

Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams

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Abstract

We measured whole-stream uptake of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (as soluble reactive phosphorus [SRP]) in two reaches of three forested headwater streams eight times from May 2003 to April 2004 ($n = 46$ measurements per nutrient type). We also measured factors that could affect uptake, including ambient nutrient concentrations, whole-stream metabolism, and organic matter standing stocks. In all three streams, we measured the highest rates of NH_4^+ and NO_3^- uptake velocity (V_f) during the spring. Low ambient NH_4^+ concentrations limited NH_4^+ uptake (U) in two streams. In one stream, when ambient NO_3^- concentrations increased during summer, $\text{NO}_3^- V_f$ decreased. Temporal patterns of SRP V_f varied among streams, but were unrelated to variability in ambient SRP concentration. However, in all three streams, seasonal variation in SRP V_f was strongly influenced by heterotrophic metabolism (as measured by community respiration; State Creek $r = 0.81$, $p = 0.03$; Shane Creek $r = 0.89$, $p < 0.01$; Walton Creek $r = 0.91$, $p < 0.01$). Although heterotrophic processes typically dominate in forested headwater streams, we found that variability in nutrient uptake among streams was also explained by variables related to autotrophic activity (i.e., proportion coverage of large inorganic substrata and gross primary production). We suggest that the unexpected influence of autotrophy in this study was a result of stream sampling frequency, which included winter and spring—seasons not typically sampled. Our study demonstrates that examining nutrient uptake across streams and during different seasons can provide insight into factors controlling nutrient uptake parameters.

Nutrient supply may limit rates of primary and microbial production in stream ecosystems (Elwood et al. 1981; Suberkropp and Chauvet 1995) and nutrient availability can have bottom-up effects on stream food webs and downstream ecosystems (Rosemond et al. 1993; Vitousek et al. 1997). Rates of in-stream nutrient processing are influenced by local factors such as riparian vegetation (Sabater et al. 2000), point-source nutrient input (e.g., treated wastewater; Martí et al. 2004), larger-scale variation in climate (Mulholland 1992), and cultural eutrophication (Alexander et al. 2000). Previous studies of nutrient uptake in streams have focused largely on controlling factors of

nutrient uptake rates that vary geographically, that is, across biomes, land-use types, or along a stream gradients (Munn and Meyer 1990; Hall and Tank 2003; Webster et al. 2003; Bernot et al. 2006). In forested headwater streams, seasonal changes in light, organic matter inputs, and temperature may also affect biotic demand for inorganic nutrients from the water column (Mulholland et al. 1985; Mulholland et al. 2000; Hill et al. 2001). Seasonal variability in nutrient uptake has been measured in grassland streams in New Zealand (Simon et al. 2005) and forested streams in the mild Mediterranean climate of Spain (Martí and Sabater 1996), but to our knowledge, no studies have conducted a similar analysis of seasonal variability in nutrient uptake rates in temperate forests where the climate is highly seasonal.

Nutrient uptake rates in streams are affected by autotrophic (i.e., algae, bryophytes, and macrophytes) and heterotrophic (i.e., fungi and bacteria) demand (Minshall 1978; Webster and Benfield 1986; Grimm 1987). Growth and activity of these organisms can be limited by inorganic nutrient availability. In addition, autotrophic activity can be limited by light availability and substratum stability (Gregory 1980; Allan 1995) and heterotrophic activity can be limited by organic carbon quantity and quality (Tank and Webster 1998). In headwater streams that drain temperate forests, changes that occur with season can influence autotrophic and heterotrophic activity; forest canopy in summer and ice cover in winter limits light, and allochthonous organic matter is highest after autumn leaf-fall (Minshall et al. 1983; Hill et al. 2001). We predicted that these seasonal changes in forested headwater streams would result in concomitant variability in nutrient uptake parameters.

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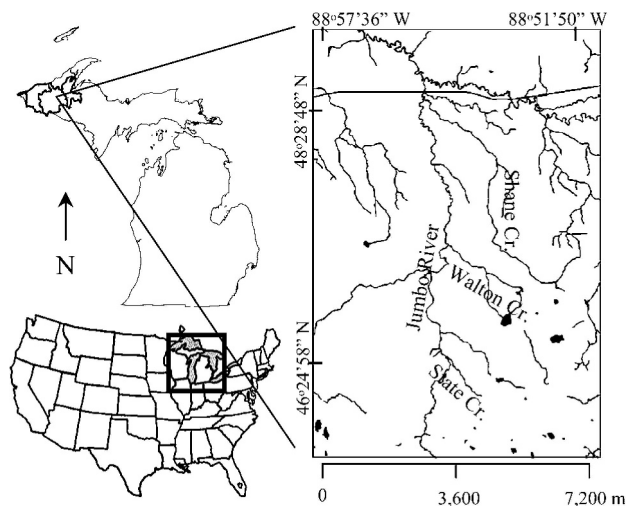


Fig. 1. Three study streams in Jumbo River drainage. The streams are tributaries of Lake Superior and are located near Kenton, Michigan (latitude $46^{\circ}48'$, longitude $88^{\circ}55'$), in the western portion of the Upper Peninsula of Michigan, within the Ottawa National Forest.

Our objective was to explore seasonal variability in ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) uptake parameters in three adjacent forested headwater streams. We predicted that streams with a high degree of seasonal variability would provide a unique opportunity to explore the factors that influence inorganic nutrient uptake. The three streams that we studied are adjacent watersheds, so we hypothesized that there would be little spatial variability in nutrient uptake parameter among streams. We predicted that the highest rates of nutrient uptake would occur in spring because of minimal canopy shading and increased primary production. In addition, we predicted high rates of nutrient uptake in autumn because of high rates of community respiration associated with organic matter entering streams during leaf-fall. We measured nutrient uptake in two reaches in three streams every month and predicted that any spatial and seasonal variability observed would help us identify factors that control nutrient uptake in these streams. Our use of spatial and temporal replication enabled statistical power unique in the study of whole-stream nutrient uptake rates. Furthermore, we conducted this study in the Upper Midwestern U.S., where there has been little research on nutrient uptake dynamics in streams. This study provides data on nutrient uptake in relatively unmodified Midwestern streams and can serve as a reference for other Midwestern streams that have been widely affected by agricultural N and P eutrophication (Alexander et al. 2000).

Methods

Study sites—We conducted this study in three forested, first-order streams (State, Shane, and Walton Creeks) in the Ontonagon River basin of Lake Superior, in the Upper Peninsula of Michigan, U.S. (Fig. 1). These streams are tributaries of the Jumbo River and are located within

approximately 4.5 km of each other. These streams were chosen as replicates for this study because they have similar orientation, geology, climate, watershed area, discharge, riparian vegetation, and logging history (Table 1).

The climate in this region has mild summers (mean July air temperature is 18.1°C) and cold winters (mean January temperature is -10°C ; Sommers 1984). Streamwater temperatures ranged from 0.0°C to 18.1°C (Table 1). Mean annual precipitation is approximately 76.2 cm, but snowfall in portions of the Ottawa National Forest can exceed 500 cm annually (<http://www.fs.fed.us/r9/ottawa/index.shtml>). Bedrock consists of the slate-containing Michigamme formation, but is not exposed in any of the stream beds. Surficial geology is composed of glacial moraine, although the texture varies by stream (Table 1). Riparian vegetation includes white pine (*Pinus strobes* L.), eastern hemlock (*Tsuga canadensis* L.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), alder (*Alnus* spp.), and paper birch (*Betula papyrifera* Marsh.), with an understory of mixed forbes and ferns.

Nutrient uptake—We conducted short-term additions of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}), in two 100-m reaches (separated by 20–50 m) of each stream monthly from May to October 2003, December 2003, and April 2004. Extreme winter conditions limited access from January to March 2004 at all sites and in December 2003 at Walton Creek.

We added nutrients in two separate short-term additions using standard methods (Stream Solute Workshop 1990; Webster and Ehrman 1996). One addition contained ammonium as NH_4Cl and NaCl as a conservative tracer measured as conductivity and the other nitrate as NaNO_3 , phosphate as KH_2PO_4 , and NaBr as a conservative tracer measured as bromide. We added NH_4^+ and NO_3^- separately to avoid the potential influence of nitrification on NO_3^- uptake, but NO_3^- samples taken during previous NH_4^+ releases showed no detectable downstream accumulation of water column NO_3^- resulting from nitrification of added NH_4^+ (Tank unpubl. data). Because of limited daylight hours during field sampling, we added two solutes in unison (NO_3^- and PO_4^{3-}) to maximize the number of reaches sampled in 1 day, but we may have potentially released the limitation of one nutrient, altering uptake of the other. Although we did not test the effect of added NO_3^- on soluble reactive phosphorus (SRP) uptake or vice versa by comparing individual and combined releases in these study streams, we have tested it previously in low-nutrient systems and the uptake of N or P was never influenced by the presence of the other during a short-term release (see Hall and Tank 2003). This is likely because the biology of these systems cannot respond quickly enough to take advantage of the short-term increase (<60 min) in nutrient supply.

Before solute additions, we collected water samples every 20 m downstream of the release site to measure ambient solute concentrations and conductivity. We added solutes at a rate of 200 mL min^{-1} (Fluid Metering, Lab pump Model RHB) to raise nutrient concentrations slightly above ambient concentrations ($+5\text{--}20 \mu\text{g NH}_4^+\text{-N}$ or $\text{PO}_4^{3-}\text{-P L}^{-1}$

Table 1. Annual mean, standard error (SE), and range of stream characteristics including physiochemical variables, organic matter standing stock, substratum percent coverage, and whole-stream metabolism. NH_4^+ = ammonium, NO_3^- = nitrate, SRP = soluble reactive phosphorus, DIN = dissolved inorganic nitrogen, DON = dissolved organic nitrogen, FBOM = fine benthic organic matter, CBOM = coarse benthic organic matter, LWD = large woody debris, GPP = gross primary production, CR = community respiration, and P:R = GPP:CR. *F* and *p* values are the result of one-way ANOVA among streams; *p*-values ≤ 0.05 are in bold.

	State			Shane			Walton			ANOVA		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	<i>n</i>	<i>F</i>	<i>p</i>
Watershed area (km ²)	24.0			45.5			35.7					
Stream gradient (%)	1.8			0.9			1					
Year last logged	1967			1967			1915					
Surficial geology												
Terminal moraine, coarse texture												
Recessional moraine, coarse texture												
Temperature (°C)	6.2		0.0-18.1	6.7		0.0-17.9	6.4		0.0-17.8	7,800	4.79	0.889
Discharge (L s ⁻¹)	67.7	6.5	43.8-104.1	39.8	7.7	12.6-79.0	46.1	14.6	17.7-125.0	23	2.32	0.124
Conductivity (μS cm ⁻¹)	141.5 ^a	9.8	83.2-164.3	83.7 ^b	9.6	37.5-110.0	71.0 ^b	8.6	34.3-93.4	23	16.01	<0.001
Wetted width (m)	2.2 ^{ab}	0	2.04-2.31	2.4 ^a	0.1	1.97-2.61	2.1 ^b	0	1.95-2.14	23	6.76	0.006
Nutrient concentrations												
NH_4^+ (μg N L ⁻¹)	5	1.1	2.0-10.8	8.3	1.3	4.3-14.6	5.9	0.6	4.3-9.0	23	3.4	0.053
NO_3^- (μg N L ⁻¹)	149.4 ^b	5.9	127.3-170.6	169.8 ^b	12.9	127.2-239.4	440.9 ^a	87.8	127.5-762.1	23	11.17	0.001
SRP (μg P L ⁻¹)	4.8 ^{ab}	0.7	2.2-8.2	7.1 ^a	0.7	1.2-10.1	4.1 ^b	0.6	1.8-6.6	23	5.45	0.013
DIN:SRP*	57.9 ^b	10.8	28.6-122.4	40.3 ^b	5.3	26.2-73.6	175.5 ^a	33.7	49.6-351.3	23	16.91	<0.001
DON (mg N L ⁻¹)	1.1 ^b	0.1	0.77-1.31	1.0 ^b	0.2	0.68-1.46	2.6 ^a	0.2	2.31-2.79	11	31.66	<0.001
Organic matter standing stock												
FBOM (g AFDM m ⁻²)	125.4	23.1	33.5-222.0	177.7	52.9	96.8-542.4	147.9	20	97.9-262.7	21	0.95	0.402
CBOM (g AFDM m ⁻²)	8.72	5.96	0.01-48.5	17.8	11.5	0.0-95.7	43	18.6	0.4-134.7	21	1.14	0.339
Wood (g AFDM m ⁻²)	145.6	81.4	0.4-673.4	99.08	28.4	29.3-240.0	175.6	47.9	15.5-406.7	21	1.38	0.273
Chlorophyll <i>a</i> (μg cm ⁻²)	1.65	0.64	0.16-4.38	1.12	0.51	0.17-4.53	1.45	0.3	0.82-3.34	21	0.97	0.396
LWD volume (m ³ m ⁻²)†	0.005	0	0.004-0.007	0.019	0.01	0.011-0.028	0.007	0	0.007-0.008	6	2.35	0.243
LWD density (no. m ⁻²)†	0.116 ^b	0.01	0.109-0.123	0.183 ^a	0.02	0.166-0.199	0.159 ^{ab}	0	0.157-0.162	6	10.45	0.044
Substrata coverage (%)												
FBOM	4.4 ^c	0.72	3.7-5.3	14.1 ^b	1	13.1-15.1	21.1 ^a	0.5	20.6-21.7	6	117.6	0.001
CBOM + wood	17	2.2	14.8-19.2	25.7	0.8	24.9-26.5	17.4	1.4	16.0-18.9	6	9.48	0.051
Sand	15	3.3	11.7-18.3	30.1	2	28.1-32.0	22.8	5	17.9-27.8	6	4.34	0.130
Gravel	30.4	1.1	19.2-31.5	24.2	3.4	20.9-27.6	23.5	0.3	23.3-23.8	6	3.37	0.171
Cobble + boulder	22.5 ^a	1.6	20.9-24.0	5.9 ^b	0.4	5.5-6.4	14.4 ^{ab}	3.2	11.3-17.6	6	16.44	0.024
Bryophyte	11.3 ^a	4.9	6.3-16.2	0.0 ^b	0	0.0-0.0	0.7 ^b	0.1	0.5-0.8	6	26.01	0.013
Whole-stream metabolism (g O ₂ m ⁻² d ⁻¹)												
GPP	0.9	0.4	0.17-2.42	0.1	0	0.02-0.13	0.6	0.2	0.19-1.17	15	2.17	1.560
CR	7.6	1.8	3.44-13.31	4.3	0.8	1.68-6.19	4.4	2.6	0.15-13.10	15	1.1	0.365
P:R	0.2	0.1	0.02-0.57	0	0	0.00-0.03	1.2	0.8	0.00-3.05	15	2.43	0.130

^{a,b,ab} Groupings from Tukey's multiple comparison test after 1-way ANOVA comparing among streams.

* DIN:SRP is the molar ratio of N in DIN to P in SRP.

† LWD volume and number of LWD pieces per stream area, from Cordova et al. (in press).

and + 6–130 $\mu\text{g NO}_3^- \text{-N L}^{-1}$), conductivity by 5–35 $\mu\text{S cm}^{-1}$, and Br^- concentration by 25–130 $\mu\text{g L}^{-1}$. When conservative tracer concentrations were uniform throughout the 100-m reach (plateau stage), we collected three replicate water samples at each of the five sites within each study reach. We filtered samples in the field through glass fiber filters (GFFs) with a pore size of 0.45 μm (Type A/E GFF, Pall Corporation), and samples were frozen until solutes were analyzed in the laboratory.

To measure NO_3^- and Br^- concentrations, we used ion chromatography (Dionex Model DX600) with AS14A analytical and guard columns and a 500- μL injection loop. We measured ammonium concentrations using the phenylhypochlorite technique (Solorzano 1969), and PO_4^{3-} concentrations as SRP using the molybdate–antimony method (Murphy and Riley 1962). SRP is generally an overestimate of PO_4^{3-} (Hudson et al. 2000), so our values for ambient SRP and SRP uptake rate (U) may overestimate actual values. We used a YSI conductivity meter (Model 30) or Hydrolab Minisonde (Model 4A) to measure specific conductivity. We used a Shimadzu TOC-V/TNM total organic carbon analyzer with a total nitrogen module to measure total nitrogen (TN), and calculated dissolved organic N (DON) concentrations as the difference between TN and $\text{NO}_3^- + \text{NH}_4^+$ concentrations.

We calculated nutrient uptake lengths (S_w) using background-corrected nutrient concentrations (enriched minus ambient concentration) divided by background-corrected tracer concentrations and plotted the natural log of this fraction against distance downstream, taking the absolute value of the inverse of the slope to calculate S_w (Stream Solute Workshop 1990). From S_w we calculated uptake velocity (V_f) as (discharge/width)/ S_w and then calculated areal nutrient uptake rate (U) as V_f * ambient nutrient concentration (Stream Solute Workshop 1990). Because S_w is highly influenced by discharge, uptake velocity and U are the most useful parameters for comparing nutrient uptake across spatial or temporal scales that have varying discharge (Davis and Minshall 1999; Hall et al. 2002). Although, in comparison to isotopic tracer measurements, short-term nutrient additions overestimate S_w because of the saturation of benthic nutrient demand (Mulholland 1992, Hall et al. 1998); we found no relationship between enrichment concentration (proportion enriched above ambient concentration) and S_w for NH_4^+ ($R^2 = 0.09$, $p = 0.57$), NO_3^- ($R^2 = 0.03$, $p = 0.26$), or PO_4^{3-} ($R^2 = 0.01$, $p = 0.55$) suggesting saturation did not occur. In addition, we assume that potential overestimates of uptake parameters in this study are equal across streams and dates.

Whole-stream metabolism—We calculated whole-stream metabolism by measuring changes in O_2 concentrations and temperature in 10-min increments for 32 h immediately before or after the short-term nutrient additions on all dates, excluding December and April, using a field-calibrated Hydrolab minisonde (Marzolf et al. 1994; Young and Huryn 1998). Reaeration was estimated by releasing a conservative gas (propane or sulfur hexafluoride) on the same day as the nutrient additions and regressing the

decline in Br^- -corrected gas concentrations at each downstream collection point (Wanninkhof et al. 1990). Propane and sulfur hexafluoride concentrations were measured on a gas chromatograph (Varian Model STAR 600, Varian Analytical Instruments) with electron capture detector (sulfur hexafluoride) or flame ionization detector (propane).

We calculated community respiration (CR) as average reaeration-corrected O_2 flux during the dark and gross primary production (GPP) as the sum of the instantaneous change in O_2 concentration (reaeration corrected) during daylight hours minus CR. This method does not include anaerobic respiration and assumes respiration in the light is equal to that in the dark (Uehlinger 2000). There was no significant dilution within our study reaches, as measured by conservative tracers, and therefore we did not correct for groundwater O_2 inputs (Hall and Tank 2005).

Organic matter standing stock and substratum distribution—We inserted a 804-cm² core (19 L plastic bucket with the bottom removed) 10 cm into the stream benthos at five randomly selected locations along each 100-m reach, vigorously stirred the substrata, removed all coarse benthic organic matter (CBOM) with a 1-mm sieve, and subsampled the remaining sediment slurry to estimate fine benthic organic matter (FBOM). In the laboratory, we separated CBOM into wood, non-wood, and bryophytes. We filtered FBOM onto a pre-ashed and weighed GFF filter, dried the filter for 3–7 days at 60°C, measured dry mass, and then determined ash-free dry mass (AFDM) after combustion (3 hours at 550°C). We extracted chlorophyll *a* (Chl *a*) from FBOM filtered onto a GFF using the non-acidification, hot ethanol method and measured Chl *a* concentrations on a Turner Designs Model TD-700 Fluorometer (Sartory and Grobbelaar 1984). All measurements of CBOM, FBOM, and Chl *a* were taken concurrently with all nutrient uptake measurements, with an additional sampling point in March 2003.

We estimated benthic coverage of various substratum types (FBOM, CBOM + wood, sand, gravel, cobble + boulder, and bryophytes) using 21 transects 5 m apart along each 100-m reach and recorded substratum type every 10 cm across each transect in May and August 2003. We quantified percent coverage for each substratum. Large woody debris volume and density in these streams were measured in the summer of 2003 (Cordova et al. 2007).

Statistical analyses—We used one-way analysis of variance (ANOVA) to compare physiochemical, organic matter, and Chl *a* standing stocks, substratum percent coverage, and whole-stream metabolism measurements among the three study streams. We used repeated measures (rm) ANOVA to analyze temporal and spatial variation of nutrient uptake metrics, using the two reaches in each stream as replicates ($n = 16$ for State and Shane, $n = 14$ for Walton for each nutrient type). If a significant interaction between stream and date resulted, we used one-way ANOVA across streams for each month using Bonferroni adjusted p -value of 0.05/8 months = 0.00625 to determine significant differences. In addition, we used rmANOVA

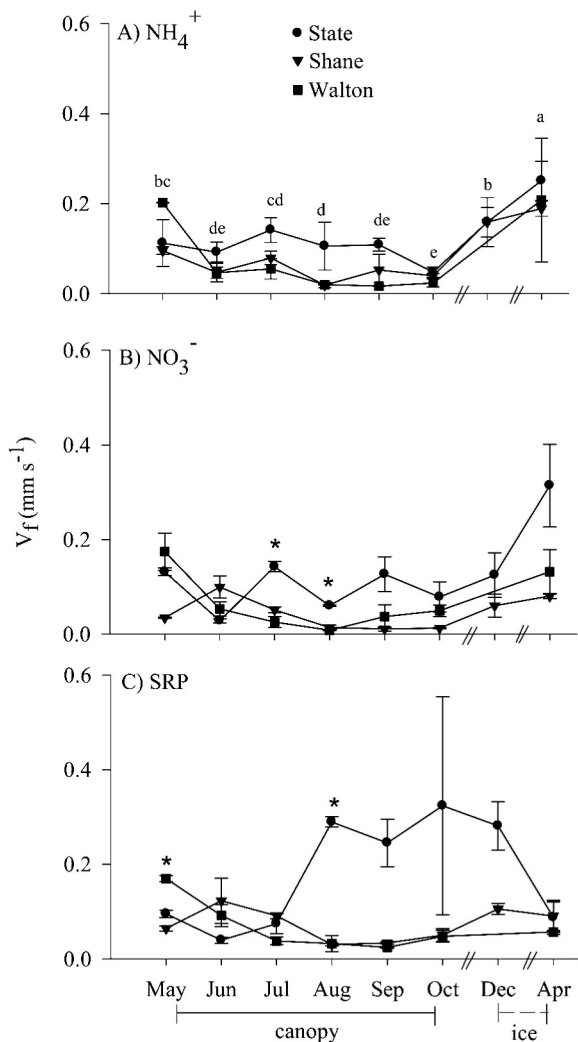


Fig. 2. Mean (\pm SE) uptake velocity (V_f) of (A) ammonium (NH_4^+), (B) nitrate (NO_3^-), and (C) soluble reactive phosphorus (SRP) in the three study streams from May 2003 to April 2004, $n = 16$ State and Shane, $n = 14$ Walton. Small letters indicate differences among dates from Tukey's multiple comparison test following rmANOVA with non-significant interaction factor. * indicates differences among sites for a specific sampling date, significant if less than a p value of 0.00625 (Bonferroni-adjusted as 0.05/8), performed after a significant interaction factor of rmANOVA.

followed by Tukey's multiple comparison test (MCT) for each individual stream to compare uptake rates among dates. For all rmANOVAs, we used a first-order autoregressive covariance structure (after Simon et al. 2005).

We used linear regression to examine the relationship between nutrient uptake and factors that directly control uptake, including temperature, whole-stream metabolism, and ambient nutrient concentration (as NH_4^+ , NO_3^- , and SRP independently and as dissolved inorganic nitrogen [DIN]:SRP ratio). Pearson's product-moment correlation was used to determine the association between nutrient uptake and metabolism and factors that indirectly control uptake, including organic matter and Chl a standing stocks, large woody debris (LWD) density, and substrata percent

coverage. We used all measurements (both reaches in each stream) as independent observations (V_f and U $n = 16$ State and Shane, $n = 14$ Walton; CR and GPP $n = 7$ State, $n = 9$ Shane and Walton). We used the coefficient of variation to compare variability in V_f between reaches, among streams, and among dates. Statistical analyses were done using SAS 9.1 (SAS Institute) or SYSTAT 10.2 (SYSTAT Software).

Results

Streamwater physiochemical conditions—In all three streams, discharge was highest in spring and lowest in summer and fall. There were no significant differences in annual discharge among streams (one-way ANOVA, $p = 0.124$; Table 1). In general, ambient water concentrations of NH_4^+ -N and SRP were low, ranging from 2–15 $\mu\text{g L}^{-1}$ and 2–10 $\mu\text{g L}^{-1}$, respectively; however, NH_4^+ -N concentrations were higher in July and December, and SRP concentrations were higher in mid-summer (Table 1). Across all streams, NO_3^- concentrations were much higher than NH_4^+ (from 21–75 times higher) and SRP (from 30–108 times higher). We did not observe differences in NH_4^+ concentrations among streams. SRP concentrations were significantly different among streams (highest in Shane Creek and lowest in Walton Creek). Nitrate concentrations were consistently higher in Walton Creek compared to the other two streams.

Nutrient uptake metrics (S_w , V_f , and U)—To examine temporal patterns in nutrient uptake we used V_f and U rather than S_w . Ammonium V_f differed among months (rmANOVA, $p < 0.0001$) and streams (rmANOVA, $p = 0.0001$), but there was no significant interaction between time and stream ($p = 0.18$). We measured the highest NH_4^+ V_f in April and in State Creek (Fig. 2A). Because concentrations were consistently low, patterns in NH_4^+ areal uptake (U) were similar to V_f .

We measured similar trends in NO_3^- V_f , with significant differences among months (rmANOVA, $p < 0.0001$) and streams (rmANOVA, $p < 0.0001$). Because there was a significant interaction between month and stream ($p < 0.0001$), we analyzed the temporal pattern for each stream individually. Similar to NH_4^+ , we observed a peak in NO_3^- V_f during April in State Creek and Walton Creek (Fig. 2B). We did not observe any seasonal patterns in Shane Creek. Although NO_3^- U generally mirrored V_f in Walton Creek, we found no differences in NO_3^- U among months (rmANOVA, $p = 0.11$). We attribute this discrepancy to the fact that when NO_3^- V_f was low in late summer and fall there was a peak in ambient NO_3^- concentration. This combination of fluctuating V_f and concentrations led to consistent NO_3^- U across months. Comparing among streams, we found that State Creek had significantly higher NO_3^- V_f than the other two streams in July and August (ANOVA, $p = 0.003$ and $p = 0.001$, respectively; Fig. 2B).

We found significant differences in SRP V_f among streams (rmANOVA, $p < 0.0001$) and among months (rmANOVA, $p = 0.001$). Because there was a significant interaction between stream and month ($p = 0.0003$), we

Table 2. Annual mean, standard error (SE), and range of nutrient uptake metrics for ammonium (NH₄⁺), nitrate (NO₃⁻), and soluble reactive phosphorus (SRP). *F* and *p* values are the result of one-way ANOVA among streams (*n* = 23); *p*-values ≤ 0.05 are in bold.

	State			Shane			Walton			ANOVA	
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	<i>F</i>	<i>p</i>
Uptake length (S _w ; m)											
NH ₄ ⁺	300	57	187–648	245	37	112–389	391	55	170–563	2.01	0.149
NO ₃ ⁻	348	74	144–718	556	164	154–1,625	476	109	241–1,056	0.77	0.478
SRP	282	67	98–532	249	46	129–476	352	36	261–530	0.96	0.400
Uptake velocity (V _f ; mm s ⁻¹)											
NH ₄ ⁺	0.127	0.021	0.048–0.251	0.085	0.021	0.019–0.189	0.081	0.032	0.017–0.189	1.06	0.364
NO ₃ ⁻	0.126	0.03	0.028–0.314	0.045	0.012	0.011–0.099	0.068	0.023	0.008–0.174	0.82	0.455
SRP	0.179 ^a	0.041	0.039–0.323	0.074 ^b	0.012	0.030–0.123	0.066 ^b	0.019	0.024–0.170	5.35	0.014
Uptake rate (U; mg m ⁻² d ⁻¹)											
NH ₄ ⁺ -N	59	16	16–131	67	22	10–202	43	18	8–131	0.73	0.494
NO ₃ ⁻ -N	1653	406	323–4,073	679	188	148–1,553	1747	331	426–2,988	3.45	0.052
SRP	67 ^a	17	16–168	42 ^a	7	23–79	21 ^b	6	148–1,553	10.35	0.001

^{a,b, ab} Groupings from Tukey's multiple comparison test after 1-way ANOVA comparing among streams.

compared spatial patterns in SRP for each month separately. In Walton Creek, SRP V_f was highest in May (ANOVA, *p* = 0.001), State Creek SRP V_f was higher than the other two streams in August (ANOVA, *p* < 0.001), and in Shane Creek there was no seasonal pattern. Because SRP concentrations were consistent among streams and months, SRP U patterns were the same as V_f.

Comparison of uptake metrics among nutrient types—We observed no differences in uptake metrics (S_w or V_f) among solutes in State and Walton Creeks. In Shane Creek S_ws were longer and V_fs were lower for NO₃⁻ than NH₄⁺ or SRP (ANOVA, *p* < 0.02). NO₃⁻ U was significantly higher than NH₄⁺ or SRP U in all three streams because of consistently high nitrate concentrations and V_f (Table 2).

Table 3. Coefficient of variation (%) in uptake velocity (V_f) for ammonium (NH₄⁺), nitrate (NO₃⁻), and soluble reactive phosphorus (SRP) across spatial and temporal scales for this study (State, Shane, and Walton Creeks), and published values. - = no measurement.

Scale	Stream	NH ₄ ⁺ V _f	NO ₃ ⁻ V _f	SRP V _f
Between reaches	State	38	30	36
	Shane	40	30	25
	Walton	33	42	25
Among streams	State, Shane, Walton	49	73	78
	10 N. American streams*	46	111	-
Among dates	State	51	70	67
	Shane	75	75	51
	Walton	110	94	78
	East Trib. Kye Burn†	54	70	37
	North Trib. Kye Burn†	76	67	57
	Reira Major‡	36	-	60

* Webster et al. (2003).

† Simon et al. (2005).

‡ Marti and Sabater (1996).

Comparison of variation in V_f at multiple scales—The coefficient of variation (CV) in V_f between reaches within streams was always less than the CV among dates or among streams (Table 3). CVs in V_f among dates and among streams were generally similar to one another. For example, the CV among dates for NO₃⁻ V_f was 70% in State Creek, 75% in Shane Creek, and 94% in Walton Creek, whereas it was 73% among streams (Table 3). Walton Creek showed the highest variation among dates in NH₄⁺, NO₃⁻, and SRP V_f (110%, 94%, and 78%, respectively).

Temperature, ambient nutrient concentrations, and nutrient uptake metrics—Temperature was unrelated to nutrient uptake rates (Table 4) except a positive relationship to SRP V_f in Walton Creek and a negative relationship to NH₄⁺ and SRP V_f in Shane Creek. We observed similar patterns with U.

We found a positive linear relationship between ambient NH₄⁺ concentration and NH₄⁺ U in State Creek (*r* = 0.87, *p* < 0.01) and Shane Creek (*r* = 0.71, *p* < 0.01), but no relationship between ambient NH₄⁺ concentration and V_f (Table 5). There were no significant relationships between ambient NO₃⁻ or SRP concentrations and NO₃⁻ U or SRP U or V_f (with the exception of State Creek; Table 5). In Walton Creek, we found a significant negative relationship between ambient NO₃⁻ and NO₃⁻ V_f.

Other studies have demonstrated that variation in inorganic N and P uptake was explained by the ratio between DIN:SRP (Munn and Meyer 1990; Simon et al. 2005). We did not find a consistent pattern in the relationships between DIN:SRP and V_f for NH₄⁺, NO₃⁻, or SRP in our study streams (Table 4). In Walton Creek, NH₄⁺ and SRP V_f were negatively related to DIN:SRP, but in Shane Creek, SRP V_f was positively related to DIN:SRP ratio. The relationships between U and DIN:SRP were identical to V_f, except in Shane Creek, where NO₃⁻ U was positively related to DIN:SRP.

Whole-stream metabolism—We measured metabolism 25 times, which included six reaches and six different months

Table 4. Linear regression of uptake velocity (V_f) of ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive phosphorus (SRP) with dissolved inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$; DIN) to SRP ratio (DIN:SRP), temperature, gross primary production (GPP), and community respiration (CR). For metabolism measurements $n = 7$ State, $n = 9$ Shane and Walton, for DIN:SRP and temperature $n = 16$ for State and Shane, $n = 14$ for Walton; p values ≤ 0.05 are in bold.

	Stream	DIN:SRP*		Temperature		GPP		CR	
		r	p	r	p	r	p	r	p
NH_4^+ V_f	State	0.020†	0.940	-0.047†	0.864	0.298	0.516	0.071	0.881
	Shane	0.112†	0.680	-0.662†	0.005	0.138	0.724	0.422	0.298
	Walton	-0.809†	<0.001	0.419†	0.136	0.876	0.002	0.837	0.005
NO_3^- V_f	State	0.150†	0.579	-0.153†	0.572	0.147	0.753	0.118	0.797
	Shane	0.478†	0.061	-0.432†	0.095	0.281	0.464	0.820	0.013
	Walton	-0.431†	0.124	0.244†	0.400	0.717	0.030	0.805	0.009
SRP V_f	State	0.374†	0.153	-0.290†	0.275	0.620	0.137	0.807	0.028
	Shane	0.508†	0.044	-0.489†	0.055	0.371	0.321	0.885	0.003
	Walton	-0.637†	0.014	0.668†	0.009	0.790	0.011	0.914	0.001

* DIN:SRP is the molar ratio of N in DIN to P in SRP.

† Dependent variable (V_f) transformed (ln) because residuals strongly differed from normal distribution (KS test, $p < 0.01$).

(May through October; Fig. 3). A complete temporal analysis of metabolism data, comparable to nutrient uptake parameters (i.e., rmANOVA; where $n = 46$), was not possible because we lacked necessary data for all reaches and all dates. However there are sufficient measurements to statically examine spatial and temporal patterns in GPP and CR. Overall, mean GPP, CR, and P:R ratio (GPP:CR) did not differ among streams (Table 1). Typically, CR exceeded GPP except in Walton Creek in July and September. GPP generally declined from May through October except in Shane Creek where it was consistently low ($<0.2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$; Fig. 3). In May, State Creek had higher GPP than Shane Creek, but was not different in Walton Creek. CR was higher in Walton Creek in May than State and Shane Creeks (Fig. 3).

Whole-stream metabolism and nutrient uptake—The strongest relationship between metabolism and nutrient

Table 5. Linear regression of ambient nutrient concentrations of ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive phosphorus (SRP) with uptake velocity (V_f) and areal uptake rate (U). $n = 16$ for State and Shane, $n = 14$ for Walton; p values ≤ 0.05 are in bold.

Solute	Stream	V_f		U	
		r	p	r	p
NH_4^+	State	0.329*	0.214	0.874*	<0.001
	Shane	0.405*	0.120	0.714*	0.002
	Walton	0.227*	0.435	0.430*	0.125
NO_3^-	State	0.428*	0.099	0.541*	0.030
	Shane	0.418*	0.107	0.395*	0.130
	Walton	-0.753*	0.002	-0.263*	0.364
SRP	State	-0.283*	0.287	0.235*	0.382
	Shane	-0.418*	0.107	0.166*	0.538
	Walton	-0.419*	0.136	0.158	0.589

* Dependent variables (V_f or U) transformed (ln) because residuals strongly differed from normal distribution (KS test, $p < 0.01$).

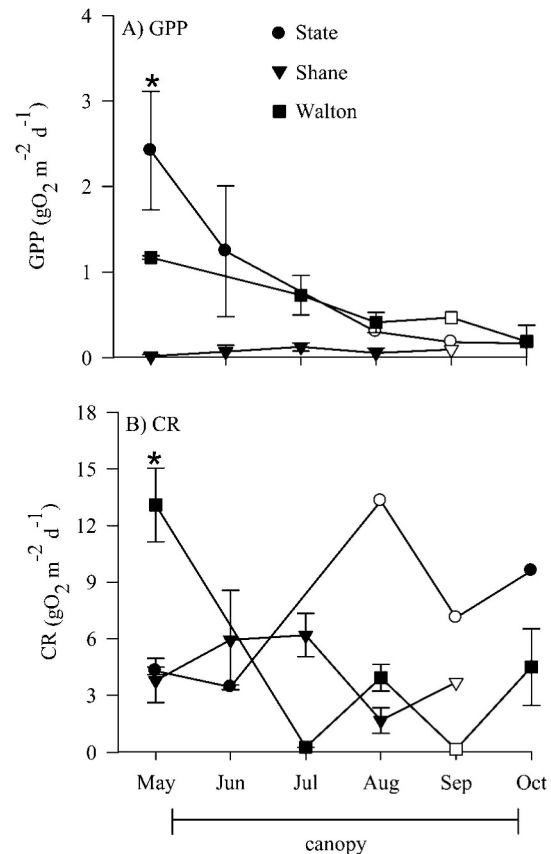


Fig. 3. Mean (\pm SE) rates of (A) gross primary production (GPP) and (B) community respiration (CR) in the three study streams from May 2003 through October 2003. Data points in white indicate only one replicate, data points in black are the mean of two replicates. * indicates that State had higher GPP than Shane in May, but GPP in Walton was not significantly different than either (ANOVA, $p = 0.05$) and Walton had higher CR than either State or Shane (ANOVA, $p = 0.03$).

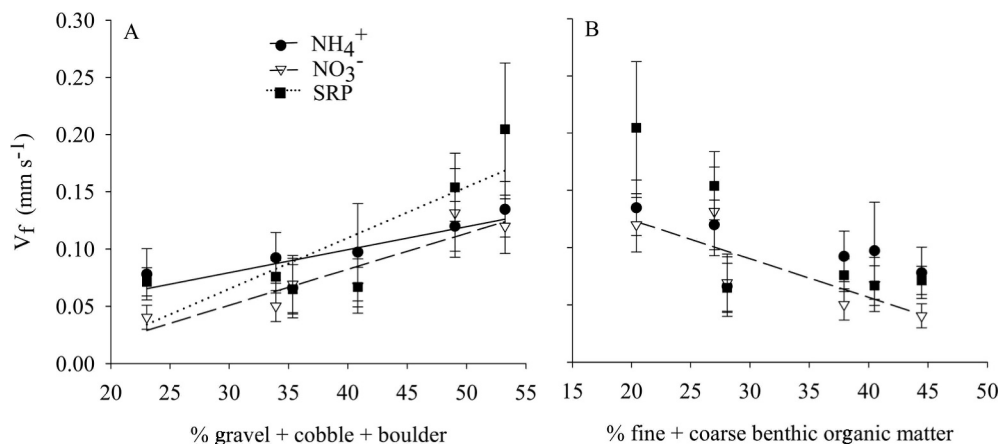


Fig. 4. Correlation between the percent coverage of (A) gravel + cobble + boulder in the six study reaches and the mean (\pm SE) uptake velocity (V_f) of ammonium (NH_4^+ ; $r = 0.859$, $p = 0.028$), nitrate (NO_3^- ; $r = 0.922$, $p = 0.009$), and soluble reactive phosphorus (SRP; $r = 0.827$, $p = 0.042$), and (B) percent coverage of organic substrata (fine + coarse; including wood) and NH_4^+ V_f ($r = -0.558$, $p = 0.223$), NO_3^- V_f ($r = -0.838$, $p = 0.037$), and SRP V_f ($r = -0.793$, $p = 0.060$).

uptake was that CR explained 65–84% of the variation in SRP V_f in all streams (Table 4). For the other two solutes, the pattern was not consistent among streams. NO_3^- V_f was related to CR only in Shane and Walton Creeks, and CR and NH_4^+ V_f were related only in Walton Creek. GPP was significantly related to V_f for all three nutrient types only in Walton Creek (Table 4). Nutrient uptake metrics were not significantly related to the ratio of GPP:CR in any case. Whole-stream uptake, U , showed similar patterns as V_f , except there was no relationship between NO_3^- U and CR in Walton Creek. Generally, there were no significant relationships between GPP or CR and any abiotic variables (i.e., NH_4^+ , NO_3^- , or SRP concentrations, DIN:SRP, or temperature; data not shown), except in Walton Creek, where we observed a positive relationship between temperature and GPP ($r = 0.97$, $p < 0.01$).

Organic matter standing stocks, substratum distribution, and nutrient uptake metrics—Among streams, standing stocks of FBOM ranged from 33.5 g AFDM m^{-2} to 542.4 g AFDM m^{-2} , CBOM ranged from 0.0 g AFDM m^{-2} to 134.7 g AFDM m^{-2} , and wood ranged from 0.4 g AFDM m^{-2} to 673.4 g AFDM m^{-2} (Table 1). There were no significant relationships between the nutrient demand and CBOM or FBOM standing stock, Chl a concentration, or LWD density (data not shown).

In all three streams, sand and gravel accounted for approximately 45% of the substrata, whereas FBOM and CBOM + wood ranged from 17% to 24% (Table 1). State Creek had significantly higher gravel + cobble + boulder and bryophyte coverage than Shane or Walton Creeks (Table 1). We found significant positive correlations between percent coverage of gravel + cobble + boulder in the six study reaches and mean NH_4^+ V_f ($r = 0.86$, $p = 0.03$), NO_3^- V_f ($r = 0.92$, $p = 0.01$), and SRP V_f ($r = 0.83$, $p = 0.04$; Fig. 4). There was no relationship between percent coverage of organic substrata (fine + coarse; including

wood) and V_f for NH_4^+ and SRP, yet there was a negative correlation with NO_3^- V_f ($r = -0.84$, $p = 0.04$; Fig. 4).

Discussion

Ambient nutrient concentrations and nutrient uptake metrics—Human land use (e.g., agriculture, urbanization) often changes nutrient concentrations in streams (Paul and Meyer 2001; Webster et al. 2003), and previous research has shown that ambient nutrient concentrations influence uptake metrics (Davis and Minshall 1999; Dodds et al. 2002). If nutrient availability (or concentration) is limiting nutrient uptake, then we would expect a positive relationship between concentration and U (either linear or Michaelis–Menten models) and if nutrient uptake is approaching saturation (i.e., on the curving portion of the Michaelis–Menten model), we would expect a negative relationship between concentration and V_f (see Fig. 2 in Davis and Minshall 1999). Ideally, testing saturation models requires multiple enrichments in the same stream over relatively short time periods to minimize variation in biotic and abiotic factors, however, other studies have successfully used data collected in different streams and at different times to explore the relationship between ambient nutrient concentrations and uptake metrics (Davis and Minshall 1999; Simon et al. 2005; Newbold et al. 2006).

Examining the relationship between U and ambient nutrient concentration can illustrate which factors (ambient concentration or V_f) more strongly affect U (Davis and Minshall 1999) even though they are autocorrelated (because U is calculated with ambient concentration). When U increases with increasing concentration, thereby driving patterns in U , the point at which increasing concentration no longer increases U is the point at which nutrient saturation should occur (Davis and Minshall 1999; Dodds et al. 2002). Because nutrient concentrations in our study streams were generally low (except for NO_3^- in

Walton Creek which peaked at $762 \mu\text{g NO}_3^- \text{-N L}^{-1}$), we did not expect to see saturation.

Ambient concentration was important in determining NH_4^+ U in State and Shane Creeks, but not in Walton (Table 5). For the relationship between NH_4^+ concentration and NH_4^+ U, there was minimal difference between the linear (Table 5) and Michaelis–Menten models (State $r = 0.71$; $p < 0.01$, Shane $r = 0.70$, $p < 0.01$), which suggested that the range of NH_4^+ concentrations was not high enough to enter the saturation portion of the Michaelis–Menten curve. Similarly, Dodds et al. (2002) found that there was little difference in the explanatory power of linear and Michaelis–Menten models in explaining the relationship between NH_4^+ concentration and NH_4^+ U in two Kansas streams. However, we hesitate to imply that there are saturation thresholds that hold across streams and biomes because of the particularly strong role that biology plays in nutrient uptake, which can vary considerably across streams of varying land use and biomes.

Although we did not find any significant relationships between NO_3^- concentration and U, the relationship between concentration and V_f can also indicate that concentrations may be nearing saturation (Davis and Minshall 1999). We found that higher NO_3^- concentrations were concurrent with decreased $\text{NO}_3^- V_f$ in Walton Creek, which had the greatest range of NO_3^- concentrations, whereas State and Shane Creeks showed no significant patterns with NO_3^- concentration and V_f . This suggests NO_3^- concentration may not affect $\text{NO}_3^- V_f$ below the maximum level recorded in these two streams, $240 \mu\text{g NO}_3^- \text{-N L}^{-1}$. In agriculturally influenced streams in southwestern Michigan, saturation of NO_3^- uptake was noted at the lowest NO_3^- concentration recorded, $\sim 400 \mu\text{g NO}_3^- \text{-N L}^{-1}$ (Bernot et al. 2006). These estimated saturation values ($< 762 \mu\text{g NO}_3^- \text{-N L}^{-1}$ in Walton Creek and $< 400 \mu\text{g NO}_3^- \text{-N L}^{-1}$ in Michigan agricultural streams) are much lower than the maximum allowable daily concentration of $10,000 \mu\text{g NO}_3^- \text{-N L}^{-1}$ for drinking water standards (Environmental Protection Agency 2002), indicating that $\text{NO}_3^- \text{-N}$ concentrations may saturate biological demand in many streams in this biome before reaching a level that initiates mitigation.

Our study streams exhibited both high background NO_3^- concentrations and relatively high $\text{NO}_3^- V_f$ values, resulting in U values that are among the highest in the literature. Our highest NO_3^- U occurred in spring, when peak GPP was measured as a result of a bloom of filamentous green algae before leaf-out, and discharge was highest because of spring runoff conditions, both contributing to higher V_f (because discharge is in the numerator in the V_f calculation; see Methods). We are not aware of other measurements of NO_3^- uptake in forested headwater streams made during post-snowmelt high flows in spring that would allow direct comparison. Some previously published values have been in the same range or higher ($686 \text{ mg NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ in Davis and Minshall 1999, $7,299 \text{ mg NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ in Webster et al. 2003, and $1,810 \text{ mg NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ in Bernot et al. 2006), and these were also associated with higher background

concentrations of nitrate and larger streams ($Q > 50 \text{ L s}^{-1}$).

To test the plausibility of our NO_3^- U values, we calculated the molar ratio of C metabolism (C respired + C fixed) to N uptake ($\text{NO}_3^- + \text{NH}_4^+ \text{-N}$), and found very low values (1–2.5; modified approach from Webster et al. 2003; Hall and Tank 2003). However, similar calculations for other sandy-bottomed, higher-nitrate streams in Michigan were highly variable, but included very low values as well (e.g., 0.9–40.6 for Bernot et al. 2006). Because previously published work containing both whole-stream metabolism and nitrate uptake is limited (Hall and Tank 2003; Webster et al. 2003; Bernot et al. 2006), more studies are needed to examine expanded ranges of NO_3^- U values.

We found little evidence that SRP concentration influenced SRP V_f in our study (Table 5). In contrast, SRP V_f declined with increasing SRP concentration in agricultural Midwestern streams (Bernot et al. 2006) and streams in upstate New York (Newbold et al. 2006); however, ambient SRP concentrations in those streams were double the range observed in our forested, upper Midwestern streams. Mulholland et al. (1990) estimated a biological saturation level of approximately $15 \mu\text{g PO}_4^{3-} \text{-P L}^{-1}$ in Walker Branch, Tennessee, a forested Appalachian stream, and the maximum SRP concentration we recorded was slightly lower than this value ($10 \mu\text{g L}^{-1}$).

The ratio of DIN : SRP has been used to explain patterns in relative DIN and SRP uptake (Munn and Meyer 1990; Simon et al. 2005), and if relative amounts of DIN or SRP were limiting uptake, we would expect that at low DIN : SRP values, DIN V_f would be high and SRP V_f low. In our study, there were no consistent relationships between V_f and DIN : SRP (Table 4). This may be because DIN values were dominated by NO_3^- , and any variability in SRP or NH_4^+ among streams or dates was obscured. Also, SRP is typically an overestimate of PO_4^{3-} , so DIN : SRP is likely an underestimate of the DIN : PO_4^{3-} ratio (Hudson et al. 2000). Dodds et al. (2003) discouraged the use of DIN : SRP to indicate stream nutrient limitation status because the ratio gives no indication of nutrient turnover rate and disregards the effect of biologically active organic N or P.

Temperature and nutrient uptake metrics—Unexpectedly, temperature was not a major factor controlling variation in nutrient uptake, even though we conducted releases in all four seasons, spanning a large range in streamwater temperatures (0–18.1°C). There was a significant negative relationship between temperature and NH_4^+ and SRP V_f in Shane Creek, no relationships in State Creek, and a significant positive relationship between temperature and SRP V_f in Walton Creek (Table 4). The lack of consistency may be due to the timing of resource availability (e.g., organic matter and light), which were higher during colder seasons in our forested headwater streams. Previous research has shown that temperature limitation of microbial growth rates were mitigated by the availability of high quality food (Wiebe et al. 1992), which may explain the patterns we see here: temperature limitation of biofilm growth can be overcome by the

availability of limiting resources such as carbon or light, which both occur at higher amounts at colder temperatures (e.g., autumn leaf-fall or just before spring leaf-out) in forested headwater streams. Additionally, the effect of temperature on stream biofilm metabolism may vary by substratum type (Tank et al. 1993; Fuss and Smock 1996), so the effects of temperature on whole-stream rates may change with the seasonal distribution and metabolic activity (or community composition) of biofilms on different substratum types.

Other investigators who have examined the relationship between temperature and whole-stream nutrient uptake have also seen equivocal results. A positive relationship was observed between temperature and P uptake (D' Angelo et al. 1991; Meals et al. 1999; Simon et al. 2005), and N uptake (Butturini and Sabater 1998; Simon et al. 2005). However, negative relationships were shown between temperature and P (Mulholland et al. 1985) and NH_4^+ uptake (Marti and Sabater 1996). A confounding factor in comparing these studies is the differences in the range of temperatures recorded, which vary by biome and the number of different seasons included. A major obstacle in elucidating the relationship between nutrient uptake and temperature is that it has not been experimentally manipulated, but rather deduced from descriptive studies, in which temperature is one of multiple interacting factors. Future studies should consider hypotheses that incorporate the complexity of resource availability and temperature effects on whole-stream nutrient uptake rates.

Seasonal variation in nutrient uptake metrics—The peaks in $\text{NH}_4^+ V_f$ in winter (December) and spring (April) were likely the result of (1) increased N demand by microbial decomposers in response to the pulse of allochthonous organic matter during autumn leaf-fall and (2) an increase in primary production caused by higher light availability just before spring leaf-out. Although we found that benthic organic matter standing stocks and Chl *a* densities were not good predictors of $\text{NH}_4^+ V_f$, we infer biotic control because the seasonal pattern of $\text{NH}_4^+ V_f$ was identical in all three streams, which did not occur for the other two nutrient types (Fig. 2). The above logic supports the results from Walton Creek, where we found significant positive relationships with metabolism and $\text{NH}_4^+ V_f$ (Table 4). We might have shown this relationship in State and Shane Creeks had we measured metabolism in December and April; data collected in subsequent years demonstrate these are periods of relatively high GPP and CR across streams (Hoellein unpubl. data).

Other studies have demonstrated links between $\text{NH}_4^+ V_f$ and GPP and CR. Synthesizing data across biomes, Meyer et al. (2005) showed a positive relationship between $\text{NH}_4^+ V_f$ and total metabolism (GPP + CR) using data from multiple studies (Hall et al. 2003; Hall and Tank 2003; Webster et al. 2003). We added our data and that of Wollheim et al. (2001), Bernot et al. (2006), and Newbold et al. (2006) to that compilation and analyzed the regressions for GPP and CR separately (rather than as total metabolism, as in Meyer et al. 2005) to distinguish autotrophic versus heterotrophic contributions to nutrient

uptake. The relationship was significant for both GPP ($R^2 = 0.22, p < 0.01$) and CR ($R^2 = 0.36, p < 0.01$; Fig. 5A,B) and consistent across (1) streams with forested, open-canopy, urban, tundra, and agricultural riparian zones, (2) streams affected by invasive species and geothermal inputs, (3) studies employing different methods to quantify nutrient uptake (including $^{15}\text{NH}_4^+$ tracer additions), and (4) studies conducted during different seasons (our data set). When U was considered the dependant variable (rather than V_f), the relationships were close to being statistically significant, explaining less variation (GPP $R^2 = 0.06, p = 0.06$; CR $R^2 = 0.08, p = 0.05$), likely because of the strong influence of widely varying background concentrations across studies.

Unlike patterns for NH_4^+ , GPP did not appear to control seasonal patterns in $\text{NO}_3^- V_f$. Although a trend of higher $\text{NO}_3^- V_f$ in spring was found in State and Walton Creeks, there was a significant interaction between stream and time, with higher values in State Creek in July and August and no relationship between $\text{NO}_3^- V_f$ and GPP (Fig. 2B; Table 4). Despite this, biotic control of $\text{NO}_3^- V_f$ can be inferred from its relationship to CR (Table 4) and for both GPP and CR when combined with previous studies (Hall and Tank 2003; Webster et al. 2003; Bernot et al. 2006; GPP $R^2 = 0.09, p = 0.05$, CR $R^2 = 0.19, p < 0.01$; Fig. 5C,D). For U, the relationship was not significant with GPP or CR. We note that there is still substantial unexplained variation for both NO_3^- and NH_4^+ uptake patterns in the combined data sets, indicating that reach-scale metabolism metrics may provide some insight into N processing across streams, but additional site-specific factors are clearly important in fully explaining variation in N V_f (Newbold et al. 2006).

Temporal patterns in SRP V_f were variable among streams and more strongly related to biotic (CR; Table 4), than abiotic factors (ambient SRP concentration, temperature, and DIN:SRP; Tables 4, 5). The dominant pattern was an increase in SRP V_f in State Creek in August, with continued high rates through December (Fig. 2C) and a parallel pattern in CR (Fig. 3), but the cause for this pattern is unknown. We could not account for this pattern by concurrent changes in organic matter standing stocks (leaf fall did not occur until mid-October), canopy cover, temperature, hydrology (i.e., discharge or groundwater input), or human influence. Although we are unsure of the underlying mechanism, the strong relationship between CR and SRP V_f , which explained 65–84% of the variation in SRP V_f across the study streams, suggests a biological pathway.

When our data for SRP V_f were combined with other studies (Mulholland et al. 1997; Meyer et al. 2005; Bernot et al. 2006), we found evidence for heterotrophic control on SRP V_f across biomes (CR $R^2 = 0.28, p < 0.01$; Fig. 5E,F). Similarly, for SRP U, there was no relationship with GPP and a significant positive relationship with CR ($R^2 = 0.21, p < 0.01$). In other forested headwater streams, researchers have observed greater P retention in December (high litter retention) relative to July (Mulholland et al. 1985) and in spring and fall relative to summer (Hall et al. 2002), indicating that seasonal variation in leaf litter retention and

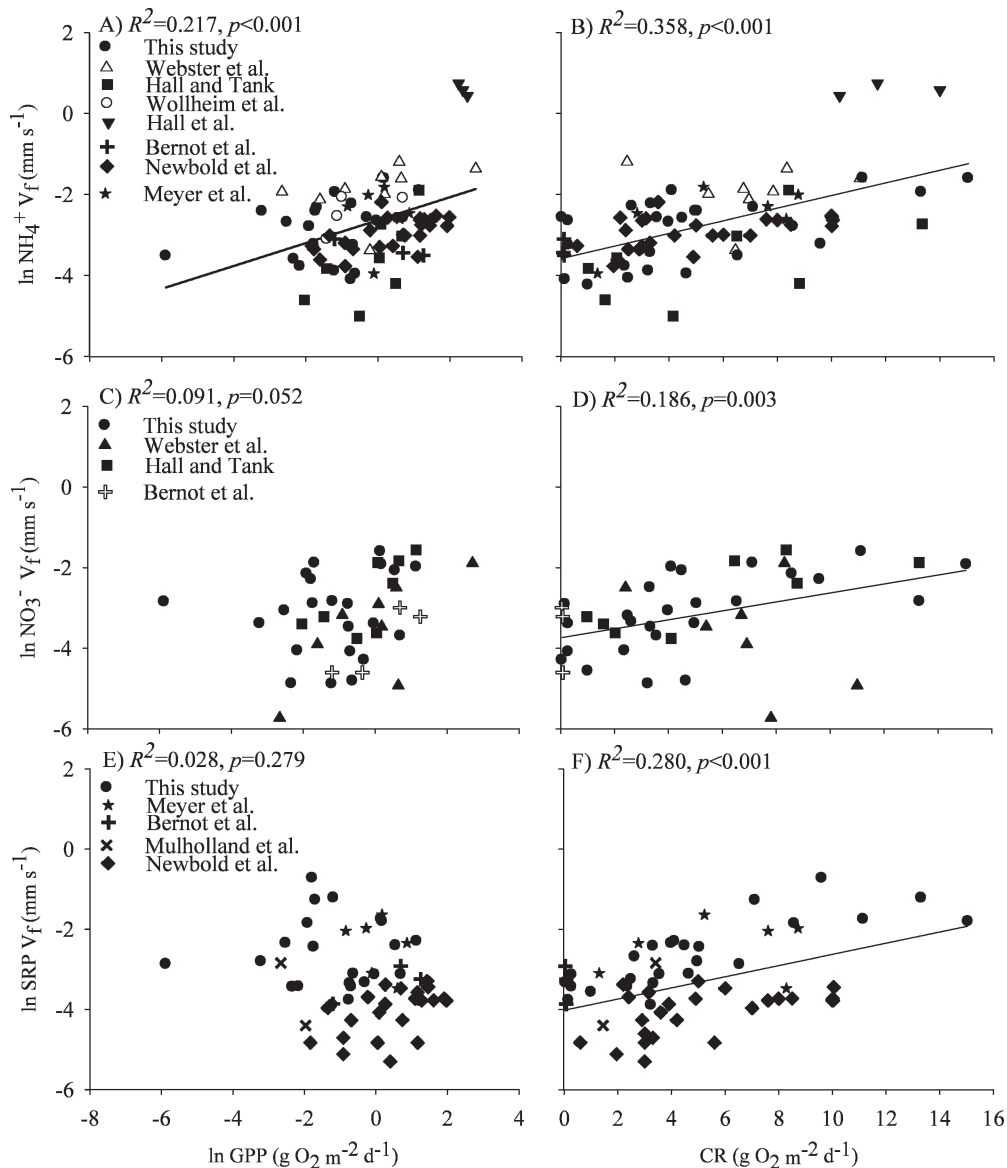


Fig. 5. Linear regression of gross primary production (GPP; natural log) or community respiration (CR) and uptake velocity (V_f ; natural log) of (A,B) ammonium (NH_4^+), (C,D) nitrate (NO_3^-), and (E,F) soluble reactive phosphorus (SRP). Data are from the present study, Mulholland et al. (1997), Wolheim et al. (2001), Webster et al. (2003), Hall and Tank (2003), Hall et al. (2003), Meyer et al. (2005), Bernot et al. (2006), and Newbold et al. (2006). Closed symbols represent whole-stream enrichment and open symbols represent isotope tracers.

irradiance were responsible for increased uptake. In contrast, we did not observe a fall/winter peak in SRP uptake, but we could not measure nutrient demand in November or December 2003 in Walton Creek because of heavy snowfall.

Interacting effects of metabolism and ambient nutrient concentration on nutrient uptake—The influence of GPP and CR on V_f may decline at higher ambient nutrient concentrations. For example, agricultural streams in Michigan with high background N (as both NH_4^+ and NO_3^-) and SRP concentrations showed evidence of

saturation of uptake and no relationship between NH_4^+ , NO_3^- , or SRP V_f and metabolism (Bernot et al. 2006). Similarly, urban streams in Georgia with high NH_4^+ and SRP concentrations also showed no relationship between V_f and GPP or CR (Meyer et al. 2005). In contrast, low nutrient streams in Wyoming (Hall and Tank 2003) and northern Michigan (this data set) showed little indication of nutrient saturation and positive relationships between V_f and GPP or CR. Finally, by measuring V_f and metabolism across a gradient of uninhabited to urban/suburbanized watersheds, Newbold et al. (2006), showed both positive relationships between V_f and metabolism, as well as

indications of saturation for both NH_4^+ and SRP V_f . In these cases, explanatory power was strengthened by considering the interaction between abiotic (nutrient concentration) and biotic (GPP and CR) factors across expanded geographic and concentration gradients.

Variation in nutrient uptake metrics among streams—Previous research in forested headwater streams suggests that the decomposition of allochthonous organic matter, as reflected in community respiration rates, would explain the majority of variation in nutrient uptake metrics (Minshall et al. 1983; Mulholland et al. 1985; Tank and Webster 1998). Our study, however, which attempted to capture both spatial (multiple streams) and seasonal variation, suggests that variation in GPP may also play an important role in explaining patterns in nutrient uptake among forested streams. State Creek had the highest mean V_f for all three nutrient types and the highest percentage of large inorganic particles (e.g., cobble/gravel) and frequency of bryophytes, resulting in generally higher GPP compared to the other streams (Table 1). We found positive correlations between streambed coverage by large inorganic substrata (a key substrate for algal biofilms and bryophytes) and mean V_f for all three nutrient types, and no positive relationships between streambed coverage of organic matter (fine + coarse) and NH_4^+ and SRP V_f (Fig. 4).

Other studies have shown that reaches with larger, more stable substrata (i.e., cobble and bedrock) have higher nutrient uptake rates than stream reaches of lower bed stability (i.e., sand and small gravel; (Munn and Meyer 1990; Marti and Sabater 1996). In these studies, reach-scale metabolism was not measured directly, but the authors inferred that moss and periphyton communities on stable substrata were responsible for higher inorganic nutrient demand. Bryophytes tend to grow in streams with stable substrata, few low flows, and higher gradient (Bowden 1999), all of which occur more in State Creek, which had bryophytes covering 6–16% of benthic surface compared to <1% in the other two streams. Bryophytes have been shown to increase particulate organic matter retention and growth of epiphytic algae, which may increase N retention (Stream Bryophyte Group 1999; Ashkenas et al. 2004), and other studies report that stream reaches containing bryophytes have higher nutrient uptake rates than reaches lacking bryophytes (Davis and Minshall 1999; Slavik et al. 2004).

Variation in nutrient uptake metrics at multiple scales—The importance of autotrophy in explaining variation in nutrient uptake rates among our three forested headwater streams was unexpected, but could be because of the seasonal breadth and sample frequency in this study. The prevailing paradigm for classification of stream ecosystems is based on a gradient with endpoints being heterotrophic, forested headwater streams versus autotrophic, open-canopy streams (Vannote et al. 1980; Dodds 2006) based on studies that examined variation in ecosystem structure and function over large and small geographic areas (i.e., across different biomes, riparian communities, and land-use types; Minshall et al. 1983; Webster et al. 2003). In our

study, seasonal variation and frequent sampling through time also provided a meaningful context to explain variation in nutrient uptake rates, which at our sites was similar to the range of variation recorded across wide geographic scales (Table 3; Fig. 5). For example, in this study, the variation in NH_4^+ V_f among our study streams was as great as variation in NH_4^+ V_f across 10 North American streams in different biomes (49% and 46%, respectively; Webster et al. 2003), and temporal variation in V_f (among dates) was generally equal to variation among streams. The use of multiple streams, replicated reaches within streams, and year-round sampling in this study incorporated gradients of controlling factors that enabled more powerful statistical analyses of spatial and temporal variation than has been seen previously in other studies. Finally, our results demonstrate that seasonal change can provide an alternative axis to geographical variation when examining factors controlling nutrient uptake rates in streams.

References

- ALEXANDER, R. B., R. A. SMITH, AND G. E. SCHWARZ. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* **403**: 758–761.
- ALLAN, J. D. 1995. *Stream ecology: Structure and function of running waters*. Kluwer.
- ASHKENAS, L. R., S. L. JOHNSON, S. V. GREGORY, J. L. TANK, AND W. M. WOLLHEIM. 2004. A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream. *Ecology* **85**: 1725–1739.
- BERNOT, M. J., J. L. TANK, T. V. ROYER, AND M. B. DAVID. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. *Freshwat. Biol.* **51**: 499–509.
- BOWDEN, W. B. 1999. Roles of bryophytes in stream ecosystems. *Journal of the North American Benthological Society* **18**: 151–184.
- BUTTURINI, A., AND F. SABATER. 1998. Ammonium and phosphate retention in a Mediterranean stream: Hydrological versus temperature control. *Can. J. Fish Aquat. Sci.* **55**: 1938–1945.
- CORDOVA, J. M., E. J. ROSI-MARSHALL, A. M. YAMAMURO, AND G. A. LAMBERTI. 2007. Quantity, controls and functions of large woody debris in Midwestern USA streams. *Riv. Res. Appl.* **23**: 21–33.
- D'ANGELO, D. J., J. R. WEBSTER, AND E. F. BENFIELD. 1991. Mechanisms of stream phosphorus retention—An experimental study. *Journal of the North American Benthological Society* **10**: 225–237.
- DAVIS, J. C., AND G. W. MINSHALL. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia* **119**: 247–255.
- DODDS, W. K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *J. N. Am. Benthol. Soc.* **22**: 171–181.
- . 2006. Eutrophication and trophic state in rivers and streams. *Limnol. Oceanogr.* **51**: 671–680.
- , AND OTHERS. 2002. N uptake as a function of concentration in streams. *Journal of the North American Benthological Society* **21**: 206–220.
- ELWOOD, J. W., J. D. NEWBOLD, A. F. TRIMBLE, AND R. W. STARK. 1981. The limiting role of phosphorus in a woodland stream ecosystem—Effects of P-enrichment on leaf decomposition and primary producers. *Ecology* **62**: 146–158.

- ENVIRONMENTAL PROTECTION AGENCY. 2002. National primary drinking water regulations. Section 141.11. U.S. Government Printing Office.
- FUSS, C. L., AND L. A. SMOCK. 1996. Spatial and temporal variation of microbial respiration rates in a blackwater stream. *Freshwat. Biol.* **36**: 339–349.
- GREGORY, S. V. 1980. Effects of light, nutrients, and grazing on periphyton communities in streams. Ph.D. thesis, Oregon State University.
- GRIMM, N. B. 1987. Nitrogen dynamics during succession in a desert stream. *Ecology* **68**: 1157–1170.
- HALL, R., J. L. TANK, AND M. F. DYBDAHL. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Front. Ecol. Environ.* **1**: 407–411.
- HALL, R. O., E. S. BERNHARDT, AND G. E. LIKENS. 2002. Relating nutrient uptake with transient storage in forested mountain streams. *Limnol. Oceanogr.* **47**: 255–265.
- , B. J. PETERSON, AND J. L. MEYER. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. *Ecosystems* **1**: 283–298.
- , AND J. L. TANK. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnol. Oceanogr.* **48**: 1120–1128.
- HILL, W. R., P. J. MULHOLLAND, AND E. R. MARZOLF. 2001. Stream ecosystem responses to forest leaf emergence in spring. *Ecology* **82**: 2306–2319.
- HUDSON, J. J., W. D. TAYLOR, AND D. W. SCHINDLER. 2000. Phosphate concentrations in lakes. *Nature* **406**: 54–56.
- MARTI, E., J. AUMATELL, L. GODE, M. POCH, AND F. SABATER. 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. *J. Environ. Qual.* **33**: 285–293.
- , AND F. SABATER. 1996. High variability in temporal and spatial nutrient retention in Mediterranean streams. *Ecology* **77**: 854–869.
- MARZOLF, E. R., P. J. MULHOLLAND, AND A. D. STEINMAN. 1994. Improvements to the diurnal upstream-downstream dissolved-oxygen change technique for determining whole-stream metabolism in small streams. *Can. J. Fish. Aquat. Sci.* **51**: 1591–1599.
- MEALS, D. W., AND OTHERS. 1999. Retention of spike additions of soluble phosphorus in a northern eutrophic stream. *Journal of the North American Benthological Society* **18**: 185–198.
- MEYER, J. L., M. J. PAUL, AND W. K. TAULBEE. 2005. Stream ecosystem function in urbanizing landscapes. *Journal of the North American Benthological Society* **24**: 602–612.
- MINSHALL, G. W. 1978. Autotrophy in Stream Ecosystems. *Bioscience* **28**: 767–770.
- , R. C. PETERSEN, K. W. CUMMINS, T. L. BOTT, J. R. SEDELL, C. E. CUSHING, AND R. L. VANNOTE. 1983. Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs* **53**: 1–25.
- MULHOLLAND, P. J. 1992. Regulation of nutrient concentrations in a temperate forest stream—Roles of upland, riparian, and instream processes. *Limnol. Oceanogr.* **37**: 1512–1526.
- , E. R. MARZOLF, J. R. WEBSTER, D. R. HART, AND S. P. HENDRICKS. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnol. Oceanogr.* **42**: 443–451.
- , J. D. NEWBOLD, J. W. ELWOOD, L. A. FERREN, AND J. R. WEBSTER. 1985. Phosphorus spiraling in a woodland stream—Seasonal variations. *Ecology* **66**: 1012–1023.
- , A. D. STEINMAN, AND J. W. ELWOOD. 1990. Measurement of phosphorus uptake length in streams – comparison of radiotracer and stable PO_4^{3-} releases. *Can. J. Fish. Aquat. Sci.* **47**: 2351–2357.
- , J. L. TANK, D. M. SANZONE, W. M. WOLLHEIM, B. J. PETERSON, J. R. WEBSTER, AND J. L. MEYER. 2000. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. *Ecol. Monogr.* **70**: 471–493.
- MUNN, N. L., AND J. L. MEYER. 1990. Habitat-specific solute retention in 2 small streams—An intersite comparison. *Ecology* **71**: 2069–2082.
- MURPHY, J., AND J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**: 31–36.
- NEWBOLD, J. D., AND OTHERS. 2006. Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds. *J. N. Am. Benthol. Soc.* **25**: 998–1017.
- PAUL, M. J., AND J. L. MEYER. 2001. Streams in the urban landscape. *Annu. Rev. Ecol. Systemat.* **32**: 333–365.
- ROSEMOND, A. D., P. J. MULHOLLAND, AND J. W. ELWOOD. 1993. Top-down and bottom-up control of stream periphyton—Effects of nutrients and herbivores. *Ecology* **74**: 1264–1280.
- SABATER, F., A. BUTTURINI, E. MARTI, I. MUNOZ, A. ROMANI, J. WRAY, AND S. SABATER. 2000. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. *Journal of the North American Benthological Society* **19**: 609–620.
- SARTORY, D. P., AND J. U. GROBBELAAR. 1984. Extraction of chlorophyll-a from fresh-water phytoplankton for spectrophotometric analysis. *Hydrobiologia* **114**: 177–187.
- SIMON, K. S., C. R. TOWNSEND, B. J. F. BIGGS, AND W. B. BOWDEN. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. *Journal of the North American Benthological Society* **24**: 1–18.
- SLAVIK, K., B. J. PETERSON, L. A. DEEGAN, W. B. BOWDEN, A. E. HERSHEY, AND J. E. HOBBI. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. *Ecology* **85**: 939–954.
- SOLORZANO, L. 1969. Determination of ammonium in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* **14**: 799–801.
- SOMMERS, L. M. 1984. Michigan: A geography. Westview Press.
- STREAM BRYOPHYTE GROUP. 1999. Roles of bryophytes in streams. *Journal of the North American Benthological Society* **18**: 151–184.
- STREAM SOLUTE WORKSHOP. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* **9**: 95–119.
- SUBERKROPP, K., AND E. CHAUVET. 1995. Regulation of leaf breakdown by fungi in streams—Influences of water chemistry. *Ecology* **76**: 1433–1445.
- TANK, J. L., AND J. R. WEBSTER. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* **79**: 2168–2179.
- , AND E. F. BENFIELD. 1993. Microbial respiration on decaying leaves and sticks in a southern Appalachian stream. *Journal of the North American Benthological Society* **12**: 394–405.
- UEHLINGER, U. 2000. Resistance and resilience of ecosystem metabolism in a flood-prone river system. *Freshwat. Biol.* **45**: 319–332.
- VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. River continuum concept. *Can. J. Fish. Aquat. Sci.* **37**: 130–137.
- VITOUSEK, P. M., H. A. MOONEY, J. LUBCHENCO, AND J. M. MELILLO. 1997. Human domination of Earth's ecosystems. *Science* **277**: 494–499.
- WANNINKHOF, R., P. J. MULHOLLAND, AND J. W. ELWOOD. 1990. Gas-exchange rates for a 1st-order stream determined with deliberate and natural Tracers. *Wat. Res. Research* **26**: 1621–1630.

- WEBSTER, J. R., AND E. F. BENFIELD. 1986. Vascular plant breakdown in fresh-water ecosystems. *Annu. Rev. Ecol. Systemat.* **17**: 567–594.
- , AND T. P. EHRMAN. 1996. Solute dynamics, p.145–160 in F. R. Hauer and G. A. Lamberti [eds.]. *Methods in stream ecology*. Academic Press.
- , AND OTHERS. 2003. Factors affecting ammonium uptake in streams—An inter-biome perspective. *Freshwat. Biol.* **48**: 1329–1352.
- WIEBE, W. J., W. M. SHELDON, AND L. R. POMEROY. 1992. Bacterial-growth in the cold—Evidence for an enhanced substrate requirement. *Appl. Environ. Microbiol.* **58**: 359–364.
- WOLLHEIM, W. M., AND OTHERS. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnol. Oceanogr.* **46**: 1–13.
- YOUNG, R. G., AND A. D. HURYN. 1998. Comment: Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Can. J. Fish Aquat. Sci.* **55**: 1784–1785.

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