

Sediment size and nutrients regulate denitrification in a tropical stream

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Abstract. Landuse changes might alter N cycling in tropical aquatic ecosystems, but understanding of N cycling in tropical streams is limited. We measured actual and potential denitrification rates during the dry season in Río Las Marías, a 4th-order Andean piedmont stream in Venezuela. Our objectives were to describe spatial and temporal variation in denitrification, quantify the effects of nutrient availability and substratum particle size on denitrification, and explore potential effects of anthropogenic sedimentation on denitrification. In 4 experiments, actual and potential denitrification rates ranged from 0 to 160 and from 0 to 740 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$, respectively. Rates were distributed approximately log-normally because of spatial variation. During a 1-mo period, actual denitrification rates decreased exponentially from 37 ± 39 to $5 \pm 7 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ (mean \pm SD), probably because of a decline in water-column $\text{NO}_3\text{-N}$ concentration from 41 ± 14 to $12 \pm 3 \mu\text{g NO}_3\text{-N/L}$. The texture (particle size) of stream substrata markedly affected denitrification rates. Actual rates were low in cobble, gravel, and fine sediments (<5 mm), but in fine sediments, rates increased in response to addition of excess $\text{NO}_3\text{-N}$ and organic C. In a 3-km stream reach, actual (but not potential) denitrification rates increased with the proportion of fine sediments (<2 mm) in mixed substrata. This increase was nonlinear, and the threshold value occurred at 37% fine particles, above which actual denitrification rates were almost always high. An experiment simulating the effects of anthropogenic sedimentation showed that topsoil inputs resulted in denitrification rates $\sim 8\times$ higher than rates in trials where excess $\text{NO}_3\text{-N}$ and organic C were supplied. Denitrification is a small but potentially significant sink for available N in this N-limited system. Anthropogenic sedimentation associated with landuse change might significantly increase denitrification rates in streams.

Key words: denitrification, stream, nitrogen cycle, tropics, anthropogenic sedimentation, land use.

Rapid landuse change in the tropics is an environmental issue of tremendous local and global concern. Among other effects, landuse change might have important consequences for N cycling in aquatic systems (Downing et al. 1999, Neill et al. 2001, Biggs et al. 2004). In general, these changes are expected to result in increased N loads to streams and rivers via

processes such as leaching, erosion, and agricultural intensification. In the temperate zone, the consequences of anthropogenic alteration of the N cycle in aquatic systems include eutrophication, reduced biodiversity, and coastal hypoxia (Vitousek et al. 1997, Carpenter et al. 1998). Yet, the consequences of landuse change for N cycling in tropical streams are poorly understood, in large part because relatively little is known about the N cycle in these ecosystems. Low N:P ratios and some experimental evidence suggest that many tropical streams are N-limited (Downing et al. 1999, Flecker et al. 2002). If so, alterations to the N cycle might be particularly important in these systems and have far-reaching consequences for the trophic basis of produc-

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tion. Better description of N cycle processes in tropical streams clearly is needed.

Denitrification, the microbially mediated transformation of NO_3 to N_2 , is the dominant pathway for losses of biologically available N from ecosystems (Seitzinger 1988, Seitzinger et al. 2006). Denitrification occurs in anaerobic conditions when organic C compounds are available as electron donors and NO_3 is present as an electron acceptor. Thus, denitrification rates might be limited by O_2 , organic C, or NO_3 availability (e.g., Piña-Ochoa and Álvarez-Cobelas 2006). Most denitrification occurs in anoxic zones or microsites in the streambed substrata in streams that are generally well oxygenated throughout their water columns. Stream denitrification rates vary widely in the temperate zone (from below detection to several hundred $\text{mg m}^{-2} \text{d}^{-1}$ or higher) in response to factors including NO_3 , organic C, and dissolved O_2 (DO) concentrations (Sheibley et al. 2003, Inwood et al. 2005, Piña-Ochoa and Álvarez-Cobelas 2006). The very few estimates available from tropical or subtropical streams indicate similar rates (Duff et al. 1996; Bartkow and Udy 2004; J. D. Potter, W. H. McDowell, J. L. Merriam [University of New Hampshire], and B. J. Peterson, and S. M. Thomas [Marine Biological Laboratory], unpublished data). However, these data are too few to provide full understanding of tropical rates or comparisons with temperate zone streams. Differences in temperature, magnitude of N yields from the watershed (Lewis et al. 1999), seasonality of precipitation and loading from the watershed, and other factors potentially could create differences in denitrification rates and controls between temperate and tropical streams.

Our 1st objective was to characterize denitrification rates in a tropical stream in the Andean piedmont. No previous estimates of denitrification rates were available for streams in this region. We focused our analysis on Río Las Marías, where we have been studying N cycling and other ecosystem properties and processes for several years (Flecker et al. 2002, Vanni et al. 2002, Taylor et al. 2006, McIntyre et al. 2007, 2008). Removal of N via denitrification could be particularly important in Río Las Marías because, as in many tropical streams, primary producers are chiefly N-limited in this system (Holmes et al. 1996, Flecker et al. 2002). Given the limited amount of information on denitrification in tropical streams, we measured actual and potential rates of denitrification and addressed fundamental questions about spatial and temporal variability in these rates.

Our 2nd objective was to explore how denitrification rates might change in response to anthropogenic sedimentation. Forest clearing is an important and

widespread landuse change in the tropics (Houghton 1994), and increased erosion following clearing often increases sediment loading to streams (Dunne 1979, Douglas et al. 1992). Denitrification requires anoxia, so fine-textured sediments with low porosity (and thus, reduced O_2 diffusion) often have high denitrification rates. This relationship between particle size and denitrification rate has been observed in terrestrial and aquatic sediments (Groffman and Tiedje 1989, Garcia-Ruiz et al. 1998b, Pinay et al. 2000). Río Las Marías resembles other watersheds in its region and throughout the tropics in that significant deforestation and landuse change currently are occurring (Karwan et al. 2001, Allan et al. 2002). We quantified the relationship between denitrification rates and substratum particle size and tested whether denitrification rates increased in response to a simulated input of terrestrial sediments.

Methods

Site description

We measured denitrification rates across a 3-km reach of Río Las Marías, Venezuela. Río Las Marías is in the Andean piedmont, in the Río Apure watershed (lat 9°01'N, long 69°45'W). The Río Las Marías watershed, like others in the region, has seen increasing landuse intensity in recent decades. Undisturbed forest represented ~60% of land cover in the watershed in 1995 (Allan et al. 2002), but forest clearing for agriculture and settlements has continued since that time (ASF, personal observation). At the study site (elevation 225 m), Río Las Marías is a 4th-order stream with a watershed area of 210 km^2 . Discharge during the dry season (December–April) ranges from $<0.1 \text{ m}^3/\text{s}$ to $0.5 \text{ m}^3/\text{s}$, and wetted width is ~10 m (Allan et al. 2006). Water temperatures are warm (~30°C during the day). The substrata are predominantly sand, gravel, and cobble, but finer particles are present in depositional zones. During the dry season, gross primary production is ~0.5 to $1.0 \text{ g C m}^{-2} \text{ d}^{-1}$, and primary producers are chiefly N-limited (Flecker et al. 2002, Taylor et al. 2006). All of the experiments reported here were conducted during the dry seasons of 2004 and 2006.

Denitrification estimates

We measured actual and potential denitrification rates using the acetylene block technique (Tiedje et al. 1989, Groffman et al. 1999). Acetylene prevents the reduction of N_2O to N_2 , allowing estimation of denitrification rate based on the accumulation of N_2O . This technique has some limitations, including

TABLE 1. Summary of methods and results for the 4 experiments exploring denitrification rates in Río Las Marías. Experiment numbers correspond to those given in Methods section. *Objective* is a summary of the experimental objective (see Methods for further details). Under *Method*, a = acetylene block, and c = chloramphenicol added to incubations to inhibit enzyme production. *N amend* and *C amend* give target concentrations above ambient of N (added as KNO₃) and C (added as dextrose or glucose) in potential denitrification assays. *Actual rates* are for unamended incubations, and *potential rates* are for incubations amended with excess C and N. Rates are given as means (range).

Experiment	Objective	Date	Method	N amend (mg N/L)	C amend (mg C/L)	Actual rate (μg N ₂ O-N m ⁻² h ⁻¹)	Potential rate (μg N ₂ O-N m ⁻² h ⁻¹)
1	Temporal variation	15 January– 18 February 2004	a	NA	NA	14.46 ^a (0–155.96)	NA
2	Effect of substratum size (cobble/gravel/fines)	12–18 January 2006	a, c	100	40	1.65 ^b (0–5.87)	5.35 ^b (0–21.20)
3	Effect of fine particles	12–16 January 2004	a	10	50	9.55 (0–46.10)	99.69 (0–744.68)
4	Effect of topsoil input	21 January 2004	a	6	30	0 (0–0)	8.11 (0–16.02)

^a Summary statistics calculated across all sample dates

^b Summary statistics reported for gravel substratum type

the fact that acetylene also inhibits the production of new NO₃⁻ via nitrification (Seitzinger et al. 1993, Groffman et al. 1999). For this reason (and several others described later), our measured denitrification rates should be considered minimum estimates. Actual denitrification rates were measured under field conditions of DO and nutrient concentrations. This approach provides a lower-bound estimate for true in situ denitrification rates because nitrification is inhibited and natural anoxic microsites might be disturbed in the process of collecting samples. Potential denitrification rates typically are measured under optimal conditions for denitrification (by supplying excess NO₃ and organic C and ensuring anoxia by flushing with O₂-free gas) and, therefore, provide an upper-bound estimate for true in situ denitrification rates. In our experiments, logistical constraints prevented us from inducing anoxia. Therefore, our potential denitrification rates approximate field rates under conditions of excess NO₃ and organic C but incomplete anoxia, and, as such, they are probably lower than those reported in other studies.

We conducted 4 experiments with different goals (Table 1). Some of our methods varied slightly among the 4 experiments; we describe these methods in detail here and summarize them in Table 1. In all of our denitrification assays, we shaded incubation chambers to reduce photosynthetic uptake of nutrients and immersed them in the stream to maintain ambient stream temperature.

In experiment 1, we measured actual denitrification rates at approximately weekly intervals for 1 mo in a single 150-m stream reach. On each sampling occasion, we used a small scoop to collect 20 samples of mixed

substratum (predominantly gravel and sand) from haphazardly selected locations across the channel. Each sample (~50 mL of substratum) was transferred to a glass canning jar (0.3 L) fitted with a butyl septum in the lid, and stream water was added to bring the total volume to 100 mL. At the beginning of the incubation, we injected 60 mL of acetylene gas into each jar and then gently shook the jar to distribute the gas throughout the sample. We also measured discharge and mean daily water-column NO₃-N concentrations approximately weekly in association with this experiment. We calculated discharge based on stream cross-sectional area and current velocity measurements taken with a Marsh–McBirney flow meter (Marsh–McBirney, Frederick, Maryland). We measured NO₃-N concentrations over a 24-h period on each sampling occasion, during which we collected 20 to 21 water samples from the thalweg. We filtered water (Pall A/E, 1-μm pore size; Pall Corporation, East Hills, New York) and stored it frozen until NO₃-N concentrations of thawed samples were determined colorimetrically on an autoanalyzer (Astoria-Pacific International, Clackamas, Oregon) in our laboratory in the US.

In experiment 2, we measured actual and potential denitrification rates in 3 substratum size classes: cobble (3–25 cm), gravel (0.5–3 cm), and fine sediments (<0.5 cm). Particle sizes were estimated in situ before collecting samples for incubations. Thus, these size class designations are not exclusive but instead reflect the dominant particle size in each sample. We chose sampling locations to maximize the representation of the nominal size class and measured particle sizes at the conclusion of incubations to confirm that each

sample did represent its nominal size class. Substrata were incubated in polycarbonate food service containers (2.170 L) with O-ring seals. We ran 8 to 17 replicate incubations for both actual and potential denitrification rates in each substratum size class. For potential denitrification assays, we added NO_3^- and glucose to target concentrations of 100 mg $\text{NO}_3\text{-N/L}$ and 40 mg C/L above background (Groffman et al. 1999). In this experiment only, we also added chloramphenicol to a target concentration of 0.3 mM to inhibit bacterial production of new enzymes in response to the excess NO_3^- and organic C available in the potential assays (Smith and Tiedje 1979).

Experiment 3 complemented experiment 2 and focused on the ways in which variation in the proportion of fine particles (<2 mm) in mixed sediments affected denitrification rates. We used the methods described for experiment 1 to collect and incubate substratum samples from several runs and pools spanning the 3-km study reach. We ran actual and potential denitrification assays from each sampling location ($n = 80$ assays total). Potential assays were amended with NO_3^- and dextrose to target concentrations of 10 mg $\text{NO}_3\text{-N/L}$ and 50 mg C/L. At the conclusion of incubations, we dried the sediments in a solar oven at $\sim 65^\circ\text{C}$ and then weighed the fine (<2 mm) and coarse (>2 mm) portions after separating them by sieving. We calculated the proportion (by mass) of fine particles in the sediments and used this proportion as a predictor of denitrification rates.

In experiment 4, we examined the effects of topsoil inputs on denitrification rates. We collected 10 substratum samples from a single gravel run using the methods described already. Each sample was split into 3 subsamples, which were randomly assigned to 1 of 3 treatments: actual denitrification assay (control), potential denitrification assay (nutrients added, target concentrations of 30 mg C/L and 6 mg $\text{NO}_3\text{-N/L}$), and topsoil-amended. For the topsoil treatment, we added 25 mg of sieved (2 mm) mineral topsoil collected from the upper 5 cm of the A horizon at a site ~ 100 m from the river. This soil was quite dry because we conducted experiment 4 during the middle of the dry season. When added to the incubation jars, this mass of soil created a thin layer (<5 mm) on top of the stream sediments. We chose the mass of soil to mimic realistic fine sediment accumulations that we had observed in nearby streams with disturbed watersheds, and we adjusted the amendment in the potential treatment to match expected N content in the soil. We extracted soil NO_3^- in the field using the KCl method (Robertson et al. 1999) and quantified it colorimetrically on an

Astoria–Pacific autoanalyzer in our laboratory in the US.

We used gas-tight syringes to collect 7.5- to 10-mL gas samples from the headspace of each chamber at the beginning and end of 4-h incubation periods and injected the samples into pre-evacuated 5-mL plastic containers with butyl stoppers (Vacutainer; Becton–Dickinson, Franklin Lakes, New Jersey). We collected additional headspace samples from subsets of incubations as replicates or to check for linearity of denitrification rate over the 4-h incubation period. We measured N_2O concentrations of samples by gas chromatography. We injected 100- μL aliquots of sample into gas chromatographs (Shimadzu GC-14A, Shimadzu Corp., Kyoto, Japan; Varian Model 3800, Palo Alto, California) equipped with electron capture detectors and Poropak Q, HayeSep R, or Unibeads C columns (Alltech, Deerfield, Illinois). We set oven temperatures at 70 to 80°C and detector temperatures at 290 to 320°C . We set flow rates of ultrapure N_2 carrier gas at 30 to 50 mL/min. Laboratory standards were made from 1, 10, and 100 ppm ultrahigh-purity N_2O gasses (Scott Specialty Gasses, Plumsteadville, Pennsylvania). We calculated denitrification rates as the linear rate of N_2O accumulation in the incubation jars (Groffman et al. 1999). We used an estimate of the surface area of streambed sampled by our scoop (or a direct measure of surface area for cobbles in experiment 2) to express denitrification rates on a per area basis. We prepared standards to check for leakage of N_2O during transport because all of our gas samples were transported by air from the field site to our laboratory. We used the same type of containers that were used for field samples and prepared 2 standards at each of 2 N_2O concentrations (5.55 and 55.5 $\mu\text{mol N}_2\text{O/L}$). Standards and samples had slightly different histories: standards had to be transported to the field site and back again, whereas samples made only one trip, and N_2O content of standards were not analyzed until the last sample had been analyzed (within 5 mo of collection). Thus, these travel standards provided an estimate of the maximum possible leakage that could have occurred, but they were not appropriate for making a leakage correction for our samples.

Statistical analyses

Some calculated denitrification rates were negative; we interpreted these as below detection limit and set them equal to 0. We $\log_{10}(x + 1)$ -transformed all data prior to analysis to normalize distributions. We analyzed the results of experiments 2 and 4 by analysis of variance (ANOVA) with the Tukey adjustment for subsequent pairwise comparisons among treatment

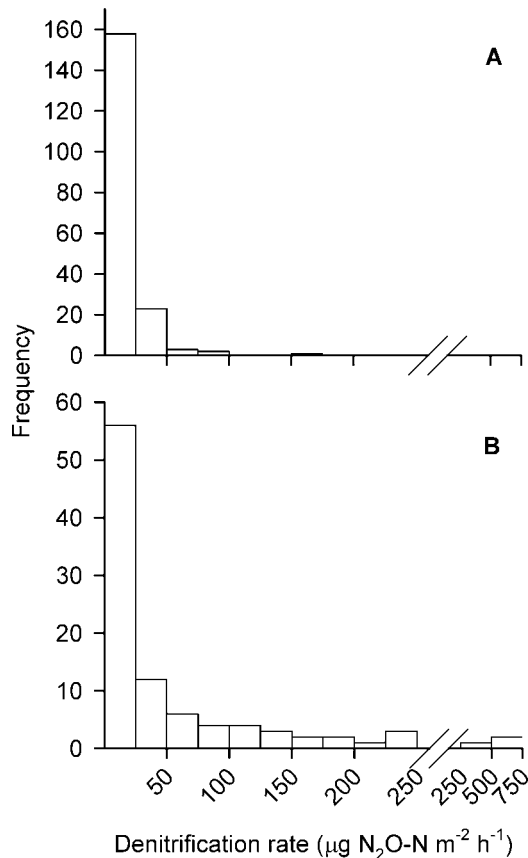


FIG. 1. Frequency distributions of actual (incubations not amended) denitrification assays (A) and potential (incubations amended with excess C and N as dextrose and NO_3) denitrification assays ($n = 86$) plus assays amended with topsoil but not inorganic nutrients ($n = 10$) (B) across all experiments in this study. One outlier (denitrification rate = $2588 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$) was omitted from panel B. Log-normal distributions of rates were observed within each experiment and treatment. Breaks in the x-axis highlight change in bin size.

means. For experiment 3, we fit linear regressions to describe the relationship between denitrification rates (actual and potential) and % fine particles in the substratum. We also used the 2-dimensional Kolmogorov–Smirnov test to identify thresholds in this relationship (Garvey et al. 1998).

Results

Denitrification rates varied substantially over time and in response to particle size and nutrient availability. Across all of the experiments, actual rates ranged between 0 and $156 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, and potential rates ranged between 0 and $745 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (excludes 1 outlier with rate = $2588 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$). Rates within treatments or dates appeared to be

approximately log-normally distributed; most rates were close to 0, although there were a few substantially larger rates (Fig. 1). Distributions of actual rates were generally narrower and centered near 0, whereas distributions of potential rates were much wider and included more high rates than did distributions of actual rates, although many low rates also were present. Hourly samples from 6 incubations demonstrated that rates of N_2O accumulation were linear. Leakage of N_2O from vial containers might have biased rate estimates toward low values and contributed to measured variability; 4 standards measured 5 mo after being transported to and from the field site lost $30 \pm 14\%$ (mean \pm SD) of their N_2O .

During the dry season, actual denitrification rates declined considerably from $36.8 \pm 39.2 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ in mid-January to $5.2 \pm 6.7 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ by mid-February (experiment 1; Fig. 2A). This decline was concomitant with a similar decline in water-column $\text{NO}_3\text{-N}$ concentrations from 41 ± 3 to $12 \pm 1 \mu\text{g NO}_3\text{-N/L}$ (Fig. 2B), and with a decline in discharge from 460 to 120 L/s (Fig. 2C).

In experiment 2, denitrification rates were near 0 in all treatments except in fine sediments when nutrients were added (Fig. 3). The interactive effect of substrate size and nutrient amendment was statistically significant ($F_{2,68} = 23.8$, $p < 0.0001$), so we did not test for main effects of substrate size and nutrient amendment but instead compared treatment-level means. Denitrification rates were greater in the potential fine sediment treatment than in any other treatments (Tukey-adjusted $p < 0.0001$ in all cases), but no other significant differences occurred among treatments ($p > 0.3$). Mean rates were $439 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ in the potential fine sediment treatment and between 1.6 and $5.3 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ in the other treatments. When we exclude 1 extreme value from the potential fine sediment treatment, the mean decreases from 439 to $132 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, but there is no qualitative change in the results of the statistical analysis.

Experiment 3 also suggested that a combination of fine particles and adequate nutrients promotes high denitrification rates. Across all of the substratum samples, the mean % fine particles (<2 mm) was $32 \pm 25\%$ ($n = 80$). A positive relationship was found between % fine particles and actual denitrification rate ($F_{1,38} = 6.0$, $p = 0.02$; Fig. 4A). This result was robust to the influence of several large residuals, but a linear model probably is not the best fit to these data; rates were variable but low at <40% fine particles (mean = $7.21 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$) and almost always high at >40% fine particles (mean = $17.62 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$; Fig. 4A). We quantified this pattern using the 2-dimensional Kolmogorov–Smirnov test, which dem-

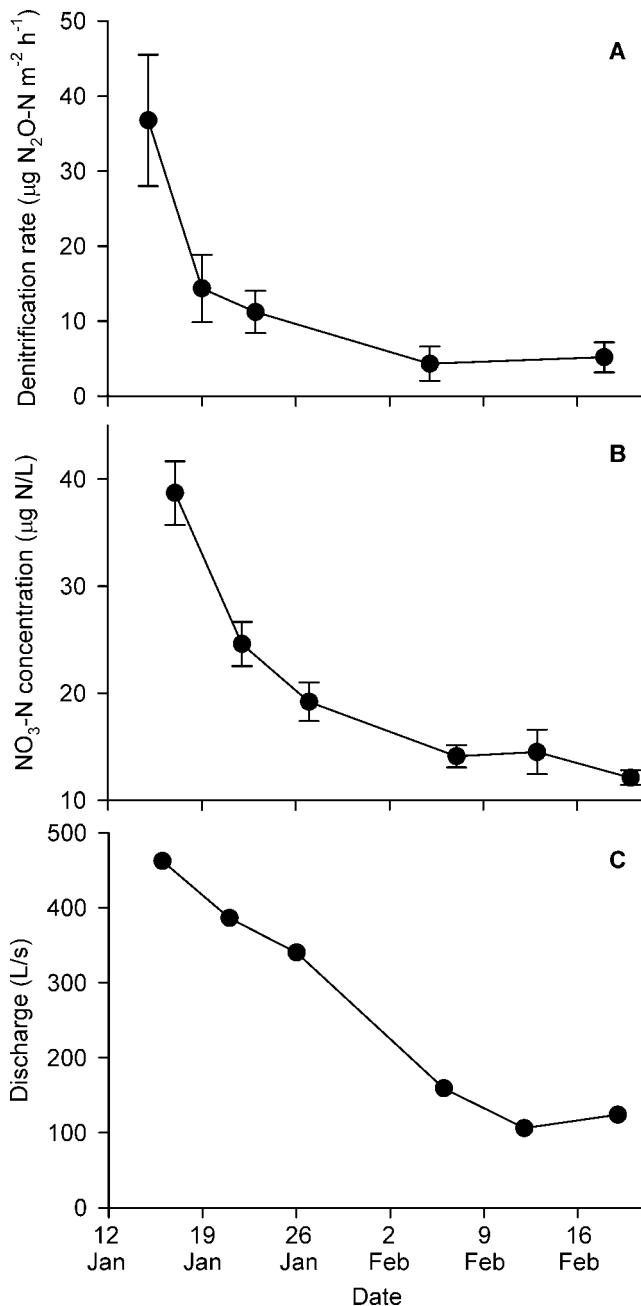


FIG. 2. Mean (± 1 SE) actual (incubations not amended) denitrification rate measured on each date (2004) in samples of mixed substrata (gravel and sand) collected from a single 150-m reach ($n = 19$ – 20) (A), water-column $\text{NO}_3\text{-N}$ concentration measured in samples spanning a 24-h period on each sampling date, in the same stream reach ($n = 20$ – 21) (B), and discharge during the dry season in Río Las Marías (C). Sampling dates differ slightly among the panels.

onstrated that denitrification rate and % fine particles were not distributed independently ($D_{\text{BKS}} = 0.11$, $p = 0.01$), and which identified a threshold value of % fine particles of 37.2%. In contrast to the results for actual

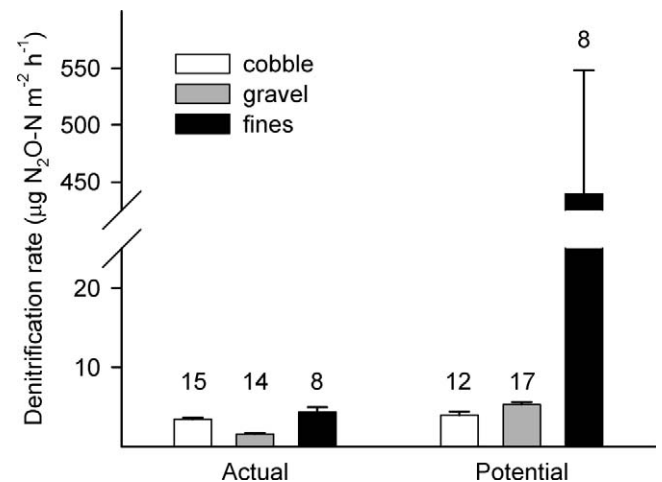


FIG. 3. Mean (± 1 SE) actual (incubations not amended) and potential (incubations amended with excess C and N as dextrose and NO_3) denitrification rates measured in 3 different substratum types in Río Las Marías: cobble (3–25 cm), gravel (0.5–3 cm), and fine sediments (< 0.5 cm). Numbers of incubations run in each treatment are indicated above the bars. The fine sediment potential treatment is significantly different from the others (Tukey's t -test, $p < 0.0001$).

denitrification rates, the relationship between potential denitrification and % fine particles was not statistically significant ($F_{1,38} = 0.8$, $p = 0.4$; Fig. 4B). Potential denitrification rates were significantly higher than actual rates (mean = 99.69 and 9.55, respectively; $t_{75} = 5.6$, $p < 0.0001$), and almost all showed non-0 rates; 0 rates were observed only at $< 46\%$ fine particles (Fig. 4B).

Topsoil addition substantially increased denitrification rates. Denitrification rate differed significantly among actual, potential, and topsoil-amended treatments ($F_{2,27} = 90.8$, $p < 0.0001$; Tukey-adjusted $p < 0.0001$ for all pairwise comparisons; Fig. 5). Actual rates in this experiment were always below detection limit. Potential rates were positive but low ($8.1 \pm 6.2 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, range = 0–16.0), whereas amendment with topsoil resulted in high rates ($67.9 \pm 28.7 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, range = 25.5–118.8). The $\text{NO}_3\text{-N}$ content of added topsoil was $15.5 \pm 6.9 \mu\text{g/g}$ ($n = 3$), so the topsoil addition introduced $\sim 390 \mu\text{g}$ of $\text{NO}_3\text{-N}$ into the incubation chambers. In comparison, $\sim 300 \mu\text{g}$ of $\text{NO}_3\text{-N}$ was added in the potential treatment.

Discussion

Actual denitrification rates in Río Las Marías varied spatially, temporally, and in response to NO_3 and organic C amendments and substratum particle size. Spatial variation in denitrification rate was consider-

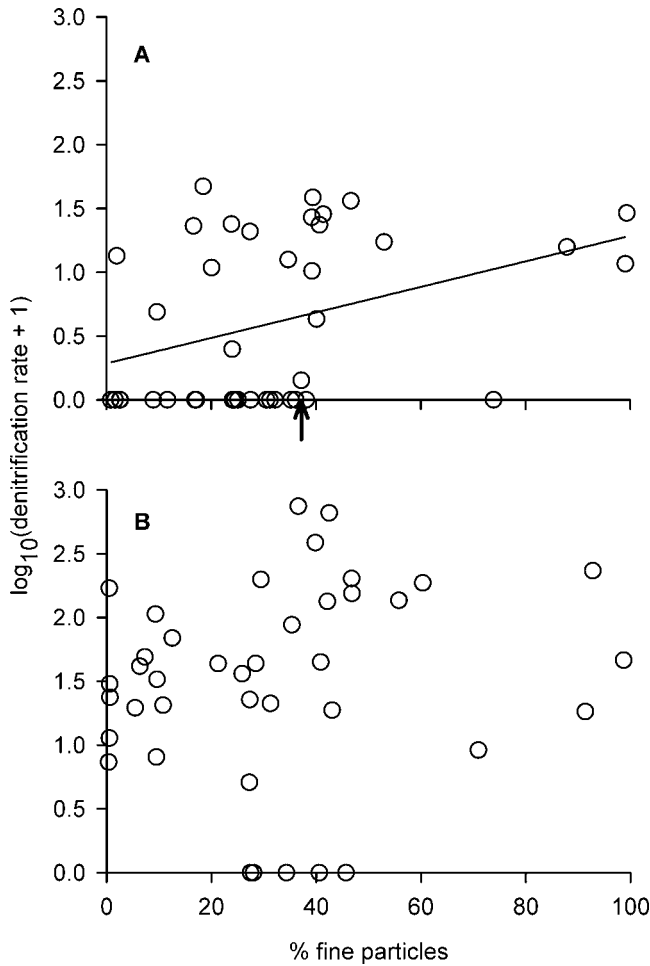


FIG. 4. Relationship between actual (incubations not amended) (A) and potential (incubations amended with excess C and N as dextrose and NO_3) (B) denitrification rates ($\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$) and substratum particle size (measured as % by mass of fine particles <2 mm). Samples were collected at multiple sites spanning a 3-km reach of Río Las Marías. Substratum texture was positively related to actual denitrification rates (solid line; $p = 0.02$) but not to potential rates ($p = 0.4$). Arrow in panel A shows the threshold value indicated by the 2-dimensional Kolmogorov–Smirnov test ($p = 0.01$). Below this value (% fine particles = 37.2%), variance in denitrification rate is not constrained by % fine particles.

able among samples collected in close proximity and subjected to the same experimental treatments. In each of our experiments, distributions of denitrification rates were extremely skewed; very low rates were found at most sites, and a few sites showed substantially higher rates (Fig. 1). Such patchiness is a common feature of denitrification; in any area, total denitrification seems to occur mostly in relatively few hotspots of activity (Parkin 1987, Royer et al. 2004, Orr et al. 2007).

The availability of NO_3 and organic C substrates is

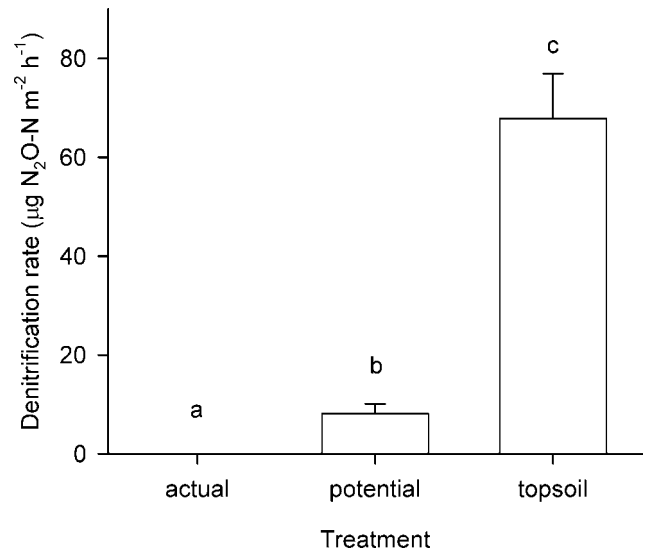


FIG. 5. Mean (+1 SE) denitrification rate in each of 3 treatments ($n = 10/\text{treatment}$): actual (incubations not amended), potential (incubations amended with excess C and N as dextrose and NO_3), and topsoil (incubations amended with 25 g of mineral topsoil). Treatments with different letters are significantly different (Tukey's t -test, $p < 0.0001$). Denitrification was below detection limit in all of the actual incubations.

an important factor limiting denitrification rates. In particular, much of the variation in denitrification rates across aquatic environments can be explained by the concentration of NO_3 in the overlying water (Piña-Ochoa and Álvarez-Cobelas 2006, Mulholland et al. 2008). The denitrification rates that we measured in Río Las Marías (mean = $0.23 \text{ mg m}^{-2} \text{ d}^{-1}$ in experiment 3, with $[\text{NO}_3^-] \approx 0.025 \text{ mg/L}$) were similar to those measured in other systems with similarly low water-column NO_3 concentrations. For instance, in 2 temperate streams, rates were $0.13 \text{ mg m}^{-2} \text{ d}^{-1}$ at $0.027 \text{ mg NO}_3/\text{L}$ and $1 \text{ mg m}^{-2} \text{ d}^{-1}$ at $0.024 \text{ mg NO}_3/\text{L}$ (Martin et al. 2001, Kemp and Dodds 2002a). Furthermore, we observed substantial increases in denitrification in mixed sediments (though not in cobble or gravel) when we added excess NO_3 and organic C (Figs 3, 4, 5).

Declining water-column NO_3 concentrations drove a $7\times$ decrease in actual denitrification rates over 1 mo early in the dry season (Fig. 2). The alternate explanation, that denitrification drove decreases in water-column NO_3 , is not consistent with our understanding of the Río Las Marías N budget. Mean denitrification rates in our survey were $0.23 \text{ mg m}^{-2} \text{ d}^{-1}$, but uptake rates for NO_3 measured by nutrient releases in 2004 averaged $27.1 \text{ mg N m}^{-2} \text{ d}^{-1}$ (McIntyre et al. 2008). Thus, very little of NO_3 uptake is attributable to denitrification, and declining NO_3 concentrations are probably the cause, not the result,

of declining denitrification rates. Few studies have measured denitrification rates at the approximately weekly intervals that we considered here, but water-column NO_3 concentrations have been observed to drive monthly to seasonal variation in denitrification rates in several cases (Martin et al. 2001, Inwood et al. 2005). Water temperature and residence time also can affect denitrification rates, but neither is a likely explanation for the temporal pattern that we observed (Sjodin et al. 1997, Garcia-Ruiz et al. 1998a). Water temperature changed minimally during the 1-mo period that we considered. Residence time influences the proportion of NO_3 that is removed from a water mass (and thus, the denitrification rate as measured by whole-stream methods), but this reach-scale attribute does not come into play in determining the rate at which NO_3 is removed, as measured by our chamber-based incubation experiments.

Declining water-column NO_3 concentrations might have been driven by the decline in surface runoff (to near 0) that occurs as the dry season progresses. This explanation assumes that loading from the watershed is a significant source of N to the stream. Low NO_3 concentrations later in the dry season (Fig. 2B) might then be maintained by instream processes such as N_2 fixation followed by nitrification. We have not always observed declines in water-column NO_3 in Río Las Marías. For instance, in 1998 and 1999, concentrations were similar to those reported here (range 18 to 60 $\mu\text{g N/L}$) but showed little change during the dry season (Flecker et al. 2002). In such years, denitrification might remain relatively high throughout the dry season.

Denitrification is a small sink for inorganic N in Río Las Marías during the dry season. It resulted in a loss rate of 2 to 8% per day of the total NO_3 load from the stream over the month-long course of experiment 1 (calculations after Royer et al. 2004). It accounts for $\sim 0.8\%$ of total NO_3 uptake based on the uptake rates cited above, which is near the lower end of recent estimates from 72 North American streams (Mulholland et al. 2008). The loss of N via denitrification is also small ($\sim 3.8\%$) compared to the gain of N via instream N_2 fixation, which contributes $\sim 6 \text{ mg N m}^{-2} \text{ d}^{-1}$ (mean of 4 dates in 2005; AJU and ASF, unpublished data). However, our data probably considerably underestimate whole-stream denitrification for at least 4 reasons. First, leakage of gas sample containers during transport and storage decreased rate estimates to some extent. Second, coupled nitrification–denitrification might be a significant pathway for N loss at water-column NO_3 concentrations similar to those that we observed (Seitzinger et al. 2006), but our measurements exclude this pathway because acetylene inhibits

nitrification as well as denitrification. Third, conversion of NO to N_2O might be incomplete when both O_2 and acetylene are present, as in our incubations (Bollmann and Conrad 1997). Fourth, we measured denitrification only in surficial sediments from the wetted channel, not in the deeper hyporheic sediments, stream banks, or parafluvial flow paths, which also contribute substantially to whole-stream denitrification (Holmes et al. 1996, Sjodin et al. 1997). This combination of directional biases means that the contribution of denitrification to the Río Las Marías N budget is probably larger than indicated here. Furthermore, even small losses of N via denitrification could cause important reductions in primary production in this N-limited system (Holmes et al. 1996). Even so, denitrification clearly is not the primary pathway for N loss from Río Las Marías under current sedimentation conditions because subsistence fisheries export large amounts of N (ASF, ROH, and B. W. Taylor, Dartmouth University, unpublished data).

Substratum particle size had pronounced effects on denitrification rates in our experiments. In experiment 2, actual denitrification rates were slightly (though not significantly) higher in fine sediments ($< 5 \text{ mm}$) than in cobble or gravel. Furthermore, potential denitrification assays amended with NO_3 and organic C showed increased denitrification rates only in fine sediments (Fig. 3), which suggests that the availability of anoxic microsites limited denitrification in cobble and gravel but not in fine sediments. In experiment 3, we considered the ways in which the proportion of even finer particles ($< 2 \text{ mm}$; i.e., sand, silt, and clay) in deposits of fine sediments influenced denitrification rates. Actual denitrification rates in this experiment were variable when the proportion of particles $< 2 \text{ mm}$ was low, but rates were almost always high when the proportion of particles $< 2 \text{ mm}$ was high (Fig. 4A). A similar threshold response to sediment texture has been observed in floodplain soils, where denitrification rates were always low when fine particles (silt and clay) constituted $< 60\%$ of the substratum, but rates increased as % fine particles increased beyond that threshold (Pinay et al. 2000).

Several mechanisms could explain the increase in actual denitrification rates in areas of substratum dominated by particles $< 2 \text{ mm}$. First, fine particles increase the prevalence of anoxia in sediments by inhibiting O_2 diffusion. Denitrification can be limited by the availability of anoxic sites in both terrestrial and aquatic sediments (Groffman and Tiedje 1989, Garcia-Ruiz et al. 1998b, Kemp and Dodds 2002b). Second, if organic matter content is high in these fine sediments, they could provide a source of NO_3 and organic C to denitrifying bacteria (Garcia-Ruiz et al. 1998b, Stein-

hart et al. 2000, Kemp and Dodds 2002b). Third, fine particles provide more surface area for bacterial colonization per volume of substratum than do larger particles (Pattinson et al. 1998, Inwood et al. 2007). The observation that rates did not increase with the proportion of particles <2 mm when excess NO_3 and organic C were added (Fig. 4B) suggests either that the 2nd of these mechanisms is the most important or that the excess nutrients saturated the denitrification response and prevented us from observing the effects of the other 2 mechanisms. In contrast to our results, Pinay et al. (2000) observed a consistent threshold effect of soil texture on denitrification rate regardless of whether excess C and N were added. Regardless of the mechanisms, denitrification rates clearly are higher in fine-textured than coarse-textured substrata in Río Las Marías.

Sedimentation and denitrification

The results of the sediment addition experiment support the hypothesis that anthropogenic sedimentation can increase denitrification rates (Fig. 5). In experiment 4, the mean denitrification rate was 8× greater in the topsoil-amended treatment than in the potential treatment, and the minimum rate in any replicate of the topsoil-amended treatment was greater than the maximum rate in any replicate of the potential treatment. Topsoil addition could have contributed to increased denitrification rates in at least 3 ways: 1) by introducing additional denitrifying bacteria, 2) by fertilizing incubations with additional NO_3 and organic C substrates, or 3) by filling substratum pores and creating more anoxic microsites. The difference in denitrification rates between the potential and topsoil-amended treatments despite the similar masses of N added in these 2 treatments (mean = 300 μg and 390 μg , respectively) suggests that the fertilization mechanism is not of primary importance. Indeed, at longer time scales, it is likely that decreased O_2 diffusion (not inputs of N, C, or denitrifying bacteria) would in fact be the predominant result from a given input of topsoil to the river. Much of the labile C and N load associated with the topsoil will be removed or taken up fairly quickly, leaving behind particles with little capacity to supply NO_3 or organic C but with undiminished capacity to fill pore spaces in the sediment. Our data suggest that decreased porosity will increase stream denitrification rates, but this effect could be buffered if decreased porosity also reduced diffusion of water-column NO_3 through the sediments. Increased stream-water NO_3 concentrations are the dominant effect of land use on denitrification rates in systems with high anthropogenic N loading (Kemp and Dodds 2002a,

Royer et al. 2004, Inwood et al. 2005, Arango and Tank 2008). However, future research should address the hypothesis that increased sedimentation is the dominant effect of land use on denitrification in both tropical and temperate systems where high anthropogenic N inputs do not occur.

Landuse change has complex effects on the N cycle in tropical streams. On one hand, deforestation alters inputs of N to streams from watersheds via changes to production and decomposition, hydrology, and soil biogeochemistry (Malmer and Grip 1994, Williams et al. 1997, Neill et al. 2001). Local conditions such as watershed size and soil type mediate these changes (Biggs et al. 2004, Thomas et al. 2004). Furthermore, substantial exports of N to streams typically are observed immediately after clearing (Malmer and Grip 1994, Williams and Melack 1997), but the long-term effects of clearing on N exports depend on subsequent land use. Landuse change also can change stream N cycles by altering instream processing of N. Increased erosion and suspended sediment loads could decrease N uptake via effects on transparency and primary production (Downing et al. 1999). In Brazil, growth of macrophytes in open-canopy pasture streams led to changes in channel morphology and sediment organic matter that promoted low DO and, therefore, might have increased denitrification rates (Thomas et al. 2004, Neill et al. 2006). Our results suggest that effects of landuse change on sediment loads and fine particle deposition might also have important consequences for N cycling by enhancing conditions for denitrification. Future work should explore these effects and incorporate them into our understanding of changing tropical aquatic N cycles.

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