

Effects of light on NO_3^- uptake in small forested streams: diurnal and day-to-day variations

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Abstract. We investigated the effects of autotrophy on short-term variations in nutrient dynamics by measuring diurnal and day-to-day variations in light level, primary productivity, and NO_3^- uptake during early and late spring in 2 forested streams, the East and West Forks of Walker Branch in eastern Tennessee, USA. We predicted that diurnal and day-to-day variations in NO_3^- uptake rate would be larger in the West Fork than in the East Fork in early spring because of higher rates of primary productivity resulting from a more stable substratum in the West Fork. We also predicted minimal diurnal variations in both streams in late spring after forest leaf emergence when light levels and primary productivity are uniformly low. Reach-scale rates of gross primary production (GPP) were determined using the diurnal dissolved O_2 change technique, and reach-scale rates of NO_3^- uptake were determined by tracer ^{15}N - NO_3^- additions. In the West Fork, significant diurnal and day-to-day variations in NO_3^- uptake were related to variations in light level and primary productivity in early spring but not in late spring, consistent with our predictions. In early spring, West Fork NO_3^- uptake rates were 2 to 3× higher at midday than during predawn hours and 50% higher on 2 clear days than on an overcast day several days earlier. In the East Fork, early spring rates of GPP were 4 to 5× lower than in the West Fork and diurnal and day-to-day variations in NO_3^- uptake rates were <30%, considerably lower than in the West Fork. However, diurnal variations in NO_3^- uptake rates were greater in late spring in the East Fork, possibly because of diurnal variation in water temperature. Our results indicate the important role of autotrophs in nutrient uptake in some forested streams, particularly during seasons when forest vegetation is dormant and light levels are relatively high. Our results also have important implications for longer-term assessments of N cycling in streams that rely on daytime measurements or measurements only under limited weather conditions (i.e., clear days).

Key words: nitrate uptake, light, diurnal patterns, tracer ^{15}N , gross primary production, nutrient spiraling.

The role of autotrophs in the structure and functioning of streams has been an important topic in stream ecology for some time. In his seminal paper, Minshall (1978) argued that autotrophy is of primary impor-

tance in maintaining the structure and function of many streams, including those in forested catchments that may have high rates of primary productivity for only a few months each year. In their interbiome comparison of stream metabolism, Bott et al. (1985) showed that rates of gross primary production (GPP) exceeded respiration in one or more seasons in most streams, even those in forested regions.

A number of studies have focused on the role of autotrophy in total metabolism and support of the food web in streams (Bott et al. 1985, Hill et al. 1995,

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Lamberti 1996). There also has been interest in the role of autotrophs in nutrient uptake and cycling in streams, particularly in recent years. Grimm (1987) showed that rates of dissolved inorganic N (DIN) uptake increased during algal regrowth following flash floods in an Arizona stream, resulting in declining DIN concentrations in stream water. Sabater et al. (2000) found that uptake rates of PO_4^{3-} (but not NH_4^+) were highly correlated with rates of primary production in a study comparing streams with logged and unlogged riparian forests in Spain. Hall and Tank (2003) reported that ~75% of the variation in NO_3^- uptake rate was explained by variation in rates of GPP in a study of N uptake and metabolism in streams in the Grand Teton National Park. McKnight et al. (2004) found higher rates of nutrient uptake by algae and lower N and P concentrations in streams where algal mats were abundant than where they were sparse in their study of Antarctic streams. Controls on N transformations in streams are of particular interest because N availability is increasing rapidly because of human activities (Vitousek et al. 1997) and streams are hot spots of N uptake and retention within landscapes (Alexander et al. 2000, Peterson et al. 2001).

Seasonal variation in primary production can result in similar variation in nutrient uptake in streams. In forested regions, this variation is related to the leaf phenology of riparian vegetation. In the West Fork of Walker Branch, eastern Tennessee, USA, analysis of long-term data records has indicated consistent seasonal changes in nutrient concentrations—decline in concentrations during late winter and early spring and subsequent increases in concentrations in late spring attributable to changes in instream uptake rates driven by leaf emergence in the riparian forest canopy (Mulholland and Hill 1997, Mulholland 2004). Hill et al. (2001) showed strong relationships between light level, periphyton photosynthesis, streamwater nutrient concentrations, and growth of the dominant herbivore in Walker Branch and a nearby forested stream during spring, indicating a tight cascade of shade effects through primary producers to biotic (food chain) as well as abiotic (nutrients) components of the ecosystem.

In addition to seasonal variations, short-term variations in nutrient uptake in streams may be caused by diurnal or day-to-day variations in light level and primary production. These light-driven, short-term variations in uptake may be particularly evident in the case of NO_3^- because energy is required for its reduction prior to its use in cellular synthesis. Several previous studies have reported diurnal variations in NO_3^- concentration in streams with minimum concentrations coinciding with maximum rates of GPP at

midday (Manny and Wetzel 1973, Grimm 1987, Mulholland 1992, Burns 1998). These studies suggest autotroph-driven variation in NO_3^- uptake in streams, even those draining forested catchments.

We investigated the effects of diurnal and day-to-day variations in light level on NO_3^- uptake during early and late spring in 2 forested streams, the East and West Forks of Walker Branch. Previous research indicated that the early spring peak in primary production in the East Fork is considerably lower than that in the West Fork (Mulholland et al. 2000, PJM, unpublished data), probably because of differences in substrata. Therefore, we predicted that diurnal and day-to-day variations in NO_3^- uptake would be more prominent in the West Fork than in the East Fork in early spring, and that diurnal and day-to-day variations would be minimal in both streams in late spring after forest leaf emergence when light levels and primary productivity are uniformly low. A previous study in the East Fork during summer indicated that day and night uptake of NO_3^- differed (Fellows et al. 2006); however, this study relied on chamber incubations of benthic substrata and may not reflect reach-scale processes. Our study used a field tracer ^{15}N addition approach to quantify diurnal and day-to-day variations in NO_3^- uptake at the stream-reach scale.

Study Sites

The study was conducted in the West and East Forks of Walker Branch Watershed (lat 35°58'N, long 84°17'W), a deciduous forest watershed in the US Department of Energy's Oak Ridge Environmental Research Park in the Ridge and Valley region of eastern Tennessee. Both streams are 1st order and originate as springs 100 to 200 m upstream from the study reaches. Mean annual precipitation is ~140 cm and mean annual temperature is ~14.5°C. The watersheds of both streams are underlain by several layers of siliceous dolomite and stream water is slightly basic. The substratum of the West Fork is primarily cobble and bedrock outcrops, whereas the East Fork substratum is primarily gravel and fine-grained organic-rich sediments. These substratum differences are the result of differences in stratigraphy of the underlying geology (Knox dolomite) and both are typical of streams in the Ridge and Valley Province of eastern Tennessee (Johnson and Van Hook 1989). Stream gradients are relatively low, 0.035 for the West Fork and 0.020 for the East Fork. More detailed descriptions of these streams are given by Mulholland et al. (2000) and Mulholland et al. (2004).

Methods

¹⁵N addition

Two series of tracer ¹⁵N addition experiments were conducted in each stream, one during the early spring before leaf emergence (5–9 April 2001) and the other during late spring well after leaf emergence (11–12 June 2001). Each experiment consisted of a continuous injection of 99% ¹⁵N-enriched KNO₃ and a conservative tracer (NaCl) for 5 to 22 h to each stream and measurement of ¹⁵N-NO₃⁻ and Cl⁻ concentrations at 2 stations downstream from the injection after steady state was achieved. The upper measurement station in both streams was ~10 m downstream from the ¹⁵N injection, a distance long enough for complete mixing of the tracer. The lower measurement stations were 120 m downstream from ¹⁵N injection in the West Fork and 90 m downstream in the East Fork. The K¹⁵NO₃ and NaCl tracers were dissolved in carboys containing ~15 L of distilled water and pumped into the streams using a battery-powered fluid metering pump (FMI, Syosset, New York). The amount of K¹⁵NO₃ and NaCl added to the carboy for each injection varied, depending on stream discharge and ambient NO₃⁻ concentration. In each injection, addition of K¹⁵NO₃ increased the ¹⁵N:¹⁴N ratio of streamwater NO₃⁻ by ~20× relative to the ambient ratio and resulted in only a small (7%) increase in NO₃⁻ concentration. Addition of NaCl increased the streamwater Cl⁻ concentration by 10 to 15 mg/L.

In April, the ¹⁵N injections in each stream were begun at 2000 h on 4 April. Stream samples were collected just before the injections (background measurements) and during the injections at ~2400 h (midnight) on 4 April, and 0600 h (predawn) and 1400 to 1500 h (midday) on 5 April. The ¹⁵N injections were terminated after the midday sampling because light levels were relatively low from overcast weather conditions. Additional ¹⁵N injections were done on 7 April and 9 April (the latter only in the West Fork) under mostly clear weather conditions. These ¹⁵N injections were begun at 0900 to 1000 h and stream samples were collected between 1400 and 1500 h (midday).

In June, the ¹⁵N injections in each stream were begun at ~2000 h on 11 June. Stream samples were collected just before the injections and during the injections at ~2400 h (midnight) on 11 June, and 0500 to 0600 h (predawn), 1000 h (midmorning), and 1300 to 1400 h (midday) on 12 June. The ¹⁵N injections were terminated after the midday sampling. In June, ¹⁵N injections were not done on different days as in April because light levels beneath the forest canopy were low and did not vary much from day to day.

Water temperature was measured, and 4 replicate water samples (~2 L each) were collected from the upper and lower sampling stations during each sampling period. All samples were immediately filtered in the field (Whatman no. 1 cellulose, nominal pore size = 11 μm) and 1-L (for analysis of ¹⁵N-NO₃⁻) and 30-mL (for analysis of NO₃⁻ and Cl⁻ concentrations) subsamples of the filtrate were returned to the laboratory within 2 h of collection.

Photosynthetically active radiation (PAR) also was measured throughout each experimental period at one location in each stream using a quantum sensor (LiCor 190SA; LiCor, Lincoln, Nebraska) and data logger (Campbell Scientific CR-10, Campbell Scientific, Logan, Utah).

Laboratory analyses

Cl⁻ concentration was measured by ion chromatography, and NO₃⁻ concentration was measured by automated Cu–Cd reduction followed by azo-dye colorimetry (Bran Luebbe Auto Analyzer 3, Seal Analytical, Mequon, Wisconsin; APHA 1992).

Additions (spikes) of unlabelled KNO₃ (200 μg N/L) were made to 1-L samples for ¹⁵N-NO₃⁻ analysis to reduce ¹⁵N:¹⁴N ratios to the ideal working range for mass-spectrometric measurement. Identical spikes also were added to 1-L samples of deionized water to calculate N recovery and to determine the ¹⁵N:¹⁴N ratio of the NO₃⁻ spike. The NO₃⁻ measurement was actually NO₃⁻ + NO₂⁻ but NO₂⁻ was assumed to be negligible in this well-oxygenated stream (Mulholland 1992). Concentrations of NO₃⁻ were expressed in terms of N (e.g., μg N/L).

Processing of samples for ¹⁵N-NO₃⁻ analysis was modified from the method of Sigman et al. (1997). Samples ranging in volume from 0.05 to 1 L (depending on NO₃⁻ concentration) were added to glass flasks containing 5 g of NaCl and 3 g of MgO. Deionized water was added to small samples (high NO₃⁻ concentrations) to bring the initial sample volume to 200 mL. The samples were then brought to a gentle boil on a hot plate until the volume was reduced to ~100 mL, thereby concentrating ¹⁵NO₃⁻ and degassing NH₃ produced from NH₄⁺ under alkaline conditions. The concentrated samples were then cooled, transferred to 250-mL high-density polyethylene bottles, and refrigerated until further processing. The ¹⁵NO₃⁻ in the concentrated samples was captured for analysis using a reduction/diffusion/sorption procedure as follows. To start, 0.5 g of MgO and 3 g of Devarda's alloy were added to each sample to reduce all NO₃⁻ to NH₃ under alkaline conditions. Immediately afterward, a filter packet consisting of a precombusted, acidified (25 μL of 2.5-mol/L KHSO₄)

glass-fiber filter (1-cm Whatman GFD) sealed between 2 Teflon® filters (Millipore white nitex LCWP 25-mm diameter, 10- μ m pore size) was placed in each sample and floated to absorb the liberated NH₃. Parafilm® was placed over the mouth of each sample bottle, and the bottle was tightly capped. Samples were then heated to 60°C for 2 d and shaken at room temperature for an additional 7 d to allow full reduction of NO₃⁻ to NH₄⁺, conversion of NH₄⁺ to NH₃, diffusion of NH₃ into the sample headspace, and absorption of NH₃ onto the GFD filter. At the end of this incubation period, filter packets were removed from the sample bottles and dried in a desiccator for 2 d, after which the Teflon filter packets were opened and the GFD filters removed. Each GFD filter with its absorbed NH₃ was encapsulated in a 5 × 9-mm aluminum tin and placed in a 96-well titer plate with each well capped. All samples were sent to the stable isotope laboratory at the University of Waterloo, Waterloo, Ontario (<http://www.science.uwaterloo.ca/research/eilab/About/inContent/Content.html>) for ¹⁵N:¹⁴N ratio analysis by mass spectrometry using a Europa Integra continuous flow isotope-ratio mass spectrometer coupled to an in-line elemental analyzer for automated sample combustion (SerCon LTD, Crewe, UK).

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

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

where R_{SAMPLE} is the ¹⁵N:¹⁴N ratio in the sample and R_{STANDARD} is the ¹⁵N:¹⁴N ratio in atmospheric N₂ ($R_{\text{STANDARD}} = 0.0036765$).

Calculation of tracer ¹⁵N flux

Tracer ¹⁵N flux was calculated from the measured $\delta^{15}\text{N}$ values in a series of steps described in Mulholland et al. (2004). First, $\delta^{15}\text{N}$ values were converted to ¹⁵N/(¹⁵N + ¹⁴N) ratios using the equation:

$$\frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} = \frac{\left(\frac{\delta^{15}\text{N}}{1000} + 1 \right) \times 0.0036765}{1 + \left[\left(\frac{\delta^{15}\text{N}}{1000} + 1 \right) \times 0.0036765 \right]} \quad [2]$$

where ¹⁵N/(¹⁵N + ¹⁴N) is the atom ratio (AR) of ¹⁵N.

¹⁵NO₃⁻ AR values were corrected for the added NO₃⁻ spike using the equation:

$$\text{AR}_i = \frac{([\text{NO}_3 - \text{N}_i] + [\text{NO}_3 - \text{N}_{\text{sp}}])(\text{AR}_{\text{mi}}) - ([\text{NO}_3 - \text{N}_{\text{sp}}])(\text{AR}_{\text{sp}})}{[\text{NO}_3 - \text{N}_i]} \quad [3]$$

where [NO₃⁻N_i] is the measured NO₃⁻ concentration at station *i* ($\mu\text{g N/L}$), [NO₃⁻N_{sp}] is the increase in NO₃⁻ concentration in the water sample resulting from the NO₃⁻ spike ($\mu\text{g N/L}$, same for all stations), AR_{mi} is the AR value at station *i* calculated from the measured $\delta^{15}\text{N}$ values on spiked samples from station *i* using equation 2, AR_{sp} is the AR value of the NO₃⁻ spike calculated from the measured $\delta^{15}\text{N}$ values of NO₃⁻ in the deionized water samples that also received the NO₃⁻ spike, and AR_i is the true AR value of NO₃⁻ at station *i*. Background-corrected AR values were then computed at each station *i* (AR_{bci}) by subtracting the background AR values (AR_b, calculated from the measured $\delta^{15}\text{N}$ values at the upstream station using equation 2) from the AR_i values calculated for samples collected at the stations downstream from the ¹⁵N injection as:

$$\text{AR}_{\text{bci}} = \text{AR}_i - \text{AR}_b. \quad [4]$$

Last, the tracer ¹⁵NO₃⁻ mass flux at each station *i* (¹⁵N_{flux i}, $\mu\text{g N/s}$) was computed by multiplying AR_{bci} by the streamwater NO₃⁻ concentration ([NO₃⁻N_i]) and stream discharge (Q_i) at each station *i* as:

$$^{15}\text{N}_{\text{flux bci}} = \text{AR}_{\text{bci}}[\text{NO}_3 - \text{N}_i]Q_i. \quad [5]$$

Q_i at each station was determined from the increase in streamwater Cl⁻ concentration during the injection as:

$$Q_i = ([\text{Cl}_{\text{inj}}]Q_{\text{pump}})/([\text{Cl}_i] - [\text{Cl}_b]) \quad [6]$$

where the Cl⁻ injection rate (mg/s) was calculated as the product of the Cl⁻ concentration in the injection solution ([Cl_{inj}]) and the solution injection rate (Q_{pump}), and the increase in Cl⁻ concentration at each station *i* was calculated as the difference between Cl⁻ concentration during the injection ([Cl_i]) and the measured Cl⁻ concentration just before the ¹⁵N injection (i.e., background concentration, [Cl_b]).

Calculation of NO₃ uptake parameters

The total uptake rate of NO₃⁻, expressed as a fractional uptake rate from water per unit distance (*k*, /m), was calculated for each sampling period from a single regression of ln(tracer ¹⁵NO₃⁻ flux) vs distance (Newbold et al. 1981, Stream Solute Workshop 1990). The inverse of *k* is the uptake length of NO₃⁻. Error associated with the calculated values of *k* was estimated as the error in each regression slope based on the 8 measurements made in each stream (4 measurements at each of 2 locations). This approach for determining *k* and its error using measurements at only 2 stations does not include error associated with longitudinal variation in uptake rate, but it does allow statistical comparisons of *k* values for the same reach

in each stream for different sampling periods (i.e., different times of the day or on different dates). Statistical differences in *k* between sampling periods were determined using the SAS General Linear Models procedure (version 8.2, SAS Institute, Cary, North Carolina).

Uptake velocity (V_f) was calculated from *k* using the equation:

$$V_f = kud \quad [7]$$

where *u* is the average water velocity and *d* is the average water depth (Stream Solute Workshop 1990). Total NO₃⁻ uptake was also calculated as a mass removal rate from water per unit area (*U*, μg N m⁻² min⁻¹) using the equation:

$$U = \frac{Fk}{w} \quad [8]$$

where *F* is the average flux of NO₃⁻ (as N) in streamwater in the experimental reach (determined as the product of average NO₃⁻ concentration and average discharge) and *w* is the average stream wetted width (Newbold et al. 1981). Error in V_f and *U* was estimated from error in *k*, assuming no error in measurements of NO₃⁻ concentration, discharge, *u*, *d*, and *w*.

Whole-stream metabolism measurements

Whole-stream rates of GPP and total respiration (*R*) were determined using the upstream–downstream diurnal dissolved O₂ change technique (Marzolf et al. 1994) with the modification suggested by Young and Huryn (1998) for calculating the air–water exchange rate of O₂. Measurements of dissolved O₂ concentration and water temperature (YSI 6000 series sondes; YSI, Yellow Springs, Ohio) were made at 5-min intervals at the 2 sampling stations in each stream over each of the experimental periods. Exchange of O₂ with the atmosphere was calculated based on the average O₂ saturation deficit or excess within the study reach and the reaeration rate determined from the decline in dissolved propane concentration during steady-state field injections of propane and a conservative tracer (Cl⁻, to account for dilution of propane by groundwater inflow) done during the measurement period in each stream. The reaeration rate of propane was converted to O₂ using a factor of 1.39 (Rathbun et al. 1978). The net rate of O₂ change caused by metabolism (equivalent to net ecosystem production [NEP]) was then calculated at 5-min intervals from the change in mass flux of dissolved O₂ between stations corrected for air–water exchange of O₂ within the reach.

The daily rate of *R* was calculated by summing the net O₂ change rate measured during the night and the daytime rate of *R* determined by a linear extrapolation between the net O₂ change rate during the 1-h predawn and postdusk periods. The daily rate of GPP was determined by summing the differences between the measured net O₂ change rate and the extrapolated value of *R* during the daylight period. All metabolic rates were converted to rates per unit area by dividing by the area of stream bottom between the 2 stations (determined from the measurement of wetted channel width at 1-m intervals over each reach).

Results

Physical and chemical conditions

Physical and chemical conditions were generally similar during ¹⁵N addition experiments in the same stream and month (Table 1). During the April experiments before leaf emergence in the forest canopy, NO₃⁻ concentrations in each stream were slightly higher in predawn samples than in samples taken at other times. Water temperatures were somewhat higher during midday sampling than during night sampling in both streams, particularly on the high-light dates. Discharge declined slightly during the sequential experiments in each stream. Discharge also was lower in June than in April, as is typical for these streams because of high evapotranspiration rates during the growing season.

Metabolism and biomass

The daily PAR fluxes to both streams were low on 5 April because of extensive cloud cover but increased substantially on 7 April and 9 April under mostly clear weather conditions (Table 2). Daily PAR values were considerably lower on 12 June after full leaf development than in April before leaf emergence. Rates of GPP generally followed PAR, with highest rates on the clear dates in April and lowest rates in June in both streams. In April, rates of GPP were 4 to 5× higher in the West Fork than the East Fork despite slightly higher PAR values in the East Fork on the same date because of higher algal and bryophyte biomass in the West Fork. For example, in April 2000, average epilithon and bryophyte biomasses were 5.7 and 2.5 g AFDM/m² in the West Fork compared with 0.7 and 0.6 g AFDM/m² in the East Fork (PJM, unpublished data). Filamentous algae also were visibly more abundant in the West Fork than the East Fork at this time (PJM, personal observation). The higher algal and bryophyte biomasses in the West Fork were probably a result of a

TABLE 1. Stream characteristics during each of the ^{15}N addition experiments in each stream.

Stream	Date (2001)	Time of sampling	Period	Discharge (L/s)	Water temperature ($^{\circ}\text{C}$)	NO_3^- concentration ($\mu\text{g N/L}$)
West Fork	4 April	2345	Midnight	6.6	13.2	16.6
	5 April	0630	Predawn	6.5	13.1	20.0
	5 April	1430	Midday	6.5	14.0	17.0
	7 April	1430	Midday	6.5	16.8	15.3
	9 April	1415	Midday	5.8	17.4	16.2
	11 June	2345	Midnight	3.4	15.2	50.5
	12 June	0540	Predawn	3.4	15.0	49.8
	12 June	0950	Midmorning	3.4	15.2	46.9
	12 June	1345	Midday	3.3	16.4	47.5
East Fork	5 April	0050	Midnight	4.1	12.0	4.8
	5 April	0710	Predawn	4.0	11.8	5.9
	5 April	1440	Midday	4.0	12.6	4.5
	7 April	1450	Midday	3.9	15.5	4.6
	12 June	0050	Midnight	0.6	17.0	46.9
	12 June	0600	Predawn	0.5	16.4	49.6
	12 June	1000	Midmorning	0.5	16.5	50.8
	12 June	1420	Midday	0.4	18.5	48.6

more stable benthic substratum in the West Fork. In June, rates of GPP were very low in both streams but, again, rates were higher in the West Fork than in the East Fork despite nearly $2\times$ higher PAR in the East Fork.

April NO_3^- uptake rates

Both streams showed significant diurnal and day-to-day variations in NO_3^- uptake rate (k) as determined from the longitudinal decline in tracer ^{15}N flux (Fig. 1A, B). These values of k corresponded to NO_3^- uptake lengths ranging from 112 to 310 m in the West Fork and from 61 to 83 m in the East Fork. NO_3^- uptake lengths were shorter in the East Fork than in the West Fork largely because discharge and NO_3^- concentrations were lower in the East Fork (Table 1).

Despite similar light regimes (Fig. 2A, B), diurnal and day-to-day variations in NO_3^- uptake parameters were considerably greater in the West Fork (Fig. 2C, E, G) than in the East Fork (Fig. 2D, F, H) in April. In the

West Fork, k was 2 to $3\times$ greater during midday periods (ranging from 0.0063–0.0090/m) than during the predawn sampling (0.0032/m), and k was $\sim 50\%$ greater on the 2 clear days (7 and 9 April) than on the overcast day (5 April) (Fig. 2C). In addition, k was nearly $2\times$ greater during the midnight sampling (0.0060/m) than during the predawn period on the same date. Values of k also were significantly greater on the 2 clear days than at midnight, although k on the overcast day did not differ from k for the previous midnight. The diurnal and day-to-day variations in k resulted in similar variations in V_f (Fig. 2E) and U (Fig. 2G) because differences in stream discharge and NO_3^- concentration were small over the 4-d experimental period (Table 1). Midday V_f (0.090–0.128 cm/min) and U (15.6–20.8 $\mu\text{g N m}^{-2} \text{min}^{-1}$) values were ~ 2 to $3\times$ greater than predawn V_f (0.046 cm/min) and U (9.2 $\mu\text{g N m}^{-2} \text{min}^{-1}$) values, and the clear-day V_f (0.125 and 0.128 cm/min) and U (19.1 and 20.8 $\mu\text{g N m}^{-2} \text{min}^{-1}$) values were $\sim 50\%$ higher than midnight V_f (0.085 cm/min) and U (14.1 $\mu\text{g N m}^{-2} \text{min}^{-1}$) values.

TABLE 2. Daily average water temperature, photosynthetically active radiation (PAR), gross primary production (GPP), and ecosystem respiration (R) on each of the dates of ^{15}N addition experiments in each stream.

Stream	Date (2001)	Water temperature ($^{\circ}\text{C}$)	Daily PAR (mol quanta $\text{m}^{-2} \text{d}^{-1}$)	Daily GPP (g $\text{O}_2 \text{m}^{-2} \text{d}^{-1}$)	Daily R (g $\text{O}_2 \text{m}^{-2} \text{d}^{-1}$)
West Fork	5 April	13.1	5.0	2.0	2.2
	7 April	13.9	12.0	4.7	4.7
	9 April	14.5	11.0	5.1	3.8
	12 June	15.4	1.0	0.2	1.9
East Fork	5 April	12.1	5.1	0.5	5.2
	7 April	13.0	15.4	0.9	6.0
	12 June	17.3	1.8	0.1	3.9

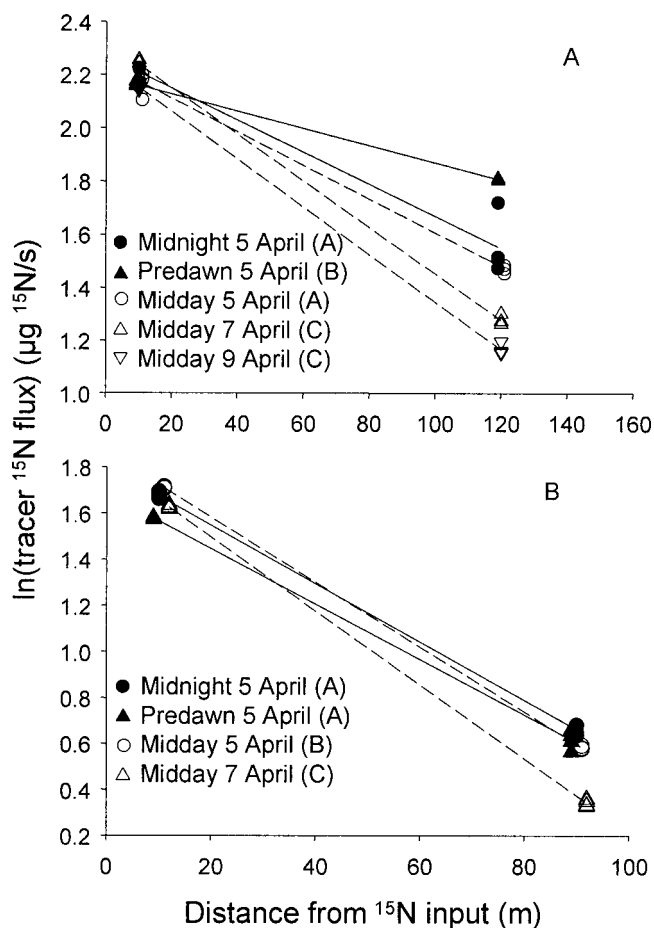


FIG. 1. Regressions of $\ln(\text{tracer } ^{15}\text{N flux})$ vs distance in the West Fork (A) and East Fork (B) of Walker Branch for experiments during the period 5 April to 9 April 2001. The slopes of each of the lines are the uptake rates (k) of NO_3^- per unit distance. Regressions with significantly different ($p < 0.05$) values of k have different letters (in parentheses next to legend).

In the East Fork, midday k values (0.014 and 0.016/m) were only ~ 10 to 30% greater than midnight and predawn k values (0.012 and 0.013/m), but the differences between midday and midnight values were significant (Fig. 2D). In addition, k was significantly greater on the clear day (7 April) than on the overcast day (5 April) but, again, the difference was relatively small (14%). Values of k did not differ between midnight and predawn in the East Fork. Diurnal and day-to-day variations in V_f (Fig. 2F) and U (Fig. 2H) also were small because differences in stream discharge and NO_3^- concentration (Table 1) were minimal between sampling periods (Table 1). Midday V_f values (0.234 and 0.267 cm/min) were only slightly greater than the midnight and predawn V_f values (0.211 and 0.200 cm/min), and midday U values (10.5

and 12.3 $\mu\text{g N m}^{-2} \text{min}^{-1}$) were similar to the midnight and predawn U values (10.1 and 11.8 $\mu\text{g N m}^{-2} \text{min}^{-1}$).

June NO_3^- uptake rates

Light regimes were similar in the West and East Forks (Fig. 3A, B). The magnitudes and diurnal variations in NO_3^- uptake parameters differed between streams (Fig. 3C–H) and differed from April values in each stream. In the West Fork, k was significantly different from 0 only at midday (0.0014/m, Fig. 3C). As in April, diurnal patterns in V_f and U (Fig. 3E, G) were similar to diurnal patterns for k because of minimal differences in NO_3^- concentrations and discharge between sampling periods (Table 1). In the West Fork, daytime V_f and U values were 5 to 10 \times greater than predawn values (Fig. 3E, G), but all values were low and error bars were large relative to the mean, suggesting that diurnal variations were not important in stream NO_3^- dynamics.

In the East Fork, k values were ~ 4 to 9 \times higher (0.0055 to 0.012/m, Fig. 3D) than in the West Fork (Fig. 3C) and values differed significantly between sampling periods. In the East Fork, k was $\sim 2\times$ higher at midday than at midnight and predawn, and mid-morning values of k were $\sim 50\%$ greater than the night values. V_f and U were considerably greater in the East Fork (Fig. 3F, H) than in the West Fork (Fig. 3E, G), and East Fork mid-morning and midday values were ~ 1.5 to 2 \times greater than night values. Although V_f was considerably lower in June than in April in the East Fork, daytime U was greater in June than in April, reflecting much higher NO_3^- concentrations in June than in April (Table 1).

Relationships between U and PAR

In April, relationships between U and daily PAR (Fig. 4A) and between U and daily GPP (Fig. 4B) were significant for the West Fork but not for the East Fork. The intercepts of both relationships were similar (11.8 $\mu\text{g N m}^{-2} \text{min}^{-1}$) and represent dark NO_3^- demands of heterotrophs and autotrophs. The slopes of these relationships (0.70 for U vs PAR and 1.68 for U vs GPP) represent the incremental daytime NO_3^- demand resulting from primary production. If we convert these slopes to equivalent units and assume a day length of 12 h, the incremental daytime NO_3^- uptake expressed per unit PAR was $1.0 \times 10^{-3} \mu\text{g N}/\mu\text{mol quanta}$ and expressed per unit of GPP was $2.4 \times 10^{-3} \mu\text{g N}/\mu\text{g O}_2$. If we assume a productivity quotient of 1.0 (mole CO_2 consumed/mole O_2 produced) and net primary production (NPP) of $\frac{1}{2}$ GPP, then the incremental daytime NO_3^- demand per unit of autotrophic C

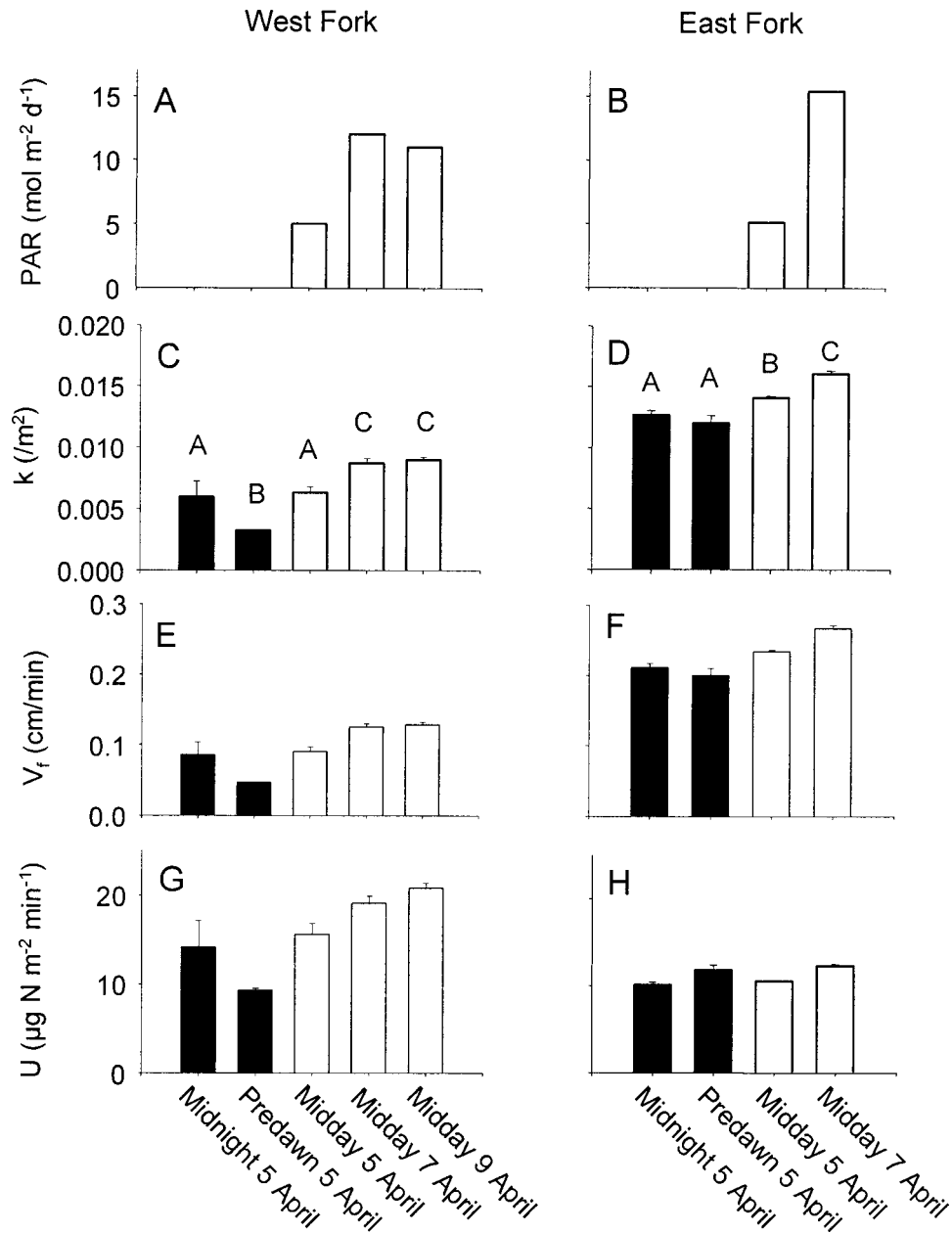


FIG. 2. Mean daily photosynthetically active radiation (PAR; A, B), NO₃⁻ uptake rate (*k*; C, D), uptake velocity (*V_f*; E, F), and uptake (*U*; G, H) for the experiments conducted during April 2001 in the West and East Forks of Walker Branch. Dark bars indicate nighttime measurements. Bars for values of *k* with different letters are significantly different (*p* < 0.05) among experiments (see Fig. 1). The error bars are upper 95% confidence intervals determined from the regressions for *k* (see Fig. 1) and from error propagation for calculations of *V_f* and *U* (see text and equations 7 and 8 for details) assuming no error in measurements of NO₃⁻ concentration, average water velocity, average width, and average water depth.

synthesis was 0.013 μg N/μg C (0.011 on a molar basis) in the West Fork. This value is only ~30% of the N:C ratio of West Fork periphyton measured in a previous study at this time of year (Mulholland et al. 2000), suggesting that a considerable amount of the autotrophic demand for N is met by uptake of NO₃⁻ at night or by uptake of other forms of N such as NH₄⁺.

Discussion

Diurnal and day-to-day variation

We showed that there were substantial diurnal and day-to-day variations in NO₃⁻ uptake related to light level and primary productivity in the West Fork of Walker Branch. We presume that these differences in

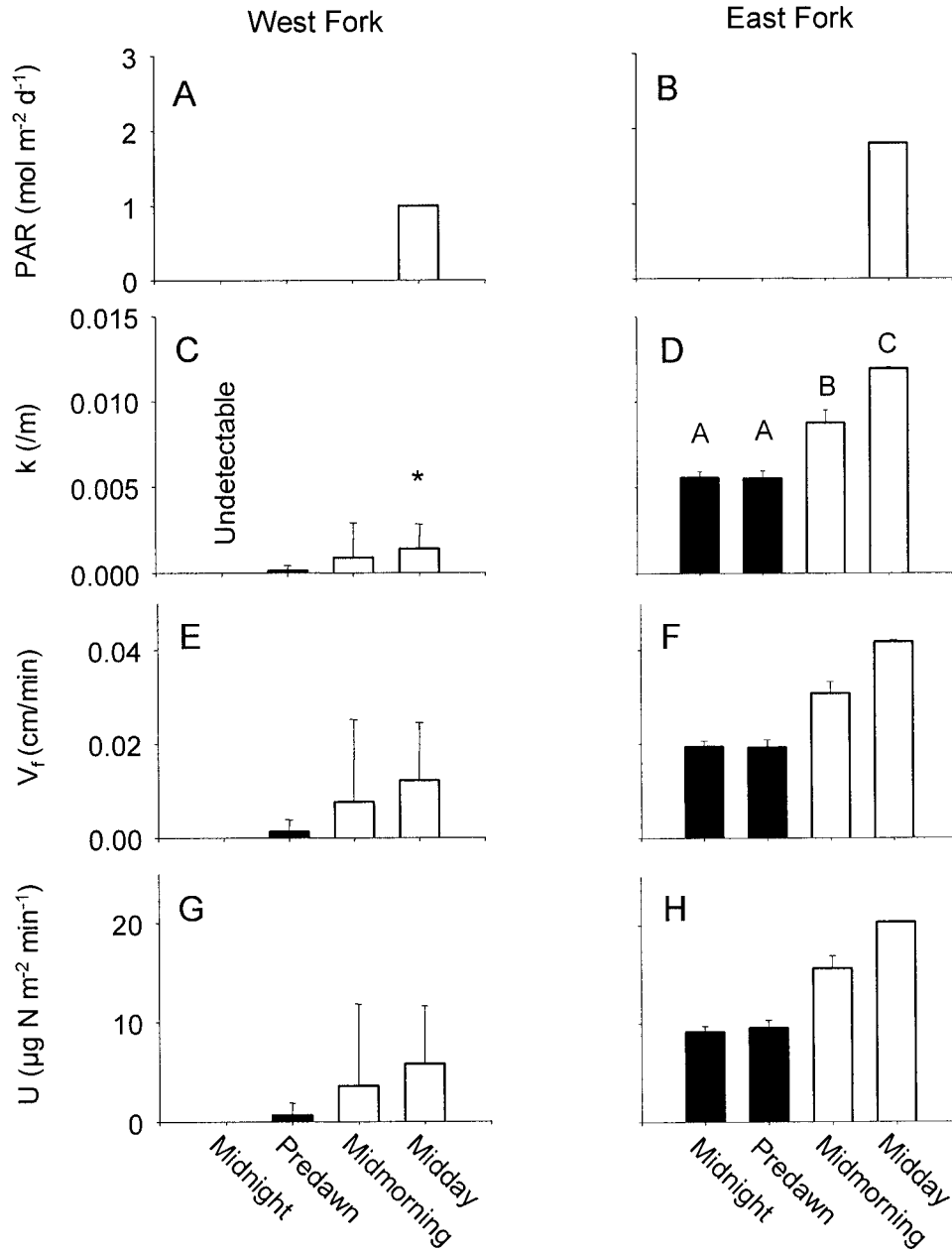


FIG. 3. Mean daily photosynthetically active radiation (PAR; A, B), NO₃⁻ uptake rate (*k*; C, D), uptake velocity (*V_f*; E, F), and uptake (*U*; G, H) for the experiments conducted during June 2001 in the West and East Forks of Walker Branch. Dark bars indicate nighttime measurements. No value for *k* differed from 0 in the West Fork on 11 to 12 June, except the midday uptake rate, which was marginally significant (*p* = 0.052; denoted by the asterisk). Bars for values of *k* in the East Fork with different letters are significantly different (*p* < 0.05) among experiments (see Fig. 1). Error bars are as in Fig. 2.

NO₃⁻ uptake were the result of differences in the demand for N and the ability of stream autotrophs (primarily benthic algae and bryophytes) to use NO₃⁻. We predicted that light level would influence whole-stream rates of NO₃⁻ uptake in seasons and streams with relatively high rates of primary production because photosynthesis provides additional energy that can be used to reduce NO₃⁻ for use in metabolism

and biosynthesis (Gutschick 1981, Huppe and Turpin 1994). We found greater diurnal and day-to-day variation in NO₃⁻ uptake rates in the West Fork than in the East Fork in April, and very low NO₃⁻ uptake rates in the West Fork in June. These results were consistent with our prediction. In early spring (before leaf emergence), the West Fork has considerably higher autotrophic biomass and rates of GPP than the East

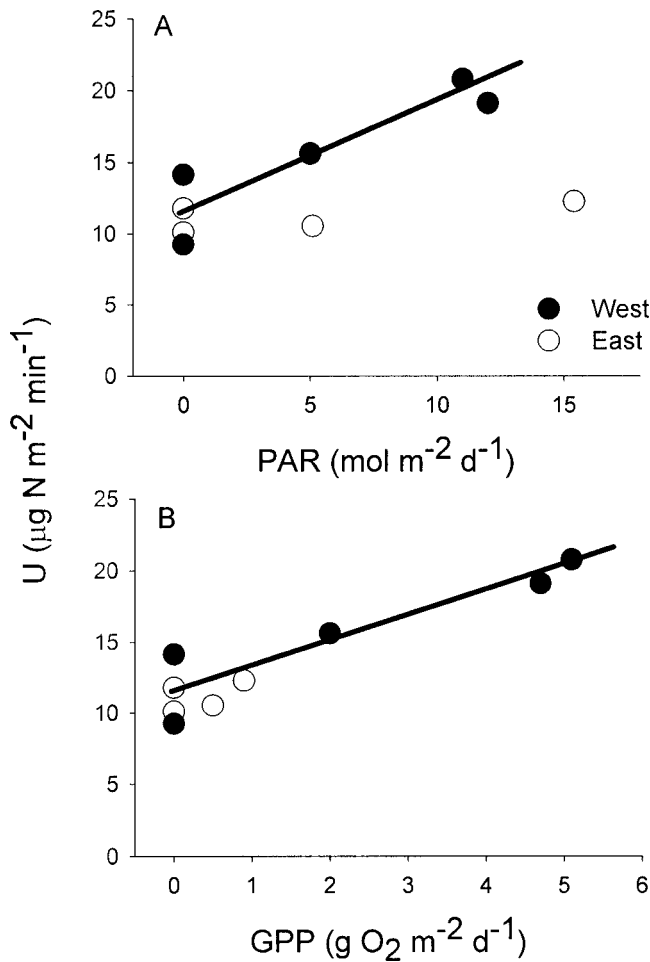


FIG. 4. Relationships between NO_3^- uptake (U) and daily photosynthetically active radiation (PAR; A) and daily gross primary production (GPP; B) for the April experiments. The regressions for the West Fork data were statistically significant ($p < 0.05$) and are shown as lines in the plots.

Fork but, in late spring (after leaf emergence), GPP drops to very low levels in both streams. Analyses of long-term NO_3^- concentration data from the West Fork indicate a consistent spring peak in instream NO_3^- uptake (Mulholland and Hill 1997, Mulholland 2004). An intensive study of stream NO_3^- concentration, light level, and primary production in the West Fork during spring showed increasing NO_3^- concentrations that coincided with declining light levels and rates of instream primary production as leaves emerged in the forest canopy (Hill et al. 2001). The significant diurnal variation in NO_3^- uptake rate that occurred under low light in the East Fork during June, however, was not in agreement with our prediction and we have no explanation for this result.

The observation of lower streamwater NO_3^- concentrations at midday than at predawn in both the West and East Forks in April (Table 1) also suggests increased NO_3^- uptake during the day. We do not have measurements of NO_3^- concentration at sufficient frequency to determine diurnal patterns in our current study, but a previous study in the West Fork of Walker Branch (10–11 April 1991) indicated diurnal variation in NO_3^- concentrations of up to $14 \mu\text{g N/L}$, or $\sim 50\%$ of the mean concentration on that date (Mulholland 1992). Mulholland (1992) also observed diurnal variation in dissolved organic C (DOC) concentrations that were opposite to those of NO_3^- concentration, suggesting that stream autotrophs were taking up NO_3^- and releasing DOC at greater rates during the day than at night.

Stream water temperatures also showed diurnal variation in both streams, particularly in April (Table 1), but this variation does not appear to account for all of the increases in NO_3^- uptake rates observed between predawn and midday periods. To estimate the potential effect of variation in stream water temperature, we calculated increases in NO_3^- uptake rates from predawn values assuming a Q_{10} of 2.0 (i.e., doubling of uptake rate per 10°C increase in temperature). In the West Fork, 56% and 54% of the increases in NO_3^- uptake rate between the 5 April predawn and 7 April and 9 April midday measurements could be explained by the 3.7 and 4.3°C increases in water temperature, respectively. In the East Fork, $>100\%$ of the April predawn to midday increases and 75% of the June predawn to midday increase in NO_3^- uptake rate could be explained by water temperature increases.

Other studies showing diurnal variation

Other researchers have reported diurnal variations in NO_3^- concentrations in streams. Manny and Wetzel (1973) reported diurnal variations in NO_3^- concentration of $\sim 200 \mu\text{g N/L}$ in Augusta Creek, Michigan, in October, but it was not clear if this variation reflected increases in the stream or in the marshes or lake upstream of the study site. Grimm (1987) reported diurnal variations in NO_3^- concentration of nearly $100 \mu\text{g N/L}$ in Sycamore Creek, Arizona, a desert stream with high GPP. Burns (1998) observed diurnal variations in NO_3^- concentration in 2 reaches of the Neversink River in the Catskill Mountains of New York throughout the spring and summer and attributed these to autotrophic uptake within the stream.

Fellows et al. (2006) reported that NO_3^- uptake rates were generally higher during the day than at night during summer in 2 forested streams in the southern Appalachian Mountains (one of which was the East

Fork of Walker Branch) and 2 forested streams in the mountains of New Mexico. Fellows et al. (2006) also reported that NO₃⁻ uptake rates were higher in the New Mexico streams (open forest canopies) where light levels were higher than in the Southern Appalachians (dense forest canopies). They used different methods (NO₃⁻ addition experiments conducted in benthic chambers and in the stream) than we did, but they found diurnal variations in NO₃⁻ uptake rate that are consistent with our observations. However, the NO₃⁻ uptake rates measured by Fellows et al. (2006) were <1/2 those reported in our study, probably because of differences in methods between their study and ours. The nutrient-addition approach used by Fellows et al. (2006) yields lower estimates of nutrient uptake than tracer approaches (Mulholland et al. 1990, 2002, Payn et al. 2005).

Nighttime variation

Algae can take up NO₃⁻ in the dark using stored C reserves (Abrol et al. 1983), but our results indicate that algal uptake is not constant through the night. We observed significantly lower NO₃⁻ uptake rates just before dawn than near midnight in the West Fork in April when algal production was high. We suggest that this result may have been caused by depletion of stored photosynthate generated during daylight hours. Thus, there may be a 24-h cycle in NO₃⁻ uptake with highest rates during the middle to latter part of the daylight period and lowest rates at night just before dawn.

Role of autotrophs and GPP

Our results showing strong relationships between NO₃⁻ uptake rates, PAR, and GPP in the West Fork during April indicate the important role of autotrophy in regulating N dynamics in streams. Hall and Tank (2003) also have reported a significant relationship between NO₃⁻ uptake, expressed as V_f , and GPP for 11 streams in Grand Teton National Park. Hall and Tank (2003) used a stoichiometric model that suggested that stream autotrophs could account for most, if not all, of the NO₃⁻ uptake in the streams, but they did not investigate diurnal variations in NO₃⁻ uptake rates.

We estimated expected periphyton uptake rates and autotrophic demand for N based on periphyton N:C stoichiometry, a productivity quotient of 1.0, and NPP = 1/2(GPP). The low incremental daytime NO₃⁻ uptake rates for the rate of GPP observed in the West Fork in April (30% of expected) suggests that a considerable amount of the autotrophic demand for N was met by uptake of NO₃⁻ at night or by uptake of other forms of N (e.g., NH₄⁺). If the average nighttime NO₃⁻ uptake

rate in the West Fork in April (11.8 μg N m⁻² min⁻¹) represents a basal uptake rate of autotrophs, then this uptake would account for ~40% of the total autotrophic N demand resulting from NPP on 9 April (highest GPP in our study). Therefore, it seems likely that other forms of N also are important sources for autotrophs.

Interactions with NH₄⁺

NH₄⁺ concentrations in the West Fork during the April experiments (2–5 μg N/L) were considerably lower than NO₃⁻ concentrations but, in a previous study, Mulholland et al. (2000) measured high rates of NH₄⁺ uptake at low NH₄⁺ concentrations using the tracer ¹⁵N addition approach. In April 1997, Mulholland et al. (2000) measured an NH₄⁺ uptake rate of 22 μg N m⁻² min⁻¹ when NH₄⁺ and NO₃⁻ concentrations were similar to those in our current study, suggesting that NH₄⁺ uptake could have accounted for a significant portion of the autotrophic demand for N in our current study.

In the earlier study, which involved a 6-wk tracer ¹⁵NH₄⁺ addition to the West Fork, Mulholland et al. (2000) observed a substantial interaction between NH₄⁺ concentration and light level in regulating NO₃⁻ uptake. Mulholland et al. (2000) calculated nitrification and NO₃⁻ uptake rates from the longitudinal distribution of tracer ¹⁵NO₃⁻ generated during the ¹⁵NH₄⁺ addition and reported that NO₃⁻ uptake rates declined from 28.8 μg N m⁻² min⁻¹ on a clear day before leaf emergence (1 April 1997) to 9 μg N m⁻² min⁻¹ after leaf emergence (12 May 1997). These values are similar to values measured in our current study in the West Fork at midday before (7 and 9 April, Fig. 2G) and after leaf emergence (12 June, Fig. 3G). However, Mulholland et al. (2000) also reported that NO₃⁻ uptake rates were undetectable on 20 April 1997 under partial leaf emergence when NH₄⁺ concentrations were ~2× greater than NH₄⁺ concentrations measured in the April experiment of our current study.

Implications for stream nutrient dynamics

Our results showing light-driven diurnal and day-to-day variations in NO₃⁻ uptake point to the potentially important role of autotrophs in nutrient uptake in forested streams, particularly during seasons when deciduous vegetation is dormant and light levels are relatively high. Our results for the West Fork showing relatively high NO₃⁻ uptake in April and minimal uptake in June suggest that nearly all NO₃⁻ uptake in April was by autotrophs. In the East Fork, autotrophs appeared to play only a minor role in NO₃⁻

uptake even under relatively high light levels in April. However, this result was consistent with the low rates of GPP measured in the East Fork where abundance of autotrophs was considerably lower than in the West Fork, presumably because the substratum in the East Fork was less stable (gravel and fine-grained sediments) than in the West Fork (bedrock and large cobble). Thus, our results suggest that substratum characteristics may play an important role in controlling the seasonal importance of autotrophs and their impact on nutrient cycling in forest streams.

Some time ago, Minshall (1978) pointed out the importance of autotrophy in regulating stream ecosystem structure and function, even for streams draining forested catchments that might be considered net heterotrophic over an annual period. Minshall (1978) noted the modeling study by McIntire (1973) showing that low algal standing stocks in streams can lead to the erroneous assumption that autotrophy is relatively unimportant. McIntire (1973) and Minshall (1978) showed that high algal productivity and turnover rates can support relatively large populations of primary consumers and rates of secondary production despite low algal standing crops. Our results indicating that autotrophs are important in controlling nutrient dynamics in the West Fork of Walker Branch lend support to Minshall's (1978) argument that autotrophy can be an important regulating factor in forested stream ecosystems.

Our results showing diurnal and day-to-day variation in NO_3^- uptake have important implications for longer-term assessments of N cycling in streams. Measurements of NO_3^- uptake usually are made during the day and may overestimate uptake when extrapolated to a 24-h period in streams with relatively high levels of autotrophy. In addition, NO_3^- uptake rates measured on relatively clear days may not be representative of rates on overcast days. Last, rates and diurnal variation in NO_3^- uptake may be much greater in late winter and spring than at other times in streams draining deciduous forests, and annual estimates of NO_3^- uptake must account for these seasonal effects.

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