2-12-2021

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Recommended Citation

Bianca M. Rodríguez-Cardona, Adam S. Wymore, and William H. McDowell (2021). Nitrate uptake enhanced by availability of dissolved organic matter in tropical montane streams, Freshwater Science, Volume 40, Number 1, https://doi.org/10.1086/713070

Nitrate uptake enhanced by availability of dissolved organic matter in tropical montane streams

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Abstract: Tropical forests store large amounts of Earth's terrestrial C, but many tropical montane streams have low dissolved organic matter (DOM). This low availability of energy likely limits certain pathways of inorganic N uptake, as evidenced by the high rates of nitrification and predominance of nitrate (NO_3^-) in the total pool of dissolved N seen in many tropical montane forests. To explore the influence of DOM availability on tropical stream N cycling, we performed nutrient pulse additions of NO_3^- with or without an added C source (acetate or urea) in streams of the Luquillo Experimental Forest, Puerto Rico. In the absence of added DOM, NO_3^- uptake was either undetectable or had very long (>3000 m) uptake lengths (S_w). When DOM was added with NO_3^-, S_w values for NO_3^- were much shorter (97–1500 m), with the shortest lengths resulting from additions of acetate. Comparing uptake metrics of the added C sources, there was greater demand for acetate compared to urea, and measurable urea uptake was detected much less frequently. During NO_3^--only additions, ambient concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) decreased in some cases, suggesting increased metabolic demand for energy from the ambient organic matter pool under elevated levels of inorganic nutrients. Collectively, these results demonstrate that pathways of inorganic N cycling are tightly tied to energy availability at this tropical site. The response of ambient DOC and DON to increases in NO_3^- concentrations points to important feedbacks between inorganic N and DOM including organic N. Understanding the controls on NO_3^- processing in these streams is important to predicting network-scale exports of N from tropical ecosystems.
Key words: nitrate uptake, tropical montane streams, dissolved organic carbon, dissolved organic nitrogen, energy limitation

Tropical forests store large amounts of Earth's terrestrial C in vegetation (Bunker et al. 2005) and soil (Drake et al. 2019), but the quantity of dissolved organic matter (DOM) is often low in montane tropical streams (McDowell and Asbury 1994). Inputs of DOM to streams are controlled by the magnitude of sources in the soil solution and the extent to which hydrologic flow paths route water through mineral soils that retain DOM through adsorption (McDowell and Likens 1988). In the Luquillo Mountains of Puerto Rico, DOM produced by upper soil horizons is retained very effectively by mineral soils, leading to low delivery of DOM to streams (McDowell 1998). These hillslope and riparian dynamics combined with low rates of instream primary production (Vannote et al. 1980, Ortiz-Zayas et al. 2005) can result in energy-limited biogeochemical reactions in streams.

In aquatic ecosystems, C and N cycles are coupled because many N transformations require an energy source.

For example, rates of nitrate (NO_3^-) uptake can be substantially increased by the addition of DOM (Bernhardt and Likens 2002), a general relationship that holds across multiple study systems (Sobczak and Findlay 2002, Bernhardt and McDowell 2008, Thouin et al. 2009). There are exceptions, however, with some experimental work finding no relationship between C availability and N uptake (Richey et al. 1985), suggesting that other factors can also limit inorganic N uptake. Thus, the relationship between the composition of DOM and N cycling is complex and highly variable across streams (Dodds et al. 2004) and multiple systems (Taylor and Townsend 2010).

Organic matter C:N ratios can be predictive of rates of N transformations because higher C:N ratios favor heterotrophic uptake of N by inhibiting nitrification (Strauss and Lamberti, 2000, 2002, Starry et al. 2005). It is unclear whether these dynamics, which have been primarily studied in

temperate systems, hold in other biomes, especially tropical ecosystems. Unlike temperate systems, tropical streams do not experience large seasonal oscillations in temperature, light availability, and inputs of organic matter that can affect the cycling of C and N. In addition, tropical forests are large exporters of inorganic N (McDowell and Asbury 1994, Hedin et al. 2009, Brookshire et al. 2012a, b), but the controls on these dynamics are not clear. In Puerto Rico's Luquillo Experimental Forest (LEF), for example, previous work has demonstrated that many biogeochemical transformations of N appear to be driven more by energy availability than nutrient limitation (Merriam et al. 2002, Potter et al. 2010, Koenig et al. 2017). Stream NO$_3^-$ concentrations correlate negatively with peak leaf fall (McDowell and Asbury 1994), and nitrification rates are relatively high compared with temperate systems (Peterson et al. 2001, Koenig et al. 2017), suggesting a link between C and N cycling, but direct experimental evidence is lacking.

The objective of this study was to understand the influence of DOM on N processing in tropical montane streams. We hypothesized that: 1) the addition of DOM would increase the uptake rates of NO$_3^-$, as measured in nutrient addition experiments, with urea having a greater influence than acetate; 2) uptake rates of added N-rich DOM (urea) would be greater than those for C-rich, N-lacking DOM (acetate), as urea can meet both energy and nutrient demands (Brookshire et al. 2005); and 3) ambient concentra-

tions of DOC and DON would decline with added NO$_3^-$ as DOM is broken down for its energetic content to fuel NO$_3^-$ uptake from the water column (Wymore et al. 2015).

METHODS

To address our study's goal, we conducted a series of nutrient pulse additions of NO$_3^-$ with or without an added source of C in streams of the Luquillo Mountains in Puerto Rico. To explore the role of organic N content in the interactions between NO$_3^-$ uptake and added DOM, we also varied the C source by providing either acetate, which is C-rich and N-lacking, or urea, a relatively N-rich form of DOM. We performed nutrient pulse additions in 2 headwater streams (treated as replicates) of the Río Espiritu Santo Watershed in the LEF located in the northeast corner of Puerto Rico. We focused on Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) (Fig. 1), which are underlain by volcaniclastic lithology with andesitic igneous materials, mudstone, and clay deposits (McDowell and Asbury 1994) and dominated by tabonuco (*Dacryodes excelsa*) and sierra palm (*Prestoea montana*). Mean annual temperature ranges between 21 and 25°C and annual precipitation from 250 to >500 cm/y (McDowell and Asbury 1994, Merriam et al. 2002). Both streams are small, steep, and highly shaded 1st- and 2nd-order streams with slopes between 8 and 16% and large rocks and boulders in the stream bed. Background
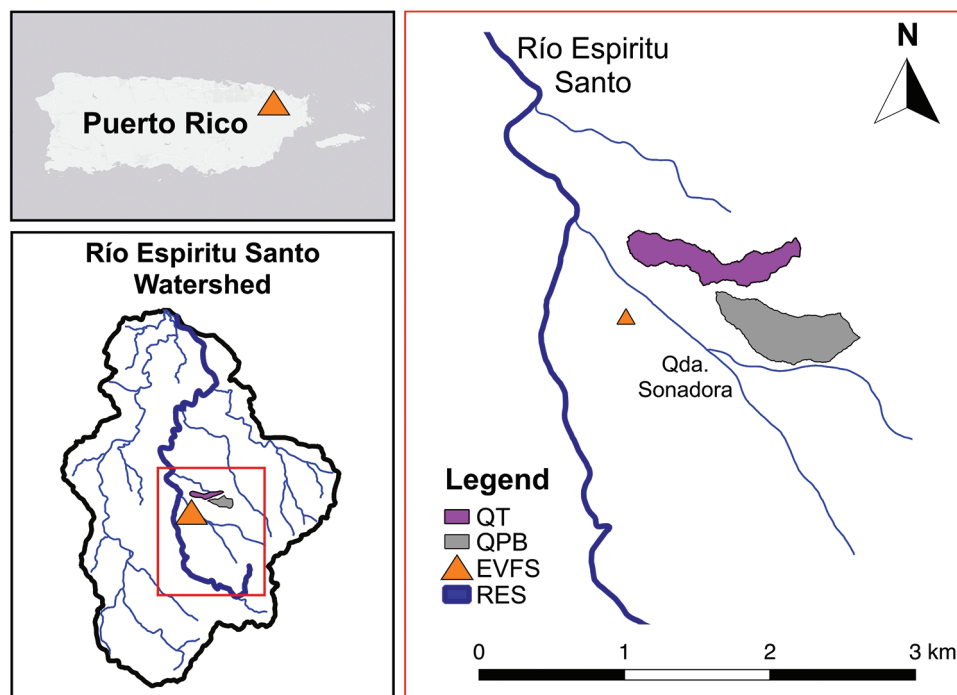

Figure 1. Map of headwaters of the Río Espiritu Santo Watershed (RES) with outlined watersheds of study sites Quebrada Toronja (QT) in purple and Quebrada Prieta-B (QPB) in gray near the El Verde Field Station (EVFS) in the Luquillo Experimental Forest in northeast Puerto Rico.

concentrations of DOC and DON are ~1 mg/L and 50 µg/L, respectively, whereas the inorganic N pool is dominated by NO_3^-, with concentrations between 65 and 100 µg/L and NH_4^+ often at or below detection limit (Table 1).

Nutrient pulse additions

We performed nutrient pulse additions (Tank et al. 2008) at both sites (i.e., QPB and QT) throughout January 2016, as droughts and hurricanes are not a concern during that time of year. We performed 4 NO_3^--only additions in QT and 3 in QPB and, at both sites, we did 3 additions of NO_3^- + acetate (potassium acetate, $C_2H_3O_2K$) and 1 addition of NO_3^- + urea (CH_4N_2O), for a total of 15 additions across both streams (Table 2). For all additions, nitrate was added in the form of sodium nitrate ($NaNO_3$). We added these solutes along with sodium chloride (NaCl) as a conservative tracer. Additions targeted an increase of Cl^-, NO_3^-, DOC, and DON concentrations 2 to 3× above ambient concentrations.

In each stream, our experimental reaches, which ranged between 50 and 75 m, were chosen to avoid large pools with excessively long retention times as well as any tributaries. The same reach was used in both streams and adjusted in length to the flow conditions of the day. We released the solutions as instantaneous additions at the top of the experimental reach. For additions done on the same date, we allowed at least 1 h between additions once the stream returned to background conditions. Background samples collected prior to additions were also used to verify there were no residual solutes from previous additions when conducted the same day. Details for individual additions can be found in Table S1.

We collected samples at a fixed point at the bottom of each experimental reach throughout the breakthrough curve (BTC). We collected between 20 and 40 samples in 125-mL acid-washed high-density polyethylene bottles (HDPE) per nutrient addition, depending on flow conditions and changes in specific conductance determined with a multiparameter sonde (model 556 MPS; Yellow Springs Instruments, Yellow Springs, Ohio). Prior to each nutrient addition, we collected duplicate background samples at the upstream addition site and downstream collection site, for a total of 4 background samples.

Uptake calculations

We calculated uptake metrics following methods from the Stream Solute Workshop (1990) and the BTC-integration method (Tank et al. 2008). We determined uptake length (S_w) as the negative inverse of the ratio between the natural logarithm of the fraction of background-corrected solute and chloride (Cl^-) retrieved and the reach length distance. Uptake length describes the average distance a molecule travels before being taken up by the biota; longer distances reflect less demand (Stream Solute Workshop 1990). We determined the mass transfer coefficient or uptake velocity (V_f) as:

$$V_f = \frac{\frac{Q}{w}}{S_w} \qquad \text{(Eq. 1)},$$

where Q is discharge, w is stream width, and S_w is the uptake length. Uptake velocity is normalized for stream velocity and depth and describes the efficiency with which the benthic community takes up nutrients; higher rates of V_f denote greater demand (Stream Solute Workshop 1990). We determined areal uptake (U) as:

$$U = V_f \times C \qquad \text{(Eq. 2)},$$

where C is background solute concentration. Areal uptake describes gross uptake on a per-area, per-time basis (Stream Solute Workshop 1990).

To determine discharge, we used the dilution gaging method (Kilpatrick and Cobb 1985) with NaCl dissolved in stream water and added to the stream as an instantaneous addition while conductivity was logged every 5 s with a HOBO® conductivity data logger (Onset®, Bourne, Massachusetts) deployed at the bottom of the experimental reach. We determined uptake of acetate by direct measurement of the acetate ion, and we determined uptake of urea by bulk DON concentrations. We did not conduct any statistical tests to compare uptake metrics between streams or the effect of adding a carbon source with nitrate due to our small sample size.

Sample analysis

We filtered all water samples (from the 125-mL bottles or collected directly from the stream) through pre-combusted Whatman GF/F filters (Maidstone, United Kingdom) and

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Table 1. Watershed area and mean dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) concentrations for the streams Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) in the Luquillo Experimental Forest, Puerto Rico, with minimum (min) and maximum (max) values in parentheses.

Site	Watershed area (km²)	DOC (mg C/L)	DOC (min, max)	DON (µg N/L)	DON (min, max)	NO_3^- (µg N/L)	NO_3^- (min, max)	NH_4^+ (µg N/L)	NH_4^+ (min, max)	PO_4^{3-} (µg P/L)	PO_4^{3-} (min, max)
QPB	0.24	0.86	(0.57, 1.29)	24.06	(7.55, 48.22)	68.69	(37, 120)	2.86	(2.5, 6.05)	2.42	(1.48, 4.33)
QT	0.18	0.99	(0.6, 1.59)	66.65	(15.66, 193.5)	115.65	(65.4, 200)	5.82	(2.5, 26.96)	4.03	(2.15, 6.65)

Table 2. Uptake metrics for all NO$_3^-$-only, NO$_3^-$ + acetate, and NO$_3^-$ + urea additions in the streams Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) in the Luquillo Experimental Forest, Puerto Rico, for uptake length (S_w), uptake velocity (V_f), areal uptake (U), and discharge (Q) from each date. – denotes undetectable uptake.

Site	Date	Addition	Uptake solute	S_w (m)	V_f (mm/min)	U (µg m^{-2} min^{-1})	Q (L/s)
QPB	1/7/16	NO$_3^-$-only	NO$_3^-$	–	–	–	6.20
QPB	1/7/16	NO$_3^-$ + acetate	NO$_3^-$	–	–	–	6.20
			Acetate	141	2.29	51.2	
QPB	1/8/16	NO$_3^-$ + urea	NO$_3^-$	147	1.72	256.3	4.89
			DON	1546	0.164	1.80	
QPB	1/12/16	NO$_3^-$-only	NO$_3^-$	3811	0.053	4.36	3.95
QPB	1/12/16	NO$_3^-$ + acetate	NO$_3^-$	1254	0.162	13.2	3.95
			Acetate	379	0.54	9.58	
QPB	1/14/16	NO$_3^-$-only	NO$_3^-$	–	–	–	3.14
QPB	1/14/16	NO$_3^-$ + acetate	NO$_3^-$	–	–	–	3.14
			Acetate	277	0.81	85.4	
QT	1/4/16	NO$_3^-$-only	NO$_3^-$	–	–	–	4.96
QT	1/5/16	NO$_3^-$-only	NO$_3^-$	–	–	–	4.96
QT	1/5/16	NO$_3^-$ + acetate	NO$_3^-$	79.7	1.73	156.9	4.96
			Acetate	97.70	1.42	35.4	
QT	1/11/16	NO$_3^-$ + acetate	NO$_3^-$	127.8	1.04	193.4	3.54
			Acetate	214	0.62	86.7	
QT	1/13/16	NO$_3^-$-only	NO$_3^-$	–	–	–	3.00
QT	1/13/16	NO$_3^-$ + urea	NO$_3^-$	1417	0.0762	10.07	3.00
			DON	–	–	–	
QT	1/15/16	NO$_3^-$-only	NO$_3^-$	–	–	–	2.87
QT	1/15/16	NO$_3^-$ + acetate	NO$_3^-$	–	–	–	2.87
			Acetate	52	2.05	83.2	
Mean uptake values		NO$_3^-$-only	NO$_3^-$	3811	0.053	4.36	
		NO$_3^-$ + acetate	NO$_3^-$	487	0.98	121.21	
		NO$_3^-$ + acetate	Acetate	193	1.29	59.60	
		NO$_3^-$ + urea	NO$_3^-$	748	0.90	133.17	
		NO$_3^-$ + urea	DON	1546	0.16	1.80	

into 60-mL acid-washed HDPE bottles. We froze samples ($-20°C$) and transported them to the Water Quality Analysis Laboratory at the University of New Hampshire for analysis. We analyzed samples for DOC and total dissolved nitrogen (TDN) by using high temperature catalytic oxidation in a Shimadzu TOC-V CPH/TNM (Kyoto, Japan). We analyzed chloride, NO$_3^-$, and acetate with ion chromatography using an Anion/Cations Dionex™ ICS-1000 with AS40 autosampler (Thermo Fisher Scientific, Waltham, Massachusetts). We analyzed ammonium (NH$_4^+$) using a SmartChem® 200 discrete automated colorimetric analyzer (Unity Scientific®, Milford, Massachusetts) and the alkaline phenate standard method. We determined DON arithmetically by subtracting the sum of NO$_3^-$ and NH$_4^+$ (DIN) concentrations from TDN.

We examined the response of the ambient DOC and DON pools to elevated concentrations of NO$_3^-$ during the NO$_3^-$-only additions by using simple linear regressions of the samples collected along the BTC. We ran all analyses in RStudio (version 1.2.1335; R Project for Statistical Computing, Vienna, Austria).

RESULTS
NO$_3^-$ uptake

The addition of a DOM source enhanced NO$_3^-$ uptake. When NO$_3^-$ was added without a DOM source, NO$_3^-$ uptake was generally undetectable (Fig. 2A–C). Only 1 (in QP) of the 7 NO$_3^-$-only additions had detectable uptake with a S_w of 3811 m. When NO$_3^-$ was added along with a DOM source in the paired additions, S_w decreased (relative to the NO$_3^-$-only additions, $n = 1$) to between 80 and 1254 m (median = 127 m, $n = 3$) for the NO$_3^-$ + acetate addition and to between 148 and 1417 m (median = 782 m, $n = 2$)
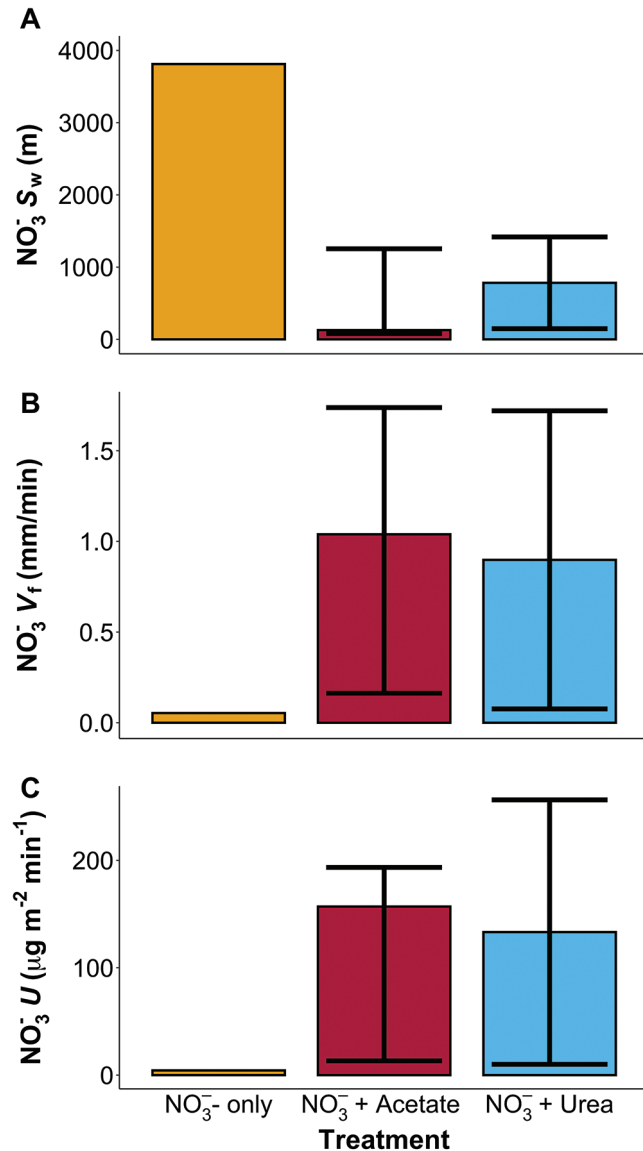
Figure 2. Median NO_3^- uptake metrics from streams Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) in the Luquillo Experimental Forest, Puerto Rico, during NO_3^--only additions (orange), NO_3^- + acetate (pink), and NO_3^- + urea (blue) for uptake length (S_w) (A), uptake velocity (V_f) (B), and areal uptake (U) (C). Bars represent minimum and maximum values. Number of additions for NO_3^--only ($n = 1$), NO_3^- + acetate ($n = 3$), and NO_3^- + urea ($n = 2$).

for the NO_3^- + urea addition (Table 2). Accordingly, V_f (mm/min) increased from 0.05 to between 0.16 and 1.74 mm/min (median = 1.04) for the NO_3^- + acetate addition and to between 0.07 and 1.72 mm/min (median = 0.90) for the NO_3^- + urea addition, whereas U ($\mu g\ m^{-2}\ min^{-1}$) increased from 4.36 to between 13.24 and 156.95 $\mu g\ m^{-2}\ min^{-1}$ (median = 157) and to between 10.07 and 256.28 $\mu g\ m^{-2}\ min^{-1}$ (median = 133), respectively (Table 2). Out of

the 6 NO_3^- + acetate additions, 3 had undetectable uptake for NO_3^- (Table 2). Based on median values, S_w declined by 79 and 97%, V_f increased by 80 and 83%, and U increased by 65 and 70% when NO_3^- was added with a C form.

DOM uptake

Acetate had greater uptake relative to urea (Fig. 3A–C). This pattern held across additions and study streams. Acetate exhibited shorter S_w and higher V_f and U than urea (Fig. 3A–C), although only 1 of the 2 urea additions had detectable uptake. Uptake length for acetate varied between 52 and 379 m across sites (median = 177 m), whereas urea had a S_w of 1546 m (Table 2). Uptake velocity for acetate was between 0.54 and 2.29 mm/min (median = 1.11), whereas urea had a V_f of 0.16 mm/min (Table 2). Areal uptake for acetate ranged between 9.58 and 86.70 $\mu g\ m^{-2}\ min^{-1}$ (median = 70.24). For urea, U was 1.80 $\mu g\ m^{-2}\ min^{-1}$ (Table 2).

Ambient DOC and DON dynamics

Ambient concentrations of DOC and DON were not always responsive to NO_3^--only additions (Fig. 4A–F). We detected substantial changes in concentrations 30% of the time (2 out of 7 NO_3^--only additions), and DOC and DON did not respond in synchrony. For example, during the NO_3^--only addition on 4 January at QT, there was no relationship between concentrations of DOC and added NO_3^- (Fig. 4A), but DON and NO_3^- showed a substantial negative relationship ($r^2 = 0.41, p < 0.001$) (Fig. 4B). Only a week later, however, we observed no relationships between NO_3^- and DOC (Fig. 4C) or DON at QT (Fig. 4D). At QPB, NO_3^- had a strong negative relationship with DOC ($r^2 = 0.84, p < 0.001$) but not with DON (Fig. 4E, D). All relationships between DOC, DON, and NO_3^- can be found in Figs S1 and S2.

DISCUSSION

The work presented here focuses on the influence of DOM availability on N uptake in tropical montane streams. Streams of the LEF provide an ideal model system for studying internal controls in tropical watersheds that export large amounts of DIN (Newbold et al. 1995, Lewis et al. 1999, Neill et al. 2001, Schrumpf et al. 2006, Hedin et al. 2009, Brookshire et al. 2012b). Our experimental data provide initial evidence for energy limitation to NO_3^- uptake, because NO_3^- uptake sometimes increased with the addition of DOM (Fig. 2A–C). The higher V_f for C-rich (acetate) over N-rich (urea) DOM provides further support for the possibility that energy density (i.e., energetic content in the added DOM), rather than N availability, drives rates of DOM uptake in these streams (Fig. 3A–C). For example, acetate has ~10× the available energy/mole than urea (based on Gibbs free energy of acetic acid vs urea). DOC and DON concentrations did not always decline
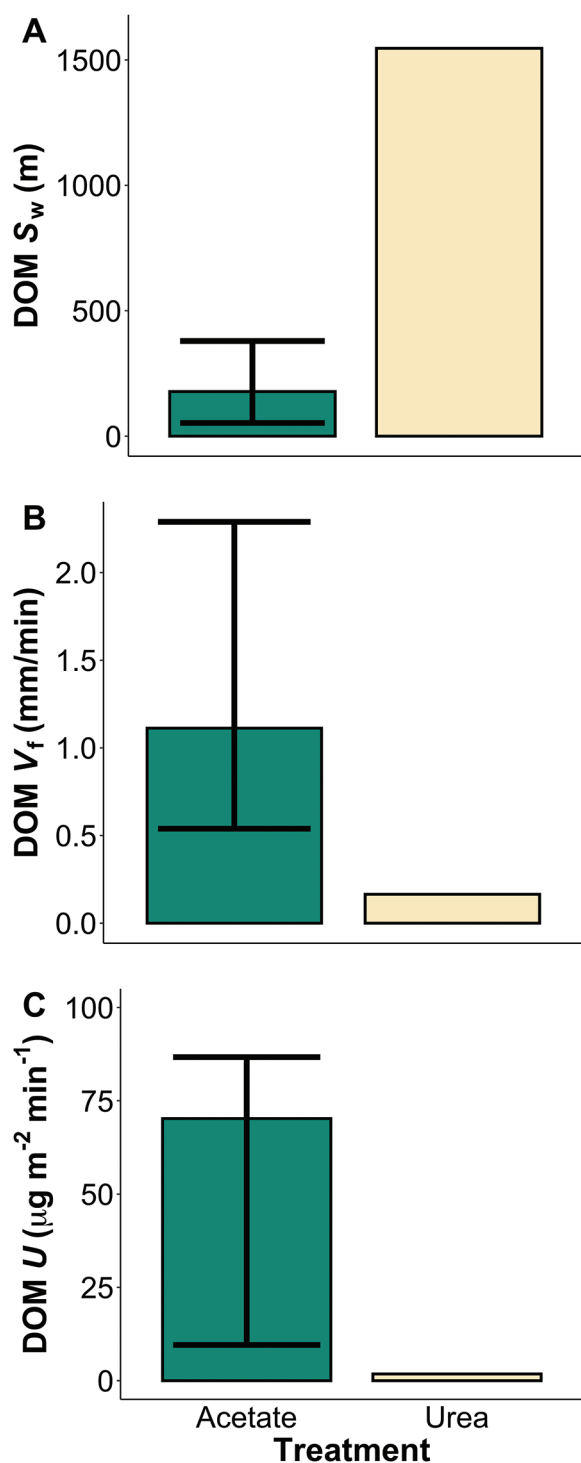
Figure 3. Median dissolved organic matter (DOM) uptake metrics from streams Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) in the Luquillo Experimental Forest, Puerto Rico, for acetate during NO$_3^-$ + acetate additions (green) and urea (calculated as bulk dissolved organic nitrogen; yellow) during NO$_3^-$ + urea additions for uptake length (S_w) (A), uptake velocity (V_f) (B), and areal uptake (U) (C). Bars represent minimum and maximum values. Number of additions for NO$_3^-$ + acetate ($n = 6$) and NO$_3^-$ + urea ($n = 1$).

during the NO$_3^-$-only additions, suggesting that the ambient pool of DOM is not very bioavailable to the microbial community.

NO$_3^-$ uptake

Because of a lack of available energy sources, uptake of NO$_3^-$ in streams of the LEF is very low to undetectable. We performed 7 individual NO$_3^-$-only additions but were only able to obtain uptake metrics for 1 of them. That addition had a S_w of 3811 m, which was considerably longer than the reach length, suggesting that uptake lengths in streams of the LEF are almost infinite because of the low demand for NO$_3^-$ under ambient conditions, as seen earlier in the Luquillo Mountains (Mulholland et al. 2000, Hall and Tank 2003, Potter et al. 2010). All NO$_3^-$ uptake values reported here (NO$_3^-$-only and NO$_3^-$ with a DOM source) fall within the range, but toward the low end, of reported values from the 2nd Lotic Intersite Nitrogen eXperiment (Hall et al. 2009). The long residence times of many streams promote removal of NO$_3^-$ from the water column (Valett et al. 1996, Brookshire et al. 2005, Zarnetske et al. 2011), but streams in the LEF and other Caribbean streams have short residence times due to their steep slopes and short travel distances from headwaters to the ocean (Merriam et al. 2002, Lloret et al. 2011, Koenig et al. 2017).

The undetectable nature of NO$_3^-$ uptake in both QT and QPB agrees with previous studies in the LEF where NO$_3^-$ uptake was much lower than NH$_4^+$ uptake, which is driven to a large extent by nitrification (Merriam et al. 2002, Potter et al. 2010, Koenig et al. 2017). Low NO$_3^-$ demand was also found in other tropical sites where there was greater retention of NH$_4^+$ than NO$_3^-$ (Finkler et al. 2018, Tromboni et al. 2018). Greater retention of NH$_4^+$ is not exclusive to tropical sites, however. NH$_4^+$ is often considered the biologically preferred form of DIN in streams because of the few reduction steps, relative to NO$_3^-$, required prior to assimilation (Martí and Sabater 1996, O'Brien and Dodds 2010, Ribot et al. 2013). In streams of the LEF, rates of nitrification are high (Merriam et al. 2002, Koenig et al. 2017), and rates of NO$_3^-$ uptake from the water column are low, as demonstrated here as well as in other studies (Hall et al. 2009, Mulholland et al. 2009, Potter et al. 2010).

The relationships we observed between NO$_3^-$ uptake and DOM availability in these tropical streams have been well documented in temperate systems (Bernhardt and Likens 2002, Bernhardt and McDowell 2008, Thouin et al. 2009) and imply that a tight linkage exist between C and N cycling in low-DOM streams in general. This finding suggests that relationships between C and N cycling can be more broadly extended to other biomes and that the controls of NO$_3^-$ dynamics may be similar across locations. Nitrate uptake was generally only detectable when additional DOM was available, consistent with our hypothesis. The increase in NO$_3^-$ uptake during the paired additions of NO$_3^-$
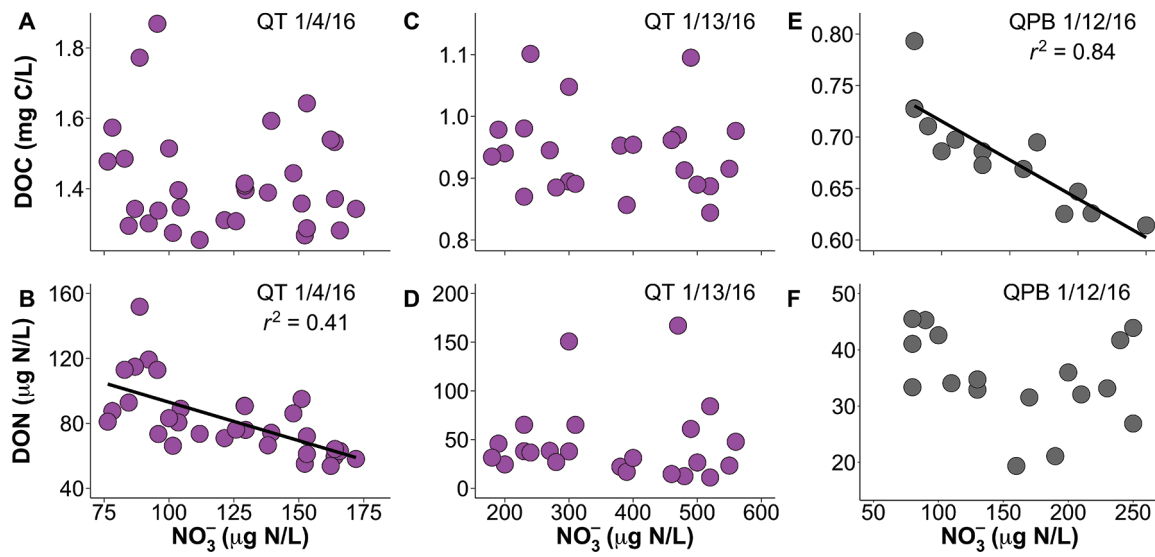
Figure 4. Relationships between ambient dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations during the NO_3^--only additions in the streams Quebrada Prieta-B (QPB) and Quebrada Toronja (QT) in the Luquillo Experimental Forest, Puerto Rico. A.—DOC and NO_3^- concentrations on 4 January in QT. B.—DON and NO_3^- concentrations on 4 January in QT. C.—DOC and NO_3^- concentrations on 13 January in QT. D.—DON and NO_3^- concentrations on 13 January in QT. E.—DOC and NO_3^- concentrations on 12 January in QPB. F.—DON and NO_3^- on 12 January in QPB. Purple points represent QT, and gray points are from QPB. Each individual point corresponds to an individual sample collected along the breakthrough curve. Background samples are not shown. Linear regressions and statistics were only included for strong relationships. See Figs S1 and S2 for figures from all 7 NO_3^--only additions.

and DOM indicates strong energy limitation in these streams that can inhibit the heterotrophic or assimilative removal of NO_3^- from the water column (Bernhardt and Likens 2002). Similarly, other studies have found an increase in NO_3^- uptake during paired stream manipulations of NO_3^- and a form of C (Bernhardt and Likens 2002, Thouin et al. 2009, Tomasek et al. 2018) or in systems with higher ambient DOC concentrations and DOC:NO_3^- ratios (Rodríguez-Cardona et al. 2016, Wymore et al. 2016). Our results, however, are some of the first to demonstrate this relationship in very low-DOC tropical montane streams through manipulations of the DOM pool.

Our study provides initial evidence that DOM availability enhances NO_3^- uptake, but the effect was not consistent over time, as several of the paired additions (3 out of 8 NO_3^- + DOM) did not yield detectable NO_3^- uptake. These paired additions coincided with those NO_3^--only additions that also had no detectable uptake. These results provide examples of the very low demand for NO_3^- in these tropical montane streams and highlight the finding that available DOM does not always stimulate the microbial community to take up NO_3^-. Nonetheless, the increased likelihood that uptake of NO_3^- will occur when DOM is available suggests that the ability of the microbial community to remove NO_3^- from the water column is ephemeral (e.g., several days) and can be enhanced by adding a C source. The ephemeral nature of NO_3^- uptake is reflected

in our data from QPB where NO_3^- uptake was enhanced by the addition of DOM on 8 and 12 January but not on the shoulder dates (Table 2). Similarly, NO_3^- uptake in QT was not detectable in the earlier and later additions in January (Table 2). This variability in uptake could be attributed to changes in nutrient demand where antecedent conditions resulted in the saturation of nutrient uptake prior to the addition of solutes. External forces acting on the stream, such as precipitation and runoff events, which themselves are temporally variable, can introduce additional allochthonous solutes (e.g., McDowell and Asbury 1994, Wymore et al. 2017), which may then reduce subsequent uptake and demand for N. Temporal variability in microbial community structure and composition may also influence solute uptake dynamics (Portillo et al. 2012).

Nutrient pulse additions are a common tool in quantifying whole stream responses to added solutes (Stream Solute Workshop 1990, Peterson et al. 2001, Mulholland et al. 2002, 2006, 2009, Payn et al. 2005, Covino et al. 2010, Day and Hall 2017). This method, however, poses some inherent issues that have not been fully addressed in the literature despite the long history of nutrient-spiraling research (Newbold et al. 1981, Stream Solute Workshop 1990). Ratios of biological-to-conservative tracers are subject to ambient noise in nutrient concentrations, making it difficult to fully constrain the variability in uptake calculations. This issue often leads to undetectable uptake rates in

which a numeric value cannot be assigned, yet valuable insight into the processing of nutrients in a stream is still obtainable from these "no detects" (Covino et al. 2018). Undetectable uptake suggests that demand for the added solute, such as NO$_3^-$ like in this study, is very low. No detect data adds to the complexity of quantifying N cycling in streams and is also a common artefact of NO$_3^-$ stream amendments (Richey et al. 1985, Mulholland et al. 2000, Dodds et al. 2002, Diemer et al. 2015, Rodríguez-Cardona et al. 2016, Covino et al. 2018). We highlight the need for a method that addresses detection limits which will aid in decisions regarding appropriate statistical methods and in quantifying uncertainty in nutrient-spiraling metrics.

DOM uptake

To further explore energy limitation as the primary mechanism regulating inorganic nutrient transformation, we also measured uptake of the added C sources. As expected, we identified a high demand for DOM in both experimental streams. Consistent with the river continuum concept (Vannote et al. 1980), high DOM demand occurs in streams low in primary productivity, such as those of the LEF (e.g., Robbins et al. 2017). The short uptake lengths for acetate obtained here using nutrient pulse additions also demonstrate how quickly certain forms of DOM can be taken up, as has been found by ^{13}C additions (Kaplan et al. 2008). The short uptake lengths of acetate compared to those of urea show an apparent preference of acetate over urea, contrary to our initial hypothesis. This potential preference for acetate is also contrary to other studies in similarly low-DOC streams where urea or other N-rich DOM molecules were taken up much more quickly than DIN or even C-rich DOM (Brookshire et al. 2005). However, because we conducted only 2 urea additions and changes in urea concentrations were estimated from changes in bulk DON, our results for uptake of acetate versus urea should be treated with caution.

Comparing uptake metrics of acetate ($n = 6$) and urea ($n = 1$) in our streams suggests a preference for C-rich over N-rich organic molecules to fuel heterotrophic processes in streams where energy density may be more important than N availability. Average reach-scale DOC uptake across a number of sites in different biomes, measured as median DOC V_f by Mineau et al. (2016), was 2.28 mm/min. Our median acetate V_f of 1.11 mm/min from the LEF streams falls well within their reported range. The acetate V_f values reported here are also similar to those from streams in Cantabrian mixed forest and Mediterranean forests, which ranged from 0.6 to 3.99 mm/min. These Cantabrian and Mediterranean streams are similar to those in the LEF, with relatively low DOC and elevated DIN concentrations (Catalán et al. 2018). Our results are suggestive of a preference for C-rich or energy-rich molecules, like acetate, but more

research on this topic is warranted, as the number of studies to date is limited.

Ambient DOC and DON dynamics

The addition of NO$_3^-$ to streams can fundamentally alter energy and nutrient demands by shifting the ratios of DOC:NO$_3^-$ (Rodríguez-Cardona et al. 2016) and DOC:DON (Wymore et al. 2015). We did not see consistent declines in DOC or DON across all NO$_3^-$-only additions, which was contrary to our initial hypothesis, but the 2 additions where changes in concentrations did occur suggest that both the ambient C-rich and N-rich fractions of the DOM pool can be used as energy sources when additional inorganic N is available (Fig. 4A–F) (Kaushal and Lewis 2005, Lutz et al. 2011, Wymore et al. 2015). Interactions between NO$_3^-$ and the ambient pool of DOM provide some support for the conclusion that the microbial community is scavenging for energy rather than N. Because ambient concentrations of DOC and DON are low, however, it is difficult to detect their uptake in response to added NO$_3^-$.

Although the relationships between NO$_3^-$ and DOC and DON were predominantly negative (when observed), the responses of DOC and DON were not always synchronous. For example, there was an instance where we detected a strong negative relationship between NO$_3^-$ and DON but did not simultaneously detect a relationship between NO$_3^-$ and DOC during the same nutrient addition. We also found an example of a substantial response of DOC to elevated NO$_3^-$ concentration with no concurrent response in concentrations of DON. This asynchrony suggests that the C-rich and N-rich fractions of the ambient DOM pool can cycle independently of each other with their own unique set of biogeochemical controls (Wymore et al. 2015, 2018). This finding is contrary to the idea that DOC and DON concentrations are coupled (Campbell et al. 2000, Goodale et al. 2000, Mann et al. 2012) and independent of DIN concentrations in streams (Brookshire et al. 2007).

The reduction in concentrations of ambient N-rich DOM that we saw periodically in QT (measured as a decline in DON concentrations with addition of NO$_3^-$-only) is likely to have occurred to meet energetic demands rather than satiating nutrient demands (Kaushal and Lewis 2005, Petrone et al. 2009, Lutz et al. 2011, Wymore et al. 2015). Although DON can be a quantitatively important fraction of dissolved N in streams that are low in DIN, in the case of streams in the LEF, NO$_3^-$ is elevated and represents most of the TDN pool (~71%). The high concentrations of NO$_3^-$ in both our study streams, when coupled with low rates of NO$_3^-$ uptake, suggest that DOM may not be sufficiently bioavailable to meet the energetic demands required for the removal of NO$_3^-$ from the water column. Indeed, DOM within LEF streams has been previously identified to be lignin-rich (Hernes et al. 2017) and highly

aromatic (Miller et al. 2016), suggesting that the DOM pool is, in fact, not very bioavailable.

Broader implications of NO_3^- and DOM dynamics

Streams of the LEF are energetically limited and only with the increased availability of low molecular weight and labile DOM will inorganic N, especially NO_3^-, be removed from the water column. Similar to temperate systems, DOM has a central role in N cycling of tropical fluvial systems. This relationship between DOM and N processing is especially important in ecosystems where major disturbances can cause large changes in ambient stream chemistry. For example, NO_3^- concentrations in the LEF streams are elevated for several years post-hurricane disturbance (McDowell et al. 1996, 2013, Schaefer et al. 2000), whereas concentrations of DOC are highly responsive to changes in flow (McDowell and Asbury 1994, Shanley et al. 2011, Wymore et al. 2017). With the potential for increased hurricane frequency and intensity (Kossin et al. 2020), understanding these interactions between DOM and DIN will be critical to building predictive frameworks of the controls on the export of inorganic N.

LITERATURE CITED

Bernhardt, E. S., and G. E. Likens. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology 83:1689–1700.

Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook Valley streams in 1979 versus 2000. Journal of Geophysical Research 113:G03032.

Brookshire, E. N. J., S. Gerber, D. N. L. Menge, and L. O. Hedin. 2012a. Large losses of inorganic nitrogen from tropical rainforests suggest a lack of nitrogen limitation. Ecology Letters 15:9–16.

Brookshire, E. N. J., L. O. Hedin, J. D. Newbold, D. M. Sigman, and J. K. Jackson. 2012b. Sustained losses of bioavailable nitrogen from montane tropical forests. Nature Geoscience 5:123–126.

Brookshire, E. N. J., H. M. Valett, S. A. Thomas, and J. R. Webster. 2005. Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology 86:2487–2496.

Brookshire, E. N. J., H. M. Valett, S. A. Thomas, and J. R. Webster. 2007. Atmospheric N deposition increases organic N loss from temperate forests. Ecosystems 10:252–262.

Bunker, D. E., F. Declerck, J. C. Bradford, R. K. Colwell, I. Perfecto, O. L. Phillips, M. Sankaran, and S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. Science 310:1029–1031.

Campbell, J. L., J. W. Hornbeck, W. H. McDowell, D. C. Buso, J. B. Shanley, and G. E. Likens. 2000. Dissolved organic nitrogen budgets for upland, forested ecosystems in New England. Biogeochemistry 49:123–142.

Catalán, N., J. P. Casas-Ruiz, M. I. Arce, M. Abril, A. G. Bravo, R. del Campo, E. Estévez, A. Freixa, P. Giménez-Grau, A. M. González-Ferreras, L. Gómez-Gener, A. Lupon, A. Martínez, C. Palacin-Lizarbe, S. Poblador, R. Rasines-Ladero, M. Reyes, T. Rodríguez-Castillo, P. Rodríguez-Lozano, I. Sanpera-Calbet, I. Tornero, and A. Pastor. 2018. Behind the scenes: Mechanisms regulating climatic patterns of dissolved organic carbon uptake in headwater streams. Global Biogeochemical Cycles 32:1528–1541.

Covino, T. P., E. S. Bernhardt, and J. B. Heffernan. 2018. Measuring and interpreting relationships between nutrient supply, demand, and limitation. Freshwater Science 37:448–455.

Covino, T. P., B. L. McGlynn, and R. A. McNamara. 2010. Tracer additions for spiraling curve characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. Limnology and Oceanography: Methods 8:484–498.

Day, N. K., and R. O. Hall. 2017. Ammonium uptake kinetics and nitrification in mountain streams. Freshwater Science 36:41–54.

Diemer, L. A., W. H. McDowell, A. S. Wymore, and A. S. Prokushkin. 2015. Nutrient uptake along a fire gradient in boreal streams of Central Siberia. Freshwater Science 34:1443–1456.

Dodds, W. K., A. J. López, W. B. Bowden, S. Gregory, N. B. Grimm, S. K. Hamilton, A. E. Hershey, E. Martí, W. H. McDowell, J. L. Meyer, D. Morrall, P. J. Mulholland, B. J. Peterson, J. L. Tank, H. M. Valett, J. R. Webster, and W. Wollheim. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society 21:206–220.

Dodds, W. K., E. Martí, J. L. Tank, J. Pontius, S. K. Hamilton, N. B. Grimm, W. B. Bowden, W. H. McDowell, B. J. Peterson, H. M. Valett, J. R. Webster, and S. Gregory. 2004. Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. Oecologia 140:458–467.

Drake, T. W., K. Van Oost, M. Barthel, M. Bauters, A. M. Hoyt, D. C. Podgorski, J. Six, P. Boeckx, S. E. Trumbore, L. Cizungu

Ntaboba, and R. G. M. Spencer. 2019. Mobilization of aged and biolabile soil carbon by tropical deforestation. Nature Geoscience 12:541–546.

Finkler, N. R., F. Tromboni, I. G. Boëchat, B. Gücker, and D. G. F. Cunha. 2018. Nitrogen and phosphorus uptake dynamics in tropical Cerrado woodland streams. Water 10:1080.

Goodale, C. L., J. D. Aber, and W. H. McDowell. 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. Ecosystems 3:433–450.

Hall, R. O. Jr, and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography 48:1120–1128.

Hall, R. O. Jr, J. L. Tank, D. J. Sobota, P. J. Mulholland, J. M. O'Brien, W. K. Dodds, J. R. Webster, H. M. Valett, G. C. Poole, B. J. Peterson, J. L. Meyer, W. H. McDowell, S. L. Johnson, S. K. Hamilton, N. B. Grimm, S. V. Gregory, C. N. Dahm, L. W. Cooper, L. R. Ashkenas, S. M. Thomas, R. W. Sheibley, J. D. Potter, B. R. Niederlehner, L. T. Johnson, A. M. Helton, and C. M. Crenshaw. 2009. Nitrate removal in stream ecosystems measured by 15N addition experiments: Total uptake. Limnology and Oceanography 54:653–665.

Hedin, L. O., E. N. J. Brookshire, D. N. L. Menge, and A. R. Barron. 2009. The nitrogen paradox in tropical forest ecosystems. Annual Review of Ecology, Evolution, and Systematics 40:613–635.

Hernes, P. J., R. Y. Dyda, and W. H. McDowell. 2017. Connecting tropical river DOM and POM to the landscape with lignin. Geochimica et Cosmochimica Acta 219:143–159.

Kaplan, L. A., T. N. Wiegner, J. D. Newbold, P. H. Ostrom, and H. Gandhi. 2008. Untangling the complex issue of dissolved organic carbon uptake: A stable isotope approach. Freshwater Biology 53:855–864.

Kaushal, S. S., and W. M. Lewis. 2005. Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. Biogeochemistry 74:303–321.

Kilpatrick, F. A., and E. D. Cobb. 1985. Measurement of discharge using tracers: U.S. Geological Survey Techniques of Water-Resources Investigations A3-16. United States Geological Survey, Washington, DC. (Available from: https://pubs.usgs.gov/twri/twri3-a16/)

Koenig, L. E., C. Song, W. M. Wollheim, J. Rüegg, and W. H. McDowell. 2017. Nitrification increases nitrogen export from a tropical river network. Freshwater Science 36:698–712.

Kossin, J. P., K. R. Knapp, T. L. Olander, and C. S. Velden. 2020. Global increase in major tropical cyclone exceedance probability over the past four decades. Proceedings of the National Academy of Sciences of the United States of America 117:11,975–11,980.

Lewis, W. M., J. M. Melack, W. H. McDowell, M. McClain, and J. E. Richey. 1999. Nitrogen yields from undisturbed watersheds in the Americas. Biogeochemistry 46:149–162.

Lloret, E., C. Dessert, J. Gaillardet, P. Albéric, O. Crispi, C. Chaduteau, and M. F. Benedetti. 2011. Comparison of dissolved inorganic and organic carbon yields and fluxes in the watersheds of tropical volcanic islands, examples from Guadeloupe (French West Indies). Chemical Geology 280:65–78.

Lutz, B. D., E. S. Bernhardt, B. J. Roberts, and P. J. Mulholland. 2011. Examining the coupling of carbon and nitrogen cycles in Appalachian streams: The role of dissolved organic nitrogen. Ecology 92:720–732.

Mann, P. J., A. Davydova, N. Zimov, R. G. M. Spencer, S. Davydov, E. Bulygina, S. Zimov, and R. M. Holmes. 2012. Controls on the composition and lability of dissolved organic matter in Siberia's Kolyma River basin. Journal of Geophysical Research: Biogeosciences 117:G01028.

Martí, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in mediterranean streams. Ecology 77:854–869.

McDowell, W. H. 1998. Internal nutrient fluxes in a Puerto Rican rain forest. Journal of Tropical Ecology 14:521–536.

McDowell, W. H., and C. E. Asbury. 1994. Export of carbon, nitrogen, and major ions from three tropical montane watersheds. Limnology and Oceanography 39:111–125.

McDowell, W. H., R. L. Brereton, F. N. Scatena, J. B. Shanley, N. V. Brokaw, and A. E. Lugo. 2013. Interactions between lithology and biology drive the long-term response of stream chemistry to major hurricanes in a tropical landscape. Biogeochemistry 116:175–186.

McDowell, W. H., and G. E. Likens. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecological Monographs 58:177–195.

McDowell, W. H., C. P. McSwiney, and W. B. Bowden. 1996. Effects of hurricane disturbance on groundwater chemistry and riparian function in a tropical rain forest. Biotropica 28:577–584.

Merriam, J. L., W. H. McDowell, J. L. Tank, W. M. Wollheim, C. L. Crenshaw, and S. L. Johnson. 2002. Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ 15N addition. Freshwater Biology 47:143–160.

Miller, M. P., E. W. Boyer, D. M. McKnight, M. G. Brown, R. S. Gabor, C. T. Hunsaker, L. Iavorivska, S. Inamdar, D. W. Johnson, L. A. Kaplan, H. Lin, W. H. McDowell, and J. N. Perdrial. 2016. Variation of organic matter quantity and quality in streams at Critical Zone Observatory watersheds. Water Resources Research 52:8202–8216.

Mineau, M. M., W. M. Wollheim, I. Buffam, S. E. G. Findlay, R. O. Hall, E. R. Hotchkiss, L. E. Koenig, W. H. McDowell, and T. B. Parr. 2016. Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. Journal of Geophysical Research: Biogeosciences 121:2019–2029.

Mulholland, P. J., R. O. Hall, D. J. Sobota, W. K. Dodds, S. E. G. Findlay, N. B. Grimm, S. K. Hamilton, W. H. McDowell, J. M. O'Brien, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, S. V. Gregory, S. L. Johnson, J. L. Meyer, B. J. Peterson, G. C. Poole, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, A. M. Helton, L. T. Johnson, B. R. Niederlehner, J. D. Potter, R. W. Sheibley, and S. M. Thomasn. 2009. Nitrate removal in stream ecosystems measured by 15N addition experiments: Denitrification. Limnology and Oceanography 54:666–680.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a 15N tracer addition. Ecological Monographs 70:471–493.

Mulholland, P. J., J. L. Tank, J. R. Webster, W. B. Bowden, W. K. Dodds, S. V. Gregory, N. B. Grimm, S. K. Hamilton, S. L. Johnson, E. Martí, W. H. McDowell, J. L. Merriam, J. L. Meyer, B. J.

Peterson, H. M. Valett, and W. M. Wollheim. 2002. Can up-take length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. Journal of the North American Benthological Society 21:544.

Mulholland, P. J., S. A. Thomas, H. M. Valett, J. R. Webster, and J. Beaulieu. 2006. Effects of light on NO3 uptake in small forested streams: Diurnal and day-to-day variations. Journal of the North American Benthological Society 25:583–595.

Neill, C., L. A. Deegan, S. M. Thomas, and C. C. Cerri. 2001. Deforestation for pasture alters nitrogen and phosphorus in small Amazonian streams. Ecological Applications 11:1817.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. Canadian Journal of Fisheries and Aquatic Sciences 38:860–863.

Newbold, J. D., B. W. Sweeney, J. K. Jackson, and L. A. Kaplan. 1995. Concentrations and export of solutes from six mountain streams in northwestern Costa Rica. Journal of the North American Benthological Society 14:21–37.

O'Brien, J. M., and W. K. Dodds. 2010. Saturation of NO_3^- uptake in prairie streams as a function of acute and chronic N exposure. Journal of the North American Benthological Society 29:627–635.

Ortiz-Zayas, J. R., W. M. Lewis, J. F. Saunders, J. H. McCutchan, and F. N. Scatena. 2005. Metabolism of a tropical rainforest stream. Journal of the North American Benthological Society 24:769–783.

Payn, R. A., J. R. Webster, P. J. Mulholland, H. M. Valett, and W. K. Dodds. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. Limnology and Oceanography: Methods 3:174–182.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

Petrone, K. C., J. S. Richards, and P. F. Grierson. 2009. Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia. Biogeochemistry 92:27–40.

Portillo, M. C., S. P. Anderson, and N. Fierer. 2012. Temporal varibility in the diversity and composition of stream bacterioplankton communities. Environmental Microbiology 14:2417–2428.

Potter, J. D., W. H. McDowell, J. L. Merriam, B. J. Peterson, and S. M. Thomas. 2010. Denitrification and total nitrate uptake in streams of a tropical landscape. Ecological Applications 20:2104–2115.

Ribot, M., D. von Schiller, M. Peipoch, F. Sabater, N. B. Grimm, and E. Martí. 2013. Influence of nitrate and ammonium availability on uptake kinetics of stream biofilms. Freshwater Science 32:1155–1167.

Richey, J. S., W. H. McDowell, and G. E. Likens. 1985. Nitrogen transformations in a small mountain stream. Hydrobiologia 124:129–139.

Robbins, C. J., R. S. King, A. D. Yeager, C. M. Walker, J. A. Back, R. D. Doyle, and D. F. Whigham. 2017. Low-level addition of dissolved organic carbon increases basal ecosystem function in a boreal headwater stream. Ecosphere 8:e01739.

Rodríguez-Cardona, B., A. S. Wymore, and W. H. McDowell. 2016. DOC:NO_3^- ratios and NO_3^- uptake in forested headwater streams. Journal of Geophysical Research: Biogeosciences 121:205–217.

Schaefer, D. A., W. H. McDowell, F. N. Scatena, and C. E. Asbury. 2000. Effects of hurricane disturbance on stream water concentrations and fluxes in eight tropical forest watersheds of the Luquillo Experimental Forest, Puerto Rico. Journal of Tropical Ecology 16:189–207.

Schrumpf, M., W. Zech, J. C. Axmacher, and H. V. M. Lyaruu. 2006. Biogeochemistry of an afrotropical montane rain forest on Mt. Kilimanjaro, Tanzania. Journal of Tropical Ecology 22:77–89.

Shanley, J. B., W. H. McDowell, and R. F. Stallard. 2011. Long-term patterns and short-term dynamics of stream solutes and suspended sediment in a rapidly weathering tropical watershed. Water Resources Research 47:W07515.

Sobczak, W. V, and S. Findlay. 2002. Variation in bioavailabtlity of dissolved organic carbon among stream hyporheic flowpaths. Ecology 83:3194–3209.

Starry, O. S., H. M. Valett, and M. E. Schreiber. 2005. Nitrification rates in a headwater stream: Influences of seasonal variation in C and N supply. Journal of the North American Benthological Society 24:753–768.

Strauss, E. A., and G. A. Lamberti. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45:1854–1859.

Strauss, E. A., and G. A. Lamberti. 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47:65–74.

Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems concepts and methods for assessing solute dynamics in stream ecosystems. Journal of the North American Benthological Society 9:95–119.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, and R. O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. Ecology 89:2935–2945.

Taylor, P. G., and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature 464:1178–1181.

Thouin, J. A., W. M. Wollheim, C. J. Vorosmarty, J. M. Jacobs, and W. H. McDowell. 2009. The biogeochemical influences of NO_3, dissolved O_2, and dissolved organic C on stream NO_3 uptake. Journal of the North American Benthological Society 28:894–907.

Tomasek, A. A., T. D. Barman, P. Wang, J. L. Kozarek, C. Staley, M. J. Sadowsky, and M. Hondzo. 2018. The effects of turbulence and carbon amendments on nitrate uptake and microbial gene abundances in stream sediment. Journal of Geophysical Research: Biogeosciences 123:1289–1301.

Tromboni, F., S. A. Thomas, B. Gücker, V. Neres-Lima, C. Lourenço-Amorim, T. P. Moulton, E. F. Silva-Junior, R. Feijó-Lima, I. G. Boëchat, and E. Zandonà. 2018. Nutrient limitation and the stoichiometry of nutrient uptake in a tropical rain forest stream. Journal of Geophysical Research: Biogeosciences 123:2154–2167.

Valett, H. M., J. A. Morrice, C. N. Dahm, and M. E. Campana. 1996. Parent lithology, surface-groundwater exchange, and

nitrate retention in headwater streams. Limnology and Oceanography 41:333–345.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, C. E. Cushing, and R. L. Vannote. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Wymore, A. S., R. L. Brereton, D. E. Ibarra, K. Maher, and W. H. McDowell. 2017. Critical zone structure controls concentration-discharge relationships and solute generation in forested tropical montane watersheds. Water Resources Research 53:6279–6295.

Wymore, A. S., A. A. Coble, B. Rodríguez-Cardona, and W. H. McDowell. 2016. Nitrate uptake across biomes and the influence of elemental stoichiometry: A new look at LINX II. Global Biogeochemical Cycles 30:1183–1191.

Wymore, A. S., J. Potter, B. Rodríguez-Cardona, and W. H. McDowell. 2018. Using in situ optical sensors to understand the biogeochemistry of dissolved organic matter across a stream network. Water Resources Research 54:2949–2958.

Wymore, A. S., B. Rodríguez-Cardona, and W. H. McDowell. 2015. Direct response of dissolved organic nitrogen to nitrate availability in headwater streams. Biogeochemistry 126:1–10.

Zarnetske, J. P., R. Haggerty, S. M. Wondzell, and M. A. Baker. 2011. Dynamics of nitrate production and removal as a function of residence time in the hyporheic zone. Journal of Geophysical Research: Biogeosciences 116:1–12.