

Nitrate reduction in sediments of lowland tropical streams draining swamp forest in Costa Rica: An ecosystem perspective

JOHN H. DUFF¹, CATHERINE M. PRINGLE² & FRANK J. TRISKA¹

¹ U.S. Geological Survey, Water Resources Division, Mail Stop 496, 345 Middlefield Road, Menlo Park, California 94025-3591; ²Institute of Ecology, University of Georgia, 308 Biological Sciences Building, Athens, Georgia 30602-2607

Received 29 July 1994; accepted 19 December 1995

Key words: NO_3^- reduction, denitrification, nitrification, swamp forest, lowland rain forest, tropical streams, Costa Rica

Abstract. Nitrate reduction and denitrification were measured in swamp forest streams draining lowland rain forest on Costa Rica's Atlantic slope foothills using the C_2H_2 -block assay and sediment-water nutrient fluxes. Denitrification assays using the C_2H_2 -block technique indicated that the full suite of denitrifying enzymes were present in the sediment but that only a small fraction of the functional activity could be expressed without adding NO_3^- . Under optimal conditions, denitrification enzyme activity averaged 15 nmoles cm^{-3} sediment h^{-1} . Areal NO_3^- reduction rates measured from NO_3^- loss in the overlying water of sediment-water flux chambers ranged from 65 to 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$. Oxygen loss rates accompanying NO_3^- depletion averaged 750 $\mu\text{moles m}^{-2} \text{h}^{-1}$. Corrected for denitrification of NO_3^- oxidized from NH_4^+ in the sediment, gross NO_3^- reduction rates increase by 130 $\mu\text{moles m}^{-2} \text{h}^{-1}$, indicating nitrification may be the predominant source of NO_3^- for NO_3^- reduction in swamp forest stream sediments. Under field conditions approximately 80% of the increase in inorganic N mass along a 1250-m reach of the Salto River was in the form of NO_3^- with the balance NH_4^+ . Scrutiny of potential inorganic N sources suggested that mineralized N released from the streambed was a major source of the inorganic N increase. Despite significant NO_3^- reduction potential, swamp forest stream sediments appear to be a source of inorganic N to downstream communities.

Introduction

Solute-rich, geothermal seeps emerge at topographically low areas along faults and where the foothills of the central mountain range merge with the coastal plain on the Atlantic slope of Costa Rica (Pringle et al. 1990). One geomorphologic result is swamp forest; tracts of water-saturated land inundated by non-point groundwater sources, seeps and springs. This water may exhibit solute composition characteristic of geothermally-modified water, including relatively high levels of SO_4^{2+} , soluble reactive phosphorus (SRP), Cl^- , Na^+ , Mg^+ , Ca^{2+} and other dissolved solids (Pringle 1991; Pringle et al. 1993).

The Salto and other small rivers draining lowland tropical wet forest pass through swamp forest. Swamp forest streams are relatively low gradient with high uniform temperatures, daily organic material inputs and fine depositional sediments. The unique chemical features and organic-rich sediments associated with swamp forest (Bourgeois et al. 1972) create a potentially complex redox environment for element cycling in lowland streams.

The organic C pool in lowland rain forest streams is almost entirely allochthonous, entering the streams either as litterfall, throughfall or dissolved in runoff from the surrounding land. Detritus-feeding fish, such as *Brycon quatemalensis* and *Cichlasoma tuba*, and nocturnal freshwater shrimp, such as *Macrobrachium carcinus*, directly consume palatable litter as it enters the channel. Algal primary production typically is low because of shading by the dense, multi-stratal canopy (Paaby & Goldman 1992). Thus, processed litter, egesta of detritivores and dissolved organic C (DOC) that enters in surface and subsurface runoff dominate the organic C pool.

Cycles of O, N, S and inorganic C are directly linked to the organic C pool via microbial pathways (Herbert & Nedwell 1990). While aerobic degradation is the most efficient means of degrading organic material, in its absence, anaerobic degradation pathways proceed through a sequence of terminal electron acceptors determined by the free energy yield. The first electron acceptor for the anaerobic respiratory chain is NO_3^- , followed by SO_4^{2+} and CO_2 .

The predominant pathways of organic C mineralization differ between marine and freshwater sediments (Capone & Kiene 1988). In marine sediments, the primary electron acceptor for the anaerobic respiratory chain is SO_4^{2+} , while in fresh water sediments including lakes, rivers and wetlands, methanogenesis predominates (except, see Seitzinger 1994) depending on the organic C load and availability of NO_3^- (Capone & Kiene 1988). In freshwater aquatic systems that receive high levels of anthropogenic N inputs, the primary electron acceptor for the anaerobic respiratory chain is NO_3^- (Hill 1981; Seitzinger 1994; Bradley et al. 1995).

Swamp forest streams have relatively high NO_3^- concentrations (Triska et al. 1993). Because of their low gradient and sustained organic C inputs, NO_3^- reduction may be important for organic matter oxidation. This study investigates NO_3^- reduction in swamp forest stream sediments, particularly rate-controlling factors including NO_3^- and C supply, and the role of stream sediments as a sink for inorganic N transported through swamp forest.

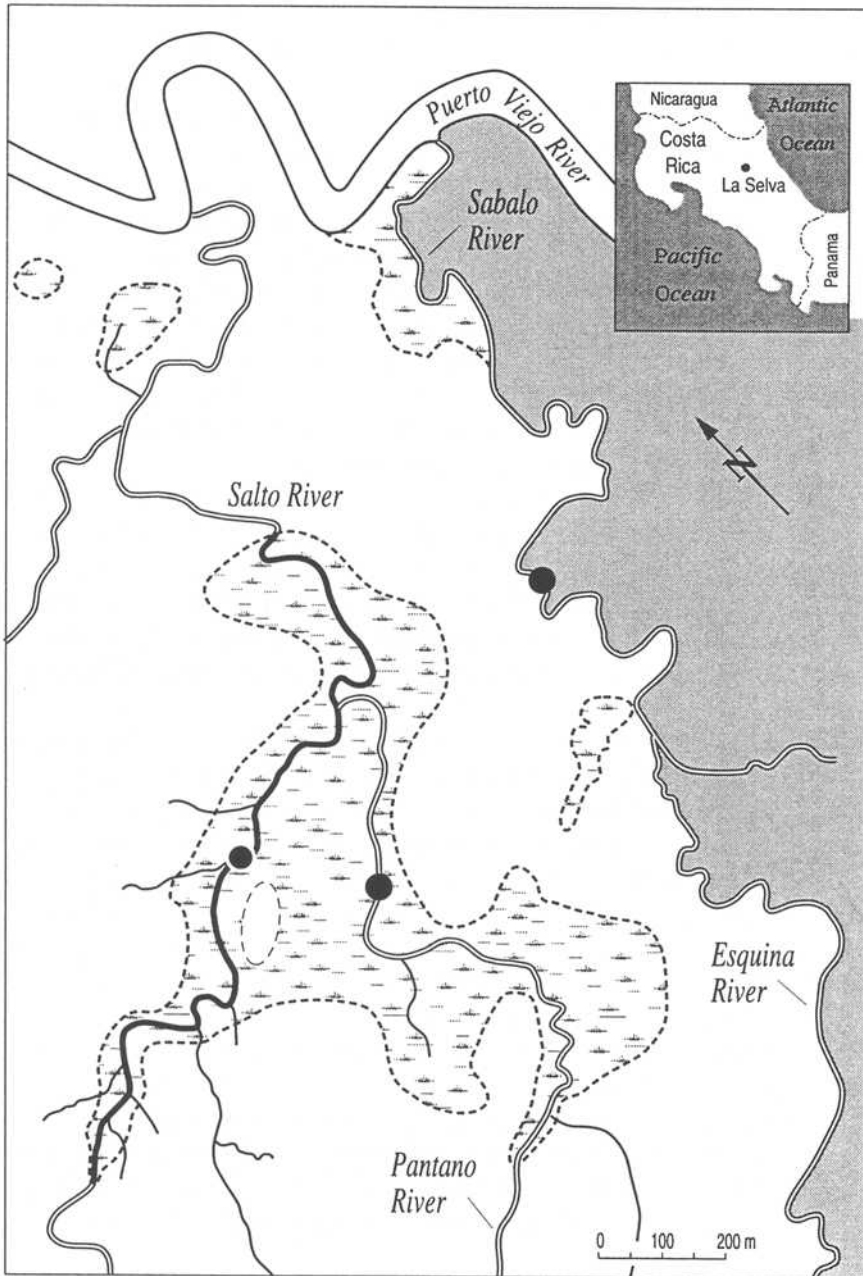


Figure 1. The study site at La Selva Biological Station depicting the Salto, Pantano and Sabalo River drainage. Major landform features include swamp forest (marsh pattern), pasture (stippled pattern) and experimental reach along the Salto River (solid stream). Study sites (●).

Site description

This study was conducted in three streams draining lowland tropical wet forest on the Atlantic slope of Costa Rica's central mountain range (Figure 1). The experiments were done in February, 1989, during the period of low precipitation (Pringle & Triska 1991). The stream sites occur in La Selva Biological Reserve (3,300 ha), owned and operated by the Organization for Tropical Studies. The Salto and Pantano are densely shaded third-order streams that drain primary, lowland forest and pass through extensive swamp forest below 100 m.a.s.l. The streams are low gradient in the swamp forest with slow, non-turbulent flow and sand and silt bottoms. By contrast, the Sabalo is a fourth-order stream that drains pasture and secondary forest, with higher light penetration than either the Salto or Pantano. The Sabalo is composed of alternating pool-riffle sequences with gravel bottom riffles and sand bottom pools. Stream and interstitial water temperatures average 25 °C throughout the year.

Large amounts of water pass through the Salto River. La Selva receives 4 m of precipitation every year, and higher elevations receive over 5 m per year (Pringle et al. 1990). Although a large percentage of water passing through swamp forest is meteoric in origin and dilute in concentration, NO_3^- levels in stream water are relatively high throughout the year. In swamp forest streams annual NO_3^- levels average 25 $\mu\text{moles L}^{-1}$ and NH_4^+ levels average 2 $\mu\text{moles L}^{-1}$. High levels of SRP are characteristic of geothermally-modified waters and levels vary among geothermally-modified and unmodified streams; SRP levels are about 3 $\mu\text{moles L}^{-1}$ in the Salto but only 0.3 $\mu\text{mole L}^{-1}$ in the Pantano and Sabalo Rivers. The concentration of DOC in the Salto River was $1.5 \pm 0.5 \text{ mg C L}^{-1}$ ($n = 15$).

Methods

Denitrification measurements in swamp forest sediments

Denitrification was assayed by the C_2H_2 -block technique (Balderston et al. 1976; Yoshinari & Knowles 1976). Sediment from the Salto, Pantano and Sabalo Rivers was collected and immediately returned to La Selva Station. Two cubic centimeters of sediment were added to 20-mL glass bottles, slurried with 8 mL of stream water, flushed for 15 min with O_2 -free N_2 and then sealed with recessed butyl rubber stoppers. Acetylene was generated from CaC_2 -saturated water and was added (2 mL) by syringe to the headspace of selected bottles. The bottles were incubated on a shaker table at 25 °C for 15 min before the headspace was subsampled (100 μL) for N_2O determination. The effects

of various amendments on denitrification also were analyzed. Nitrate, glucose and NO_3^- plus glucose (1 mM final concentration per substrate) treatments differentiated C from NO_3^- limitation. Stock solutions were flushed with O_2 -free N_2 prior to amendment. Some sediment slurries were incubated with air in place of O_2 -free N_2 . Selected slurries were amended with chloramphenicol, an inhibitor of protein synthesis (Brooks et al. 1992), to assess denitrifying enzyme activity (Tiedje et al. 1982). Chloramphenicol was injected as a slurry at about twice its solubility to maintain saturation (final concentration = 4 mg mL^{-1}). Denitrification also was measured in unfiltered stream water enriched with NO_3^- and glucose.

Sediment-water nutrient fluxes in swamp forest sediments

Nitrate reduction rates by the sediment microbial community were estimated in situ in the Salto River from sediment-water fluxes in benthic chambers. The tapered base of cylindrical polycarbonate chambers (21 cm long by 19 cm diameter) was inserted into the sediment to a depth of 6 cm. Sodium nitrate, nitrapyrin and C_2H_2 were added individually or in combination to selected chambers through a needle canicula into a stream of chamber water recirculated through a closed-loop silicone tube with a 12-volt peristaltic pump. Nitrapyrin and C_2H_2 were employed as inhibitors of NH_4^+ oxidation. Acetylene was generated from CaC_2 and water and added to chambers. Acetylene-saturated chamber water was recirculated for 45 min after which time gas bubbles in the chamber were replaced with stream water by opening top and side ports simultaneously. The chambers were fitted with combination O_2 -temperature probes mounted on stirring platforms so that O_2 flux could be measured. Samples of the water were withdrawn by syringe for analysis of nutrients and dissolved gases. Subsamples for nutrients (65 mL) were filtered through 0.45- μm membrane filters and frozen in HCl-cleaned polyethylene bottles. Subsamples for dissolved N_2O and C_2H_2 (5 mL) were injected into sealed serum bottles (14 mL) and subsequently analyzed by headspace equilibrium. Oxygen concentration, temperature and pH were recorded at times of sampling. The small amount of water withdrawn from the chamber was replaced with fresh stream water drawn into an opposing port. In subsequent samples, chamber water was circulated for 5 min to insure mixing. Stream water was analyzed for the same parameters measured inside the chambers at each time point.

Dissolved inorganic N transport through swamp forest streams

Dissolved inorganic N (DIN) transport through swamp forest was determined along a 1250-m reach of the Salto River (Figure 1) under background condi-

tions using whole-stream tracer injection techniques (Cooper & Cooke 1984; Triska et al. 1993). Rhodamine WT was used to accurately calculate discharge at the upstream and downstream stations. A complete description of the injection techniques can be found in Triska et al. (1993). Prior to the 3-h injection, water samples were collected at both stations and analyzed for background NO_3^- , NO_2^- and NH_4^+ in order to calculate the mass flux between the stations. DIN mass at each station was calculated by multiplying the discharge rate times the inorganic N concentration (Equation 1):

$$M_{\text{IN}} = Q * C_{\text{IN}} \quad (1)$$

where M_{IN} = mass of inorganic N (mol N h^{-1}), Q = discharge (L s^{-1}) and C_{IN} = concentration of inorganic N (umol N L^{-1}). DIN transport was calculated from the mass flux between upstream and downstream stations (Equation 2):

$$F_{\text{IN}} = M_{\text{U}} - M_{\text{D}} \quad (2)$$

where F_{IN} = inorganic N flux between upstream and downstream stations (mmol N h^{-1}), M_{U} = mass of inorganic N at the upstream station (mol N h^{-1}) and M_{D} = mass of inorganic N at the downstream station (mol N h^{-1}). Mass fluxes between the stations were converted to the area of the streambed ($\text{umol N m}^{-2} \text{h}^{-1}$) by estimating an areal conversion factor of 7680 m^2 per stream reach.

Analytical procedures

Oxygen and temperature were measured directly in chambers using an O_2 meter (Yellow Springs Model 57, Yellow Springs Instrument Company, Yellow Springs, OH) with a probe mounted on a stirring platform. Nitrate plus NO_2^- and NH_4^+ were measured on an Autoanalyzer II (Technicon Corp. Inc., Tarrytown, NY) using a cadmium-reduction method and a phenol-hypochlorite method, respectively. Samples containing 0.05 to 25 $\mu\text{L N}_2\text{O}$ (C_2H_2 -block experiments) were determined on a gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a ^{63}Ni electron capture detector. The gases were separated with a Poropak R column (inner diameter 0.22 cm) at 90°C . The column was divided into two lengths (66 and 133 cm) separated by a six-port backflush valve to vent the C_2H_2 . Carrier gas was a P-5 mixture of CH_4 (5%) and Ar (95%) at a flow rate of 30 mL min^{-1} .

Table 1. Nitrous oxide formation rates (C_2H_2 block technique) in sediments from lowland tropical streams draining swamp forest and pasture in La Selva Biological Reserve, Costa Rica, nmoles cm^3 sediment $^{-1}$ h $^{-1}$ (1 SD), $n = 3$. NM indicates not measured.

Treatment	Swamp forest			Pasture	
	Salto	Pantano	Pantano	Sabalo River	
		Sand, fine silts		Sand	Gravel
	2/23/89	2/20/89	2/21/89	2/22/89	2/22/89
Endogenous	3.4 (0.4)	0	0.3 (0.3)	1.0 (0.0)	0.3 (0.6)
Glucose	3.1 (0.3)	0	0.1 (0.1)	0.7 (0.1)	1.6 (1.2)
Nitrate	14.9 (0.7)	14.7 (0.4)	28.5 (9.5)	19.6 (0.8)	2.5 (1.4)
Nitrate, glucose	22.4 (3.5)	21.1 (1.2)	41.0 (3.4)	24.4 (3.5)	1.8 (2.4)
Nitrate, glucose, chloramphenicol	11.4 (2.3)	13.8 (1.8)	23.0 (2.4)	8.8 (1.2)	0.1 (0.2)
Boiled sediments	0	NM	NM	NM	NM
Stream water only	0	NM	NM	NM	NM

Results

Denitrifying activity

When the C_2H_2 -block was applied to Salto River sediments, N_2O production occurred in flasks supplemented with NO_3^- , with NO_3^- plus glucose, and with NO_3^- plus glucose plus chloramphenicol (Figure 2). Neither glucose-amended nor unsupplemented control flasks sustained production of N_2O following a small, short pulse of activity. In fact, N_2O declined in the presence of C_2H_2 in unamended flasks after 2.5-h incubations, whereas flasks supplemented with NO_3^- and NO_3^- plus glucose sustained N_2O production throughout the incubations. Initial rates of N_2O formation in flasks supplemented with NO_3^- and NO_3^- plus glucose were 14.9 and 22.4 nmoles cm^3 sediment $^{-1}$ h $^{-1}$, respectively (Table 1). In C_2H_2 -block experiments with Salto sediments, N_2O production was not observed without C_2H_2 or after heat treatment, and N_2O production did not occur in stream water alone.

Denitrifying activity was compared in sediment from five locations with the C_2H_2 -block method (Table 1). Adding glucose without NO_3^- had no stimulatory effect at any location. Denitrifying activity was stimulated by NO_3^- at all four sites. Denitrifying activity with added NO_3^- , NO_3^- plus glucose and NO_3^- plus glucose plus chloramphenicol differed less than two-fold among the sand and fine-grain sediments from the three streams. N_2O production was stimulated in coarse-grain sediment from the Sabalo River but had activity rates 8 to 15 times lower than sediments from the depositional environments.

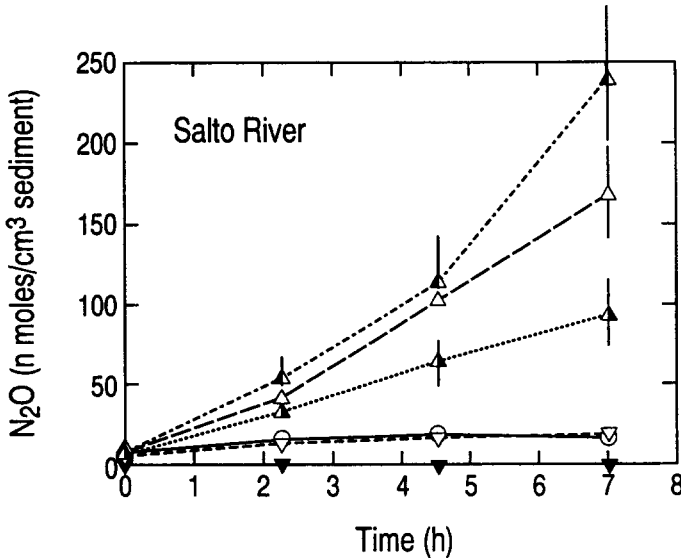


Figure 2. Acetylene block assay applied to triplicate Salto River sediment slurries. No additions (—○—), glucose (—▽—), NO₃⁻ (—△—), NO₃⁻ + glucose (—▲—), NO₃⁻ + glucose + chloramphenicol (···▲···), stream water with NO₃⁻ + glucose (—▼—).

Sediment-water nutrient fluxes

The direction of NO₃⁻ flux in all chamber incubations was from the overlying water into the sediment. Nitrate loss rates from the overlying water measured in six chambers ranged between 9 and 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$ (Table 2). The rate of NO₃⁻ loss in the endogenous chamber was 65 $\mu\text{moles m}^{-2} \text{h}^{-1}$. Acetylene decreased the NO₃⁻ loss rate in the endogenous chambers by 86%. The rate of NO₃⁻ loss after adding NO₃⁻ to the overlying water (initial concentrations in the overlying water of the NO₃⁻-amended chambers were about 75 $\mu\text{mol N L}^{-1}$) was 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$ (Table 2), more than 7 times higher than in the endogenous chamber. In the NO₃⁻-amended chambers that also had C₂H₂ or nitrapyrin added, NO₃⁻ loss rates were lessened 2–5 fold (Table 2). Acetylene inhibited NO₃⁻ loss rates to a much greater extent than did nitrapyrin.

Nitrous oxide increased in the overlying water of chambers incubated with C₂H₂. The recovery of NO₃⁻ as N₂O was 40% in an endogenous incubation, 18% with NO₃⁻ and 24% with NO₃⁻ and nitrapyrin (Table 2). Nitrate was measured as NO₃⁻ plus NO₂⁻, therefore the balance of reduced NO₃⁻ was not NO₂⁻.

Loss of NO₃⁻ from the overlying water in benthic chambers was accompanied by linear decreases of dissolved O₂ (Table 2). Oxygen loss in the overlying water ranged from 230 to 780 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in endogenous and treated chambers (Table 2). Chambers without added C₂H₂ had significantly

Table 2. Nitrate, nitrous oxide and oxygen flux in water overlying Salto River sediments measured in benthic chambers. Negative numbers mean the flux is from the overlying water to the sediment. NM indicates not measured.

Treatment	NO ₃	N ₂ O	O ₂
	umol m ⁻² h ⁻¹		
Nitrate	-470	NM	-725
Nitrate, Nitrapyrin	-263	NM	-742
Nitrate, Acetylene	-97	9	-300
Nitrate, Nitrapyrin, Acetylene	-106	13	-254
Endogenous	-65	NM	-779
Endogenous, Acetylene	-9	2	-229

Table 3. Discharge, inorganic N concentrations, inorganic N mass and inorganic N flux from the streambed to the overlying water in the 1250-m experimental reach of the Salto River.

	Discharge L s ⁻¹	NO ₃	NH ₄	DIN	NO ₃	NH ₄	DIN	NO ₃	NH ₄	DIN
		Conc			Mass			Flux		
		umol N L ⁻¹			mol N h ⁻¹			umol N m ⁻² h ⁻¹		
Upstream	328	13.5	0.8	14.3	15.9	0.9	16.9			
Downstream	544	12.1	1.3	13.4	23.7	2.5	26.2			
Difference	216	-1.4	0.5	-0.9	7.8	1.6	9.4	1010	209	1218

higher O₂ loss rates (avg = 750 umoles m⁻² h⁻¹) than chambers with added C₂H₂ (avg = 260 umoles m⁻² h⁻¹). Nitrapyrin had little effect on O₂ loss rates.

DIN transport through swamp forest streams

Discharge in the Salto River was significantly higher at the downstream station than at the upstream station. Discharge increased by about 216 L s⁻¹ along the reach, from 328 L s⁻¹ to 544 L s⁻¹, an increase of 40% (Table 3). The Pantano River, the major tributary entering this reach, had a discharge of about 36 L s⁻¹ (Triska et al. 1993) contributing no more than about 15% to the increase. The remainder of the increase was due to numerous small springs and groundwater discharge entering the reach.

The concentrations of DIN at the top and bottom of the reach are presented in Table 3. Nitrate concentrations were 13.5 and 12.1 umol N L⁻¹, and NH₄⁺ concentrations were 0.8 and 1.3 umol N L⁻¹ at the top and bottom of the reach, respectively. Collectively, DIN decreased by 0.9 umol N L⁻¹ between up and downstream stations.

DIN mass was calculated to be $16.9 \text{ mol N h}^{-1}$ at the upstream station and $26.2 \text{ mol N h}^{-1}$ at the downstream station for a net increase of 9.4 mol N h^{-1} (Table 3). Assuming this reach of the Salto River contained 7680 m^{-2} of streambed, the downstream increase of DIN per unit area streambed was $1218 \text{ umol N m}^{-2} \text{ h}^{-1}$, 80% of which was NO_3^- .

Discussion

Denitrification measurements

When assayed by the C_2H_2 -block technique, sediment slurries from the Salto River displayed a significant potential for denitrification when NO_3^- was added (Figure 2). Relative to the quantity of functional denitrifying enzymes in these sediments, only a fraction of the activity was expressed without adding NO_3^- , suggesting N, but not C, limitation. These assays demonstrate that simple organic molecules are available in swamp forest stream sediments to support heterotrophic microbial activities including denitrification.

The C_2H_2 -block assays can not accurately assess the status of NO_3^- -reducing activity in sediments with low NO_3^- concentrations because C_2H_2 uncouples processes that generate NO_3^- in stream sediments (Seitzinger et al. 1993). Adding C_2H_2 to sediment slurries to inhibit N_2O reductase activity, or removing O_2 from the flasks to favor NO_3^- reduction over O_2 both uncouple NH_4^+ oxidation to NO_3^- by nitrifying bacteria. In sediments where redox conditions favor NO_3^- as the terminal oxidant, denitrification rates in flasks would be severely underestimated when uncoupled from nitrification. Similarly, in stream habitats where denitrification rates are controlled by advection or diffusion of NO_3^- from groundwater or stream water, incubating the sediments at in situ NO_3^- concentrations would underestimate denitrification as NO_3^- would be rapidly depleted. In order to compare denitrifying enzyme activity between habitats, factors that affect denitrifying enzyme activity were optimized so that none were limiting, including NO_3^- , organic C and O_2 .

When denitrifying enzyme activity was compared per unit volume sediment using the C_2H_2 -block technique, swamp forest sediments from the Pantano and Salto Rivers were comparable with sandy sediments from the Sabalo River, a stream that drains pasture adjacent to rain forest (Table 1). Denitrifying activity was restricted to the finer sands in the Sabalo River, suggesting that sediment size and distribution, which determine hydraulic conductivity, play a role in the distribution of denitrifying activity. Although activity rates were comparable between swamp forest and open pasture streams, the lower availability of fine sands in the coarser Sabalo streambed probably limited the role of denitrification in the surficial sediments.

Swamp forest sediment denitrification rates were significantly higher than rates reported for stream sediments at Little Lost Man Creek, California (Duff & Triska 1990), a coastal, redwood forest stream with gravel-cobble, when both were estimated with the C_2H_2 -block technique. Even when supplemented with NO_3^- and glucose, denitrifying activity was below detection in channel sediment from Little Lost Man Creek. The relatively high concentrations of DIN (28 vs 4 $\mu\text{moles L}^{-1}$ total DIN) and lower interstitial dissolved O_2 in swamp forest streams contrasts with Little Lost Man Creek and imparts an advantage to heterotrophs capable of denitrification in swamp forest streams.

Swamp forest denitrification rates were equivalent to summer rates (25°C) measured in sediments from the Shingobee River, a sand and gravel bottom stream in north central Minnesota (Duff, unpublished data). Although surface water DIN in the Shingobee River during summer (about 4 $\mu\text{moles L}^{-1}$) was lower than in the Salto River, interstitial water less than 2.5 cm below the sediment-water interface contained about 25 $\mu\text{moles L}^{-1}$ (as NH_4^+), suggesting that in the Shingobee River denitrification was closely coupled to nitrification in surficial sediments.

Sediment-water fluxes of nutrients

An areal estimate of denitrification based on N_2O formed in the overlying water in benthic chambers incubated with C_2H_2 ranged from 2–13 $\mu\text{moles m}^{-2} \text{h}^{-1}$ for endogenous, NO_3^- , and NO_3^- plus nitrapyrin-supplemented chambers. These rates are equivalent to 4–26 $\mu\text{moles m}^{-2} \text{h}^{-1}$ based on the 2:1 stoichiometry of NO_3^- reduced to N_2O (Table 4). These rates are lower than estimates from other lotic sediments in which inorganic N levels were elevated compared with the pristine swamp forest streams (Table 4). Cooper & Cooke (1984) reported the highest rates measured in New Zealand stream sediments at approximately 2100 $\mu\text{moles m}^{-2} \text{h}^{-1}$. Christensen & Sorensen (1988) measured rates from 42–458 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in lowland Danish streams. This range in rates resulted from seasonal temperature variations within the sediments with the highest rates associated with high NO_3^- agricultural runoff. Seitzinger (1988) found rates from 210–235 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in Potomac River sediments and from 166–345 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in Delaware River. Seitzinger (1988) considered both rivers impacted by eutrophication. In stream sediments from riparian wetlands, Seitzinger (1994) measured rates less than 20 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in streams draining undisturbed wetland ($NO_3^- = 1 \mu\text{M}$) and 250–405 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in streams draining disturbed wetland ($NO_3^- = 55\text{--}130 \mu\text{M}$).

Nitrate loss rates measured in the overlying water of flux chambers were far higher than NO_3^- loss rates estimated from N_2O formation in nutrient flux

Table 4. Denitrification rates measured by whole-core N_2 flux and the C_2H_2 block methods in stream sediments.

Stream/river name	Location	Watershed characteristic	Denitrification rate $umol\ m^{-2}\ h^{-1}$	DIN conc uM	Method	Comment	Reference
Salto River	Costa Rica	Lowland swamp forest	5-25	25-75	C_2H_2	1	This study
	New Zealand	Pasture, pine catchment	2100	325-1000	C_2H_2	2	Cooper & Cooke 1984
Gelbaek	Denmark	Lowland, agriculture	125-375	50-500	C_2H_2	3	Christensen & Sorensen 1988
Rabis Baek	Denmark	Lowland	40-165	100-150	C_2H_2	3	Christensen & Sorensen 1988
Rabis Baek	Denmark	Lowland	40-460	100-150	C_2H_2	3	Christensen & Sorensen 1988
Potomac	MD	Mainstem	230	>70	N_2	4	Seitzinger 1988
Delaware	NJ	Tidal freshwater portion	165-345	>70	N_2	4	Seitzinger 1988
Skit	NJ	Forested watershed	<20	1	N_2	4	Seitzinger 1994
Hammonton	NJ	Agricultural	250-450	130	N_2	4	Seitzinger 1994

1. Sediment-water flux in benthic chambers with C_2H_2 in the overlying water.

2. Whole cores with C_2H_2 .

3. Flow-thru chambers with C_2H_2 .

4. Whole-cores, N_2 flux.

chambers. Nitrate loss rates were 65 and 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in endogenous and NO_3^- -amended chambers, respectively (Table 2). These estimates compare far more favorably with the estimates cited from other lotic environments (see Table 5). For example, Robinson et al. (1979) found rates from 121–302 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in Swifts Brook by measuring NO_3^- decrease in water overlying cores. It must be kept in mind that NO_3^- lost from the overlying water is not necessarily denitrified since it can be reduced to NH_4^+ by dissimilatory microbial processes (Nishio et al. 1982) and incorporated into biomass.

There are three sources of NO_3^- for denitrification in aquatic sediments including nitrate diffusing into the sediments from the water column, nitrate produced in the sediments from nitrification of NH_4^+ released during organic N mineralization and NO_3^- advected through the sediments from groundwater (Slater & Capone 1987; Christensen & Sorensen 1988; Seitzinger 1988). Nitrate loss measured from the overlying water in this study may have underestimated gross denitrification in the sediment if nitrification was also a source of NO_3^- . It is possible to calculate the amount of NO_3^- produced by nitrification and subsequently denitrified in sediments using 35% of the benthic O_2 consumption measured in flux chambers as an upper limit for NH_4^+ oxidized to NO_3^- (Christensen & Rowe 1984; Henriksen & Kemp 1988; Seitzinger 1988) and a theoretical O_2 requirement for nitrification of 4.33 mg O_2 consumed for each 1.0 mg NH_4^+ -N oxidized to NO_3^- -N (Wezernak & Gannon 1967). This exercise increases the estimate of gross denitrification by approximately 132 $\mu\text{moles m}^{-2} \text{h}^{-1}$, or from 65 and 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$ to 197 and 605 $\mu\text{moles m}^{-2} \text{h}^{-1}$ in endogenous and NO_3^- -amended chambers (no inhibitors), respectively. Thus, in the endogenous chamber, 65% of the gross NO_3^- that was reduced (measured in the overlying water + calculated) could have been formed through nitrification, indicating nitrification may be the predominant NO_3^- source for denitrification in swamp forest sediments. With the addition of NO_3^- to the overlying chamber water, diffusion from the overlying water became the predominant NO_3^- source.

Using the rate for denitrifying enzyme activity from the denitrification assays with chloramphenicol (15 $\text{nmoles cm}^3 \text{sediment}^{-1} \text{h}^{-1}$) and the endogenous estimate of NO_3^- loss from the overlying water in benthic flux chambers (197 $\mu\text{moles m}^{-2} \text{h}^{-1}$), we estimate that streambed sediment 1.3 cm deep was sufficient to reduce all of the NO_3^- transported across the sediment-water interface.

One potential effect of C_2H_2 on microorganisms is inhibition of NH_4^+ oxidation in nitrifying bacteria (Hynes & Knowles 1978). In systems where denitrification is coupled to nitrification, adding C_2H_2 blocks the NO_3^- supply and underestimates gross denitrification. The same is true for nitrapyrin, an inhibitor of nitrification. In swamp forest sediments, adding C_2H_2 or

Table 5. Nitrate reduction rates measured by nitrate loss in water overlying stream sediments.

Stream name	Location	Watershed/stream type	Denitrification rate umol m ⁻² h ⁻¹	DIN conc uM	Comment	Reference
Salto River	Costa Rica	Swamp forest	65-470	25-75	1	This study
Swifts Brook	Ontario	Spring-fed	180-495	700	2	Sain et al. 1977
	The Netherlands	Irrigation ditch	280-480	360	3	van Kessel 1977
Swifts Brook	Ontario	Spring-fed	120-300	120	3	Robinson et al. 1979
Duffin Creek	Ontario	Forest, agricultural	300-745	3-80	2	Hill 1981
Duffin Creek	Ontario	Forest, agricultural	45-180	3-80	3	Hill 1981

1. Sediment-water flux in benthic chambers.

2. Packed sediment columns.

3. Sediment-water flux in cores.

nitrapyrin should have resulted in higher rates of NO_3^- disappearance from the overlying water because NO_3^- formation (and its preferential utilization by sediment NO_3^- -reducing bacteria) in the sediments would be blocked.

Addition of nitrification inhibitors lowered the rate of NO_3^- loss in endogenous and NO_3^- -amended chambers (Table 2); C_2H_2 being more effective than nitrapyrin (97 vs. 263 $\mu\text{moles m}^{-2} \text{h}^{-1}$ compared with 470 $\mu\text{moles m}^{-2} \text{h}^{-1}$). These results are counter intuitive. They instead indicate that NO_3^- formed in the sediments in the absence of inhibitor increased the instantaneous concentration available to denitrifiers and thus the rate of NO_3^- reduction. In addition, NO_3^- produced in the absence of inhibitors may have saturated reducing sites that otherwise might not be saturated by NO_3^- diffusing from the overlying water, thus increasing the instantaneous concentration and the rate of reduction. Because C_2H_2 also inhibited O_2 uptake by 65%, withholding C_2H_2 may have decreased the thickness of the aerobic surface layer by increasing respiration thereby facilitating NO_3^- diffusion from the overlying water. Although chamber studies indicate that NO_3^- does diffuse from the overlying water into the sediments, placing the chambers over the sediments may change the hydrologic and chemical dynamics of the surface sediments (e.g. flow patterns, chemical profiles and, possibly, the depth of the aerobic-anaerobic interface), changing the predominant source of NO_3^- .

DIN transport through swamp forest streams

Under field conditions, DIN mass in the Salto River increased by about 9.4 mol N h^{-1} between upstream and downstream stations (Table 3). Approximately 80% of the increase was in the form of NO_3^- with the balance in NH_4^+ . The increase in DIN mass between stations was associated with increased discharge along the reach in since DIN concentration decreased nominally.

Increase in mass of transported DIN can be attributed to four sources: (1) the Pantano River, (2) small surface seeps or springs along the reach, (3) direct groundwater discharge or transformation of groundwater N and (4) mineralization of organic matter in the streambed.

The Pantano River, the major tributary entering this reach, supplied only a small percentage of the DIN mass as it contributed only 16% of the measured increase in discharge between upstream and downstream stations. The mass of DIN delivered by Pantano River water was calculated to be 0.2 mol N h^{-1} , equivalent only to about 2% of the increased mass.

Assuming the increase in DIN mass was from small surface seepages or springs, the DIN concentration required to elevate the mass by about 9.4 mol N h^{-1} would be 10.3 $\mu\text{mol N L}^{-1}$. This concentration is close to background DIN concentrations observed in 22 seeps and springs along the banks of the Salto (avg = $8.4 \pm 2.8 \mu\text{mol N L}^{-1}$, range 5–15 $\mu\text{mol N L}^{-1}$) (Triska et al.

1993). Although at least five seeps formed small tributaries to the Salto River (Triska et al. 1993), it is unlikely that these springs alone could contribute up to 84% of the increase in discharge observed along the Salto River, which is equivalent to six times the Pantano's discharge.

Rather, the source of increased discharge was likely non point source groundwater passed through the streambed. Preliminary calculations indicate that an increase of 216 L s^{-1} would be equivalent to about 84 L m^{-2} streambed h^{-1} (excluding discharge contributed from the Pantano), approximately twice that measured in a temperate stream in north central Minnesota (Jackman et al. in press).

Triska et al. (1993) measured DIN concentrations in groundwater wells on the banks adjacent to the Salto River. In 9 out of 15 wells, all on the west bank, NH_4^+ concentrations ($22 + 10 \text{ umol N L}^{-1}$) exceeded those required to raise the DIN mass in the channel ($10.3 \text{ umol N L}^{-1}$). Nitrate concentrations averaged only $0.6 + 0.6 \text{ umol N L}^{-1}$ in the same wells. If bankside groundwater is a major source of DIN mass to the channel, and 80% of the downstream export is NO_3^- (Table 3), then most NH_4^+ observed in groundwater would have to be oxidized to NO_3^- during subsurface transport. An analagous situation was observed in groundwater chemistry of the floodplain, bank and stream water in a tropical rain forest watershed in Puerto Rico (McDowell et al. 1992). Six wells on the east bank had low DIN levels. Total DIN was significantly lower ($\text{NH}_4^+ = 2 + 0.5 \text{ umol N L}^{-1}$; $\text{NO}_3^- = 1 + 0.5 \text{ umol N L}^{-1}$) than in west bank wells and insufficient to constitute the increase in DIN mass during channel transport.

While the sparse hydrologic data presents definitive conclusions, the relatively low discharge and DIN concentrations measured from potential surface and groundwater sources suggest that in addition to inorganic N derived from west bank groundwater, mineralized N from organic matter in the streambed is also a possible source of the increase in DIN transport.

Total inorganic N flux from the sediments (release of NH_4^+ , NO_2^- and NO_3^-) can be equated to organic N mineralization in the sediment (Seitzinger 1994). This source can be calculated as the downstream increase of the measured total inorganic N mass divided by the area of the streambed. Nitrification rates for this reach can be calculated as the downstream increase of measured NO_3^- mass (Seitzinger 1994). Both calculations omit any NO_3^- lost by denitrification in the sediments and thus underestimate total mineralization or nitrification rates. Assuming this reach of the Salto River contained approximately 7680 m^{-2} of streambed, organic N mineralization and nitrification rates were $1220 \text{ umol N m}^{-2} \text{ h}^{-1}$ and $1010 \text{ umol N m}^{-2} \text{ h}^{-1}$, respectively.

Despite significant NO_3^- reduction potential, the swamp forest stream reach was a source of DIN to down channel communities during low rainfall

periods. Eighty percent of the DIN potentially released from mineralization of organic N in the streambed or derived from bankside groundwater was nitrified. The large proportion of NO_3^- released to the channel indicated that nitrification in interstitial environments was a major source of NO_3^- for streambed denitrification and for downstream production.

Acknowledgments

This work was supported by National Science Foundation (NSF) grant BSR-91-07772. We thank the Organization for Tropical Studies for infrastructural support. We also would like to thank JM Caffrey and RL Smith for critical review of this manuscript.

References

- Balderston WL, Sherr B & Payne WJ (1976) Blockage by acetylene of N_2O reduction in *Pseudomonas perfectomarinus*. *Appl. Environ. Microbiol.* 31: 504–508
- Bourgeois WW, Cole DW, Riekert H & Gessel SP (1972) Geology and soils of comparative ecosystem study area, Costa Rica. Contribution No. 11, Institute of Forestry Production, University of Washington, Seattle WA
- Bradley PM, McMahon PB & Chapelle FH (1995) Effects of carbon and nitrate on denitrification in bottom sediments of an effluent-dominated river. *Water Resour. Res.* 31: 1063–1068
- Brooks MH, Smith RL & Macalady DL (1992) Inhibition of existing denitrification enzyme activity by chloramphenicol. *Appl. Environ. Microbiol.* 58: 1746–1753
- Capone DG & Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. *Limnol. Oceanogr.* 33: 725–749
- Christensen PB & Rowe GT (1984) Nitrification and oxygen consumption in northwest Atlantic deep-sea sediments. *J. Mar. Res.* 42: 1099–1116
- Christensen PB & Sorensen J (1988) Denitrification in sediment of lowland streams: Regional and seasonal variation in Gelbaek and Rabis Baek, Denmark. *FEMS Microbiol. Ecol.* 53: 335–344
- Cooper AB & Cooke JG (1984) Nitrate loss and transformation in 2 vegetated headwater streams. *N.Z. J. Mar. Freshwater Res.* 18: 441–450
- Duff JH & Triska FJ (1990) Denitrification in sediments from the hyporheic zone adjacent to a small forested stream. *Can. J. Fish. Aquat. Sci.* 47: 1140–1147
- Herbert RA & Nedwell DB (1990) Role of environmental factors in regulating nitrate respiration in intertidal sediments. In: Revsbech NP & Sorensen J. (Eds.) *Denitrification in Soil and Sediment* (pp 77–90). Plenum Press, New York
- Henricksen K & Kemp WM (1988) Nitrification in estuarine and coastal marine sediments. In: Blackburn TH & Sorensen J (Eds) *Nitrogen Cycling in Coastal Marine Environments* (pp 207–249). John Wiley, Chichester
- Hill AR (1981) Nitrate-nitrogen flux and utilization in a stream ecosystem during low summer flows. *Can. Geogr.* 25: 225–239
- Hynes RK & Knowles R (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. *FEMS Microbiol. Lett.* 4: 319–321
- Jackman AP, Triska FJ & Duff JH (in press) Hydrologic examination of groundwater discharge into the upper Shingobee River. In: Averett RC & Winter TC (Eds) *Interdisciplinary*

- Research Initiative: Hydrologic and Biogeochemical Research in the Shingobee River Headwaters Area, North Central Minnesota. *Water Resour. Invest.* (pp)
- McDowell WH, Bowden WB & Asbury CE (1992) Riparian nitrogen dynamics in two geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns. *Biogeochemistry* 18: 53–75
- Nishio T, Koike I & Hattori A (1982) Denitrification, nitrate reduction, and oxygen consumption in coastal and estuarine sediments. *Appl. Environ. Microbiol.* 43: 648–653
- Paaby P & Goldman CR (1992) Chlorophyll, primary productivity, and respiration in a lowland Costa Rican stream. *Rev. Biol. Trop.* 40: 185–198
- Pringle CM & Triska FJ (1991) Effects of geothermal groundwater on nutrient dynamics of a lowland Costa Rican stream. *Ecology* 72: 951–965
- Pringle CM, Triska FJ & Browder G (1990) Spatial variation in basic chemistry of streams draining a volcanic landscape on Costa Rica's Caribbean slope. *Hydrobiologia* 206: 73–85
- Pringle CM (1991) Geothermally-modified waters surface at La Selva Biological Station, Costa Rica: volcanic processes introduce chemical discontinuities into lowland tropical streams. *Biotropica* 23: 523–529
- Pringle CM, Rowe L, Triska FJ, Fernandez JF & West J (1993) Landscape linkages between geothermal activity and solute composition and ecological response in surface waters draining the Atlantic slope of Costa Rica. *Limnol. Oceanogr.* 38: 753–774
- Robinson JB, Whiteley HR, Stammers WN, Kaushik NK & Sain P (1979) The fate of nitrate in small streams and its management implications. In: Loehr RC, Haith DA, Walter MF, & Martin CS (Eds) *Best Management Practices for Agricultural and Silviculture* (pp 247–259). Ann Arbor Science Publishers Inc., Ann Arbor, MI
- Sain P, Robinson JB, Stammers WN, Kaushik NK & Whitley HR (1977) A laboratory study of the role of stream sediment in nitrogen loss from water. *J. Environ. Qual.* 6: 274–278
- Seitzinger SP (1988) Denitrification in freshwater and coastal ecosystem: Ecological and geochemical significance. *Limnol. Oceanogr.* 33: 702–724
- Seitzinger SP (1994) Linkages between organic matter mineralization and denitrification in eight riparian wetlands. *Biogeochemistry* 25: 19–39
- Seitzinger SP, Nielsen LP, Caffrey J & Christensen PB (1993) Denitrification measurements in aquatic sediments: A comparison of three methods. *Biogeochemistry* 23: 147–167
- Slater JM & Capone DG (1987) Denitrification in aquifer soils and nearshore marine sediments influenced by groundwater nitrate. *Appl. Environ. Microbiol.* 53: 1292–1297
- Tiedje JM, Sexstone AJ, Myrold DD & Robinson JA (1982) Denitrification: ecological niches, competition & survival. *Antonie van Leeuwenhoek* 48: 569–583
- Triska FJ, Pringle CM, Zellweger GW, Duff JH & Avanzino RJ (1993) Dissolved inorganic nitrogen composition, transformation, retention, and transport in naturally phosphate-rich and phosphate-poor tropical streams. *Can. J. Fish. Aquat. Sci.* 50: 665–675
- van Kessel JF (1977) Removal of nitrate from effluent following discharge on surface water. *Water Res.* 11: 533–537
- Wezernak PP & Gannon PP (1967) Theoretical oxygen requirements of nitrification. *Appl. Environ. Microbiol.* 15: 1211–1215
- Yoshinari T & Knowles R (1976) Acetylene inhibition of nitrous oxide reductase by denitrifying bacteria. *Biochem. Biophys. Res. Commun.* 69: 705–710