was expressed as nitrate produced per unit dry sediment mass. Although we measured *net* nitrification, the values obtained approximated *gross* nitrification, as microbial immobilization of nitrate and denitrification were insignificant using this procedure with Sycamore Creek sediments (unpublished data).

## Results

### *System configuration and hydrology*

The 2500 m<sup>2</sup> study reach (100 × 25 m) consisted predominately of parafluvial zone, with hyporheic and bank/riparian zones occupying smaller areas (Figure 1). Subsurface flowpaths determined by examination of piezometric surface (Figure 1a) and dye injections (Figure 1b) were in close agreement. Interstitial flow rate through parafluvial gravel bars averaged 2 m/h. Hyporheic upwelling zones occurred at the upstream margin of the study reach and midway along the reach in the right channel, whereas downwelling zones were predominately midway through the study reach in the left channel (Figure 1b).

### *Physico-chemical characteristics of interstitial water*

Physico-chemical attributes of interstitial water in the three subsurface zones and in surface water at the upstream and downstream extremes of the study reach are summarized in Table 1. Nitrate concentration ranged from 0.3 to 120 µg NO<sub>3</sub>-N/L and generally increased along hyporheic and parafluvial flowpaths. In contrast, dissolved oxygen was highest in surface water and declined along hyporheic and parafluvial flowpaths. Patterns for other parameters were less apparent (eg., DOC, temperature, conductivity), except that interstitial water in bank sediments typically was chemically distinct from water in other locations.

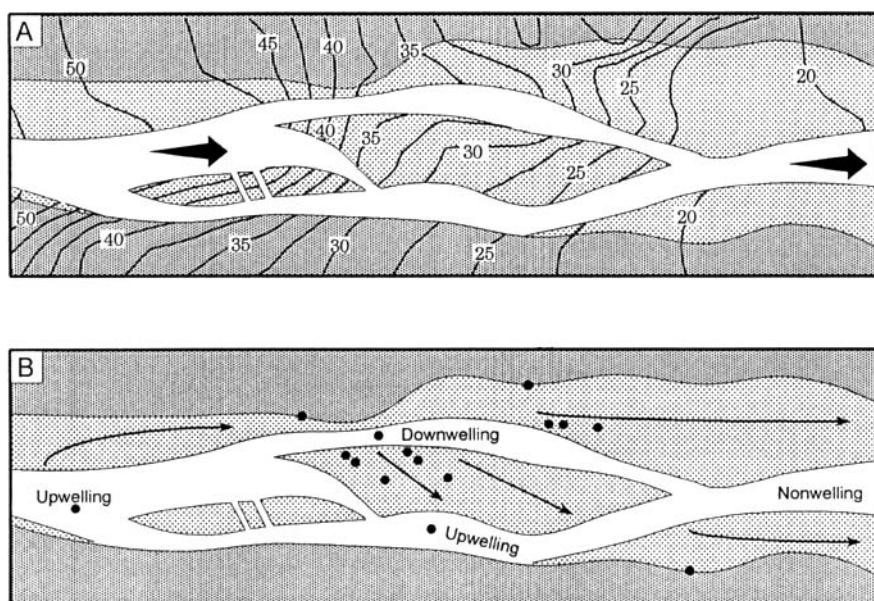
### *Denitrification in Sycamore Creek sediments*

Denitrification potential (C & N amended, anaerobic incubation) varied among subsystems ( $p < 0.01$ ) but not between dates (Figure 2a;  $p \geq 0.05$ ). Denitrification potential was significantly higher in bank sediments than in parafluvial or hyporheic sediments ( $p < 0.05$ ). Unamended denitrification

*Table 1.* Physico-chemical attributes ( $\pm 1$  SE) of water in three interstitial subsystems and in the surface stream at the upstream and downstream limits of the study reach. Surface water was collected at only one upstream and one downstream location on each date so standard errors are not given.

	Subsystem							
	Surface Stream		Hyporheic		Parafluvial Flowpath			Bank
	Up-stream	Down-stream	Down-Welling	Up-Welling	0.5 m	1 m	5 m	
						18 August 1993		
NO <sub>3</sub> -N (μg/L)	26.00	30.00	81.00 (2.50)	120.00 (6.90)	58.00 ( 4.10)	81.00 (14.00)	88.00 (10.00)	3.10 ( 1.70)
NH <sub>4</sub> -N (μg/L)	0.00	0.00	0.00 (0.00)	0.00 (0.00)	0.00 ( 0.00)	0.00 ( 0.00)	0.00 ( 0.00)	14.00 (14.00)
O <sub>2</sub> (mg/L)	8.60	8.10	4.30 (0.38)	3.43 (0.52)	7.73 ( 0.13)	7.83 ( 0.23)	6.50 ( 0.50)	0.00 ( 0.00)
DOC (mg/L)	2.13	1.73	1.58 (0.08)	1.56 (0.06)	1.67 ( 0.02)	1.69 ( 0.04)	1.78 ( 0.03)	4.52 ( 0.75)
TEMP (C)	29.00	27.80	23.90 (0.10)	25.10 (0.60)	25.40 ( 0.20)	24.80 ( 0.40)	24.10 ( 0.00)	27.60 ( 1.90)
COND (μS/cm)	514.00	464.00	473.70 (2.00)	476.00 (1.00)	468.30 ( 1.70)	467.30 ( 1.70)	468.00 ( 3.50)	570.30 (37.60)
pH	8.09	8.08	7.74 (0.03)	7.69 (0.02)	7.97 ( 0.01)	7.92 ( 0.01)	7.78 ( 0.02)	7.23 ( 0.06)
						23 October 1993		
NO <sub>3</sub> -N (μg/L)	19.00	14.00	51.00 (4.20)	90.00 (7.10)	66.00 (13.00)	76.00 (13.00)	62.00 (17.00)	0.30 ( 0.30)
NH <sub>4</sub> -N (μg/L)	0.00	0.00	0.00 (0.00)	0.00 (0.00)	0.00 ( 0.00)	0.00 ( 0.00)	0.00 ( 0.00)	2.20 ( 2.20)
O <sub>2</sub> (mg/L)	9.50	9.70	6.63 (0.19)	5.63 (0.75)	8.33 ( 0.35)	8.07 ( 0.03)	7.53 ( 0.17)	0.00 ( 0.00)
DOC (mg/L)	3.54	3.12	3.67 (0.08)	3.59 (0.16)	3.26 ( 0.04)	3.24 ( 0.11)	3.19 ( 0.22)	4.57 ( 0.50)
TEMP (C)	21.90	22.30	17.60 (0.30)	19.20 (0.50)	17.00 ( 0.10)	16.50 ( 0.15)	17.30 ( 0.20)	20.50 ( 0.20)
COND (μS/cm)	483.00	479.00	485.30 (0.30)	484.00 (1.50)	484.00 ( 1.50)	486.00 ( 0.60)	488.30 ( 1.50)	519.70 (19.10)
pH	8.04	8.13	7.68 (0.03)	7.64 (0.01)	7.83 ( 0.02)	7.84 ( 0.01)	7.88 ( 0.02)	7.06 ( 0.13)





**Figure 1.** Overhead view of the study reach ( $100 \times 25$  m). Unshaded region is the surface stream/hyporheic zone, lightly shaded area is the parafluvial zone, and darkly shaded region is the bank/riparian zone. A. Contour lines showing water table in parafluvial zone and bank sediments. Contour interval = 2.5 cm. Solid circles show typical sampling locations. B. Subsurface hydrologic flowpaths (determined by dye injections) and locations of hyporheic upwelling and downwelling.

rate was significantly lower than denitrification potential, and did not vary among locations or between dates (Figure 2b;  $p \geq 0.05$ ).

Denitrification rate calculated from nitrous oxide accumulation in field chambers ranged from greater than  $150 \text{ ug N} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$  at the stream-parafluvial interface to less than  $5 \text{ ug N} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$  in bank sediments (Figure 3); however, variance within each location was high and consequently differences among locations were not significant ( $p \geq 0.05$ ). All nitrous oxide produced could be attributed to denitrification, as none accumulated in chambers that did not contain acetylene, indicating that nitrification was not a source of nitrous oxide.

### *Factors controlling denitrification rate*

Nutrient amendment increased denitrification rate ( $p < 0.001$ ), whereas purging bottles with  $\text{N}_2$  to make sediments anoxic had no effect (Figure 4;  $p \geq 0.05$ ). Carbon amendment alone had no effect, while nitrate amendment alone

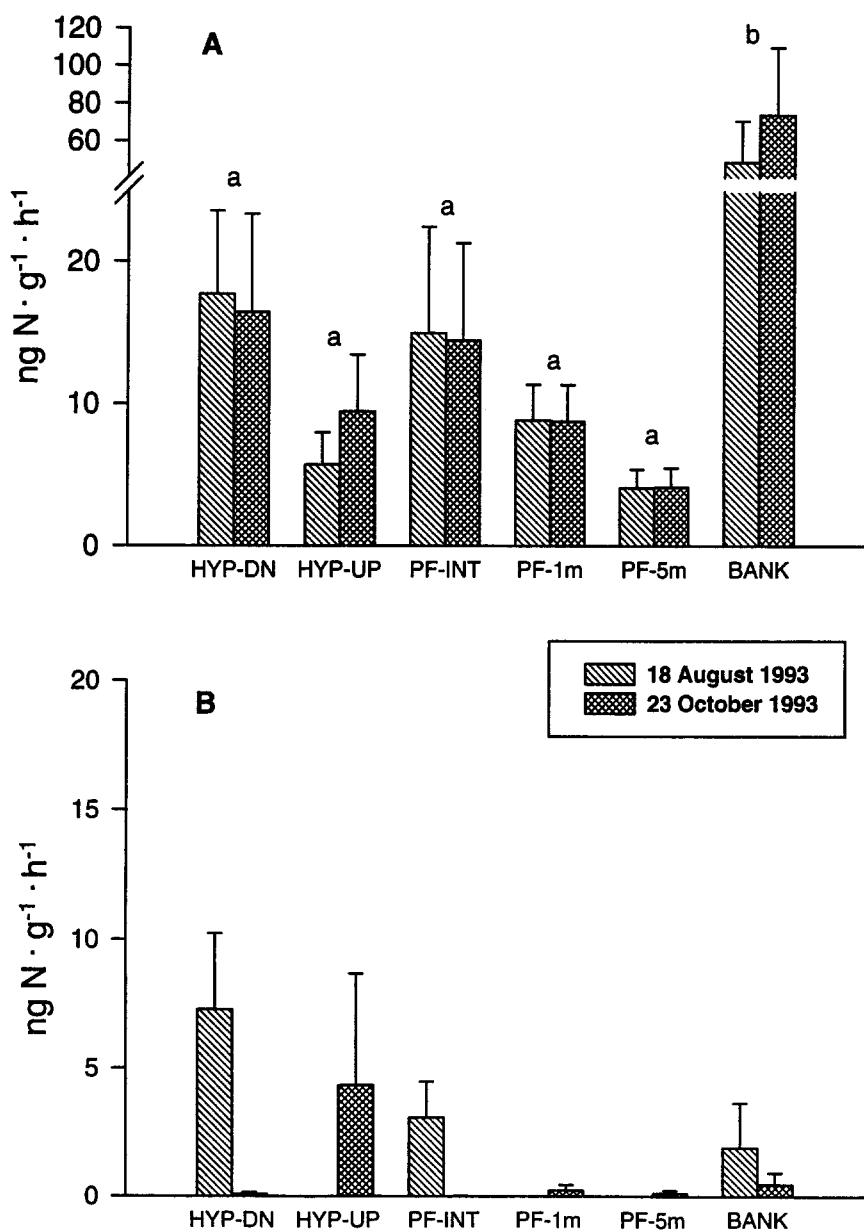


Figure 2. Denitrification rates in subsurface components of Sycamore Creek. (A) Denitrification potential (C and N amended, anoxic incubations). (B) Unamended denitrification (not amended with C or N, oxic incubations). HYP-DN = hyporheic downwelling zone, HYP-UP = hyporheic upwelling zone, PF-INT = parafluvial zone at the stream-parafluvial interface, PF-1m = 1m along parafluvial flowpath, PF-5m = 5 m along parafluvial flowpath, BANK = bank/riparian zone. Error bars indicate +1 SE and statistically significant differences are indicated by different lower case letters.

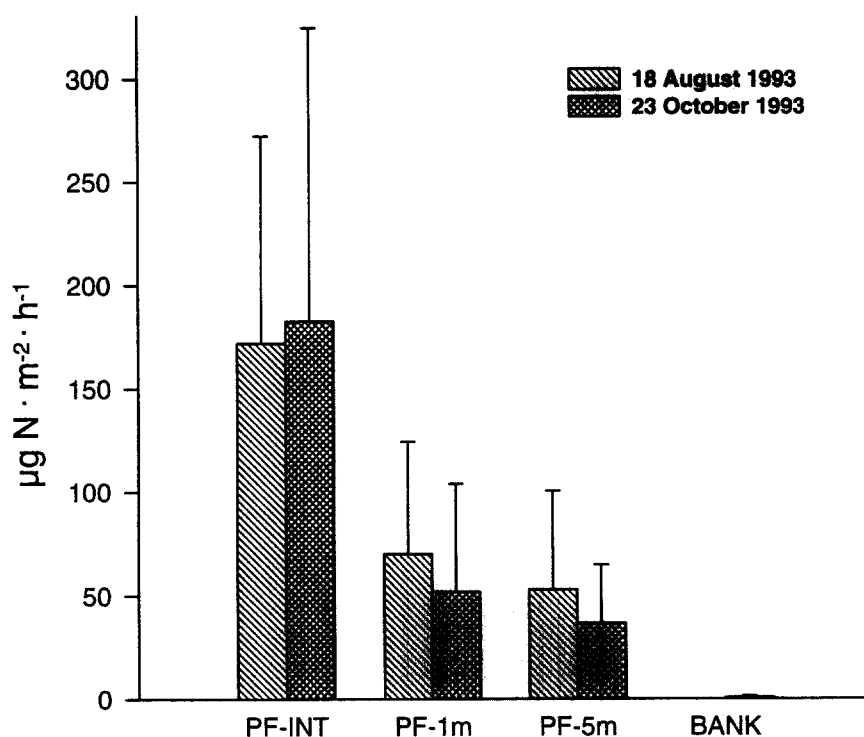
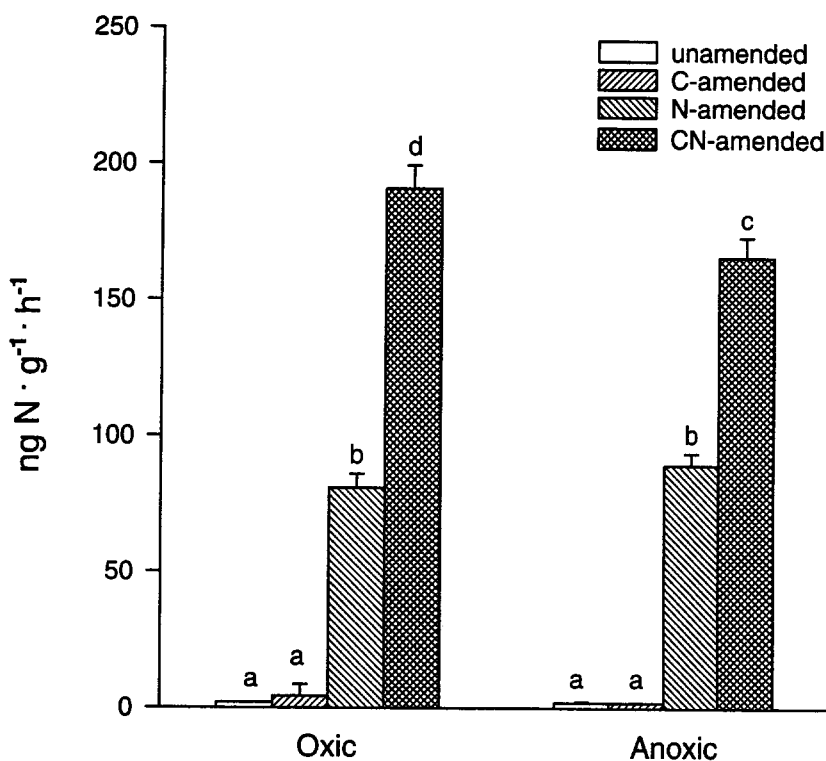


Figure 3. Denitrification rates determined using static chambers in the field. Data shown are for chambers with acetylene added to their headspace to block  $\text{N}_2\text{O}$  conversion to  $\text{N}_2$ . Chambers without acetylene never accumulated  $\text{N}_2\text{O}$ . See Figure 2 for site abbreviations. Error bars indicate +1 SE.

increased denitrification rate ( $p < 0.05$ ). Denitrification rate further increased when nitrate and carbon were added together (Figure 4).

Temperature was related to denitrification rate in parafluvial sediments ( $p < 0.05$ ; Figure 5). Sediments incubated at  $10^\circ\text{C}$  had low denitrification rate, whereas denitrification rate for sediments incubated at  $24$  or  $35^\circ\text{C}$  were higher but not significantly different from one another.

Whereas a previous experiment showed that denitrification rate increased with nitrate amendment (Figure 4), the third experiment demonstrated that denitrification rate also varied as a function of nitrate amendment concentration ( $p < 0.05$ ; Figure 6). Denitrification rate in sediments incubated with nitrate-N concentrations up to  $1.0\text{ mg/L}$  did not differ from sediments incubated with distilled water, but concentrations above  $1\text{ mg/L}$  greatly increased denitrification rate.



**Figure 4.** Results of experiment addressing factors controlling denitrification rate in parafluvial sediments. Amendments were glucose (3.0 g C/L as glucose) and nitrate (200 mg  $\text{NO}_3\text{-N/L}$  as  $\text{KNO}_3$ ) in combination or alone, and oxic versus anoxic incubations. Error bars indicate +1 SE, and statistically significant differences are indicated by different lower case letters.

### *Nitrification*

Net nitrification rate varied between locations and dates (Figure 7). Rates were higher at hyporheic downwelling ( $11.6\text{--}13.8 \text{ ng N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) than upwelling zones ( $3.5\text{--}5.7 \text{ ng N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ), and also declined along parafluvial flow-paths. Net nitrification rates in bank sediments averaged  $4.3 \text{ ng N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ , but actual rates are probably much less (or zero) because bank sediments are typically anoxic (Table 1) and our incubation procedure introduced oxygen.

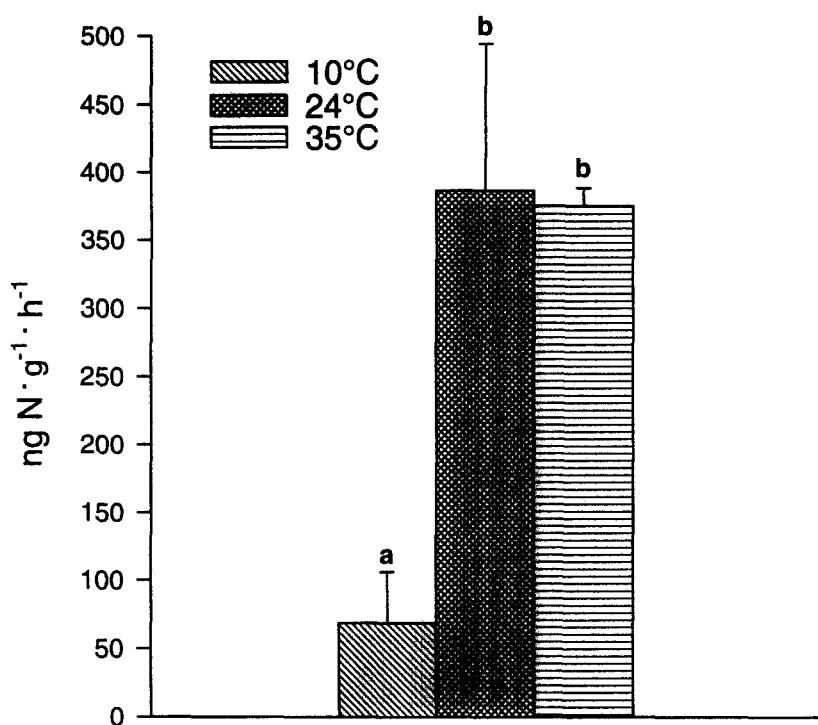


Figure 5. Effect of temperature on denitrification potential of parafluvial sediments. Error bars indicate +1 SE, and statistically significant differences are indicated by different lower case letters.

## Discussion

### *Denitrification in Sycamore Creek sediments*

To transform nitrate to nitrous oxide or dinitrogen, denitrifying organisms require a suitable organic carbon source, sufficient nitrate to use as a terminal electron acceptor, and typically an anoxic or hypoxic environment; consequently, denitrification is normally highest in environments rich in nitrate and organic carbon. In contrast, sediments of Sycamore Creek are generally well-oxygenated, low in organic matter, and have relatively low interstitial nitrate concentration (Grimm & Fisher 1984; Holmes et al. 1994a; Jones et al. 1995a). These factors all suggest that denitrification should not be widespread in Sycamore Creek. However, even modest rates of denitrification may significantly influence the functioning of this or other nitrogen-limited ecosystems.

While oxidizing conditions prevail, hypoxia does occur in Sycamore Creek (Valett et al. 1990). Stanley and Boulton (1995) report widespread anoxia

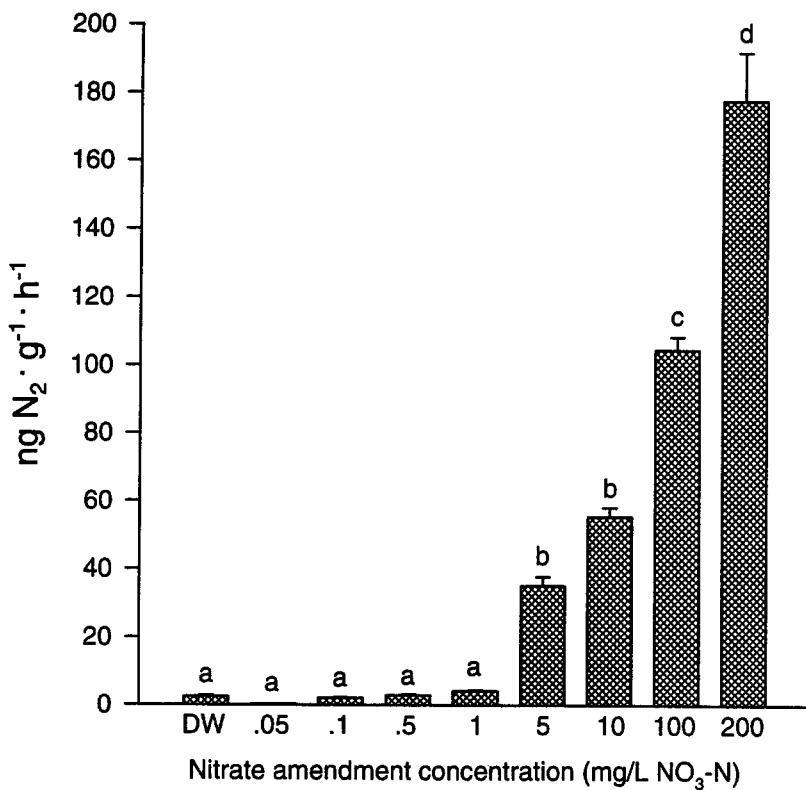


Figure 6. Effect of nitrate concentration on denitrification rate. All incubations were amended with 40 ml solution containing 3.3 g/L C as glucose and were incubated anaerobically. Error bars represent +1 SE, and statistically significant differences are indicated by different lower case letters.

during drying, and Jones et al. (1995b) found anoxia to be common in bank sediments. Based upon subsurface respiration rates, Jones et al. (1995b) calculated that surface water would be depleted of its oxygen after moving 7 m along hyporheic or parafluvial flowpaths. Although this depletion is seldom observed, it does suggest that anoxia may be more common than our measurements suggest. For example, anoxic microsites may be present in otherwise oxic regions, and these sites might not have been identified by our coarser scale sampling methods.

Actual denitrification rate is difficult to establish for a number of reasons. For example, most sampling procedures disrupt sediment structure and anoxic microsites, as well as eliminate natural hydrologic flow through sediments (Tiedje et al. 1989). Moreover, acetylene blockage of nitrification is problematic in low-nitrate systems, and estimation of denitrification rate is

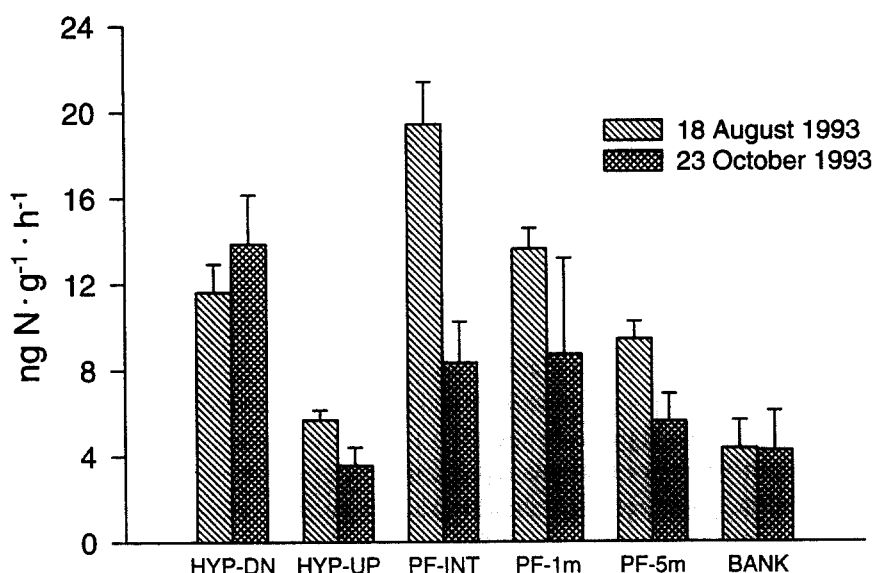


Figure 7. Net nitrification rate (per unit sediment dry mass). Error bars represent  $\pm 1$  SE. Statistically significant differences among locations (Tukey's multiple comparison,  $p < 0.05$ ) are denoted by different lower case letters.

further complicated by its high degree of spatial variability (Tiedje et al. 1989; Seitzinger 1988). In spite of these complications, estimates of actual denitrification rates are necessary to assess the importance of denitrification to the functioning of an ecosystem.

In this study, three independent measures of denitrification rate are used to bracket actual denitrification rates. Denitrification potential overestimates actual denitrification because substrates (organic carbon and nitrate) are supplied in excess (and greatly above field concentrations), while the unamended treatment probably underestimates true rates because incubations were performed using distilled water which dilutes natural substrate concentrations. Further, acetylene blocks nitrification, which is likely the primary source of nitrate to denitrifiers in systems with low ambient nitrate concentrations. For this reason, unamended and field accumulation measures probably underestimate denitrification. While either method taken individually would be inconclusive with regard to actual denitrification rate, in combination they allow us to produce upper and lower rate estimates, which can then be used to determine the potential significance of denitrification in this stream.

Counter to expectations, denitrification rate did not increase along parafuvial and hyporheic flowpaths (Figures 2, 3). In fact, denitrification was gener-

ally highest at hyporheic downwelling and parafluvial inwelling zones. This was unexpected given the relatively high oxygen and low nitrate at these locations (Table 1). The fact that denitrification occurs in these regions indicates that anoxic microsites are probably present in these otherwise oxic zones, and raises the question as to why denitrification would be higher in these seemingly unlikely locations. Previous research has shown that respiration is highest at the upstream ends of hyporheic and parafluvial flowpaths (Jones et al. 1995a), perhaps because labile organic matter is imported from the surface stream and is therefore more available at points of surface-to-subsurface hydrologic linkage (Holmes et al. 1994a, Jones et al. 1995a). Higher denitrification rates at these locations may similarly be explained by greater availability of labile organic matter. Additionally, whereas nitrate concentration is low at hyporheic downwelling and parafluvial inwelling zones, nitrate supply is relatively high given the high nitrification rates (Figure 7). Since interstitial nitrate concentrations represent a net effect of nitrification versus denitrification, future research should focus on surface-subsurface interfaces to further examine the role of labile carbon and nitrification-denitrification coupling.

#### *Factors controlling denitrification rate*

Laboratory experiments indicated that nitrate was the primary factor limiting denitrification, but carbon became limiting following nitrate amendment (Figure 4). This result seems inconsistent with our observation of decreasing denitrification along flowpaths, as nitrate typically increases along flowpaths (Table 1; Holmes et al. 1994a). However, the nitrate increase along flowpaths ( $\sim 0.1$  mg/L  $\text{NO}_3\text{-N}$  for 5 m long flowpaths) is small compared with the range of nitrate concentrations used in the laboratory experiment (0–200 mg/L  $\text{NO}_3\text{-N}$ ), and denitrification rate did not increase until nitrate amendment exceeded 1.0 mg/L  $\text{NO}_3\text{-N}$ . Therefore, the magnitude of nitrate increase along flowpaths may be inconsequential with respect to denitrification rate. Further, whereas ambient nitrate concentration was low at the upstream ends of flowpaths, nitrate supply via nitrification was high (Figure 7), which could account for the higher denitrification rate at these locations.

Purging flasks with  $\text{N}_2$  to render them anoxic had no effect on denitrification rate (Figure 4). Apparently either all flasks went anoxic during the incubation or denitrification occurred in microsites that were anoxic regardless of whether the flask had been purged of oxygen. This result does not mean that oxygen concentration is unimportant in controlling denitrification in Sycamore Creek, as incubation bottles differ from in situ sediments in several ways, including the fact that dissolved oxygen is continually supplied to subsurface sediments by inflowing water.



Temperature influenced denitrification in Sycamore Creek sediments (Figure 5,  $p < 0.05$ ), and has been reported as a controlling factor in other studies (e.g., Dawson & Murphy 1972, Messer & Brezonik 1984, Seitzinger 1988, Peterjohn 1991). Temperature ranged from  $\sim 5$  to  $32^\circ\text{C}$  in interstitial water in Sycamore Creek; therefore, denitrification is expected to show a seasonal periodicity based on temperature, although other factors may confound this relationship.

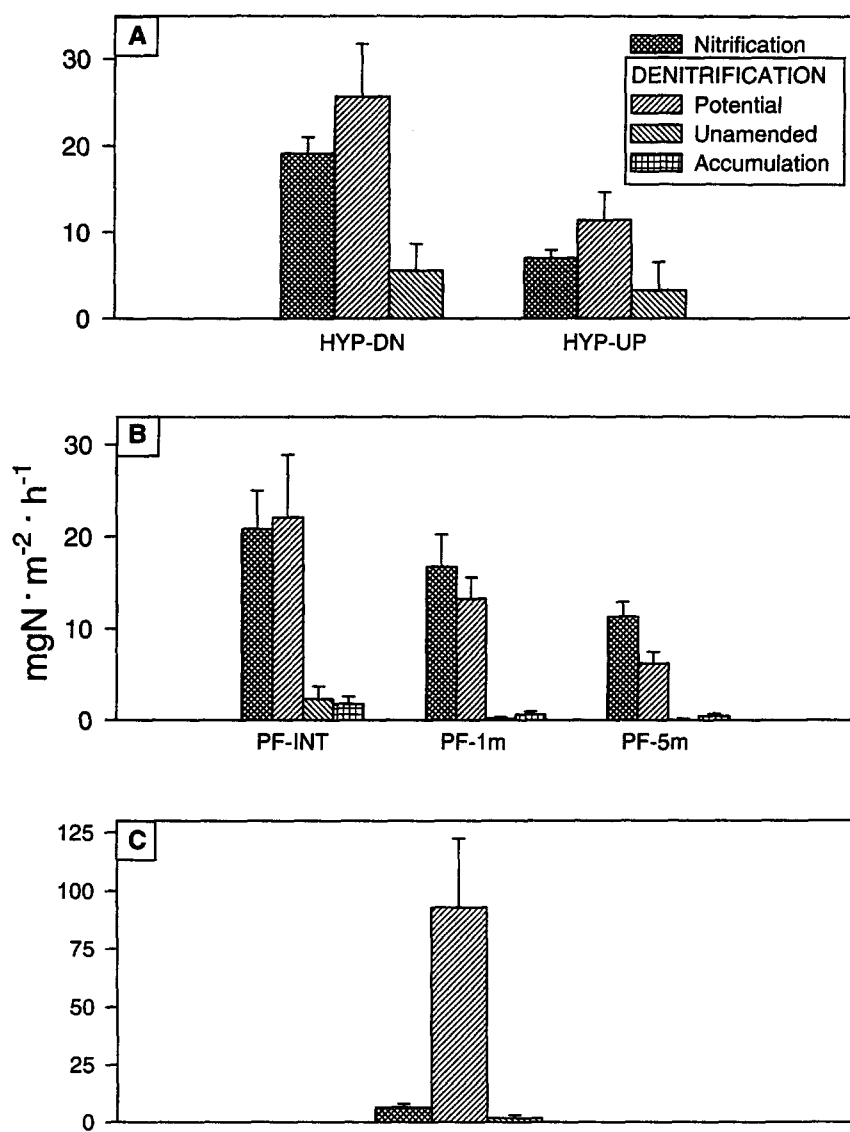
### *Importance to the ecosystem*

In order to assess the importance of denitrification to ecosystem functioning, we compared our measures of nitrate loss through denitrification to nitrate production through nitrification. All units were converted to  $\text{mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ . Conversion from mass-specific to area-specific units were made assuming  $1.5\text{ kg/L}$  sediments and a sediment depth of  $1\text{ m}$ .

In the hyporheic zone, denitrification potential exceeded nitrification in both upwelling and downwelling zones, while the unamended rate was  $\sim 30\text{--}40\%$  of nitrate production (Figure 8a). Therefore, if denitrification was occurring at its potential rate, the hyporheic zone would be a sink for nitrate. Because nitrate typically increases along hyporheic flowpaths (Table 1), denitrification must be occurring at less than the potential rate. However, even using our conservative estimate of denitrification (unamended treatment), a significant amount of nitrate produced in hyporheic sediments is denitrified.

Similar patterns are seen at the stream-parafluvial interface (Figure 8b). Denitrification potential does not differ from nitrification rate, while unamended and field chamber accumulation rates are lower. Again, even a conservative estimate of denitrification rate suggests that  $\sim 10\%$  of nitrate produced is lost to denitrification. Farther along parafluvial flowpaths, nitrification and denitrification potential declined, and unamended and accumulation rates dropped to near zero (Figure 8b).

Bank sediments differ from hyporheic and parafluvial sediments in that denitrification potential is very high, whereas nitrification, unamended denitrification, and field accumulation rates are very low (Figure 8c). Nitrate is typically absent from interstitial water in bank sediments (Table 1); therefore, actual denitrification rates would be expected to be low due to nitrate limitation. However, high denitrification potential indicates that denitrifier populations are relatively large (Jørgensen & Tiedje 1993). McCarty (1972) and Payne (1973) estimate that the energy efficiency of denitrification is about  $60\%$  that of aerobic metabolism, and therefore aerobic metabolism is the favored pathway when oxygen is present. Most denitrifying bacteria are facultative anaerobes, allowing them to exploit environments that alternate between oxic and anoxic conditions. Thus, denitrification must at times be



**Figure 8.** Comparison of nitrate production rate (nitrification) to nitrate loss through denitrification in different subsystems of Sycamore Creek. All rates have been converted to an areal basis assuming a sediment depth of 1 m. A. Hyporheic zone. B. Parafluvial zone. C. Bank/riparian zone. Location abbreviations correspond to those given for Figure 2. Error bars represent +1 SE.

important in bank sediments, or denitrifier populations would not be expected to persist, unless they can utilize alternative electron acceptors or fermentation (Jørgensen & Tiedje 1993). Alternatively, low interstitial nitrate concentrations in bank sediments could be due to high denitrification rate. However, interstitial flow is very slow in bank sediments and consequently nitrate supply is limited, suggesting that actual denitrification rate is also relatively low.

For the entire 2500 m<sup>2</sup> study reach, based on subsystem-specific rates and the areal extent of subsystems, nitrate production by nitrification was approximately 465 g N/d, while denitrification removed 40–568 g N/d. For comparison, nitrate flux in the surface stream during baseflow is about 430 g N/d. Since the hyporheic and parafluvial zones are nitrate sources, we know that nitrification exceeds denitrification and therefore denitrification is occurring at less than its potential rate. However, even our lower estimate of 40 g N/d lost to denitrification (8.6% of nitrate produced by nitrification) is significant given that primary productivity is limited by nitrogen availability in Sycamore Creek.

Carbon and nitrogen cycles are often considered independently, although they are linked and may interact in important ways (Likens 1981; Schlesinger 1991). For example, denitrification can be an important respiratory pathway in anoxic environments. We calculated that denitrification in our study reach of Sycamore Creek consumes 2.3–114.6 mg C·m<sup>-2</sup>·d<sup>-1</sup> (assuming a stoichiometry of 106 C oxidized per 94.4 N reduced; Krumbein & Swart 1983), compared to about 3250 mg C·m<sup>-2</sup>·d<sup>-1</sup> consumed by aerobic respiration (Jones et al. 1995a). Thus, while of significance for nitrogen cycling, denitrification is of little importance to whole-system respiration.

## Conclusions

Peterjohn and Schlesinger (1990) estimate that 77% of atmospheric inputs of nitrogen to catchments in the desert southwest of the United States eventually return to the atmosphere, much by denitrification. Denitrification has been reported in Sonoran Desert soils (Virginia et al. 1982; Peterjohn 1991), but our study is the first to assess denitrification in Sonoran Desert stream ecosystems. Infiltration of precipitation into desert soils is minimal due to their hydrophobic character and the presence of a continuous, nearly impermeable caliche layer near the soil surface (Fuller 1975; Peterjohn & Schlesinger 1990). As a result, runoff is rapidly transported by overland flow to stream channels, and lateral removal of nitrate by denitrification in the riparian zone is probably insignificant. However, we have demonstrated the potential for substantial longitudinal removal of nitrate once water has entered the stream channel.

While denitrification in desert soils is restricted to brief periods following rainstorms when soils are moist (Virginia et al. 1982; Peterjohn 1991), stream sediments are potentially active sites for denitrification throughout the year. Consequently, streams ecosystems may be hot-spots for nitrogen retention via denitrification in arid catchments.

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