

Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study

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Abstract. Nutrient uptake length is an important parameter for quantifying nutrient cycling in streams. Although nutrient tracer additions are the preferred method for measuring uptake length under ambient nutrient concentrations, short-term nutrient addition experiments have more frequently been used to estimate uptake length in streams. Theoretical analysis of the relationship between uptake length determined by nutrient addition experiments (S_w') and uptake length determined by tracer additions (S_w) predicted that S_w' should be consistently longer than S_w , and that the overestimate of uptake length by S_w' should be related to the level of nutrient addition above ambient concentrations and the degree of nutrient limitation. To test these predictions, we used data from an interbiome study of NH_4^+ uptake length in which $^{15}\text{NH}_4^+$ tracer and short-term NH_4^+ addition experiments were performed in 10 streams using a uniform experimental approach. The experimental results largely confirmed the theoretical predictions: S_w' was consistently longer than S_w and $S_w':S_w$ ratios were directly related to the level of NH_4^+ addition and to indicators of N limitation. The experimentally derived $S_w':S_w$ ratios were used with the theoretical results to infer the N limitation status of each stream. Together, the theoretical and experimental results showed that tracer experiments should be used whenever possible to determine nutrient uptake length in streams. Nutrient addition experiments may be useful for comparing uptake lengths between different streams or different times in the same stream, however, provided that nutrient additions are kept as low as possible and of similar magnitude.

Key words: stream, ammonium, uptake length, nutrient cycling, nutrient spiraling, nitrogen limitation.

Nutrient cycling is an important ecosystem process. In streams, nutrient availability often limits the growth rate of stream algae and heterotrophic microbes, and the decomposition rate of allochthonous organic matter (Elwood et al.

1981, Rosemond et al. 1993, Suberkropp and Chauvet 1995). The concept of nutrient spiraling has been developed to describe the simultaneous processes of nutrient cycling and downstream transport (Webster and Patten 1979, Newbold et al. 1981). One of the principal indices of nutrient spiraling is nutrient uptake

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length, defined as the mean distance traveled by a nutrient atom dissolved in water before uptake by biota or sorption to particulate matter (Newbold et al. 1981).

Uptake length is a measure of nutrient use efficiency in streams (i.e., nutrient uptake relative to supply), rather than of nutrient uptake rate per se. Uptake length is the inverse of the fractional rate of nutrient uptake from water per unit stream length and, thus, has units of distance. The preferred method for measuring uptake length is by nutrient tracer additions that maintain ambient nutrient concentrations (Newbold et al. 1981, 1983a, Mulholland et al. 1985). Tracers (e.g., radioisotopes such as ^{32}P , stable isotopes such as ^{15}N) can be difficult or expensive to use, however. As a result, short-term nutrient additions have been used as an alternative to tracer additions for estimating uptake length (Stream Solute Workshop 1990).

A growing number of studies have used short-term nutrient additions for comparisons of uptake length within and among streams (Munn and Meyer 1990, Martí and Sabater 1996, Davis and Minshall 1999, Webster et al. 2000, Hall et al. 2002). In previous studies comparing tracer and nutrient addition approaches, however, Mulholland et al. (1990, 2000a) showed that the nutrient addition approach results in overestimates of uptake length for PO_4^{-3} , NH_4^+ , and NO_3^- . In addition, Hart et al. (1992) showed that estimates of PO_4^{-3} uptake length were longer when concentrations were increased during PO_4^{-3} addition experiments. These studies also showed that the degree of overestimation of uptake length was positively related to the increase in nutrient concentration during the nutrient additions.

In this paper, we evaluate how closely uptake lengths determined by nutrient additions approximate uptake lengths determined by tracer additions. We present an analysis showing the theoretical relationship between uptake length determined by short-term nutrient additions and uptake length determined by tracer additions. Then, we compare measurements of NH_4^+ uptake length using nutrient and tracer additions in 10 streams in different biomes to determine what characteristics influence the magnitude of the difference between the different approaches (i.e. the overestimate of uptake length using the nutrient addition approach).

Theoretical Analysis

The relationship between uptake length estimated using short-term nutrient additions and uptake length determined by tracer additions can be understood by considering nutrient spiraling theory. Uptake length under ambient nutrient concentrations (S_w) is related to the downstream flux of nutrient in water (F_w , in units of mass/time) and uptake rate of nutrients from water (U , in units of mass area $^{-1}$ time $^{-1}$):

$$S_w = \frac{F_w}{wU} \quad (1)$$

where w is the average stream width (Newbold et al. 1981). To estimate uptake length using short-term nutrient additions (S_w'), nutrients are added to stream water, thereby increasing F_w and U , and the loss rate of the added nutrients from water per unit stream length is determined from the decline in the added nutrient concentration over the experimental stream reach (Mulholland et al. 1990). Because F_w is simply the product of nutrient concentration and discharge, it is evident from equation 1 that S_w' will be equivalent to S_w only if U increases in direct proportion to the increase in nutrient concentration during nutrient addition experiments. Thus, it is likely that S_w' would be equivalent to S_w only for those streams in which nutrients are strongly limiting and only for relatively low levels of nutrient addition.

The effects of nutrient limitation and the magnitude of nutrient addition on the relationship between S_w' and S_w are demonstrated by considering a model stream under different nutrient limitation scenarios. Nutrient uptake rate was assumed to follow Michaelis–Menten kinetics as follows:

$$U = \frac{CU_{\text{MAX}}}{C + K_s} \quad (2)$$

where C is nutrient concentration, U_{MAX} is the maximum nutrient uptake rate, and K_s is the half-saturation constant (i.e., the nutrient concentration at which U is $\frac{1}{2} U_{\text{MAX}}$). Although the Michaelis–Menten equation describes the relationship between nutrient uptake rate and concentration for individual species of algae and bacteria (Tilman 1982), it has been shown to approximate nutrient uptake kinetics for entire stream ecosystems as well (Bothwell 1989, Dodds et al. 2002).

Both S_w and S_w' can be calculated from equation 1, except that for calculation of S_w' the incremental values of F_w (ΔF_w) and U (ΔU) resulting from the nutrient addition are used instead of the ambient values for these terms. These incremental values are calculated as:

$$\Delta F_w = Q\Delta C \quad (3)$$

$$\Delta U = \frac{C'U_{MAX}}{C' + K_s} - \frac{CU_{MAX}}{C + K_s} \quad (4)$$

where Q is stream discharge, C' is the nutrient concentration in stream water during the nutrient addition (ambient concentration + added concentration), C is the ambient nutrient concentration, and ΔC is the increase in nutrient concentration resulting from the nutrient addition ($\Delta C = C' - C$). The substitution of ΔF_w and ΔU into equation 1 to calculate S_w' reflects the fact that S_w' is determined experimentally from the longitudinal rate of decline of the added nutrient concentration (ΔC) as described in Stream Solute Workshop (1990).

The model stream has a discharge rate of 10 L/s, an average width of 3 m, and a biological community with a U_{MAX} of $5 \mu\text{g m}^{-2} \text{s}^{-1}$ and a K_s of $20 \mu\text{g/L}$. The degree of nutrient limitation is defined by the value of C relative to K_s (Tilman 1982). Strong nutrient limitation exists when $C \ll K_s$ because U will increase sharply with an increase in C (equation 2). In contrast, weak nutrient limitation exists when $C > K_s$ because U will increase very little with an increase in C . In this analysis, we consider 3 nutrient limitation scenarios established by varying the ambient nutrient concentration, C :

Scenario 1 (strong limitation):

$$C = 2 \mu\text{g/L} \quad (=0.1 \times K_s)$$

Scenario 2 (moderate limitation):

$$C = 10 \mu\text{g/L} \quad (=0.5 \times K_s)$$

Scenario 3 (weak limitation):

$$C = 40 \mu\text{g/L} \quad (=2 \times K_s)$$

The effects of the magnitude of the nutrient concentration increase and nutrient limitation scenario on S_w' are shown in Fig. 1A. The relationship between S_w' and magnitude of the nutrient concentration increase (i.e., ΔC at the point of the nutrient addition) is linear for a given

nutrient limitation scenario, with the slope increasing with reduction in the severity of nutrient limitation. Values of S_w' are longer than those for S_w for all nutrient concentration increases and all nutrient limitation scenarios, although S_w' is only slightly longer than S_w for strong limitation with low nutrient concentration increases. This consistent over-prediction of uptake length by the nutrient addition approach is the result of nutrient uptake kinetics. As noted above, S_w' will be equivalent to S_w only if the increase in nutrient uptake with nutrient addition (ΔU) is directly proportional to the increase in nutrient concentration (ΔC). However, under Michaelis–Menten kinetics (or any saturation-type nutrient uptake kinetics) ΔU will be directly proportional to ΔC (i.e., $\Delta U/U = \Delta C/C$) only for very small values of C ($C \ll K_s$) near the origin of the Michaelis–Menten curve.

The relative over-prediction of nutrient uptake length using the nutrient addition approach can be quantified as the $S_w':S_w$ ratio, with ratios >1 indicating over-prediction of uptake length. The relationships between $S_w':S_w$ ratio and the magnitude of nutrient concentration increase during nutrient addition experiments relative to ambient nutrient concentration ($\Delta C/C$) for each nutrient limitation scenario are shown in Fig. 1B. The y-intercepts shown in Fig. 1B are the $S_w':S_w$ ratios when extrapolating to 0 nutrient addition. The slopes of the lines and the y-intercepts in Fig. 1B are both a function of the nutrient limitation scenario, with steeper slopes and larger intercepts under reduced nutrient limitation. Only in the case of strong nutrient limitation does the $S_w':S_w$ ratio approach 1 (y-intercept of 1.1) at relatively low nutrient additions. The over-prediction of uptake length is less than a factor of 2 for nutrient concentration increases of <9 times ambient concentration ($\Delta C/C < 9$) under strong nutrient limitation, and for nutrient concentration increases of <0.5 times ambient concentration ($\Delta C/C < 0.5$) under moderate nutrient limitation (Fig. 1B). For weak nutrient limitation, the over-prediction of uptake length by the nutrient addition approach is greater than a factor of 3, with the over-prediction increasing sharply with increases in the magnitude of nutrient addition. The relationships between the over-prediction of uptake length and relative increase in nutrient concentration presented in Fig. 1B are robust with re-

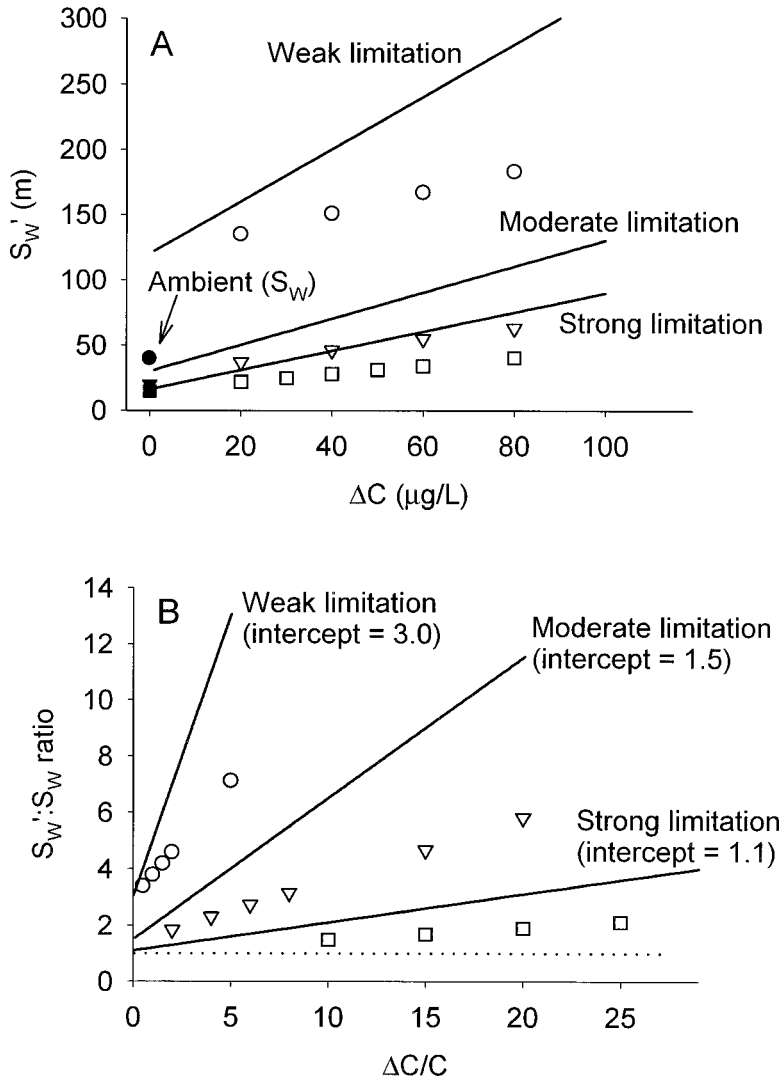


FIG. 1. Theoretical relationships between (A) uptake length determined from nutrient addition experiments (S_w') and nutrient concentration increase above ambient levels (ΔC), and (B) the ratio of uptake length determined from nutrient additions to uptake length determined by tracer additions ($S_w':S_w$ ratio) and nutrient concentration increase relative to the ambient nutrient concentration ($\Delta C/C$) for 3 nutrient limitation scenarios (see text). In panel A, nutrient uptake lengths under ambient nutrient concentrations (S_w) are shown as solid points, increasing with reduction in nutrient limitation severity (S_w is 14.7 m for strong limitation, 20 m for moderate limitation, and 40 m for weak limitation). In panel B, y-intercepts for each nutrient limitation scenario are given in parentheses. The dashed line indicates a ratio of 1. The open symbols in panels A and B are the results of simulations of field experiments under strong (open squares), moderate (triangles), and weak (circles) nutrient limitation.

spect to K_s and U_{MAX} (i.e., relationships are independent of the values of K_s and U_{MAX} chosen).

The theoretical analysis assumes that S_w' is measured over an infinitesimally short distance at the point of nutrient addition. In actual field

experiments, however, S_w' is measured over a sufficiently long distance to produce a measurable decline in nutrient concentration. Thus, C' and ΔC decline with distance, and measured S_w' is somewhat shorter than the theoretical val-

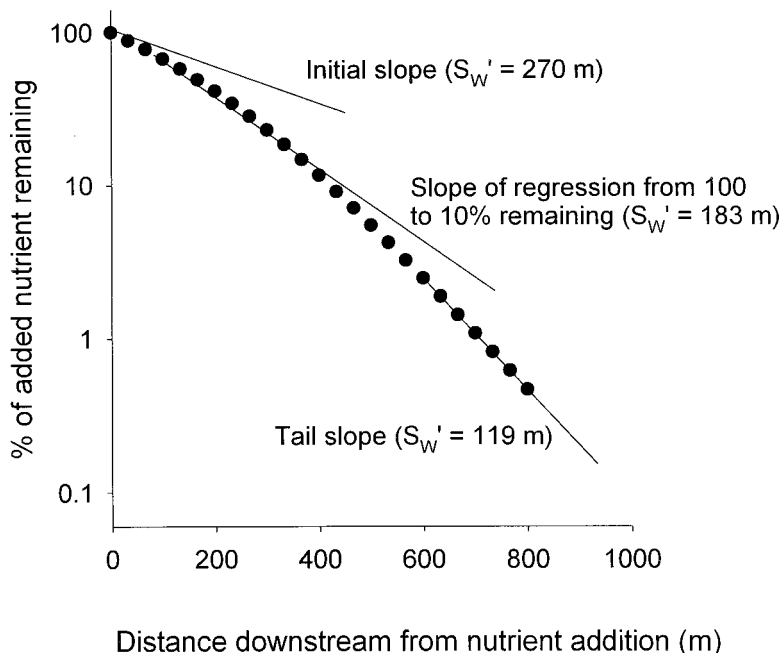


FIG. 2. Results of a simulation based on a scenario of weak nutrient limitation (ambient nutrient concentration $[C] = 40 \mu\text{g/L}$) with an upstream (release site) concentration of $120 \mu\text{g/L}$ (increase in nutrient concentration resulting from nutrient addition $[\Delta C] = 80 \mu\text{g/L}$). The weak nutrient limitation scenario was used because it most clearly shows the nonlinearity of the data as a result of the decline in ΔC , and consequently uptake rate, with distance.

ue for a given ΔC at the point of nutrient addition. To show how measured S_W' might differ from theoretical S_W' , we simulated field experiments using the same model stream scenarios. Assuming temporal steady state and no increase in discharge, the differential equation for the change in nutrient concentration (C') with distance downstream from the nutrient addition (x) is:

$$\frac{dC'}{dx} = \frac{Q}{w}Z - \frac{Q}{w}U \quad (5)$$

where Q is discharge, w is width, Z is nutrient mineralization rate, and U is nutrient uptake rate. Mineralization rate was assumed to be constant and equal to uptake rate at ambient nutrient concentration. Nutrient uptake rate was calculated from equation 2. Solution to this differential equation was approximated using a FORTRAN program with the Runge-Kutta numerical integration technique. The natural logarithm of the simulated added nutrient remaining was then regressed against distance downstream and the slope of this line was used

to calculate uptake length (S_W'). Based on what might be appropriate for an actual field experiment, we used data from the release site downstream to the point where 10% of the added nutrient remained in stream water.

In general, the results of the simulations produced uptake lengths somewhat shorter than the theoretical calculations but with the same y-intercepts for each nutrient limitation scenario (Fig. 1). The shorter uptake lengths result from our arbitrary choice of regressing data from 100 to 10% of added nutrient remaining in stream water. As shown in Fig. 2, if uptake follows Michaelis-Menton kinetics, a plot of the logarithm of added nutrient remaining is not linear. Thus, the regression slope (and consequently S_W') is partially dependent on the portion of the curve used. We picked 100 to 10% as representative of a real field experiment. If we used just the points at the very upstream part of the curve, our estimates of uptake length are essentially identical to the theoretical calculations. On the other hand, if we selected points from the tail of the curve, the results become independent of

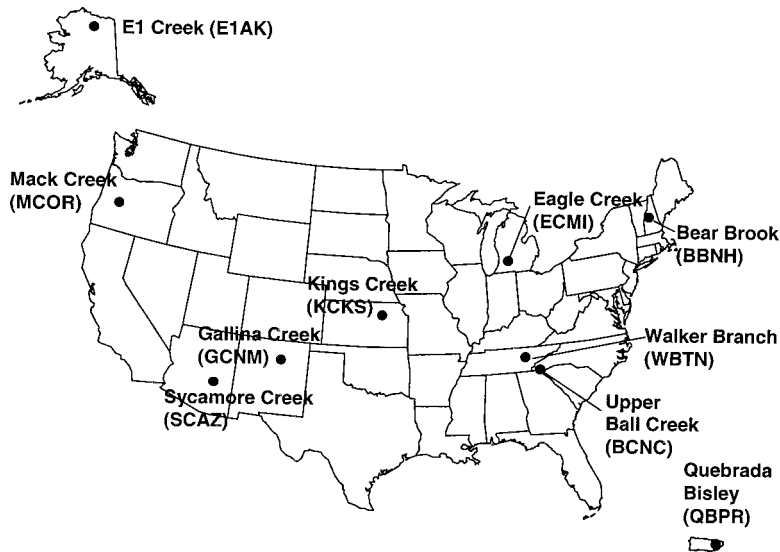


FIG. 3. Locations of streams used in this study (abbreviations in parentheses).

the upstream concentration ($C + \Delta C$), that is, the lines in Fig. 1 would be horizontal but with the same y-intercepts as the theoretical calculations (with some difference because of the error of the numerical approximation at very low values).

Study Sites

Uptake length of NH_4^+ was measured using tracer and nutrient addition experiments in 10 streams across the United States (Fig. 3). These measurements were made as part of the Lotic Intersite Nitrogen experiment (LINX) to determine rates and mechanisms of N cycling in streams. The study streams ranged from tropical (Quebrada Bisley, Puerto Rico) to tundra (E1, Alaska) and from humid to arid climates within the temperate zone. Streams were 1st to 3rd order and were relatively undisturbed by current human activities. Seven of the 10 streams were in forested catchments, and riparian canopies of these streams were partial to complete. The time chosen for the experiments in each stream was designed to represent a period of relatively high rates of N uptake and cycling. More detailed discussions of the characteristics and results of the ^{15}N tracer experiments in many of these streams are available in site-specific reports (Ball Creek, North Carolina: Tank et al. 2000; Walker Branch, Tennessee: Mulholland et al.

2000b; Quebrada Bisley, Puerto Rico: Merriam et al. 2002; Kings Creek, Kansas: Dodds et al. 2000; Eagle Creek, Michigan: Hamilton et al. 2001; E1 Creek, Alaska: Wollheim et al. 2001).

Methods

We measured NH_4^+ uptake length using both short-term NH_4^+ additions and tracer $^{15}\text{NH}_4^+$ additions in each of the study streams. Ammonium additions were performed 1 to 9 d prior to the tracer additions in each stream. A concentrated NH_4Cl solution was pumped into a well-mixed section of each stream at a rate designed to increase the NH_4^+ concentration by ~ 10 to $50 \mu\text{gN/L}$ above ambient levels. A concentrated solution of NaCl or NaBr was added simultaneously to determine discharge rate at the point of the addition and any downstream increase in discharge (Webster and Ehrman 1996).

Water samples were collected at 5 to 7 locations over a distance of 100 to 500 m downstream from the NH_4^+ addition point. Samples were collected immediately prior to the NH_4^+ addition and during the addition after conservative-tracer steady state was achieved at all sampling locations (~ 2 – 3 h after the NH_4^+ addition began). The NH_4^+ addition was terminated after all water samples were collected. Water samples were immediately filtered in the

field (0.45 μm membrane filters), returned to the laboratory and refrigerated, and analyzed for NH_4^+ using either phenate colorimetry (APHA 1995) or fluorometry (Holmes et al. 1999). In most cases, specific conductance (YSI model 30) or Br^- (ion-specific electrode) were measured in the field on unfiltered water at each station to determine discharge (Webster and Ehrman 1996); Br^- was measured by ion chromatography at Eagle Creek.

Ammonium uptake length determined using the nutrient addition approach (S_w') was calculated as -1 times the inverse of the slope of the regression of the natural logarithm of the concentration of added NH_4^+ (i.e., steady state NH_4^+ concentration in stream water during the NH_4^+ addition minus NH_4^+ concentration before the addition) versus distance. The concentration of added NH_4^+ was corrected for dilution using the conservative tracer data (Stream Solute Workshop 1990).

To determine NH_4^+ uptake length under ambient NH_4^+ concentrations (S_w), tracer levels of $^{15}\text{NH}_4^+$ (10% ^{15}N -enriched NH_4Cl solution) were added at a constant rate to each stream over a 3- to 6-wk period at the same location as used for the NH_4^+ addition experiment. The tracer additions were designed to achieve a 50% increase in the $^{15}\text{N}:^{14}\text{N}$ ratio in the dissolved NH_4^+ pool based on expected discharge and NH_4^+ concentration in each stream. The $^{15}\text{NH}_4^+$ additions resulted in negligible increases in the NH_4^+ concentration in stream water ($<0.2\%$). A conservative tracer (NaCl or NaBr) was periodically added with the $^{15}\text{NH}_4^+$ to determine discharge.

Samples of stream water were collected ~ 10 m upstream and at 5 to 7 locations downstream from the $^{15}\text{NH}_4^+$ addition point ~ 8 h after commencement of $^{15}\text{NH}_4^+$ addition in each stream. All samples were immediately filtered (0.45 μm membrane filters), returned to the laboratory and refrigerated, and chemical analyses were performed within 2 wk. For most streams, NH_4^+ concentration was determined as described above. In streams where the NH_4^+ concentration was below detection, it was determined from the mass spectrometry analysis (see below). Nitrate was determined by Cu–Cd reduction followed by azo dye colorimetry (NO_2^- was assumed to be negligible in all streams because of high dissolved oxygen levels) or by ion chromatography (APHA 1995). Total dissolved in-

organic N (DIN) was calculated as the sum of NH_4^+ and NO_3^- N. Soluble reactive P (SRP) was determined by the molybdenum blue method (APHA 1995). To determine discharge at each station, measurements of specific conductance or Br^- were made in the field as described above.

Ammonium was isolated for ^{15}N analysis from 4-L samples of filtered water using an NH_3 diffusion procedure (Sorensen and Jensen 1991, Holmes et al. 1998). Briefly, this method involved addition of MgO to convert dissolved NH_4^+ to NH_3 , diffusion of the NH_3 into the headspace of a sealed container, and sorption of the headspace NH_3 onto a precombusted, acidified (25 μL of 2 M H_2SO_4) glass fiber filter (Whatman GF/D) sealed within a packet formed by 2 Teflon filters and floating on the surface of the water. Following 14 d of diffusion at 40°C on a shaker table, the filter packets were removed from the sample bottles and dried in a desiccator prior to ^{15}N analysis. The $^{15}\text{N}:^{14}\text{N}$ ratio in NH_4^+ absorbed on each filter was determined by automated high temperature combustion with cryogenic separation of gases followed by measurement of $^{15}\text{N}:^{14}\text{N}$ ratio using a Finnigan Delta S mass spectrometer at the Ecosystems Center laboratory, Marine Biological Laboratory, Woods Hole, Massachusetts. Total mass of N sorbed on the filter was also determined and used to calculate NH_4^+ concentrations in stream water based on experimentally derived efficiencies of NH_4^+ recovery determined from standards.

Measurements of $^{15}\text{N}:^{14}\text{N}$ ratio were expressed as $\delta^{15}\text{N}$ values (units of ‰) according to the following equation:

$$\delta^{15}\text{N} = \left[\left(\frac{R_{\text{SAMPLE}}}{R_{\text{STANDARD}}} \right) - 1 \right] (1000) \quad (6)$$

where R_{SAMPLE} is the $^{15}\text{N}:^{14}\text{N}$ ratio in the sample and R_{STANDARD} is the $^{15}\text{N}:^{14}\text{N}$ ratio in standard air ($R_{\text{STANDARD}} = 0.003663$). All $\delta^{15}\text{N}$ values were corrected for background levels of ^{15}N by subtracting $\delta^{15}\text{N}$ values of samples collected upstream from the ^{15}N addition point from $\delta^{15}\text{N}$ values of samples collected downstream from the ^{15}N addition. Therefore, all $\delta^{15}\text{N}$ values represent ratios of tracer $^{15}\text{N}:^{14}\text{N}$ only (i.e., do not include naturally occurring ^{15}N).

Tracer-based NH_4^+ uptake length in each stream (S_w) was calculated as -1 times the in-

verse of the slope of the regression of the natural logarithm of tracer $^{15}\text{NH}_4^+$ flux in stream water versus distance (Newbold et al. 1981). Tracer $^{15}\text{NH}_4^+$ flux ($\mu\text{g } ^{15}\text{N/s}$) in stream water at each station i was calculated using the background-corrected measurements of ammonium $\delta^{15}\text{N}$ ($\delta^{15}\text{NH}_4^+$), $\text{NH}_4^+\text{-N}$ concentration ($[\text{NH}_4^+]$) determined from total N recovered during mass spectrometer analysis of $\delta^{15}\text{NH}_4^+$ (corrected for the reagent blank) and stream discharge (Q) using the equation:

$$^{15}\text{NH}_4\text{flux}_i = \left(\frac{\delta^{15}\text{NH}_4}{1000} \right) (0.003663)(Q_i)([\text{NH}_4]_i) \quad (7)$$

A number of other physical and biological characteristics were measured in each stream to identify possible relationships with the ratio of uptake lengths determined by nutrient addition and tracer methods. We determined average stream water velocity from the conservative tracer profiles during the ^{15}N addition at each station (Webster and Ehrman 1996). Average stream width was determined from measurements made at intervals of 1 to 5 m along the study reach, and average depth was calculated from measurements of discharge, average water velocity, and average width.

Standing stocks of different types of benthic organic matter were measured 1 to 2 wk prior to the beginning of the $^{15}\text{NH}_4^+$ tracer experiment in each stream, and these standing stocks were assumed to be the same for both NH_4^+ addition and tracer experiments in each stream. To measure detritus standing stock, an open-ended metal cylinder (0.07 m^2) was placed into the stream bottom at 10 locations and coarse particulate organic matter (CPOM, $>1 \text{ mm}$ diameter) was collected and separated into leaves and wood. To estimate fine particulate organic matter (FPOM), the sediments were vigorously agitated within the cylinder to a depth of $\sim 10 \text{ cm}$, the slurry was pumped through a 1-mm screen into a container of known volume, and the pumped slurry was subsampled. Material was returned to the laboratory, dried (60°C), weighed, combusted (550°C), and reweighed to determine ash-free dry mass (AFDM) per unit area sampled. Total benthic detritus was the sum of CPOM (leaves and wood) and FPOM,

expressed as a standing stock per unit area of stream bottom.

Epilithon standing stock was measured by collecting rocks randomly from 5 to 6 locations in each stream, placing a 5-cm diameter PVC cylinder with a neoprene gasket attached to one end on the rock surface, scrubbing material from the enclosed area with a brush, and rinsing the scraped material into a vial. The scraped material was then filtered (Whatman GFF), extracted in 90% acetone overnight, and analyzed spectrophotometrically for chlorophyll a , using the method of Lorenzen (1967). Biomass of the algal component of the epilithon was estimated as $100 \times$ chlorophyll a mass per unit area of rock surface scraped (Reynolds 1984).

The areal coverage of filamentous algae and bryophytes was determined by establishing transects across each stream every 5 m along the study reach and determining presence/absence every 10 to 20 cm across the transects. Biomass per unit area was estimated by scraping or coring material from known areas of substratum containing 100% coverage of filamentous algae or bryophytes, and AFDM was determined as the difference between dry mass (60°C) and ash mass (550°C). The standing stock of filamentous algae and bryophytes was calculated as the product of average % cover and biomass per unit area in areas of 100% cover. Total autotrophic standing stock was calculated as the sum of the algal component of epilithon, filamentous algae, and bryophyte standing stocks.

The data were analyzed using correlation and stepwise multiple regression with a model entry criterion of $p < 0.05$ (SAS, 1985. SAS user's guide statistics, SAS Institute, Cary, North Carolina). The multiple regression analyses were used to determine whether the ratio of uptake lengths determined from NH_4^+ addition and tracer methods ($S_w':S_w$ ratio) was predictable from stream characteristics.

Results

The streams varied considerably in physical and chemical characteristics (Table 1). Discharge varied from 3.5 to 208 L/s and average stream width, depth, and water velocity varied by about an order of magnitude among streams on the date the ^{15}N -tracer experiments began. Discharge in most streams was similar between dates of the NH_4^+ addition and tracer experi-

TABLE 1. Stream characteristics. The physical and chemical characteristics are for the dates of the ^{15}N tracer additions. If discharge on the data of the NH_4^+ addition differed by $>20\%$, it is listed in parentheses. The date listed is the beginning of the tracer addition and "Days prior" refers to the length of time the NH_4^+ addition was conducted prior to the ^{15}N tracer addition. DIN = dissolved inorganic N, SRP = soluble reactive P, ΔC = increase in NH_4^+ concentration resulting from nutrient addition, AFDM = ash-free dry mass, nm = not measured. Stream abbreviations as in Fig. 3.

Stream	Physical characteristics				Chemical characteristics				Standing stocks (g AFDM/m ²)						
	Discharge (L/s)	Width (m)	Velocity (cm/s)	Depth (cm)	Water temp. (°C)	NH_4^+ ($\mu\text{g N/L}$)	DIN ($\mu\text{g N/L}$)	DIN:SRP (molar)	ΔC ($\mu\text{g N/L}$)	Total autotrophs ^a	Epilithon + filamentous algae	Total detritus	Leaves	$^{15}\text{NH}_4^+$ addition date	Days prior
BCNC	51.4 (36.4)	2.7	10.6	18.0	8.0	3.0	4.0	4.4	14	0.1	1.3	109	62	4 Nov. 1996	9
WBTN	9.8	3.1	6.8	4.6	12.2	2.7	18.3	16.9	20	41	3.8	385	76	1 Apr. 1997	1
SCAZ	70	5.8	28.6	4.2	19.5	1.9	18.7	3.2	58	178	191	20	0	1 May 1997	3
BRNH	3.5	2.1	1.8	9.0	13.0	4.0	61.4	16.8	67	11	2.7	53	4.2	17 Jun. 1997	6
GCNM	4.3	1.3	9.9	3.3	10.1	5.2	12.7	4.0	31	42	3.5	120	6.9	26 Aug. 1997	5
QBPR	17.9 (28.9)	4.7	3.0	12.7	21.9	4.2	184	30.8	61	1	3.5	44	6.0	15 Jan. 1998	6
KCKS	41.8	2.4	11.3	15.4	10.5	2.4	5.5	5.5	32	9	78	215	1.4	16 Apr. 1998	2
ECMI	208 (276)	5.0	23.6	17.6	20.0	23.4	51.1	50.3	12	0.7	5.8	362	0.9	16 Jun. 1998	4
MCOR	55.8	5.1	7.6	15.9	13.3	3.6	57.9	21.4	18	18	2.9	112	0.4	21 Jul. 1998	4
E1AK	nm	1.5	16.0	6.4	10.0	1.4	37.1	45.6	98	0.6	nm	nm	3.5	30 Jun. 1997	3

^a Calculated as the sum of bryophytes, filamentous algae, and the algal component of epilithon (determined as 100 times the chlorophyll *a* content of epilithon)

ments; however, discharge in Ball Creek was $\sim 1/3$ lower, and discharges in Quebrada Bisley and Eagle Creek were $\sim 1/3$ higher during the NH_4^+ addition compared with the tracer experiment. Ambient concentrations of NH_4^+ varied from ~ 1 to $23 \mu\text{g N/L}$ and differed by $< 1.5 \mu\text{g N/L}$ between dates of the experiments. DIN:SRP ratios were < 6 in 4 streams, suggesting the potential for strong N limitation in these streams.

Standing stocks of benthic organic matter also varied considerably among the streams (Table 1). Standing stock of autotrophs was high in Sycamore Creek (dominated by filamentous algae), and in Walker Branch and Gallina Creek (dominated by bryophytes). Detritus standing stock was highest in Walker Branch and Eagle Creek and was dominated by FBOM (data not shown) in all streams but Ball Creek. Standing stocks of epilithon plus filamentous algae (combined as a single compartment) and decomposing leaves were considered separately in the analysis because these compartments have relatively high rates of NH_4^+ uptake per unit mass (Mulholland et al. 2000b, Tank et al. 2000, Hamilton et al. 2001, Dodds et al. 2002) and, thus, might have particularly strong effects on uptake length. Standing stock of epilithon plus filamentous algae was high in Sycamore Creek and Kings Creek; however, the high value in Kings Creek was primarily caused by a thick epilithon mat that appeared to be undergoing senescence. Standing stock of epilithon plus filamentous algae exceeded that of total autotrophs in several streams because epilithon included nonalgal and nonliving organic matter. Standing stock of decomposing leaves was high in 2 streams, Ball Creek and Walker Branch.

S_w values were considerably shorter than S_w' values for all streams where both could be calculated (Fig. 4A). For Eagle Creek, S_w' could not be determined because no decline in the concentration of added NH_4^+ could be detected over the stream reach during the experiment, suggesting that S_w' for Eagle Creek was very long. Values of S_w' were about twice values of S_w for 5 of the 9 streams for which ratios could be calculated (Fig. 4B). $S_w':S_w$ ratios varied from ~ 4 to 18 for the other 4 streams.

Among all the physical, chemical, and biological characteristics measured (Table 1), $S_w':S_w$ ratios were significantly correlated only with NH_4^+ concentration increase during the NH_4^+

additions (Fig. 5A), and with ambient DIN:SRP concentration ratio (Fig. 5B). However, there was substantial covariation between NH_4^+ concentration increase and DIN:SRP ratio among our streams ($r = 0.657$, $p = 0.054$) and, excluding E1 Creek, Alaska, $S_w':S_w$ ratio was significantly correlated only with the NH_4^+ concentration increase ($r = 0.731$, $p = 0.039$). Nearly all of the variation in $S_w':S_w$ ratio was accounted for by a multiple regression model with NH_4^+ concentration increase, water temperature, and DIN:SRP ratio as independent variables (Table 2). Excluding E1 Creek, $S_w':S_w$ ratio was predicted by a model based only on NH_4^+ concentration increase and total autotroph standing stock.

Discussion

Comparison of methods

Ammonium uptake lengths determined in this study ranged over almost 2 orders of magnitude (23 to 1350 m). This range in uptake length encompassed most of the range of values reported for other streams, all of which have been determined using NH_4^+ additions. Other reports of NH_4^+ uptake length include 82 and 190 m on 2 dates for an alpine stream in Slovakia (Kopacek and Blazka 1994), 10 to 693 m during different seasons and in different reaches of 2 streams in Spain (Martí and Sabater 1996, Butturini and Sabater 1998, Sabater et al. 2000), 3 to 25 m for 2 small mountain streams in North Carolina (Wallace et al. 1995, Webster et al. 2000), and 5 to 277 m for 13 small mountain streams in New Hampshire (Hall et al. 2002). Ammonium uptake lengths are positively related to stream discharge (Kopacek and Blazka 1994, Butturini and Sabater 1998, Webster et al. 2000, Hall et al. 2002), and negatively related to riparian vegetation density (Martí and Sabater 1996, Sabater et al. 2000) and the standing stocks of algae and detritus in streams (Wallace et al. 1995, Martí and Sabater 1996). Based on an analysis of the ^{15}N tracer experiment data only, NH_4^+ uptake lengths in our streams were primarily a function of stream discharge (Peterson et al. 2001).

S_w' values were considerably longer than S_w values in all streams. This result appears to be robust given the wide range of streams we studied. Our theoretical analysis indicated that S_w' should be greater than S_w and that the relative

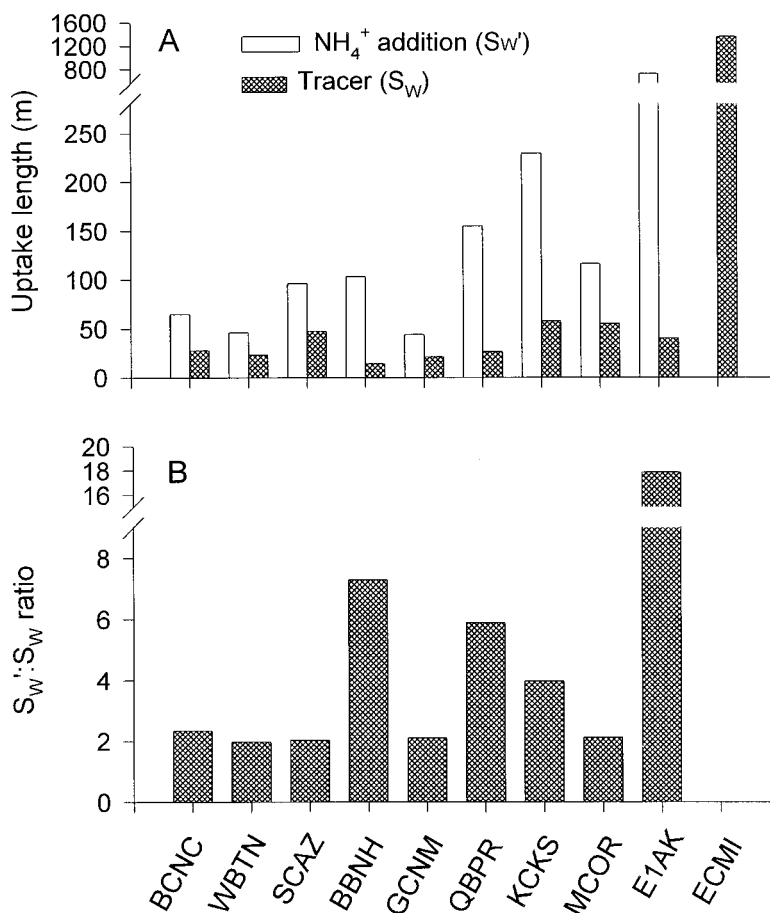


FIG. 4. Ammonium uptake lengths computed from NH₄⁺ additions (S_{w'}) and from ¹⁵N tracer additions (S_w) (A), and S_{w'}:S_w ratios for each stream (B). Stream abbreviations as in Fig. 3.

over-prediction of S_w by S_{w'} (i.e., S_{w'}:S_w ratio) should be a function of the increase in nutrient concentration during the addition and the degree of nutrient limitation. Our experimental results showing significant correlations between S_{w'}:S_w ratio and the NH₄⁺ concentration increase during the NH₄⁺ additions and between S_{w'}:S_w ratio and ambient DIN:SRP ratio provide empirical evidence in support of the theoretical results. Despite the high degree of covariance between NH₄⁺ concentration increase and DIN:SRP ratio, stepwise multiple regression analysis indicated that these 2 variables were both significant predictors of S_{w'}:S_w ratio (at least when all streams were included). Although DIN:SRP ratio alone does not necessarily indicate the likelihood of N or P limitation, DIN and SRP concentrations in our study streams were relatively

low (<200 μg N/L and <15 μg P/L, respectively), suggesting that N or P could be limiting. Our experimental results are also consistent with previous reports comparing nutrient addition and tracer methods for measuring uptake lengths of PO₄⁻³ (Mulholland et al. 1990, Hart et al. 1992) and NO₃⁻ (Mulholland et al. 2000a) in individual streams. In these earlier studies, uptake lengths determined by nutrient additions were 1.5 to 3 times longer than those determined by tracer additions, and were positively related to the concentration increase during the nutrient addition.

Mechanisms of nutrient uptake

In the theoretical analysis, we assumed that nutrient uptake followed Michaelis-Menten ki-

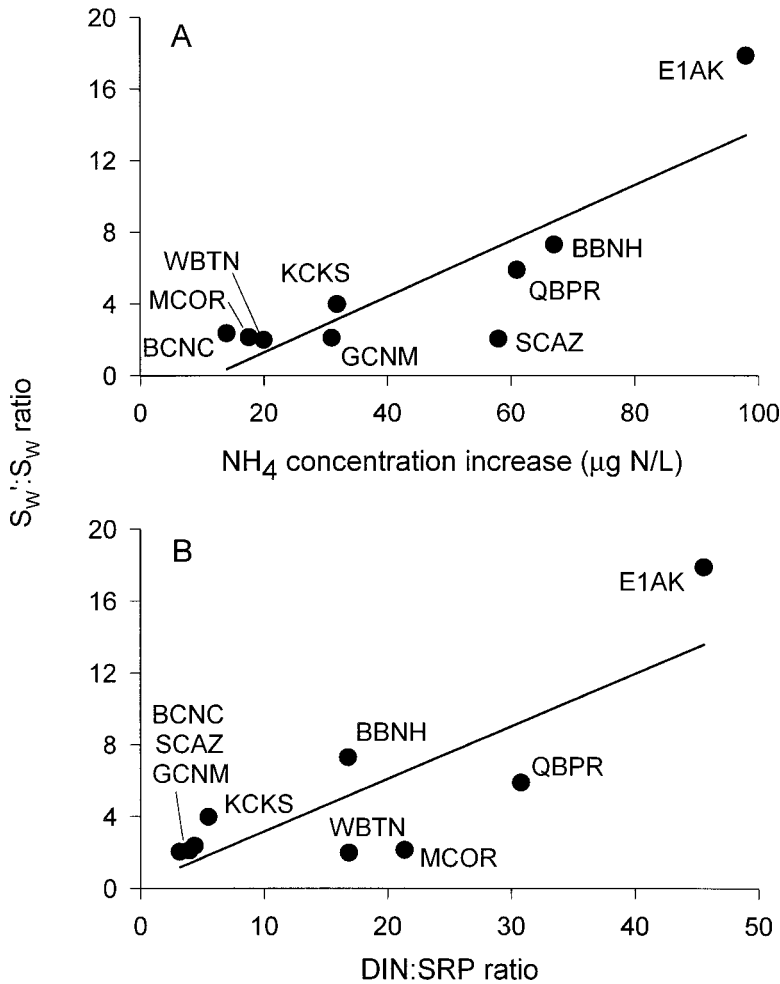


FIG. 5. Correlations of $S_w':S_w$ ratio with (A) the NH_4^+ concentration increase during the NH_4^+ additions ($r = 0.853$, $p = 0.004$), and (B) ambient streamwater DIN:SRP ratio ($r = 0.819$, $p = 0.007$). Stream abbreviations as in Fig. 3.

netics. This assumption seems reasonable for biologically controlled uptake of a limiting nutrient, although there have been few studies examining the mathematical form of the relationship between nutrient uptake and nutrient concentration in streams. Using nutrient addition experiments in streamside flumes, Bothwell (1988, 1989) reported that stream periphyton growth became saturated as PO_4^{-3} concentrations increased, suggesting PO_4^{-3} uptake also followed saturation kinetics. Several other studies of stream periphyton have also shown that nutrient uptake follows Michaelis–Menten, or more generally, saturation kinetics (Horner et al. 1990, Kim et al. 1990). Mulholland et al. (1990)

compared nutrient addition and tracer experiments for determining PO_4^{-3} uptake length in Walker Branch, and showed that whole-stream rates of PO_4^{-3} uptake appeared to follow Michaelis–Menten kinetics at low P concentrations ($<10 \mu\text{g P/L}$), but sorption kinetics at higher concentrations ($>40 \mu\text{g P/L}$). In an earlier NH_4^+ addition experiment in Walker Branch, Newbold et al. (1983b) reported that NH_4^+ uptake appeared to involve an initial adsorption followed by biotic uptake exhibiting saturation kinetics. Dodds et al. (2002) performed a series of NH_4^+ additions to Kings Creek, and found that Michaelis–Menten kinetics described NH_4^+ uptake in some experiments but not in others.

TABLE 2. Results of stepwise multiple regression for $S_w':S_w$ ratio for all streams for which both the uptake length determined by NH_4^+ addition (S_w') and the uptake length determined by ^{15}N tracer addition (S_w) were measured ($n = 9$) and without E1 Creek, Alaska ($n = 8$).

Independent variable	Parameter estimate (SE)	r^2	F	p
All streams:				
Intercept	2.793 (0.837)			
NH_4^+ concentration increase (ΔC)	0.126 (0.013)	0.727	18.67	0.004
Water temperature	-0.440 (0.061)	0.158	8.22	0.029
DIN:SRP ratio	0.151 (0.025)	0.102	37.32	0.002
Full model		0.986	120.90	0.0001
Excluding E1 Creek:				
Intercept	0.948 (0.386)			
NH_4^+ concentration increase (ΔC)	0.091 (0.010)	0.535	6.90	0.039
Total autotroph standing stock	-0.024 (0.003)	0.422	49.24	0.0009
Full model		0.957	55.80	0.0004

It is possible that some of the uptake of added NH_4^+ that we observed in the NH_4^+ addition experiments could have been the result of increased adsorption under higher NH_4^+ concentrations. Increased NH_4^+ adsorption would have resulted in temporarily shorter S_w' values until a new steady state under higher NH_4^+ concentrations was established. We sampled 2 to 3 h after the beginning of the NH_4^+ additions, and this delay may not have been long enough to allow steady state to be reached. Thus, the overestimates of ambient uptake length ($S_w':S_w$ ratio) that we observed might be even larger under steady state conditions if adsorption increased during the NH_4^+ addition experiments.

Adsorption was probably not an issue with the ^{15}N tracer experiments because NH_4^+ concentrations were increased only marginally (<0.2%). There was undoubtedly some isotopic exchange of ^{15}N and ^{14}N in adsorbed NH_4^+ pools during the tracer experiment because ^{15}N concentration was increased by ~50%. However, this exchange should occur relatively rapidly (within minutes) and we did not collect water samples until ~8 h after the beginning of the ^{15}N additions. Thus, our S_w values are unlikely to be influenced by adsorption.

It is possible that transient biological responses to increases in NH_4^+ concentration affected the NH_4^+ addition results. If there was short-term, luxury uptake of NH_4^+ with increases in concentration, then uptake rates would be initially higher than under steady state. This situation would result in shorter values of S_w' than would be measured in longer NH_4^+ additions. It is unlikely that there was a delay in biological

response to added NH_4^+ in our study streams because NH_4^+ is the form of N most readily available to algae and microbes.

Nutrient demand and N limitation

Our experimental data also suggested that factors influencing nutrient demand were important determinants of $S_w':S_w$ ratio. The multiple regression results indicated that water temperature (all streams) and standing stock of autotrophs (excluding E1 Creek, Alaska) were significant predictors of $S_w':S_w$ ratio, with higher temperatures and greater standing stocks resulting in lower $S_w':S_w$ ratios. Higher water temperatures and greater standing stock of autotrophs can result in greater nutrient demand and, presumably, greater nutrient deficiency in streams with low nutrient concentrations such as those we studied. As shown in the theoretical analysis, $S_w':S_w$ ratios in more strongly nutrient-limited streams should be lower than $S_w':S_w$ ratios in less nutrient-limited streams for a given level of nutrient increase.

We can combine the theoretical analysis and the experimental results to provide an indication of the degree of N limitation in each of our study streams. Assuming the same nutrient limitation scenarios used in the theoretical analysis and adding the experimental data to the plot of $S_w':S_w$ ratio versus the relative nutrient concentration increase (Fig. 1B), we observe that the experimentally measured values generally fall between the lines for strong and moderate nutrient limitation (Fig. 6). Sycamore Creek falls below the strong limitation line, indicating very

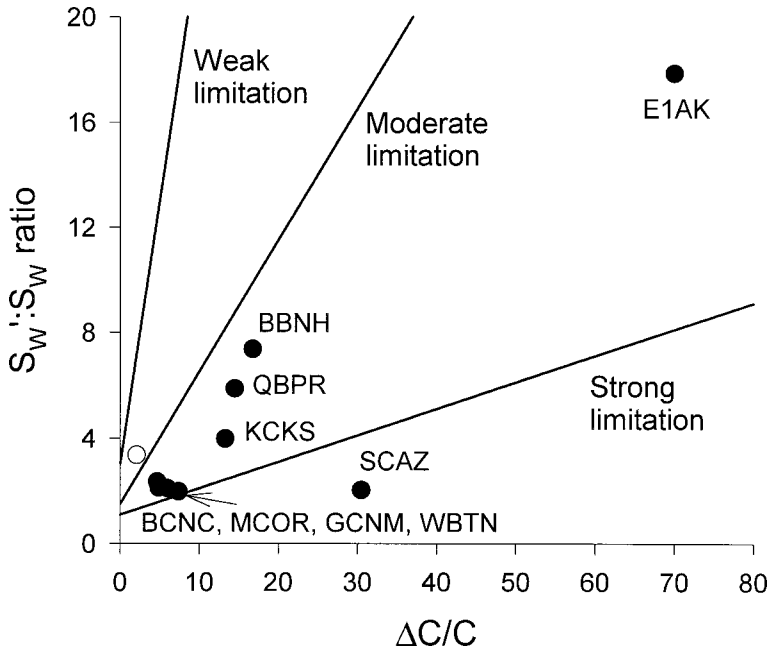


FIG. 6. Relationships between the ratio of uptake lengths determined by short-term NH_4^+ additions and uptake lengths determined by ^{15}N tracer additions ($S'_W:S_W$), and the nutrient concentration increase relative to the ambient nutrient concentration ($\Delta C/C$). The lines show the theoretical relationships for strong ($C = 2 \mu\text{g/L} = 0.1$ half-saturation constant [K_s]), moderate ($C = 10 \mu\text{g/L} = 0.5 K_s$), and weak ($C = 40 \mu\text{g/L} = 2 K_s$) N limitation. The solid points are $S'_W:S_W$ ratios determined by NH_4^+ additions and ^{15}N tracer additions in each of our streams. The open circle is $\text{NO}_3^- S'_W:S_W$ ratio determined by NO_3^- and ^{15}N tracer additions in Walker Branch within several days of the NH_4^+ addition experiments (Mulholland et al. 2000a). Stream abbreviations as in Fig. 3.

strong N limitation, which is consistent with its very low DIN:SRP ratio (3.2) and results of previous studies showing high rates of N fixation and strong N limitation of algae in this stream (Grimm and Fisher 1986, Grimm and Petrone 1997). Relatively low DIN:SRP ratios also suggest at least moderate N limitation for most of our other streams (particularly Ball Creek, Galina Creek, and Kings Creek where DIN:SRP ratios were <6). However, relatively high DIN:SRP ratios for E1 Creek and Quebrada Bisley (31 and 46, respectively) suggest weak if any N limitation, and yet the $S'_W:S_W$ ratios for these streams plot in the region between moderate and strong N limitation in Fig. 6.

A similar analysis of N limitation can be performed using measurements of NO_3^- uptake length. For Walker Branch, NO_3^- uptake length was determined using NO_3^- addition and ^{15}N tracer approaches within a few days of the NH_4^+ experiments reported here (Mulholland et al. 2000a). A plot of $\text{NO}_3^- S'_W:S_W$ ratio versus

relative NO_3^- concentration increase falls between the moderate and weak N limitation lines (Fig. 6). The discrepancy in N limitation indicated by the NH_4^+ and NO_3^- measurements in Walker Branch may be a result of delayed biological response (e.g., NO_3^- reductase synthesis) to added NO_3^- during NO_3^- additions and thus longer $\text{NO}_3^- S'_W$, or may be an indication that abiotic processes (e.g., increased adsorption) contribute to uptake of added NH_4^+ and thus shorter $\text{NH}_4^+ S'_W$ than would result from biological uptake alone. If abiotic processes contribute substantially to uptake of added NH_4^+ , then the degree of N limitation suggested by the data in Fig. 6 would be overestimated.

The analysis presented in Fig. 6 also may overestimate the severity of N limitation because of constraints inherent in field measurements of S'_W . As shown by the simulation of field experiments presented in Fig. 1 (open symbols), the decline in ΔC with distance downstream from the addition point results in some-

what shorter measured values of S_w' and, consequently, lower $S_w':S_w$ ratios than predicted by theory for a given initial ΔC . In addition, there may be some variability in our $S_w':S_w$ ratios resulting from the length of reach over which measurements were made, as shown in Fig. 2.

Nutrient addition approach

Despite our results showing that S_w' values consistently overestimate S_w values, the nutrient addition approach may still be a useful tool for comparing nutrient cycling within or between streams if several precautions are taken. Nutrient additions should be as low as possible while still allowing for accurate measurement of concentrations at several distances downstream. Our theoretical and empirical results showed that smaller increases in nutrient concentration resulted in lower overestimates of ambient S_w .

Multiple nutrient additions might be useful for more closely approximating ambient uptake length than single addition experiments. We have shown theoretically that the overestimation of uptake length (i.e., $S_w':S_w$ ratio) is linearly related to ΔC . Several short-term nutrient addition experiments could be conducted under similar conditions (e.g., successively on the same day or over successive days with similar weather conditions) but using increasingly higher nutrient concentrations. Extrapolating the relationship between measured S_w' and ΔC back to 0 nutrient increase would provide an estimate of uptake length that more closely approximates S_w . Although this multi-addition, extrapolation approach is more time consuming than single addition experiments, this approach should produce estimates of S_w' that are only ~10% longer than S_w for strongly nutrient-limited streams ($C = 0.1 K_s$) (or ~50% longer for moderately nutrient-limited streams, $C = 0.5 K_s$).

In conclusion, nutrient uptake length is an important parameter for understanding nutrient cycling in streams. Our results suggest that tracer addition experiments should be used to determine nutrient uptake length whenever possible because they maintain ambient nutrient concentrations during the measurement. When tracer experiments are impractical, however, short-term nutrient additions might be used to estimate uptake length. However, one must keep in mind that uptake length measured using nutrient additions will overestimate ambient up-

take length, with the degree of overestimation related inversely to the degree of nutrient limitation and the magnitude of the nutrient addition.

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