

## Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming

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### Abstract

Streams and rivers regulate nitrogen transport (N) to downstream ecosystems. Rates of N uptake can be high in streams, but controls on the variation in uptake rates of N among streams are not known. We measured ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) uptake velocities ( $V_f$ ) and compared these with whole-reach estimates of gross primary production (GPP) and community respiration (CR) in 11 low-nitrogen streams in Grand Teton National Park, Wyoming. We predicted that increased metabolism would positively relate to higher N demand because of stoichiometric N requirements associated with carbon fixation. Rates of GPP and CR explained 82% of variation in  $\text{NH}_4^+$   $V_f$ . Nitrate  $V_f$  was controlled by GPP, not CR, with GPP explaining 75% of variation in  $\text{NO}_3^-$   $V_f$ . Nitrate concentrations did not increase downstream during  $\text{NH}_4^+$  addition in all streams, including streams with zero  $\text{NO}_3^-$  uptake, suggesting low nitrification rates relative to  $\text{NH}_4^+$  uptake. Using a stoichiometric model, we show that areal N uptake estimated from microbial and algal production was similar to measured areal N uptake. High primary production could be a prerequisite for streams exhibiting high  $\text{NO}_3^-$  uptake rates.

Streams and rivers are important avenues for N transport, yet they can also remove and transform dissolved N (Burns 1998; Peterson et al. 2001) and retain N in the terrestrial landscape (Howarth et al. 1996; Alexander et al. 2000). Hence, N cycling in streams has received much recent attention (Alexander et al. 2000; Sabater et al. 2000; Peterson et al. 2001). Ecologists have described nutrient dynamics in streams with the concept of spiraling (or uptake) length, which is the average distance traveled by dissolved nutrient before biotic uptake (Newbold et al. 1981). Using uptake length, areal uptake rates of N can be calculated from the water column to benthic biota (Newbold et al. 1981). Peterson et al. (2001) used a nutrient spiraling approach and found that headwater streams can remove and transform dissolved N inputs rapidly, presumably because of high rates of biological activity combined with high sediment–water contact time. Dissolved nitrogen uptake rates from the water column are especially high in shallow streams, thereby de-

creasing N loading to downstream ecosystems (Alexander et al. 2000).

Although N uptake velocities and areal uptake rates can be high in streams (Tank et al. 2000; Peterson et al. 2001), no studies have quantitatively addressed what factors control variation in nitrogen uptake beyond geomorphic features of streams such as water velocity or depth (Alexander et al. 2000; Wollheim et al. 2001) or measures of transient storage (Valett et al. 1996; Hall et al. 2002). We expect that nutrient uptake by streams is high when net production is high (Grimm 1987). For example, a relationship between biological processes and nutrient uptake was implied when increased light from riparian vegetation removal was linked to higher ammonium ( $\text{NH}_4^+$ ) uptake in a stream in Spain (Sabater et al. 2000). Physical attributes of streams can only indirectly control N uptake; it is the biotic demand by algae and microbes in attached biofilms that will ultimately determine N uptake and transformation in a stream. Given that many freshwater ecosystems are N limited (Elser et al. 1990; Francoeur 2001), we predict that variation in biological demand for dissolved N will explain most of the variation in stream uptake of N in low-nutrient streams. Streams with high autotrophic and heterotrophic production should remove more dissolved N because of simple stoichiometry: increased C fixation or heterotrophic C uptake will increase demand for N. Highly productive streams should transform N at higher rates, and are thereby more likely to control form and timing of watershed export of N.

To test this hypothesis, we quantified  $\text{NH}_4^+$  and nitrate ( $\text{NO}_3^-$ ) uptake in 11 low-nutrient, N-limited streams in northwest Wyoming and related N uptake to whole-reach estimates of community respiration (CR) and gross primary production (GPP). Both N uptake and metabolism (GPP and CR) were measured at the segment scale (cf. Frissell et al. 1986), thereby integrating spatial variation over a 100–400-

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m length of stream. We define N uptake in this paper as gross removal of dissolved inorganic N from the water column, which is one component of overall retention (Newbold et al. 1981; Mulholland et al. 1997; Peterson et al. 2001). We express N uptake in three ways. First, we measured uptake length—the distance that a molecule travels before removal from the water column. However, this measure is strongly dependent on scale and is influenced by variance in stream discharge (Davis and Minshall 1999; Hall et al. 2002). We correct for the effect of stream size by calculating an uptake velocity that represents the biotic demand for a nutrient relative to its concentration in the water column. Finally, uptake velocity multiplied by stream water N concentration gives an areal N uptake rate from the water column to the benthos expressed as mass N per unit area per unit of time. Because stream size varied across our 11 study streams, we focus on uptake velocity and the areal N uptake rates to compare uptake among streams.

## Methods

**Study sites**—We used 11 streams in Grand Teton National Park (GTNP), Wyoming, which were chosen to maximize variation in organic matter inputs. The 11 streams were located at nearly the same elevation (2,120 m) and were close in proximity (furthest distance among streams was 43 km) (Fig. 1). Streams varied in basin geology: seven streams were located on the east side of GTNP and drained mixed sedimentary rock (sandstone, siltstone, limestone) watersheds, three streams drained crystalline metamorphic rocks of the Teton Range, and one stream (Glade Creek tributary) drained the South Boundary area of Yellowstone National Park and John D. Rockefeller National Parkway, which is composed of rhyolite lava bedrock (Table 1; Fig. 1). Stream discharge ranged from 4 to 230 L s<sup>-1</sup> (Table 1). Canopy cover on these streams ranged from no canopy to shading by streamside willows and open coniferous forests. For each stream, we chose a 100–400-m study reach, which was used for both nitrogen uptake and metabolism measurements. Reach length depended on stream velocity, and the travel time for each reach was 15–30 min. All 11 low-nutrient streams are relatively unaffected by human disturbance. Nine of 11 streams were N limited according to data from nutrient-diffusing bioassays using a factorial N and P design (Tank and Webster 1998; J.L.T. and R.O.H. unpubl. data).

**Nitrogen uptake and metabolism**—We measured whole-reach metabolism and nitrogen uptake once for each stream; eight streams were measured in July and early August 1999 and the remaining three streams in July 2000. To measure nitrogen uptake lengths, we conducted short-term (1–3 h) additions of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, in conjunction with a conservative tracer (Webster and Ehrman 1996). After collecting six to eight background samples of stream solute concentrations along the study reach, a solution of NH<sub>4</sub><sup>+</sup>Cl or NaNO<sub>3</sub> and a conservative tracer (Cl<sup>-</sup> as NaCl or Br<sup>-</sup> as NaBr) were pumped steadily into the stream. We added NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> separately to avoid the possible influence of nitrification on estimating NO<sub>3</sub><sup>-</sup> uptake length, and we started the NO<sub>3</sub><sup>-</sup> addition at least 1 h following the NH<sub>4</sub><sup>+</sup> addition to

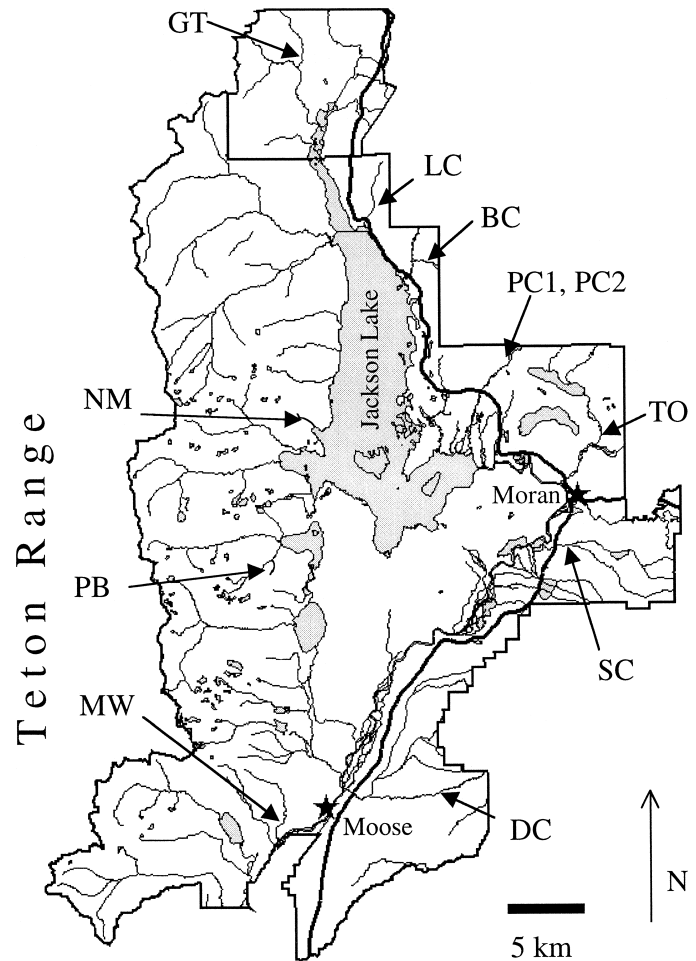


Fig. 1. The 11 study streams within Grand Teton National Park, Wyoming. MW, Moose-Wilson Creek; PB, Paintbrush Canyon tributary; NM, North Moran Bay Creek; GT, Glade Creek Tributary; LC, Lizard Creek; BC, Bailey Creek; PC1 and PC2, Pilgrim Creek channels 1 and 2; TO, Two Ocean lake outlet; SC, Spread Creek; DC, Ditch Creek.

insure that water column N concentrations had returned to background levels. Target enrichments of dissolved N were 12  $\mu\text{g NH}_4^+\text{-N L}^{-1}$  and 20  $\mu\text{g NO}_3^-\text{-N L}^{-1}$ . By increasing stream water concentration of N to measure uptake, it is possible that we underestimated uptake velocity relative to using isotope additions (Mulholland et al. 2002) because of saturation of microbial uptake. However, this effect might have been low because these streams were N limited (Mulholland et al. 2002). Additionally, we use the data to compare among streams rather than measure absolute rates. Target concentrations of conservative tracers were 5 mg Cl L<sup>-1</sup> and 40  $\mu\text{g Br L}^{-1}$ . When the conservative tracer concentration was constant through time at the downstream end of the study reach, we collected water samples at each of eight sites spaced along the study reach. To estimate if any of the added NH<sub>4</sub><sup>+</sup> was nitrified, we collected NO<sub>3</sub><sup>-</sup> samples at each of the sampling stations during the NH<sub>4</sub><sup>+</sup> addition and fit these data to a two-compartment model estimating nitrification rates (Mulholland et al. 2000; Bernhardt et al. 2002).

Ammonium concentration in water samples was measured

Table 1. Descriptions of the 11 streams used in this study.

Stream	Watershed location	Bed-rock geology*	Discharge (L s <sup>-1</sup> )	Velocity (m min <sup>-1</sup> )	Metabolism		Nitrogen uptake velocity		Concentration		
					CR (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	GPP (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mm min <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mm min <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (μg N L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (μg N L <sup>-1</sup> )	
Ditch Creek	Mt. Leidy highlands/Jackson Hole	S	231	17	5.8	0.14	98.0	1.94	9.6	2.9	5
Spread Creek	Mt. Leidy highlands/Jackson Hole	S	87	9.7	5.5	0.10	49.9	3.11	12.6	9.0	13.3
Two Ocean Lake outlet	Pacific Creek valley	S	144	16.7	4.1	0.13	44.1	8.77	5.5	0.9	9.9
Pilgrim Creek channel 1	Wildcat Peak	S	46	12.1	4.1	0.06	66.6	0.97	2.4	1.3	<5
Pilgrim Creek channel 2	Wildcat Peak	S	12	6.8	2.5	0.04	108.1	1.59	2.0	0.6	<5
Lizard Creek	Wildcat Peak	S	25	5.7	2.5	0.11	47.6	4.10	1.4	0.4	5.5
Bailey Creek	Wildcat Peak	S	118	12.7	5.4	0.10	69.7	2.02	1.6	1.7	5.2
Glade Creek tributary	Yellowstone South Boundary	R	149	19.7	3.0	0.15	126.3	1.08	9.2	3.9	<5
North Moran Bay Creek	Teton range	CM	9	4.6	0.8	0.14	135.0	5.76	1.9	0.0	43
Moose-Wilson Road Creek	Teton range	CM	35	11.3	2.2	0.09	143.8	6.05	1.1	0.0	89
Paintbrush Canyon Creek	Teton range	CM	4	3.0	1.3	0.06	58.7	1.87	1.1	0.0	169

\* S, mixed sedimentary rock; R, rhyolite lava; CM, crystalline metamorphic.

† Normalized to 20°C.

on unfiltered samples using a sensitive fluorometric assay with a detection limit of 0.5 μg N L<sup>-1</sup> (Holmes et al. 1999). Water samples and standards were fixed in the field with the fluorescent reagent and analyzed within 4 h at the laboratory. Water samples for NO<sub>3</sub><sup>-</sup>, chloride, and bromide were immediately filtered in the field and frozen the same day. These anions were analyzed using a Dionex ion chromatograph with an AS4A anion column (detection limit = 5 μg NO<sub>3</sub><sup>-</sup> N L<sup>-1</sup>).

We calculated nutrient uptake lengths for each injection using the linear form of an exponential model.

$$\ln N_x = \ln N_0 - ax \quad (1)$$

$N_0$  and  $N_x$  are nitrogen concentrations at the addition site (0 m) and  $x$  m downstream from the addition site, and  $a$  is the per-meter uptake rate (Newbold et al. 1981). Uptake length  $S$  (m) equals  $a^{-1}$ . We corrected nutrient concentrations for dilution from groundwater inputs (assuming groundwater and stream water N were equal) with downstream changes in concentrations from the concurrent conservative tracer additions (Webster and Ehrman 1996), and we used linear regression to estimate parameters for Eq. 1 from field data. Because stream depth and velocity strongly influence uptake length (Hall et al. 2002), we calculated a nutrient uptake velocity ( $V_f$ ), also referred to as a mass transfer coefficient (Stream Solute Workshop 1990), to make nutrient uptake metrics comparable among streams of different size.

$$V_f \text{ (m min}^{-1}\text{)} = Qa/w \quad (2)$$

$Q$  is stream discharge (m<sup>3</sup> min<sup>-1</sup>), and  $w$  is wetted channel width (m). Discharge was estimated based on mass balance of chloride in stream water, and width was measured at 10 transects along the study reach. The nutrient uptake velocity can be interpreted as the velocity at which a nutrient moves through the water column toward the benthos and represents the biotic demand for nutrients relative to concentration in the water column. Areal uptake rate of N ( $U$ , mg N m<sup>-2</sup> min<sup>-1</sup>) was calculated as

$$U = V_f N_a \quad (3)$$

where  $N_a$  equals the ambient N concentration in the stream based on eight prerelease measurements.

Whole-reach CR and GPP were estimated using the open-channel method refined by Marzolf et al. (1994) by using the corrected measure of oxygen (O<sub>2</sub>) flux via reaeration (Young and Huryn 1998). Metabolism measurements occurred 1 d after the nutrient uptake measures. Weather conditions for each metabolism measurement were always sunny to partly cloudy with no storms either during or before the measurements. We measured O<sub>2</sub> concentrations and stream temperature at 10-min intervals at the top and bottom of each study reach of known travel time (~20 min) with Hydrolab recording O<sub>2</sub> meters. We calibrated these meters in the field and ensured that each probe read within 1% of the other. We calculated instantaneous metabolism on the basis of differences in O<sub>2</sub> concentrations (upstream vs. downstream) while accounting for the reaeration flux of O<sub>2</sub> with the atmosphere (Marzolf et al. 1994). By integrating these measurements during a 33-h period, we estimated daily CR and GPP.

We estimated reaeration rates of  $O_2$  by measuring the reaeration coefficient of sulfur hexafluoride ( $SF_6$ ), a tracer gas (Wanninkhof et al. 1990).  $SF_6$  was added to streams by bubbling at the solute addition site at a flow rate of 50–150 ml  $min^{-1}$ . Concurrent with the N sampling, we collected 45 ml of water in 60-ml plastic syringes at 8–10 locations downstream of the addition site. We shook water samples for 10 min to equilibrate with a 15-ml air headspace, injected the headspace into 10-ml evacuated serum vials, and analyzed for  $SF_6$  on a gas chromatograph with an electron capture detector using the same approach as Cole and Caraco (1998). We calculated the reaeration coefficient of  $SF_6$  ( $k_{SF_6}$ ,  $min^{-1}$ ) as

$$k_{SF_6} = a_{SF_6} \times V \quad (4)$$

where  $a_{SF_6}$  is the per-meter loss rate of  $SF_6$  and  $V$  is stream velocity (m  $min^{-1}$ ). Per-meter loss rate of  $SF_6$  was calculated the same way as for nutrients. With Eq. 1, we converted  $k_{SF_6}$  to  $k_{O_2}$  by the ratio of their Schmidt numbers (1.4) following Wanninkhof et al. (1990) (Table 1). We estimated stream velocity by fitting the solute concentration curve to a one-dimensional advection, dispersion, transient storage model and solving iteratively (Hart 1995).

We used simple linear regression to relate whole-reach metabolism with nitrogen uptake velocities (for both  $NH_4^+$  and  $NO_3^-$ ). We used multiple linear regression to relate GPP and CR to  $NH_4^+$  and  $NO_3^-$  uptake velocities with  $\alpha = 0.1$  for variables in the model.

*Predicting areal N uptake rates based on metabolism*—We predict that if assimilatory uptake of N by benthic algae and biofilm microbes is the dominant mechanism for N removal from the water column, then areal N uptake by the benthos should be a function of autotrophic and heterotrophic production. We can test this prediction by estimating areal N uptake rates based on the stoichiometry of C and N in the biota; if measured rates are similar to predicted rates, then it provides evidence that stream metabolism is driving variation in N uptake rates.

Predicted areal N uptake rates were derived by assuming an autotrophic and heterotrophic demand for N based on measured C production values (i.e., from metabolism assuming respiratory quotient = 1) and a molar C:N = 20. This ratio was chosen on the basis of the average of epilithon and filamentous algae C:N from several open-canopy streams: Kings Creek (Dodds et al. 2000), Sycamore Creek (E. Marti et al. unpubl. data), Eagle Creek, (Hamilton et al. 2001), Walker Branch prior to leaf out (Mulholland et al. 2000) and Hubbard Brook (R.O. Hall unpubl. data). Net autotrophic production was assumed to be  $0.5 \times GPP$  (Odum 1957; Webster and Meyer 1997); therefore, the respiratory contribution by autotrophs was also  $0.5 \times GPP$ . Consequently, heterotrophic respiration (HR) could be calculated as CR less the calculated autotrophic respiration (i.e.,  $HR = CR - 0.5GPP$ ). Although heterotrophic production was not measured directly, we conservatively estimated heterotrophic production on the basis of heterotrophic respiration with both a moderate- and low-growth efficiency (0.2 and 0.05, respectively), where heterotrophic growth efficiency (HGE) is calculated as

$$\begin{aligned} \text{HGE} &= \text{heterotrophic production} \\ &\div (\text{heterotrophic production} \\ &\quad + \text{heterotrophic respiration}) \end{aligned}$$

by using del Giorgio et al. (1997) and values for HGE therein. We used two growth efficiencies because we do not know growth efficiency of heterotrophs, and a high and low efficiency will bound our estimate. Our other assumptions (fraction of respired GPP, and C:N), are unlikely to vary fourfold as growth efficiency might. Finally, with our stoichiometric approach, predicted areal N uptake was compared to measured areal N uptake from our nutrient additions, where total measured areal N uptake rates ( $mmol\ m^{-2}\ min^{-1}$ ) were calculated as  $V_f$  multiplied by ambient nutrient concentration for both  $NH_4^+$  and  $NO_3^-$  (Newbold et al. 1981). For streams where  $NO_3^-$  concentrations were lower than the detection limit of  $5\ \mu g\ N\ L^{-1}$ , we assumed that  $NO_3^-$  concentrations were 0. We used correlation analysis to compare modeled and measured values.

## Results

Metabolism varied in the 11 streams, with 24-fold variation in GPP and 13-fold variation in CR (Table 1), but CR was only weakly positively related to GPP ( $r^2 = 0.28$ ,  $p = 0.1$ ). Even though most of our study streams were unshaded, with an open canopy, GPP/CR was less than 1 for all streams indicating net heterotrophy in all 11 streams on the days we measured metabolism.

Nitrogen concentrations in our study streams were low;  $NH_4^+$  averaged  $2.2\ \mu g\ NH_4\text{-}N\ L^{-1}$  (range 0.5–10  $\mu g\ NH_4\text{-}N\ L^{-1}$ ), and  $NO_3^-$  averaged only  $9\ \mu g\ NO_3\text{-}N\ L^{-1}$  in streams draining watersheds north and east of GTNP, whereas the three streams draining the Teton Range had higher  $NO_3^-$  concentrations (mean  $100\ \mu g\ NO_3\text{-}N\ L^{-1}$ , range 43–170  $\mu g\ NO_3\text{-}N\ L^{-1}$ ) (Table 1).

Uptake lengths of  $NH_4^+$  and  $NO_3^-$  were not related to stream size when size was expressed as specific discharge ( $Q/w$ ) (Fig. 2). In three streams,  $NO_3^-$  uptake lengths could not be estimated because concentrations did not significantly decline downstream, which is interpreted as an infinitely long uptake length. These three streams had low  $Q/w$  (arrows on Fig. 2); yet, uptake lengths were greater than 1,000 m on the basis of variation in the slope estimate in the regression models. Although increased stream depth and velocity should transport nutrients farther downstream before removal, we found no relationship between specific discharge and N uptake length ( $p > 0.6$  for both  $NH_4^+$  and  $NO_3^-$ ), highlighting that variation in N uptake length was controlled by other factors in these streams.

Water-column  $NH_4^+$  was unrelated to metabolism, suggesting that concentration of  $NH_4^+$  was not driving variation in metabolism. In contrast, ammonium demand expressed as  $V_f$  was positively related to rates of GPP and CR (Fig. 3), individually explaining 78% (GPP) and 50% (CR) of the variability in  $NH_4^+ V_f$ . Using multiple regression analysis, GPP and CR explained 82% of the variation in a  $NH_4^+ V_f$ ; ( $NH_4^+ V_f = 0.0032GPP + 0.00036CR - 0.00059$ , GPP  $p = 0.0017$ , CR  $p = 0.064$   $R_{adj}^2 = 0.82$  and  $n = 11$ ).

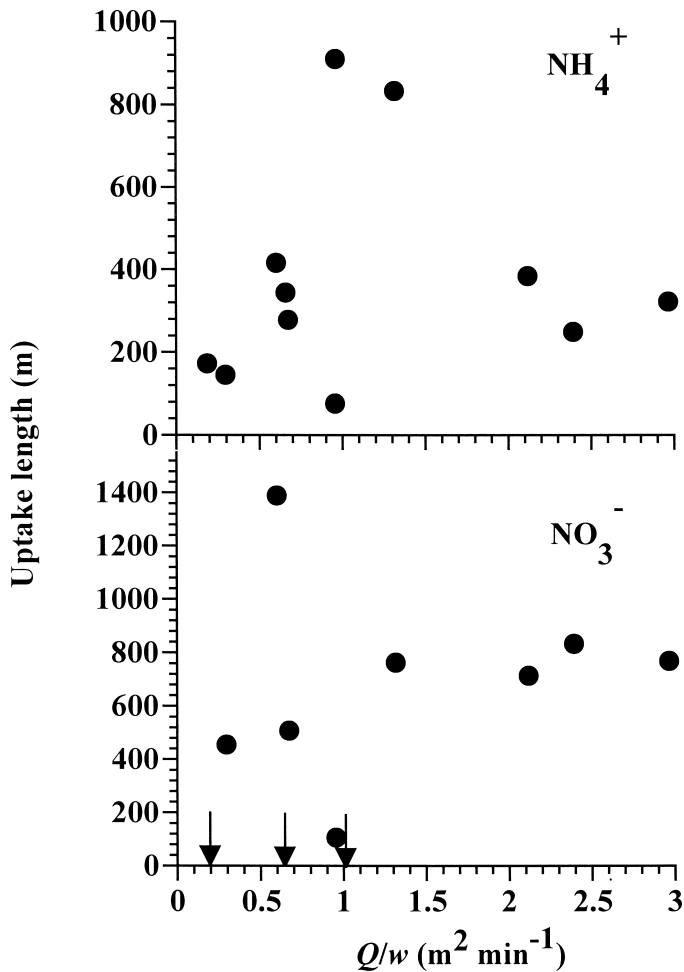


Fig. 2. Uptake length of  $\text{NH}_4^+$  (top panel) and  $\text{NO}_3^-$  (bottom panel) does not relate with specific discharge. Specific discharge is discharge ( $Q$ ) per meter of stream width ( $w$ ). The slopes of a least-squares linear regressions are not significantly different from zero. Arrows on the  $x$ -axis for the  $\text{NO}_3^-$  panel indicate the specific discharge for three streams for which we could not measure  $\text{NO}_3^-$  uptake length.

During our short-term  $\text{NH}_4^+$  additions in our low-nutrient study streams, we expected to see some evidence of in-stream nitrification. However, little of the added  $\text{NH}_4^+$  appeared to be nitrified. In the North Moran Bay tributary, any nitrification should have been readily detectable because there was no  $\text{NO}_3^-$  uptake, and high  $\text{NO}_3^-$  is correlated with high nitrification (Bernhardt et al. 2002); yet, only 6% of added  $\text{NH}_4^+$  was nitrified. Because background  $\text{NO}_3^-$  was so low in each of these streams, we would have readily been able to detect an increase in  $\text{NO}_3^-$  if even a fraction of the added  $\text{NH}_4^+$  was nitrified. Using the model in Bernhardt et al. (2002), we found that if 10% of the ammonium had been nitrified and appeared as  $\text{NO}_3^-$  in the water column, the increase would have been detectable over the actual  $\text{NO}_3^-$  concentrations.

In contrast to  $\text{NH}_4^+$ , only GPP, not CR, predicted  $\text{NO}_3^- V_f$  with GPP explaining 75% of the variation (Fig. 3). This relationship also remains statistically significant without the leveraging point from the most productive stream (Spread

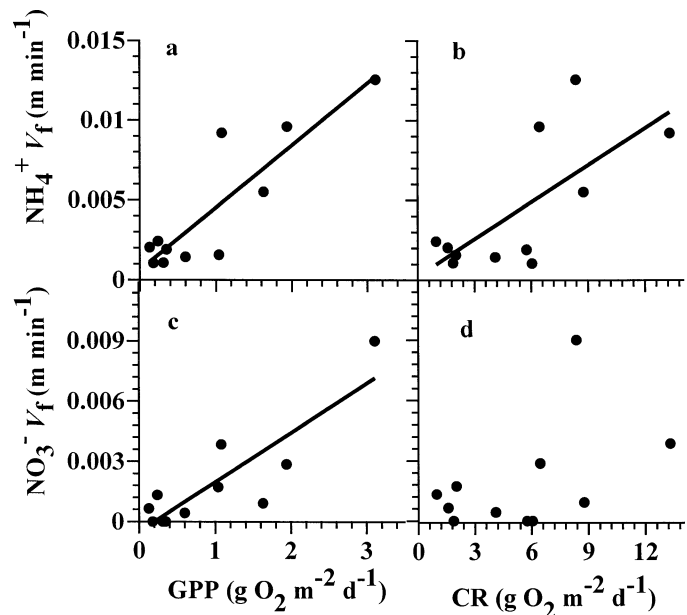


Fig. 3. Metabolism is related to nitrogen uptake velocity ( $V_f$ ) in 11 streams. Lines are least-squares linear regressions and are only presented when the slope is significantly different from zero ( $p < 0.05$ ). Equations are (a)  $V_f \text{NH}_4^+ = 0.000619 + 0.00392\text{GPP}$  ( $r^2 = 0.78$ ), (b)  $V_f \text{NH}_4^+ = 0.00024 + 0.000773\text{CR}$  ( $r^2 = 0.50$ ), (c)  $V_f \text{NO}_3^- = -0.000486 + 0.00246\text{GPP}$  ( $r^2 = 0.75$ ).

Creek) ( $p = 0.046$ ), despite lowering the predictive power of this relationship to  $r^2 = 0.41$ . GPP/CR explained 80% of the variation in  $\text{NO}_3^- V_f / \text{NH}_4^+ V_f$ , indicating that as GPP/CR increases,  $\text{NO}_3^-$  demand increases relative to  $\text{NH}_4^+$  (Fig. 4).

Estimating N demand based on C and N stoichiometry appears to support our prediction that assimilatory N uptake

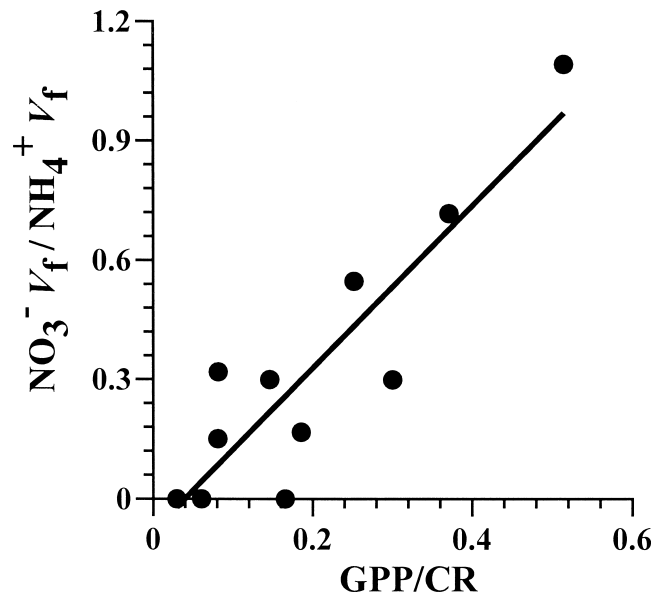


Fig. 4.  $\text{NO}_3^- / \text{NH}_4^+ V_f$  is positively related to GPP/CR. The line is the prediction from a least-squares regression. The equation is  $\text{NO}_3^- V_f / \text{NH}_4^+ V_f = -0.081 + 2.05(\text{GPP}/\text{CR})$ , ( $p = 0.002$ ,  $r^2 = 0.80$ ).

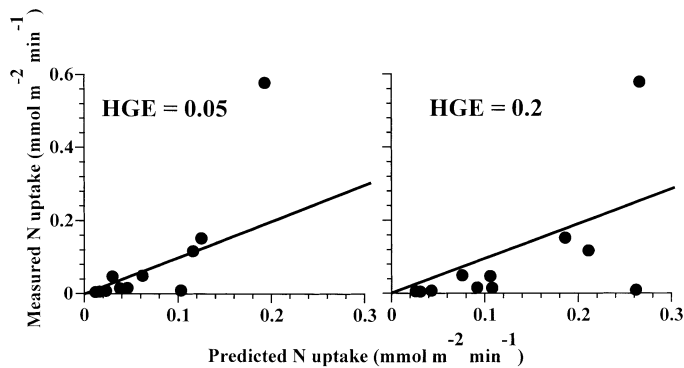


Fig. 5. Predicted areal N fluxes relate to observed N fluxes in 11 streams. Predictions were derived from estimates of autotrophic and heterotrophic demand for N based on assumed C production values and C:N = 20. Autotrophic production was assumed to be 0.5GPP. Heterotrophic production was not measured but was estimated on the basis of respiration for two heterotrophic growth efficiencies (HGE), where  $HGE = (\text{heterotrophic production} / [\text{heterotrophic production} + \text{respiration}])$ . Heterotrophic respiration was estimated as whole-stream CR = 0.5GPP. The line is the 1:1 line where predicted and measured N uptake rates are equal.

by autotrophs and heterotrophs is the dominant mechanism for N removal in our study streams. Using either the low (HGE = 0.05) or high (HGE = 0.2) growth efficiencies, predicted areal N uptake rates were significantly correlated with measured N uptake rates (HGE = 0.05,  $r = 0.84$ ; HGE = 0.2,  $r = 0.62$ ). When HGE = 0.05, most of the points fall near the 1:1 line, suggesting that predicted and measured uptake was close for all but two streams (Fig. 5). When HGE is four times higher, predicted areal N uptake rates (i.e., ambient N concentrations  $\times V_f$ ) were higher than measured rates for most streams. In both scenarios, Spread Creek, the stream with the highest measured GPP, had a higher areal N uptake rate than would have been predicted from its already high production and respiration (Table 1).

## Discussion

*Linking metabolism and N uptake*—Using whole-reach estimates of metabolism and N uptake, we quantified an explicit biological mechanism to explain variation in N uptake velocity among our streams. A substantial fraction variation in N uptake velocities in our 11 streams could be explained by variation in metabolism. We emphasize that we have only measured one component of N retention: removal of dissolved N from the water. To estimate actual retention would require measuring residence time using  $^{15}\text{N}$  as an isotopic tracer (e.g., Tank et al. 2000) or quantifying an annual nutrient budget for a reach (Meyer and Likens 1979). However, measuring N uptake characterizes a stream's ability to remove and transform dissolved inorganic N (Peterson et al. 2001).

To date, many studies that examine variation in nutrient uptake among streams have focused on physical variables (e.g., stream depth [Alexander et al. 2000], transient storage [Valett et al. 1996; Hall et al. 2002], riparian vegetation [Sabater et al. 2000], or the influence of biome [Munn and Mey-

er 1990]). Although these studies are able to explain part of the variation in nutrient uptake, physical attributes only indirectly control nutrient uptake by influencing biological processes in some way. For example, increased transient storage should increase water residence time, thereby increasing water contact time with the sediments and facilitating uptake by microbial biofilms. We suggest that after correcting for the effects of water velocity and depth (e.g., Alexander et al. 2000) and nutrient concentration (Dodds et al. 2002), biological variables will be more predictive of N uptake velocity than physical variables. For example, Hall et al. (2002) were able to explain only 46% of  $\text{NH}_4^+ V_f$  by combining measures of transient storage and  $\text{NO}_3^-$  concentration in eastern deciduous forest streams, whereas we were able to explain 82% of the variation in  $\text{NH}_4^+ V_f$  with whole-stream metabolism in this study.

Our results are based on experiments performed in mid-summer, when discharge was low in these snowmelt-dominated streams, and correspondingly, production was most likely to be high. Because we have not measured metabolism during winter or spring snowmelt runoff, we cannot predict N uptake velocity at these times. However, we suggest that the mechanism observed here (i.e., ecosystem metabolic control over N uptake) would hold for other times of the year. Because uptake velocity ( $V_f$ ) is independent of discharge, increasing  $Q$  should not affect  $V_f$ . Therefore, if metabolism is lower during spring runoff or in winter, we suggest that  $V_f$  will also be lower.

When we predicted areal N uptake using a stoichiometric approach based on measured whole-stream metabolism and an assumed HGE of 0.05, predicted rates were both strongly correlated with and close to our measured areal N uptake rates. In contrast, using a higher HGE of 0.2 predicted an areal uptake that was slightly higher than measured areal uptake. Our goal with this model was to provide evidence that higher demand for C leads to higher demand for N. The predictions for any one stream vary considerably, most likely as a result of our three main assumptions: that C:N equals 20 for primary producers, that autotrophic respiration equals 50% of GPP, and that our assumed heterotrophic growth efficiency lies near 0.05–0.2. The first two assumptions are reasonably sound. However, because HGE can vary fourfold or greater, we performed this calculation using two HGE values. In addition, we must also consider that some fraction of the N uptake in biofilms is via recycling within the biofilm itself; thus, the areal N uptake from the water column represents a fraction of the total microbial uptake of N. This difference might be especially true for the 11 very low nitrogen streams in our study, where we might expect that, because of low N availability in the water column, recycling of N within biofilms is critical for maintaining the relatively high rates of primary production in some streams. Finally, C:N ratios in biomass might not equal C:N ratios of uptake because algae and bacteria make carbon-rich exopolymers and leak dissolved organic carbon. Because of the assumptions involved in predicting N uptake rate from metabolism, we hesitate to use metabolism to predict N uptake rates, especially given that measuring N uptake is not easier than measuring metabolism. However, despite discrepancies between measured and predicted areal N uptake rates, their

strong correlation and predicted rates that are nearly equal to measured rates suggests that autotrophic and heterotrophic production are driving removal of dissolved N in these streams.

*Other evidence for biological control of N uptake*—Other studies have related nutrient uptake to biological variables in streams, however the common thread of these studies has been an alteration or manipulation of biological structure in streams (e.g., excluding leaves, killing microbes), as opposed to comparing rates among streams. Phosphorus uptake lengths were negatively correlated with increasing standing stocks of decaying leaves, presumably a result of increased microbial demand as leaves decompose (Mulholland et al. 1985). In a study using replicate artificial streams, phosphorus uptake length was 2.5 times longer immediately after sediment microbes were killed using chlorine, confirming that biotic uptake mechanisms were important to P uptake (D'Angelo et al. 1990). Removal of first leaf litter, then wood, from a mountain stream in North Carolina increased nutrient uptake lengths compared to a reference stream containing natural inputs of allochthonous organic matter (Webster et al. 2001).

By quantifying whole-reach metabolism in this study, we were able to establish a tight link between carbon-fixation/degradation and nitrogen demand. As opposed to static estimates of biofilm standing stocks, the process of metabolism, expressed as a rate, accounts for rapid turnover of algal and microbial biomass. Streams are spatially heterogeneous (Palmer and Poff 1997) and biofilm biomass or production estimates from substrata are likely too variable to relate to reach-scale estimates of nutrient uptake. Additionally, our metabolism measurements integrate over a large stream reach and include processes occurring in the hyporheic zone. Few previous studies have linked metabolism to nutrient uptake. Mulholland et al. (1997) used two streams that varied greatly in CR and showed that the high-CR stream had an increased demand for phosphorus. Results from a multisite, multibiome  $^{15}\text{N}$  tracer study comparing  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake velocity with whole-stream estimates of metabolism did not show a strong relationship (Mulholland et al. 2001; Peterson et al. 2001; Webster et al. unpubl. data). We suggest two reasons why we found a strong relationship in the GTNP streams. First, the variation in N uptake velocity was higher in our 11 study streams in GTNP ( $\text{CV} = 0.94$  for  $\text{NH}_4^+$  and  $1.41$  for  $\text{NO}_3^-$ ) compared to the 11 streams located across the country in the interbiome study ( $\text{CV} = 0.80$  for  $\text{NH}_4^+$  and  $0.99$  for  $\text{NO}_3^-$ ). Second, despite the higher variation in N uptake velocity, our streams in GTNP were located in close proximity and had similar climates, albeit different geology. Variation in N uptake in 11 streams in 8 biomes could be caused by latent variables, such as climate, that are hard to quantify in the context of examining stream channel N processing.

Unlike other studies (Butterini and Sabater 1998; Wolheim et al. 2001; Hall et al. 2002), we found no relationship between stream size ( $Q/w$ ) and N uptake lengths ( $S$ ). In our streams, variation in metabolism is about as high as variation in stream size, and many of the larger streams had high GPP and therefore shorter N uptake lengths. Perhaps if we had

used sites with a larger range of stream size we would find that as stream size increased, N uptake lengths would also increase, as in other studies. Our estimated  $\text{NH}_4^+$  uptake velocities were similar in magnitude to those from other streams; the mean of  $4.4 \text{ mm min}^{-1}$  compares with rates at Hubbard Brook, New Hampshire, Walker Branch, Tennessee, and Hugh White Creek, North Carolina, and is lower than two streams in Spain (Hall et al. 2002).

*Nitrification, and control of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake*—We were unable to quantify “net” nitrification in any of our study streams. We say net nitrification because it is possible that the  $\text{NH}_4^+$  we added could have been converted to  $\text{NO}_3^-$  and taken up immediately. However, because there was no measurable  $\text{NO}_3^-$  uptake in three of the streams, there would have been apparent  $\text{NO}_3^-$  generation if there were substantial nitrification in these streams. Our results differ from those of Bernhardt et al. (2002) and Peterson et al. (2001), who both found that in many streams a large fraction of immobilized ammonium is nitrified. Bernhardt et al. (2002) showed that as ambient  $\text{NO}_3^-$  concentrations increased, nitrification also increased, and they suggest that this relationship was because high  $\text{NO}_3^-$  mediated competition for  $\text{NH}_4^+$  between heterotrophs and nitrifiers. Nitrification rate is increased by  $\text{NH}_4^+$  availability and decreased by high labile organic matter availability (Strauss and Lamberti 2000). Ammonium was always  $<10 \mu\text{g N L}^{-1}$ ; thus, its low availability might have decreased nitrification rates. Additionally, high GPP can lead to high input of high-quality algal-derived dissolved organic matter, which could decrease nitrification. Given the low  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations and N limitation in our study streams, we suggest that heterotrophs and photoautotrophs are responsible for most of the ammonium demand; thus, ammonium uptake is primarily assimilatory rather than dissimilatory nitrification.

For low-nitrogen streams in GTNP, nitrate  $V_f$  was solely related to GPP, whereas  $\text{NH}_4^+$   $V_f$  was related to both GPP and CR. This pattern suggests that the relative demand of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  might depend on the constituents of the stream biofilm assemblage, with heterotrophic microorganisms primarily using  $\text{NH}_4^+$  and photoautotrophs using both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Studies in marine systems show that phytoplankton will take up  $\text{NO}_3^-$  when  $\text{NH}_4^+$  concentrations are low (Dortch 1990) but that heterotrophic bacteria do not normally use  $\text{NO}_3^-$  as a preferred dissolved inorganic nitrogen source (Kirchman 1994). Heterotrophic bacteria might be colimited by the carbon and nitrogen limitation in our streams, as has been found elsewhere (Tank and Webster 1998) and thus less likely to reduce  $\text{NO}_3^-$ , which is an energetically expensive process (Dortch 1990). In contrast, algae are presumably less limited by energy in these high-light streams and might be able to reduce nitrate using abundant solar energy (Gutschick 1981). Despite high primary production rates in some streams, all 11 streams had  $\text{GPP/CR} < 1$ , indicating net heterotrophy on the days we measured metabolism. The low  $\text{GPP/CR}$  ratios suggest a strong heterotrophic (bacterial or fungal) component to stream metabolism that contributes to  $\text{NH}_4^+$  removal from the water column (Tank et al. 2000). We suggest that variation in metabolic processes (GPP vs. CR) in streams will determine

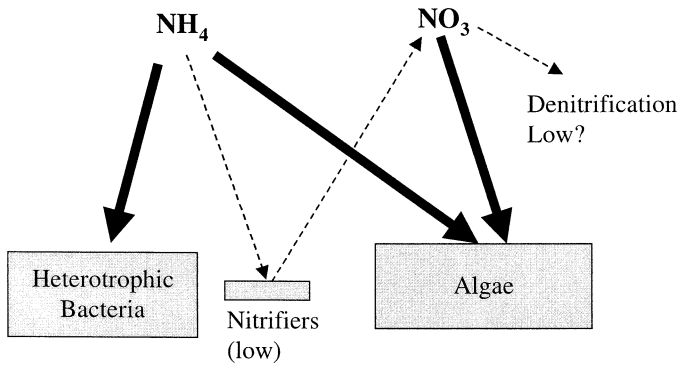


Fig. 6. Model of N cycling in streams within Grand Teton National Park. Solid arrows indicate relatively large fluxes of nitrogen. Dotted arrows are fluxes that are too small to measure (in the case of nitrification) or are presumed to be small (i.e., denitrification).

which form of N is preferentially removed in a stream reach, thereby linking biofilm heterotrophy and autotrophy with  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake.

We present a conceptual model of N cycling in our low-N streams (Fig. 6). Ammonium is removed by both photoautotrophs and heterotrophs, whereas  $\text{NO}_3^-$  is taken up primarily by photoautotrophs. Nitrification is low, presumably because of extremely low  $\text{NH}_4^+$  concentrations and high heterotrophic demand. In streams with higher  $\text{NH}_4^+$ , or even higher  $\text{NO}_3^-$ , concentrations, we might expect higher nitrification, as has been found elsewhere (Peterson et al. 2001; Bernhardt et al. 2002). We could not measure  $\text{NH}_4^+$  regeneration, but we assume it was similar to areal uptake because we never observed a change in  $\text{NH}_4^+$  concentration along our stream reaches. Given the low  $\text{NO}_3^-$  concentrations, oxic conditions, and little accumulation of organic-rich sediment, we assume that denitrification rates are extremely low and limited by nitrate and carbon availability, as well as anoxic conditions. Lab denitrification assays on stream sediments in anoxic conditions show that when nitrate concentrations fall below  $\sim 750 \mu\text{g N L}^{-1}$ , denitrification rates are nitrate limited (Royer et al. pers. comm.).

Streams recently have been recognized as important landscape features; they are responsible for much of the retention, transformation, and transport of nutrients such as N (Fisher et al. 1998; Alexander et al. 2000; Peterson et al. 2001). Our research shows that dissolved nitrogen uptake velocity was strongly related to whole-stream estimates of metabolism, thereby providing one mechanistic explanation for dissolved N removal in low-nitrogen streams. Given that  $\text{NO}_3^-$  uptake velocity was primarily linked to rates of GPP, we suggest that maintenance of high GPP is one means that streams have to effectively remove  $\text{NO}_3^-$ , outside of denitrification, which is a permanent sink for N. Disturbances such as sedimentation might lower GPP and lower  $\text{NO}_3^-$  demand by autotrophs. Future work in these streams will examine the fate of  $\text{NO}_3^-$  loss from the water by both assimilatory and dissimilatory pathways using tracers that will allow us to examine overall patterns of retention.

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