Measuring and interpreting relationships between nutrient supply, demand, and limitation

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Abstract: Stream nutrient uptake and limitation are interconnected by relationships between nutrient supply and demand. We used multiple approaches, including estimates of nutrient supply, measures of stream metabolism derived from dissolved O_2 curves, and nutrient-enrichment experiments, as complementary measures of nutrient supply, demand, and limitation in New Hope Creek, a 3rd-order stream in the Duke Forest of North Carolina. Over the course of 1 y of sampling, NO_3^--N supply relative to demand (S:D) was large during winter (S:D = 133.4), spring (S:D = 62.0), and summer (S:D = 108.32). Potential N demand, estimated based on ecosystem metabolic rates, was measurable during winter, spring, and summer months, but we measured no N uptake in response to N addition from nutrient-enrichment experiments. In contrast, during autumn, declines in stream NO_3^--N concentrations to annual minima (autumn concentration range 0.009–0.034 mg/L NO_3^--N), and low S:D (1.84) induced transient N limitation, and NO_3^--N enrichment stimulated increased uptake. We demonstrate that nutrient-enrichment experiments fail to detect uptake when nutrient concentrations are nonlimiting even when ambient biological uptake remains an important sink for the nutrient of interest. In addition, we present a conceptual model of 'supply–demand space' to aid interpretation of nutrient-enrichment data. Last, we suggest that simultaneous measures of nutrient supply, demand, uptake, and limitation can help define supply–demand relationships and provide a step toward a more robust understanding of biogeochemical cycling in streams.
Key words: ecosystem limitation, carbon and nutrient supply, ecosystem demand, nutrient uptake, nutrient enrichment

Nutrient uptake and associated retention in streams is ultimately the result of interactions between nutrient supply and demand. When nutrient supply exceeds assimilatory demand the nutrient becomes nonlimiting (Liebig 1855) and downstream export can be enhanced (Bouwman et al. 2005). This relationship has been the focus of considerable research, in part because N saturation in many streams (Earl et al. 2006) has enhanced downstream export (Alexander et al. 2009) with deleterious effects for receiving bodies (Rabalais 1998, Alexander et al. 2000, Levin et al. 2009). Nutrient-limitation status in streams, which influences downstream export and loading, varies along a continuum from strong limitation to oversaturation (Newbold et al. 1982, Stanley et al. 1990, Tank et al. 2006, King et al. 2014). Stream nutrient-limitation status can be assessed with nutrient-enrichment experiments, in whole-stream (Stream Solute Workshop 1990) or nutrient-diffusing substrate formats (Fairchild et al. 1985), wherein supply is manipulated. In these experiments, addition of a nonlimiting nutrient is unlikely to stimulate an increase in uptake of the added nutrient (Newbold et al. 1981). However, this lack of response does not necessarily indicate low demand for, or low ambient uptake of, the nutrient of interest. High ambient uptake rates can be satisfied by commensurate supply, which results in no measurable uptake of nutrients supplied in even greater excess during an enrichment experiment (Mulholland et al. 2002). Thus, nutrient-enrichment experiments are predominantly tests of limitation.

The inference available from nutrient-enrichment experiments varies based on where the study system is situated

in what we refer to as 'supply–demand space' (Fig. 1). For example, under saturating conditions, nutrient-enrichment experiments can reveal that ambient nutrient load is saturating, but not by how much (Fig. 1). Under nonsaturating conditions, enrichment techniques become increasingly sensitive to enrichment level as the difference between unsatisfied demand increases relative to supply (left side of supply–demand space; Fig. 1). The relationship between uptake and enrichment level can be assessed by means of multiple constant-rate enrichments (Payn et al. 2005) or via pulse enrichments (Tracer Additions for Spiraling Curve Characterization [TASCC]; Covino et al. 2010). These enrichment techniques can be used to estimate ambient uptake and potential demand as long as supply is below demand, but fail to stimulate uptake under saturating conditions. TASCC was originally developed in highly oligotrophic, high-light streams in the intermountain western USA, which, in retrospect, were positioned in the lower left region of supply–demand space (Fig. 1) that enabled straightforward interpretation of results from the approach. Interpreting results from pulse or constant-rate enrichments in systems that are occasionally, seasonally, or permanently nutrient saturated has proven far more challenging. Therefore, we suggest that simultaneous measures of nutrient supply, demand, uptake, and limitation, evaluated in a supply–demand framework, will help improve interpretation of nutrient-enrichment data and provide a more robust understanding of biogeochemical cycling in streams.
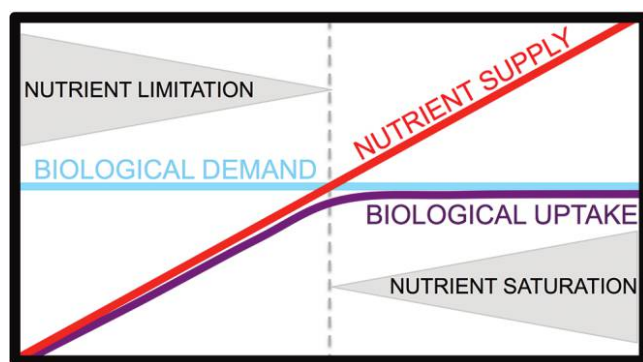

Figure 1. Conceptual diagram of relationships among nutrient supply, demand, uptake, and limitation. In the lower left region where biological demand exceeds supply, the nutrient is limiting, and even low uptake rates are measureable with enrichment. However, uptake estimates in the lower left region are very sensitive to enrichment level. On the right side, where supply exceeds biological demand, enrichment approaches will fail to detect nutrient uptake even when uptake rates are quite high. Stream systems can move vertically between the upper and lower regions as biological demand increases and decreases, and can move between left and right regions as supply changes. As such, stream ecosystems can occupy different regions of supply–demand space through time.

METHODS
Study site description

This research occurred on New Hope Creek (NHC) in the Korstian Division of the Duke Forest, central North Carolina (Fig. 2A). Our experimental reach along NHC is a 3rd-order stream and has a contributing watershed area of 72.4 km^2 (Fig. 2B). Peaks in stream discharge are rain-event driven, and flows range from ~30 to 4200 L/s. Land cover in the basin includes forested, agricultural, and suburban developments. Our experimental stream reach was near the Wooden Bridge in the Korstian Division and stretched 175 and 200 m up- and downstream, respectively, from the bridge (Fig. 2C). Channel morphology along this reach consists of a series of pools and riffles with considerable amounts of bedrock channel bed. As with many streams in the region, NHC was used historically to generate mill power and relict millponds are evident.

Stream physical and chemical measurements

We recorded stream temperature, specific conductivity (SC) (CS-547 temperature and conductivity probes connected to CR-1000 data loggers; Campbell Scientific, Logan, Utah), and dissolved O_2 (DO) (EXO-1 sondes; YSI, Yellow Springs, Ohio) at 15-min intervals from sensor platforms installed at both the up- (NHC1) and downstream (NHC2) boundaries of our experimental reach. At the upstream (NHC1) site only, we measured both stream stage (capacitance rod; TruTrack, Christchurch, New Zealand) and precipitation (TE525MM tipping bucket rain gauge; Texas Electronics, Dallas, Texas) at 15-min intervals. We also measured NO_3^--N (SUNA-V1 sensors; Satlantic, Halifax, Canada), and fluorescing dissolved organic material (fDOM) concentrations (EXO-1) at each station on a less-continuous basis (e.g., shorter-term deployments). To develop a rating curve, we measured stream discharge at weekly to biweekly intervals based on velocity–area gauging (Dingman 2002) across all manageable flow conditions. We were unable to gauge stream discharge at the highest flow states because of safety considerations. We converted real-time stage values to stream discharge for all water heights within the range of our rating curve.

Stream metabolism, nutrient demand, and limitation

We calculated daily gross primary production (GPP; g O_2 m^{-2} d^{-1}) and ecosystem respiration (ER; g O_2 m^{-2} d^{-1}) based on the single-station diel DO change method (Odum 1956), as implemented by the StreamMetabolizer software package (https://github.com/USGS-R/streamMetabolizer). StreamMetabolizer uses inverse modeling to estimate daily metabolic rates from diel changes in DO (as in Holtgrieve et al. 2010). We used StreamMetabolizer's hierarchical Bayesian model to estimate gas exchange at a daily time-step. This model estimates gas exchange by predicting the diurnal DO concentrations given depth, water temperature, an estimate
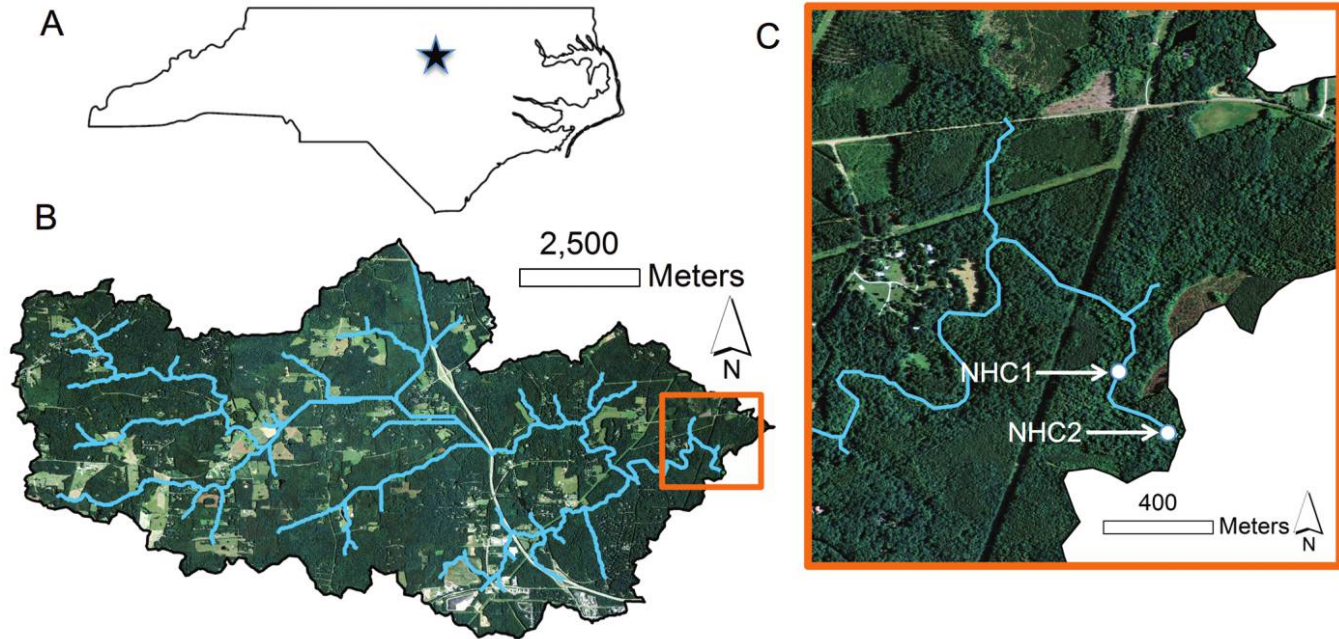
Figure 2. A.—Location of the study site in Duke Forest, North Carolina, USA. B.—Delineation of the New Hope Creek Watershed and satellite land-cover imagery with an orange box indicating the experimental stream reach. C.—Up- (NHC1) and downstream (NHC2) sampling locations along the experimental stream reach used for nutrient enrichments.

of solar insolation based on geographic location and date, and stream discharge. The model was fit through R (version 3.2.3; R Project for Statistical Computing, Vienna, Austria) with Markov Chain Monte Carlo (MCMC) sampling (Carpenter et al. 2017). We included model process error and observation errors in DO. Prior distributions for GPP, ER, gas exchange, and the error parameters were weakly informative to constrain estimates to possible values (e.g., GPP was positive), but ensured that the data were guiding the model fit. Gas-exchange estimates were made with 500 MCMC samples from the posterior distribution after parameter convergence. Areal measures of GPP and ER (g O_2 m^{-2} d^{-1}) were obtained by dividing volumetric GPP and ER (g O_2 m^{-3} d^{-1}) by stream stage (m). Last, we estimated daily net ecosystem productivity (NEP, g O_2 m^{-2} d^{-1}) as the balance of daily GPP and ER values.

We used GPP and ER values to develop estimates of daily NO_3^--N demand. First, we quantified the daily gross autotrophic NO_3^--N assimilation (Heffernan and Cohen 2010) from daily GPP values based on a photosynthetic coefficient of 1, an autotrophic respiration coefficient (r_a) of 0.5 (Hall and Tank 2003), and an autotrophic molar C:N of 12:1 (approximate median value published by Stelzer and Lamberti [2001]). We quantified daily gross heterotrophic NO_3^--N assimilation from heterotrophic respiration (R_h = ER − r_aGPP) assuming a heterotrophic growth rate of 0.2 and molar C:N of 20:1 (Hall and Tank 2003). We calculated total daily NO_3^--N demand as the sum of daily gross autotrophic and heterotrophic NO_3^--N assimilation. We then compared

daily NO_3^--N demand to daily NO_3^--N supply, which we calculated as:

$$NO_{3supply} = {}^{86,400QC}/_{wL}, \qquad \text{(Eq. 1)}$$

where $NO_{3supply}$ is the daily NO_3^--N supply (g O_2 m^{-3} d^{-1}), Q is stream discharge (L/s), C is NO_3^--N concentration (g/L), w is stream width (m), L is stream length (m), and 86,400 is to scale from seconds to day (86,400 s/d). We used a stream length of 100 m (King et al. 2014). Ideal estimates of supply to the stream bed would exceed the stream length over which the water column mixes vertically and, therefore, solutes would be available to the bed, but we lacked hydrodynamic information to estimate this length. From daily demand and supply measures, we evaluated NO_3^--N limitation status as the ratio of supply to demand (S:D). For a single stream, relative estimates of S:D over time will be relatively insensitive to assumptions about effective mixing length or error in metabolism estimates and conversions from metabolism to demand.

Nutrient-enrichment experiments

We quantified nutrient uptake and limitation based on nutrient enrichments across environmental conditions (i.e., February–November) during 2013. For each nutrient enrichment, we injected potassium nitrate (KNO$_3$, biological tracer) and sodium chloride (NaCl, conservative tracer) as an instantaneous injection 25 to 175 m upstream from site NHC1 and monitored NO_3^--N and SC breakthrough curves

(BTCs) in real time at NHC2. Real-time SC measurements were converted to [Cl⁻] using developed calibrations between SC and [Cl⁻]. We collected grab samples during each BTC to verify in-situ measurements. NO_3^--N and Cl^- were analyzed on a Dionex ICS-2100 (Dionex, Sunnyvale, California) with AS-18 analytical and AG-18 guard columns and an AS-40 autosampler (Dionex) with detection limits of 3 and 10 µg/L for NO_3^--N and Cl^-, respectively.

We evaluated the NO_3^--N:Cl^- ratio and estimated mass recovery of each tracer at the downstream sampling location. Under saturating conditions both tracers will be transported conservatively. Conservative transport results in nearly equal mass recovery of both tracers and stable nutrient to conservative tracer ratios during the BTC. We quantified tracer mass recoveries for both Cl^- and NO_3^--N for the injections as the product of Q and the integral of the BTC:

$$T_{MR} = Q \int_0^t T_c(\tau)d\tau, \qquad \text{(Eq. 2)}$$

where T_{MR} is the tracer mass recovery, and T_c is the time-integrated background-corrected tracer concentration for either Cl^- or NO_3^--N. Here we focused on NO_3^--N because it is the main component of dissolved inorganic N in our system.

RESULTS
Seasonal patterns of stream physical and chemical variables

We tracked stream physical and chemical variables in NHC from February to November 2013. The precipitation pattern had no strong seasonal structure, except that most of the large rain events occurred between June and September. Stream temperature had a clear seasonal trend (Table 1) with highest temperatures in July and August and lowest temperatures in autumn (November) and late winter/early spring (February–April). Patterns in DO concentrations were opposite of stream temperature trends with highest DO concentrations associated with cold stream temperatures. In-stream NO_3^--N concentrations were lowest during litterfall (October–November) and highest in July and August (Table 1, Fig. 3A).

Stream metabolism, nutrient demand, and limitation

GPP was less variable than ER. GPP ranged from 0.02 to 2.48 g O_2 m^{-2} d^{-1}, and ER ranged from 0.51 to 11.79 g O_2 m^{-2} d^{-1} from February to November (Fig. 3B). During this period, median GPP was 0.51 g O_2 m^{-2} d^{-1}, mean GPP was 0.59 g O_2 m^{-2} d^{-1}, median ER was 4.30 g O_2 m^{-2} d^{-1}, and mean ER was 4.63 g O_2 m^{-2} d^{-1} (Fig. 3B). Net ecosystem productivity (NEP; the balance between GPP and ER) was strongly related to ER dynamics because ER was variable, whereas GPP was more stable (Fig. 3A). NEP was generally negative, indicating net heterotrophy along the stream reach (Fig. 3A).

Response to nutrient enrichment was related to patterns of S:D, and we measured the lowest S:D in autumn (Table 1). Daily NO_3^--N demand did not fluctuate drastically across seasons and was comparable during the April 17 (0.0997 g N m^{-2} d^{-1}) and November 21 (0.0953 g N m^{-2} d^{-1}) experiments, but supply varied widely among seasons (Table 1). As a result, supply far exceeded demand during winter, spring, and summer, but supply and demand were more balanced in autumn. NO_3^--N limitation status was more

Table 1. Summary of environmental conditions during nutrient-enrichment experiments. Date is formatted mm/dd.

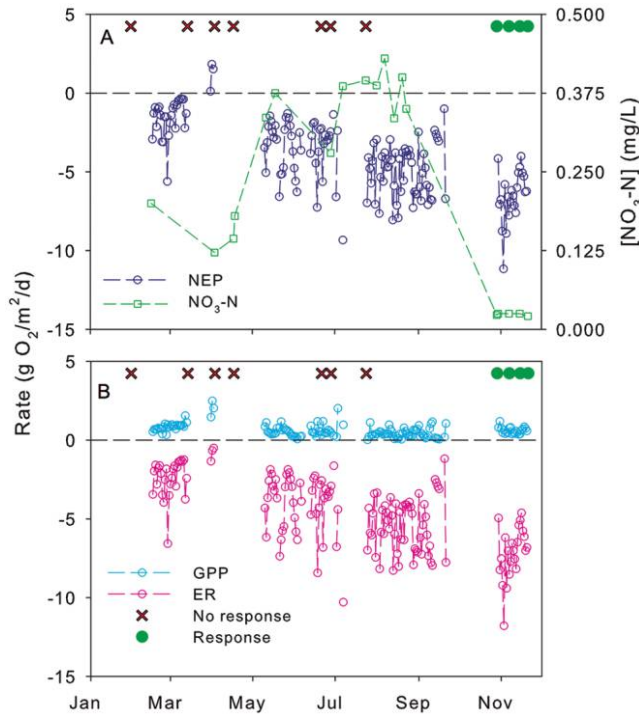| Date	Discharge (L/s)	Temperature (°C)	Ambient [NO_3^--N] (mg/L)	NO_3^--N supply (g N m^{-2} d^{-1})	NO_3^--N demand (g N m^{-2} d^{-1})	Supply: demand	Uptake detected?
2/15	491.4	9.5	0.200	7.85	0.0588	133.4	No
4/17	276.1	9.7	0.144	3.18	0.0997	31.9	No
5/11	423.8	18.6	0.336	11.37	0.1135	100.2	No
5/18	149.0	19.2	0.375	4.49	0.0456	98.4	No
6/28	228.4	24.1	0.280	5.13	0.0510	100.4	No
7/7	770.7	23.1	0.386	23.66	0.1482	159.7	No
8/1	104.5	23.1	0.387	3.25	0.0520	62.5	No
8/7	158.9	22.8	0.430	5.48	0.0593	92.5	No
8/14	392.2	23.6	0.335	10.49	0.0629	166.7	No
8/20	247.3	20.7	0.400	7.92	0.0615	128.9	No
8/23	228.4	23.2	0.350	6.40	0.0596	107.5	No
10/29	78.6	21.7	0.023	0.15	0.1265	1.2	Yes
11/7	95.8	13.2	0.025	0.19	0.1211	1.6	Yes
11/21	101.5	9.3	0.021	0.17	0.0953	1.8	Yes

Figure 3. Net ecosystem productivity (NEP) from March–November, NO_3^--N concentration, and indication of stream response to NO_3^--N enrichment and timing of nutrient-enrichment experiments (A) and gross primary productivity (GPP) and ecosystem respiration (ER) calculated from real-time dissolved O_2 data (B).

closely related to variation in supply than demand, and we observed uptake response to NO_3^--N enrichment only when S:D was low.

Nutrient-enrichment experiments

We performed 14 pulse nutrient-enrichment experiments across a range of environmental conditions from February to November (Table 1). Nutrient enrichment did not stimulate additional nutrient uptake during winter, spring, or summer experiments, resulting in failure to detect uptake during these periods. However, nutrient enrichment did stimulate enhanced uptake during our autumn experiments (Fig. 3A). To demonstrate these patterns we present representative experimental data from spring (April) and autumn (November) (Table 1, Fig. 4A, B). In enrichment experiments where we were unable to detect uptake (winter, spring, and summer), we observed conservative transport behavior for both Cl^- and NO_3^--N as indicated by the constant ratio of NO_3^--N:Cl^- across the BTC (Fig. 4A). In contrast, we observed enhanced uptake in response to nutrient enrichment during autumn as demonstrated by the changing NO_3^--N:Cl^- across the BTC (Fig. 4B). During the November experiment, the NO_3^--N:Cl^- ratio began low, rose to highest levels near the peak of the tracer BTC, decreased through the peak and tail of the BTC,

and then became variable as sensitivity truncation occurred (Fig. 4B). Sensitivity truncation (sensu Drummond et al. 2012) results in strong variability in the NO_3^--N:Cl^- ratio on the BTC tails. Thus, the central portion of the BTC contains the highest signal-to-noise ratio, and analyses of pulse nutrient-injection data should focus on this high-signal region.

Assessment of tracer mass recovery of both tracers (conservative, Cl^-; and biologically active, NO_3^--N) can be used as a measure of uptake response to nutrient enrichment. When both tracers are transported nearly conservatively, the mass recovery of each will be similar, whereas mass recovery will depart more strongly as uptake response increases. We observed high NO_3^--N mass recovery when S:D was high (April; Fig. 5A), and lower NO_3^--N mass recoveries when S:D was low (November; Fig. 5A). The result is that when S:Ds were high, NO_3^--N mass recovery also was high, and nutrient enrichment failed to detect uptake. In fact, NO_3^--N mass recovery during the April enrichment injection was only 4% less than Cl^- recovery, which probably is within margins of error (Fig. 5B). Conversely, during the November injection S:D was low, NO_3^--N recovery was
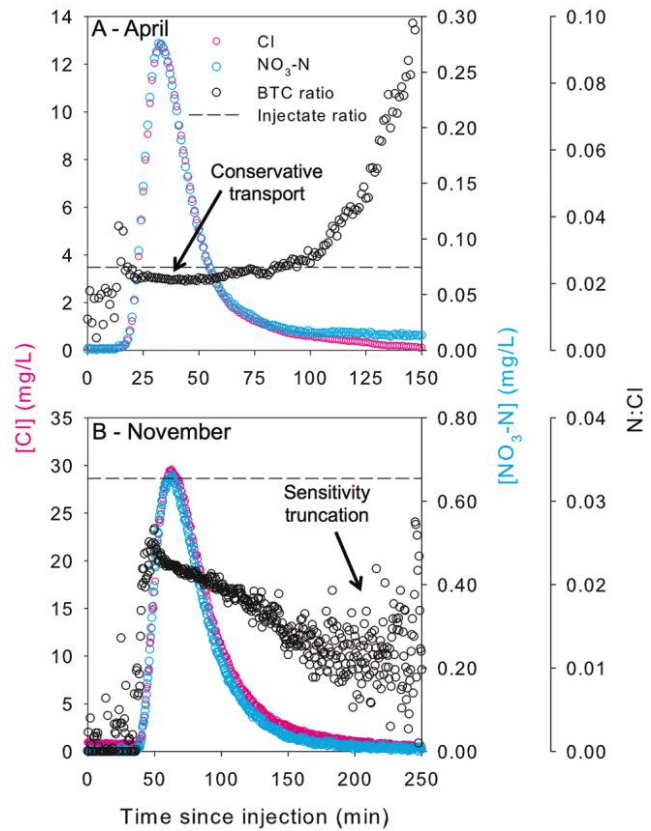

Figure 4. Cl^- and NO_3^--N tracer breakthrough curves (BTCs) for representative pulse-enrichment experiments that occurred during spring (A) and autumn (B). The tracer ratio (N:Cl) through time indicates whether the stream responded to nutrient enrichment.
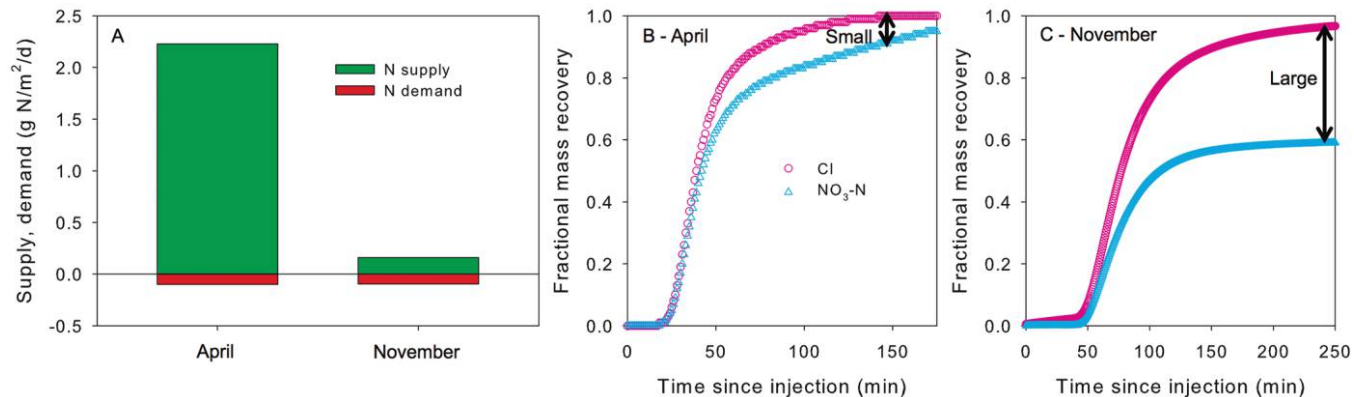
Figure 5. NO_3^--N supply and demand for the April and November experiments (A), mass recovery for Cl^- and NO_3^--N tracers during April enrichment experiment (B), and mass recovery for Cl and NO_3^--N tracers during November enrichment experiment (C).

38% less than Cl^- recovery (Fig. 5C), and we were able to detect uptake with nutrient enrichment.

DISCUSSION

Biological demand for N in NHC was consistently measureable throughout the year, but supply was sufficient to meet/exceed this demand in all but the autumn months (Table 1). Only during our autumn enrichment experiments, when supply was low relative to demand, did we detect increased N uptake in response to NO_3^--N enrichment. Demand did not vary drastically throughout the year, but temporal dynamics in S:D were driven by variability in NO_3^--N supply. Seasonal patterns of NO_3^--N demonstrated highest concentrations in summer and lowest in autumn, similar to patterns observed in other streams of the eastern USA (Mulholland 1992, Swank and Vose 1997, Band et al. 2001). Numerous mechanisms to explain the summer maxima–autumnal minima have been proposed. These mechanisms include changes in watershed hydrology (Mulholland and Hill 1997), increased in-stream heterotrophic respiration associated with autumnal litterfall (Roberts and Mulholland 2007), and microbial response to seasonal temperature fluctuation (Brookshire et al. 2011). We are unable to explain the driver of the autumnal NO_3^--N minima we observed in NHC. For example, we observed slightly elevated in-stream heterotrophic respiration during autumn, but the modest increase is unlikely to account for the drastic decrease in stream NO_3^--N concentration. In fact, autumnal NO_3^--N demand was on the high end of our measured demand values, but it was not the highest that we observed (Table 1). Regardless of the mechanism driving the autumnal NO_3^--N minima, this variability in supply strongly controlled S:D and associated stream response to nutrient enrichment.

Demand was nearly equivalent between the April and November experiments, but the supply decreased $14\times$ (Table 1, Fig. 5A). This example suggests that temporal variability in supply can have strong, even dominant, controls on our ability to measure nutrient uptake and, thus, on our interpretation of what limits stream ecosystem productivity. For example, uptake can be detected in enrichment experiments only when the nutrient of concern is limiting or colimiting (Mulholland et al. 2002, Payn et al. 2005, Earl et al. 2007). Under nonlimiting conditions, the added nutrient will be transported in a near-conservative fashion, and enrichment experiments generally will fail to detect uptake. Many researchers have discarded observations where this occurred. However, discard may not be the appropriate use for such observations. These results indicate the nutrient of interest is nonlimiting and may be of increased utility when evaluated in a context of the relations between supply, demand, and limitation.

To help improve interpretation of results obtained from nutrient-enrichment experiments, we present a conceptual model of potential supply–demand relationships (Fig. 1). On the left side of this plot, demand exceeds supply, and nutrient-enrichment approaches can detect uptake. Because supply is low relative to demand, the ecosystem will be responsive to nutrient enrichment (Kim et al. 1990), and uptake will be detectable even at low rates (Earl et al. 2006). However, on the left side of supply–demand space, uptake estimates will be very sensitive to the magnitude of enrichment. For example, uptake derived from a single plateau enrichment will overestimate ambient uptake (Mulholland et al. 2002) as a function of the shape of the concentration–uptake relationship (Earl et al. 2007) with little information on the magnitude of overestimation. To help quantify the relationship between the magnitude of enrichment and enhanced uptake, Payn et al. (2005) proposed the use of multiple-plateau enrichments. Multiple-plateau enrichments can define the shape of the concentration–uptake curve as long as the nutrient of interest is limiting and other environmental variables that influence demand are not changing between experiments. If environmental conditions change between consecutive enrichments, kinetic assumptions are

not met because more than concentration alone is changing (Voet and Voet 1995). To reduce the amount of time required to define the concentration–uptake curve, we introduced a pulse-enrichment technique (TASSC; Covino et al. 2010). TASSC can shorten the length of time of the experiment and limit the opportunity for changing environmental conditions, but presents other challenges. One limitation is that the concentration experienced across the experimental reach is generally unknown, which can lead to errors in characterization of the concentration–uptake curve and derived estimates of ambient uptake. Multiple-plateau and pulse-enrichment approaches can characterize the concentration–uptake curve only when supply is low relative to demand (left side of supply–demand space; Fig. 1). As supply exceeds demand, nutrient-enrichment techniques will reveal evidence of saturation but will not provide any information on the magnitude of saturation (right side of supply–demand space; Fig. 1). Nutrient enrichments, estimates of demand, and measures of supply provide information on different aspects of stream biogeochemical cycling, so we suggest that they need to be used in concert to determine where a stream is situated in supply–demand space. None of these measures will be able to answer that question alone.

Conclusions

We used multiple approaches, including estimates of daily nutrient supply, stream metabolism, and nutrient-enrichment experiments, as complementary measures of nutrient supply, demand, and limitation in NHC, a 3rd-order stream in the Duke Forest of North Carolina. We found that NO_3^--N enrichment did not stimulate increased uptake during winter, spring, and summer months. NO_3^--N enrichment enhanced uptake only in autumn when NO_3^--N supply was particularly low. We were unable to detect uptake from winter, spring, and summer nutrient-enrichment experiments, but stream metabolic variables indicated considerable productivity during these times. This productivity indicates that NO_3^--N supply satisfied demand and NHC was situated in the right-hand side of our conceptual model of supply–demand space during winter, spring, and summer. Conversely, the stream was operating in the lower-left region of supply–demand space during autumn when supply was low. The left side of supply–demand space, where demand exceeds supply, is well-suited for enrichment techniques to detect uptake and quantify concentration–uptake relationships. However, under nonlimiting conditions, enrichment techniques will fail to detect uptake. Under these conditions, nutrient enrichment will reveal that ambient nutrient load is saturating, but not by how much. Therefore, we suggest that information from multiple approaches, evaluated within a supply–demand conceptual framework, can help improve interpretation of experimental results and understanding of stream biogeochemical dynamics.

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