ARE RIVERS JUST BIG STREAMS? A PULSE METHOD TO QUANTIFY NITROGEN DEMAND IN A LARGE RIVER

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Abstract. Given recent focus on large rivers as conduits for excess nutrients to coastal zones, their role in processing and retaining nutrients has been overlooked and understudied. Empirical measurements of nutrient uptake in large rivers are lacking, despite a substantial body of knowledge on nutrient transport and removal in smaller streams. Researchers interested in nutrient transport by rivers (discharge >10,000 L/s) are left to extrapolate riverine nutrient demand using a modeling framework or a mass balance approach. To begin to fill this knowledge gap, we present data using a pulse method to measure inorganic nitrogen (N) transport and removal in the Upper Snake River, Wyoming, USA (seventh order, discharge 12,000 L/s). We found that the Upper Snake had surprisingly high biotic demand relative to smaller streams in the same river network for both ammonium (NH₄⁺) and nitrate (NO₃⁻). Placed in the context of a meta-analysis of previously published nutrient uptake studies, these data suggest that large rivers may have similar biotic demand for N as smaller tributaries. We also found that demand for different forms of inorganic N (NH₄⁺ vs. NO₃⁻) scaled differently with stream size. Data from rivers like the Upper Snake and larger are essential for effective water quality management at the scale of river networks. Empirical measurements of solute dynamics in large rivers are needed to understand the role of whole river networks (as opposed to stream reaches) in patterns of nutrient export at regional and continental scales.

Key words: ammonium; nitrate; nutrient spiraling; river; Snake River, Wyoming, USA; stream; uptake length; uptake velocity.

INTRODUCTION

River networks regulate the export of nutrients from the terrestrial landscape, making them critical for mitigating eutrophication of downstream ecosystems (Alexander et al. 2000) and we must understand the role of entire river systems (as opposed to stream reaches) in affecting regional and continental nutrient export patterns. Foundational research examining the contribution of rivers to inorganic nitrogen (N) export to coastal systems has shown that simple models can predict N export based on human-influenced point and non-point source loading from fertilizer application and NO₃ deposition (Caraco and Cole 1999) or even more simply from population density alone (Peierls et al. 1991, Howarth et al. 1996). These relationships have shaped our worldview on the dominant external edaphic factors that control river nutrient export but provide no indication of the role of internal nutrient uptake and transformation that may occur in rivers.

Our current empirical understanding of fluvial nutrient dynamics is based mainly on research conducted in small, headwater streams (Ensign and Doyle 2006). Particularly for nitrogen, the take-home message is that small streams are processing hotspots with the potential to transform and retain dissolved nutrients (Peterson et al. 2001), and thus may control N exports from river networks because they make up the majority of catchment river miles (Alexander et al. 2000, 2007). Current research on the biogeochemistry of small streams is now focusing on what controls nutrient uptake, how land use modifies those patterns, and what constitutes effective stream restoration (Doyle et al. 2003, Meyer et al. 2005, Bernot et al. 2006, Hoellein et al. 2007, Mulholland et al. 2008).

Despite comparatively high N uptake rates in headwater streams, excess nutrients are nevertheless exported to downstream ecosystems (Alexander et al. 2007). Nutrient export from large rivers is receiving considerable attention, for example N loading in the Mississippi River causes periodic hypoxic zones in the Gulf of Mexico (Rabalais et al. 2002, Dodds 2006), and this phenomenon may not be limited solely to river networks with intensive upstream agriculture (Monteiro et al. 2006), as previously assumed. Most often nutrient uptake in rivers is inferred using models based on data from small streams (Wollheim et al. 2006) or estimated from nutrient mass balances (Howarth et al. 1996, Alexander et al. 2000, 2007). Results from such studies...
suggest that larger streams and rivers may be responsible for the majority of nitrogen removal because long transport distances result in increased water residence time (Seitzinger et al. 2002, Wollheim et al. 2006). Nevertheless there remains a lack of empirical data on nutrient uptake in larger systems (e.g., discharge >1000 L/s). Given the focus on entire river networks as conduits for excess nutrients into coastal zones, the ability of large rivers to process and retain nutrients has been overlooked and understudied.

In part, this knowledge gap reflects the current methods for measuring nutrient uptake in situ that are impractical for quantifying nutrient cycling in large rivers. Nutrient spiraling theory was developed in headwater streams and has a long history in stream ecology (e.g., Webster and Patten 1979, Newbold et al. 1981). Spiraling theory represents a conceptual framework for understanding solute dynamics in fluvial systems with a strength being that it combines both hydrological and biological controls on nutrient removal in lotic systems. Nutrient spiraling parameters are typically measured using steady-state solute releases of isotopic tracers (e.g., Newbold et al. 1981, Peterson et al. 2001) or low-level short-term nutrient enrichments (e.g., Mulholland et al. 2002). In either case, nutrient fluxes (a result of discharge and nutrient concentration) are generally too high in rivers to use short term additions delivered using pumps. These methods cannot increase concentrations sufficiently to quantify subsequent decline without great difficulty or prohibitive cost. To date, the majority (~90% of N = 625) of nutrient uptake measurements have been made in streams with discharge <200 L/s, with almost half of these made in streams <20 L/s (Fig. 1).

To address this lack of data, we used a nutrient pulse addition approach to quantify N uptake in a large river. Using methods adapted from small-stream ecology we were able to measure N uptake in the 12 000 L/s Upper Snake River in northwest Wyoming. Here, we report our data from this technique in the context of a larger meta-analysis of nutrient uptake measurements published to date, and we address the following questions in our analysis: (1) How does nutrient uptake in a large western river compare to smaller streams for which we have numerous measurements? and (2) How does the relative role of biology vs. hydrology in nutrient uptake vary with stream size and form of inorganic N? Surprisingly, we found that the Upper Snake had similar biotic demand for N as smaller streams in the same river network. The Snake River data combined with our meta-analysis suggests that riverine nutrient uptake may scale with stream size; large rivers may have similar demand compared to small tributaries. Yet different forms of inorganic N show different uptake parameters and thus may scale differently with stream size, which may have ramifications for uptake and delivery of N to downstream ecosystems.

**Materials and Methods**

**Study site**

In July 2005, we conducted solute releases in a 3-km reach of the Upper Snake River (width = 41 m, Q = 12 000 L/s) in John D. Rockefeller National Parkway, Wyoming, USA upstream of Jackson Lake (seventh order, catchment area = 1376 km²). We chose to quantify N uptake in the Snake River during summer because we wanted to test the nutrient pulse method in a larger system where we had previous nutrient uptake

![Graph](image-url)

**Fig. 1.** Distribution of stream nutrient uptake studies for NH₄⁺ and NO₃⁻ grouped by stream size (based on discharge). The majority of estimates are from streams with discharge <200 L/s. Numbers above each bar indicate sample size. Data are summarized from the Supplement.
data from small tributaries in the same river network. During summer, the cobble/boulder bottom of the Upper Snake supports an active algal assemblage (e.g., filamentous green streamers), which we predicted would result in high rates of biotic N demand, particularly because ambient concentrations were very low (dissolved inorganic N, DIN < 10 µg/L).

**Pulse addition method**

We conducted pulse additions using a conservative tracer (chloride, Cl\(^-\)) plus a reactive solute (ammonium, NH\(_4^+\) or nitrate, NO\(_3^-\) conducted separately). We added the nutrient pulse by filling a 610-L cattle tank with river water and 276 kg of NaCl and 4.3 kg KNO\(_3\) or 5.7 kg (NH\(_4\))\(_2\)SO\(_4\), depending on the release and mixing until increasing biotic demand. From sampling station below a large eddy created from a pool reactor (based on dispersion). The mixed across the channel, and to fine tune how much travel distance required for the solute solution to be well downstream where(1330, 1430, 1750, and 2610 m from the release point) with conductivity meters to measure travel time, dispersion, and mixing, allowing us to measure the travel distance required for the solute solution to be well mixed across the channel, and to fine tune how much reactive solute we had to add (based on dispersion). The key to mixing in the Snake River was placing the first sampling station below a large eddy created from a pool located at a river bend. Considering previous research that has shown that enrichment-type releases can saturate nutrient demand, and thus underestimate nutrient uptake (Mulholland et al. 2002, Payn et al. 2005), we aimed to raise the peak concentrations of NH\(_4^+\) and NO\(_3^-\) during the peak of the pulse to ~50 µg/L above background concentrations. This increase was analytically detectable, but the highest concentration during the pulse was likely not high enough to saturate demand (Dodds et al. 2002, Earl et al. 2006), and only lasted for a few minutes at most. During each pulse, we collected water samples every two minutes at each station to characterize the peak as it passed by a station, while also measuring specific conductance. Upon return to the laboratory, we quantified NO\(_3^-\) using ion chromatography (Dionex Model DX600) with AS14A analytical and guard columns (Dionex Corporation, Sunnyvale, California, USA) and a 500-µL injection loop, and NH\(_4^+\) using the phenylhydrazine technique (Solorzano 1969, APHA 1998).

**Pulse release calculations**

Nutrient spiraling theory (e.g., Webster and Patten 1979, Newbold et al. 1981) uses interrelated metrics to quantify different aspects of nutrient transport and removal in flowing waters. Uptake length (\(S_u\)) is the average distance a solute molecule travels prior to removal from the water column. As such, it combines both hydrologic and biological processes, because \(S_u\) lengthens with increasing depth and velocity, and shortens with increasing biotic demand. From \(S_u\) (in meters), we calculated biotic demand relative to concentration (uptake velocity, \(V_t\) in mm/s) using stream discharge (\(Q\) in m\(^3\)/s) and width (\(w\), in meters); it is calculated as \(V_t = (Q/w)/S_u\) (Stream Solute Workshop 1990, Davis and Minshall 1999). Uptake velocity (\(V_t\)) normalizes \(S_u\) for the effects of depth and velocity (\(Q\) divided by width, \(w\)), so streams of different sizes can be directly compared.

For each pulse release, we calculated uptake parameters (described in the last paragraph) using a mass balance approach, by calculating the mass of reactive solute passing over each station, relative to the mass of conservative tracer (i.e., area under each curve in Fig. 2A, B; Chapra 1997). We assumed that any reduction in the mass of N relative to conservative tracer at downstream stations was a result of biological uptake between stations as sorption is balanced by desorption throughout the pulse (Stream Solute Workshop 1990). We calculated uptake length (\(S_u\)) using background-corrected mass of solute passing over a station divided by background-corrected mass of conservative tracer passing over a station and plotted the natural log of this fraction versus distance downstream where \(S_u\) is the absolute value of the inverse of the slope (Stream Solute Workshop 1990). Uptake velocity (\(V_t\)) was calculated using equations described above. We independently confirmed our results from the pulse mass balance approach using a one-dimensional advection, dispersion, transient storage solute transport model (OTIS-P [Runkel 1998, 2007]) to estimate first-order decay rate (\(\lambda\), min\(^{-1}\)) for the solute, using NH\(_4^+\) as an example.

**RESULTS**

**Quantifying inorganic N uptake in the Upper Snake River**

Using the pulse release method we were able to quantify declines (corrected for dilution and dispersion with the conservative tracer) in the mass of NH\(_4^+\) and NO\(_3^-\) passing by stations downstream of the release point (Fig. 2A, B, respectively), allowing us to successfully calculate the uptake length (\(S_u\)) and uptake velocity (\(V_t\)) for each reactive solute. In the Upper Snake, the uptake length \(S_u\) for NH\(_4^+\) was 2000 m (linear regression, \(r^2 = 0.85\), \(P = 0.07\), Fig. 2A). When scaled for discharge and width, the NH\(_4^+\) uptake velocity, \(V_t\), was 9.3 mm/min (Table 1). For NO\(_3^-\), the \(S_u\) was somewhat longer at 2500 m (linear regression, \(r^2 = 0.80\), \(P = 0.10\), Fig. 2B) and we calculated a slightly lower \(V_t\) at 7.4 mm/min (Table 1). To corroborate the linear regression approach based on mass loss, the first-order decay rate (\(\lambda\)) estimated using OTIS-P was 2.20 × 10\(^{-4}\) min\(^{-1}\), roughly equivalent to the value (2.33 × 10\(^{-4}\) min\(^{-1}\)) obtained using the mass-balance calculated \(S_u\) (Stream Solute Workshop 1990).

We can place our NH\(_4^+\) and NO\(_3^-\) uptake estimates in the context of the smaller tributaries in the Upper Snake River basin using data from Hall and Tank (2003); discharge in the smaller tributaries ranged from 9–231 L/s (Table 1). For both NH\(_4^+\) and NO\(_3^-\), \(S_u\) estimates in the Upper Snake were generally longer than in the...
smaller tributaries, reflecting the influence of depth and velocity on $S_w$. However, when we scaled for discharge by calculating $V_f$ (i.e., biotic demand relative to concentration), $\text{NH}_4^+$ and $\text{NO}_3^-$ demand were similar, and fell among the higher values, compared to the smaller tributaries (Table 1). Using the nutrient pulse method, $\text{NH}_4^+$ and $\text{NO}_3^-$ demand in the Upper Snake River are comparable to biotic demand for small streams in the same catchment (Hall and Tank 2003).

**Snake River nitrogen uptake in context of meta-analysis**

Using inorganic N uptake data from previously published studies (Appendix A), plus our data for the Upper Snake River (this study), we examined the relationship between $S_w$ and stream size (discharge, $Q$ in L/s) for both $\text{NH}_4^+$ and $\text{NO}_3^-$. There was a significant relationship between $\text{NH}_4^+ S_w$ and $Q$, with stream size explaining almost half of the variation in uptake length (linear regression, $r^2 = 0.47, P = 0.0001$, Fig. 3A). For

**Table 1.** Comparison of physical characteristics and ammonium and nitrate uptake metrics in the Snake River, Wyoming, USA, and its smaller tributaries (data from Hall and Tank [2003]).

<table>
<thead>
<tr>
<th>Stream</th>
<th>$Q$ (L/s)</th>
<th>Width (m)</th>
<th>$W:D$ ratio</th>
<th>$\text{NH}_4^+$ (µg/L)</th>
<th>$\text{NH}_4^+ S_w$ (m)</th>
<th>$\text{NH}_4^+ V_f$ (mm/min)</th>
<th>$\text{NO}_3^-$ (µg/L)</th>
<th>$\text{NO}_3^- S_w$ (m)</th>
<th>$\text{NO}_3^- V_f$ (mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snake River (this study)</td>
<td>12,000</td>
<td>41.0</td>
<td>84</td>
<td>5</td>
<td>2,000</td>
<td>9.3</td>
<td>5</td>
<td>2,500</td>
<td>7.4</td>
</tr>
<tr>
<td>Ditch Creek</td>
<td>231</td>
<td>5.8</td>
<td>41</td>
<td>2</td>
<td>249</td>
<td>9.6</td>
<td>5</td>
<td>821</td>
<td>2.9</td>
</tr>
<tr>
<td>Glade Creek</td>
<td>149</td>
<td>3.0</td>
<td>20</td>
<td>1</td>
<td>322</td>
<td>9.2</td>
<td>&lt;5</td>
<td>758</td>
<td>3.9</td>
</tr>
<tr>
<td>Two Ocean Creek</td>
<td>144</td>
<td>4.1</td>
<td>32</td>
<td>3</td>
<td>384</td>
<td>5.5</td>
<td>10</td>
<td>2,412</td>
<td>0.9</td>
</tr>
<tr>
<td>Bailey Creek</td>
<td>118</td>
<td>5.4</td>
<td>52</td>
<td>2</td>
<td>833</td>
<td>1.6</td>
<td>5</td>
<td>747</td>
<td>1.7</td>
</tr>
<tr>
<td>Spread Creek</td>
<td>87</td>
<td>5.5</td>
<td>55</td>
<td>1</td>
<td>76</td>
<td>12.6</td>
<td>13</td>
<td>108</td>
<td>9.0</td>
</tr>
<tr>
<td>Pilgrim Creek</td>
<td>46</td>
<td>4.1</td>
<td>74</td>
<td>1</td>
<td>278</td>
<td>2.4</td>
<td>&lt;5</td>
<td>558</td>
<td>1.3</td>
</tr>
<tr>
<td>Paintbrush Creek</td>
<td>39</td>
<td>1.3</td>
<td>20</td>
<td>2</td>
<td>172</td>
<td>1.1</td>
<td>169</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Moose Creek</td>
<td>35</td>
<td>2.2</td>
<td>25</td>
<td>10</td>
<td>910</td>
<td>1.1</td>
<td>89</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lizard Creek</td>
<td>25</td>
<td>2.5</td>
<td>24</td>
<td>1</td>
<td>416</td>
<td>1.4</td>
<td>6</td>
<td>1,568</td>
<td>0.4</td>
</tr>
<tr>
<td>Moran Bay</td>
<td>9</td>
<td>0.8</td>
<td>6</td>
<td>2</td>
<td>344</td>
<td>1.9</td>
<td>43</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Note:** NA signifies that no significant uptake was measurable; $S_w$, uptake length; $Q$, stream discharge; $V_f$, uptake velocity; $W:D$ ratio, width to depth ratio.
the largest streams in the data set, there was the least variation in uptake length, with data points falling very close to the regression line (Fig. 3A), recognizing this pattern is confounded by the difference in the number of data points between small and large streams. The Upper Snake River ($S_w = 2000\ m$, $Q = 12\ 000\ L/s$) had the same uptake length as the Kansas River, Kansas ($S_w = 2000$, $Q = 14\ 350\ L/s$ [Dodds et al., unpublished manuscript]), but both were shorter than the Lower Kuparuk River, Alaska ($S_w = 5360$, $Q = 18\ 300\ L/s$ [Wollheim et al.].

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**Figure 3.** Meta-analysis of previously published results plus this study shows that uptake length ($S_w$) of (A) NH$_4^+$ and (B) NO$_3^-$ increases with stream discharge $Q$ (L/s). Note that we excluded studies not using stable isotopes where $S_w > 5000\ m$ for NH$_4^+$ or NO$_3^-$ from the data set in the Supplement. (C) NH$_4^+$ $S_w$ plotted vs. NO$_3^-$ $S_w$ for streams where both solutes were collected ($N = 132$); the dashed line represents the 1:1 relationship. We note that plotting NH$_4^+$ $V_f$ vs. NO$_3^-$ $V_f$ would be identical because $Q$ and width are the same for both releases; thus for either plot, biological demand relative to concentration for NH$_4^+$ is higher than for NO$_3^-$. 

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How does nutrient concentration interact with stream size in controlling uptake length?

Although stream size is a major driver of $S_w$, increasing nutrient concentration also lengthens $S_w$ (Stream Solute Workshop 1990), therefore unexplained variation in the $S_w$ vs. $Q$ relationship may be explained by N availability, either as background or plateau concentration (for those measured using short-term nutrient additions). For NH$_4^+$, Fig. 3A identifies estimates made using $^{15}$N isotope additions which do not raise background NH$_4^+$ concentrations; these estimates fall below the regression line, being the least likely to overestimate $S_w$ due to a saturation effect (sensu Mulholland et al. 2002). The $^{15}$N tracer estimates contrast with those $S_w$ estimates made with short-term enrichment methods where background enrichment was $\geq$50 $\mu$g NH$_4^+$-N/L; these estimates fall above the regression line indicating that excess NH$_4^+$, particularly in smaller streams, can result in longer $S_w$ estimates (Fig. 3A). We compared the slope of the regression of $Q$ vs. $S_w$ for all data (Fig. 3A) with the regression of $Q$ vs. $^{15}$N tracer data only, and although the regression was also significant for $Q$ vs. $^{15}$NH$_4^+$ $S_w$ (linear regression, $y = 0.69x + 0.79$, $r^2 = 0.71$, $P < 0.0001$), the slope of 0.69 was significantly greater than that from the $Q$ vs. $S_w$ regression for all data (ANCOVA, $P = 0.579$, Fig. 3B). In summary, NO$_3^-$ $S_w$ does not appear to be as sensitive to variation in NO$_3^-$ availability, which contrasts with results from the NH$_4^+$ $S_w$ meta-analysis.

We can further explore the secondary influence of ambient concentration by examining it in the context of the residuals of the $S_w$ vs. $Q$ relationship for each solute. For NH$_4^+$, the residuals of the $Q$ vs. $S_w$ relationship were significantly related to background NH$_4^+$ concentration ($r^2 = 0.13$, $P < 0.0001$). In contrast, this was not the case for the residuals of the $Q$ vs. NO$_3^-$ $S_w$ relationship and NO$_3^-$ concentration ($P = 0.316$). For the meta-analysis, the range in background concentration of NH$_4^+$ and NO$_3^-$ varied considerably between the two data sets; background NH$_4^+$ ranged from $\sim$1 to 160 $\mu$g N/L, whereas the NO$_3^-$ range was almost 40x larger ($\sim$1–6100 $\mu$g N/L). There has been some support in the literature that shows that NH$_4^+$ is used preferentially over NO$_3^-$ to satisfy inorganic N demand (e.g., Dortch 1990), supporting our results indicating that background concentration of NH$_4^+$ was a major driver in the deviation from the $Q$ vs. $S_w$ relationship, but the same was not true for NO$_3^-$ $S_w$.

The results from the meta-analysis suggest that NH$_4^+$ is tightly cycled; after stream size is accounted for, NH$_4^+$ $S_w$ may be under strong biological control because concentration explains the residual variation in the NH$_4^+$ $S_w$ vs. $Q$ relationship. From our limited data it may be that the influence of biological demand on uptake is less variable among rivers with increasing size. Alternatively the relationship may be a response to changing sorption kinetics in larger systems. We acknowledge either mechanism may be biased by the lack of data from larger systems, nevertheless, we present this hypothesis of strong biological control to fuel future research.

We also plotted NH$_4^+$ $S_w$ vs. NO$_3^-$ $S_w$ from the subset of studies ($N = 132$) that quantified both solutes on the same stream (Fig. 3C). If NH$_4^+$ and NO$_3^-$ were biologically used interchangeably, we would predict that the data would show a 1:1 relationship (dashed line, Fig. 3C), but in fact this is not the case (91% of the data fall on or above the 1:1 line). In particular, for the smallest streams (shaded triangles, Fig. 3C), when NH$_4^+$ $S_w$ is low (i.e., highest demand), NO$_3^-$ $S_w$ is longer, and it appears that NH$_4^+$ is meeting the inorganic N demand. For the largest systems, (closed boxes, Fig. 3C), the data (including that for the Upper Snake River) approach the 1:1 line. In streams $>200$ L/s, the demand for NH$_4^+$ compared to NO$_3^-$ is similar. These results suggest that larger systems appear to behave distinctly different than smaller systems in regards to NH$_4^+$ vs. NO$_3^-$ cycling.

Discussion

Identification of factors that control nutrient retention in the full size range of fluvial systems in a river network is essential for determining the relative role that biological activity and subsequent nutrient uptake may
play in reducing export of elevated nutrient loads to downstream ecosystems. Key papers have pointed to the importance of small streams as locations for high rates of nutrient cycling (Alexander et al. 2000, Peterson et al. 2001, Bernot and Dodds 2005). Alexander et al. (2007) applied their spatially explicit, mass-balance SPARROW model to stream networks in the northeastern United States and concluded that first-order streams contribute approximately 40% of the nitrogen flux to downstream (above fourth order) rivers, emphasizing the importance of small streams in N removal and retention, but also highlighting the remaining role of downstream rivers in influencing/preventing export to sensitive coastal ecosystems. Using our empirical approach to quantify inorganic N uptake combined with OTIS-P modeling of the pulse addition, we found the in-stream loss rate (λ) for NH₄⁺ in the Snake River to be 2.20 × 10⁻⁴ min⁻¹ (or 0.32 d⁻¹). From Alexander et al. (2000), we can compare our in-stream loss rate to a meta-analysis of 112 U.S. rivers that used SPARROW modeling to predict the relationship between in-stream N loss rate and depth. For a river with a depth = 0.45 m, similar to the Snake River, SPARROW predicts a mean in-stream loss rate of 0.45 d⁻¹ which is slightly higher than our empirical estimate for NH₄⁺ alone. If we add NO₃⁻ demand to that estimate, the Snake River estimate is higher (0.63 d⁻¹) than predicted via the SPARROW analysis (Alexander et al. 2000), but we acknowledge that our rates could be considered an estimate of gross inorganic N removal and the SPARROW modeling represents net N removal. Modeling efforts have suggested that large rivers are important sites of nutrient removal, but the empirical work lags far behind. The lack of information results from the difficulties of applying empirical methodologies for solute dynamics to large river systems. Our results suggest that mass-balance modeling (e.g., SPARROW) may underestimate the potential for river N removal and further study is needed to determine what aspects of rivers promote higher N removal rates (e.g., shallow depth and increased light penetration in the Snake River).

Although modeling approaches suggest that larger rivers can potentially play an important role in N removal (Seitzinger et al. 2002, Wollheim et al. 2006), most empirical measurements have been made in smaller systems (i.e., <200 L/s, Fig. 1). Using the nutrient pulse method described here, we show that it is possible to empirically measure nutrient cycling across a range of stream sizes within a river network, allowing us to fill an important gap in our understanding of how nutrient retention and removal scales with increasing size. The pulse addition technique we present here is practical for larger systems because it requires less solute than steady state releases, which are not feasible in systems with high discharge or high nutrient concentrations or both. A second advantage of the pulse addition method is that it is unbiased by transient storage of solutes (Runkel 2007); this bias may lengthen estimates of uptake length when using steady state additions. The pulse addition method will allow us to extend the scaling approaches of Ensign and Doyle (2006) and Wollheim et al. (2006) to examine nutrient uptake in larger rivers.

Three scenarios of large river biotic demand

We present three possible scenarios of how river size would influence N uptake: (1) As river size increases, the relative role of biological demand in nutrient uptake decreases as invoked by Alexander et al. (2000) in their analysis of N export via the Mississippi River network to the Gulf of Mexico. Their modeling results corroborated earlier studies using mass balance approaches (e.g., Howarth et al. 1996). To put it simply, rivers function as “pipes.” (2) Alternatively, river size has no effect on biological demand thus it remains constant with increasing size. Wollheim et al. (2006) used this assumption in their model of river N export and concluded that nutrient uptake is mainly a function of river length, which is somewhat analogous to longer travel time. In other words, rivers are just big streams. (3) Finally, biotic demand may increase with increasing river size, potentially due to the simultaneous demand by benthic and water column biotic processes. We could find no previous empirical or modeling efforts that would support or reject this scenario.

What empirical estimates do we have of nutrient uptake in larger systems?

To date, we know of only three rivers with Q > 10000 L/s where N uptake was empirically measured using whole-system techniques comparable to measurements made in smaller systems (Fig. 1). While NH₄⁺ S₉ was long in all three rivers, despite their geographic separation, NH₄⁺ S₉ in the Lower Kuparuk River was more than twice as long (S₉ = 5360 m, Wollheim et al. 2001), compared to the NH₄⁺ S₉ = 2000 m for both the Kansas River, Kansas (Dodds et al., unpublished manuscript) and the Upper Snake River, Wyoming (this study; Fig. 3A). When we account for differences in discharge among the three rivers (Q = 12000–18300 L/s), NH₄⁺ demand (expressed as an uptake velocity, Vf) was also lowest in the arctic Lower Kuparuk at 3.7 mm/min, compared to the high of 9.3 mm/min in the Upper Snake River during summer (this study); the low-gradient Kansas River fell in between (Vf = 5.7 mm/min). Both the Lower Kuparuk River and the Upper Snake River have very low background inorganic N concentrations (NH₄⁺ + NO₃⁻ = 10–20 μg N/L), which would be expected to result in fairly high Vf estimates, yet the NH₄⁺ Vf from the Upper Snake River was ~2.5 times higher than that of the arctic Lower Kuparuk River, perhaps reflecting differences in biotic activity influenced by lower (albeit summer) water temperatures (Wollheim et al. 2001). Notably, these NH₄⁺ Vf from three rivers are comparable to those found in smaller streams both when we compare streams of varying size within a catchment (e.g., Upper Snake...
River vs. estimates in Hall and Tank (2003) or among small streams in general (Ensign and Doyle 2006). Data summarized from Appendix A (N = 297 data points) indicate a mean $NH_4^+$ $V_f = 7.58$ mm/min and the median $NH_4^+$ $V_f = 3.75$ mm/min. Results from this meta-analysis lend support to the idea that biological demand may remain constant or even increase with increasing river size, but clearly more systematic testing of these predictions are needed (sensu Ensign and Doyle 2006). Recent models have assumed that $V_f$ remains constant with increasing stream size, which means that large rivers can play an important role in mitigating N export, because of the longer travel time (and thus processing time) associated with larger systems (Wollheim et al. 2006). As for $NO_3^-$ uptake in particular, our estimate from the Upper Snake River is the only one available, despite the relevance of $NO_3^-$ export to downstream and coastal eutrophication issues (Seitzinger et al. 2002, Bernot and Dodds 2005). Clearly, more empirical estimates are needed.

The effect of river size on N uptake may be solute specific

Our analyses suggest that $NH_4^+$ and $NO_3^-$ uptake respond differently to river size (Fig. 3A, B). Our data show that $NH_4^+$ and $NO_3^-$ are not interchangeable forms of N; the slopes of the relationship of each vs. $Q$ were statistically different from each other (0.51 for $NH_4^+$ and 0.32 for $NO_3^-$). Further, $S_w-NO_3^-$ was far longer (often orders of magnitude) than $S_w-NH_4^+$ measured in the same stream at the same discharge (Fig. 3C). The results from the meta-analysis of those streams that had concomitant measurements for both $S_w-NO_3^-$ and $S_w-NH_4^+$ (N = 132) are consistent with a smaller subset of data for 10 streams from different biomes presented by Peterson et al. (2001) showing that $S_w-NO_3^-$ was 10x greater than $S_w-NH_4^+$. Interestingly, the difference between $S_w-NO_3^-$ and $S_w-NH_4^+$ becomes smaller as streams get larger (Fig. 3C, $Q > ~200$ L/s), which may suggest that either form of inorganic N may be able to meet biotic demand in rivers, while $NH_4^+$ is preferred in small streams. This finding is similar to that of Ensign and Doyle (2006) who found that cumulative uptake flux of $NO_3^-$ increased with stream order while the latter is primarily controlled by benthic dynamics (that dominate smaller streams). Further study of this hypothesis is needed.

Although researchers often assume that $NH_4^+$ and $NO_3^-$ are interchangeable with respect to meeting N demand by stream biota (hence measuring only one or the other in N uptake studies), there are several reasons that explain the differences we report here. First, $NH_4^+$ is a preferred N substrate for both heterotrophic microbes and algae (Rice and Tiedje 1989, Dortch 1990), largely because less energy is required for its assimilation into biomass (Hildebrand 2005). This preference is so strong, that the addition of $NH_4^+$ can suppress $NO_3^-$ uptake via repression of nitrate reductase (Van’t Riet et al. 1968) and/or inhibition of $NO_3^-$ transport into cells (Creswell and Syrett 1979).

Second, $^{15}$N-$NH_4^+$ tracer studies have documented that in some streams nitrification can account for a large (~45%) fraction of apparent $NH_4^+$ removal (reflected in $S_w-NO_3^-$ [Peterson et al. 2001, Simon et al. 2007]). Thus some portion of the $S_w-NH_4^+$ in the meta-analysis represents $NH_4^+$ that was nitrified to $NO_3^-$, which may lead to increased $NO_3^-$ background concentrations (e.g., Bernhardt et al. 2002) and higher variation and magnitude of $S_w-NO_3^-$ values. For $NO_3^-$, dissimilatory nitrate reduction to ammonium (DNRA) could generate ammonium; however, our understanding of this process is lacking, and its prevalence in streams is currently being examined (Burgin and Hamilton 2007).

Differences in $NH_4^+$ and $NO_3^-$ uptake may be related to whole-stream metabolism. For example, if large rivers become more heterotrophic due to larger sediment loads (Vannote et al. 1980), we would expect demand for $NO_3^-$ to decline relative to $NH_4^+$ because $NO_3^-$ uptake has been shown to be correlated with photosynthesis in systems where primary producers dominate (Hall and Tank 2003). The opposite is likely true: most of the small streams in our meta-analysis are light, forested systems with uptake likely more driven by heterotrophic processes, hence tightly cycled $NH_4^+$.

An alternative explanation for the pattern seen in comparing $NH_4^+$ and $NO_3^-$ uptake is that $NH_4^+$ uptake can also be influenced by abiotic sorption processes; $NH_4^+$ uptake could be higher than $NO_3^-$ because the former is subject to cation exchange as well as biological uptake while the latter is primarily controlled by biological mechanisms. In moving from small streams to larger rivers, abiotic controls may change thus shifting the relationship between $NH_4^+$ $S_w$ and $Q$. Rivers transporting more particulates may increase the potential for water column driven cation exchange: perhaps replacing benthic exchange as a mechanism for $NH_4^+$ uptake. This mechanism may work in concert with an increase in water column biotic demand described above. In summary, location in the river network may dictate solute-specific uptake and deserves further study.

Conceptual framework for scaling nutrient spiraling metrics as streams get larger

Going back to our three scenarios of large river biotic demand, we can summarize the relative influence of hydrology vs. biology on N uptake in a conceptual diagram using the relationships between size and N uptake based on our meta-analysis (Fig. 4). In small streams, the relative demand of $NH_4^+$ is greater than the demand for $NO_3^-$, reflected in shorter $S_w$. Our meta-analysis indicates that as size (as $Q$) increases, the lines for $NH_4^+$ and $NO_3^-$ converge, indicating that in larger fluvial systems the demand for these solutes may be similar. But this pattern may be biased by the fact that
we have very few estimates of $S_w$ for larger systems, and indeed the slopes may be strongly influenced by the predominance of data for small streams. Conceptually, this pattern allows us to address the three scenarios of large river nutrient uptake. For example, when nutrient uptake is controlled more by hydrologic processes, $S_w$ should fall well above these lines indicating that the N demand is lower than predicted by its discharge (scenario 1: rivers as pipes). The Upper Snake River data (this study) illustrates scenario 2 in which biological N demand in rivers is similar to small streams, and equal for NH$_4^+$ and NO$_3^-$, and therefore falls on the intersection of the two lines. Conversely, when biological activity is greater than the influence of hydrology in a river (scenario 3), it should fall well below these lines, exemplified by the geothermal Polecat Creek (Hall et al. 2003) and the fertilized Kuparuk River (Wollheim et al. 2001) (Fig. 3A). We predict that riverine conditions, such as productivity and sediment type, will determine where a given river falls on this plot; however, the relative role of hydrology and biology in other large rivers is not currently known due to the lack of empirical measurements (Fig. 4).

The river continuum concept and nutrient cycling

While untested with respect to nutrient dynamics, the river continuum concept (RCC) (Vannote et al. 1980, Minshall et al. 1985) gives us an additional conceptual framework for predicting how nutrient demand should change with stream/river size. Previous research across a range of small streams has shown that nutrient demand is tightly coupled with instream metabolism (Hall and Tank 2003, Meyer et al. 2005, Fellows et al. 2006), and we predict that metabolism should continue to regulate nutrient uptake even as streams become rivers. According to the RCC, gross primary production (GPP) relative to community respiration (CR) increases in mid-order rivers (GPP/CR higher); we would predict that mid-order rivers would have higher assimilative nutrient demand than small streams (i.e., fall below lines, Fig. 4). Furthermore, in even larger systems (e.g., sixth to eighth order), the RCC predicts that heterotrophic demand by suspended sediments and/or plankton begin to influence the biology of rivers (GPP/CR lower). Our results from the Upper Snake River in comparison to its small tributaries suggest that nutrient demand is similar regardless of stream order. Turbid rivers with high sediment load or suspended plankton may be metabolically dominated by heterotrophic respiration, which again may increase nutrient demand, but for a very different reason than that which would be predicted from clear water streams such as the Snake and its tributaries where uptake is more strongly related to benthic production (Hall and Tank 2003, Fellows et al. 2006). In addition to biological shifts associated with increasing size, channel complexity may further influence nutrient demand in large rivers. Stanford and Ward (1993) extend the RCC to include hyporheic zones, postulating greater surface/subsurface exchange in aggraded river valleys than in headwater streams. Exchange between surface water and subsurface sediments can increase solute uptake (Ensign and Doyle 2006, Runkel 2007, but see Hall et al. 2002). Unregulated western rivers, such as the Upper Snake River, which drain glacial alluvium have gravel bars and side channels which may increase hyporheic exchange (Fernald et al. 2001, Hauer and Lorang 2004) and may result higher biotic nutrient demand, compared to small
streams (this study), but there are currently not sufficient empirical data to address this hypothesis.

Summary: large river nutrient cycling

Quantifying the potential for large rivers to process nutrients is essential to elucidate the role of large rivers in controlling nutrient export to downstream ecosystems, such as the Gulf of Mexico, reservoirs, and estuaries. Modeling efforts have suggested that large rivers are important sites of nutrient removal (e.g., Wollheim et al. 2006), but empirical estimates lag far behind, mainly as a result of the methodological difficulties in applying small-stream approaches to larger river systems. We have presented a method to quantify nutrient uptake in large rivers and we place our results in the context of a meta-analysis of previous research thereby providing a context in which to place future studies. Empirical measurements of solute dynamics in large rivers are needed to understand the role of whole river networks (as opposed to stream reaches) in patterns of nutrient export at regional and continental scales and ultimately, to manage water quality effectively.

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APPENDIX
Full citations associated with column 1 author_date_code of the data in the Supplement (Ecological Archives E089-164-A1).

SUPPLEMENT
Inorganic nitrogen (NH₄-N and NO₃-N) uptake parameters and associated hydrologic data from 54 published studies used to
evaluate relationships between uptake and discharge (Ecological Archives E089-164-S1).