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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 (NO₃⁻) 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 NO₃⁻ 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, NO₃⁻ uptake was either undetectable or had very long (>3000 m) uptake lengths (Sw). When DOM was added with NO₃⁻, Sw values for NO₃⁻ 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 NO₃⁻-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 NO₃⁻ concentrations points to important feedbacks between inorganic N and DOM including organic N. Understanding the controls on NO₃⁻ 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 (NO₃⁻) 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 ecosystems, 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, Hedlin 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 concentrations 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
concentrations of DOC and DON are ~1 mg/L and 50 µg/L, respectively, whereas the inorganic N pool is dominated by \( \text{NO}_3^- \), with concentrations between 65 and 100 µg/L and \( \text{NH}_4^+ \) 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 \( \text{NO}_3^- \)-only additions in QT and 3 in QPB and, at both sites, we did 3 additions of \( \text{NO}_3^- \) + acetate (potassium acetate, \( \text{C}_2\text{H}_3\text{O}_2\text{K} \)) and 1 addition of \( \text{NO}_3^- \) + urea (\( \text{CH}_4\text{N}_2\text{O} \)), for a total of 15 additions across both streams (Table 2). For all additions, nitrate was added in the form of sodium nitrate (\( \text{NaNO}_3 \)). We added these solutes along with sodium chloride (\( \text{NaCl} \)) as a conservative tracer. Additions targeted an increase of \( \text{Cl}^- \) 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:

\[
V_f = \frac{Q}{S_w} \quad (\text{Eq. 1}),
\]

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:

\[
U = V_f \times C \quad (\text{Eq. 2}),
\]

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 \( \text{NaCl} \) 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

<table>
<thead>
<tr>
<th>Site</th>
<th>Watershed area (km²)</th>
<th>DOC (mg C/L) (min, max)</th>
<th>DON (µg N/L) (min, max)</th>
<th>( \text{NO}_3^- ) (µg N/L) (min, max)</th>
<th>( \text{NO}_2^- ) (µg N/L) (min, max)</th>
<th>( \text{NH}_4^+ ) (µg N/L) (min, max)</th>
<th>( \text{NH}_4^+ ) (µg N/L) (min, max)</th>
<th>PO₄³⁻ (µg P/L) (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPB</td>
<td>0.24</td>
<td>0.86 (0.57, 1.29)</td>
<td>24.06 (7.55, 48.22)</td>
<td>68.69 (37, 120)</td>
<td>2.86 (2, 6.05)</td>
<td>2.42 (1.48, 4.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>0.18</td>
<td>0.99 (0.6, 1.59)</td>
<td>66.65 (15.66, 193.5)</td>
<td>115.65 (65.4, 200)</td>
<td>5.82 (2.5, 26.96)</td>
<td>4.03 (2.15, 6.65)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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, NO₃⁻, and acetate with ion chromatography using an Anion/Cations Dionex™ ICS-1000 with AS40 autosampler (Thermo Fisher Scientific, Waltham, Massachusetts). We analyzed ammonium (NH₄⁺) 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 NO₃⁻ and NH₄⁺ (DIN) concentrations from TDN.

We examined the response of the ambient DOC and DON pools to elevated concentrations of NO₃⁻ during the NO₃⁻-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

#### NO₃⁻ uptake

The addition of a DOM source enhanced NO₃⁻ uptake. When NO₃⁻ was added without a DOM source, NO₃⁻ uptake was generally undetectable (Fig. 2A–C). Only 1 (in QP) of the 7 NO₃⁻-only additions had detectable uptake with a \( S_w \) of 3811 m. When NO₃⁻ was added along with a DOM source in the paired additions, \( S_w \) decreased (relative to the NO₃⁻-only additions, \( n = 1 \)) to between 80 and 1254 m (median = 127 m, \( n = 3 \)) for the NO₃⁻ + acetate addition and to between 148 and 1417 m (median = 782 m, \( n = 2 \))

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Addition</th>
<th>Uptake solute</th>
<th>( S_w ) (m)</th>
<th>( V_f ) (mm/min)</th>
<th>( U ) (l g⁻¹ min⁻¹)</th>
<th>( Q ) (L/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPB</td>
<td>1/7/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.20</td>
</tr>
<tr>
<td>QPB</td>
<td>1/7/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>141</td>
<td>2.29</td>
<td>51.2</td>
<td>6.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetate</td>
<td>NO₃⁻</td>
<td>147</td>
<td>1.72</td>
<td>256.3</td>
<td>4.89</td>
</tr>
<tr>
<td>QPB</td>
<td>1/8/16</td>
<td>NO₃⁻ + urea</td>
<td>NO₃⁻</td>
<td>1546</td>
<td>0.164</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>QPB</td>
<td>1/12/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>3811</td>
<td>0.053</td>
<td>4.36</td>
<td>3.95</td>
</tr>
<tr>
<td>QPB</td>
<td>1/12/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>1254</td>
<td>0.162</td>
<td>13.2</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetate</td>
<td>NO₃⁻</td>
<td>379</td>
<td>0.54</td>
<td>9.58</td>
<td></td>
</tr>
<tr>
<td>QPB</td>
<td>1/14/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.14</td>
</tr>
<tr>
<td>QPB</td>
<td>1/14/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>277</td>
<td>0.81</td>
<td>85.4</td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>1/4/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.96</td>
</tr>
<tr>
<td>QT</td>
<td>1/5/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.96</td>
</tr>
<tr>
<td>QT</td>
<td>1/5/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>97.70</td>
<td>1.42</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>1/11/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>127.8</td>
<td>1.04</td>
<td>193.4</td>
<td>3.54</td>
</tr>
<tr>
<td>QT</td>
<td>1/13/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>214</td>
<td>0.62</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td>QT</td>
<td>1/13/16</td>
<td>NO₃⁻ + urea</td>
<td>NO₃⁻</td>
<td>1417</td>
<td>0.0762</td>
<td>10.07</td>
<td>3.00</td>
</tr>
<tr>
<td>QT</td>
<td>1/15/16</td>
<td>NO₃⁻-only</td>
<td>NO₃⁻</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.87</td>
</tr>
<tr>
<td>QT</td>
<td>1/15/16</td>
<td>NO₃⁻ + acetate</td>
<td>NO₃⁻</td>
<td>52</td>
<td>2.05</td>
<td>83.2</td>
<td></td>
</tr>
</tbody>
</table>

Mean uptake values

| NO₃⁻-only | NO₃⁻ | 3811 | 0.053 | 4.36 |
| NO₃⁻ + acetate | NO₃⁻ | 487  | 0.98  | 121.21 |
| NO₃⁻ + acetate | Acetate | 193  | 1.29  | 59.60 |
| NO₃⁻ + urea | NO₃⁻ | 748  | 0.90  | 133.17 |
| NO₃⁻ + urea | DON  | 1546 | 0.16  | 1.80  |
for the \( \text{NO}_3^- + \text{urea} \) 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 \( \text{NO}_3^- + \text{acetate} \) addition and to between 0.07 and 1.72 mm/min (median = 1.11) for the \( \text{NO}_3^- + \text{urea} \) addition, whereas \( U \) (l gm\(^{-2}\) min\(^{-1}\)) increased from 4.36 to between 13.24 and 156.95 l gm\(^{-2}\) min\(^{-1}\) (median = 157) and to between 10.07 and 256.28 l gm\(^{-2}\) min\(^{-1}\) (median = 133), respectively (Table 2). Out of the 6 \( \text{NO}_3^- + \text{acetate} \) additions, 3 had undetectable uptake for \( \text{NO}_3^- \) (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 \( \text{NO}_3^- \) 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 l gm\(^{-2}\) min\(^{-1}\) (median = 70.24). For urea, \( U \) was 1.80 l gm\(^{-2}\) min\(^{-1}\) (Table 2).

**Ambient DOC and DON dynamics**

Ambient concentrations of DOC and DON were not always responsive to \( \text{NO}_3^- \)-only additions (Fig. 4A–F). We detected substantial changes in concentrations 30% of the time (2 out of 7 \( \text{NO}_3^- \)-only additions), and DOC and DON did not respond in synchrony. For example, during the \( \text{NO}_3^- \)-only addition on 4 January at QT, there was no relationship between concentrations of DOC and added \( \text{NO}_3^- \) (Fig. 4A), but DON and \( \text{NO}_3^- \) showed a substantial negative relationship \( (r^2 = 0.41, p < 0.001) \) (Fig. 4B). Only a week later, however, we observed no relationships between \( \text{NO}_3^- \) and DOC (Fig. 4C) or DON at QT (Fig. 4D). At QPB, \( \text{NO}_3^- \) 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 \( \text{NO}_3^- \) 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 \( \text{NO}_3^- \) uptake, because \( \text{NO}_3^- \) 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 \( \sim 10 \times \) the available energy/mole than urea (based on Gibbs free energy of acetic acid vs urea). DOC and DON concentrations did not always decline
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 may 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 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 ($n = 1$).
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$_3^-$, despite the availability of DOM. 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

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.
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, Rodriguez-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 (Catálan 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 DON 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, Goode 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
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Streams of the LEF are energetically limited and only with the increased availability of low molecular weight and labile DOM will inorganic N, especially NO₃⁻, 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, NO₃⁻ 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.

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