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Nitrification increases nitrogen export from a tropical river network

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Abstract: Scaling aquatic ecosystem processes like nutrient removal is critical for assessing the importance of streams and rivers to watershed nutrient export. We used pulse NH4+ enrichment experiments and measured net NH4+ uptake in 7 streams throughout a mountainous tropical river network in Puerto Rico to assess spatial variability in NH4+ uptake and to infer the physical, chemical, and biological characteristics that most influence its variation. Across 14 experiments, NH4+ uptake velocity (v) ranged from 0.3 to 8.5 (mean = 2.7) mm/min and was positively related to algal biomass standing stock, measured as chlorophyll a. On average, 49% of experimentally added NH4+ was immediately transformed to NO3−, suggesting that nitrification can rival microbial and algal assimilation as a fate of streamwater NH4+. We considered the implications of our empirical results at the river-network scale based on a simple mass-balance model parameterized for the Río Mameyes watershed. Most catchment NH4+ inputs are delivered to 1st-order streams. Therefore, model results indicated that high NH4+ uptake rates in headwater streams limit NH4+ inputs to downstream reaches, thereby decreasing the role of larger streams in NH4+ removal at the river-network scale. In-stream nitrification resulted in additional NO3− inputs, which were more likely than NH4+ to be transported downstream because of lower biological demand for NO3− relative to NH4+. Given our estimates of catchment N loading to streams and rivers, we estimated that 39% of modeled watershed NO3− export was produced within the river network by nitrification. Together, these results suggest that streams and rivers can significantly transform the N load from their catchments.

Key words: nitrification, nutrient spiraling, ammonium uptake, river network model, gross primary production, TASC, tropical, stream, Puerto Rico

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and rivers across different watersheds, land uses, and biomes (Peterson et al. 2001, Hall et al. 2009). As a result, a fundamental understanding of the patterns and controls on nutrient uptake within individual river networks is lacking (Ensign and Doyle 2006), and it remains difficult to scale aquatic ecosystem processes from individual stream reaches to whole river networks and infer the role of aquatic ecosystems in global nutrient cycles (Wollheim et al. 2008b).

These scaling challenges are exacerbated in tropical environments, where fewer studies of aquatic nutrient cycling have been conducted compared to temperate ecosystems. Anthropogenic N deposition and N inputs from urbanization are increasing throughout the tropics (Downing et al. 1999, Matson et al. 1999). Therefore, understanding N removal in river networks is important for predicting the effects of anthropogenic activities on watershed N export, especially in tropical mountainous watersheds like those in Puerto Rico, where short transit times from land to the ocean make downstream estuaries vulnerable to eutrophication (Ortiz-Zayas et al. 2006). We know from previous research in Puerto Rico that stream NO$_3^-$ uptake rates are relatively low across streams draining forested, agricultural, and urban lands, but that NO$_3^-$ uptake increases with aquatic gross primary production (Potter et al. 2010). In addition, nitrification is thought to be an important fate of streamwater NH$_4^+$, as reported by Merriam et al. (2002) for one stream in northeastern Puerto Rico. However, no research to date has investigated the spatial variability in either stream NH$_4^+$ uptake or the relative importance of in-stream nitrification to NH$_4^+$ uptake in these river networks, which contain considerable heterogeneity in their topography, underlying geology, and hydrologic flowpaths over relatively small watershed areas (Pike et al. 2010, McDowell et al.1992, 2012).

The primary objectives of our study were to examine the spatial variability in stream NH$_4^+$ uptake and nitrification in a tropical mountain river network in Puerto Rico and to use a mass-balance model to analyze the implications of measured N uptake rates for the spatial distribution of N removal at the river-network scale. We predicted that NH$_4^+$ uptake would increase with stream metabolism, notably gross primary productivity (GPP), which should increase biological demand for N in streams with sufficient light availability. Furthermore, we expected that the relative importance of nitrification to stream NH$_4^+$ uptake would be highest in shaded headwaters, where streams may be energy-limited by lack of light inputs, high rates of decomposition, and low dissolved organic C (DOC) concentrations (Merriam et al. 2002, Helton et al. 2015). Better understanding of the patterns in N uptake and the processes influencing its variation within individual river networks will contribute to development of ecological scaling relationships that inform the fate of energy and nutrients in catchments.

**METHODS**

**Study sites**

This study was conducted in the Luquillo Experimental Forest in northeastern Puerto Rico (Fig. 1). We selected seven 1st- to 3rd-order streams within the upper Rio Mameyes watershed. Mean daily air temperature in the Luquillo mountains ranges from 19 to 26°C and annual precipitation ranges from 2500 to 4500 mm/y, with greater rainfall observed at higher elevations (100–1000 m asl) (Brown et al. 1983). The months of January to April typically have lower rainfall than the rest of the year (Heartsill-Scalley et al. 2007), but these tropical forests are not characterized by marked seasonality in precipitation, soil-solution chemistry, or stream chemistry (McDowell 1998, McDowell et al. 2012). The dominant soils are highly weathered, clay-rich

![Figure 1. Location of study sites within the upper Río Mameyes network, Puerto Rico. B1 = Bisley 1, B3 = Bisley 3, ANG = Angelito, PC = Piscina, JD = Juan Diego, QM = Máquina, and QC = Caimitillo. The hollow triangle denotes the location of the US Geological Survey gaging station on the Río Mameyes (USGS 50065500).](image-url)
Our study streams were heavily shaded but varied in aspect, catchment topography, and geology depending on their position in the watershed (Table 1). Four streams were situated in the western headwaters at higher elevation (300–800 m asl). Two of these streams drain hornfels facies, outcrops of contact metamorphosed volcaniclastic bedrock. Three streams were situated at lower elevation and drained north-facing slopes underlain by unmetamorphosed volcaniclastic bedrock, which is less resistant to erosion than the hornfels facies rock (Fig. 1) (Pike et al. 2010). Three of the 7 streams were identified as intensive study sites where we replicated our NH$_4^+$ enrichment experiments (described in detail below) in 2 reaches (Bisley 1, Bisley 3, Angelito) or at 2 points in time during a 6-wk period (Bisley 1, Bisley 3) to assess within-stream variability in NH$_4^+$ uptake. In each of the 7 streams, we chose study reaches that contained no tributary inflows and had a mean hydrologic travel time between 20 and 60 min to ensure sufficient distance and time for proper mixing and detectable uptake of solutes. The resulting reach lengths were typical of small streams (40–80 m; Webster and Valett 2006).

Stream metabolism and algal biomass

We estimated whole-stream metabolism for each stream reach by measuring changes in dissolved O$_2$ concentration (DO), water temperature, and light intensity at 10-min intervals over 1- to 5-d deployment periods with YSI (Yellow Springs Instruments, Yellow Springs, Ohio) ProODO optical dissolved O$_2$ sondes and Odyssey (Christchurch, New Zealand) light loggers. We estimated daily gross primary production (GPP) and ecosystem respiration (ER) rates by fitting a 1-station Lagrangian model to diel O$_2$ curves. We modified the approach taken by Riley and Dodds (2013) by describing the temperature dependence of GPP and ER with an Arrhenius equation, and we used a Bayesian approach with a uniform prior distribution to estimate daily rates of GPP and ER (Song et al. 2016). For multiday deployments, we allowed parameters (GPP, ER, and the gas-exchange coefficient [$K$-O$_2$]) to vary from day to day, but we assumed that $K$-O$_2$ is relatively constant between days if flow conditions do not change dramatically. Therefore, for days where GPP, ER, and $K$-O$_2$ were not uniquely estimable, we constructed a gamma distribution based on $K$-O$_2$ estimated from other deployment days in the same stream and used it as the prior distribution for $K$-O$_2$.

Logistical constraints required that we offset our stream metabolism measurements by as much as 3 to 9 d at 3 study sites (Piscina, Juan Diego, and Caimitillo). We present the metabolism data from these sites because no significant precipitation occurred between the metabolism and nutrient uptake measurements, and because climatic conditions, such as light and air temperature, remained similar (González 2015). In 3 other streams where multiday deployments overlapped the offset time period in Piscina, Juan Diego, and Caimitillo, variability in daily stream metabolism was lower than variability observed over the 6-wk experimental window (e.g., Bisley 1 reach 1, GPP CV = 68% during overlapping offset period; CV = 119% all observations; LEK, unpublished data). Therefore, we included stream metabolism data for Piscina, Juan Diego, and Caimitillo, but we acknowledge that short-term variability in daily GPP and ER at these sites may limit this comparison. In 6 of the 7 stream sites, we measured chlorophyll $a$ (Chl $a$) standing stock once within 0 to 4 d of the nutrient-enrichment experiment as a proxy for autotrophic biomass. We sampled benthic substrata for Chl $a$ at 10 evenly spaced transects throughout the stream reach either by scraping biofilm from a known surface area or by whole-rock extraction (Murdock and Dodds 2007). We analyzed Chl $a$ after hot-ethanol extraction on a Thermo Scientific (Waltham, MA) Genesys 6 spectrophotometer (Sartory and Grobbelaar 1984) and corrected for acidity time (Parker et al. 2016).

Nutrient-enrichment experiments

We performed 14 NH$_4^+$ enrichment experiments in 7 streams during January to March 2013. Prior to each reach-scale experiment, we collected background samples

<table>
<thead>
<tr>
<th>Study site</th>
<th>Stream order</th>
<th>Bedrock lithology</th>
<th>Upstream area (km$^2$)</th>
<th>Streambed slope (°)</th>
<th>Mean catchment slope (°)</th>
<th>Mean canopy cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisley 1</td>
<td>1</td>
<td>VC</td>
<td>0.07</td>
<td>13.2</td>
<td>27.9</td>
<td>95</td>
</tr>
<tr>
<td>Bisley 3</td>
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<td>VC</td>
<td>0.35</td>
<td>13.1</td>
<td>27.9</td>
<td>94</td>
</tr>
<tr>
<td>Angelito</td>
<td>2</td>
<td>VC</td>
<td>0.40</td>
<td>8.9</td>
<td>25.0</td>
<td>93</td>
</tr>
<tr>
<td>Piscina</td>
<td>2</td>
<td>HF</td>
<td>0.31</td>
<td>12.5</td>
<td>20.2</td>
<td>77</td>
</tr>
<tr>
<td>Juan Diego</td>
<td>2</td>
<td>VC</td>
<td>0.50</td>
<td>12.4</td>
<td>36.1</td>
<td>85</td>
</tr>
<tr>
<td>Máquina</td>
<td>3</td>
<td>VC</td>
<td>0.68</td>
<td>14.1</td>
<td>26.9</td>
<td>86</td>
</tr>
<tr>
<td>Caimitillo</td>
<td>2</td>
<td>HF</td>
<td>0.50</td>
<td>13.7</td>
<td>18.3</td>
<td>88</td>
</tr>
</tbody>
</table>

Table 1. Physical characteristics of study streams in the Luquillo Experimental Forest. VC = unmetamorphosed volcaniclastic bedrock, HF = hornfels facies (contact metamorphosed volcaniclastic bedrock).
of stream water for chemical analyses and measured stream discharge by dilution-gauging of short-term NaCl releases (Kilpatrick and Cobb 1985, Payn et al. 2009). We monitored specific conductance to ensure that Cl\(^-\) concentrations had returned to background levels between NaCl dilution-gauging releases and nutrient-enrichment experiments.

During each experiment we added reactive NH\(_4^+\) (as NH\(_3\)Cl) and a conservative tracer (as NaCl) to the stream as a single, pulse addition (Tank et al. 2008, Covino et al. 2010). We collected 20 to 35 samples over time (solute breakthrough curves) at the downstream end of each reach, where we monitored specific conductance with a YSI 556 conductivity probe logging at 5-s intervals. We filtered samples in the field through a 0.7-μm filter (Whatman GF/F) into 60-mL, acid-washed, high-density polyethylene (HDPE) bottles and shipped them frozen to the Water Quality Analysis Laboratory at the University of New Hampshire. Samples were analyzed for: 1) NH\(_4^+\)Cl) and a conservative tracer (as NaCl) to the stream discharge by dilution-gauging of short-term NaCl releases (Kilpatrick and Cobb 1985, Payn et al. 2009). We collected 20 to 35 samples over time (solute breakthrough curves) at the downstream end of each reach, where we monitored specific conductance with a YSI 556 conductivity probe logging at 5-s intervals. We filtered samples in the field through a 0.7-μm filter (Whatman GF/F) into 60-mL, acid-washed, high-density polyethylene (HDPE) bottles and shipped them frozen to the Water Quality Analysis Laboratory at the University of New Hampshire. Samples were analyzed for: 1) NH\(_4^+\) by the phenol–hypochlorite method (Solórzano 1969) on a Westco (Brookfield, Connecticut) SmartChem 200 discrete auto-analyzer, 2) Cl\(^-\) with a Dionex (Waltham, Massachusetts) ICS-1000 ion chromatograph, and 3) NO\(_3^–\)-N with the Cdrduction technique on a Seal Analytical (Mequon, Wisconsin) discrete auto-analyzer. Background water-chemistry samples also were analyzed for DOC and total dissolved N (TDN) by the high temperature catalytic oxidation method on a Shimadzu (Columbia, Maryland) TOC-V CPH with TNM N unit. We calculated dissolved organic N (DON) by subtracting dissolved inorganic N (DIN = NO\(_3^–\) + NH\(_4^+\)) from measured TDN concentrations.

### Estimating NH\(_4^+\) uptake and NO\(_3^–\) production

We used 2 approaches to assess stream response to experimental NH\(_4^+\) enrichments and to estimate rates of NH\(_4^+\) uptake. For each approach, we quantified nutrient-spiraling parameters from the breakthrough curve of added solutes, including the areal NH\(_4^+\) uptake rate (U; μg N m\(^{-2}\) min\(^{-1}\)) and NH\(_4^+\) uptake velocity (v\(_f\); N m/min), which represents uptake relative to NH\(_4^+\) availability (Stream Solute Workshop 1990). We estimated U of added N (hereafter referred to as net uptake [Unet]; Payn et al. 2005) for each sample across the breakthrough curve by using an approach modified from the tracer addition for spiraling curve characterization (TASCC) method (Covino et al. 2010). We calculated the 1st-order kinetic rate (K\(_f\); 1/min) for each sample by assuming an exponential decline in nutrient concentration over the stream reach (Stream Solute Workshop 1990):

\[
K_f = \frac{\ln \left( \frac{N_{BC}}{N_{BC}} \right) _{Unet} - \ln \left( \frac{N_{BC}}{N_{BC}} \right) _{ClBC}}{t},
\]  

(Eq. 1)

where N\(_{BC}\), Cl\(_{INJ}\), N\(_{BC}\), and Cl\(_{BC}\) are the NH\(_4^+\)-N and Cl\(^-\) concentrations of the injected solution and the background-corrected NH\(_4^+\)-N and Cl\(^-\) concentrations of each sample, respectively, and t is time elapsed since injection of the nutrient solution. Our use of Eq. 1 enabled us to account for variable travel times rather than using a single, average residence time throughout the breakthrough curve. This approach was advantageous because a single, average residence time likely is not representative of our study streams and relatively short reach lengths.

We converted K\(_f\) for each sample to net v\(_f\) and Unet:

\[
v_f = K_f z,
\]  

(Eq. 2)

\[
Unet = v_f N_{add},
\]  

(Eq. 3)

\[
N_{add} = \sqrt{N_{cons} N_{BC}},
\]  

(Eq. 4)

where z is reach-averaged depth and N\(_{add}\) represents the reach-averaged concentration of added NH\(_4^+\) for a given sample, calculated as the geometric mean of the background-corrected NH\(_4^+\) concentration and the estimated NH\(_4^+\) concentration given conservative transport (\(N_{cons}\), Eq. 4). We calculated \(N_{cons}\) by multiplying the background-corrected Cl\(^-\) concentration by the NH\(_4^+\) : Cl\(^-\) ratio of the injected solution. We used the geometric mean to represent the added nutrient concentration during the experiment because the background-corrected NH\(_4^+\) concentration at the measurement location probably underestimates the N concentration experienced by stream biofilms along the length of the experimental reach. In this sense, the geometric mean concentration represents a best approximation of background-corrected NH\(_4^+\) concentration throughout the stream reach during the enrichment experiment (Covino et al. 2010). For each experiment, we used the slope of the best-fit line between Unet and the total reach-averaged NH\(_4^+\) concentration in each sample (N\(_{total}\), equal to the sum of background NH\(_4^+\)-N concentration and N\(_{add}\) to estimate the average net v\(_f\). Use of this approach carried the inherent assumption that net v\(_f\) is constant with concentration (i.e., 1st-order kinetics), and therefore, net NH\(_4^+\) v\(_f\) represents a reach-averaged v\(_f\) during the experiment.

We also used a transport-based modeling approach to estimate NH\(_4^+\) uptake. We observed significant hysteresis in the relationship between Unet and reach-averaged NH\(_4^+\) concentration during all of our pulse nutrient-enrichment experiments (Fig. 2). This consistent pattern suggested that NH\(_4^+\) Unet might vary with flowpath in our study reaches and motivated our use of an advection–dispersion modeling approach to estimate separate NH\(_4^+\) uptake rates for the stream channel and transient storage zone, respectively. We used a 1-dimensional advection–dispersion model with transient storage, and assumed 1st-order uptake kinetics (Stream Solute Workshop 1990, Runkel 2007). Specifically, we modeled solute dynamics as:
transient storage zone, and represent the cross-sectional area of the stream channel and values for the relative size of transient storage (as NO₃⁻-N) respectively.

The kinetic rate (1/min) in the main channel and transient storage zone, respectively, for stream velocity, where sample shading indicates the elapsed time since injection (resulting in NO₃⁻-N recovered at the downstream sampling station, NH₄⁺-N injected, and NH₄⁺-N recovered at the downstream sampling station during the experiment (sensu Snyder and Bowden 2014). Estimates of % NH₄⁺-N nitrified based on Eq. 7 represent net nitrification and, therefore, are conservative because we do not account for any NO₃⁻-N that may have been consumed in the stream reach.

Statistical analyses

We used net NH₄⁺-νᵣ to compare NH₄⁺-uptake rates across streams because νᵣ enables comparison of streams with different hydrologic characteristics and background N concentrations (Hall et al. 2002, Ensign and Doyle 2006). We present results from the advection-dispersion model method because estimates of net νᵣ were similar between the 2 approaches presented above (Table 2) and because the advection-dispersion method enabled us to isolate NH₄⁺-uptake in the main stream channel (hereafter, net stream NH₄⁺-νᵣ) and in transient storage zones. We used Pearson correlations to relate net stream NH₄⁺-νᵣ and % NH₄⁺-nitrified to physical, chemical, and biological variables that we hypothesized would influence their variation in streams draining the Luquillo Mountains. We examined bivariate relationships between net stream NH₄⁺-νᵣ and % NH₄⁺-nitrified and 9 potential covariates, including light intensity, Tᵣ, GPP, ER, Chl a, and N and C availability (as NO₃⁻-N, DON, DOC, and the DOC : DIN molar ratio). To avoid bias toward the intensive study sites where nutrient-addition experiments were performed in multiple reaches, we averaged values for νᵣ, % NH₄⁺-nitrified, and all covariates when experiments were performed in multiple reaches within a stream on the same day (resulting in n = 9). All statistical analyses were conducted in R (version 3.3.2; R Project for Statistical Computing, Vienna, Austria).

River-network N dynamics and export

Our 2nd objective was to analyze the implications of our empirical results at the river-network scale. We applied an N mass-balance model to a statistical representation of the 5th-order Río Mameyes network following the approach described by Wollheim et al. (2006), although we added an
Table 2. Mean background characteristics and uptake metrics for streams in the Río Mameyes network during 14 NH₄⁺ enrichment experiments. Data from reaches 1 and 2 within the same study site were averaged for each experiment date. Whole-stream metabolism was not measured in Quebrada Máquina because of logistical constraints (–). Reach light intensity is presented as mean photosynthetically active radiation (PAR) over 24 h. NH₄⁺ background concentrations were below the laboratory method detection limit. Q = discharge, Aᵢ/A = relative size of the transient storage zone, α = storage exchange-rate coefficient, DOC = dissolved organic C, DON = dissolved organic N, Chl a = chlorophyll a, GPP = gross primary production, ER = ecosystem respiration, νᵢ = uptake velocity, reg app = regression approach.

| Study site | Date       | Q (L/s) | Width (m) | Depth (m) | PAR (µE m⁻² s⁻¹) | Aᵢ /A (1/min) | NH₄⁺ (µg N/L) | NO₃⁻ (µg N/L) | DOC (mg C/L) | DON (µg N/L) | Chl a (mg/m²) | GPP (g O₂ m⁻² d⁻¹) | ER (g O₂ m⁻² d⁻¹) | NH₄⁺ νᵢ (mm/min)| Storage zone NH₄⁺ νᵢ (mm/min) | NH₄⁺ νᵢ (reg app) | % nitrified |
|------------|------------|---------|-----------|-----------|------------------|---------------|---------------|---------------|---------------|---------------|----------------|-----------------|----------------|----------------|----------------|----------------|
| Bisley 1   | reach 1    | 30 Jan 2013 | 1.0       | 1.1       | 0.04            | 2.0           | 0.45           | 0.03          | <5            | 139           | 0.6            | 23              | –               | 0.8            | 3.1            | 2.1            | 0.02            | 1.7           | 18            |
|            |            | 6 Mar 2013  | 1.7       | 1.3       | 0.07            | 2.7           | 0.29           | 0.08          | <5            | 134           | 0.5            | 15              | 4.6             | 5.8            | 21.2           | 3.8            | 0.00            | 2.6           | 78            |
| Bisley 1   | reach 2    | 30 Jan 2013 | 1.0       | 1.4       | 0.03            | 7.0           | 0.33           | 1.42          | <5            | 108           | 0.8            | 25              | –               | 0.1            | 0.5            | 1.4            | 0.00            | 0.9           | 14            |
|            |            | 6 Mar 2013  | 1.7       | 1.4       | 0.06            | 2.8           | 0.55           | 0.13          | <5            | 93            | 0.8            | 10              | 3.3             | 3.8            | 5.5            | 3.3            | 0.00            | 1.9           | 49            |
| Bisley 3   | reach 1    | 5 Feb 2013  | 9.5       | 2.8       | 0.10            | 6.5           | 0.67           | 0.19          | <5            | 87            | 0.7            | 22              | –               | 2.8            | 7.6            | 2.7            | 0.00            | 2.0           | 24            |
|            |            | 7 Mar 2013  | 9.1       | 3.5       | 0.08            | 7.0           | 0.68           | 0.20          | <5            | 66            | 0.8            | 20              | 0.7             | 2.2            | 16.6           | 2.1            | 0.00            | 1.5           | 48            |
| Bisley 3   | reach 2    | 5 Feb 2013  | 8.7       | 3.3       | 0.07            | 9.8           | 0.97           | 0.30          | <5            | 83            | 0.7            | 46              | –               | 0.4            | 6.5            | 1.7            | 0.00            | 1.4           | 34            |
|            |            | 7 Mar 2013  | 9.1       | 3.5       | 0.08            | 6.2           | 0.76           | 0.22          | <5            | 67            | 0.6            | 16              | 2.4             | 0.7            | 9.9            | 1.7            | 0.00            | 1.7           | 71            |
| Angelito   | reach 1    | 3 Feb 2013  | 22.0      | 5.0       | 0.09            | 16.1          | 0.36           | 0.14          | <5            | 82            | 1.2            | 50              | –               | 1.2            | 3.5            | 1.7            | 0.00            | 1.2           | 70            |
| Angelito   | reach 2    | 3 Feb 2013  | 27.5      | 4.9       | 0.11            | 11.0          | 0.33           | 0.15          | <5            | 78            | 1.0            | 62              | –               | 2.5            | 0.6            | 2.6            | 0.05            | 2.7           | 48            |
| Piscina    | 22 Feb 2013 | 15.5      | 3.8       | 0.15       | 53.8           | 0.49           | 0.14          | <5            | 57            | 1.2            | 39              | 8.2             | 2.7            | 12.9           | 3.5            | 0.00            | 3.2           | 69            |
| Juan Diego | 19 Feb 2013 | 10.7      | 2.9       | 0.08       | 22.0           | 0.50           | 0.12          | <5            | 104           | 0.7            | 18              | 5.7             | 0.8            | 6.1            | 2.5            | 0.00            | 2.0           | 38            |
| Máquina    | 18 Feb 2013 | 7.5       | 2.9       | 0.12       | –              | 1.68           | 0.32           | <5            | 161           | 1.7            | 122             | 2.3             | –              | –              | 0.3            | 0.00            | 1.9           | 60            |
| Caimitillo | 23 Feb 2013 | 13.0      | 3.0       | 0.12       | 66.6           | 0.78           | 0.20           | <5            | 100           | 0.6            | 39              | 24.9            | 1.7            | 0.2            | 8.5            | 0.00            | 4.6           | 63            |
additional source of NO$_3^-$ from in-stream nitrification. We estimated mean length, drainage area, and number of individual streams for each stream order in the river network based on the stream-order area ($R_0$), number ($R_0$), and length ($R_L$) ratios developed by Horton (1945). The empirical values of these ratios for the Río Mameyes network are 3.5, 3.3, and 1.7, respectively, and were obtained from a digital topological river network developed from a 10-m digital elevation model. We estimated mean river width and depth for each stream order from hydraulic scaling relationships (Leopold and Maddock 1953) based on downstream scaling exponents and coefficients developed specifically for the Río Mameyes network (Pike et al. 2010). Direct drainage from land to each river order was estimated based on the proportion of total river-network length represented by that river order, assuming that inputs enter the upstream end of a stream reach, as described by Wollheim et al. (2006).

In our model, we assumed that NH$_4^+$ and NO$_3^-$ in streams of a given order are sourced from: 1) local groundwater inputs and 2) upstream river reaches. N input from the catchment ($N_{LOAD_i}$, mg/d) into each stream order $i$ is calculated as:

$$N_{LOAD_i} = RO \times D_i \times N_{GW}.$$  
(Eq. 8)

$RO$ is the median daily runoff (4.9 mm/d; USGS 50065500, 38-y period of record), $D_i$ is the watershed area that drains directly into each stream order, and $N_{GW}$ is the concentration of dissolved NH$_4^+$ or NO$_3^-$ in groundwater. We assigned $N_{GW}$ as 0.14 mg NH$_4^+$·L$^{-1}$ and 0.10 mg NO$_3^-$·L$^{-1}$, respectively, based on the average concentration from soil lysimeters and shallow groundwater wells within the Río Mameyes and the neighboring Río Sonadora watersheds (McDowell et al. 1992, 1996, McDowell 1998, McDowell and Liptzin 2014, Cusack et al. 2016). In applying Eq. 8, we assumed spatially uniform runoff and $N_{LOAD}$ to isolate the hydrological and biological processes that affect the distribution of nutrient removal throughout the river network.

A proportion of N inputs to an individual stream of order $i$ is removed by in-stream processing ($R_i$, unitless) associated with benthic sediments:

$$R_i = 1 - e^{-\nu_i/HL_i},$$  
(Eq. 9)

$\nu_i$ represents either the net NH$_4^+$ uptake ($\nu_i$; mean; our study) or the net NO$_3^-$ uptake ($\nu_i$; 0.41 mm/min; mean, $n = 9$ streams; Potter et al. 2010), respectively. $HL_i$ is the hydraulic load, equivalent to mean discharge over streambed area ($Q/wl$) for each stream order $i$ of mean width $w$ and length $l$. We conservatively assumed that neither NH$_4^+$ nor NO$_3^-$ uptake vary with stream size because: 1) the mean $\nu_i$ encompasses the observed range, which was not strongly related to most of the covariates we considered (see Results below) and 2) across streams, syntheses of empirical measurements of nutrient uptake processes little evidence that $\nu_i$ changes systematically with stream size (Ensign and Doyle 2006, Tank et al. 2008, Hall et al. 2013). We also assumed that a uniform fraction of immobilized NH$_4^+$ is converted to NO$_3^-$ via nitrification (mean; our study), and any N in excess of benthic demand is delivered downstream to larger stream reaches. We tested the sensitivity of model results to these assumptions, especially variability in NH$_4^+$/NO$_3^-$ and nitrification by: 1) testing all combinations of parameter values representing the observed mean ± 1 SE for NH$_4^+$, NO$_3^-$ and % NH$_4^+$ nitrified, and 2) testing a model scenario where NH$_4^+$ and NO$_3^-$ uptake increased by 20% with each stream order from the mean $\nu_i$ rates described above to simulate increasing N demand with stream size, as might be expected with downstream opening of the canopy and increased assimilative N demand (Tank et al. 2008).

This modeling exercise necessarily simplifies real systems and is not meant to predict or forecast N exports from the Río Mameyes watershed. Rather, we used this heuristic mass-balance approach to ask: what are the implications of observed rates of NH$_4^+$ uptake and nitrification when applied to an entire river network? In this way, our empirical observations of stream NH$_4^+$ uptake and nitrification enabled us to assess the potential for these tropical streams to alter the amount and form of N that is exported from the river network.

**RESULTS**

Physicochemical characteristics and stream metabolism

Mean transient storage residence time ($T_i$) was short and ranged from ~0.5 to 16 min. Background NH$_4^+$ concentrations were consistently below analytical detection, although NO$_3^-$ concentrations were relatively high for an undisturbed watershed (57–161 µg N/L). Background DOC concentrations were low, ranging from 0.5 to 1.7 mg C/L, and DOC : DIN molar ratios varied from 3.4 to 16.6. Daily average light intensity ranged from 2.0 to 66.6 µE m$^{-2}$ s$^{-1}$ (Table 2), but was not significantly related to GPP over the observed ranges in our study ($r = -0.25$, $p = 0.413$). Chl $a$ standing stock ranged from 0.7 to 24.9 mg/m$^2$. Log$_e$(Chl $a$) was not significantly correlated with stream GPP ($r = -0.01$, $p = 0.982$), but Chl $a$ was greatest at Caimitillo, the stream site with the highest daily average light intensity (Table 2).

NH$_4^+$ uptake across streams

In each of our 14 NH$_4^+$ enrichment experiments, we observed significant hysteresis in the relationship between $U_{net}$ and reach-averaged NH$_4^+$ concentration. NH$_4^+$ $U_{net}$ was consistently greater for samples on the rising limb than on the falling limb of the breakthrough curve, as shown for an experiment conducted in the Angelito stream (Fig. 2). Our estimates of $\nu_i$ from the advection–dispersion transport model...
corroborate these patterns because net stream $\text{NH}_4^+$ $\nu_f$ was much greater than net $\text{NH}_4^+$ $\nu_f$ in transient storage zones, which was undetectable (i.e., not different from 0) for most of our experiments (Table 2).

Mass removal of added $\text{NH}_4^+$ ranged from 25 to 80% in our 14 experiments (data not shown), and net stream $\text{NH}_4^+$ $\nu_f$ ranged from 0.3 to 8.5 mm/min (mean = 2.7 mm/min; Table 2). Variability in $\text{NH}_4^+$ $\nu_f$ was greater among streams (coefficient of variation [CV] = 71%) than within intensive study streams with >2 measurements (Bisley 1 CV = 42%; Bisley 3 CV = 24%). Net stream $\text{NH}_4^+$ $\nu_f$ was not significantly correlated with most physical, chemical, and biological covariates that we considered (Fig. 3A–I, Table 3) with the exception of a positive correlation between net stream $\text{NH}_4^+$ $\nu_f$ and Chl $a$ (Fig. 3B, Table 3), suggesting that stream $\text{NH}_4^+$ demand increases with autotrophic biomass.

**Nitrification**

On average, 49% of added $\text{NH}_4^+$ was converted to $\text{NO}_3^-$, which suggests that nitrification was a significant fate of added $\text{NH}_4^+$. Across all experiments, 14 to 78% of added $\text{NH}_4^+$ was nitrified immediately (Table 2), resulting in nitrification rates ranging from 0.2 to 16.9 $\mu$g N m$^{-2}$ min$^{-1}$. Percent $\text{NH}_4^+$ nitrified was not significantly correlated with net stream $\text{NH}_4^+$ $\nu_f$, background concentrations of dissolved N and C, or daily average light intensity over their observed range of variability (Table 3). Percent $\text{NH}_4^+$ nitrified varied

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**Figure 3.** Scatterplots showing the correlations between net stream $\text{NH}_4^+$ uptake velocity ($\nu_f$) and photosynthetically active radiation (PAR) (A), algal biomass standing stock (chlorophyll $a$) (B), gross primary production (GPP) (C), ecosystem respiration (ER) (D), transient storage zone residence time ($T_s$) (E), $\text{NO}_3^-$ (F), dissolved organic N (DON) (G), dissolved organic C (DOC) (H), and the DOC:dissolved inorganic N (DIN) molar ratio (I). If experiments were performed in multiple reaches within a stream on the same day, we used mean values of covariates and net stream $\nu_f$ (resulting in $n = 9$), although sample size for individual bivariate relationships varied depending on data availability (Table 3).
at the network scale results from the combined in
biotic demand and the distribution of NO₃ to 0.9 kg N/d in the 5th-order mainstem (Fig. 4A). NO₃
the 5th-order mainstem river (Fig. 4B).

NH₄ occurs because: 1) most NH₄ was 0.041, 0.033, 0.026, 0.021, and 0.017 km², respectively.
was 45, 23, 12, 6, and 3 km, and total benthic surface area
work enters small streams
catchment, although NO₃
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Table 3. Pearson correlation coefficients for the relationships between net stream channel NH₄⁺ uptake velocity (ν₁) and % NH₄⁺ nitrified vs physical, chemical, and biological covariates. Photosynthetically active radiation (PAR), chlorophyll a, transient storage residence time (Tᵣ), ambient dissolved organic C (DOC), ambient dissolved organic N (DON), and DOC:DIN molar ratio were ln(x-transformed) to meet normality assumptions. Stream metabolism was not measured at the Máquina site, and chlorophyll a was not measured during the January/early February experiments at Bisley 1, Bisley 3, and Angelito. Bold indicates (p < 0.05).

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>n</th>
<th>Net stream NH₄⁺ ν₁</th>
<th>% NH₄⁺ nitrified</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR (µE m⁻² s⁻¹)</td>
<td>8</td>
<td>0.106</td>
<td>0.61</td>
</tr>
<tr>
<td>Chlorophyll a (mg/m²)</td>
<td>6</td>
<td>0.019</td>
<td>0.89</td>
</tr>
<tr>
<td>GPP (g O₂ m⁻² d⁻¹)</td>
<td>8</td>
<td>0.622</td>
<td>0.21</td>
</tr>
<tr>
<td>ER (g O₂ m⁻² d⁻¹)</td>
<td>8</td>
<td>0.460</td>
<td>−0.31</td>
</tr>
<tr>
<td>Tᵣ (min)</td>
<td>9</td>
<td>0.680</td>
<td>−0.16</td>
</tr>
<tr>
<td>Ambient NO₃⁻ (µg N/L)</td>
<td>9</td>
<td>0.506</td>
<td>−0.26</td>
</tr>
<tr>
<td>Ambient DON (µg N/L)</td>
<td>9</td>
<td>0.630</td>
<td>−0.19</td>
</tr>
<tr>
<td>Ambient DOC (mg C/L)</td>
<td>9</td>
<td>0.137</td>
<td>−0.54</td>
</tr>
<tr>
<td>DOC:DIN molar ratio</td>
<td>9</td>
<td>0.417</td>
<td>−0.31</td>
</tr>
</tbody>
</table>

within intensive study streams (Bisley 1 CV = 76%, Bisley 3 CV = 46%), but we are unable to explain this variation at the individual stream or landscape levels with our data.

River-network N dynamics and export

Based on stream hydrography and hydraulic measurements throughout the upper Río Mameyes network, stream length and benthic surface area were skewed toward the headwaters. Total stream length for 1st- to 5th-order streams was 45, 23, 12, 6, and 3 km, and total benthic surface area was 0.041, 0.033, 0.026, 0.021, and 0.017 km², respectively. Direct inputs to the river network were distributed more heavily toward 1st- and 2nd-order streams, which together receive 76% of the direct drainage from land. Network-scale NH₄⁺ uptake declined from 6 kg N/d integrated across all 1st-order streams to 0.6 kg N/d in the 5th-order mainstem river (Fig. 4A). This decline in NH₄⁺ uptake with stream order occurs because: 1) most NH₄⁺ delivered to the river network enters small streams first, and 2) biological demand for NH₄⁺ is relatively high compared to the length and residence time of small streams (Fig. 4B), resulting in rapid removal near the point where NH₄⁺ enters the network. Proportional removal of NH₄⁺ inputs to a stream of a given order declined from 0.30 for 1st-order streams to 0.11 in the 5th-order mainstem (Fig. 4B). For inputs to a given stream order, proportional N removal was lower for NO₃⁻ than NH₄⁺ because of the low NO₃⁻ νᵢ relative to NH₄⁺ ν₁ (Ensign and Doyle 2006). Total inputs of NH₄⁺ and NO₃⁻ to the river network were similar (13.4 and 15.9 kg N/d, respectively, with 6.3 kg N/d of NO₃⁻ inputs from in-stream nitrification), but differences in biotic demand resulted in lower estimated NH₄⁺ export (0.5 kg N/d) than NO₃⁻ export (7.5 kg N/d). At the network scale, model results indicated that 2.9 kg N/d of the total NO₃⁻ export was produced within the river network by nitrification.

The proportion of N inputs removed within individual streams, and therefore, the magnitude of network-scale NH₄⁺ and NO₃⁻ uptake were only slightly sensitive to the parameter values for NH₄⁺ νᵢ NO₃⁻ νᵢ and % NH₄⁺ nitrified, based on ranges in field measurements (Fig. 4A, B). Small streams were relatively more important than larger streams for N uptake in the Río Mameyes network across all parameter combinations (Fig. 4A, B) regardless of our a priori assumption of constant νᵢ with stream size (Fig. S1A, B vs Fig. 4A, B). Network-scale NH₄⁺ uptake was relatively unchanged despite increasing NH₄⁺ νᵢ with stream order (<7% increase in NH₄⁺ uptake for each stream order; Fig. S1A) because NH₄⁺ demand in small streams (based on measured νᵢ) is high enough to limit downstream transport of NH₄⁺ to larger streams. Increased NO₃⁻ νᵢ with stream size

Ambient NO₃⁻ (µg N/L) 9 0.506 −0.26
Ambient DON (µg N/L) 9 0.630 −0.19
Ambient DOC (mg C/L) 9 0.137 −0.54
DOC:DIN molar ratio 9 0.417 −0.31

did result in slightly higher network-scale NO₃⁻ uptake rates compared to the base model scenario (e.g., NO₃⁻ uptake increased by 18% across 1st-order streams and increased by 62% in the 5th-order mainstem). However, NO₃⁻ uptake in this increasing NO₃⁻ vᵢ scenario was greatest in 2nd-order streams, and small streams remained the more important sites of NO₃⁻ removal at the network scale (Fig. S1A).

**DISCUSSION**

NH₄⁺ uptake rates are high in the mountainous tropical streams we studied. NH₄⁺ vᵢ was consistently greater than NO₃⁻ vᵢ previously measured in 9 streams spanning a range of human land uses in Puerto Rico (Potter et al. 2010). Our habitat-partitioned estimates of NH₄⁺ vᵢ suggest that the stream channel is very reactive compared to transient stor-

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**NH₄⁺ uptake**

NH₄⁺ vᵢ spanned a range commonly observed in streams (Hall et al. 2002, Ensign and Doyle 2006). However, despite differences in watershed topography and geology (Table 1) as well as stream chemistry and hydrology (Table 2), NH₄⁺ vᵢ was relatively uniform across streams, and the variability was driven largely by 1 stream with a disproportionately high NH₄⁺ vᵢ (Caimitillo). Stream metabolism should influence NH₄⁺ uptake (Wollheim et al. 2001, Hall and Tank 2003, Newbold et al. 2006, Hoellein et al. 2007). However, NH₄⁺ vᵢ was positively correlated with Chl a standing stock but not GPP (Fig. 3B, C). This result could be a consequence of the offset between metabolism and nutrient uptake in some streams because, despite similar weather conditions between measurements, other factors including turbidity or time since scour can affect daily variation in GPP (Uehlinger 2000). Our observation that benthic Chl a was related to NH₄⁺ uptake is consistent with high rates of epilithon uptake of isotopically labeled NH₄⁺ in the Bisley 3 stream (Merriam et al. 2002), suggesting that algae in biofilms can contribute to inorganic N uptake even in relatively shaded streams. Similar to previous findings in temperate streams, we did not observe a strong influence of ER on NH₄⁺ uptake (Webster et al. 2003, Fellows et al. 2006). In these tropical streams, leaf litter is decomposed rapidly (Heartsill-Scalley et al. 2012), so microbial heterotroph N demand might be satisfied by recycling high-energy organic N during decomposition (Sinsabaugh and Follstad-Shah 2012) rather than by using inorganic N from the water column. Our measurements were limited to small streams, and the variability in light intensity, GPP, and ER across our sites probably underestimates the variability that might be observed at the scale of a larger river network. Further measurements in larger rivers may help statistically examine the influence of metabolism on NH₄⁺ uptake.

NH₄⁺ uptake represents the net balance between multiple processes that consume and produce NH₄⁺ and integrates multiple habitats within a stream reach. We observed
clockwise hysteresis patterns in $U_{\text{net}}$ during each of our experiments. This observation suggests that: 1) hydrologic exchange with transient storage is significant, and 2) $K_t$ differs between the main channel and transient storage zones (e.g., subsurface hyporheic flow and surface pools). One explanation for the clockwise hysteresis pattern is preferential uptake in the stream channel combined with downstream flowpaths through less-reactive hyporheic zones. Alternatively, calculations of sample-specific uptake rates (sensu Covino et al. 2010) are sensitive to abiotic sorption of NH$_4^+$ to sediments. Concentration-driven changes in net adsorption could result in NH$_4^+$ sorption (and overestimation of NH$_4^+$ removal) on the rising limb of the breakthrough curve followed by desorption (and underestimation of NH$_4^+$ removal) on the falling limb as sediment–water concentration gradients shift with NH$_4^+$ concentration in the water column.

We observed increasing NH$_4^+$ : Cl ratios on the falling limb of the breakthrough curve in 4 of our experiments, as would be expected given desorption of added NH$_4^+$ (Day and Hall 2017). However, we did not observe this pattern in most experiments with clockwise hysteresis in $U_{\text{net}}$, so we assume that the adsorption effect was limited across streams. Hysteresis patterns have been observed by numerous investigators conducting pulse nutrient-enrichment experiments (Gibson et al. 2015, Trentman et al. 2015) and may provide useful information about habitat-specific uptake rates in streams, although abiotic adsorption to sediments must be considered as well.

**Nitrification**

Nitrification was an important mechanism of NH$_4^+$ removal during downstream transport. Our observation that on average, 49% of added NH$_4^+$ was converted to NO$_3^-$ is consistent with previous work in Bisley 3, where Merriam et al. (2002) found that nitrification accounted for 57% of total NH$_4^+$ uptake during a 6-wk $^{15}$NH$_4^+$ tracer experiment. In streams across the USA, Peterson et al. (2001) found that on average, 20 to 30% of NH$_4^+$ uptake was nitrified, which, taken together with our findings, indicates that nitrification can rival assimilation as a short-term sink for dissolved NH$_4^+$. Our study confirms the generality of high nitrification rates in mountainous tropical streams, where microbes are probably energy-limited because decomposition of terrestrial leaf litter is rapid (Heartstill-Scalley et al. 2012), dissolved organic C is strongly retained by mineral soils (McDowell 1998, Neff and Asner 2001), and light availability limits primary production (Ortiz-Zayas et al. 2002, Tank and Dodds 2003). However, our observations of nitrification as a percentage of NH$_4^+$ uptake (14–78%) span a larger range than was observed for 12 streams representing diverse biomes throughout the USA (Peterson et al. 2001). Much of the variation in our study occurred within the same stream, suggesting that nitrification rates in streams can vary temporally as much as spatially. In Bisley 1, for example, nitrification varied from 18% of NH$_4^+$ uptake in late January to 78% when measured again in early March. This pattern was repeated in the 4 stream reaches in which we performed multiple NH$_4^+$ enrichment experiments over a 6-wk period, suggesting that conditions were more favorable for nitrification later in the spring. We cannot explain the temporal variability in net nitrification with our data, although we speculate that leaf litter and particulate organic C standing stocks may have become depleted during the late winter when litterfall rates are lowest (Silver et al. 2014).

Percent NH$_4^+$ nitrified did not increase with background inorganic N concentration, as has been previously observed in temperate streams (Bernhardt et al. 2002, Newbold et al. 2006). Nitrifiers are thought to be poor competitors for NH$_4^+$ relative to heterotrophic bacteria and autotrophs, and Bernhardt et al. (2002) hypothesized that higher levels of inorganic N may alleviate heterotrophic demand and allow nitrifiers to use available NH$_4^+$.

However, streams draining tropical forests in general, and the Luquillo Mountains specifically, have high dissolved inorganic N concentrations relative to forested streams in temperate biomes (Lewis et al. 1999, Brookshire et al. 2012), and nitrifiers may not be in competition with heterotrophs for NH$_4^+$. We also found no relationship between nitrification rates and DOC concentration despite the expectation that low C availability favors nitrifiers over heterotrophic microbes that rely on organic C as an energy source (Strauss and Lamberti 2000, Helton et al. 2015). High inorganic N and low DOC availability may favor nitrification in tropical mountainous streams in general, but other factors, such as microbial community composition, litterfall, or variable redox state and hydrologic connectivity of groundwater flowpaths likely regulate stream nitrification rates.

**River-network N dynamics and export**

High nitrification potential in streams of the Luquillo Mountains suggests that a significant amount of NO$_3^-$ may be produced in tropical streams themselves. At the river-network scale, model results indicate that 39% of watershed NO$_3^-$ export is produced in streams and rivers, challenging the idea that streamwater NO$_3^-$ provides an integrated signal of plant–soil biogeochemical processes (Brookshire et al. 2012). NH$_4^+$ uptake and nitrification in streams transforms the catchment N load into NO$_3^-$, which is less likely to be consumed during the short transit times from land to ocean. Thus, in-stream nitrification results in “shunting” (sensu Raymond et al. 2016) of N to the coastal zone.

Small streams removed a greater proportion of N inputs than large streams in the Río Mameyes network. The importance of small streams for regulating watershed nutrient export has been well documented (Alexander et al. 2000, Peterson et al. 2001), although recent research has highlighted an increased role for large rivers as important sites of nutrient removal (Seitzinger et al. 2002, Ensign and
These conclusions appear contradictory, but the role of small vs large streams in river-network nutrient removal is not mutually exclusive, and the patterns in the Río Mameyes network are governed by the distribution of nutrient inputs, nutrient-removal efficiency, and river-network morphology. Direct N inputs from land are distributed toward headwater streams in the Río Mameyes network, so high $\text{NH}_4^+$ uptake efficiency in small streams means that little $\text{NH}_4^+$ is transported downstream, producing a steep negative relationship between $\text{NH}_4^+$ uptake and stream size (Fig. 4A). Therefore, despite our a priori assumption of constant $\text{NH}_4^+$ with stream size, $\text{NH}_4^+$ removal in larger streams is limited by $\text{NH}_4^+$ availability. Larger streams remained relatively more important for $\text{NO}_3^-$ uptake because low $\text{NO}_3^-$ and in-stream nitrification allow $\text{NO}_3^-$ transport to downstream reaches. $\text{NO}_3^-$ uptake in larger streams also would increase if $\text{NO}_3^-$ uptake were to increase with stream size (e.g., with downstream opening of the canopy and increased assimilative N demand in downstream reaches) (Fig. S1A). Because uptake velocities typically differ considerably for $\text{NH}_4^+$ and $\text{NO}_3^-$ (Ensign and Doyle 2006), the roles of small vs large rivers in regulating watershed exports also shift with the strength of biotic demand (Wollheim et al. 2006).

Geomorphology is also a large driver of nutrient removal in river networks. Wollheim et al. (2006) found that the proportion of $\text{NO}_3^-$ removal as a function of stream size was sensitive to channel-width scaling and shifted from small- to large-stream dominance when the hydraulic width exponent increased from 0.36 to 0.52. In the Río Mameyes network, headwater streams are relatively wide, and width increases slowly in the downstream direction (hydraulic width exponent $= 0.35$), probably because of high stormflows and steep slopes that shape channel geometry (Pike et al. 2010). As a result of the slow increases in channel width in the Río Mameyes, the hydraulic load increases rapidly with increasing stream size, limiting the contact with reactive benthic sediments compared to smaller streams (Seitzinger et al. 2002).

The relationship between nutrient removal and river size varies depending on the distribution of nutrient inputs, biological uptake rates, and hydraulic loads. The latter is also influenced by daily runoff rates, the distribution of stream length, and the downstream scaling of channel width (Ensign and Doyle 2006, Mulholland et al. 2008). Therefore, patterns in the spatial distribution of aquatic nutrient removal would be expected to differ in river networks with similar N uptake rates but different geomorphology, and our results highlight the need for further research comparing nutrient spiraling in river networks with varying hydrologic and geomorphic characteristics. The modeling exercise that we presented here represents steady-state conditions and considers only a snapshot in time during base flow. We expect that high-flow events would decrease the proportion of $\text{NH}_4^+$ removed by headwater streams, allowing a greater role of larger rivers in network N uptake (Wollheim et al. 2008a, Raymond et al. 2016). Further empirical work quantifying N uptake and nitrification under different flow conditions and in larger rivers would help refine these expectations, but our approach is an important step toward understanding nutrient spiraling in a tropical river network. We suggest that systematic study of biogeochemical cycling throughout river networks will help bridge the gap between our understanding of site-level processes and large-scale biogeochemical patterns.

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Author contributions: LEK performed the research, analyzed the data, and wrote the paper. CS contributed new methods and analyzed the data. WMW, JR, and WHM performed the research. All authors discussed the results and contributed to development of the manuscript.

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