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 K15NO3 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, V1 [cm/s], and whole-stream nitrate uptake rate, U [μg N·m⁻²·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 μg N·m⁻²·min⁻¹; median = 15 μg N·m⁻²·min⁻¹), were dominated by the end product N2 (rather than N2O), and were best predicted by whole-stream respiration rates and stream NO3 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 NO3 uptake is limited by delivery of substrate (NO3⁻) 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 N2O 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 N2O emissions. Conservation of small streams is thus critical in tropical ecosystem management.

Key words: denitrification; N loading; N2O 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 (NO3⁻) 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 NO3 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, Neil 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$_2$O). 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$_2$O and N$_2$ during denitrification is also critical because of the potent greenhouse effects of N$_2$O (Wang et al. 1976). The ratio of N$_2$O:N$_2$ is related to oxygen, H$_2$S, and NO$_3^-$ availability and is lower in aquatic systems than soils (Firestone et al. 1980, Seitzinger and Kroeze 1998). N$_2$O emissions from rivers are thought to be a significant global source of N$_2$O (Seitzinger and Kroeze 1998), and there is a critical need to quantify stream N$_2$O 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$_2$O 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 (MTribs), and Quebrada Ceiba are mostly gravel-bottomed. Petunia and MTribs 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. MTribs 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.

<table>
<thead>
<tr>
<th>Stream name</th>
<th>Watered area (ha)</th>
<th>Native Agri</th>
<th>Cultivation Urban</th>
<th>Sub. type</th>
<th>Reach length (m)</th>
<th>Slope (%)</th>
<th>Q (L/s)</th>
<th>Width (m)</th>
<th>Depth (m)</th>
<th>Hydraulic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisley</td>
<td>58</td>
<td>99.7</td>
<td>0</td>
<td>0.3</td>
<td>Bl/Be</td>
<td>385</td>
<td>12.9</td>
<td>12.5</td>
<td>3.20</td>
<td>0.07</td>
</tr>
<tr>
<td>RIT</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>S/Fg</td>
<td>365</td>
<td>3.1</td>
<td>20.0</td>
<td>1.18</td>
<td>0.27</td>
</tr>
<tr>
<td>Pared</td>
<td>64</td>
<td>99.8</td>
<td>0.2</td>
<td>0</td>
<td>G/Co</td>
<td>525</td>
<td>14.2</td>
<td>5.2</td>
<td>2.74</td>
<td>0.04</td>
</tr>
<tr>
<td>Grande</td>
<td>95</td>
<td>28.3</td>
<td>69.3</td>
<td>2.4</td>
<td>S/Fg</td>
<td>690</td>
<td>1.4</td>
<td>12.3</td>
<td>1.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Maizales</td>
<td>265</td>
<td>61.7</td>
<td>34.3</td>
<td>4</td>
<td>G</td>
<td>450</td>
<td>2.5</td>
<td>25.0</td>
<td>3.53</td>
<td>0.35</td>
</tr>
<tr>
<td>Vaca</td>
<td>172</td>
<td>60.5</td>
<td>32.2</td>
<td>7.4</td>
<td>G</td>
<td>490</td>
<td>2.5</td>
<td>80.7</td>
<td>1.96</td>
<td>0.16</td>
</tr>
<tr>
<td>Petunia</td>
<td>110</td>
<td>6.7</td>
<td>0</td>
<td>92.3</td>
<td>G</td>
<td>350</td>
<td>4.0</td>
<td>4.7</td>
<td>1.80</td>
<td>0.11</td>
</tr>
<tr>
<td>MTRib</td>
<td>160</td>
<td>42</td>
<td>1.5</td>
<td>56.7</td>
<td>G</td>
<td>375</td>
<td>2.4</td>
<td>23.2</td>
<td>2.46</td>
<td>0.41</td>
</tr>
<tr>
<td>Ceiba</td>
<td>505</td>
<td>44.2</td>
<td>2.7</td>
<td>53.2</td>
<td>G</td>
<td>625</td>
<td>1.6</td>
<td>49.5</td>
<td>2.73</td>
<td>0.07</td>
</tr>
</tbody>
</table>

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, x, F_MTrib). 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 x is a measure of the lateral movement of solutes in a stream channel, which is primarily controlled by turbulence. F_MTrib 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 Icaos Tributary; and MTRib, Rio Mameyes Tributary.

Experimental procedures

Tracer enrichment experiments with 15NO₃ 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 15N 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 15N flux value (corrected for background and dilution) against distance below the 15N injection. The slope of this regression is the distance-normalized NO3 uptake rate and the inverse of the slope is the NO3 uptake length (S_w). Whole stream nitrate uptake rate (U; μg N·m⁻²·s⁻¹) was calculated by the following equation:

\[ U = F / (S_w \times w) \]  

where w is the average wetted width and F is the nutrient flux. The mass transfer coefficient (V1) was calculated by dividing U by the stream water NO3 concentration (Newbold et al. 1981, Stream Solute Workshop 1990). Assimilatory 15N uptake by biomass was calculated from the mass of 15N tracer found in each biomass compartment.

Denitrification rates (production of N₂ and N₂O) were determined from the production of 15N₂ and 15N₂O in the study reach using the approach described in Mulholland et al. (2004). First, tracer 15N₂ and 15N₂O MF values were computed and 15N₂ and 15N₂O flux were calculated with the MF values and the N₂ and N₂O mass values (statistically corrected for incomplete headspace mixing and air contamination). We solved for the 15N₂ and 15N₂O production rates by fitting the following relationship to the longitudinal pattern in tracer 15N₂ and 15N₂O flux (A) with distance x (in units of meters) downstream from the 15N₂ addition point:

\[ A = \left( \frac{k_{den} \times N_0}{k_2 - k_1} \right) \times (e^{-k_1 x} - e^{-k_2 x}) \]  

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

<table>
<thead>
<tr>
<th>Stream name</th>
<th>Experiment date (Feb–Mar)</th>
<th>NO₃ (μg N/L)</th>
<th>NH₄ (μg N/L)</th>
<th>DON (μg N/L)</th>
<th>SRP (μg P/L)</th>
<th>Water temperature (°C)</th>
<th>PAR (m²·µmol·m⁻²·d⁻¹)</th>
<th>Daily GPP (g O₂·m⁻²·d⁻¹)</th>
<th>Daily ER (g O₂·m⁻²·d⁻¹)</th>
<th>GPP/ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisley</td>
<td>2004</td>
<td>171</td>
<td>3</td>
<td>10</td>
<td>22</td>
<td>21</td>
<td>0.15</td>
<td>0.01</td>
<td>2.42</td>
<td>0.00</td>
</tr>
<tr>
<td>RIT</td>
<td>2005</td>
<td>131</td>
<td>7</td>
<td>23</td>
<td>0</td>
<td>19</td>
<td>0.50</td>
<td>0.47</td>
<td>4.49</td>
<td>0.10</td>
</tr>
<tr>
<td>Pared</td>
<td>2006</td>
<td>105</td>
<td>4</td>
<td>280</td>
<td>7</td>
<td>22</td>
<td>1.55</td>
<td>0.39</td>
<td>0.42</td>
<td>0.92</td>
</tr>
<tr>
<td>Grande</td>
<td>2004</td>
<td>276</td>
<td>11</td>
<td>70</td>
<td>13</td>
<td>23</td>
<td>6.39</td>
<td>5.18</td>
<td>7.64</td>
<td>0.68</td>
</tr>
<tr>
<td>Maizales</td>
<td>2005</td>
<td>206</td>
<td>7</td>
<td>78</td>
<td>12</td>
<td>23</td>
<td>13.95</td>
<td>7.29</td>
<td>5.31</td>
<td>1.37</td>
</tr>
<tr>
<td>Vaca</td>
<td>2006</td>
<td>440</td>
<td>3</td>
<td>167</td>
<td>9</td>
<td>23</td>
<td>1.52</td>
<td>3.08</td>
<td>15.69</td>
<td>0.20</td>
</tr>
<tr>
<td>Petunia</td>
<td>2004</td>
<td>997</td>
<td>15</td>
<td>53</td>
<td>26</td>
<td>24</td>
<td>0.02</td>
<td>0.34</td>
<td>4.60</td>
<td>0.07</td>
</tr>
<tr>
<td>MTRib</td>
<td>2005</td>
<td>174</td>
<td>2204</td>
<td>847</td>
<td>310</td>
<td>21</td>
<td>0.82</td>
<td>7.13</td>
<td>7.41</td>
<td>0.96</td>
</tr>
<tr>
<td>Ceiba</td>
<td>2006</td>
<td>512</td>
<td>50</td>
<td>11</td>
<td>22</td>
<td>25</td>
<td>1.98</td>
<td>9.33</td>
<td>11.74</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Note: Abbreviations are: DON, dissolved organic nitrogen; SRP, soluble reactive phosphorus; PAR, photosynthetically available radiation; GPP, gross primary productivity; ER, ecosystem respiration.
where $k_{\text{den}}$ 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 $\text{m}^{-1}$); and $k_2$ is the $N_2$ or $N_2O$ gas exchange rate through the air–water interface per unit distance (units of $\text{m}^{-1}$). 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 $k_{\text{den}}$ from fitting the model to $A$. Denitrification rate $k_{\text{den}}$ was then multiplied by stream NO$_3$ flux and divided by average stream width to give the areal denitrification rate. N$_2$O emission rates were estimated from dissolved N$_2$O concentrations in stream water and the N$_2$O 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 $S_w$, $V_f$, $A_{\text{den}}$, and $U_{\text{den}}$) were $Q/w$, NO$_3$ concentration, ER, and $F^{200}_{\text{med}}$ (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 $U_{\text{den}}$) 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·m$^{-2}$·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 ($\alpha$) ranged from 0.00002 (Pared) to 0.01875 s$^{-1}$ (Grande), but most were...
below 0.001 s\(^{-1}\), and \(F_{\text{med}}\) (Table 1) ranged from 0 (Maizales) to 0.24 (RIT).

NO\(_3\) concentrations varied from 105 \(\mu\)g N/L in Pared to 997 \(\mu\)g 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 \(\mu\)g N/L, with the highest concentrations in Ceiba (50 \(\mu\)g N/L) and MTRib (2204 \(\mu\)g 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 nine orders of magnitude from a mean of 27 \(\mu\)g N m\(^{-2}\) min\(^{-1}\) at RIT to 251 \(\mu\)g N m\(^{-2}\) min\(^{-1}\) at Ceiba. \(U\) had a significant positive relationship with 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 \(\mu\)g N m\(^{-2}\) min\(^{-1}\) at RIT to 251 \(\mu\)g N m\(^{-2}\) 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 m\(^{-2}\) d\(^{-1}\)) was generally higher than assimilation by heterotrophs associated with detrital pools (FBOM and CBOM; mean = 12.7 mg N m\(^{-2}\) 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 \(S_{\text{W}}\) and NO\(_3\) concentration, which also indicates that total NO\(_3\) uptake more closely follows first-order kinetics.

Denitrification

Areal denitrification rates (\(U_{\text{den}}\)) ranged from a stream day/night average of 0 \(\mu\)g N m\(^{-2}\) min\(^{-1}\) (Pared) to 133 \(\mu\)g N m\(^{-2}\) 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 \(N_2O\). \(U_{\text{den}}\) 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 (\(V_{\text{f,den}}\)) and uptake length due to denitrification (\(S_{\text{W,den}}\)) 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 (\(U_{\text{den}}\)) 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.

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°C to 25.3°C (Table 2). Light varied almost three orders of magnitude, from 0.02 mol quanta m\(^{-2}\) d\(^{-1}\) in an urbanized stream with very thick riparian cover (Petunia) to 13.95 mol quanta m\(^{-2}\) 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\(_2\)m\(^{-2}\) d\(^{-1}\) (Bisley) to 9.33 g O\(_2\) m\(^{-2}\) 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\(_2\) m\(^{-2}\) 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 (\(S_{\text{W}}\), Fig. 1) for NO\(_3\) was shortest in Pared (mean = 315 m), while \(S_{\text{W}}\) was longest in Vaca (day = 8480 m). \(S_{\text{W}}\) had a significant positive relationship with \(Q/W\) (\(r^2 = 0.72, P = 0.00\)). The average uptake velocity (\(V_U\)), ranged from 0.13 mm/min (Petunia; Fig. 1) to 0.94 mm/min (Maizales). \(V_U\) 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 \(\mu\)g N m\(^{-2}\) min\(^{-1}\) at RIT to 251 \(\mu\)g N m\(^{-2}\) 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; \(r^2 = 0.80, P < 0.01\)).
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$_4^+$. Nitrate 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$_2$O 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$_2$O 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$_2$O emission rates (from stream N$_2$O 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. 2006; 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,
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. 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.](image)
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\textsubscript{3} uptake varying with physical characteristics of the stream channel.

Across a spatial gradient of streams receiving chronic NO\textsubscript{3} inputs in Puerto Rico, first-order uptake kinetics better explained the biotic response to increasing NO\textsubscript{3} concentration for total NO\textsubscript{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\textsubscript{3} concentrations has been found in a single prairie reach in Kansas with short experimental fertilizations with NO\textsubscript{3} (Dodds et al. 2002), but saturation or efficiency loss is the typical response across streams with chronic NO\textsubscript{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 (Rio Piedras), mean NO\textsubscript{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\textsubscript{3} uptake in tropical streams. Denitrification accounted for over 35% of total NO\textsubscript{3} uptake in five of our nine streams, and averaged 33% across all nine streams. In their study on the importance...
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 ($V_f$) 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 (Bohle et al. 2004, Mulholland et al. 2004, 2008, 2009, O’Brien et al. 2007). Stream $V_f$ in Puerto Rico varies almost as much as does stream $V_f$ 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 $V_f$ 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 $U_{\text{den}}$ (Fig. 2a, c) indicating a stimulation of overall NO$_3$ uptake and denitrification, but we surprisingly found no significant relationship between $V_f$ or $V_f \text{den}$ 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$_2$O degassing in a landscape context

Concentrations of N$_2$O 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$_2$O to the atmosphere by tropical streams appears to be a small component of total watershed N$_2$O emissions. Watershed emissions of N$_2$O are high in tropical sites (Matson and Vitousek 1990), yet N$_2$O emissions from our streams (0.85–14.27 ng N cm$^{-2}$ h$^{-1}$) were similar to those found in temperate streams and rivers (Cole and Caraco 2001, Laursen and Seitzinger 2004). Areal N$_2$O 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 cm$^{-2}$ h$^{-1}$ in Bisley stream compared to 3.24 ng N cm$^{-2}$ h$^{-1}$ in Bisley soils; 3.74 ng N cm$^{-2}$ h$^{-1}$ in Icacos compared to 8.31 ng N cm$^{-2}$ h$^{-1}$ in Icacos soils). Total N$_2$O 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$_2$O from the stream surface amounts to only 0.1% of the total N$_2$O 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$_2$O 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$_2$O 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$_2$O production, but masks the important role played by streams in watershed-scale estimates of denitrification. The ratio of N$_2$O 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$_2$O:N$_2$ is higher in tropical soils than streams probably due to the lower oxygen availability in stream sediments (Seitzinger and Kroez 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$_2$O: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$_2$O 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 $\text{N}_2$:$\text{N}_2\text{O}$ 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 ($\text{N}_2\text{O}$) 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 $\text{N}_2\text{O}$ production alone under field conditions. Direct measurement of the $\text{N}_2$ produced during denitrification is essential for accurate measure of stream denitrification, which means that isotopic approaches to understanding N dynamics in streams are particularly important in tropical biomes.

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