

Hydrologic and biogeochemical drivers of dissolved organic carbon and nitrate uptake in a headwater stream network

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Abstract Headwater streams are foci for nutrient and energy loading from terrestrial landscapes, in situ nutrient transformations, and downstream transport. Despite the prominent role that headwater streams can have in regulating downstream water quality, the relative importance of processes that can influence nutrient uptake have not been fully compared in heterotrophic aquatic systems. To address this research need, we assessed the seasonality of dissolved organic carbon (DOC) and nitrate (NO_3^-) uptake, compared the relative influence of hydrologic and biogeochemical drivers on observed seasonal trends in nutrient uptake, and estimated the influence of these biological transformations on watershed scale nutrient retention and export. We determined that seasonal reductions in DOC and NO_3^- concentrations led to decreases in the potential for the biotic community to take up nutrients, and that seasonality of DOC and NO_3^- uptake was consistent with the

seasonal dynamics of ecosystem metabolism. We calculated that that during the post-snowmelt period (June to August), biotic retention of both dissolved organic carbon and nitrate exceeded export fluxes from this headwater catchment, highlighting the potential for biological processes to regulate downstream water quality.

Keywords Dissolved organic Carbon · Nitrogen · Nutrient uptake · TASCC · Residence time · Watershed export

Introduction

Streams are biogeochemically dynamic areas within landscapes, where material inputs from hillslopes, riparian zones, and upstream contributing areas are integrated, transported, and transformed by biotic and abiotic processes. Biogeochemical transformations that occur in stream networks are the result of a diverse array of biological and geochemical processes that can regulate stream solute concentrations and export dynamics. Despite the numerous studies that have investigated nutrient uptake in streams and rivers (Webster et al. 2003; Ensign and Doyle 2006; Hall et al. 2009b) and the importance of these biogeochemical retention processes for downstream water quality and ecosystem health, the drivers that influence

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the magnitude of stream nutrient uptake (and subsequently the net impact of in-stream retention on watershed scale fluxes) remain an active and critically important area of research.

Headwater streams can be important sites of temporary nitrogen retention or nitrogen removal (Grimm 1987; Peterson et al. 2001; Mulholland 2004; Gardner et al. 2011; Bernal et al. 2012). Empirical and model based studies of headwater streams have shown that aquatic retention can significantly reduce concentrations and export fluxes from catchments (Peterson et al. 2001; Bernhardt et al. 2005), with the highest rates of retention occurring in the smallest streams (Alexander et al. 2000; Gomez-Velez et al. 2015). The potential for aquatic systems to remove nitrogen inputs early in the in the stream network could be particularly important given the numerous anthropogenic alterations to nitrogen cycling like land use change and agriculture (Vitousek et al. 1997). Decades of research has shown that streams alter the timing, magnitude, and form of dissolved solutes (Meyer and Likens 1979), suggesting that the ultimate impact of land use changes on water quality may be further mediated by biological processing.

A growing body of research over the past decade has also established that headwater streams play an influential role in the cycling and processing of carbon (C) inputs from the terrestrial landscape (Jones et al. 2003; Cole et al. 2007; Johnson et al. 2008; Crawford et al. 2014), and that aquatic carbon and nitrogen cycles are inherently coupled in aquatic ecosystems (Bernhardt et al. 2002; Brookshire et al. 2005). Co-limitation has been found to be a nearly ubiquitous phenomenon, where primary producers across diverse terrestrial and aquatic ecosystems have been shown to be co-limited by both nitrogen (N) and phosphorus (P), as well as other micronutrients such as iron, potassium, and silica (Elser et al. 2007; Harpole et al. 2011). While most studies have focused on co-limitation by two macro- or micronutrients, biological communities in oligotrophic and heterotrophic stream systems may experience limited supplies of both energy and nutrients. Despite this, a limited number of studies have directly assessed the capacity for both carbon and nitrogen uptake (but see Bernhardt and Likens 2002; Hall and Tank 2003; Brookshire et al. 2005; Fellows et al. 2006; Johnson et al. 2009; Lutz et al. 2011), and

potential energy and macronutrient co-limitation in headwater streams.

Our current understanding of the processes that drive nutrient uptake in aquatic systems reflects the focus of early studies on understanding ecosystem limitation by macronutrients, particularly on N or P limitation of biomass growth and production. Consequently, parameters such as the rate of biomass accumulation and magnitude of gross primary production at the ecosystem level have been identified as important drivers of nutrient uptake rates in streams (Grimm 1987; Hall and Tank 2003; Elser et al. 2007). Given the dynamic seasonal phenology of primary production in most streams and the susceptibility of primary producers to disturbance (Resh et al. 1988; Grimm and Fisher 1989), these studies have also drawn attention to the role of disturbance and succession as indirect drivers of nutrient dynamics in streams (Grimm 1987; Mulholland et al. 2009). While these studies highlight important ecological processes regulating aquatic nutrient uptake, they focus predominantly on autotrophic processes and do not typically consider how these processes may differ in largely heterotrophic systems.

Heterotrophic systems are common globally (Duarte and Prairie 2005; Hoellein et al. 2013), and CO₂ emissions resulting from metabolism of terrestrially derived organic matter are significant for global C budgets (Raymond et al. 2013). Unlike predominantly autotrophic systems, primary production and biomass growth may not drive nutrient dynamics in heterotrophic streams. Instead, the availability of terrestrial organic matter inputs as well as the amount of contact time between solutes and the benthos have both been identified as important ecological variables for determining the magnitude of nutrient uptake rates. Dissolved organic carbon from upland and riparian zones provide substrate for respiration and heterotrophic assimilation (Fisher and Likens 1973; Wilkinson et al. 2013). Therefore the availability of organic carbon substrates may be an important driver of the amount of carbon uptake and respiration (Findlay and Sobczak 1996; Tank et al. 2010). Additionally, numerous studies have identified that extended contact time between solutes and the benthos can enhance opportunities for dissolved solutes to be taken up from the water column. Confirming this, reach-scale hydrologic drivers including residence time and transient storage have been identified as important controls on

the magnitude of nutrient uptake and nutrient retention (Valett et al. 1996; Mulholland et al. 1997; Hall et al. 2002; Ensign and Doyle 2005; Lautz and Siegel 2007; Battin et al. 2008; Harvey 2016). Therefore the availability of dissolved organic carbon substrates as well as streamwater residence time can be critical variables influencing the magnitude of nutrient uptake rates in predominantly heterotrophic systems.

While numerous studies have identified processes that may influence nutrient uptake in streams, fewer have empirically linked nutrient uptake to retention or export at the watershed scale (but see Alexander et al. 2000; Peterson et al. 2001; Wollheim et al. 2005; Hall et al. 2009a; Gardner et al. 2011; Pennino et al. 2014). In fact, the relative importance of streams in regulating solute export to downstream rivers, lakes, or estuaries remains unresolved, with some studies arguing for a significant degree of biotic control over watershed exports (Mulholland 1992; Bernhardt et al. 2005; Roberts and Mulholland 2007; Mulholland et al. 2009; Bernal et al. 2015; Lupon et al. 2015), while others emphasize the dominance of transport processes and the minimal role of in situ processing, particularly on a mass basis (Likens et al. 1970; Kothawala et al. 2015; Raymond et al. 2016). Others still have suggested that streams may operate at a biogeochemical steady state where rapid cycling is balanced by re-release, thus leading to terrestrial losses controlling the magnitude of fluxes from aquatic systems (Brookshire et al. 2009; Creed et al. 2015). This conceptual divergence may in part be due to the limited number of studies that have empirically compared the magnitude of nutrient uptake at the stream ecosystem level with measured watershed scale fluxes.

The limited number of studies directly comparing in-stream retention and downstream export over daily or even seasonal time scales is partially due to the challenge of using observed concentration dynamics to infer both retention and watershed fluxes. The challenge stems from the fact that export fluxes calculated from ambient concentration dynamics have the biotic signal of retention embedded within them, and thus represent an integrated signal of terrestrial loading and in-stream biological processes. The concentration of a solute in streamwater at any given time is a reflection of uptake or retention that has already occurred, as well as a control on the magnitude of uptake at future time points. This creates equifinality in the number of uptakes scenarios that could

generate a given solute concentration. For example, low nitrate concentrations in a stream may be the result of low inputs and a low rate of uptake, or high inputs that are masked by a high rate of uptake also resulting in low concentrations. Given the latter case, reduced concentration due to biotic uptake then sets a limit on how much further uptake and modification will occur. In this way, the same ambient concentration dynamic can be achieved via multiple uptake regimes, limiting the use of flux dynamics as an indicator of biological processing. This further suggests that in situ nutrient uptake rates must be experimentally derived for a more accurate comparison with export fluxes. At the very least, it is critical to recognize the biotic signatures embedded within the concentration dynamic used to estimate physically driven export fluxes.

We believe that new understanding of the magnitude and seasonal evolution of dissolved organic carbon and nitrate uptake kinetics and their relationship to changing physical and biological drivers is required to better understand the dynamics of nutrient demand and influences on watershed nutrient export. To address this, we conducted reach-scale DOC and nitrate tracer additions using the TASC method (Covino et al. 2010b; Piper et al. 2017) across the summer growing season in a forested, subalpine catchment while monitoring a suite of hydrologic and biogeochemical dynamics. This allowed us to address the following questions:

Q1a How do DOC and NO_3^- uptake kinetics change seasonally?

Q1b How do DOC and NO_3^- uptake kinetics respond to seasonal variations in biogeochemical drivers (ambient DOC and NO_3^- concentrations, i.e. substrate availability) and hydrologic drivers (i.e. residence time)?

Q2 What is the role of biological uptake in regulating streamwater DOC and NO_3^- concentrations and downstream fluxes?

We had two hypotheses for how the capacity of the stream to take up nutrients (Q1a) could change over time. First, we hypothesized that the capacity of a stream to take up DOC and NO_3^- over time could be characterized by single kinetic curve that captures the response of the stream to changing concentrations on a seasonal or annual time scale (Fig. 1a). In this case, as

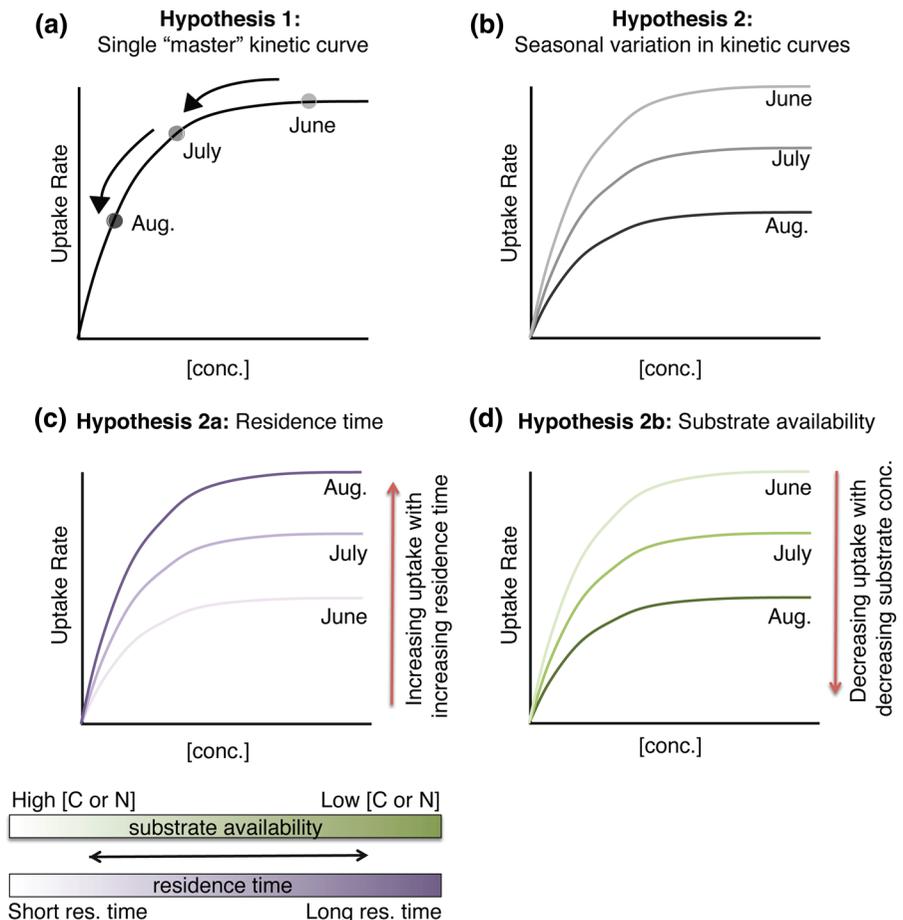
ambient concentrations change in the stream, the uptake rate would move along its’ “master” kinetic curve. This type of kinetic response would suggest that the maximum or ambient capacity of the stream community to take up DOC and NO_3^- is not changing over time, but rather that temporal variation in uptake kinetics is driven by equilibration of demand to available concentration levels following Michaelis–Menten kinetics.

Our second, alternative hypothesis was that the capacity of the stream to take up DOC and NO_3^- would change as the community up- or down-regulates uptake or growth in response to shifting environmental conditions or as the community changes seasonally. In this case, we would expect that the shape and magnitude of the kinetic curve, as an indicator of system responsiveness to nutrient availability, to evolve over seasonal or annual timescales (Fig. 1b). Under this scenario, as DOC and NO_3^- concentrations

increase during the spring snowmelt, uptake rates would increase due to growth or up-regulation and then decrease as solutes are flushed out of the system and ambient concentrations decline. At each time point, the uptake capacity of the stream (which is characterized by a distinct kinetic curve) would be unique. With this study, we sought to test these two hypotheses of how uptake rates may change over time, and to determine whether the temporal patterns in uptake were driven by kinetic equilibration along a “master” kinetic curve for the site (Fig. 1a) or whether the capacity of the stream to take up DOC and NO_3^- was variable over time (Fig. 1b).

If we found the uptake capacity of the stream to be variable over time, we were interested in identifying the environmental variables that drove the seasonal changes in DOC and NO_3^- uptake (Q1b). We hypothesized that temporal changes in nutrient uptake may be influenced by two important variables—

Fig. 1 Conceptual figure describing hypothesized seasonality in DOC and NO_3^- uptake rates, and potential drivers of these seasonal trends. Hypothesis 1 predicts that the system will have static uptake kinetics and move along a single “master” kinetic curve over the course of the season, whereas hypothesis 2 predicts that uptake kinetics will be variable over time in response to changing environmental drivers. Hypothesis H2a and H2b described the predicted relationships between 2 potential drivers of DOC and NO_3^- uptake kinetics: residence time (H1) and ambient substrate availability (H2)



substrate (DOC and NO_3^-) availability and residence time. Many oligotrophic, snowmelt-dominated catchments provide valuable laboratories to address Q1b. In much of the Rocky Mountain West, stream hydrographs are dominated by snowmelt and discharge decreases consistently throughout the summer, often with few additional precipitation inputs. This seasonal decrease in streamflow volume is frequently accompanied by coincident increases in stream network residence time (Mallard et al. 2014; Bergstrom et al. 2016). The majority of stream solutes in these highly oligotrophic headwater systems are terrestrially derived and delivered via subsurface runoff (Jencso et al. 2010; Pacific et al. 2010), so seasonally declining GW levels lead to reductions in solutes associated with shallow soils such as DOC and NO_3^- . While streamflow and solute concentrations are characterized by strong seasonality, other critical environmental variables display less variability over the summer period (light availability, water temperature). This provides an ideal experimental, hypothesis-testing framework, given that one hypothesized hydrologic driver of uptake (residence time) increases throughout the summer growing season, while another hypothesized biogeochemical driver (DOC and NO_3^- concentrations, which will henceforth referred to as substrate availability) decreases over the same time period. We sought to take advantage of these divergent seasonal trends to assess whether either variable was a clear predictor of DOC and/or NO_3^- uptake. We hypothesized that if residence time was the dominant influence on uptake rates, then uptake rates would increase over the study period (H2a, Fig. 1c). In contrast, if substrate availability and ambient concentrations were more crucial determinants of uptake rates, then we hypothesized that uptake rates would decrease over the summer growing season (H2b, Fig. 1d).

In this study, we take advantage of the natural seasonal variation in two hypothesized drivers of dissolved organic carbon and nitrate uptake to develop new understanding about the relative influence of residence time and substrate availability on uptake kinetics. We then place in-stream nutrient retention estimates in the context of measured catchment scale nutrient fluxes to assess its potential to mediate catchment scale solute fluxes.

Methods

Study site and data collection

The Tenderfoot Creek Experimental Forest (TCEF) is located in central Montana (Fig. 2a, latitude $46^\circ 55' \text{N}$, longitude $110^\circ 55' \text{W}$). This study focuses on Stringer Creek, a 555 ha first-order sub-catchment located within TCEF (Fig. 2b). Stringer Creek is a meandering, open-canopy stream located in a wide riparian corridor dominated by sedges (*Carex* spp.) and rushes (*Juncaceae* spp.) (Mincemoyer and Birdsall 2006). The benthic substrate is predominantly coarse gravel (median diameter of 5.4 cm) interspersed with larger cobbles with minimal standing stocks of organic matter in the stream channel. Ambient nutrient concentrations are low, with nitrate concentrations ranging from 10–30 $\mu\text{g N L}^{-1}$ and total phosphorus concentrations ranging from 20 to 50 $\mu\text{g N L}^{-1}$.

Stream discharge in Stringer Creek is strongly dominated by snowmelt, which occurs between late May and early June, and then decreases throughout the summer growing season with few additional precipitation inputs (Fig. 3). Previous work at the site has demonstrated that the organic rich soils in the near-stream riparian area are important sources of DOC and NO_3^- to the stream (Pacific et al. 2010). The hydrologic connection between the riparian area and stream channel diminishes as discharge decreases over the summer growing season, resulting in seasonal reductions in solute loading (Jencso et al. 2010, Pacific et al. 2010).

Day length during the study period from mid-June to mid-August remained relatively constant, only changing by approximately 1 h (15–16 h daylight) over the study period. The relatively constant day length and minimal riparian shading suggest that light availability was not likely to influence the seasonality in uptake rates. Maximum daily water temperature increased from 5 to 15.5 °C while night-time minimum water temperature remained constant at approximately 4–5 °C. If water temperature were a key driver of nutrient uptake rates, we would expect the higher temperatures in the late season to increase growth rates and enzyme activity, thus leading to elevated late season nutrient uptake rates.

We conducted a 4 month field campaign in the summer of 2013 during which we monitored carbon and nitrogen cycling in Stringer Creek. We conducted

Fig. 2 Map of Stringer Creek (a), a subwatershed in the Tenderfoot Creek watershed (c) located in south central Montana (b). In-stream monitoring sites were located in the headwaters (green circles) and the watershed outlet (purple square)

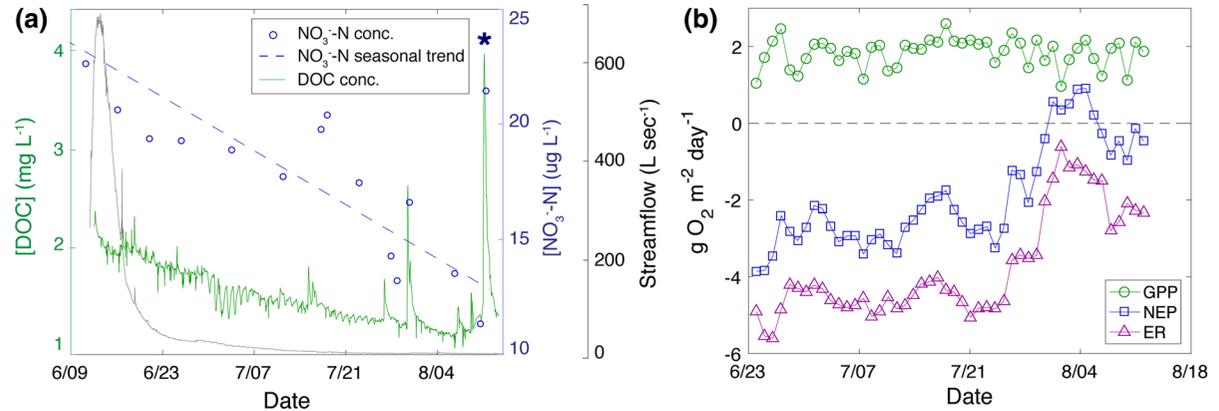
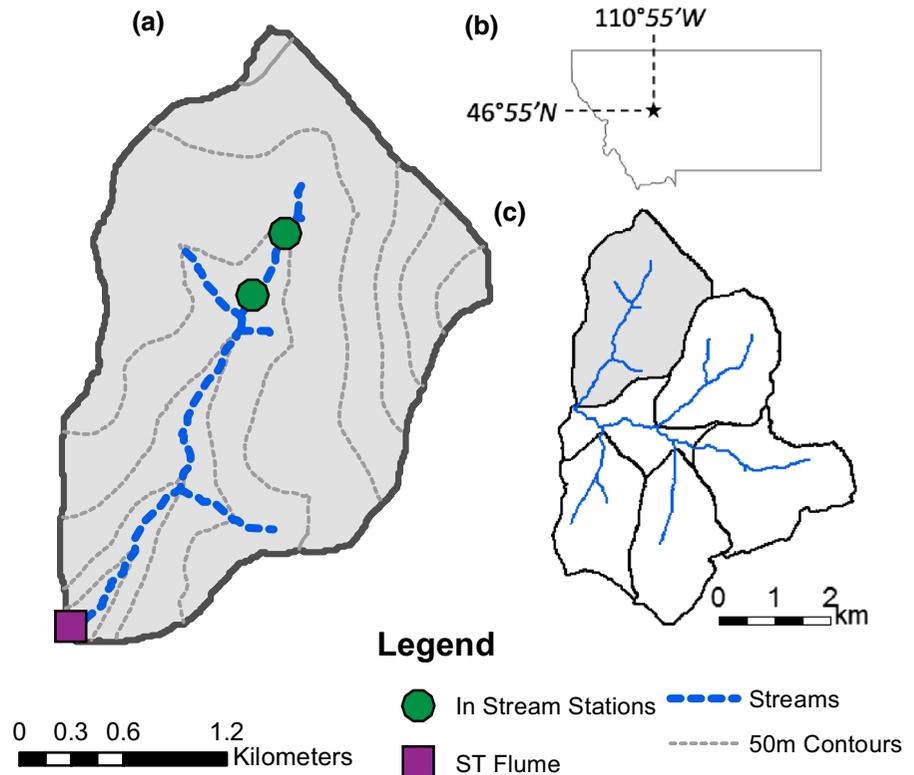


Fig. 3 **a** Time series of continuous DOC concentrations (green), $\text{NO}_3\text{-N}$ grab sample concentrations and fitted seasonal trend (blue), and continuous discharge (grey). **b** Seasonal trends in gross primary production (GPP, green), ecosystem respiration

(ER, purple), and net ecosystem productivity (NEP, blue). Negative rates of NEP indicate heterotrophy, and positive rates indicate autotrophy. The asterisk over 8/13–8/14 indicates the date of a late season hailstorm

in situ monitoring and experimental nutrient additions following the start of the snowmelt period in early May through the end of the baseflow period in late August. For the purposes of this study, we focus on a 2 month period following snowmelt starting in mid-June through August (6/20–8/20), which represents

the primary growing season (approximately 45–75 days) in both the terrestrial and aquatic ecosystems at TCEF (Mincemoyer and Birdsall 2006).

We installed two in-stream monitoring stations in the headwater reaches of Stringer Creek. The most upstream station was located immediately

downstream of the first point of perennial flow, and the second station was located approximately 500 m downstream of the first station (Fig. 2a). This focal reach bracketed a forested section of the stream that is characteristic of streams in the area, with a wide, grassy riparian zone and very little shading.

At each in-stream station, we installed in situ sensors to measure a range of water quality parameters, including dissolved oxygen (DO) (Campbell Scientific CS511-L) and electrical conductance (EC) and temperature (Campbell Scientific CS547A). We measured photosynthetically active radiation (PAR) above the water surface (Campbell Scientific LI190SB Quantum Sensor) to determine the photoperiod for each day. We used Turner Cyclops 7 sensors to measure fluorescent dissolved organic matter (fDOM), chlorophyll A, and turbidity. All sensors took measurements every 15 min for the duration of the study period. Sensors were maintained and checked for fouling every 2–3 days, and visible fouling was never observed on the sensors. This was confirmed by no significant change in sensor signals after cleaning. Sensor calibration was checked and recalibrated if necessary every 1–2 weeks.

Each station was co-located with a capacitance rod that recorded streamwater stage (TruTrac WT-VO) with ± 1 mm resolution. We conducted dilution gauging (Kilpatrick and Cobb 1985; Covino et al. 2011) on a weekly basis and used the observed stage: discharge relationship to estimate continuous stream discharge. Dilution gauging experiments were conducted over short < 35 m reaches to minimize groundwater exchange. We also simultaneously conducted mass recovery experiments over the full 500 m reach (Covino and McGlynn 2007; Payn et al. 2009; Covino et al. 2011), which were used to determine the gross gains and losses of groundwater occurring over the stream reach in addition to the net change in discharge. The breakthrough curves from these conservative tracer mass recovery experiments were also used to calculate residence time metrics, described in more detail below.

Continuous in situ stream solute monitoring was supplemented and corroborated with streamwater grab samples collected on a weekly to sub-weekly basis to create a time series of NO_3^- concentrations in the headwater reach, and to generate a relationship between fDOM and DOC concentrations that could be used to convert continuous measures of fDOM into

DOC concentrations. All samples were collected in HDPE bottles and filtered using $0.45 \mu\text{m}$ polyetherene sulfonate (PES) filters (Whatman Puradisc 25 mm syringe filters). DOC samples were filtered into opaque brown bottles, acidified to a pH of 1–2 using 6 N HCl acid, and kept refrigerated during transport until analysis at Duke University using a Shimadzu total organic carbon (TOC-V) analyzer (Shimadzu TOC-V CPH Total Organic Carbon Analyzer with ASI-V autosampler, Kyoto, Japan). Major cations and anions (NO_3^- , Cl^- , etc.) were filtered, refrigerated during transport, and frozen until analysis at Duke University using ion chromatography (Dionex ICS 2000 with eluent generator and AS40 autosampler, anion column AS18, Sunnyvale, CA). For the remainder of this study, when we present DOC and NO_3^- concentrations, they are referring to concentrations of DOC-C (mg C L^{-1}) and NO_3^- -N ($\mu\text{g N L}^{-1}$).

Ecosystem metabolism calculations

Ecosystem metabolism estimates were calculated using the 1-station approach (Demars et al. 2015). We calculated net ecosystem productivity (NEP) based on temporal changes in DO, and corrected this change in DO (ΔDO) for evasion occurring over the 500 m stream reach (Eq. 1, Marzolf et al. 1994). We estimated the reaeration coefficient (k) using experimental propane injections (Genereux and Hemond 1992; Marzolf et al. 1994) at three time points that represented a range of streamflow conditions over the study period. We then used the discharge data and experimentally derived gas exchange results to fit the parameters on a number of hydraulically derived equations that estimate k based on discharge and channel geometry or slope (Genereux and Hemond 1992; Melching and Flores 1999). We then used these empirically fitted hydraulic equations to model k , and selected the equation that best fit the experimentally derived k at our 3 experimental time points (Bennett and Rathburn 1972). This allowed us to use our empirically-derived reaeration values to inform our continuous estimation of k over the entire study period.

$$\text{NEP} = \Delta\text{DO} - k \quad (1)$$

We utilized the day-night separation method (Marzolf et al. 1994) to calculate NEP, which assumes that all NEP at night was due to ecosystem respiration

(ER). We differentiated between day and night using a PAR threshold of $400 \mu\text{mol photon s}^{-1} \text{m}^{-2}$; this threshold recreated day lengths that matched manual measurements made in the field of the photoperiod for the streambed, which was determined by when direct sunlight was over the ridge line and at an angle that vegetation shading was minimized. Nighttime NEP was attributed entirely to ecosystem respiration (ER), and GPP was assumed to be zero during that time. We then applied an average nighttime ER rate ($ER_{\text{night-avg}}$) to sunlight periods to calculate gross primary production (GPP, Eq. 2; Marzolf et al. 1994).

$$GPP = NEP + ER_{\text{night-avg}} \quad (2)$$

TASCC additions

The TASCC method (Tracer Additions for Spiraling Curve Characterization) is a recent evolution of traditional nutrient spiraling methods, which typically utilize a steady-state plateau injection at a single enrichment level to calculate a single set of uptake rates corresponding to that enrichment concentration (Covino et al. 2010b). Instead of generating a single measure of nutrient uptake at a given tracer plateau concentration, TASCC utilizes the full range of concentrations experienced by the stream across a tracer breakthrough curve to parameterize a Michaelis–Menten (most common) or efficiency loss uptake kinetics model that provide a suite of uptake metrics from ambient to saturating streamwater concentrations (Piper et al. 2017; Covino et al. 2010a, b, 2012)

We conducted nitrate TASCC additions on a ~ weekly basis (9 total) using KNO_3 as a reactive tracer and NaCl as a conservative tracer. We conducted labile dissolved organic carbon TASCC additions using potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$) as the reactive tracer at three time points, in sequence with nitrate enrichment additions. Enrichments increased background nutrient concentrations 1–2 orders of magnitude above background to ensure that kinetic saturation was reached. Because we were utilizing a labile carbon tracer, we will henceforth refer to this carbon uptake rate as a “labile carbon uptake rate.” The labile carbon uptake rates measured should be interpreted carefully when being used to make inferences about uptake rates of the native carbon pool.

These enrichment additions were conducted over the focal stream reach described previously. Solutes were injected above a small riffle to facilitate complete mixing and assessed visually using Rhodamine RWT. Prior to the reactive solute TASCC injection, discharge was measured using dilution gauging at the top and the bottom of the reach and background nutrient samples were collected. Data loggers measuring streamwater specific conductance at the top and bottom of the reach were used to monitor the solute breakthrough curve and facilitate timing of grab samples along the entirety of the added conservative and nutrient tracer breakthrough curves.

Nutrient spiraling additions measure the uptake rate of added tracer (i.e. U_{add}), which can be summed with the ambient uptake rate (i.e. U_{amb}) to estimate the total uptake rate (i.e. U_{tot}). Added and ambient nutrient uptake rates were calculated following the methods developed by Covino et al. (2010b). For each sample taken along the solute breakthrough curve, we first calculated the added nutrient uptake metrics (added spiraling length (S_w), uptake rate (U), and uptake velocity (V_f)). The added longitudinal uptake rate ($k_{w\text{-add-dyn}}$) was calculated by plotting the logged N:Cl or C:Cl ratio of the injectate and each grab sample against stream distance, and then calculating the slope between each pair of points (injectate sample and each grab sample). The added nutrient spiraling length ($S_{w\text{-add-dyn}}$, L) was calculated as the negative inverse of $k_{w\text{-add-dyn}}$.

The added areal uptake rate ($U_{\text{add-dyn}}$, $\text{M L}^{-2} \text{T}^{-1}$) for each grab sample was calculated using Eq. 3 (below). We utilized the same equations for the tracer tests using acetate and nitrate, and for clarity in the equations below C is used to represent DOC or NO_3^- -N concentration.

$$U_{\text{add-dyn}} = Q \times [C_{\text{add-dyn}}] / S_{w\text{-add-dyn}} \times w \quad (3)$$

Where Q is discharge ($\text{L}^3 \text{T}^{-1}$) and $[C_{\text{add-dyn}}]$ (Eq. 4, below) is the geometric mean of the background-corrected observed concentration ($C_{\text{add-obs}}$) and the background-corrected tracer concentration expected if the tracer traveled conservatively [C_{cons} , see Covino et al. (2010b) for more details], and w is stream width (L).

$$[C_{\text{add-dyn}}] = \sqrt{[C_{\text{add-obs}}] \times [C_{\text{cons}}]} \quad (4)$$

Another final way of representing the uptake rate in the stream is to normalize $U_{add-dyn}$ by its associated concentration, which then represents the uptake velocity ($L T^{-1}$) or uptake efficiency. Because the uptake velocity metric (V_f) represents a concentration-normalized uptake rate, it is inter-related with the areal uptake rate via concentration. We calculated the added uptake velocity ($V_{f-add-dyn}$, $L T^{-1}$) using Eq. (5):

$$V_{f-add-dyn} = U_{add-dyn} / C_{add-dyn} \quad (5)$$

The added spiraling parameters were then used to estimate ambient spiraling metrics. We plotted the $S_{w-add-dyn}$ values against the total dynamic concentration of each sample ($[C_{tot-dyn}]$, Eq. 6), and used linear regression to back-extrapolate to the ambient concentrations and derive the corresponding ambient uptake length (S_{w-amb}).

$$[C_{tot-dyn}] = \sqrt{[C_{tot-obs}] * ([C_{cons}] + [C_{amb}])} \quad (6)$$

Using this S_{w-amb} estimate, we calculated U_{amb} and V_{f-amb} using the following equations (Eqs. 7, 8):

$$U_{amb} = Q \times [C_{amb}] / S_{w-amb} \times w \quad (7)$$

$$V_{f-amb} = U_{amb} / C_{amb} \quad (8)$$

Finally, we estimated a total areal uptake rate (U_{tot}) or total uptake velocity (V_{f-tot}) by combining the added nutrient uptake from the enrichment addition and the ambient uptake into a single metric by summing the added uptake rate/velocity ($U_{add-dyn}$ or $V_{f-add-dyn}$) with the ambient uptake rate/velocity (U_{amb} or V_{f-amb}).

Fitting Michaelis–Menten kinetics and estimating benchmark uptake rates

The areal uptake rate data (U_{tot}) was used to parameterize linear, efficiency loss, and Michaelis–Menten (M–M) models. In all cases, the M–M model performed the best (based on R^2) and was therefore selected to estimate the maximum areal uptake rate parameter (U_{max}) and half saturation concentration parameter (K_m) for each tracer test (Eq. 9), where $[C]$ represents the solute concentration of each sample or time point ($mg L^{-1}$).

$$U = \frac{U_{max} \times [C]}{K_m + [C]} \quad (9)$$

The fitted Michaelis–Menten parameters for each tracer test were then used to calculate total areal uptake (U_{tot}) at a set of benchmark concentrations. For each tracer test (each with a unique set of Michaelis–Menten parameters), we calculated areal uptake rate at 3 standard concentration values that represent the average range of observed ambient concentrations at our study site (henceforth called benchmark concentrations, Covino et al. 2012). For NO_3^- , these concentrations were 10, 25, and $50 \mu g N L^{-1}$. For DOC the benchmark concentrations were 1.5, 3, and $5 mg C L^{-1}$. We used these benchmark uptake rates to compare ambient-level uptake in our stream across the growing season.

Ambient areal uptake is a useful metric to compare over time and between sites, because it can be scaled up to calculate reach scale or network scale fluxes. We used the benchmark uptake rate approach to standardize this analysis and avoid potential biases incorporated in the traditional ambient areal uptake rate (Eq. 7). The ambient areal uptake rate calculation (Eq. 7) is sensitive to the ambient concentration associated with that sample, thus ambient uptake comparisons between high and low concentration sites or time points can be challenging to interpret. For example at a high N site, is greater ambient uptake because of greater N availability or greater demand at the site? The benchmark uptake approach allows us to standardize concentrations and compare areal uptake rates from different time points or sites at the same ambient concentration, thus more equitably and robustly comparing the magnitude of ambient areal uptake occurring across space and time. We utilize this benchmark uptake metric in combination with V_f , the traditional spiraling metric that describes uptake efficiency (concentration-normalized) for comparison across time points or sites (Eqs. 5, 8).

The initial estimates of the benchmark areal uptake rates and ambient spiraling metrics (U_{amb} , V_{f-amb}) for our dissolved organic carbon tracer tests assume that the entire carbon pool is labile carbon (100% labile fraction). To address potential bias introduced by tracer lability, we downscaled the benchmark areal uptake rates, ambient uptake rate (U_{amb}), and ambient uptake velocity (V_{f-amb}) by 80–90% to assess what the benchmark areal uptake rates would be if the total carbon pool only contained 10–20% labile carbon substrates (Cory and Kaplan 2012). A similar

approach was suggested in Mineau et al. (2016), where a correction factor was applied to V_f efficiencies that were measured using labile carbon substrates to adjust for the effect of tracer lability (Mineau et al. 2016). This approach allows us to adjust for the effects of tracer lability and compare more conservative benchmark and ambient carbon uptake metrics.

Residence time metrics

We calculated stream reach residence time metrics of recovered conservative solutes from mass recovery additions conducted over the full reach length. The breakthrough curves from these instantaneous conservative tracer additions represent the residence time distributions of recovered tracer in the stream reach (Harvey et al. 1996; Mason et al. 2012) and thus the potential contact time for nutrient uptake. Studies have shown that the peak of the breakthrough curve (or modal travel time) can be used to describe the advective portion of flow in the channel, whereas the tailing behavior can be related to transient storage within the channel (Harvey et al. 1996; Mason et al. 2012; Patil et al. 2013). We calculated the modal travel time (modal TT) and the travel time at which 99% of the solute mass had been recovered (99% MRTT) as metrics describing transient storage for each set of mass recovery additions. These metrics describe the travel time of the recovered solutes that we injected into the stream. Thus we use these metrics (hereafter collectively referred to as metrics of residence time) to refer to the amount of contact time between solutes and zones where they may be taken up by the biotic community.

Retention/export ratios

We sought to compare the magnitude of in-stream retention across the 0.5 km headwater stream reach to the total amount of dissolved organic carbon and nitrate being exported from the forested 137 ha sub-watershed draining to that point. To estimate total in-stream nutrient retention on a daily basis, we linearly interpolated the Michaelis–Menten (MM) parameters (U_{max} and K_m) estimated from each tracer test to create a daily time series of U_{max} and K_m . We then estimated the areal uptake rate ($\text{mg C or NO}_3\text{-N m}^{-2} \text{ day}^{-1}$) for each day by inserting the interpolated daily MM parameter values and the mean daily DOC or NO_3^-

concentration into the Michaelis–Menten model equation (Eq. 9). We scaled the daily areal labile DOC or nitrate uptake rate ($\text{mg DOC or NO}_3\text{-N m}^{-2} \text{ day}^{-1}$) over the 500 m headwater study reach area (m^2) to estimate the total mass of solute (kg) retained by biological uptake processes. To adjust the DOC retention estimates for the potential effects of tracer lability, we downscaled the retention rates by 80–90% of their original values to better address the potential bias introduced by our experimental methods. We show the carbon retention rates as a shaded region with the upper boundaries representing the magnitude of retention with a 100% labile carbon fraction, and the lower boundaries representing the magnitude of DOC retention at 10% labile carbon fraction. This approach represents an approximation of short-term solute retention that assumes the uptake rate remains constant over a day, and does not account for uptake that is later translated into export through biogeochemical cycling (e.g. NO_3^- that is taken up, converted into organic matter and later re-mineralized or exported as dissolved organic N or particulate organic matter, etc.).

We compared this estimate of solute retention with the total amount of DOC and NO_3^- exported from the reach downstream. We calculated export fluxes by multiplying the average daily DOC and NO_3^- (mg L^{-1}) concentration by the instantaneous discharge (L s^{-1}) and integrating to a daily time step to obtain a daily solute mass flux. We then calculated the ratio of the mass of NO_3^- and labile DOC retained by in-stream processes to the mass of NO_3^- and DOC exported downstream, hereafter referred to as the retention/export ratio. In the case of DOC retention versus export, we estimated this ratio using the retention estimates for both a 10% labile carbon fraction and a 100% labile carbon fraction. This ratio is conceptually related to the Damköhler number, a dimensionless number that relates the time scale of chemical reactions to the transport time scale. We used this retention/export ratio to assess the relative magnitude of biological and physical processes, how this balance changed seasonally, and how this ratio compared for dissolved organic carbon relative to nitrate.

Results

Seasonal trends in biogeochemical and hydrologic variables: ambient concentrations, metabolism, and residence time

Ambient DOC and NO₃⁻ concentrations

DOC concentrations decreased by approximately 1 mg C L⁻¹ over the growing season (– 56%), and exhibited variation on diel and seasonal time scales (Fig. 3). The highest DOC concentrations observed during the study period of mid June to mid-August (6/20–8/20) occurred on 8/14, when an intense summer hailstorm (up to 4 cm diameter hailstones) occurred early on the evening of 8/13. The impact of the large hailstones damaged vegetation, perturbed the streambed and near stream soil, deposited riparian vegetation in the stream, and hydrologically activated DOC-rich near stream areas. Due to the very dry antecedent conditions in the watershed, this event only increased stream discharge minimally. While this event elevated DOC and NO₃⁻ concentrations for approximately 36 h, it had very little influence on total growing season DOC and NO₃⁻ fluxes from the system.

Nitrate concentrations also decreased over the summer growing period. The absolute magnitude of the decrease in NO₃⁻ concentrations was much smaller than the seasonality observed in DOC concentrations, but the relative percent decrease was similar (– 41%). Nitrate concentrations remained between 12 and 25 µg N L⁻¹ for the entire study period, declining 10–12 µg N L⁻¹ over the summer growing season (Fig. 3). NO₃⁻ concentrations also showed a moderate amount of variability on a day-to-day basis, demonstrated by the residuals of measured grab samples in relation to the seasonal trend line (Fig. 3a). The mid-August hailstorm also impacted NO₃⁻ concentrations, resulting in an 85% increase in NO₃⁻ concentrations from 13 to 24 µg N L⁻¹ on 8/14, the day after the storm.

Residence time metrics

Both metrics of residence time (modal TT and 99% MRTT) were negatively correlated with discharge (Fig. 4a). As discharge decreased over the study period, the modal travel time over the study reach

increased 6 times (from 32 to 194 min) and the travel time associated with 99% mass recovery increased 5.7 times (from 51 to 294 min, Fig. 4b, Table 1). The increase in residence time suggests that the amount of contact time between solutes and benthic communities was greatly enhanced over the study period. As we hypothesized, our two potential drivers (residence time and solute availability) exhibited opposing seasonal trends (Figs. 1, 4b). While residence times increased, both DOC and NO₃⁻ concentrations declined by ~ 40–55% (Table 1, Fig. 4b). This allowed us to compare the temporal trends in labile DOC and NO₃⁻ uptake with our hypothesized drivers to determine whether uptake was more strongly correlated with hydrologic (residence time) or biogeochemical (solute availability) variables.

Ecosystem metabolism dynamics

Net ecosystem productivity (NEP) rates increased (e.g. became less negative) and became less heterotrophic over the growing season. NEP was strongly heterotrophic at the beginning of the study period (– 4 g O₂ m⁻² day⁻¹), but declined until reaching nearly balanced amounts of heterotrophy and autotrophy in early August (NEP ≈ 0; Fig. 3b). Seasonality in NEP was predominantly driven by variation in ER, which decreased while GPP remained quite stable and low (on average 1.5 g m⁻² h⁻¹) throughout the entire study period (Fig. 3b).

Labile DOC and NO₃⁻ uptake kinetics

Nitrate areal uptake rates decreased strongly over the study period (Fig. 5a). At each successive experimental time point (n = 7), nitrate areal uptake was significantly lower than the previous time point, as is evidenced by the lack of overlap between the 95% confidence intervals on the Michaelis–Menten model uptake kinetic curves. This seasonal trend was reflected in the maximum areal uptake rates (U_{max}), which declined consistently over the summer growing season (Fig. 5a), and the ambient uptake rates (U_{amb} , Fig. 7a). This suggests a decrease in uptake capacity across a range of concentrations from ambient to saturation as the growing season progressed.

To more consistently assess how ambient range nutrient uptake was changing seasonally, we also compared areal nitrate uptake rates for the set of

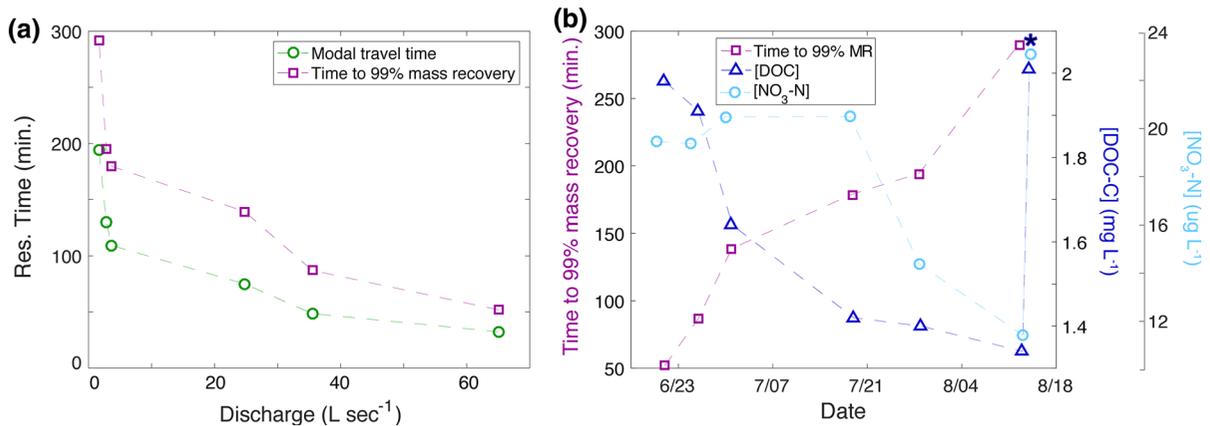


Fig. 4 Seasonal trends in modal travel time (indicative of advective flow travel time) and time to 99% mass recovery (indicative of longer travel times and transient storage). **a** Both residence time metrics are inversely correlated with discharge, with shorter residence times in the early growing season. **b** The opposing seasonal trends in residence time (purple) and ambient

[DOC] (dark blue) and [NO₃-N] (light blue). Concentration data from 8/14 (noted with the asterisk) are elevated due to intense summer storm that caused flushing of riparian nutrients, but did not significantly increase discharge. Residence time data for 8/14 is not available

Table 1 Discharge (Q), ambient DOC and NO₃-N concentrations, modal travel times (TT), and time to 99% mass recovery (MR) in Stringer Creek during each tracer experiment

Date	Q (L s ⁻¹)	[NO ₃ -N] _{amb} (µg L ⁻¹)	[DOC-C] _{amb} (mg L ⁻¹)	Modal T.T. (h:min:s)	99% MR T.T. (h:min:s)
6/21/13*	65.05	19.30	1.98	00:32:19	00:51:52
6/26/13*	35.53	19.20	1.91	00:48:28	01:26:58
7/1/13*	24.74	20.31	1.64	01:15:02	02:19:18
7/19/13*	3.67	20.33	1.42	01:49:16	02:59:36
7/29/13*	2.83	14.21	1.40	02:09:57	03:15:06
8/13/13	1.66	11.25	1.34	03:14:15	04:52:00
8/14/13	6.92	22.92	2.01	n/a	n/a

Nitrate additions were performed on all dates listed, and carbon additions were conducted on dates noted with an asterisk

benchmark concentrations (U_{tot} , Fig. 8) and ambient nitrate uptake efficiencies (V_{f-amb} , Fig. 7a). This allowed us to control for the effects of changing ambient stream concentrations over the growing season. Even when controlling for seasonal concentration dynamics, ambient-level nitrate uptake rates were highest in the early season and decreased until mid-August (Fig. 8). The same trend was observed in the ambient nitrate uptake efficiency (V_{f-amb}) data, which also decreased by $\sim 80\%$ over the summer growing season (Fig. 7a). This suggests that the temporal patterns in nutrient uptake we observed were not explained by a simple kinetic response (H1, Fig. 1a), but rather that the capacity of the system to

take up nutrients at or near ambient concentrations changed over the growing season (H2, Fig. 1b).

Nitrate uptake responded strongly to increased NO₃⁻ and DOC concentrations following the hail-storm on 8/13. In response to an approximate doubling of background nitrate concentrations (85% increase), the nitrate areal uptake rates measured on 8/14 were up to 4 times greater than the previous day (Fig. 6). The $V_{f-amb-N}$ data confirm this trend and show an increase in the nitrate uptake velocity from 1 to 2.5 mm min⁻¹ on 8/14 in response to the hail storm and subsequent solute loading (Fig. 7a). This uptake response occurred despite no significant change in discharge or residence time, and occurred extremely rapidly. We were not able to conduct follow up experiments aside

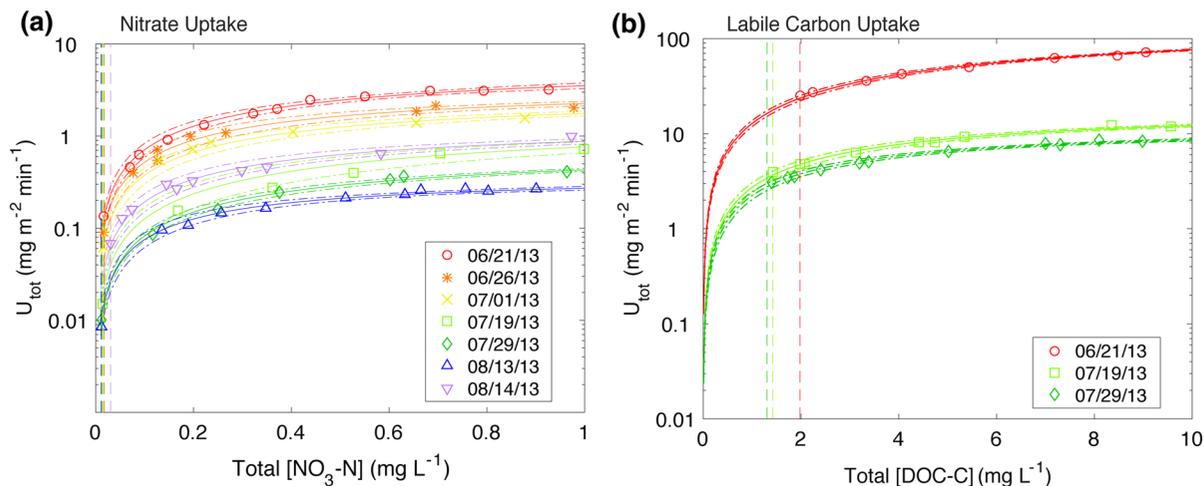


Fig. 5 Seasonal variation in total areal uptake rates (U_{tot}) for **a** nitrate and **b** labile DOC in Stringer Creek versus the geometric mean of total nitrate concentration and total DOC concentration, respectively. The Michaelis–Menten model fits

and 95% confidence intervals are indicated with solid and dashed lines. The number of grab samples for all tracer tests was between 14 and 20 individual samples (not all shown within the \times limits of these figures)

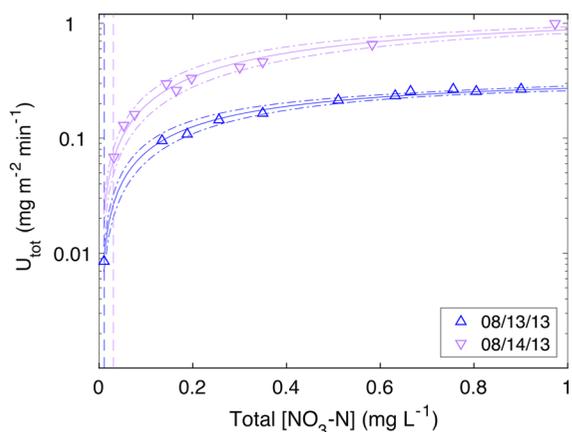


Fig. 6 Total nitrate uptake rate (U_{tot}) for the day immediately preceding the summer hailstorm (8/13/13, shown in blue triangles) and following the disturbance (8/14/13, shown in purple triangles). Ambient nitrate concentrations (denoted by the vertical dotted lines) increased 85% (from 13 to 24 $\mu\text{g N L}^{-1}$) in response to the storm, and nitrate uptake increased by nearly a factor of 4 over the 12 h following the disturbance

from the day after the disturbance to determine how transient or long-lived this response was, but given the rapid up-regulation of uptake rates it seems possible that the re-equilibration to a state of lower uptake may have been equally rapid.

Labile DOC uptake displayed a similar seasonal trend to nitrate uptake, with strong decreases over the study period (Fig. 5b). Maximum areal uptake rates (Fig. 5b) and ambient uptake metrics (S_{w-amb} , U_{amb} ,

and V_{f-amb} , Fig. 7b) for labile DOC declined over the study period. As with nitrate uptake, the declines in ambient labile DOC uptake were not solely a kinetic response driven by equilibration to ambient DOC concentrations over the growing season. As Figs. 7b and 9 demonstrate, benchmark labile DOC uptake rates and labile DOC uptake efficiencies (V_{f-C}) decreased substantially from mid June to late July, suggesting that the capacity of the community to take up labile DOC diminished over the study period and that this response was not merely a kinetic response to changes in ambient concentration.

Labile DOC areal uptake rates were approximately two orders of magnitude greater (on a mass basis) than areal nitrate uptake rates across all three experimental time points where both nitrate and labile dissolved organic carbon uptake were measured (Figs. 5, 8, 9). Even the more conservative ($\sim 20\%$ labile dissolved organic carbon fraction) benchmark areal uptake rates for carbon were 12–34 times higher than the benchmark nitrate uptake rates at each time point (Figs. 8, 9). V_{f-amb} for labile DOC (assuming a 20% labile DOC fraction) was higher than nitrate V_{f-amb} in mid-June (Fig. 7), but by mid and late July the ambient uptake efficiencies for nitrate ($V_{f-amb-N}$) were slightly higher than for labile DOC (1.02 and 0.79 mm min^{-1} vs. 0.55 and 0.43 mm min^{-1}). The labile DOC: NO_3^- ratios of ambient areal uptake (assuming a 20% labile DOC fraction) were 43.6, 61.7, and 70.7, which was

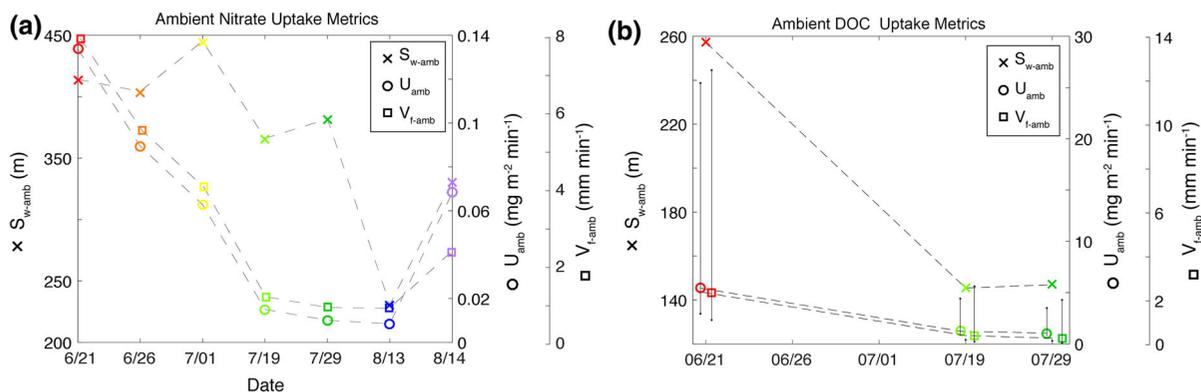
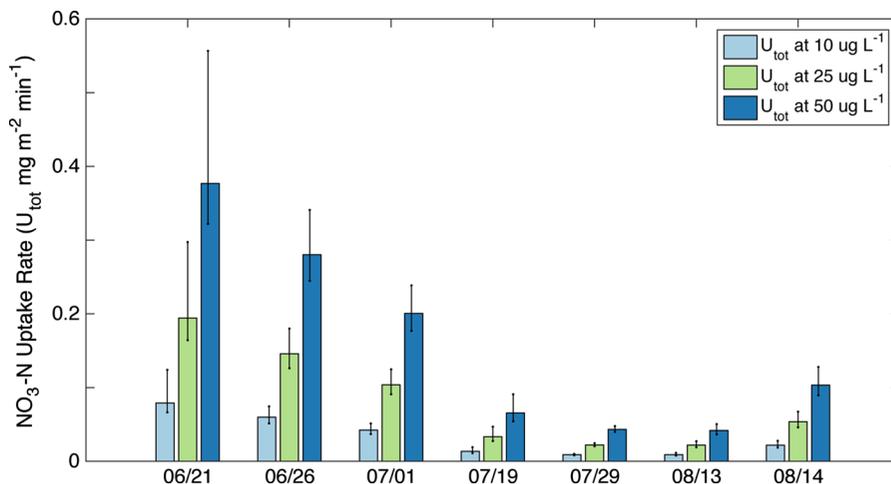


Fig. 7 Ambient spiraling length (S_{w-amb}), ambient areal uptake rate (U_{amb}), and ambient uptake velocity (V_{f-amb}) for **a** nitrate and **b** dissolved organic carbon. For U_{amb-C} and $V_{f-amb-C}$ (panel **b**), colored symbols are placed at the downscaled uptake rate corresponding with a 20% labile C fraction, with error bars

noting the range between areal uptake metrics for 10% (lower bar) and 100% (upper bar) labile DOC fraction. The DOC:NO₃⁻ ratios of areal ambient uptake rates assuming a 20% labile C fraction (in molar terms) on 06/21, 07/19, and 07/29 were 43.6, 61.7, and 70.7 respectively

Fig. 8 Total areal nitrate uptake rates at benchmark concentrations of 10, 25, and 50 $\mu\text{g N L}^{-1}$. Benchmark uptake rates and 95% confidence intervals were calculated using the fitted Michaelis–Menten parameters and 95% confidence intervals of those parameter estimates from tracer experiments on each date



below the labile DOC:NO₃⁻ ratio of ambient streamwater (~ 93).

Retention/export ratio

The DOC retention/export ratio displayed strong seasonality, as did the status of the stream reach as a net source or sink of DOC (Fig. 10). The retention/export ratio corresponding with a 10% labile C fraction (lower boundary of green shaded region, Fig. 10b) remained below 1 the entire growing season, suggesting greater export than in situ processing of carbon at lower lability fractions. In contrast, at the maximum lability fraction (100% labile C fraction, upper boundary), more DOC was retained than exported.

Across all lability fractions, the dissolved organic carbon retention/export ratio peaked in mid-July, highlighting that the greatest amount of retention (relative to export) occurred in mid summer as discharge decreased and concentrations remained elevated. In contrast to the strong seasonality observed in the dissolved organic carbon retention/export ratio, nitrate retention and export were balanced throughout the study period (Fig. 10d), resulting in a retention/export ratio that remained near 1 throughout the summer (Fig. 10e).

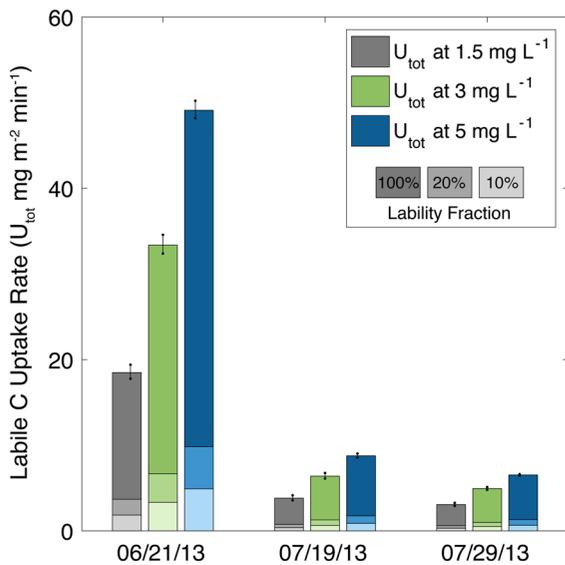


Fig. 9 Total areal dissolved organic carbon uptake at benchmark concentrations of 1.5, 3 and 5 mg C L⁻¹. Benchmark uptake rates and 95% confidence intervals were calculated using the fitted Michaelis–Menten parameters and 95% confidence intervals of those parameter values from tracer experiments on each date

Discussion

One of the most striking results from this study is the high degree of seasonality in labile DOC and NO₃⁻ uptake kinetics we observed within a single stream. Although prior studies have demonstrated seasonal variation in ambient uptake rates (Marti and Sabater 1996; Simon et al. 2005; Hoellein et al. 2007), they lacked kinetic and residence time information. Our study documented substantial changes in uptake across a range of concentrations from ambient to saturation and a range of instream residence times. As a result, these data can be also be used to explore potential mechanisms driving seasonality in nutrient uptake.

We hypothesized that uptake kinetics could change seasonally in two ways: the total capacity of the stream to remove DOC and NO₃⁻ could remain static and any seasonal changes in DOC or NO₃⁻ uptake would be a function of concentration and Michaelis–Menten kinetics (i.e. moving along a “master kinetic curve”, H1, Fig. 1a), or the capacity of the stream to take up nutrients could be dynamic in time, and thus the shape and magnitude of the kinetic curve would change over the summer growing season (H2, Fig. 1b). The results

from our study demonstrate that the capacity of the biological community in this oligotrophic headwater stream to take up DOC and NO₃⁻ changed dramatically over a 2 month period (Figs. 1, 5). Rather than traversing the same master kinetic curve over the season or year as ambient concentrations change (H1, Fig. 1a), we found that the functional capacity of the stream to take up labile DOC and NO₃⁻ changed over time. This was reflected in shifts in the shape and magnitude of the kinetic curve (H2, Figs. 1b, 5). Furthermore, we found that the most immediate driver of these changes in the uptake capacity of the stream were seasonal reductions in the availability of dissolved organic carbon (energy) and nitrate substrates. Our results suggest that in response to reductions in dissolved organic carbon and nitrate availability, the biological community began “shutting down” and down-regulating metabolic or growth processes. Thus the decreases in dissolved organic carbon and nitrate uptake were not a purely kinetic response, but rather were driven by changes in the capacity of the biological community to take up nutrients.

Our results reinforce the findings of previous studies that have highlighted the high degree of temporal variation in uptake rates (Marti and Sabater 1996; Simon et al. 2005; Hoellein et al. 2007), and demonstrate that we would have missed the dynamics of nutrient retention in this system if we had not captured its strong seasonality in uptake kinetics. Studies using a kinetic curve measured at a single time point or a curve generated from another site to extrapolate uptake over an entire growing season or even another point in time likely cannot accurately portray the capacity of the stream to take up nutrients. Here, had we used a single set of kinetic parameters to estimate seasonal nutrient uptake, we would have grossly under or over-estimated the total amount of labile dissolved organic carbon and nitrate taken up by the biotic community. This issue is highly pertinent for water quality modeling, since applying a single removal rate spatially (over a river network) and temporally over growing seasons are standard practices when modeling nutrient retention in stream networks. We suggest that a temporally explicit approach to measuring uptake and modeling retention must be taken, especially in highly dynamic systems.

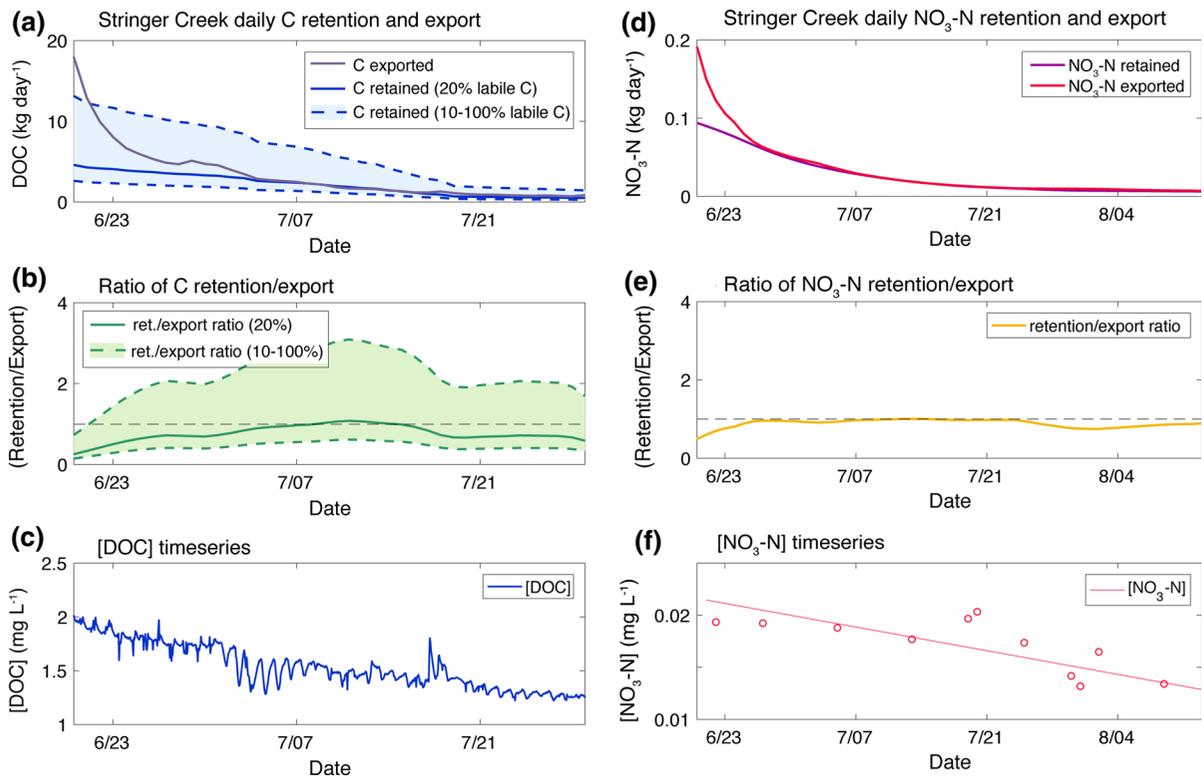


Fig. 10 **a** Dissolved organic carbon retention and export from Stringer Creek. Solid blue line indicates DOC retention assuming a 20% labile C fraction, while the lower boundary of the blue shaded region represents the amount of dissolved organic carbon retention with a 10% labile C fraction and the upper boundary represents the amount of dissolved organic carbon retention with a 100% labile C fraction. **b** The ratio of dissolved organic carbon retention to export. The solid green line indicates the retention/export associated with a 20% labile C fraction, and the lower and upper boundary of the shaded green region represent the ratio assuming 10 and 100% labile C

fraction, respectively. The ratio of dissolved organic carbon retention to export increased until early July, and then declined until the end of the study period. **c** The shifting balance of export and retention led to a [DOC] time series with strong seasonality. In contrast, **d** nitrate retention and export were closely balanced throughout the study period, **e** leading to a nitrate retention to export ratio that was approximately one for the entire summer growing season. **f** Because of the consistent balance between NO_3^- export and retention, [NO_3^- -N] exhibited low seasonality, only decreasing by 0.01 mg N L^{-1} (**f**)—an order of magnitude less variation than the seasonal trend in [DOC]

Drivers of DOC and NO_3^- uptake

One of the initial goals of this study was to determine how DOC and NO_3^- uptake responded to seasonal variation in two potential drivers—substrate availability (biogeochemical) and residence time (hydrologic). Our results confirm that, as hypothesized, these two basic drivers had divergent seasonal trends. Residence time increased over the growing season while ambient concentrations of both DOC and NO_3^- declined over the same interval (Fig. 4). We also found that all metrics describing DOC and NO_3^- uptake, from ambient to saturation, decreased substantially over the summer growing season. The opposing seasonal trends in uptake metrics and residence time do not

support Hypothesis 2a (Fig. 1c), and suggest that residence time was not a primary driver of labile DOC or NO_3^- uptake in our system. Instead, all labile DOC and NO_3^- uptake metrics (Figs. 5, 7, 8, 9) appear to follow the same seasonal trend as ambient concentrations (Figs. 3, 4), lending support to Hypothesis 2b and suggesting that substrate availability is a key control on the capacity of streams to take up and transform nutrients and energy (Fig. 1d).

Our results suggest that in Stringer Creek, the growth and respiration of the heterotrophic community closely followed trends in the availability of terrestrially derived organic matter and nitrate (Berggren et al. 2009, 2010; Lapierre et al. 2013). We found that net ecosystem productivity and

ecosystem respiration (the dominant metabolic process in our heterotrophic system) both decreased over the growing season, leading to the stream becoming less heterotrophic as the growing season progressed. This seasonal decrease was also mirrored in both DOC and NO_3^- concentrations and uptake rates. Interestingly, there was very little seasonality in GPP, which remained consistently low throughout the summer. The similarity between the seasonal respiration (ER) rates and uptake rates (and the lack of similarity between the primary production rates and uptake) suggests that dissolved organic carbon and nitrate uptake is more coupled to respiration of organic matter or heterotrophic biomass growth than autotrophic growth and primary production in this oligotrophic, heterotrophic system.

The results of this study suggests a tight coupling between nutrient uptake, respiration, and metabolism at the ecosystem level, and suggests that the magnitude of metabolism may be influenced by the availability of terrestrially-derived carbon and nitrogen. We posit that these changes in the community to take up DOC and NO_3^- represent a down-regulation or decline in the heterotrophic community size or function as the availability of terrestrially-derived energetic substrates and macronutrients became more limited. Despite more favorable environmental conditions at the end of the growing season (longer residence times and higher temperatures), the heterotrophic community was limited by the amount of labile DOC and NO_3^- available to fuel respiration and assimilatory processes.

The second line of evidence that supports the importance of substrate availability as a driver of nutrient uptake is the response of nutrient uptake rates to increased DOC and NO_3^- concentrations following the hailstorm event. The rapid, four-fold increase in NO_3^- uptake rates (Fig. 6) following the hailstorm on 8/13 in response to a doubling of nitrate concentrations (and the nearly tripling of DOC concentrations) provides strong support for the hypothesis that substrate availability is a key control on setting the magnitude of ambient and saturation level uptake rates. The disproportionate increase in uptake relative to concentration highlights that this was not only a kinetic response, but rather an increase in biological activity in response to the increased availability of fresh organic matter and nitrate flushed into the stream from riparian areas. This lends support to hypothesis

2b and strengthens the importance of substrate availability in determining the magnitude of nitrogen and carbon uptake at our site.

We do not suggest that residence time is an unimportant influence on nutrient transformation in streams. Numerous studies have shown that the amount of in-channel or subsurface contact time is a key control on the magnitude of nutrient uptake and retention (Battin et al. 2008; Hanley et al. 2013; Cory et al. 2014; Ågren et al. 2014; Cory et al. 2015; Kothawala et al. 2015; Wollheim et al. 2015; Lambert et al. 2016; Raymond et al. 2016). Despite its importance in many systems, our results demonstrate that in the oligotrophic headwaters of Stringer Creek, residence time was of secondary influence relative to substrate availability for determining the temporal trends in labile DOC and NO_3^- uptake. We suggest that it is important to consider the “regulatory hierarchy” (Sinsabaugh 1997) or multiple layers of influences that reduce the potential or maximum retention rates of in-stream communities, and to recognize that the primacy of these various drivers may shift between systems and in response to environmental change. In the oligotrophic headwater of Stringer Creek, the most proximate control on the seasonality of DOC and NO_3^- uptake was the availability of substrates for uptake and respiration. If seasonal trends in DOC and NO_3^- concentrations were to shift due to hypothetical land use change or disturbance at Tenderfoot Creek that alleviated substrate limitation, then it is likely that residence time would be a more influential control on stream nutrient retention at this site.

The idea that there are competing controls on nutrient uptake rates has further relevance for the water quality modeling studies, which typically scale uptake rates based upon temperature. We found that our highest uptake rates occurred early in the growing season when temperature was lowest, due to the elevated substrate availability at that time. This suggests that perhaps relying on temperature to model uptake rates seasonally may not account for other more proximal controls on uptake (e.g. substrate availability) that determine the seasonal trends in nutrient uptake. The results from our study demonstrate that although multiple processes have the potential to influence nutrient uptake, in strongly oligotrophic, heterotrophic systems the most immediate control on the magnitude of nutrient retention may

be the availability of substrates for uptake and respiration.

We acknowledge that the lability of our dissolved organic carbon tracer (acetate) influenced the magnitude of our observed labile carbon (acetate) uptake rates. The uptake rates that we measured using acetate as the carbon tracer represent a *labile* dissolved organic carbon uptake rate and not the uptake rate of the ambient or native DOC pool. A recent meta-analysis by Mineau et al. (2016) combined studies conducted at the reach and assay scale and found that DOC uptake velocities (V_f) measured using simple compound additions (e.g. acetate) could be as much as 3.7 times greater than the estimated ambient-level uptake velocities from more conservative leachate experiments. Therefore these rates represent the upper bounds of potential DOC uptake that would be observed at this site, and only allow us to confidently make inferences about labile DOC uptake and limitation. However, the issue of tracer lability should have little to no impact on the seasonality that we observed in uptake metrics because the same carbon tracer was used across all experiments. While we cannot use these data to directly assess the ambient uptake rates of the native DOC pool at our site, these results document the high demand for labile carbon substrates and highlight that our system can be limited by labile DOC despite having relatively high ambient DOC concentrations. This reinforces the idea that carbon lability is an important control on energetics in many heterotrophic systems (Berggren and Giorgio 2015).

C versus N limitation in oligotrophic systems

Most established uses of the nutrient spiraling theory have approached nutrient limitation from a singular perspective—not only have they typically focused on single element limitation (but see Bernhardt and Likens 2002; Hall and Tank 2003; Brookshire et al. 2005; Fellows et al. 2006; Schade et al. 2011, 2016), but they have also tended to use single metrics that reflect the *magnitude* of uptake to determine or define limitation at the ecosystem level. Traditionally, when comparing two macronutrients such as nitrogen or phosphorus, the element with the shortest spiraling length (S_{w-amb}), highest uptake rate (U_{amb}), and highest nutrient uptake efficiency (V_{f-amb}) is thought to be the limiting nutrient (Newbold et al. 1981; Stream Solute

Workshop 1990). The slope of the relationship between concentration and S_w has also been suggested to be an indicator of the strength of nutrient limitation, with lower slope coefficients (i.e. flatter slopes) indicating stronger nutrient limitation (Mulholland et al. 2002; Schade et al. 2011).

Based on this paradigm and given our results, one would conclude that this system, despite being characterized by low nitrate concentrations and (relatively) higher DOC concentrations, is limited by the amount of labile dissolved organic carbon. $S_{w-amb-C}$ and the slope of S_{w-C} are all consistently lower than the same metrics from the nitrate tracer experiments (Table 2). Even when accounting for potential bias due to tracer lability, U_{amb-C} and $V_{f-amb-C}$ are consistently higher than U_{amb-N} and $V_{f-amb-N}$ across sampling dates (Fig. 7) and the benchmark nitrate areal uptake rates (Fig. 8) are over an order of magnitude lower than the benchmark labile DOC uptake rates (Fig. 9). The spiraling length slope coefficients for labile DOC were 7–25 times smaller than the slope coefficients for the nitrate spiraling lengths, suggesting much more rapid turnover of labile DOC in this experimental stream reach (Table 2). All these metrics reflect a system in which labile DOC is in very high demand, is being cycled rapidly by the biotic community, and could be limiting growth.

In response to theoretical and empirical advances in recent years, numerous studies have begun to take a multi-element approach to understanding nutrient uptake and limitation. A number of studies have explored the stoichiometry of N and P uptake (Simon et al. 2005; Schade et al. 2011, 2016; Piper et al. 2017), while also linking N and P uptake to metabolic processes (Hall and Tank 2003; Heffernan and Cohen 2010; Cohen et al. 2013). We suggest that this move towards a stoichiometric framework for assessing limitation is a critical step towards a more complete, ecosystem-level understanding of limitation. Instead of relying solely on the *magnitude* of ambient uptake rates as an indicator of limitation, we suggest two additional approaches that can be used to provide a more nuanced assessment of nutrient demand and limitation—assessment of the *stoichiometry* of ambient uptake rates, and comparisons of the *responsiveness* of a system to added nutrient.

First, we suggest that comparison of the stoichiometry of ambient uptake and ambient C and N pools may be another useful indicator of limitation that describes

Table 2 Michaelis–Menten parameters from the three sampling dates with both carbon and nitrogen tracer additions

Date	K_{m-C} (mg L ⁻¹)	U_{max-C} (mg m ⁻² min ⁻¹)	K_{m-N} (mg L ⁻¹)	U_{max-N} (mg m ⁻² min ⁻¹)	S_{w-C} slope	S_{w-N} slope
6/21/13	12.2	169	0.78	6.29	25.3	616.9
7/19/13	6.3	20	1.27	1.73	27.8	216.9
7/29/13	4.6	13	0.88	0.81	35.6	374.2

the deviation of the system from expected stoichiometric ratios, and which may lead to different conclusions regarding limitation than traditional approaches. Threshold element ratios reflect the threshold past which a system theoretically switches from limitation by one element to another due to inadequate supply of a given macronutrient or energy (Sterner and Elser 2002). The C:N threshold ratio therefore would separate energy-limited growth (C:N ratio lower than threshold ratio) from nutrient limited growth (C:N ratio higher than the threshold ratio; Sinsabaugh et al. 2016). The most basic C:N threshold ratio can be derived from the Redfield ratio which gives a molar threshold ratio of 6.6, while a meta-analysis of DOC:NO₃⁻ ratios from a wide range of systems found that the DOC:NO₃⁻ threshold ratio was between 2.2 and 5.2 (Redfield 1958; Taylor and Townsend 2010). We compared this range in DOC:NO₃⁻ threshold ratios to the DOC:NO₃⁻ ratios of ambient streamwater at TCEF and to DOC:NO₃⁻ ratios of ambient uptake. Our results show that the molar DOC:NO₃⁻ ratio of streamwater was 119, 81, and 118 on the 3 sampling dates where both DOC and NO₃⁻ uptake were measured. These ambient streamwater DOC:NO₃⁻ ratios far exceeded the DOC:NO₃⁻ threshold ratio and indicate that NO₃⁻ was in far shorter supply than DOC, suggesting that this system should be more N limited. We also observed labile DOC:NO₃⁻ uptake rate ratios at benchmark concentrations that far exceeded any expected or theoretical stoichiometric ratios (DOC:NO₃⁻ ratios of uptake ranging from 43 to 70). These results suggest that although more labile dissolved organic carbon was being consumed than nitrate in our study reach, nitrate was much more scarce than dissolved organic carbon and therefore may have been limiting growth.

The potential N limitation in this system as suggested by the stoichiometric comparison of DOC:NO₃⁻ threshold ratios and the DOC:NO₃⁻ of

the ambient streamwater is also reinforced by examination of the K_m values of DOC and NO₃⁻ uptake. The half saturation constant (K_m) represents the substrate concentration ([NO₃⁻] or [DOC]) at which the total uptake rate is at half its maximum rate (U_{max}). We hypothesize that systems characterized by a low K_m value typically have a steeper ascending limb of their kinetic curve, indicating that uptake rates can react quickly at low concentrations to take up added nutrient but may have lower total maximum uptake rates (U_{tot}). Several studies have used K_m as a metric to describe the *responsiveness of the biotic community* to elevated substrate concentrations (Covino et al. 2012; Piper et al. 2017). In our study stream, K_{m-N} was consistently several times lower than K_{m-C} (Table 2) suggesting that while this stream had lower total demand for nitrate (relative to dissolved organic carbon), nitrate uptake was more responsive to added solute and responded more rapidly at low concentrations than dissolved organic carbon uptake.

How can these seemingly contradictory findings (both labile DOC and NO₃⁻ limitation) that are dependent on the choice of metric be explained? It is clear that labile DOC uptake in this system occurs at a much higher magnitude than NO₃⁻ uptake, but also that in-stream communities are experiencing environmental conditions outside the stoichiometric ratio required for balanced growth and as such are highly responsive to added N. Therefore, we hypothesize that the high labile DOC uptake we observe could be due to elevated metabolic demand for energy that is not coupled to growth. Bacterial growth efficiency (BGE) represents the amount of new microbial biomass produced per unit of organic carbon assimilated (del Giorgio and Cole 1998). Several studies have documented that in highly oligotrophic systems with high C:N resource stoichiometry, bacterial growth efficiency is inversely correlated with resource C:N stoichiometry. BGE values for these communities have been found to be as low as 5–20% (del Giorgio

and Cole 1998; Taylor and Townsend 2010). This suggests that demand for DOC could be elevated due to high maintenance energy costs, thus not translating assimilated organic carbon into biomass due to N limitation. This would further suggest that in highly oligotrophic systems with low BGE and a large fraction of uptake that does not translate into growth, use of traditional spiraling metrics to compare potential macronutrient and energy limitation may be challenging or could be inappropriate.

We suggest that there could be conceptual shortcomings to relying on the language of “limitation” to discuss how dissolved organic carbon and macronutrients are cycled in heterotrophic streams. Because limitation is conceptually linked to growth of new biomass, it makes comparisons and differentiation between uptake that fuels metabolic versus growth processes challenging. Despite this, there are many scenarios in which one would want to be able to compare the uptake of organic carbon to other macronutrients to assess their relative importance in observed ecosystem dynamics and nutrient transformation, retention and export.

We propose that one potentially productive and fruitful approach would integrate new and existing methods to describe and compare the magnitude of nutrient uptake and the regulation of nutrient cycles in aquatic systems. In particular, we propose using three metrics to describe the relative importance of a given nutrient for regulating ecosystem function or the cycling of multiple elements in streams. In addition to the traditional spiraling metrics that use the *magnitude of uptake* to determine limitation status (i.e., S_{w-amb} , U_{amb} , V_{f-amb} , or benchmark uptake rates), we suggest adding metrics that represent the *responsiveness of the system* to changes in concentration (K_m) and the *deviation of uptake from expected stoichiometry* (C:N ratios of ambient uptake). These all provide different perspectives of nutrient demand even within a single stream, as demonstrated by our data. The traditional spiraling metrics that compare the total *magnitude of uptake* (S_{w-amb} , U_{amb} , V_{f-amb}) will provide information on which element the biotic community is cycling most rapidly and in the greatest amounts (on a mass basis), whereas the *responsiveness metrics* (K_m) and *stoichiometry metrics* (C:N ratios) may illuminate how the responsive the system will be to additional available nutrients and which elements are present at less than ideal stoichiometric levels for growth. In the

case of our study system, we found greater demand for dissolved organic carbon than nitrate (higher $S_{w-amb-C}$, U_{amb-C} , $V_{f-amb-C}$) suggesting rapid cycling of organic carbon, but we also find that the system is far more depleted in nitrate relative to organic carbon (high DOC:NO₃⁻ ratios of streamwater and uptake) and subsequently more responsive to additions of nitrate (lower K_{m-N} values) than dissolved organic carbon. We suggest that this type of multiple metric approach will be necessary to separate the effects of metabolism and growth to compare stream element cycling more comprehensively.

We acknowledge that in our study, we assume that the heterotrophic community is responsible for the majority of measured labile DOC and NO₃⁻ uptake. We make this assumption because of the low and temporally stable primary production rates observed at our site, and the higher rates of respiration and net ecosystem productivity. However, future work could attempt to determine the fraction of uptake driven by heterotrophic and autotrophic communities and establish whether the same environmental drivers regulated these different fractions.

Comparing dissolved organic carbon and nitrate retention/export

The final objective of our study was to assess the relative influence of biological uptake on the regulation of watershed scale solute export. Our results suggest that both dissolved organic carbon and nitrate fluxes were subject to a high degree of biological control. In-stream retention of labile DOC equaled or exceeded DOC export for nearly the entire study period, which resulted in a retention/export ratio greater or equal to 1 (Fig. 10a). The magnitude of DOC retained was greater when assuming a higher labile C fraction (upper boundaries of Fig. 10a), but even at lower labile C fractions (10% labile C, lower boundaries of Fig. 10a) retention was nearly equal to export. The dissolved organic carbon retention/export ratio peaked in mid-July, at which point in-stream retention was as much as three times greater than downstream export from the system (Fig. 10b), suggesting that biological retention of dissolved organic carbon exerts a significant influence on watershed scale fluxes.

While residence time does not appear to be the primary driver of the seasonal trends in DOC and

NO_3^- uptake rates, it is an important determinant of the seasonal patterns observed in the retention/export ratio. Numerous studies have demonstrated that net nutrient retention is enhanced by longer residence times (Valett et al. 1996; Alexander et al. 2000; Battin et al. 2008) and have effectively modeled nutrient retention as a function of uptake rate and concentration (or uptake efficiency), residence time, and reach length (Wollheim et al. 2006; Aguilera et al. 2013). We observed that despite decreasing dissolved organic carbon uptake rates throughout the summer, the ratio of DOC retention/export increased until mid-July (Fig. 10b). We suggest that the increase in the DOC retention/export ratio was due to a more rapid increase in residence time relative to the simultaneous decrease in uptake rates. This allowed for more DOC to be retained than exported from the headwater reaches despite decreasing uptake rates.

Furthermore, we hypothesize that the decrease in the DOC retention/export ratio at the end of the summer represents a shift to substrate limitation. As ambient DOC and NO_3^- concentrations declined in late July and early August, the biological community became substrate limited and began shutting down, thus pushing the retention/export ratio towards more balanced amounts of hydrologic and biological influence on export fluxes (ratio of 1). Despite higher residence times at the end of the summer, reaction rates were sufficiently low (due to substrate limitation) such that retention decreased relative to export.

The shifting influence of export and retention over time was also reflected in the DOC concentration time series, which displayed a high degree of seasonality (Fig. 10c). We suggest that the variability in DOC concentrations reflects the changing influence of watershed and in-stream processes over the growing season. As the strong influence of physical export processes weakened following the snowmelt period and stream loading from the terrestrial landscape decreased, in-stream processes began to exert more influence on observed concentration dynamics, subsequently reducing the amount of DOC exported from the catchment. This suggests that DOC concentrations and export dynamics may be subject to a shifting balance of physical and biological control.

In contrast to DOC dynamics, the nitrate time series indicated a different concentration regulation regime (Fig. 10f). Nitrate retention and export were closely balanced throughout the study period (Fig. 10d),

resulting in a stable retention/export ratio of 1 with very little seasonality. In comparison to the DOC concentration time series, the NO_3^- concentration time series displayed nearly chemostatic behavior (Fig. 10e). Concentrations declined by $\sim 10 \mu\text{g L}^{-1}$ ($\sim 40\%$) while discharge decreased by $\sim 800\%$ over the same time period (Table 1). We posit that uptake kinetics could be one mechanism to explain chemostatic behavior in some streams and rivers. As concentrations became elevated, uptake rates increased, effectively lowering concentrations down toward the ambient baseline. On the other end of the spectrum, as ambient concentrations decreased, uptake rates also decreased thus allowing ambient concentrations to move up toward the ambient baseline. We suggest that the concentration buffering effect of nutrient uptake kinetics could lead to muted streamwater concentrations through time, especially in systems with significant biological uptake and moderate streamwater residence times. Chemostatic behavior has often been attributed to flow proportional mobilization of solutes from the terrestrial landscape (Godsey et al. 2009; Basu et al. 2010), however, our results suggest that uptake kinetics may be another or additional mechanism that could explain and generate chemostatic solute dynamics in aquatic systems.

Conclusions

This study sought to (1) explore whether the temporal patterns in DOC and NO_3^- uptake kinetics were driven by equilibration to “master kinetic curve” (H1) or whether the capacity of the biological community to take up nutrients changed over the growing season (H2), (2) understand the relative influence of two potential drivers, residence time (H2a) and substrate availability (H2b), on nutrient uptake in a heterotrophic stream system, and (3) assess how influential nutrient uptake could be in regulating solute fluxes from a headwater stream network. We found that the capacity of the biological community to retain solutes changed over the post-snowmelt summer season, and this was reflected by seasonal changes in the shape and magnitude of the kinetic curves characterizing DOC and NO_3^- uptake. We also determined that the declining temporal trends in both labile DOC and NO_3^- uptake mirrored the seasonal decreases in ambient [DOC] and [NO_3^-], and were inversely

related to residence time metrics that described both advective transport and the degree of transient storage.

Results from this study also highlighted challenges associated with assessing nutrient limitation based upon a single metric, and as a result we suggest that multiple metrics describing different aspects of how a nutrient is cycled should be considered when determining what element(s) limit stream productivity and function. We suggest three possibilities (1) the *magnitude of ambient uptake*, (2) the *responsiveness of the system* to near-ambient changes in concentration, and (3) the *deviation of uptake from expected stoichiometry* as a suite of potential metrics to assess limitation, and encourage future studies to determine the utility and value of this approach for illuminating the complex interactions of nutrients at the ecosystem level, and advancing our understanding of multi-element limitation in aquatic systems.

Lastly, we determined that the magnitude of biological DOC and NO_3^- uptake and retention was equal to or exceeded watershed solute export throughout the post-snowmelt study period, thus indicating the potential for the biotic community to regulate downstream water quality in headwater stream networks. These results do not suggest a complete dominance of biological processes for regulating water quality, but instead suggest a two-step mechanism whereby watershed and in-stream physical processes set a baseline which biological processes can further modify, thus creating multiple layers of physical and biological influence on downstream concentrations. We suggest that consideration of these coupled biophysical processes is critical for understanding observed solute dynamics in headwater streams, and for recognizing how perturbations to either set of processes will impact water quality.

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References

- Ågren AM, Buffam I, Cooper DM et al (2014) Can the heterogeneity in stream dissolved organic carbon be explained by contributing landscape elements? *Biogeochemistry* 11:1199–1213. <https://doi.org/10.5194/bg-11-1199-2014>
- Aguilera R, Marcé R, Sabater S (2013) Modeling nutrient retention at the watershed scale: does small stream research apply to the whole river network? *J Geophys Res Biogeosci* 118(2):728–740
- Alexander RB, Smith RA, Schwarz GE (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758–762. <https://doi.org/10.1038/35001562>
- Basu NB, Destouni G, Jawitz JW et al (2010) Nutrient loads exported from managed catchments reveal emergent biogeochemical stationarity. *Geophys Res Lett* 37:1–5. <https://doi.org/10.1029/2010GL045168>
- Battin TJ, Kaplan LA, Findlay S et al (2008) Biophysical controls on organic carbon fluxes in fluvial networks. *Nat Geosci* 2:595. <https://doi.org/10.1038/ngeo602>
- Bennett JP, Rathburn RE (1972) Reaeration in open-channel flow. Geological Survey Professional Paper 737, U. S. Government Printing Office, Washington, DC
- Berggren M, Giorgio PA (2015) Distinct patterns of microbial metabolism associated with riverine dissolved organic carbon of different source and quality. *J Geophys Res Biogeosci*. <https://doi.org/10.1002/2015JG002963>
- Berggren M, Laudon H, Jansson M (2009) Hydrological control of organic carbon support for bacterial growth in boreal headwater streams. *Microb Ecol* 57:170–178. <https://doi.org/10.1007/s00248-008-9423-6>
- Berggren M, Laudon H, Haei M, Stro L (2010) Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. *ISME J*. <https://doi.org/10.1038/ismej.2009.120>
- Bergstrom A, McGlynn B, Mallard J, Covino T (2016) Watershed structural influences on the distributions of stream network water and solute travel times under baseflow conditions. *Hydrol Process* 30:2671–2685. <https://doi.org/10.1002/hyp.10792>
- Bernal S, Von Schiller D, Martí E, Sabater F (2012) In-stream net uptake regulates inorganic nitrogen export from catchments under base flow conditions. *J Geophys Res Biogeosci* 117:1–10. <https://doi.org/10.1029/2012JG001985>
- Bernal S, Lupon A, Ribot M et al (2015) Riparian and in-stream controls on nutrient concentrations and fluxes in a headwater forested stream. *Biogeosciences* 12:1941–1954. <https://doi.org/10.5194/bg-12-1941-2015>
- Bernhardt ES, Likens GE (2002) Dissolved organic carbon enrichment alters stream nitrogen dynamics in a forest stream. *Ecology* 83:1689–1700. [https://doi.org/10.1890/0012-9658\(2002\)083\[1689:DOCEAN\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1689:DOCEAN]2.0.CO;2)
- Bernhardt ES, Hall RO Jr, Likens GE (2002) Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook experimental forest. *Ecosystems* 5:419–430. <https://doi.org/10.1007/s10021-002-0179-4>
- Bernhardt ES, Likens GE, Hall RO et al (2005) Can't see the forest for the stream? In-stream processing and terrestrial nitrogen exports. *Bioscience* 55:219–230. [https://doi.org/10.1641/0006-3568\(2005\)055\[0219:ACSTFF\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0219:ACSTFF]2.0.CO;2)
- Brookshire ENJ, Valett HM, Thomas SA et al (2005) Coupled cycling of dissolved organic nitrogen and carbon in a forest

- stream. *Ecology* 86:2487–2496. <https://doi.org/10.1890/04-1184>
- Brookshire ENJ, Valett HM, Gerber S (2009) Maintenance of terrestrial nutrient loss signatures during in-stream transport. *Ecology* 90:293–299. <https://doi.org/10.1890/08-0949.1>
- Cohen MAJ, Kurz MJ, Heffernan JB et al (2013) Diel phosphorus variation and the stoichiometry of ecosystem metabolism in a large spring-fed river. *Ecol Monogr* 83:155–176
- Cole JJ, Prairie YT, Caraco NF et al (2007) Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10:172–185. <https://doi.org/10.1007/s10021-006-9013-8>
- Cory RM, Kaplan LA (2012) Biological lability of streamwater fluorescent dissolved organic matter. *Limnol Oceanogr* 57:1347–1360. <https://doi.org/10.4319/lo.2012.57.5.1347>
- Cory RM, Ward CP, Crump BC, Kling GW (2014) Sunlight controls water column processing of carbon in arctic fresh waters. *Science* 345(80):925–928. <https://doi.org/10.1126/science.1253119>
- Cory RM, Harrold KH, Neilson BT, Kling GW (2015) Controls on dissolved organic matter (DOM) degradation in a headwater stream: the influence of photochemical and hydrological conditions in determining light-limitation or substrate-limitation of photo-degradation. *Biogeoosci Discuss* 12:9793–9838. <https://doi.org/10.5194/bgd-12-9793-2015>
- Covino T, McGlynn BL (2007) Stream gains and losses across a mountain-to-valley transition: Impacts on watershed hydrology and stream water chemistry. *Water Resour Res* 43:1–14. <https://doi.org/10.1029/2006WR005544>
- Covino T, McGlynn BL, Baker M (2010a) Separating physical and biological nutrient retention and quantifying uptake kinetics from ambient to saturation in successive mountain stream reaches. *J Geophys Res Biogeosci* 115:1–17. <https://doi.org/10.1029/2009JG001263>
- Covino T, McGlynn BL, McNamara RA (2010b) Tracer additions for spiraling curve characterization (TASCC): quantifying stream nutrient uptake kinetics from ambient to saturation. *Limnol Oceanogr Methods* 8:484–498. <https://doi.org/10.4319/lom.2010.8.484>
- Covino T, McGlynn BL, Mallard J (2011) Stream-groundwater exchange and hydrologic turnover at the network scale. *Water Resour Res* 47:1–11. <https://doi.org/10.1029/2011WR010942>
- Covino T, McGlynn BL, McNamara R (2012) Land use/land cover and scale influences on in-stream nitrogen uptake kinetics. *J Geophys Res* 117:1–13. <https://doi.org/10.1029/2011JG001874>
- Crawford JT, Lottig NR, Stanley EH et al (2014) CO₂ and CH₄ emissions from streams in a lake-rich landscape: patterns, controls, and regional significance. *Global Biogeochem Cycles*. <https://doi.org/10.1002/2013GB004661>
- Creed IF, McKnight DM, Pellerin BA et al (2015) The river as a chemostat: fresh perspectives on dissolved organic matter flowing down the river continuum. *Can J Fish Aquat Sci* 72:1–37
- del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. *Annu Rev Ecol Syst* 29:503–541
- Demars BOL, Thompson J, Manson JR (2015) Stream metabolism and the open diel oxygen method: Principles, practice, and perspectives. *Limnol Oceanogr Methods* 13:356–374. <https://doi.org/10.1002/lom3.10030>
- Duarte CM, Prairie YT (2005) Prevalence of heterotrophy and atmospheric CO₂ emissions from aquatic ecosystems. *Ecosystems* 8:862–870. <https://doi.org/10.1007/s10021-005-0177-4>
- Elser JJ, Bracken MES, Cleland EE et al (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Ensign SH, Doyle MW (2005) In-channel transient storage and associated nutrient retention: evidence from experimental manipulations. *Limnol Oceanogr* 50:1740–1751. <https://doi.org/10.4319/lo.2005.50.6.1740>
- Ensign SH, Doyle MW (2006) Nutrient spiraling in streams and river networks. *J Geophys Res Biogeosci* 111:1–13. <https://doi.org/10.1029/2005JG000114>
- Fellows CS, Valett HM, Dahm CN et al (2006) Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems* 9:788–804. <https://doi.org/10.1007/s10021-006-0005-5>
- Findlay S, Sobczak WV (1996) Variability in removal of dissolved organic carbon in hyporheic sediments. *J North Am Benthol Soc* 15:35–41
- Fisher SG, Likens GE (1973) Energy flow in bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecol Monogr* 43:421–439
- Gardner KK, McGlynn BL, Marshall LA (2011) Quantifying watershed sensitivity to spatially variable N loading and the relative importance of watershed N retention mechanisms. *Water Resour Res* 47:1–21. <https://doi.org/10.1029/2010WR009738>
- Genereux DP, Hemond HH (1992) Determination of gas exchange rate constants for a small stream on walker branch watershed, Tennessee. *Water Resour Res* 28:2365–2374. <https://doi.org/10.1029/92WR01083>
- Godsey SE, Kirchner JW, Clow DW (2009) Concentration—discharge relationships reflect chemostatic characteristics of US catchments. *Hydro Process* 23:1844–1864. <https://doi.org/10.1002/hyp.7315>
- Gomez-Velez JD, Harvey JW, Cardenas MB, Kiel B (2015) Denitrification in the Mississippi River network controlled by flow through river bedforms. *Nat Geosci*. <https://doi.org/10.1038/ngeo2567>
- Grimm NB (1987) Nitrogen dynamics during succession in a desert stream. *Ecology* 68:1157–1170. <https://doi.org/10.2307/1939200>
- Grimm NB, Fisher SG (1989) Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. *J North Am Benthol Soc* 8:293–307
- Hall RO, Tank JL (2003) Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnol Oceanogr* 48:1120–1128. <https://doi.org/10.4319/lo.2003.48.3.1120>
- Hall RO, Bernhardt ES, Likens GE (2002) Relating nutrient uptake with transient storage in forested mountain streams. *Limnol Oceanogr* 47:255–265. <https://doi.org/10.4319/lo.2002.47.1.0255>

- Hall RO, Baker MA, Arp CD, Koch BJ (2009a) Hydrologic control of nitrogen removal, storage, and export in a mountain stream. *Limnol Oceanogr* 54:2128–2142. <https://doi.org/10.4319/lo.2009.54.6.2128>
- Hall RO, Tank JL, Sobota DJ et al (2009b) Nitrate removal in stream ecosystems measured by Total uptake 15 N addition experiments: total uptake. *Limnol Oceanogr* 54:653–665
- Hanley KW, Wollheim WM, Salisbury J et al (2013) Controls on dissolved organic carbon quantity and chemical character in temperate rivers of North America. *Global Biogeochem Cycles* 27:492–504. <https://doi.org/10.1002/gbc.20044>
- Harpole WS, Ngai JT, Cleland EE et al (2011) Nutrient co-limitation of primary producer communities. *Ecol Lett* 14:852–862. <https://doi.org/10.1111/j.1461-0248.2011.01651.x>
- Harvey J (2016) Hydrologic exchange flows and their ecological consequences in river corridors. In: *Stream ecosystems in a changing environment*, pp 1–83. <https://doi.org/10.1016/B978-0-12-405890-3.00001-4>
- Harvey JW, Wagner BJ, Bencala KE (1996) Evaluating the reliability of the stream tracer approach to characterize stream-subsurface water exchange. *Water Resour Res* 32:2441–2451. <https://doi.org/10.1029/96WR01268>
- Heffernan JB, Cohen MJ (2010) Direct and indirect coupling of primary production and diel nitrate dynamics in a subtropical spring-fed river. *Limnol Oceanogr* 55:677–688. <https://doi.org/10.4319/lo.2009.55.2.0677>
- Hoellein TJ, Tank JL, Rosi-Marshall EJ et al (2007) Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams. *Limnol Oceanogr* 52:1964–1977. <https://doi.org/10.4319/lo.2007.52.5.1964>
- Hoellein TJ, Bruesewitz DA, Richardson DC (2013) Revisiting Odum (1956): a synthesis of aquatic ecosystem metabolism. *Limnol Oceanogr* 58:2089–2100. <https://doi.org/10.4319/lo.2013.58.6.2089>
- Jencso KG, McGlynn BL, Gooseff MN et al (2010) Hillslope hydrologic connectivity controls riparian groundwater turnover: implications of catchment structure for riparian buffering and stream water sources. *Water Resour Res*. <https://doi.org/10.1029/2009wr008818>
- Johnson MS, Lehmann J, Riha SJ et al (2008) CO₂ efflux from Amazonian headwater streams represents a significant fate for deep soil respiration. *Geophys Res Lett* 35:L17401. <https://doi.org/10.1029/2008GL034619>
- Johnson LT, Tank JL, Arango CP (2009) The effect of land use on dissolved organic carbon and nitrogen uptake in streams. *Freshw Biol* 54:2335–2350. <https://doi.org/10.1111/j.1365-2427.2009.02261.x>
- Jones JB, Stanley EH, Mulholland PJ (2003) Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophys Res Lett* 30:1495. <https://doi.org/10.1029/2003GL017056>
- Kilpatrick FA, Cobb ED (1985) Measurement of discharge using tracers. Report of the U.S. Geological Survey, Techniques of Water Resources Investigations Book 3, Chap A16, pp 6–15
- Kothawala DN, Ji X, Laudon H et al (2015) The relative influence of land cover, hydrology and in-stream processing on the composition of dissolved organic matter in boreal streams. *J Geophys Res Biogeosciences* 120:1–15. <https://doi.org/10.1002/2015JG002946>
- Lambert T, Teodoru CR, Nyoni FC et al (2016) Degradation of dissolved organic matter in a large tropical river. *Biogeoosci Discuss* 13:2727–2741. <https://doi.org/10.5194/bg-2016-9>
- Lapierre J-F, Guillemette F, Berggren M, del Giorgio PA (2013) Increases in terrestrially derived carbon stimulate organic carbon processing and CO₂ emissions in boreal aquatic ecosystems. *Nat Commun* 4:2972. <https://doi.org/10.1038/ncomms3972>
- Lautz LK, Siegel DI (2007) The effect of transient storage on nitrate uptake lengths in streams: an inter-site comparison. *Hydrol Process* 21:3533–3548. <https://doi.org/10.1002/hyp.6569>
- Likens GE, Bormann FH, Johnson NM et al (1970) Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. *Ecol Monogr* 40:23–47. <https://doi.org/10.2307/1942440>
- Lupon A, Martí E, Sabater F, Bernal S (2015) Green light: gross primary production influences seasonal stream N export by controlling fine-scale N dynamics. *Ecology* 97:133–144. <https://doi.org/10.1890/14-2296.1>
- Lutz BD, Bernhardt ES, Roberts BJ, Mulholland PJ (2011) Examining the coupling of carbon and nitrogen cycles in Appalachian streams: the role of dissolved organic nitrogen. *Ecology* 92:720–732. <https://doi.org/10.1890/10-0899.1>
- Mallard J, McGlynn B, Covino T (2014) Lateral inflows, stream-groundwater exchange, and network geometry influence stream water composition. *Water Resour Res* 50:4603–4623. <https://doi.org/10.1002/2013WR014944>
- Marti E, Sabater F (1996) High variability in temporal and spatial nutrient retention in Mediterranean streams. *Ecology* 77:854–869. <https://doi.org/10.2307/2265506>
- Marzolf ER, Mulholland PJ, Steinman AD (1994) Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Can J Fish Aquat Sci* 51:1591–1599. <https://doi.org/10.1139/f94-158>
- Mason SJK, McGlynn BL, Poole GC (2012) Hydrologic response to channel reconfiguration on Silver Bow Creek, Montana. *J Hydrol* 438–439:125–136. <https://doi.org/10.1016/j.jhydrol.2012.03.010>
- Melching CS, Flores HE, Flores HE (1999) Reaeration equations derived from U.S. Geological Survey database. *J Environ Eng*. [https://doi.org/10.1061/\(asce\)0733-9372\(1999\)125:5\(407\)](https://doi.org/10.1061/(asce)0733-9372(1999)125:5(407))
- Meyer JL, Likens GE (1979) Transport and transformation of phosphorus in a forest stream ecosystem. *Ecology* 60:1255. <https://doi.org/10.2307/1936971>
- Mincemoyer SA, Birdsall JL (2006) Vascular flora of the tenderfoot creek experimental forest, Little Belt Mountains, Montana. *Madrono* 53:211–222. [https://doi.org/10.3120/0024-9637\(2006\)53](https://doi.org/10.3120/0024-9637(2006)53)
- Mineau MM, Wollheim WM, Buffam I et al (2016) Dissolved organic carbon uptake in streams: a review and assessment of reach-scale measurements. *J Geophys Res Biogeosci*. <https://doi.org/10.1002/2015JG003204>
- Mulholland PJ (1992) Regulation of nutrient concentrations in a temperate forest stream: roles of upland, riparian, and

- instream processes. *Limnol Oceanogr* 37:1512–1526. <https://doi.org/10.4319/lo.1992.37.7.1512>
- Mulholland PJ (2004) The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. *Biogeochemistry* 70:403–426. <https://doi.org/10.1007/s10533-004-0364-y>
- Mulholland PJ, Marzolf ER, Webster JR, Hart DR (1997) Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnol Oceanogr* 42:443–451. <https://doi.org/10.4319/lo.1997.42.3.0443>
- Mulholland PJ, Tank JL, Webster JR et al (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. *J North Am Benthol Soc* 21:544–560. <https://doi.org/10.2307/1468429>
- Mulholland PJ, Roberts BJ, Hill WR, Smith JG (2009) Stream ecosystem responses to the 2007 spring freeze in the southeastern United States: unexpected effects of climate change. *Glob Chang Biol* 15:1767–1776. <https://doi.org/10.1111/j.1365-2486.2009.01864.x>
- Newbold JD, Elwood JW, O'Neill RV, van Winkle W (1981) Measuring nutrient spiralling in streams. *Can J Fish Aquat Sci* 38:860–863. <https://doi.org/10.1139/f81-114>
- Pacific VJ, Jencso KG, McGlynn BL (2010) Variable flushing mechanisms and landscape structure control stream DOC export during snowmelt in a set of nested catchments. *Biogeochemistry* 99:193–211. <https://doi.org/10.1007/s10533-009-9401-1>
- Patil S, Covino TP, Packman AI et al (2013) Instream variability in solute transport: hydrologic and geomorphic controls on solute retention. *J Geophys Res Earth Surf* 118:413–422. <https://doi.org/10.1029/2012JF002455>
- Payn RA, Gooseff MN, McGlynn BL et al (2009) Channel water balance and exchange with subsurface flow along a mountain headwater stream in Montana, United States. *Water Resour Res* 45:1–14. <https://doi.org/10.1029/2008WR007644>
- Pennino MJ, Kaushal SS, Beaulieu JJ et al (2014) Effects of urban stream burial on nitrogen uptake and ecosystem metabolism: implications for watershed nitrogen and carbon fluxes. *Biogeochemistry* 121:247–269. <https://doi.org/10.1007/s10533-014-9958-1>
- Peterson BJ, Wollheim WM, Mulholland PJ et al (2001) Control of nitrogen export from watersheds by headwater streams. *Science* 292(80):86–90
- Piper LR, Cross WF, McGlynn BL (2017) Colimitation and the coupling of N and P uptake kinetics in oligotrophic mountain streams. *Biogeochemistry*. <https://doi.org/10.1007/s10533-017-0294-0>
- Raymond PA, Hartmann J, Lauerwald R et al (2013) Global carbon dioxide emissions from inland waters. *Nature* 503:355–359. <https://doi.org/10.1038/nature12760>
- Raymond PA, Saiers JE, Sobczak WV (2016) Hydrological and biogeochemical controls on watershed dissolved organic matter transport: pulse-shunt concept. *Ecology* 97:5–16. <https://doi.org/10.1890/07-1861.1>
- Redfield AC (1958) The biological control of chemical factors in the environment. *Am Sci* 46:205–221
- Resh VH, Brown AV, Covich AP et al (1988) The role of disturbance in stream ecology. *J North Am Benthol Soc* 7:433–455. <https://doi.org/10.2307/1467300>
- Roberts BJ, Mulholland PJ (2007) In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. *J Geophys Res* 112:G04002. <https://doi.org/10.1029/2007JG000422>
- Schade JD, MacNeill K, Thomas SA et al (2011) The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. *Freshw Biol* 56:424–436. <https://doi.org/10.1111/j.1365-2427.2010.02509.x>
- Schade JD, Seybold EC, Drake T et al (2016) Variation in summer nitrogen and phosphorus uptake among Siberian headwater streams. *Polar Res* 35:1–28. <https://doi.org/10.3402/polar.v35.24571>
- Simon KS, Townsend CR, Biggs BJB, Bowden WB (2005) Temporal variation of N and P uptake in 2 New Zealand streams. *J North Am Benthol Soc* 24:1–18. [https://doi.org/10.1899/0887-3593\(2005\)024<0001:TVONAP>2.0.CO;2](https://doi.org/10.1899/0887-3593(2005)024<0001:TVONAP>2.0.CO;2)
- Sinsabaugh RL (1997) Large-scale trends for stream benthic respiration. *J North Am Benthol Soc* 16:119–122
- Sinsabaugh RL, Turner BL, Talbot JM et al (2016) Stoichiometry of microbial carbon use efficiency in soils. *Ecol Monogr* 86:172–189. <https://doi.org/10.1017/CBO9781107415324.004>
- Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton
- Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. *J North Am Benthol Soc* 9:95–119
- Tank JLL, Rosi-Marshall EJE, Griffiths NA et al (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *J North Am Benthol Soc* 29:118–146. <https://doi.org/10.1899/08-170.1>
- Taylor PG, Townsend AR (2010) Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. *Nature* 464:1178–1181. <https://doi.org/10.1038/nature08985>
- Valett HM, Morrice JA, Dahm CN, Campana ME (1996) Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnol Oceanogr* 41:333–345. <https://doi.org/10.4319/lo.1996.41.2.0333>
- Vitousek PM, Aber JD, Howarth RW et al (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750
- Webster JR, Mulholland PJ, Tank JL et al (2003) Factors affecting ammonium uptake in streams—an inter-biome perspective. *Freshw Biol* 48:1329–1352
- Wilkinson GM, Pace ML, Cole JJ (2013) Terrestrial dominance of organic matter in north temperate lakes. *Global Biogeochem Cycles* 27:43–51. <https://doi.org/10.1029/2012GB004453>
- Wollheim WM, Pellerin BA, Vörösmarty CJ, Hopkinson CS (2005) N retention in urbanizing headwater catchments. *Ecosystems* 8:871–884. <https://doi.org/10.1007/s10021-005-0178-3>
- Wollheim WM, Vörösmarty CJ, Peterson BJ, Seitzinger SP, Hopkinson CS (2006) Relationship between river size and

nutrient removal. *Geophys Res Lett* 33(6). <https://doi.org/10.1029/2006GL025845>.

Wollheim WM, Stewart RJ, Aiken GR et al (2015) Removal of terrestrial DOC in aquatic ecosystems of a temperate river

network. *Geophys Res Lett* 42:6671–6679. <https://doi.org/10.1002/2015GL064647>