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Partitioning assimilatory nitrogen uptake in streams: an analysis of stable isotope tracer additions across continents

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Abstract. Headwater streams remove, transform, and store inorganic nitrogen (N) delivered from surrounding watersheds, but excessive N inputs from human activity can saturate removal capacity. Most research has focused on quantifying N removal from the water column over short periods and in individual reaches, and these ecosystem-scale measurements suggest that assimilatory N uptake accounts for most N removal. However, cross-system comparisons addressing the relative role of particular biota responsible for incorporating inorganic N into biomass are lacking. Here we assess the importance of different primary uptake compartments on reach-scale ammonium (NH_4^+-N) uptake and storage across a wide range of streams varying in abundance of biota and local environmental factors. We analyzed data from 17 ^{15}N-NH_4^+ tracer addition experiments globally, and found that assimilatory N uptake by autotrophic compartments (i.e., epilithic biofilm, filamentous algae, bryophytes/macrophytes) was higher but more variable than for heterotrophic microorganisms colonizing detrital organic matter (i.e., leaves, small wood, and fine particles). Autotrophic compartments played a disproportionate role in N uptake relative to their biomass, although uptake rates were similar when we rescaled heterotrophic assimilatory N uptake associated only with live microbial biomass. Assimilatory NH_4^+-N uptake, either estimated as removal from the water column or from the sum uptake of all individual compartments, was four times higher in open- than in closed-canopy streams. Using Bayesian Model Averaging, we found that canopy cover and gross primary production (GPP) controlled autotrophic assimilatory N uptake while ecosystem respiration (ER) was more important for the heterotrophic contribution. The ratio of autotrophic to heterotrophic N storage was positively correlated with metabolism (GPP:ER), which was also higher in open- than in closed-canopy streams. Our analysis shows riparian canopy cover influences the relative abundance of different biotic uptake compartments and thus GPP:ER. As such, the simple categorical variable of canopy cover explained differences in assimilatory N uptake among streams at the reach scale, as well as the relative roles of autotrophs and heterotrophs in N storage. Finally, this synthesis links cumulative N uptake by stream biota to reach-scale N demand and provides a mechanistic and predictive framework for estimating and modeling N cycling in other streams.

Key words: ammonium; assimilation; ^{15}N; nitrogen; riparian canopy cover; stable isotopes; storage; stream; uptake.

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Introduction

In pristine freshwaters, low concentrations of dissolved inorganic N (DIN) can limit algal and microbial production with small increases in N availability leading to shifts in food web structure (Wang et al. 2007). Human activity has more than doubled reactive nitrogen (N) inputs to the global N cycle over the last century (Galloway et al. 2008, 2014), and excess N loading to surface waters is now widespread (Carpenter et al. 1998, Sobota et al. 2013). Headwater streams are particularly effective at removing and storing inorganic N delivered from their surrounding watersheds (Alexander et al. 2000), and during seasons of high biological activity, headwaters typically store or biologically transform more than one-half of the DIN inputs (Peterson et al. 2001). Yet excessive N availability in surface waters alters ecosystem function and eventually saturates N removal capacity (Dodds et al. 2002, Mulholland et al. 2008). Most previous research has focused on characterizing reach-scale N removal from the water column over short time periods, but cross-system comparisons addressing the relative roles of specific biota responsible for incorporating inorganic N into biomass (i.e., assimilatory uptake) are still lacking. Mulholland et al. (2008) found that assimilatory N uptake accounts for ~80% of N removal from the water column but that uptake can vary substantially across systems. Moreover, mechanisms and controls on assimilatory N uptake likely vary across broad scales (i.e., biomes; Dodds et al. 2015). For example, inorganic N availability can control N uptake, as shown in headwater streams where N can be limiting and ammonium (NH_4^+-N) is tightly cycled (Tank and Dodds 2003). In contrast, nitrate (NO_3^--N) availability is strongly influenced by surrounding land use and in urban and agricultural watersheds nitrate is often available in excess, resulting in saturation of N uptake (Helton et al. 2011). A synthetic framework characterizing and quantifying the factors and mechanisms regulating assimilatory N uptake in streams is needed, especially to improve predictive modeling to help assess, maintain, and restore stream structure and function in the face of anthropogenic change.

Organisms responsible for assimilatory N uptake in streams are generally associated with the streambed, growing on sediments and other surfaces located in the benthic zone. From an ecosystem perspective, these benthic biota are considered primary uptake compartments because they meet their N requirements through direct uptake of DIN from the water column, and include autotrophic microorganisms that colonize inorganic substrata (i.e., epilithic biofilm dominated by microalgae), as well as macrophytes, filamentous algae, and bryophytes in many streams. Primary uptake compartments also include heterotrophic microorganisms (i.e., bacteria and fungi) in biofilms developed on colonizing organic substrata including fine benthic organic matter (FBOM), decomposing leaves, and wood. Inorganic N demand among compartments may vary with biotic (e.g., biomass and metabolic activity), physical, and chemical conditions (Kemp and Dodds 2002) and in turn may create a local environmental context unique to each stream (Janetski et al. 2009, Peipoch et al. 2014). For example, stream flow conditions can generally influence nutrient availability for biofilms (Biggs et al. 2005), while organic matter quality (e.g., C:N or C:P) also influences nutrient uptake by heterotrophic biofilms associated with detritus (Melillo et al. 1984, Greenwood et al. 2007, Kominoski et al. 2011). Nevertheless, while we understand the controls on nutrient uptake for individual organisms, dynamics of the collective responses in a multi-species context are less well known.

Local environmental context can influence the relative abundance of primary uptake compartments, which may then influence assimilatory N uptake at the level of the stream reach. The role of riparian canopy cover and the dichotomy of closed- vs. open-canopy streams were some of the early focus areas in comparing stream ecosystems (Minshall et al. 1985), and canopy cover, through changes in light availability and supply of organic matter, controls the distribution and abundance of autotrophic and heterotrophic organisms. Furthermore, experimental studies have confirmed that changes in riparian canopy cover will alter stream ecosystem function (Wallace et al. 1997, Sabater et al. 2000, Collins et al. 2016). For example, light availability drives autochthonous production, while the riparian canopy supplies organic matter inputs (e.g., leaves, wood, dissolved organic matter) colonized by decomposers. Changes in the relative abundance of heterotrophs vs. autotrophs have been documented with shifts in riparian canopy (Gurtz and Wallace 1984, Sabater et al. 1998, Riley and Dodds 2012), along with responses in stream metabolism, which has been linked to reach-scale N uptake (Hall and Tank 2003, Webster et al. 2003, Bernot et al. 2010). While the role of canopy cover in driving ecosystem function in flowing waters has been proposed previously (Vannote et al. 1980), a multi-site test of theory is needed to explicitly link canopy cover to N uptake from the water column, while also contributing to broader questions around the relative role of autotrophic and heterotrophic production in fueling stream ecosystems and food webs.

Experimental additions of trace levels of ^{15}N have allowed the partitioning of N uptake into specific ecosystem compartments (see Table 1 for references) in addition to quantifying rates of reach-scale N uptake and transformation (Peterson et al. 2001, Hall et al. 2009*b*, Mulholland et al. 2008). This approach was originally developed and applied through the first Lotic Intersite Nitrogen eXperiment (LINXI) using identical multi-week ^{15}N-NH_4^+ tracer additions conducted in 10 headwater streams in different North American biomes (Peterson et al. 2001, Webster et al. 2003). The ^{15}N tracer approach has been used in additional streams worldwide, but no synthesis has addressed the relative role of primary uptake compartments across this larger group

of streams. Assessment of the contribution of different biota to assimilatory N uptake has been limited primarily to single streams (see references in Table 1; Kemp and Dodds 2002, von Schiller et al. 2009, Sobota et al. 2012), along with a few studies comparing the role of selected biota across sites (O'Brien and Dodds 2008, Hoellein et al. 2009, Murdock et al. 2010).

Our goal was to develop a synthetic understanding about how N is partitioned among autotrophic and heterotrophic organisms that are responsible for dissolved N removal from the water column, typically measured using reach-scale nutrient spiraling techniques that have been the currency for previous cross-system analyses (Ensign and Doyle 2006, Tank et al. 2008, Hall et al. 2013). We examine how assimilatory N uptake is influenced by both stream biology and local environmental drivers, focusing on the role of riparian canopy cover, which influences light availability, allochthonous organic matter inputs, and the composition of biotic uptake compartments within streams (Fisher and Likens 1973). We analyze data from ^{15}N tracer addition experiments in 17 headwater streams, located in different biomes across the globe, all draining catchments with low levels of human influence, but with differing ecological characteristics (e.g., ecosystem metabolism and food web composition). Specifically, we predicted that canopy cover is a major determinant of N assimilation because it controls the relative abundance and activity of primary uptake compartments, coupling N demand by individual biota to reach-scale N uptake. Our study approach allows us to link organismal characteristics to ecosystem-scale dynamics using N as a common currency, thereby generating a synthetic understanding of controls on N uptake in streams and the ability to predict compartment-specific N demand across systems.

Methods

Site description and ^{15}N tracer addition approach

We analyzed data from 17 different ^{15}N-NH_4^+ tracer addition experiments conducted in streams in the United States, Puerto Rico, Panama, New Zealand, Denmark, Iceland, and Spain; results from 7 of these tracer additions have not yet been published (Table 1). This analysis allowed for the comparison across continents and terrestrial biomes and included new ^{15}N tracer experiments conducted after the initial LINXI project (Simon et al. 2004, Riis et al. 2012, 2014).

The biogeoclimatic regions covered by these experiments range from arctic to tropical, with the majority being in the temperate zone. In general, ^{15}N tracer addition experiments were conducted at baseflow (i.e., summer in temperate and arctic systems, dry season in tropical systems). All study streams were relatively small (discharge range = 4–202 L/s), and mean stream temperature during the ^{15}N tracer addition experiments ranged

Table 1. Site description including stream name, location, biogeoclimatic region, and canopy cover for the 17 different ^{15}N-NH_4^+ tracer addition experiments used in this analysis. Data from this study are referenced as unpublished data.

Stream ID	Stream name	Country	Latitude†	Longitude‡	Biogeoclimatic region	Canopy cover (%)	Reference
PRPN	El Valle	Panama	8.6°	−80.0°	tropical	80	unpublished data
POPN	El Valle	Panama	8.6°	−80.0°	tropical	80	unpublished data
BBNH	Bear Brook, New Hampshire	USA	43.9°	−71.8°	temperate	81	unpublished data
GCNM	Gallina Creek, New Mexico	USA	36.6°	−105.6°	arid	60	unpublished data
SCSP	Santa Colona	Spain	41.9°	2.6°	semi-arid	85	unpublished data
ECMI	Eagle Creek, Michigan	USA	42.3°	−85.3°	temperate	89	Hamilton et al. (2001)
WBTN	Walker Branch, Tennessee	USA	36.0°	−84.3°	temperate	80	Mulholland et al. (2000)
UBNC	Upper Ball Creek, North Carolina	USA	35.1°	−83.4°	temperate	93	Tank et al. (2000)
MCOR	Mack Creek, Oregon	USA	44.2°	−122.2°	temperate	75	Ashkenas et al. (2004)
QBPR	Quebrada Bisley	Puerto Rico	18.3°	−65.8°	tropical	88	Merriam et al. (2002)
SCAZ	Sycamore Creek, Arizona	USA	33.7°	−111.5°	arid	0	unpublished data
SBIC	Steinbogalaekur	Iceland	65.5°	−17.0°	arctic	0	unpublished data
E1AK	E1, Alaska	USA	68.6°	−149.6°	arctic	0	Wollheim et al. (2001)
KCKS	Kings Creek, Kansas	USA	39.1°	−96.6°	temperate	7	Dodds et al. (2000)
LIDK	Lilleaa	Denmark	56.3°	10.1°	temperate	6	Riis et al. (2012, 2014)
KTNZ	Kyeburn Stream	New Zealand	−45.0°	170.4°	temperate	0	Simon et al. (2004)
KGNZ	Kyeburn Stream	New Zealand	−45.0°	170.4°	temperate	0	Simon et al. (2004)

Notes: Based on the distribution of canopy cover data, we grouped streams as closed canopy (streams with >60% canopy cover) and open canopy (streams with <10% canopy cover). Where available, we include references for previously published data.
†Negative signs indicate degrees south.
‡Negative signs indicate degrees west.

from 6° to 23°C across streams (Table 2). Concentrations of NO_3^-, NH_4^+, and soluble reactive phosphorus (SRP) were generally low in our systems, as reflected in the mean concentrations across sites that fall below 10 μg/L for both NH_4^+ and SRP, and below 150 μg/L for NO_3^- (Table 2). Moreover, in 11 streams where we had previously assessed nutrient limitation status of stream biofilms, we found that 9 were limited by the availability of inorganic N (Table 2; Tank and Dodds 2003, von Schiller et al. 2007, Johnson et al. 2009). In contrast, there was a binary distribution of canopy cover for the 17 streams (Table 1). We assessed canopy cover using visual assessment, densiometer, and aerial photography. The distribution of canopy cover in our data set is bimodal, with open-canopy streams having <10% cover, and most none, while closed-canopy systems ranging from >60% to 93% riparian cover. Water velocity was higher in open- than closed-canopy streams; otherwise, there were no significant differences in physicochemical variables between categories of canopy cover (Table 2).

Detailed methods for ^{15}N tracer addition experiments have been published elsewhere (Table 1) and follow methods first published by Mulholland et al. (2000) based on the protocols used in the LINXI project. The experiments we compare here used the ^{15}N tracer addition approach where sufficient ^{15}N-NH_4^+ was added to increase the ^{15}N:^{14}N ratio of available N in stream water, while minimizing the increase in total NH_4^+ concentration. Assimilatory NH_4^+-N uptake was quantified via the movement of tracer ^{15}N from the water column into primary uptake compartments, reflected by ^{15}N enrichment above natural isotope abundance (Peterson et al. 1997, 2001, Dodds et al. 2014). Briefly, as was prescribed in the original LINX protocols, we conducted ^{15}N tracer additions for up to 42d, except in Spain (SCSP), where high temperatures, rapid N assimilation, and quick ^{15}N labeling in primary uptake compartments facilitated a shorter release time (1 d). We combined the observed ^{15}N enrichment with N standing stock estimates of each primary uptake compartment to quantify compartment-specific N assimilatory uptake. For these measurements, a 100–300 m reach was selected at each site to conduct the ^{15}N tracer addition and to track the flow of ^{15}N into uptake compartments. Generally, measurements were made at five to seven stations spaced along each study reach and at a reference site upstream of the ^{15}N release point.

During the ^{15}N tracer additions, we also measured reach-scale metabolism (i.e., gross primary production [GPP] and ecosystem respiration [ER]) in each study stream, bracketing the same experimental reach used for the ^{15}N tracer addition. We used the two-station, upstream- downstream diurnal dissolved oxygen change technique (Marzolf et al. 1994) incorporating the modifications suggested by Young and Huryn (1998) for accurately estimating the air-water oxygen exchange rate. Detailed results and analysis of stream metabolism estimates for eight of the study streams are described in Mulholland et al. (2001). Briefly, we measured dissolved oxygen concentration and water temperature at two stations in each study reach at 5-min intervals over a 24-h period during each ^{15}N tracer addition. In one stream (Sycamore Creek, Arizona, USA), dissolved oxygen concentration and water temperature were measured at hourly intervals using Winkler titration methods. The distance between the two stations depended on stream velocities and ranged from 35 to 300 m, resulting in water travel times ranging from of 10–40 min. We also estimated reaeration using steady-state additions of propane and measuring the downstream decline in the dissolved gas relative to a conservative tracer (Mulholland et al. 2001). We calculated the net rate of dissolved oxygen change at 5-min intervals using the change in mass between stations corrected for air-water exchange, with ER estimated by summing the net oxygen change rate during the night and extrapolated for daylight hours, and GPP estimated by summing the difference between measured net oxygen change rate and ER. We converted metabolism rates to areal units using the area of stream bottom between the two stations and wetted width measurements made at 1-m intervals over each study reach.

N biomass of primary uptake compartments

We sampled dominant primary uptake compartments at each stream site generally following methods described by Mulholland et al. (2000), and we analyzed each primary uptake compartment for biomass, %N content, and percent reach cover. We sampled leaves, small wood (i.e., twigs and sticks), and fine benthic organic matter (FBOM) using an open-ended PVC cylinder pushed into the sediments. Epilithon was sampled by scrubbing and washing all gravel and rocks within a known area. Filamentous algae, bryophytes, and macrophytes were sampled from a known area with 100% coverage. All primary uptake compartment samples were dried and ground before analysis of N content and then scaled to reach-weighted N biomass using percent cover.

^{15}N-NH_4^+ tracer additions

Detailed methods for the ^{15}N-NH_4^+ tracer additions have been previously described for some of the study sites (Table 1). Briefly, ^{15}N-NH_4^+ was added to each stream at a constant rate for a given period along with a conservative hydrologic tracer (Br^- or Cl^-), and subsequent tracer ^{15}N enrichment of primary uptake compartments was tracked. The total mass of ^{15}N-NH_4Cl (99% isotopically enriched) added to each study stream was based on discharge, ambient NH_4^+-N concentration, and duration of the experiment and generally resulted in an increase in the $\delta^{15}N$ of stream water NH_4^+-N to 500–1000‰. Stream water was sampled about 1 d after the experiment started at several downstream stations and samples were analyzed for $\delta^{15}N$-NH_4^+, which allowed estimation of reach-scale NH_4^+-N uptake and nitrification with minimal interference due

TABLE 2. Physical and chemical characteristics of the study streams during the $^{15}N\text{-}NH_4^+$ tracer additions, with sites grouped into categories of closed- and open-canopy streams.

Stream ID	Slope (m/m)	Discharge (L/s)	Velocity (cm/s)	Width (m)	Depth (cm)	Temp (°C)	NH_4^+ (μg N/L)	NO_3^- (μg N/L)	SRP (μg P/L)	DIN : SRP (molar)	Inorganic N- limitation?
Closed canopy											
PRPN	—	22	3.4	3.5	17.6	20.0	3	123	4	64.9	—
POPN	—	23	3.4	3.7	17.6	20.0	3	123	4	64.9	—
BBNH	0.14	9	1.9	2.1	9.0	14.3	4	54	4	36.4	yes
GCNM	0.12	4	9.9	1.3	3.3	7.2	5	4	8	2.5	yes
SCSP	0.02	4	1.5	4.5	6.1	22.5	13	772	23	34.1	no
ECMI	0.01	202	23.6	5.0	17.6	23.0	16	18	3	21.0	no
WBTN	0.04	18	6.8	3.1	4.6	12.4	4	19	3	15.3	yes
UBNC	0.17	130	10.6	2.7	18.0	7.2	3	2	3	4.3	yes
MCOR	0.10	57	7.6	5.1	15.9	13.1	2	59	13	22.3	yes
QBPR	0.13	20	3.0	4.7	12.7	22.0	3	129	14	26.6	yes
Mean (SE)	0.09 (0.02)	49 (21)	7.2 (2.1)	3.6 (0.4)	12.2 (1.9)	16.2 (1.9)	6 (2)	130 (73)	8 (2)	29.2 (6.9)	
Open canopy											
SCAZ	0.003	43	28.6	5.8	8.3	23.0	6	9	14	2.3	yes
SBIC	0.13	156	59.0	1.8	15.0	6.9	5	19	10	5.3	—
E1AK	0.06	134	13.0	1.5	10.3	9.8	3	14	2	20.3	yes
KCKS	0.02	16	11.3	2.4	15.4	15.5	3	2	3	3.6	yes
LIDK	0.01	63	10.0	2.6	23.0	12.4	64	1,433	63	15.3	—
KTNZ	0.09	35	14.0	1.4	19.0	6.2	3	5	1	14.9	—
KGNZ	0.04	22	12.5	1.4	14.0	5.9	4	4	1	17.9	—
Mean (SE)	0.05 (0.02)	67 (21)	21.2 (6.7)	2.4 (0.6)	15.0 (1.9)	11.4 (2.4)	13 (9)	212 (204)	14 (9)	11.4 (2.8)	
P	0.301	0.201	**0.007**	0.114	0.332	0.118	0.605	0.297	0.662	0.108	

Notes: Mean and standard error of the mean (SE) for each variable are given for each canopy-cover category, and statistical results from independent *t* tests comparing the two stream categories are shown in the bottom row with significant differences indicated in bold ($P < 0.05$). We note previously measured N-limitation status for 10 of the streams via nutrient diffusing substrata, as reported by Tank and Dodds (2003), von Schiller et al. (2007), and Johnson et al. (2009). Temp, stream temperature; NH_4^+, ammonium; NO_3^-, nitrate; SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen; —, not measured.

to remineralization of assimilated tracer ^{15}N (Peterson et al. 2001). Analyses of the ^{15}N content of NH_4^+-N and NO_3^--N followed procedures adapted from Holmes et al. (1998) and Sigman et al. (1997), respectively. At sites in Panama and Iceland, due to methodological limitations, water column ^{15}N-NH_4^+ was inferred from ^{15}N label of actively growing biofilms colonizing clay tiles (Whiles et al. 2013).

All uptake compartments were sampled within the first week of the ^{15}N tracer addition to estimate compartmental ^{15}N-NH_4^+ uptake (except for SCSP where the shorter release was conducted). Using values from the first week minimized interference from ^{15}N mineralized to the water column after uptake. Biotic uptake compartments were sampled again on the last day of each experiment to calculate the tracer ^{15}N stored in each compartment over the duration of the experiment. Samples for ^{15}N analysis of each compartment were collected as described for N biomass (see *N biomass of primary uptake compartments*), and then dried and finely ground before isotopic analysis by stable-isotope mass spectrometry. All ^{15}N values for compartments were background-corrected for ^{15}N natural abundance in order to estimate tracer ^{15}N content.

Calculation of N uptake and storage based on ^{15}N

Nitrogen spiraling metrics were estimated in two ways: from longitudinal, reach-scale declines of ^{15}N-NH_4^+ in the water column and from specific ^{15}N labeling of primary uptake compartments based on calculations from Mulholland et al. (2000). Reach-scale uptake length (S_w, in m) of NH_4^+ was directly calculated from the exponential decline in background-corrected ^{15}N-NH_4^+ flux downstream from the ^{15}N addition point; S_w was then converted to areal uptake (U, in mg $N{\cdot}m^{-2}{\cdot}d^{-1}$) using equations from the Stream Solute Workshop (1990). Reach-scale nitrification was estimated from the longitudinal profile of ^{15}N-NO_3^- concentrations along the reach observed during the ^{15}N-NH_4^+ tracer addition experiment (Mulholland et al. 2000). We then calculated the reach-scale assimilatory NH_4^+-N uptake ($U_{assim\text{-}WAT}$, mg $N{\cdot}m^{-2}{\cdot}d^{-1}$) by subtracting nitrification from NH_4^+ U. We note that in four streams (Panama [two streams], Iceland, Denmark) nitrification was not measured directly, and in Upper Ball Creek (North Carolina, USA), nitrification was below the limit of detection (Peterson et al. 2001).

Compartment-specific ^{15}N labeling was used to calculate NH_4^+-N uptake and ^{15}N storage for each primary uptake compartment following Mulholland et al. (2000). Although some of these data were published as part of site-specific studies (Table 1), we re-calculated compartmental NH_4^+-N uptake and storage de novo for all 17 streams to ensure analytic consistency. Briefly, areal NH_4^+-N uptake (mg $N{\cdot}m^{-2}{\cdot}d^{-1}$) for each compartment was calculated from paired values of the ^{15}N tracer content in the primary uptake compartment and overlying water column ^{15}N-NH_4^+ on the first sampling date. We used samples from the first three stations downstream from the addition point to minimize the potential influence of tracer ^{15}N-NH_4^+ regenerated along the reach (Mulholland et al. 2000, Tank et al. 2000). In calculating NH_4^+-N uptake for each compartment, we accounted for the loss of ^{15}N due to N turnover during the same period, which was estimated using the exponential decline in compartmental ^{15}N content hours to days after the ^{15}N-NH_4^+ addition was terminated. We also calculated biomass-specific NH_4^+-N uptake (mg $N{\cdot}mg\ N^{-1}{\cdot}d^{-1}$), by dividing areal uptake by N biomass for each compartment, which equates to a compartment-specific N turnover rate. Finally, for each stream, we summed areal uptake for each primary uptake compartment to calculate a reach-scale assimilatory NH_4^+-N uptake ($U_{assim\text{-}PUC}$), which we then compared to $U_{assim\text{-}WAT}$.

In addition to compartment-specific NH_4^+-N uptake, we also used ^{15}N labeling to calculate reach-scale N storage in different biotic uptake compartments. We compared the mass of ^{15}N added during the addition and ^{15}N stored in each compartment along the study reach at the end of the addition, expressing N storage as a percentage of total ^{15}N added that was retained along the reach. For each compartment, the total mass of stored ^{15}N was based on an integration of the downstream decline in compartment-specific ^{15}N-biomass along the reach. If the slope of the regression of ^{15}N biomass vs. distance was not significant ($P > 0.05$), we used the mean ^{15}N biomass for the entire reach. For this calculation, we used a reach length equal to five times the measured NH_4^+ S_w to standardize estimates for variable reach lengths across the 17 streams. This standardization ensured that, for the systems we considered, >99% of the ^{15}N tracer was removed, so our calculations fully encompass the reach length where biota were exposed to ^{15}N-NH_4^+ from the water column (Mulholland et al. 2000). Finally, we examined the relative contribution of autotrophic and heterotrophic compartments to assimilatory N uptake and storage by grouping them as follows: autotrophic compartments were defined as being dominated by primary producers and included epilithic biofilm, bryophytes, macrophytes, and filamentous algae, whereas heterotrophic compartments included biofilms on leaves, small wood, and FBOM.

Statistical analyses

We used t tests (significance level $\alpha = 0.05$) to compare closed- and open-canopy streams for all physicochemical characteristics, stream metabolism metrics (GPP, ER, GPP:ER), N uptake metrics from water column measurements (S_w, $U_{assim\text{-}WAT}$), and data from measurements of compartmental uptake ($U_{assim\text{-}PUC}$, N storage) and N biomass. We used analysis of variance (ANOVA; $\alpha = 0.05$) to examine differences among compartments for NH_4^+-N uptake and N storage. We used Tukey's HSD post-hoc comparisons ($\alpha = 0.05$) to determine specific differences for statistically significant

results from the ANOVA analyses. We used simple linear regression to relate $U_{assim\text{-}PUC}$ to $U_{assim\text{-}WAT}$, and we explored the correlation between autotrophic:heterotrophic N storage with GPP:ER, testing the differences in autotrophic:heterotrophic N storage between closed- and open-canopy streams using a *t* test. When necessary, we log-transformed data to meet the assumptions of normality and equal variance, and the statistical analyses were performed using Statistica (Version 7.0; Statsoft, Tulsa, Oklahoma, USA).

We also explored potential predictors of total compartmental uptake ($U_{assim\text{-}PUC}$), uptake by autotrophic primary uptake compartments ($U_{assim\text{-}auto}$), and uptake by heterotrophic primary uptake compartments ($U_{assim\text{-}hetero}$) using Bayesian Model Averaging (BMA; Raftery 1995). First, we specified initial models with all potential predictor variables using the bayesglm function in the arm package (Gelman and Su 2015) in R (version 3.2.2; R Core Development Team 2015). Potential predictor variables included canopy cover, discharge (Q), stream width (w), stream depth (d), specific discharge (Q/w), stream velocity (v), stream temperature, background levels of NH_4^+-N, NO_3^--N, DIN, SRP, the ratio of DIN:SRP, GPP, and ER. After running the initial model, we analyzed multi-collinearity for all variables by calculating variance inflation factors (VIF) using the car package (Fox and Weisberg 2011) in R. We iteratively removed the predictor variable with the highest VIF, until no variable selected had a VIF > 20. Conservatively, we used a VIF threshold of 20 to determine which variables we excluded from the model, allowing us to exclude variables that exhibited high degrees of multicollinearity, but still preserving the majority of our variables in the model. This iterative approach of variable selection was performed separately for $U_{assim\text{-}PUC}$, $U_{assim\text{-}auto}$, and $U_{assim\text{-}hetero}$. For each response variable, the final model included canopy cover, w, d, Q/w, v, water temperature, NH_4^+-N, SRP, DIN:SRP, GPP, and ER. After finalizing the final full model, we performed BMA using the bic.glm function in the BMA package (Raftery et al. 2015) in R. Using the Bayesian Information Criterion (BIC), which is similar to the traditional Akaike Information Criterion, we selected the best possible model out of all subset models. Next, we calculated the approximate posterior probability of the best model and retained all candidate models with a posterior probability ≥0.05 of the best model. Finally, we performed model averaging over the remaining models to quantify the contribution of each predictor variable to the remaining models, providing a probability of inclusion, an estimate, and a standard deviation for each predictor variable. We performed BMA separately for $U_{assim\text{-}PUC}$, $U_{assim\text{-}auto}$, and $U_{assim\text{-}hetero}$.

Results

Reach-scale metabolism and N biomass

Among the 17 streams, GPP varied across four orders of magnitude, ranging from 0.001 to 15 g $O_2 \cdot m^{-2} \cdot d^{-1}$ (Table 3), and was approximately seven times higher in open-canopy compared to closed-canopy streams (*t* test, $P = 0.013$; Fig. 1A). Similarly, ER showed substantial variation, ranging from 0.30 to 29 g $O_2 \cdot m^{-2} \cdot d^{-1}$ (Table 3), but was not different between open- and closed-canopy streams (*t* test, $P = 0.126$; Fig. 1B). In closed-canopy streams, GPP:ER showed low variability and averaged 0.07 indicating a predominance of heterotrophic activity. However, in open-canopy streams, GPP:ER varied 10-fold (range = 0.3–3.7, Table 3) and was significantly higher than in closed-canopy streams (*t* test; $P = 0.0002$, Fig. 1C).

Reach-scale N biomass, summed from individual uptake compartments, was highly variable among streams (range = 0.7–9.6 g N m^{-2}, Fig. 2A) and did not differ with canopy cover (*t* test; $P = 0.493$). Heterotrophic compartments dominated N biomass in closed-canopy streams, whereas the relative contribution of filamentous algae and epilithic biofilm was more apparent in open-canopy streams (Fig. 2A). The percentage of contribution of epilithic biofilm to reach-scale N biomass was significantly higher in open-canopy streams (Fig. 2A, *t* test, $P = 0.048$). Nevertheless, FBOM was the dominant fraction of reach-scale N biomass in the streams regardless of canopy cover (Figs. 2A, 3A, ANOVA, $P < 0.0001$).

Assimilatory NH_4^+-N uptake and N storage

The sum of all compartment-specific NH_4^+-N uptake ($U_{assim\text{-}PUC}$) was higher in open-canopy streams than in closed-canopy (*t* test, $P = 0.023$; Fig. 2B, Table 3), reflecting a similar trend in total assimilatory NH_4^+-N uptake from the water column ($U_{assim\text{-}WAT}$), which was four times higher in open- than in closed-canopy streams (Table 3, *t* test, $P = 0.029$). By compartment, NH_4^+-N uptake for epilithic biofilm was also higher in open- vs. closed-canopy streams (*t* test, $P = 0.026$; Fig. 2B), whereas there were no significant differences for any other compartments (*t* tests, all $P > 0.05$). The percentage of added ^{15}N tracer stored in primary uptake compartments at the end of the ^{15}N addition experiments was not significantly different between closed- and open-canopy streams (*t* test, $P = 0.09$; Fig. 2C) and the reach-scale N storage was highly variable among the 17 streams, ranging from 4% to 84% (Table 3). Although not included directly in this analysis, reach-scale N storage by macroinvertebrate consumers was also variable, and averaged 9.0% across sites (range = 0.3–25%; Norman et al. 2017).

Pooling data across all 17 streams, regardless of canopy cover, emphasized differences in assimilatory N dynamics between autotrophs and heterotrophs, and autotrophic compartments showed higher N demand and faster turnover. Although N Biomass was variable across compartments (Fig. 3A), NH_4^+-N uptake by epilithic biofim, filamentous algae, and bryophytes/macrophytes was generally higher, yet more variable, than for heterotrophs (i.e., leaves, wood biofilm, and FBOM; Fig. 3B). Additionally,

TABLE 3. Functional metrics of the study streams during the ^{15}N-NH_4^+ tracer addition experiments.

	Stream metabolism			Data from water column measurements		Data from PUC measurements	
Stream ID	GPP ($g\ O_2 \cdot m^{-2} \cdot d^{-1}$)	ER ($g\ O_2 \cdot m^{-2} \cdot d^{-1}$)	GPP : ER	S_w (m)	$U_{assim\text{-}WAT}$ ($mg\ N \cdot m^{-2} \cdot d^{-1}$)	$U_{assim\text{-}PUC}$ ($mg\ N \cdot m^{-2} \cdot d^{-1}$)	N storage (% of added ^{15}N)
Closed canopy							
PRPN	0.001	0.71	0.001	63	22.9	11.1	50.7
POPN	0.01	0.32	0.04	108	13.3	8.3	75.8
BBNH	0.20	6.90	0.03	14	39.1	11.5	71.3
GCNM	0.40	6.70	0.06	21	59.5	57.9	26.2
SCSP	0.42	7.31	0.06	66	10.4	13.2	84.4
ECMI	0.80	6.40	0.13	1,351	35.0	24.8	10.2
WBTN	1.20	5.40	0.22	23	25.7	21.9	44.1
UBNC	0.06	29.00	0.002	30	192.7	35.1	5.0
MCOR	1.90	11.00	0.17	55	34.3	19.7	27.8
QBPR	0.07	7.80	0.01	26	22.2	17.2	29.2
Mean (SE)	0.51 (0.20)	8.15 (2.53)	0.07 (0.02)	176 (131)	45.5 (16.9)	22.1 (4.7)	42.5 (8.7)
Open canopy							
SCAZ	15.00	8.30	1.81	47	39.2	88.3	72.8
SBIC	1.91	2.02	0.95	123	349.2	342.8	42.2
E1AK	1.10	0.30	3.67	40	15.1	6.3	5.1
KCKS	1.80	2.40	0.75	56	55.4	56.9	18.7
LIDK	1.65	5.29	0.31	303	447.0	71.4	4.2
KTNZ	1.29	1.31	0.98	25	185.8	51.7	6.3
KGNZ	1.11	0.63	1.77	22	135.4	40.7	10.8
Mean (SE)	3.41 (1.94)	2.89 (1.09)	1.46 (0.42)	88 (38)	175.3 (62.5)	94.0 (42.6)	22.9 (9.7)
P	**0.013**	0.126	**0.0002**	0.900	**0.029**	**0.023**	0.090

Notes: Stream metabolism is reported as gross primary production (GPP), ecosystem respiration (ER), and their ratio (GPP:ER). Nitrogen spiraling metrics at the reach-scale were derived in two ways: from water column measurements (uptake length [S_w] and assimilatory NH_4^+-N uptake [$U_{assim\text{-}WAT}$]) and from summed primary uptake compartments (PUC) expressed as assimilatory NH_4^+-N uptake ($U_{assim\text{-}PUC}$) and N storage (percentage of added ^{15}N). Mean and standard error of the mean (SEM) for each variable are given for each stream category, and statistical results from independent *t* tests comparing the two stream categories are shown in the bottom row with significance highlighted in boldface type.

when we scaled NH_4^+-N uptake by N biomass in each compartment ($mg\ N \cdot mg\ N^{-1} \cdot d^{-1}$), which equates to a compartment-specific turnover rate (d^{-1}), there were even clearer differences between autotrophic and heterotrophic uptake compartments; biomass-specific NH_4^+-N uptake by primary producers, was significantly higher than compartments dominated by heterotrophic organisms colonizing allochthonous detritus (ANOVA, $P < 0.0001$; Fig. 3C). Despite differences in NH_4^+-N uptake between autotrophs and heterotrophs, N storage as a percentage of total ^{15}N stored was generally similar among uptake compartments (Fig. 3D). Data pooled across streams showed no strong patterns in the relative contribution to reach-scale N storage between autotrophic and heterotrophic compartments, although there was a significant difference between FBOM and leaves (ANOVA, $P = 0.010$; Fig. 3D).

Delving deeper into the trends among the three major heterotrophic uptake compartments, estimates of biomass-specific NH_4^+-N uptake (i.e., turnover) were strongly influenced by the mass of "dead N" in these pools. As an alternative to the bulk estimate of compartmental N turnover, we estimated the role of active microbial N in ^{15}N-NH_4^+ uptake using data from chloroform fumigation applied in three of the closed-canopy, forested streams (UBNC, WBTN, and BBNH; Sanzone et al. 2001). Isolating the ^{15}N signature in the microbial N colonizing detritus showed that microbial N as percentage of total N averaged 12% for leaves, 4% for FBOM, and 8% for wood biofilms (in outer 2 mm of small wood; Sanzone et al. 2001). We then applied these percentages to estimate assimilatory NH_4^+-N uptake associated only with the "live N" microbial biomass in heterotrophic compartments. Rescaled microbial biomass-specific NH_4^+-N uptake rates, and hence N turnover rates, were similar to those for autotrophic compartments (leaves = 0.09, wood = 0.04, and FBOM = 0.14 mg N per mg microbial N per day; Fig. 3C, noted as colored dashes).

Summed compartmental NH_4^+-N uptake accounted for the majority of water-column NH_4^+-N removal, and we found a significant relationship between $U_{assim\text{-}WAT}$ and $U_{assim\text{-}PUC}$ (Fig. 4). In general, $U_{assim\text{-}PUC}$ values were close to or slightly lower than $U_{assim\text{-}WAT}$, with the closed-canopy streams grouping in the lower end of this continuum. The slope of the relationship (linear regression; $\log U_{assin\text{-}WAT} = 0.41 + 0.89 \log U_{assim\text{-}PUC}$, $r^2 = 0.62$, $P = 0.0002$; Fig. 4) was not significantly different from a

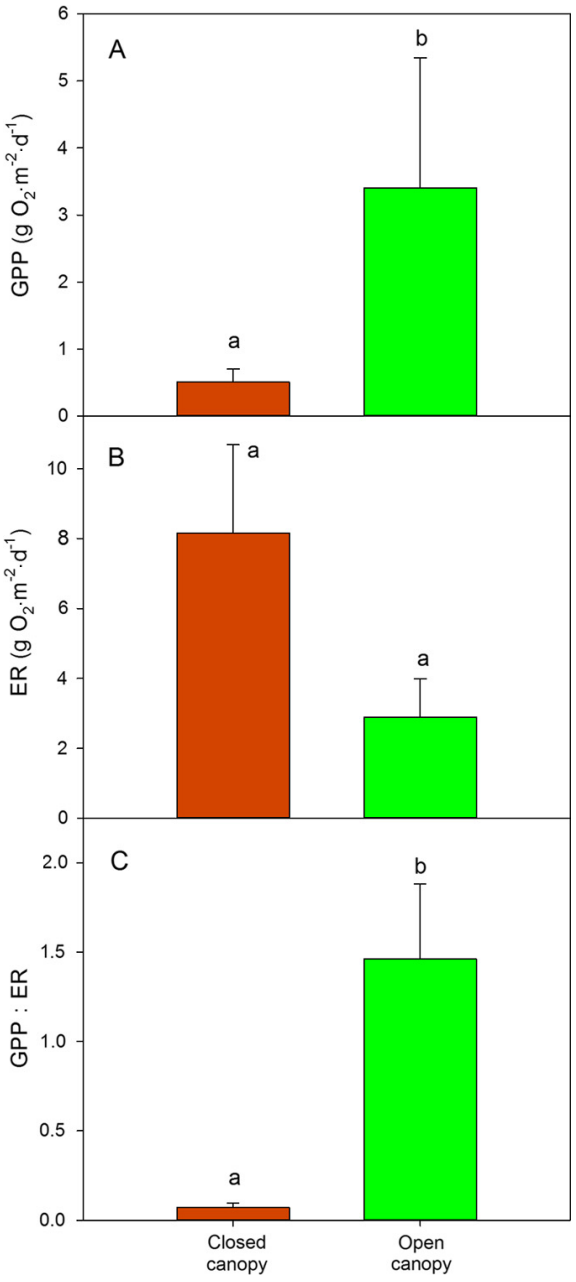

FIG. 1. Comparison of (A) gross primary production (GPP), (B) ecosystem respiration (ER), and (C) GPP:ER between open- and closed-canopy streams reported as mean (+SE). Different lowercase letters above bars indicate significant differences ($P \leq 0.05$) as reported in Table 3.

slope of 1 (ANCOVA $P = 0.519$) but the relationship is shifted above the 1:1 suggesting that $U_{\text{assim-WAT}}$ was generally higher than that accounted for by $U_{\text{assim-PUC}}$.

The results from the BMA analysis and model selection for $U_{\text{assim-PUC}}$, $U_{\text{assim-auto}}$, and $U_{\text{assim-hetero}}$ (Fig. 5A–C) show that predictor variables differed depending on the N uptake metric considered. We found that the magnitude of $U_{\text{assim-PUC}}$ was negatively related to Q/w, DIN: SRP, and water temperature, while it was positively related to water velocity, NH_4^+-N, and depth (Fig. 5A; Table 4). When considering $U_{\text{assim-PUC}}$ by autotrophic and heterotrophic compartments separately, we found contrasts in significant predictors included in each model. For autotropic uptake, as $U_{\text{assim-auto}}$, Q/w and water velocity remained as important negative and positive predictors, respectively, but canopy cover was also negatively related to $U_{\text{assim-auto}}$, and as such, GPP emerged as an important driver for autotrophic assimilation compared to the model for all compartments (i.e., $U_{\text{assim-PUC}}$; Fig. 5B, Table 4). In contrast, partitioning only heterotrophs, $U_{\text{assim-hetero}}$ did not have any strong predictors, although a positive relationship with ER had the most support from the BMA analysis (Fig. 5C, Table 4). In general, the drivers of $U_{\text{assim-PUC}}$ were more similar to the drivers of $U_{\text{assim-auto}}$ than $U_{\text{assim-hetero}}$ (Fig. 5).

To summarize the relationship between metabolism and N assimilation, we examined the ratio of autotrophic to heterotrophic N storage relative to metabolism; more N was stored in autotrophic compared to heterotrophic uptake compartments when GPP:ER was high (Pearson correlation, $r = 0.80$, $P < 0.05$; Fig. 6). Additionally, the ratio of autotrophic:heterotrophic N storage was also significantly higher in open- compared to closed-canopy streams (t test, $P = 0.034$; Fig. 6 inset).

To summarize the results, we partitioned metabolism and N demand by autotrophic and heterotrophic uptake compartments for open- and closed-canopy streams via a quantitative conceptual model (Fig. 7). As might be expected, the synthesis revealed that autotrophs were key drivers of assimilatory N uptake and storage in open-canopy streams where they dominated assimilatory uptake (80%) and storage (66%; Fig. 7). Yet their role was also prominent in closed-canopy streams. For example, in these shaded systems (reflected in GPP:ER), only 10% of N biomass was found in autotrophs, however, they comprised almost one-half (42%) of the assimilatory NH_4^+-N uptake. Regardless of canopy type, autotrophic uptake compartments play a disproportionate role in assimilatory NH_4^+-N uptake relative to their biomass, which likely impacted N storage, as we ultimately found no differences in closed- vs. open-canopy streams (Fig. 7).

DISCUSSION

Inorganic N is generally stored and tightly cycled in ecosystems where there is high biological demand relative to supply (Vitousek et al. 1997, Galloway et al. 2008). Previous research suggests that assimilatory N uptake can account for a large fraction of the inorganic N removal from the water column in streams (Peterson et al. 2001, Mulholland et al. 2008). Specifically, for NH_4^+-N uptake, assimilatory uptake generally exceed nitrification (Peterson et al. 2001). Thus examining the magnitude and controls on assimilatory NH_4^+-N uptake and storage, as described in this manuscript, as well as its transfer to stream consumers (Norman et al., *in press*) is

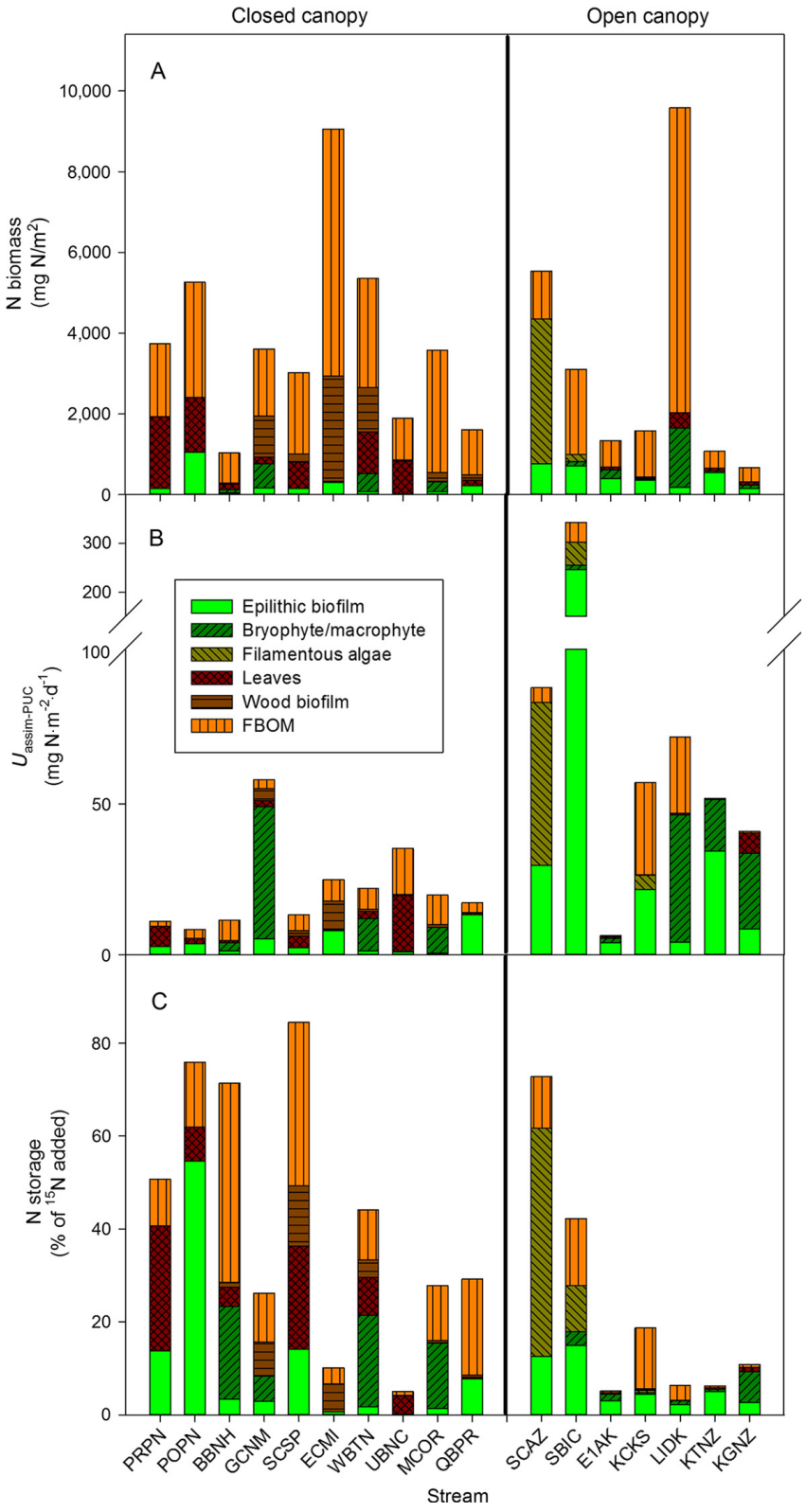

FIG. 2. Reach-scaled values for (A) N biomass, (B) assimilatory NH_4^+-N uptake for primary uptake components ($U_{assim-PUC}$), and (C) N storage as a percentage of total added ^{15}N in each stream. Different fill in each bar represents the relative contribution of each primary uptake compartment to reach-scaled totals in each stream as reported in Table 3 for $U_{assim-PUC}$ and N storage. Streams are grouped into open- and closed-canopy categories. See Table 1 for stream IDs. FBOM, fine benthic organic matter.

critical for understanding how streams regulate downstream N transport. This synthesis from 17 ecosystem-scale ^{15}N tracer additions quantifies the contribution of autotrophic and heterotrophic uptake compartments to assimilatory N uptake in headwater streams and shows that autotrophic compartments account for a larger proportion of N uptake than predicted based on standing stocks alone. Additionally, the simple categorical variable of canopy cover explained differences in assimilatory N uptake among streams at the reach scale, as well as how stream metabolism (as GPP and ER) mediates the relative roles that autotrophs and heterotrophs play in N storage. This analysis links cumulative N uptake by stream biota to reach-scale N demand and provides a mechanistic and predictive framework for estimating and modeling N cycling across stream ecosystems.

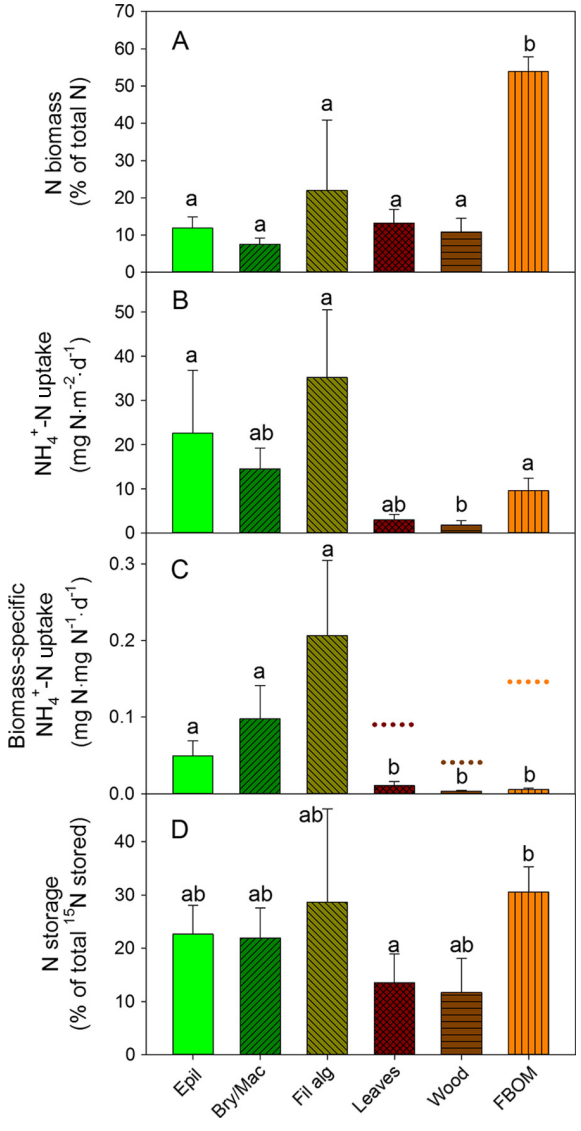

FIG. 3. Comparison of compartment-specific uptake and contributions to (A) reach-scaled total N biomass, (B) NH_4^+-N uptake, (C) biomass-specific NH_4^+-N uptake, and (D) relative contribution to reach-scale N storage, expressed as a percentage of total ^{15}N stored, and reported as mean (+SE) across all streams. Different letters over bars indicate significant differences among primary uptake compartments based on ANOVA with post hoc Tukey test ($P < 0.05$). Uptake compartments are abbreviated as Epil, epilithic biofilm; Bry/Mac, bryophytes and macrophytes; Fil alg, filamentous algae; Leaves, biofilm on decomposing leaves; Wood, biofilm on small wood; FBOM, fine benthic organic material. Additionally, for panel C, dashed lines above bars for Leaves, Wood, and FBOM indicate recalculated biomass-specific NH_4^+-N uptake based on microbial N biomass (in mg N·[mg microbial N]$^{-1}$·d^{-1}) using estimates from Sanzone et al. (2001).

Reach-scale assimilatory NH_4^+-N uptake, expressed as the sum of all compartmental uptake ($U_{assim\text{-}PUC}$), was on average four times higher in open- than in closed-canopy streams (Fig. 7) and our analysis shows that the factors controlling $U_{assim\text{-}PUC}$ are similar to those explaining autotrophic uptake (i.e., $U_{assim\text{-}auto}$; Fig. 5A, B). Previous studies have linked inorganic N uptake to primary producers, even in low-light, closed-canopy streams. For example, algal blooms occurring prior to leaf-out and canopy closure in early spring increased N uptake in forested streams (Hoellein et al. 2007, Roberts and Mulholland 2007). Short-term solute addition experiments quantifying reach-scale N removal from the water column also found that open-canopy systems had higher NH_4^+-N uptake (Sabater et al. 2000), but the role of autotrophs was only inferred by correlative analyses with standing stocks and GPP. Hall and Tank (2003) correlated GPP with reach-scale NO_3^- demand in multiple open-canopy streams in northwest Wyoming, yet they could not identify significant correlations with any estimate of biomass (e.g., chlorophyll *a*, epilithon AFDM), likely because structural metrics are often a poor indicator of biological activity. Additionally, the Wyoming study was limited to open-canopy streams, and the constrained environmental context highlights the need for broader cross-site comparisons, while simultaneously offering a theoretical foundation for the current synthesis linking metabolism to assimilatory N demand.

Ecosystem metabolism and N biomass

In this synthesis, the difference in GPP among the study streams spans the ranges reported in previously published stream metabolism studies (Mulholland et al. 2001, Bernot et al. 2010, Marcarelli et al. 2011, Hoellein et al. 2013, Hall et al. 2016), suggesting that these 17 systems are representative of the wide metabolic spectrum found in headwater streams. Not surprisingly, GPP was highest in open-canopy streams, and this was reflected in higher N biomass of autotrophic uptake compartments (Fig. 7), a pattern also reflected in comparisons of biomass-specific N uptake (i.e., turnover rate) between autotrophs and heterotrophs. In contrast to GPP, ER did not differ significantly between open- and closed-canopy streams (Table 3), likely due to the dominant contribution of FBOM at all sites. Moreover, the similarity in ER likely reflects similarities in N biomass and storage in open- and closed-canopy streams, even though the

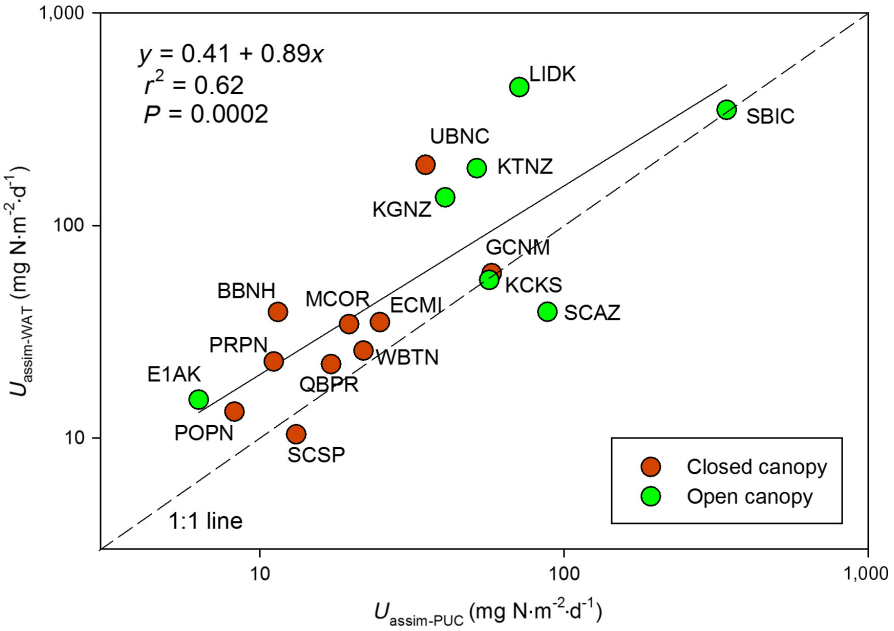

FIG. 4. Log-log relationship between reach-scale assimilatory NH_4^+-N uptake measured from the water column ($U_{assim\text{-}WAT}$) and assimilatory NH_4^+-N uptake summed from primary uptake compartments ($U_{assim\text{-}PUC}$). Solid line represents results from linear regression, with equation for the regression model based on log-transformed data noted on the graph. The dashed line represents the 1:1 relationship, and brown (dark) and green (light) symbols represent open- and closed-canopy streams, respectively. See Table 1 for stream IDs.

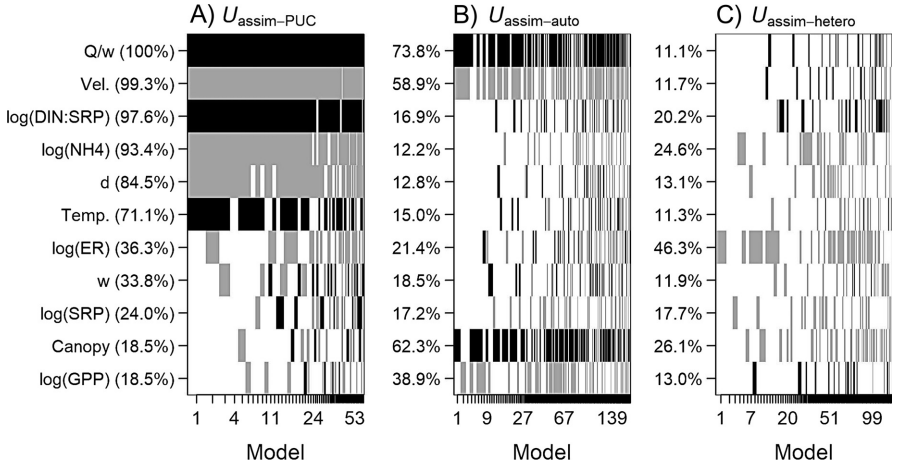

FIG. 5. Model structure of Bayesian Model Averaging output for (A) $U_{assim\text{-}PUC}$, (B) $U_{assim\text{-}auto}$, and (C) $U_{assim\text{-}hetero}$. The *x*-axis is model number ordered by decreasing posterior probability (i.e., model 1 is the "best" model based upon Bayesian Information Criterion, BIC). The number of models included is based on the number of models falling within 1/20th of the posterior probability of the best model. The *y*-axis represents all variables included in the full model, ordered by the probability of inclusion in the final subset of $U_{assim\text{-}PUC}$ models. The identity of the variable, whether or not it was transformed, and the probability of inclusion in the final subset of models are listed in *y*-axis for panel A. Cells within the plot are shaded based on their status in the model structure: black cells denote the variable is included and has a negative estimate, gray cells indicate the variable is included and has a positive estimate, and white cells indicate the variable is not included in that specific candidate model. For panels B and C, variables are arranged in the same order as for panel A on the *y*-axis to highlight differences among the models, with the probability of inclusion provided for each variable. *Q*, discharge; *w*, stream width; DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; *d*, water depth; Temp., temperature; ER, ecosystem respiration; GPP, gross primary production.

primary uptake compartments contributing to ER (i.e., autotrophs vs. heterotrophs) may change with riparian canopy cover (Bernot et al. 2010, Riley and Dodds 2012).

The range of GPP:ER we observed (0.001–3.7) was similar to the range reported by Marcarelli et al. (2011) based on a review of data from 229 streams. In our analysis,

TABLE 4. Results from Bayesian Model Averaging (BMA) for summed compartment-specific NH_4^+-N uptake ($U_{assim\text{-}PUC}$), autotrophic uptake ($U_{assim\text{-}auto}$), and heterotrophic uptake ($U_{assim\text{-}hetero}$).

	$U_{assim\text{-}PUC}$			$U_{assim\text{-}auto}$			$U_{assim\text{-}hetero}$		
Variable	Pr (Inclusion) (%)	Estimate	SD	Pr (Inclusion) (%)	Estimate	SD	Pr (Inclusion) (%)	Estimate	SD
Intercept	**100**	**1.6**	**0.17**	**100**	**1.33**	**0.44**	**100**	**0.61**	**0.44**
Q/*w*	**100**	**−0.54**	**0.09**	**73.8**	**−0.59**	**0.49**	11.1	−0.02	0.14
Velocity	**99.3**	**0.04**	**0.01**	58.9	0.03	0.03	11.7	0	0.01
log (DIN:SRP)	**97.6**	**−0.37**	**0.14**	16.9	−0.04	0.16	20.2	−0.07	0.22
log(NH_4^+)	**93.4**	**0.23**	**0.11**	12.2	0.02	0.12	24.6	0.12	0.29
Depth	**84.5**	**0.01**	**0.01**	12.8	0	0.01	13.1	0	0.01
Temperature	**71.1**	**−0.01**	**0.01**	15	0	0.01	11.3	0	0.01
log(ER)	36.3	0.04	0.07	21.4	0	0.14	46.3	0.21	0.29
Width	33.8	0	0.04	18.5	−0.01	0.05	11.9	0	0.04
log(SRP)	24	0	0.07	17.2	0.04	0.14	17.7	0.05	0.18
Canopy cover	18.5	0	0.06	**62.3**	**−0.5**	**0.5**	26.1	0	0.51
log(GPP)	18.5	0	0.02	38.9	0.1	0.16	13	−0.01	0.07

Notes: We list all variables that could potentially be included in the BMA analysis, along with the probability of each variable being included in the models of the best subset and the model-averaged estimate and standard deviation (SD) for each variable. Variables with a SD that did not overlap zero are denoted in boldface type. *Q*, discharge; *w*, stream width; DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; ER, ecosystem respiration; GPP, gross primary production.

GPP:ER differed between the two stream types, being very low (i.e., highly heterotrophic) in closed-canopy streams, and higher (i.e., more autotrophic) in open-canopy streams (Fig. 7). In an expanded meta-analysis by Hall et al. (2016) that included rivers, they showed increasing GPP:ER as canopy cover opens with increasing system size. Shaded headwater streams generally have ER ≫ GPP and theory predicts that ER should be lower in open-canopy systems as allochthonous organic matter inputs decline. Yet for open canopy systems, it has been recently suggested that that the degree to which GPP is greater than ER is constrained by an upper limit, with most systems, even open-canopy ones, having GPP:ER < 1. As GPP increases, so does ER via the additional contribution of autotrophic respiration along with that of system heterotrophs (Hall and Beaulieu 2013). We saw a similar compensatory dynamic influencing other ecosystem metrics as well. For example, there were no differences in total N biomass of primary uptake compartments between open- and closed-canopy streams. Looking closer, in closed-canopy streams N biomass was dominated by heterotrophs, while in open-canopy streams, the distribution of N biomass was more equally-partitioned between autotrophs and heterotrophs (Fig. 7).

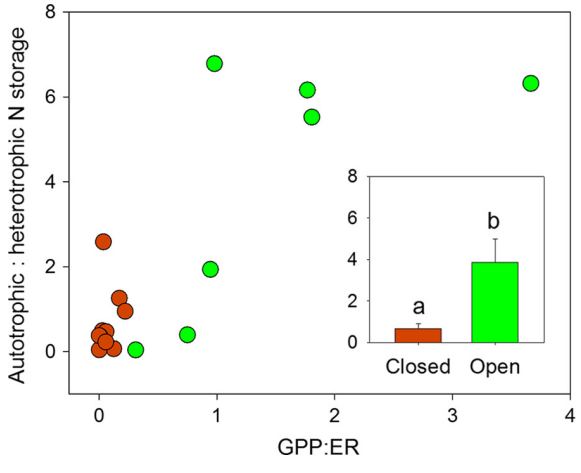

FIG. 6. Relationship between the ratio of autotrophic:heterotrophic N storage and GPP:ER. The inset shows the mean (+SE) of the ratio of autotrophic:heterotrophic N storage for open- and closed-canopy streams. Different letters indicate differences in the mean (+SE) between categories of stream canopy cover (*t* test, $P = 0.003$, $t = -3.406$) with brown (dark) and green (light) symbols representing open- and closed-canopy streams, respectively. See Table 1 for stream IDs.

Patterns of compartmental NH_4^+-N uptake

Synthesis across streams also showed that autotrophic uptake compartments play a disproportionate role in assimilatory NH_4^+-N uptake relative to their biomass regardless of canopy cover (Fig. 7). Higher N demand in autotrophic primary uptake compartments was also shown in previous studies of compartment-specific metabolism (Fellows et al. 2006, Acuña et al. 2011). For heterotrophic compartments, most of the N biomass consists of detrital organic matter colonized by a thin layer of live microbial biofilm, and as such, only a small proportion of total N biomass of heterotrophic compartments is actively assimilating inorganic N from the water column (Suberkropp and Chauvet 1995). This contrasts with autotrophic uptake compartments, which are dominated by live biomass actively assimilating N from the water column rather than deriving it from organic matter (Dodds et al. 2014). Autotrophic compartments generally have much lower C:N ratios (i.e., higher N content), and much of the N content is actively cycled, hence N turnover in autotrophs is generally faster (Dodds et al. 2004, but see Ashkenas et al. 2004).

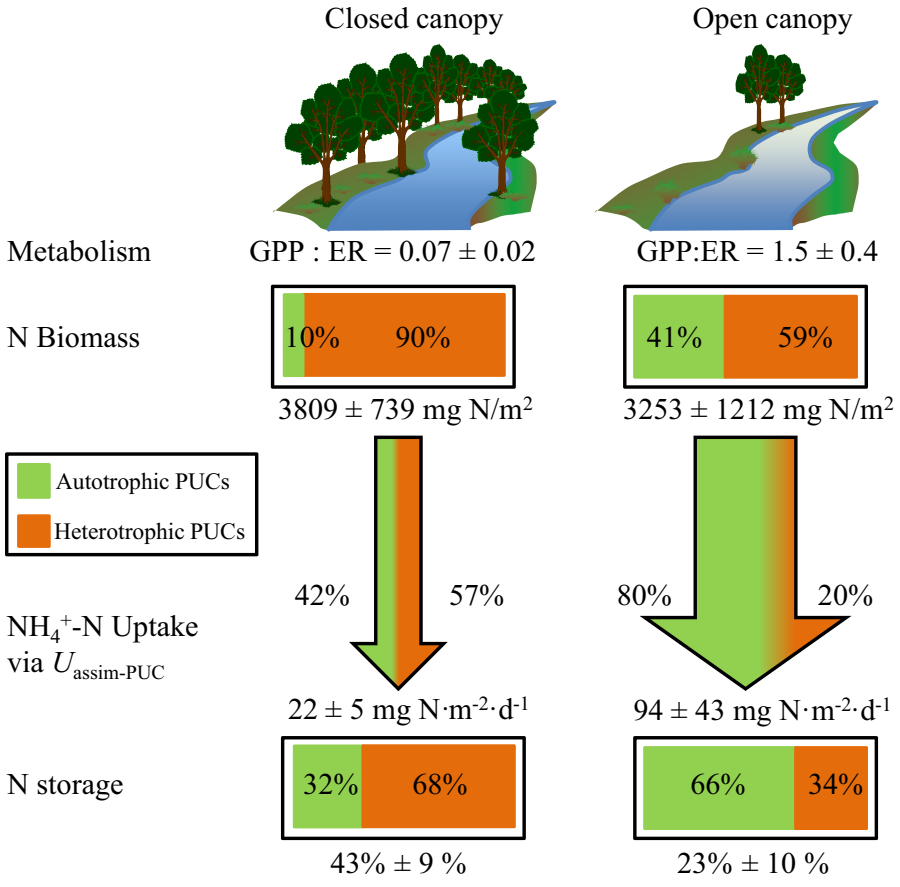

FIG. 7. Summarized differences (mean ± SEM) between open- and closed-canopy streams for reach-scale metabolism as GPP : ER, N biomass (mg N/m^2 from Fig. 2A), assimilatory NH_4^+-N uptake by primary uptake compartments ($U_{assim\text{-}PUC}$ from Table 3), and N storage (percentage of added ^{15}N, from Table 3). For each metric, orange and green shading, and the associated percentages, reflect relative contribution of autotrophic and heterotrophic uptake compartments. In general, autotrophic biota play a disproportionate role in assimilatory N uptake and storage relative to their biomass.

We also showed how the high proportion of detrital N in heterotrophic compartments strongly influences estimates of compartmental turnover rates, as reflected in biomass-specific N uptake. Based on data from Sanzone et al. (2001), we estimated biomass-specific N uptake rates for only the live microbial N (Fig. 3C) and found that rescaled assimilatory N uptake for heterotrophic compartments is similar to autotrophic compartments. This reframing to account for the role of microbial N assimilation alters our interpretation of the relative activity of heterotrophic compartments compared to autotrophs who appear to be the "biological engines" of stream N cycling. Yet accounting for only live N in turnover estimations results in a biological parity between autotrophic and heterotrophic uptake compartments.

Finding environmental or physicochemical predictor variables that explained variation in $U_{assim\text{-}PUC}$ across the 17 streams was surprisingly challenging. For example, despite our inclusion of sites from a wide range of biomes, stream temperature did not explain variation in assimilatory N uptake, which was unexpected given that temperature has been shown to influence stream metabolism (Acuña et al. 2008, Valett et al. 2008, Demars et al. 2011). Nevertheless, our BMA approach allowed us to find predictive relationships that would be expected given the biology of these systems. For example, $U_{assim\text{-}PUC}$ increased with NH_4^+-N but decreased with DIN : SRP. Increased NH_4^+-N availability should lead to increased $U_{assim\text{-}PUC}$ only until another factor, such as P availability or organic matter quality becomes limiting, thus NH_4^+-N uptake under those environmental conditions is "saturated." A loss in efficiency of inorganic N uptake, approaching saturation, has been identified previously using both empirical (Dodds et al. 2002, Newbold et al. 2006, O'Brien et al. 2007, Ribot et al. 2013) and modeling approaches (Helton et al. 2011), but here we isolate the apparent saturation effect by specifically linking it to assimilatory N uptake by primary uptake compartments.

The saturation of inorganic N uptake is also important in the context of watershed management because when assimilatory demand is saturated, downstream export of excess DIN is higher. Nevertheless, assimilatory N uptake

by biotic compartments should be considered as a temporary storage of bioreactive N. Eventually, much of the assimilated N spirals downstream, as an organic particle, dissolved organic N (Johnson et al. 2009), or once again mineralized as inorganic N. As such, assimilatory N uptake is a "transient storage of N" that delays and alters the form of downstream N export. This delay and change in form also affects local conditions, influencing the degree of inorganic N limitation, and can result in stimulation of N fixation (Grimm and Petrone 1997) when N availability is low due to this assimilatory demand. Our modelling also emphasized the importance of physical and hydraulic conditions in controlling assimilatory N uptake. For example, $U_{\text{assim-PUC}}$ increased with increasing contact between overlying streamwater and biota (i.e., negative relationship with Q/w and positive relationship with velocity, suggesting an overall negative relationship with water depth). Previous research has shown that increased velocity reduces the thickness of the diffusive boundary layer (Bishop et al. 1997) with concurrent increases in assimilatory N uptake (Arnon et al. 2013, Peipoch et al. 2016). Finally, by altering the timing of N export downstream, assimilatory uptake can potentially influence the trophic status of coastal ecosystems that are often sensitive to delivery of excess N from upstream watersheds (Diaz and Rosenberg 2008, Rabalais et al. 2010).

We also show differences across streams in N removal from the water column ($U_{\text{assim-WAT}}$), which was likely a response to differences in physicochemical conditions such as ambient nutrient concentrations (Dodds et al. 2002, Newbold et al. 2006), stream-specific biota, and riparian canopy cover (Sabater et al. 2000). Summing compartment-specific NH_4^+-N uptake to the reach-scale (i.e., $U_{\text{assim-PUC}}$) matched reasonably well with $U_{\text{assim-WAT}}$ (Fig. 4), although $U_{\text{assim-WAT}}$ was generally higher than $U_{\text{assim-PUC}}$. Given that we already accounted for NH_4^+-N uptake due to dissimilatory processes (e.g., nitrification), this mismatch was likely due to inaccuracies in the upscaling of assimilatory uptake, as well as the omission of the probable role of microbial uptake in the hyporheic zone (Hall et al. 2009*a*) and/or in the water column (Reisinger et al. 2015). These two uncertainties are deserving of further study. Nevertheless, the upscaling of compartment-specific uptake has been successfully applied in the past; denitrification assays conducted by habitat type in microcosms were scaled to the stream reach, and approximated whole-reach denitrification determined using ^{15}N-NO_3^-tracer additions (Findlay et al. 2011). We conclude that compartment-specific measurements are insightful for exploring mechanisms and identifying controls on reach-scale phenomena as reflected in water column nutrient removal.

Contribution of primary uptake compartments to N storage: mass balance and metabolic drivers

Using ^{15}N tracer data, we tracked the outcome of assimilatory NH_4^+-N uptake into reach-scale N storage. Across the 17 streams, which included most of the ^{15}N-NH_4^+ tracer addition experiments conducted to date, the percentage of ^{15}N-NH_4^+ temporarily stored via assimilatory N uptake into biomass was variable, ranging from 4% to 84% (mean = 36%), with no apparent pattern in the variation in ^{15}N storage (Table 3). The ^{15}N that is not stored in biomass can be exported downstream in various forms: ^{15}N-NH_4^+ that moves through the system untransformed; ^{15}N-NO_3^- as a product of nitrification, which can range from <5% in UBNC to 57% in QBPR (Peterson et al. 2001); or dissolved or particulate organic ^{15}N export, which has been challenging to quantify due to often episodic transport (Mulholland et al. 2000, Tank et al. 2000). Nevertheless, the lack of in stream retention (rather than "recovery") emphasizes that streams are dominated by flow-through processes, and fueled by consistent delivery from upstream.

Similar variability in ^{15}N storage was found using ^{15}N-NO_3^- tracer additions in 72 streams for the LINXII study (Mulholland et al. 2008), where <50% of the ^{15}N added was accounted for in almost half of the study streams (LINXII project, *unpublished data*). In addition, Hall et al. (2009*a*) could only account for 13% and 42% of ^{15}N-NO_3^- added in two different 14-d additions in an Idaho mountain stream, conducted during snowmelt and baseflow conditions, respectively. In this case, low ^{15}N recoveries were attributed in part to storage in the hyporheic zone. In addition, some studies have shown that assimilated N is stored in the adjacent riparian zone, either via uptake by plant roots (Ashkenas et al. 2004, Schade et al. 2005) or via transfer into riparian food webs by predators (Sanzone et al. 2003). To accurately account for the importance of assimilatory N uptake in streams, we not only need to quantify compartment-specific N biomass and activity, but we also need to understand the role of riparian and hyporheic storage, as well as N mineralization and subsequent export. At longer time scales, future research is needed in order to understand the ultimate fate of stored N in light of seasonal differences in biological processes (e.g., canopy leaf out or abscission) and to explore the role of hydrologic extremes (e.g., floods or drought).

Patterns in N storage in autotrophic and heterotrophic compartments could not be explained with any single predictor variable related to environmental conditions (e.g., Table 2) while integrative functional metrics such as reach-scale metabolism (as GPP:ER) were best at explaining differences. The ratio of autotrophic:heterotrophic N storage was related to the relative role of autotrophic to heterotrophic metabolism (Fig. 6), so more N was stored in autotrophic compared to heterotrophic primary uptake compartments when GPP:ER was high. In fact, the ratio of autotrophic to heterotrophic N storage was significantly higher in open- compared to closed-canopy streams (Fig. 6 inset). We also suggest that this trend may hold for inorganic N in general, not just for NH_4^+-N. For example, in the 72 LINXII streams where 24-h ^{15}N-NO_3^- tracer addition experiments were conducted, N storage in autotrophic compartments was also positively related to

GPP (Hall et al. 2009*b*). The storage of inorganic N removed from the water column, partitioned between autotrophic and heterotrophic organisms, is thus a reflection of reach-scale metabolic activity (i.e., GPP and ER) rather than by compartmental biomass per se, and we show here that metabolism in turn is strongly influenced by riparian canopy cover.

Conclusion

Recent research has shown that assimilatory N uptake into stream biomass can account for most of the N removal from the water column (Mulholland et al. 2008) but the relative roles of autotrophic vs. heterotrophic biota in assimilatory N uptake had not been comprehensively analyzed to derive broad generalizations. In addition, most of the studied systems were closed-canopy streams and drivers such as GPP and canopy cover were limited in their range (Webster et al. 2003). We demonstrate that assimilatory N uptake is driven by metabolism, autotrophs disproportionately contribute to N assimilation relative to their biomass, and that heterotrophs must be separated from the bulk organic matter in order for their N assimilation rates to be similar to primary producers. This unique synthesis of data from 17 ^{15}N tracer additions conducted in a wide range of streams around the world provides a framework that links N demand by individual biota to reach-scale N uptake, and that canopy cover is a major determinant of N assimilation. The resulting conceptual model (Fig. 7) provides a foundation for comparison of N cycling in other stream ecosystems or to support future modeling efforts implemented from regional to global scales.

Our analysis highlights unanswered questions regarding the return of N to the water column following senescence and turnover of various biotic compartments (Dodds et al. 2004) as well as the role of consumers in trophic N transfer within food webs (Whiles et al. 2013). Understanding the impact of biota on temporal patterns in stream N concentrations is dependent on N assimilation, release, and trophic transfer, and these remain poorly linked in stream ecosystems (but see Norman et al. 2017).

We also found significant variation both among streams and among compartments in assimilatory NH_4^+-N uptake and N storage, yet there was a tight coupling between ecosystem metabolism and assimilatory N uptake through primary uptake compartments (Fig. 6). In future, the strength (and potential generality) of this relationship should be tested with forthcoming ^{15}N tracer additions perhaps using other forms of dissolved N (e.g., $^{15}N\text{-}NO_3^-$, $DO^{15}N$). Nevertheless, the relationship between ecosystem metabolism and assimilatory N uptake opens the door for the use of long-term, low-cost deployments of oxygen sensors to measure continuous metabolism, which could be used to estimate and partition assimilatory N storage in stream and river networks.

Finally, the relative role of autotrophs vs. heterotrophs is mediated by canopy cover via influences on light and organic matter availability, emphasizing the importance of environmental context (sensu Janetski et al. 2009) in interpreting patterns in assimilatory N uptake. Human-induced changes in local environmental conditions, such as the alteration of canopy cover (e.g., logging or riparian planting), should be considered when modeling and managing N dynamics in stream and river networks, as they will modify assimilatory N uptake thereby altering the form and timing of downstream N export.

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Data Availability

Data are available from CurateND, a service of the Hesburgh Libraries at the University of Notre Dame: https://doi.org/10.7274/r0mg7mjp