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Denitrification and total nitrate uptake in streams of a tropical landscape

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Abstract. Rapid increases in nitrogen (N) loading are occurring in many tropical watersheds, but the fate of N in tropical streams is not well documented. Rates of nitrate uptake and denitrification were measured in nine tropical low-order streams with contrasting land use as part of the Lotic Intersite Nitrogen eXperiment II (LINX II) in Puerto Rico using short term (24-hour) additions of $K^{15}NO_3$ and NaBr. Background nitrate concentrations ranged from 105 to 997 μg N/L, and stream nitrate uptake lengths were long, varying from 315 to 8480 m (median of 1200 m). Other indices of nitrate uptake (mass transfer coefficient, V_f [cm/s], and whole-stream nitrate uptake rate, U [$\mu g\ N \cdot m^{-2} \cdot s^{-1}$]) were low in comparison to other regions and were related to chemical, biological, and physical parameters. Denitrification rates were highly variable (0–133 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$; median = 15 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$), were dominated by the end product N_2 (rather than N_2O), and were best predicted by whole-stream respiration rates and stream NO_3 concentration. Denitrification accounted for 1–97% of nitrate uptake with five of nine streams having 35% or more of nitrate uptake via denitrification, showing that denitrification is a substantial sink for nitrate in tropical streams. Whole-stream nitrate uptake and denitrification in our study streams closely followed first-order uptake kinetics, indicating that NO_3 uptake is limited by delivery of substrate (NO_3) to the organisms involved in uptake or denitrification. In the context of whole-catchment nitrogen budgets, our finding that in-stream denitrification results in lower proportional production of N_2O than terrestrial denitrification suggests that small streams can be viewed as the preferred site of denitrification in a watershed in order to minimize greenhouse gas N_2O emissions. Conservation of small streams is thus critical in tropical ecosystem management.

Key words: denitrification; N loading; N_2O emissions; nitrate uptake; Puerto Rico; tropical streams; tropics.

Introduction

Until recently, most anthropogenic N inputs occurred in the industrialized, temperate regions of the world. This situation is rapidly changing with economic expansion in the tropics, where nearly two-thirds of Earth's anthropogenic energy-related N inputs will take place by 2020 (Galloway et al. 1994). Current rates of nitrogen export in tropical watersheds with minimal disturbance are about five-fold greater than in temperate regions with similar runoff (Downing et al. 1999). This export often has a high percentage of dissolved organic nitrogen (DON; ~35%), while dissolved inorganic N also makes up about 35% of that N export and particulate N making up the rest (McDowell and Asbury 1994, Lewis et al. 1999). Human activities induce a shift from organic to inorganic forms of exported N, with mobile nitrate (NO_3) dominating outputs (Cole et al. 1993). Nitrate loading is known to reduce biotic efficiency of N uptake in streams (O'Brien et al. 2007, Mulholland et al. 2008) and to cause coastal eutrophication from stream and river inputs (Turner and Rabalais 1994, Vitousek et al. 1997). Understanding the fate of NO_3 in stream and river systems is thus critical in efforts to maintain ecological health in aquatic ecosystems. This is especially true in a rapidly urbanizing region such as Puerto Rico, where N export in highly urbanized basins is higher than the most impacted zones in Northern Europe (Ortiz-Zayas et al. 2006).

Studies of in-stream N dynamics are largely lacking in the tropics and in urban tropical streams in particular, with most of the published work focused on reference and agricultural streams in Costa Rica, Puerto Rico, and Amazonian Brazil. The few studies that have been published suggest that the high inorganic N concentrations typically found in tropical streams result in little or no limitation to primary production (Pringle et al. 1986, Neill et al. 2006) with no studies to date indicating the functional response of tropical streams in response to anthropogenic N loading (Earl et al. 2006, O'Brien et al. 2007). In temperate streams biotic (Hall and Tank 2003, Webster et al. 2003, Mulholland et al. 2006) and

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hydrologic controls (Valett et al. 1996, Wollheim et al. 2001) on inorganic N have been documented, but they have not been examined in detail in the tropics. Understanding controls on nitrate uptake and denitrification is a critical research need for Puerto Rico, where short transit times from land to the ocean, as well as high population densities, make estuaries and the coastal zone particularly sensitive to nitrogen-induced eutrophication (Ortiz-Zayas et al. 2006).

To protect coastal water quality, the most desirable fate of bioavailable N in streams is denitrification, the microbial reduction of NO_3 to unavailable gaseous N (N_2 and N_2O). In temperate streams denitrification can be a significant sink of total N inputs (20–35%) with higher rates occurring in systems that receive substantial anthropogenic N (Seitzinger 1988). Denitrification in the near-stream zone of streams in Puerto Rico has been found to be important in regulating nitrogen flux (Bowden et al. 1992, McDowell et al. 1992, 1996, Chestnut and McDowell 2000), but in-stream denitrification has been largely ignored. Differentiating between production of N_2O and N_2 during denitrification is also critical because of the potent greenhouse effects of N_2O (Wang et al. 1976). The ratio of $N_2O:N_2$ is related to oxygen, H_2S, and NO_3 availability and is lower in aquatic systems than soils (Firestone et al. 1980, Seitzinger and Kroeze 1998). N_2O emissions from rivers are thought to be a significant global source of N_2O (Seitzinger and Kroeze 1998), and there is a critical need to quantify stream N_2O production and emission (and their controls) in the tropics (Seitzinger et al. 2000).

We hypothesize that the high ambient nitrate concentrations, warm water temperatures, and large organic matter inputs associated with high rates of terrestrial primary production would make denitrification particularly important as a sink for NO_3^- in tropical streams even though overall NO_3 uptake is low. We test this hypothesis using ^{15}N tracers that allow quantification of whole stream denitrification rates without the physical and chemical perturbations of the acetylene block method (Mulholland et al. 2004). We performed these experiments in small tropical streams of Puerto Rico in various types of land use with a range of chemical, biological, and physical characteristics that allows us to analyze the relationships between these characteristics and rates of various N cycling processes. The specific questions we ask include: (1) What is the relative importance of assimilatory NO_3 uptake and denitrification in tropical streams with widely varying land use? (2) Are tropical streams different than their temperate counterparts in terms of the rates and controls on NO_3 uptake and denitrification? (3) What factors regulate NO_3 uptake and denitrification? (4) What are the functional responses of these streams to the increased NO_3 concentrations caused by human activities in their watersheds? (5) What proportion of stream denitrification results in N_2O as the gaseous end product, and does this differ from denitrification in tropical soils?

METHODS

Site description

This study was conducted in and around the Luquillo Experimental Forest (LEF), on the Caribbean island of Puerto Rico. The area is characterized by steep slopes in forested areas at higher altitudes and moderate to steep slopes in the lower, more human-impacted regions. The elevation of our study watersheds ranged from 10 to 675 m. Annual rainfall ranges from 150 cm in metropolitan San Juan to 250 cm in the lower altitudes of the forest and increases with elevation (McDowell et al., *in press*). The mean annual temperature ranges from 19°C to 26°C. Total agricultural land in Puerto Rico has decreased significantly in the last 50 years, while forested and urban lands have increased due to socioeconomic factors (Grau et al. 2003). Much of the agricultural land is divided into small parcels and is interspersed with forest fragments, while urban land is developed intensively. Nine streams (first to third order) were selected in the study area in three different classes of land use: reference (tropical rainforest), agriculture, and urban. Land-use categories were assigned based on visual observation of the dominant land use adjacent to the study reach.

The reference streams were located in the Luquillo Mountains of northeastern Puerto Rico. The three streams, Quebrada Bisley, Rio Icacos Tributary (RIT), and Quebrada Pared, are steep, confined and very shaded (Tables 1 and 2). Percent forested land was not below 99.7% in any of the three watersheds. They have steeper channel slopes than the other streams in this study.

The agricultural streams were located at lower elevations in the coastal plains. Quebrada Grande is a sandy-bottomed stream draining horse and cattle pasture. It has an incised stream channel that is heavily disturbed by frequent rain events. Quebrada Maizales is mostly cobble and boulder, draining banana plantation and horse pasture as well as suburban housing developments. Portions of its stream bank have been stabilized with concrete to maintain the stream course. Quebrada Vaca flows through cattle pasture and patchy forest. The streambed consists mostly of gravel and the riparian zone is mostly broadleaf trees. Grande and Maizales have very few trees in their riparian zones.

The three urban streams Quebrada Petunia, Rio Mameyes Tributary (MTrib), and Quebrada Ceiba are mostly gravel-bottomed. Petunia and MTrib have thick, tree-lined riparian cover, while Ceiba has a more open canopy. Petunia drains a residential area in metropolitan San Juan and contained the highest NO_3 concentrations of any of the study sites. MTrib and Ceiba also drain residential areas in the towns of Palmer and Ceiba, respectively. Evidence of periodic sewage leaks directly into the streams was observed in all three of our urban streams.

TABLE 1. Characteristics of streams used in the study.

Stream name	Watershed area (ha)	Watershed cover type (%)			Sub. type	Reach length (m)	Slope (%)	Q (L/s)	Width (m)	Depth (m)	Hydraulic characteristics			Velocity (cm/s)
		Native	Agriculture	Urban							A_S/A	α (s^{-1})	F_{med}^{200} (fraction)	
Bisley	58	99.7	0	0.3	Bl/Be	385	12.9	12.5	3.20	0.07	0.380	0.00013	0.078	5.366
RIT	30	100	0	0	S/Fg	365	3.1	20.0	1.18	0.27	0.131	0.00005	0.242	6.159
Pared	64	99.8	0.2	0	G/C	525	14.2	5.2	2.74	0.04	0.236	0.00002	0.030	1.852
Grande	95	28.3	69.3	2.4	S/Fg	690	1.4	12.3	1.02	0.06	0.318	0.01875	0.010	20.000
Maizales	265	61.7	34.3	4	G	450	2.5	25.0	3.53	0.35	0.000	0.00576	0.000	2.011
Vaca	172	60.5	32.2	7.4	G	490	2.5	80.7	1.96	0.16	0.164	0.00004	0.031	6.073
Petunia	110	6.7	0	92.3	G	350	4.0	4.7	1.80	0.11	0.128	0.00004	0.038	2.315
MTrib	160	42	1.5	56.7	G	375	2.4	23.2	2.46	0.41	0.151	0.00005	0.021	2.278
Ceiba	505	44.2	2.7	53.2	G	625	1.6	49.5	2.73	0.07	0.045	0.00005	0.006	6.755

Notes: Dominant stream substrate (sub.) types are: Bl, boulder; Be, bedrock; S, sand; Fg, fine gravel; G, gravel; and C, cobble. Other variables are Q, discharge and transient storage parameters (A_S/A, α, F_{med}). A_S/A is the size of the transient storage zone adjusted to stream area. Transient storage is the area of the stream where flow paths are moving much more slowly than the average velocity of the stream channel (Bencala 1983). All the hydraulic characteristics were determined by using the OTIS-P model (Runkel 1998) from the NaCl injections. The dispersion coefficient α is a measure of the lateral movement of solutes in a stream channel, which is primarily controlled by turbulence. F_{med}^{200} is a measure of the fraction of median water travel time due to storage within the stream reach (Runkel 2002). Abbreviated stream names are: RIT, Rio Icacos Tributary; and MTrib, Rio Mameyes Tributary.

Experimental procedures

Tracer enrichment experiments with $^{15}NO_3$ experiments were conducted in the nine streams as part of the Lotic Intersite Nitrogen eXperiment II (LINX II; Mulholland et al. 2008). Experiments were conducted in February and March over three years (2004–2006). The ^{15}N addition experiments, sampling, and analysis followed the methods of Hall et al. (2009) and Mulholland et al. (2009) and will not be explained in detail here.

Uptake lengths (S_w; in the water compartment) were calculated using regressions of the natural log of the ^{15}N flux value (corrected for background and dilution) against distance below the ^{15}N injection. The slope of this regression is the distance-normalized NO_3 uptake rate and the inverse of the slope is the NO_3 uptake length (S_w). Whole stream nitrate uptake rate (U; μg $N \cdot m^{-2} \cdot s^{-1}$) was calculated by the following equation:

$$U = F/(S_w \times w) \tag{1}$$

where w is the average wetted width and F is the nutrient flux. The mass transfer coefficient (V_f) was calculated by dividing U by the stream water NO_3 concentration (Newbold et al. 1981, Stream Solute Workshop 1990). Assimilatory ^{15}N uptake by biomass was calculated from the mass of ^{15}N tracer found in each biomass compartment.

Denitrification rates (production of N_2 and N_2O) were determined from the production of $^{15}N_2$ and $^{15}N_2O$ in the study reach using the approach described in Mulholland et al. (2004). First, tracer $^{15}N_2$ and $^{15}N_2O$ MF values were computed and $^{15}N_2$ and $^{15}N_2O$ flux were calculated with the MF values and the N_2 and N_2O mass values (statistically corrected for incomplete headspace mixing and air contamination). We solved for the $^{15}N_2$ and $^{15}N_2O$ production rates by fitting the following relationship to the longitudinal pattern in tracer $^{15}N_2$ and $^{15}N_2O$ flux (A) with distance x (in units of meters) downstream from the ^{15}N addition point:

$$A = \left(\frac{k_{den} \times N_0}{k_2 - k_1}\right) \times (e^{-k1x} - e^{-k2x}) \tag{2}$$

TABLE 2. Stream chemical and biological characteristics during each of the ^{15}N addition experiments.

Stream name	Experiment date (Feb–Mar)	NO_3 (μg N/L)	NH_4 (μg N/L)	DON (μg N/L)	SRP (μg P/L)	Water temperature (°C)	PAR (mol quanta·$m^{-2} \cdot d^{-1}$)	Daily GPP (g O_2·$m^{-2} \cdot d^{-1}$)	Daily ER (g O_2·$m^{-2} \cdot d^{-1}$)	GPP/ER
Bisley	2004	171	3	10	22	21	0.15	0.01	2.42	0.00
RIT	2005	131	7	23	0	19	0.50	0.47	4.49	0.10
Pared	2006	105	3	280	7	22	1.55	0.39	0.42	0.92
Grande	2004	276	11	70	13	23	6.39	5.18	7.64	0.68
Maizales	2005	206	7	78	12	23	13.95	7.29	5.31	1.37
Vaca	2006	440	3	167	9	23	1.52	3.08	15.69	0.20
Petunia	2004	997	15	53	26	24	0.02	0.34	4.60	0.07
MTrib	2005	174	2204	847	310	21	0.82	7.13	7.41	0.96
Ceiba	2006	512	50	11	22	25	1.98	9.33	11.74	0.79

Note: Abbreviations are: DON, dissolved organic nitrogen; SRP, soluble reactive phosphorus; PAR, photosynthetically available radiation; GPP, gross primary productivity; ER, ecosystem respiration.

where k_{den} is the denitrification rate; N_0 is the flux of ^{15}N-nitrate calculated at the point of the injection; k_1 is the measured rate of decline in streamwater ^{15}N-nitrate flux with distance due to all processes (in units of m^{-1}); and k_2 is the N_2 or N_2O gas exchange rate through the air–water interface per unit distance (units of m^{-1}). Values of k_2 were determined from a propane or SF_6 injection experiment. A least-squares fitting procedure (Solver Tool, Microsoft Excel 2003; Microsoft, Redmond, Washington, USA) was used to determine the values of k_{den} from fitting the model to A. Denitrification rate k_{den} was then multiplied by stream NO_3 flux and divided by average stream width to give the areal denitrification rate. N_2O emission rates were estimated from dissolved N_2O concentrations in stream water and the N_2O gradient between stream water and atmosphere.

Physical, chemical, and biological variables were measured within three days of the ^{15}N experiment. Average stream width, velocity, and discharge were measured each day of the experiment. Water travel time and transient storage zone size and exchange rates were determined once during the experiment from a NaCl injection. The conductivity data were entered in the OTIS-P model, which was used to quantify the hydrologic parameters affecting solute transport (Runkel 1998). Whole stream gross primary productivity (GPP) and ecosystem respiration (ER) were measured concurrently with the ^{15}N addition experiment using the two-station dissolved oxygen (DO) method with data logging sondes (YSI 6920; YSI, Yellow Springs, Ohio, USA). Air–water gas exchange was measured by addition of a conservative gas (propane or sulfur hexafluoride) either during or immediately after the experiment. Gas exchange rate was calculated in terms of the fractional decline in conservative gas (corrected for dilution due to groundwater input between stations) between two measurement stations. Stream water chemistry (Br, Cl, NO_3, NH_4, SRP, and DOC) was also collected at all stations before, during, and after the experiment. For more detailed descriptions for these experimental methods see Hall et al. (2009) and Mulholland et al. (2009).

Statistical analysis

Simple linear regression using ordinary least squares was performed on relationships between measured hydrological, biological, and chemical variables and N uptake parameters that were the primary relationships found across North American streams (Hall et al. 2009, Mulholland et al. 2009). The explanatory variables used to assess controls on total NO_3 removal (described by S_W, V_f, and U) were specific discharge (discharge divided by stream wetted width; Q/w), NO_3 concentration, and GPP (Hall et al. 2009). The explanatory variables used to assess controls on denitrification (described by $S_{W\ den}$, $V_{f\ den}$, and U_{den}) were Q/w, NO_3 concentration, ER, and F^{200}_{med} (a transient water storage metric, standardized to a stream length of 200 m; Mulholland et al. 2009). Relationships were considered significant where $P < 0.05$. All total N removal and denitrification parameters were $\log_{10}$-transformed prior to statistical analysis to improve normality, except fractional parameters that were arcsine-square-root transformed. Diel differences in NO_3 cycling metrics were examined using paired *t* tests, but subsequent simple linear regression analyses were performed on the average of the day/night measures for each stream. Statistical analyses were performed with SPSS 17 (SPSS Inc., Chicago, Illinois, USA).

The statistical models examining the functional response of stream biota to increasing nutrient concentration were done with the following statistical tests. The relationship between U (and U_{den}) and NO_3 was considered to follow first-order response if there was a significant regression found with log transformed independent and dependent variables and if the slope was equal to 1, which was determined by a *t* test of the slope of the regression. The relationship was considered to follow the efficiency loss model (O'Brien et al. 2007) if this same regression had a slope between 0 and 1. The relationship would be considered saturated if there was a significant fit with the Michaelis-Menten model on non-transformed data and calculated K_S was within the range of NO_3 concentrations in our study. Saturation was also tested using S_W by linear regression between S_W and NO_3 concentration. In this case Michaelis-Menten would be valid if there was a linear relationship, first-order kinetics would be valid if there was no relationship, and the efficiency loss model would be valid if there was a power relationship with the slope significantly less than 1. A full explanation of these models can be found in O'Brien et al. (2007).

RESULTS

Physical and chemical parameters

Stream discharge (Q) ranged from 4.7 L/s (Petunia) to 80.7 L/s (Vaca) during the ^{15}N additions (Table 1; median = 20.3 L/s) and discharge was relatively constant throughout the experiments in each stream. The nine streams ranged in mean width from 1 m (Grande) to 3.5 m (Maizales) and in mean depth from 4 cm (Pared) to 35 cm (Vaca). Velocity was unrelated to discharge and was highest in a sandy bottom stream (Grande, 20 cm/s). PAR (Table 2) ranged from 0.02 (Petunia) to 13.95 mol quanta$\cdot m^{-2}\cdot d^{-1}$ (Maizales) and was related to canopy cover.

Dispersion coefficients (D) ranged from 0.024 (Maizales) to 0.455 m^2/s (Grande) and were highly dependent on water velocity ($r^2 = 0.61$, $P = 0.01$). The area of the transient storage zone (A_S) varied between 0 (Maizales) and 0.13 m^2 (Vaca), while the size of the transient storage zone normalized for stream size (A_S/A; Table 1) ranged from 0 (Maizales) to 0.38 (Bisley). Storage zone exchange coefficient (α) ranged from 0.00002 (Pared) to 0.01875 s^{-1} (Grande), but most were below 0.001 s^{-1}, and F_{med}^{200} (Table 1) ranged from 0 (Maizales) to 0.24 (RIT).

NO_3 concentrations varied from 105 μg N/L in Pared to 997 μg N/L in Petunia (Table 2) and were an indication of the severity of anthropogenic impacts on the streams. Concentrations of NH_4 had a median of 7 μg N/L, with the highest concentrations in Ceiba (50 μg N/L) and MTrib (2204 μg N/L). NO_3 was the largest proportion of dissolved N, with the exceptions of Pared, where DON was the dominant dissolved form of N, and MTrib, where NH_4 dominated. Concentrations of NH_4 during the ^{15}N experiment in MTrib were about seven to eight times higher than when measured prior to the experiment due to a temporary input of raw sewage entering directly into the stream. This was not realized until after the experiment had begun.

Metabolism and biomass

Fine benthic organic matter (FBOM) was the dominant organic matter compartment (mean = 57% of total ash-free dry mass [AFDM]) in all streams except RIT, where coarse benthic organic matter (CBOM) was dominant (44% of total AFDM) and Grande where epilithon was 35% of total AFDM. Ceiba had the highest total standing stock of organic matter (140 g AFDM/m^2) and the mean was 82 g AFDM/m^2 across all streams.

Stream water temperature ranged from 19.0° to 25.3°C (Table 2). Light varied almost three orders of magnitude, from 0.02 mol quanta$\cdot m^{-2}\cdot d^{-1}$ in an urbanized stream with very thick riparian cover (Petunia) to 13.95 mol quanta$\cdot m^{-2}\cdot d^{-1}$ in an agricultural stream with no riparian vegetation (Maizales). All streams were net heterotrophic except Maizales, which had a P/R above 1. GPP varied three orders of magnitude, ranging from 0.01 g $O_2\cdot m^{-2}\cdot d^{-1}$ (Bisley) to 9.33 g $O_2\cdot m^{-2}\cdot d^{-1}$ (Ceiba). GPP was positively correlated with PAR (log scale, $r^2 = 0.46$, $P = 0.046$) and watershed area ($r^2 = 0.67$, $P = 0.01$). Ecosystem respiration (ER) was highly variable among streams, ranging from 0.42 (Pared) to 15.7 g $O_2\cdot m^{-2}\cdot d^{-1}$ (Vaca). ER was negatively correlated with stream gradient ($r^2 = 0.47$, $P = 0.04$) and positively correlated with depth ($r^2 = 0.62$, $P = 0.01$) and discharge ($r^2 = 0.79$, $P = 0.01$).

Nitrogen dynamics

The uptake kinetics of NO_3 varied considerably among streams and there was no statistically significant pattern in the day to night differences in any of the NO_3 uptake parameters. Three streams had poor model fits for one of their sampling periods (RIT night, MTrib day, and Vaca night), so only one sampling period was used in the following summary and regression relationships. The uptake length (S_W, Fig. 1) for NO_3 was shortest in Pared (mean = 315 m), while S_W was longest in Vaca (day = 8480 m). S_W had a significant positive relationship with Q/w ($r^2 = 0.72$; $P = 0.00$). The average uptake velocity (V_f), ranged from 0.13 mm/min (Petunia; Fig. 1) to 0.94 mm/min (Maizales). V_f was not related to NO_3 concentration (Fig. 2b) but it had a significant positive relationship with GPP (Fig. 3c; $r^2 = 0.44$, $P = 0.05$). Areal uptake rate (U, Fig. 1) varied over an order of magnitude from a mean of 27 μg N$\cdot m^{-2}\cdot min^{-1}$ at RIT to 251 μg N$\cdot m^{-2}\cdot min^{-1}$ at Ceiba. U had a significant positive relationship with NO_3 concentration (Fig. 2a; $r^2 = 0.47$, $P = 0.04$) and GPP (Fig. 3a; $r^2 = 0.80$, $P < 0.01$).

Total autotrophic assimilation of NO_3 (mean = 15.62 mg N$\cdot m^{-2}\cdot d^{-1}$) was generally higher than assimilation by heterotrophs associated with detrital pools (FBOM and CBOM; mean = 12.7 mg N$\cdot m^{-2}\cdot d^{-1}$). The two biomass compartments with the most rapid NO_3 uptake (per gram organic matter or AFDM) were roots attached to riparian vegetation (Pared, RIT, and Vaca) and CBOM as leaves (Ceiba, Petunia, Grande, and MTrib), with CBOM as wood (Bisley) and filamentous algae (Maizales) highest in the other two streams. This was despite the fact that FBOM was the dominant organic matter compartment across streams. Assimilation by roots might not be due to autotrophic uptake at our sites, since we observed significant biofilm on the roots at every stream where we did not attempt to separate the biofilm from the bulk roots.

A linear regression model of U and NO_3 (log transformed data) produced a statistically significant relationship with a slope not significantly different from 1, indicating that uptake more closely followed first-order kinetics than Michaelis-Menten saturation or the efficiency loss model. There was also no significant relationship between S_W and NO_3 concentration, which also indicates that total NO_3 uptake more closely follows first-order kinetics.

Denitrification

Areal denitrification rates (U_{den}) ranged from a stream day/night average of 0 μg N$\cdot m^{-2}\cdot min^{-1}$ (Pared) to 133 μg N$\cdot m^{-2}\cdot min^{-1}$ (Vaca; Fig. 1) and as was the case with total NO_3 uptake, denitrification varied unpredictably between night and day (only the night denitrification rates were used from Bisley due to poor model fit). ^{15}N in N_2 and N_2O was detectable in all nine streams and N_2 was the dominant end product of denitrification by two orders of magnitude compared to N_2O. U_{den} had a significant positive relationship with NO_3 concentration (Fig. 2c; $r^2 = 0.46$; $P = 0.04$) and ER (Fig. 3d; $r^2 = 0.57$; $P = 0.02$). Denitrification velocity ($V_{f\ den}$) and uptake length due to denitrification ($S_{W\ den}$) were not related to NO_3 concentration (Fig. 2d) or any of the other parameters measured. First order kinetics better explains the relationship between denitrification rate (U_{den}) and NO_3 concentration across streams on log transformed data compared to Michaelis-Menten saturation or the efficiency loss model.

Denitrification accounted for 1–97% of nitrate uptake, with five of the nine streams having denitrification accounting for greater than 35% of total nitrate uptake.

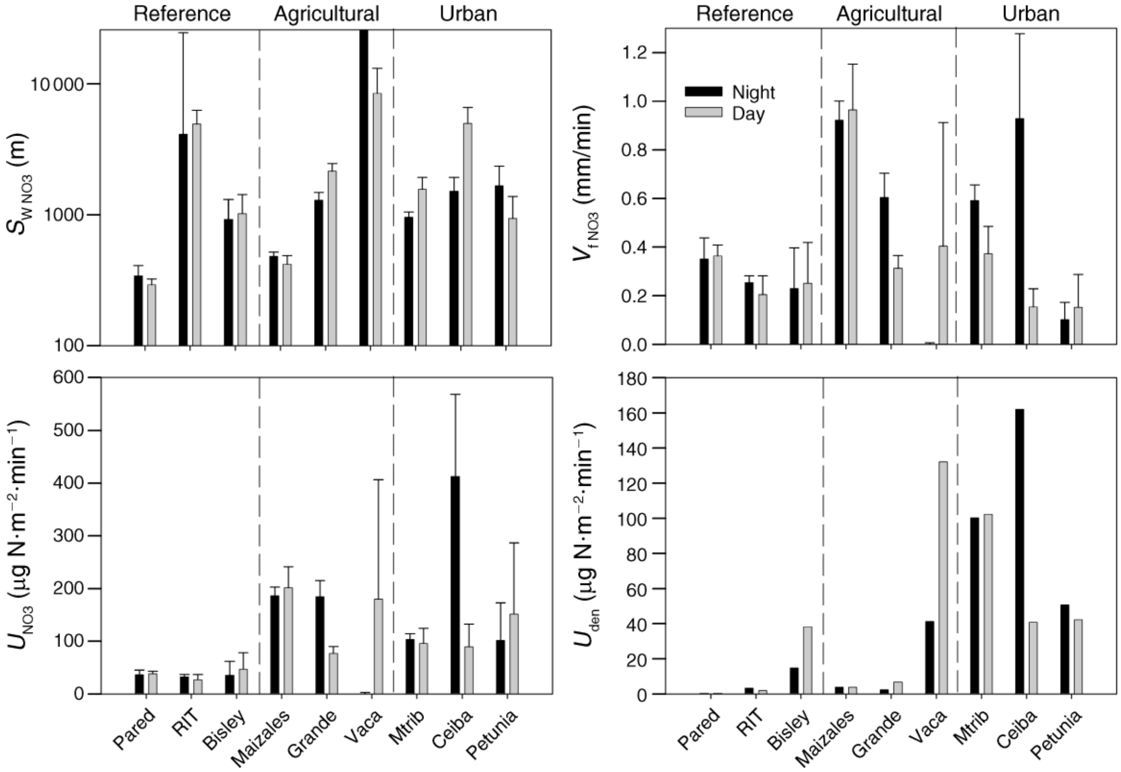

FIG. 1. NO_3 uptake length (S_W), mass transfer velocity (V_f), areal uptake rate (U_{NO3}), and denitrification rate (U_{den}) in the nine streams for both day and night sampling periods. Error bars are the upper 95% confidence interval from the linear regression of the decline in $\ln^{15}NO_3$ over distance downstream. (Uptake length for Vaca at night is infinite and therefore off scale.) Dotted lines separate streams of different land use.

This indicates that denitrification has the potential to be a substantial sink for nitrate. This average was slightly skewed by MTrib where sewage input increased NH_4 concentrations from a background of 34 μg N/L to 5658 μg N/L at the start of the reach. This ammonium was removed from solution quickly, through both assimilation and nitrification, and benthic N demand was mostly met by NH_4. NO_3 assimilation was only 2% of uptake, so the only uptake of NO_3 was due to denitrification. Without MTrib, denitrification still averaged 25% of NO_3 uptake in the remaining eight streams.

Production of N_2O ranged from 0 (Vaca) to 1.8 μg N·m^{-2}·h^{-1} (Petunia) and was strongly related to NO_3 concentration ($r^2 = 0.61$, $P = 0.01$). The ratio of N_2O to N_2 produced by denitrification was not correlated with NO_3 concentration or any of the other variables, but was highest in the sandy-bottomed streams (RIT and Grande). N_2O emission rates (from stream N_2O concentration and air-water gas exchange) ranged from 8.5 (Pared) to 142.7 μg N·m^{-2}·h^{-1} (MTrib).

DISCUSSION

Total NO_3 uptake in tropical streams

Nitrate uptake rates are poorly constrained in tropical streams, and our data are some of the first to examine the variability in stream NO_3 uptake across a broad range of land use and ambient NO_3 concentrations. Uptake lengths (S_W) in our streams were relatively long, which was expected given the high NO_3 concentrations we observed even in relatively pristine reference watersheds (105–171 μg N/L). Among the few studies that have been published, our data fall within the range of previously reported values of V_f for tropical streams (Duff et al. 1996, Merriam et al. 2002, Neill et al. 2006). Our results show that values of V_f in tropical streams are similar to those in streams from other biomes with riparian forests that tend to reduce primary production (and presumably N uptake) by benthic algae, and have similar NO_3 concentrations (e.g., streams from North Carolina, Massachusetts, Oregon, and Michigan, USA in LINX II; Mulholland et al. 2008 and Fig. 4). Nitrate uptake rates in our tropical streams are below those found in highly autotrophic, N-limited streams in other biomes such as the desert streams of Arizona (Grimm et al. 2005, Mulholland et al. 2008; Fig. 4).

Tropical stream NO_3 uptake was correlated with a combination of physical, chemical, and biological factors (Figs. 2 and 3), similar to results obtained in most temperate zone streams (Hall and Tank 2003, Webster et al. 2003, Fellows et al. 2006, Mulholland et al. 2006, Hall et al. 2009). Biological nitrogen demand in Puerto Rican streams appears to be mostly autotrophic,

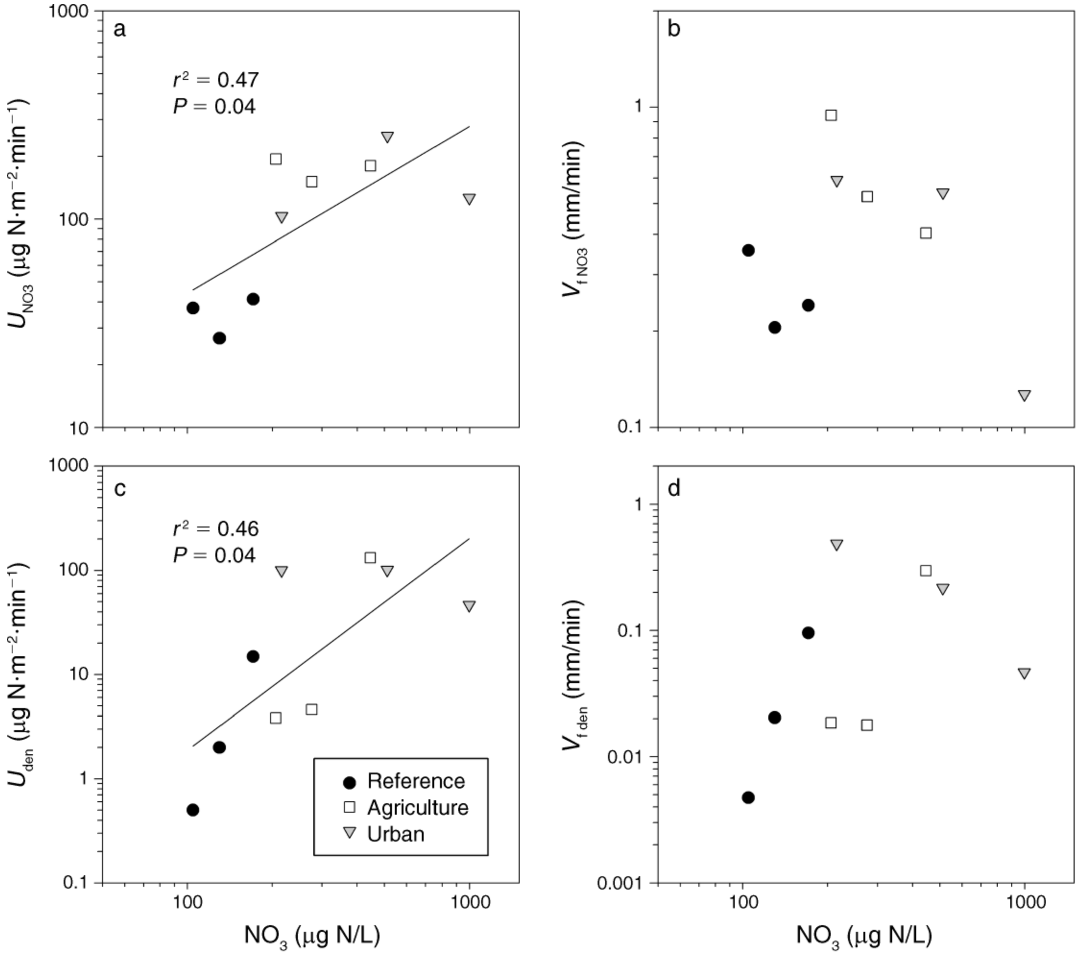

FIG. 2. (a) Total areal NO_3 uptake (U_{NO3}), (b) total NO_3 uptake velocity ($V_{f\ NO3}$), (c) areal denitrification rate (U_{den}), and (d) denitrification velocity ($V_{f\ den}$) as a function of stream NO_3 concentration in all nine study streams. Lines indicate significant relationships using simple log-linear regression.

based on the strong relationships we observed between nitrate uptake (as both V_f and U) and measures of GPP (Fig. 3). In streams in the Grand Teton National Park with comparable variation in both V_f and stream light levels, Hall and Tank (2003) also concluded that autotrophic production could account for most NO_3 uptake. The importance of autotrophy in the regulation of diurnal variation in NO_3 concentration has been shown in Walker Branch, Tennessee (Mulholland et al. 2006), but we did not see a discernable diurnal pattern in our streams. Despite the lack of evidence of diurnal patterns, the importance of autotrophic N demand is apparent in Puerto Rico in comparisons among streams.

Association of N demand with autotrophs suggests that light limitation is a major driver of biological N assimilation in streams across a variety of landscapes in Puerto Rico. In a comparison of stream metabolism in all the watersheds with forested riparian zones in LINX II, Bernot et al. (*in press*) found that GPP was the highest in Puerto Rico. They concluded that epilithon in Puerto Rican streams appears to have higher affinity for low light conditions, resulting in higher than predicted production and therefore greater potential for N assimilation than reference streams from other forested biomes. Energy limitation may also be an important driver of N dynamics in heavily forested streams in the Luquillo Experimental Forest (LEF) of Puerto Rico where there is little light reaching the stream and nitrification is the biggest major sink for added tracer NH_4 (Merriam et al. 2002).

Physical controls on NO_3 uptake were also evident in our results, as S_W was the shortest in streams that had low Q/w. Low Q/w primarily enhances contact time of stream water with the stream bottom, thus enhancing benthic uptake. Comparison of two streams (Pared and Vaca) provides an example of the impacts of physical conditions on S_W. In contrast to Pared, Vaca was especially deep relative to its flow (specific discharge was 30 times higher) and this translated into NO_3 S_W that was over 27 times longer. Nutrient S_W for streams in other biomes is also related to stream size (Peterson et al. 2001, Wollheim et al. 2001, Hall et al. 2009),

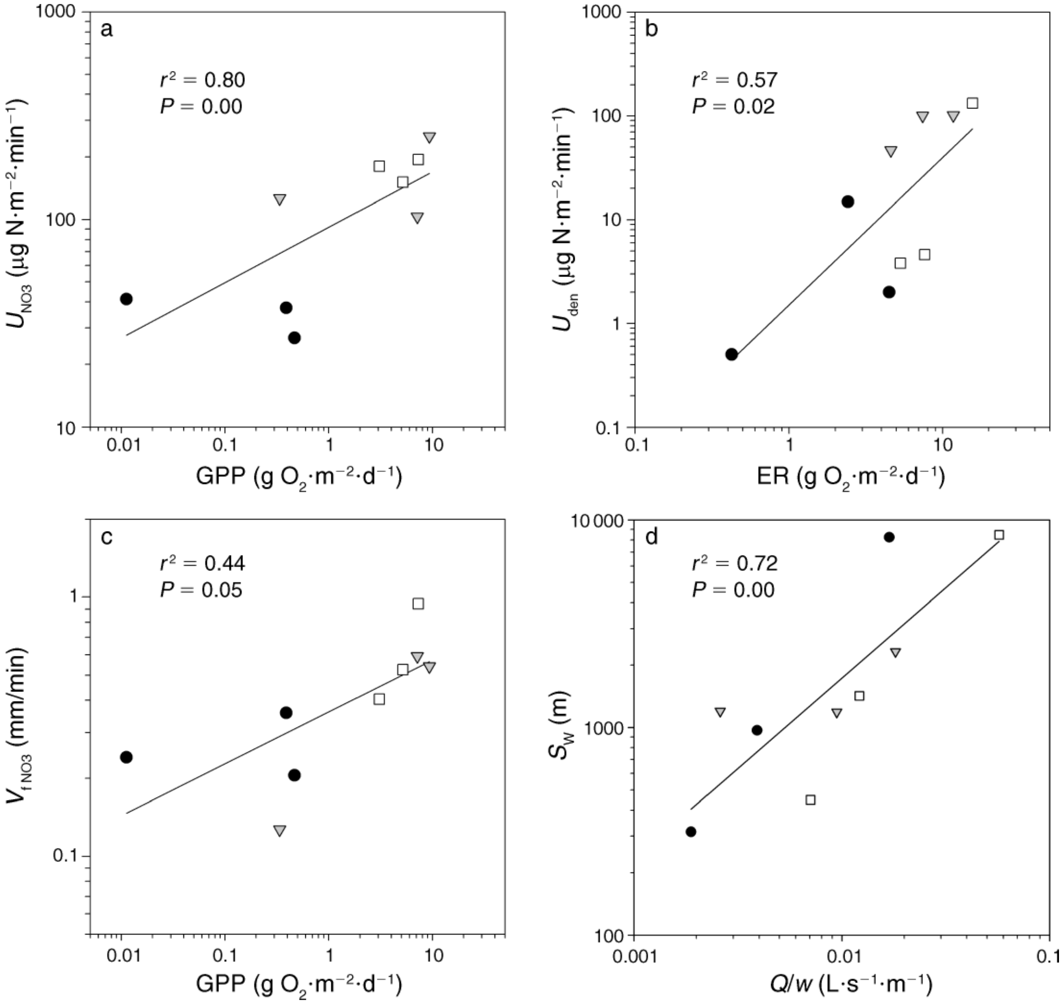

FIG. 3. (a) Total NO_3 uptake rate (U_{NO3}) as a function of gross primary production (GPP), (b) areal denitrification rate (U_{den}) as a function of ecosystem respiration (ER), (c) total NO_3 uptake velocity (V_f) as a function of GPP, and (d) total NO_3 uptake length (S_W) as a function of specific discharge (discharge divided by stream wetted width [Q/w]) in all nine streams. All plots shown are significant relationships in simple log-linear regression.

indicating that the distance that a nutrient travels is strongly dependent on the stream's physical characteristics. The evidence provided here indicates that tropical streams behave in a similar manner, with stream NO_3 uptake varying with physical characteristics of the stream channel.

Across a spatial gradient of streams receiving chronic NO_3 inputs in Puerto Rico, first-order uptake kinetics better explained the biotic response to increasing NO_3 concentration for total NO_3 uptake (Fig. 2a) than did either Michaelis-Menten saturation or the efficiency loss model. This implies that uptake is limited by mass transport into the stream benthos rather than being limited by inherent limitations of the biota to assimilate or transform nitrate. A first-order response to increasing NO_3 concentrations has been found in a single prairie reach in Kansas with short experimental fertilizations with NO_3 (Dodds et al. 2002), but saturation or efficiency loss is the typical response across streams with chronic NO_3 inputs (O'Brien et al. 2007, Mulholland et al. 2008). In a survey of 42 streams over three years in an urban watershed in Puerto Rico (Río Piedras), mean NO_3 concentrations were 736 μg N/L and several streams had concentrations between 1000 and 2500 μg N/L (J. D. Potter et al., *unpublished data*). Similar studies in streams with this chronic level of N loading likely would help fill in the details about the occurrence of N saturation in tropical streams.

Denitrification in tropical streams

The data reported here provide some of the first empirical evidence that denitrification is a significant fraction of total NO_3 uptake in tropical streams. Denitrification accounted for over 35% of total NO_3 uptake in five of our nine streams, and averaged 33% across all nine streams. In their study on the importance

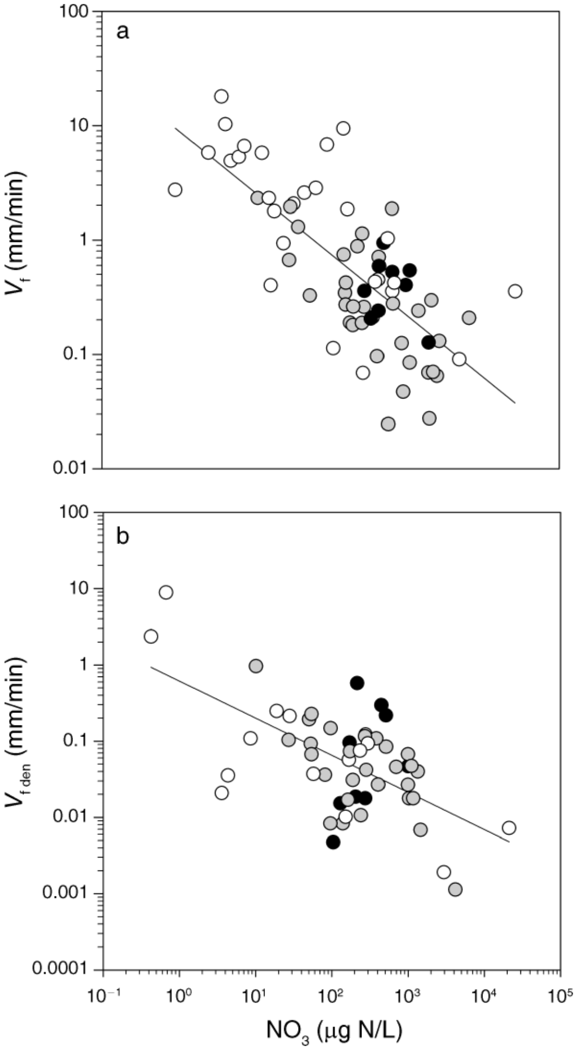

FIG. 4. Comparison of NO_3 dynamics in our tropical sites and all watersheds studied in LINX II (Mulholland et al. 2008). (a) Total NO_3 uptake velocity (V_f) and (b) NO_3 uptake velocity due to denitrification ($V_{f\,den}$) as a function of average stream NO_3 concentration. Black circles are from Puerto Rico, gray circles from other riparian forest based ecosystems, and open circles from ecosystems where riparian vegetation is not forest.

of N removal in headwater streams in the Mississippi River basin, Alexander et al. (2000) estimated a mean annual loss rate of N (k_t) via denitrification of 45.5% d^{-1}. In headwater streams of Puerto Rico, the average k_t was 67% d^{-1} across streams.

The nitrate uptake velocities resulting from denitrification ($V_{f\,den}$) in our Puerto Rican streams were highly variable, but within the range observed in other regions. There are several published studies that have used the reach-scale ^{15}N tracer technique to quantify ambient rates of denitrification in biomes other than the tropics (Bohlke et al. 2004, Mulholland et al. 2004, 2008, 2009, O'Brien et al. 2007). Stream $V_{f\,den}$ in Puerto Rico varies almost as much as does stream $V_{f\,den}$ from all the other biomes, but this variation in denitrification V_f occurs over a smaller range in NO_3 concentrations (Fig. 2d). Three of our streams (Ceiba, MTrib, and Vaca) have higher $V_{f\,den}$ than streams with similar nitrate concentrations located in other regions (Fig. 4). This suggests that tropical streams have the potential for highly efficient denitrification efficiency, although this potential was only realized for a third of the streams studied.

Areal rates of tropical stream denitrification exhibited strong relationships with NO_3 concentration (Fig. 2c) and whole stream ER (Fig. 3b). These relationships have also been found in several studies in temperate streams (Seitzinger et al. 2006, Mulholland et al. 2009), consistent with the physiological constraints on denitrification, with adequate supplies of NO_3 and organic carbon, and zones of reduced oxygen concentrations. The correlation we observed between ER and U_{den} indicates that energy availability may be a key driver of variation in denitrification rates among tropical streams. In areas of the stream with high organic matter, oxygen levels are likely reduced by aerobic respiration, and subsequently NO_3 is used by anaerobic or facultative bacteria as an electron acceptor. Incoming groundwater may also provide a low-oxygen environment in many of our streams; groundwater entering Icacos tributaries averages 1.4 mg oxygen/L (McDowell et al. 1992).

Areal rates of denitrification (U_{den}) were higher in our study streams than those measured in other biomes at similar stream water NO_3 concentrations, and had the highest average U_{den} of all the LINX II streams, despite not having the highest NO_3 concentrations. These results suggest that denitrification rates in small tropical streams are not primarily dependent upon NO_3 supply, as they are across biomes (Mulholland et al. 2008), but rather are more closely related to stream respiration and the presence of anaerobic zones. The high rates of denitrification in tropical streams are likely due to denitrifying organisms evolving in an environment where climatic factors lead to high rates of R (year round warm water temperatures, large inputs of organic matter, high rainfall) and high ambient NO_3. Although our study was conducted under conditions of base flow and our values represent rates at only one time of year, it illustrates the importance of denitrification in the removal of N in Puerto Rican streams. This is especially true when one considers that these denitrification rates are minimum estimates. They only include denitrification of water column NO_3, and do not include the coupled nitrification–denitrification pathway, the denitrification of NO_3 originally removed from the water column via assimilatory pathways (Seitzinger et al. 2006), or the denitrification associated with riparian N retention, which can be substantial (Chestnut and McDowell 2000).

Tropical stream NO_3 cycling and land use

Several recent studies have shown that the effects of land use on stream NO_3 cycling are primarily indirect, through impacts on NO_3 concentrations and riparian cover and function (Hall et al. 2009, Mulholland et al. 2009). Our results indicate that tropical watersheds also follow these continental-scale patterns, since stream NO_3 concentrations and GPP (correlated with percent canopy cover) were generally higher in urban and agricultural streams. Increased NO_3 concentrations led to higher U and U_{den} (Fig. 2a, c) indicating a stimulation of overall NO_3 uptake and denitrification, but we surprisingly found no significant relationship between V_{f} or $V_{\mathrm{f\ den}}$ and NO_3 concentration among our nine study streams (Fig. 2b, d). We expected that as NO_3 concentrations increased with anthropogenic impacts, there would be a reduction in the ability of these streams to retain N, which has been seen in other regions (Bernot and Dodds 2005, O'Brien et al. 2007) and at the continental scale (Mulholland et al. 2008, Hall et al. 2009). The absence of this relationship may be due to the relatively limited number of sites in our study (e.g., relative to Mulholland et al. 2008), or the small range in NO_3 concentrations, with even the reference streams having abundant NO_3, or it may indicate a fundamental difference in the NO_3 dynamics of tropical and temperate streams. This latter interpretation is made more plausible by the large differences in N cycling between tropical and temperate forests (e.g., Matson et al. 1999).

Changes in riparian cover and function associated with land use change appear to have different effects on overall NO_3 uptake and denitrification. In the case of total NO_3 uptake, the removal of riparian vegetation stimulates GPP (as indicated by correlation with PAR and percent canopy cover) and subsequently V_{f} (through assimilation). In the case of denitrification, the presence and function of the riparian zone is critical to stimulation of NO_3 removal as indicated by its relationship with ER. In our tropical streams, ER increases with increasing stream depth, discharge, and FBOM standing stocks, but increases with decreasing stream gradient. This indicates that it is the presence of riparian zones that provide OM and the conditions that generate anaerobic zones that drives variation in rates of stream denitrification across the landscape. The majority of streams where these physical attributes are present in Puerto Rico are in watersheds with anthropogenic impacts (and elevated NO_3 inputs), since forested areas are typically limited to the mountainous interior where stream velocities flush OM standing stocks and steep slopes limit the presence of riparian zones except in specific geologies (McDowell et al. 1992). Effectively, land use change occurs in places where denitrification can respond better to elevated inputs. If a goal of watershed management is ultimately to remove NO_3 via denitrification, then management efforts to increase denitrification should take priority over an increase in assimilation, which may be only a temporary removal pathway. Management activities that focus on enhancing the riparian inputs of organic matter that help fuel stream respiration, and maintaining the channel geomorphology that facilitates the retention and decomposition of organic matter, should be a top priority for tropical ecosystem management.

Stream denitrification and N_2O degassing in a landscape context

Concentrations of N_2O in stream water reflect the combined effects of nitrification and denitrification occurring in ground water that is transported into the stream, as well as in the stream itself. Venting of N_2O to the atmosphere by tropical streams appears to be a small component of total watershed N_2O emissions. Watershed emissions of N_2O are high in tropical sites (Matson and Vitousek 1990), yet N_2O emissions from our streams (0.85–14.27 ng $N \cdot cm^{-2} \cdot h^{-1}$) were similar to those found in temperate streams and rivers (Cole and Caraco 2001, Laursen and Seitzinger 2004). Areal N_2O emission rates from our streams were similar to those of soils (Macy 2005) in two of the same watersheds used in this study (6.53 ng $N \cdot cm^{-2} \cdot h^{-1}$ in Bisley stream compared to 3.24 ng $N \cdot cm^{-2} \cdot h^{-1}$ in Bisley soils; 3.74 ng $N \cdot cm^{-2} \cdot h^{-1}$ in RIT compared to 8.31 ng $N \cdot cm^{-2} \cdot h^{-1}$ in Icacos soils). Total N_2O loss is thus mostly from soils at the watershed scale, because streams contribute only a small fraction (0.6%) of watershed surface area. Emission of N_2O from the stream surface amounts to only 0.1% of the total N_2O emissions measured in the Icacos watershed, and 1.3% in Bisley. This estimate of 1.3% in the Bisley watershed is the same value that was found in the Hudson River basin (Cole and Caraco 2001). In agricultural (predominantly pasture) watersheds areal rates of N_2O emission are about 10-fold higher in the stream than in soils (Erickson et al. 2001), but still only average about 3% of overall N_2O emissions in the watershed.

The efficiency with which streams convert denitrification end products to N_2 results in a small role for stream emissions in watershed-scale N_2O production, but masks the important role played by streams in watershed-scale estimates of denitrification. The ratio of N_2O to N_2 produced by soils in Puerto Rico as a result of denitrification is about 1:1 (Chestnut et al. 1999), which is much greater than the range found in our streams (0.001–0.025) and streams from other studies (Seitzinger 1988, Seitzinger et al. 2000, Mulholland et al. 2004). The N_2O:N_2 is higher in tropical soils than streams probably due to the lower oxygen availability in stream sediments (Seitzinger and Kroeze 1998) rather than NO_3 availability, since NO_3 is abundant in both systems (McDowell and Asbury 1994, Chestnut et al. 1999). The large difference in N_2O:N_2 indicates that denitrification in tropical streams proceeds to the most reduced end product (N_2) much more often than it does in tropical soils. It also suggests that a disproportionately large amount of denitrification in tropical watersheds may be occurring in stream channels, rather than on the landscape, even though N_2O fluxes in tropical watersheds are considerable. In the Bisley watershed, for example, total denitrification in streams (29 kg N/yr) is about 15% of total soil denitrification (195 kg N/yr; Chestnut et al. 1999), even though water surface area accounts for less than 1% of the total land surface. If we apply the same rate of soil denitrification from the reference watershed to our urban watersheds ($n = 3$), then 40–56% of total watershed denitrification occurs in the stream channel in our urban study watersheds. This estimate is likely conservative, since these watersheds have extensive impervious surfaces and hydrologic routing often bypasses soil infiltration, thereby limiting rates of soil denitrification. Small streams thus appear to play a particularly significant role in the biogeochemical cycles of urbanizing watersheds in the changing Puerto Rican landscape.

The extraordinarily high ratio of N_2:N_2O in denitrification end products has additional important implications for understanding denitrification at watershed and landscape scales. From the standpoint of minimizing greenhouse gas emissions, denitrification that occurs in streams is much more desirable than that which occurs in soils, as it produces much less of the radiatively important end product (N_2O) per mole of nitrate that is denitrified. Protecting stream ecosystem function thus should be given high priority in urban land management in the tropics, and practices such as ditching and piping streams should be avoided. Secondly, the extraordinarily high and variable ratio means that measurement of denitrification in tropical streams cannot be accomplished through measurement of N_2O production alone under field conditions. Direct measurement of the N_2 produced during denitrification is essential for accurate measurement of stream denitrification, which means that isotopic approaches to understanding N dynamics in streams are particularly important in tropical biomes.

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