

Ammonium uptake and mineralization in prairie streams: chamber incubation and short-term nutrient addition experiments

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SUMMARY

1. We used two separate approaches to estimate ambient ammonium cycling in the north and south branches of Kings Creek, a prairie stream. Chamber experiments were conducted to determine ammonium uptake and mineralization rates associated with epilithic biofilms and filamentous algae collected from the streams. A series of short-term whole-stream ammonium addition experiments were also conducted to estimate the rate of uptake at ambient stream concentrations, based on the relationship between ammonium concentrations and uptake rates.
2. Chamber experiments were scaled up to whole-stream levels, resulting in ambient gross uptake estimates of $0.08 \mu\text{g}^{-2} \text{s}^{-1}$ for the north branch and $0.16 \mu\text{g}^{-2} \text{s}^{-1}$ for the south branch. The substrata-specific estimates of mineralization were higher than uptake in both streams.
3. Substrata-specific measurements indicated that ammonium uptake is higher in riffle habitats than in pools habitats. The results of the short-term ammonium addition experiments support these findings.
4. Short-term ammonium addition experiments show that uptake rates saturate with increasing ammonium concentrations. The observed saturation of uptake rates is consistent with a Michaelis–Menten relationship.
5. Scaled estimates of uptake from the chamber experiments were similar to estimates of ambient ammonium uptake based on the whole-stream experiments, and were comparable with previous estimates of ammonium uptake and mineralization made by using stable isotope tracer methods in Kings Creek.

Keywords: mineralization, nitrogen, prairie, streams, uptake

Introduction

Nutrient spiralling (Webster & Patten, 1979) describes a key aspect of processing of available nutrients in streams (Newbold *et al.*, 1981), and is a central component of stream ecosystem function. Researchers have employed a variety of methods to quantify related parameters used to describe nutrient spiralling

particularly those elements of nutrient spiralling that involve nutrient retention. There are three interrelated parameters useful to describe nutrient retention. The average distance a molecule travels downstream is indicated by the uptake length (S_w). The nutrient mass retained per unit time and area of stream bottom is the uptake rate (U_t). The rate of movement of molecules out of the water column (V_t) is the mass transfer coefficient (Stream Solute Workshop, 1990). These parameters are used to quantify changes in nutrient retention over time in a single stream (e.g. Simon *et al.*, 2005) or compare differences in nutrient retention among streams (e.g. Peterson *et al.*, 2001).

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Short-term nutrient addition experiments are commonly used to measure gross nutrient retention in streams (e.g. Newbold *et al.*, 1983; Mulholland, Steinman & Elwood, 1990; Hall & Tank, 2003; Niyogi, Simon & Townsend, 2004). These experiments generally include release of an inorganic nutrient (NH₄⁺, NO₃⁻ or PO₄⁻) at a constant rate into a stream to elevate the concentration above ambient (Webster & Ehrman, 1996). The resulting decline in nutrient concentration with distance downstream (net uptake) is considered to be proportional to the gross rate of uptake. The advantage of short-term nutrient addition experiments over isotopic methods (e.g. Dodds *et al.*, 2000; Mulholland *et al.*, 2000) is that they are much less expensive and the procedures are less complex than for isotopic tracers (such as ¹⁵N). The disadvantage is that a single short-term nutrient addition typically underestimates ambient uptake rates (Dodds *et al.*, 2002) because the S_w at elevated experimental concentrations is longer than the S_w at ambient concentrations (Mulholland *et al.*, 1990, 2002).

Although a single short-term nutrient addition may not accurately predict S_w at ambient conditions, these kinds of experiments can still be used to predict ambient uptake rates, if the relationship between uptake and concentration can be established (Dodds *et al.*, 2002). It is typically assumed that the relationship between uptake and concentration is best described by the Michaelis–Menten equation (Stream Solute Workshop, 1990; Mulholland *et al.*, 2002), although this is not always found to be the case (Dodds *et al.*, 2002). Payn *et al.* (2005) proposed a method in which the linear relation between S_w from multiple- or single-nutrient addition experiments and the concentration of added nutrient were used to project S_w at ambient stream conditions, assuming the Michaelis–Menten model is valid.

In many streams, nutrient concentration is relatively stable over a time frame of hours during base-flow conditions, and there is little net uptake. Under these conditions, gross uptake rate must be balanced by mineralization (Dodds, 1993). Mineralization, the regeneration of NH₄⁺ from the benthic zone to the water column, has been often investigated in marine (Rysgaard *et al.*, 2000), estuarine (Anderson, McGlathery & Tyler, 2003) and wetland (Zhu & Ehrenfeld, 1999) ecosystems. Although uptake of NH₄⁺ has been extensively studied in streams, mineralization has seldom been directly reported for lotic ecosystems. An

exception is Peterson *et al.* (2001), in which regeneration (mineralization) was expressed as a proportion of ammonium uptake.

Sediment-scale assays have also been used to predict whole-stream processes. Such assays have been used to predict nitrification, denitrification and uptake of ammonium and nitrate in streams (Kemp & Dodds, 2002a,b). They are also commonly used in marine and wetland systems (e.g. Stanley & Ward, 1997). The advantage of these assays is that they are relatively easy to conduct and replicate. The disadvantage is the difficulty in scaling measurements up to the whole system.

We conducted a series of short-term NH₄⁺ addition experiments in a whole stream and concurrently performed incubation experiments on substrata taken from that stream. The objectives of this study were: (i) measure mineralization and gross ammonium uptake rates associated with stream substrata; (ii) compare ammonium uptake and mineralization rates occurring in the scale of stream habitats (pools and riffles); (iii) measure the reach scale ambient ammonium uptake and the ecosystem response to short-term changes in NH₄⁺ concentration (whole-stream uptake kinetics) and (iv) compare two independent, non-isotopic measurements of ecosystem NH₄⁺ retention at ambient concentrations in two prairie streams.

Methods

Study area

Our study was conducted on tributaries of the Kings Creek catchment at the Konza Prairie Biological Station, located in the Flint Hills region of north-eastern Kansas, U.S.A.. The experiments were conducted on two streams draining subcatchments, north branch (39°06.008'N and 96°34.454'W) and south branch (39°05.271'N and 96°35.067'W) of the Kings Creek system. Land use in both catchments is composed entirely of native tallgrass prairie. Prairie streams in this region are typically oligotrophic, with low concentrations of dissolved and total N and P (Gray *et al.*, 1998). Gallery forests are found in the riparian areas of some streams, which provide the streams with some shade and small amounts of wood and leaf materials; however, large woody debris is usually absent from these headwater streams. The stream bottom is characterized by cobble and occa-

Table 1 Hydrologic and nitrogen characteristics of the two streams in this study during the stepwise short-term NH_4^+ addition experiments

| Stream | South branch stream | North branch stream |
|---|---------------------|---------------------|
| Discharge (L s^{-1}) | 4.7 | 7.4 |
| Wetted width (m) | 2.1 | 2.1 |
| Average depth (m) | 0.125 | 0.118 |
| NH_4^+ -N ($\mu\text{g L}^{-1}$) | 2.9 | 0.6 |
| NO_3^- -N ($\mu\text{g L}^{-1}$) | 2.5 | 1.5 |
| Stream area as pool (%) | 30 | 40 |
| Stream area as riffle (%) | 70 | 50 |
| Stream area as filamentous algae (%) | 0 | 10 |

sional bedrock. Primary producers within the stream channel are dominated by epilithic diatoms and filamentous green algae (*Cladophora* and *Spirogyra*). Kings Creek is an intensively studied system with published descriptions of the nitrogen cycle (Dodds *et al.*, 2000), hydrology (Gray *et al.*, 1998) and ecology (Gray & Dodds, 1998).

The two streams we investigated had similar physical and biological characteristics. Stream discharge was similar and average width was the same for both streams (Table 1). NH_4^+ concentrations were also similar between the two streams, with the south branch stream (SBS) having a slightly greater concentration than the north branch stream (NBS). The predominant biota in the two experimental reaches was epilithic biofilms that cover the cobble in the stream bottom. The north branch reach also had streamers of filamentous algae (*Cladophora*) present in the riffle habitats. The two streams were considered replicates for the purposes of this study.

Chamber experiments

Chamber experiments were conducted to estimate NH_4^+ uptake and mineralization rates at substrata-specific scale by incubating substrata/cobbles colonized by the dominant nutrient uptake biological compartments (i.e. epilithic biofilm and filamentous algae). These experiments were performed with substrata collected from the two study streams over 4 days in July 2004. Cobbles were selected randomly from several pools and riffles within the reach and transported in stream water to the laboratory for analysis within 2 h. Cobbles collected from the stream were minimally disturbed during transport.

Substrata were incubated in the light (mixed fluorescent and halogen sources with an average intensity of $11.75 \text{ mol quanta m}^{-2} \text{ day}^{-1}$) at 20°C with 22 L of stream water in open-topped recirculating chambers (Dodds & Brock, 1998) located inside controlled environmental chambers. Multiple cobbles were placed in the chambers (3–4 cobbles per chamber) sufficient to cover the bottom area (c. 0.05 m^2). Water velocity was held constant during and across the experiments and approximated average in-stream velocities. Four replicate incubations were conducted for each habitat for both streams (16 total chamber incubations). Stream water in the chambers was then diluted with de-ionized water amended with sodium bicarbonate and HCl (to approximate the osmotic balance and pH of ambient stream water). The resulting change in NH_4^+ concentration was measured over time (c. 2.5 h, samples taken every 30 min) and used to establish the steady state base concentration (C'). Concentration of NH_4^+ was then increased to approximately $25 \mu\text{g L}^{-1}$ by adding a spike of 5.0 mM NH_4Cl to each chamber. Samples for NH_4^+ concentration (10 mL each) were taken every 30 min for 3 h after the spike and the resulting decline in concentration was used to calculate the uptake coefficient.

This method assumes that, over the short period of the experiment in the closed chambers, a steady state NH_4^+ concentration (C') will become established at which the rate of uptake equals the rate of mineralization. Raising the NH_4^+ concentration above C' will yield a net uptake over time (Fig. 1) because, at least over short periods of time, uptake is dependant on concentration while mineralization is not. Dilution of NH_4^+ below base concentration will result in net

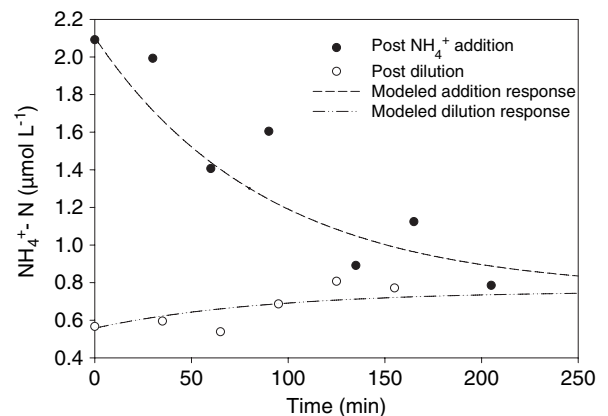


Fig. 1 An example of the response of NH_4^+ concentration to dilution and addition of NH_4^+ in chamber experiments.

mineralization, because gross uptake will become less than mineralization, causing NH₄⁺ concentration to return to base concentration. By identifying base concentration and rate of net uptake, rates of mineralization and uptake can be determined.

Uptake is assumed to be a first-order kinetic reaction, in the narrow range of concentrations in the chamber experiment. Net change in substrate concentration over time can be described by the equation:

$$dC/dt = -K_t \times C + M \quad (1)$$

where t is time, K_t is chamber uptake coefficient and mineralization (M) is assumed to be independent of substrate concentration. Note that in this paper, we define the solid rock and materials on the bottom of the streams that are colonized by organisms as substrata and the chemicals they metabolize as substrates. K_t was calculated as the slope of C' corrected, ln-transformed, NH₄⁺ concentrations over time after the spike. M was then calculated as:

$$M = K_t \times C' \quad (2)$$

Chamber uptake rates at ambient stream conditions were calculated by the equation:

$$U_t = C_0 \times V \times K_t \times A^{-1} \quad (3)$$

in which V is volume of the chamber (22.0 L) and A is total substrata area (*c.* 0.05 m²). Substrata-specific uptake rates were then weighted by the proportion at which each substrata type occurred in the stream as measured by stream transects described below. Substrata uptake rates were scaled up to whole-reach levels for comparisons with short-term nutrient addition experiments. The U_t for each stream was calculated as the sum of the weighted substrata-specific uptake rates.

Stepwise short-term whole-stream NH₄⁺ addition experiments

Short-term NH₄⁺-enrichment experiments were conducted on the two streams in July 2004 (Webster & Ehrman, 1996). The experiments consisted of four stepwise NH₄Cl-enrichment treatments (with NaBr as a hydrologic tracer) per day at each stream, in which the NH₄⁺ concentration was incrementally increased with each subsequent release (Payn *et al.*, 2005). One set of stepwise additions was conducted on each

stream, and we assume that no change in NH₄⁺ mineralization occurred during the experiment. The concentration of Br⁻ tracer was monitored at the bottom of the reach using an ion selective electrode, and the addition of the solute was continued at a constant rate until the concentration of the reached plateau (increased at <1% each 5 min). Once the Br⁻ concentration reached plateau (usually after 1.5 h), water samples were taken every 10 m along the length of the reach (80 both streams, eight sampling stations per stream). After each sampling, the concentration of NH₄⁺ added to the stream was increased and the stream was sampled again after the period of time required to reach plateau.

Cross-sectional transects were used to measure water width and depth, and percentage coverage of the selected substrata types along the study reach. Transects were conducted every 5 m along a 100 m that encompassed the experimental reaches to measure wetted width, habitat and benthic biota type (10 points per transect) in the stream ($n = 20$ transects or 200 points per stream). Average stream depth was measured every 10 m using similar transects ($n = 10$ transects or 100 points per stream).

Water chemistry samples were returned to the laboratory in a cooler, filtered immediately with 47-mm Whatman GF/F glass fibre filters (Whatman International Ltd., Maidstone, England), and frozen until analysis. The NH₄⁺ concentration of each sample was measured colorimetrically using the phenol-hypochlorite method on a Hitachi U-2000 spectrophotometer (Hitachi Ltd. Tokyo, Japan) equipped with a 5-cm cell (APHA, 1995). The Br⁻ tracer concentration of each sample was measured with an Orion ion-specific electrode, with all samples measured at the same temperature as the standards and stirred at the same rate.

Ammonium concentration for each sample (C) was corrected for background NH₄⁺ concentration (C_0) and dilution by correcting for the change in Br⁻ concentration. The slope of the ln-transformed concentration of added NH₄⁺ over distance (k_x) was calculated by linear regression.

Parameters of nutrient retention were calculated for each addition using the k_x coefficient experiment, as outlined by the Stream Solute Workshop (1990). Uptake length (S_w) for each experiment was defined as the negative inverse of the linear uptake coefficient ($S_w = -1/k_x$). The NH₄⁺ flux (F_w) for each addition was calculated as the stream discharge multiplied by

the plateau NH_4^+ concentration. The area-weighted rate of N uptake (U_t) was calculated from S_w , F_w and stream width (w):

$$U_t = \frac{F_w}{S_w \times w} \quad (4)$$

Prediction of U_t at ambient NH_4^+ concentrations for each stream was based on the relationship between U_t and NH_4^+ concentration established by the stepwise short-term NH_4^+ addition experiments. Regression equations for the Michaelis–Menten model were based on the pooled data from the north and south branches. Estimates of U_t at ambient conditions were made by solving the regression equations with the C_0 from each stream. Additional estimates of S_w at ambient concentrations were made using the S_w projection method of Payn *et al.* (2005). In this method, linear regression is used to establish a relationship between S_w and the added NH_4^+ concentration ($C - C_0$). From this relationship, projected S_w was calculated for each stream by entering negative ($-1 \times C_0$) ambient NH_4^+ concentration into the regression equation. The resulting S_w was used to calculate U_t using eqn 1.

Statistics

The Michaelis–Menten model was fit to U_t and C by using least squares (LS) regression with the Levenberg–Marquardt estimation algorithm, and 95% CI for ambient U_t based on the variance associated with this model. The relationship between S_w and added NH_4^+ concentration was fit using linear regression and 95% CI for ambient S_w were calculated based on the variance associated with this model. Differences in NH_4^+ uptake coefficients, as well as mineralization rates, between substrata types were tested by using one-way factorial ANOVA with Tukey HSD contrasts. Differences between substrata-specific uptake and mineralization rates were tested by using an ANOVA with LS means contrasts. All statistical analyses were performed using the STATISTICA 6 (Statsoft, Tulsa, OK, U.S.A.) statistical package.

Results

Substrata-specific uptake and mineralization

Chamber experiments produced K_t that ranged from 0.0027 min^{-1} in the south branch pool epilithon

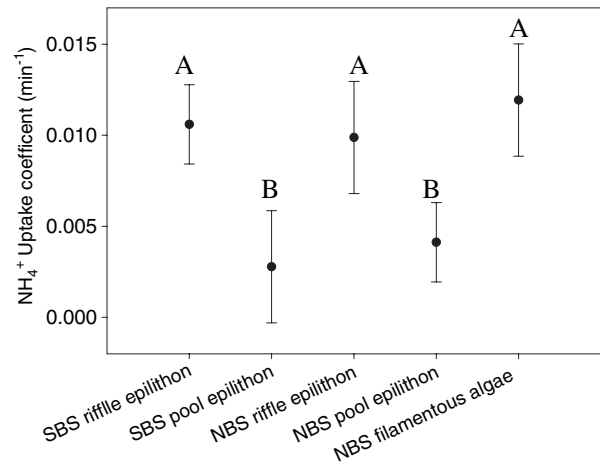


Fig. 2 NH_4^+ uptake coefficients from chamber experiments by stream and substrata type. Letters represent statistical difference between substrata types using Tukey HSD at an $\alpha = 0.05$. Error bars indicate 95% CI.

samples to 0.012 min^{-1} in the filamentous algae samples (Fig. 2). Based on the uptake coefficients, substrata-specific uptake rates at ambient stream NH_4^+ ranged from $0.042 \mu\text{g N m}^{-2} \text{ s}^{-1}$ in the south branch pool epilithon samples to $0.12 \mu\text{g N m}^{-2} \text{ s}^{-1}$ in the filamentous algae samples. Mineralization rates ranged from $0.08 \mu\text{g N m}^{-2} \text{ s}^{-1}$ in NBS pool epilithon to $0.51 \mu\text{g N m}^{-2} \text{ s}^{-1}$ in south branch riffle epilithon (Fig. 3).

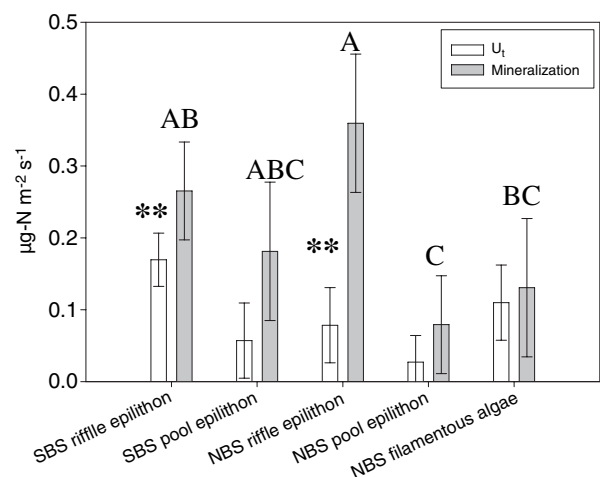


Fig. 3 NH_4^+ uptake and mineralization rates from chamber experiments by stream and substrata type. Letters represent statistical difference in mineralization rate between substrata types using Tukey HSD at an $\alpha = 0.05$. Asterisks represent statistical difference between uptake and mineralization rates at an $\alpha = 0.01$. Error bars indicate 95% CI.

Uptake coefficients for riffle epilithon, from both north and south branches, and filamentous algae were significantly higher (ANOVA, $F_{1,8} = 12.0$, $P = 0.001$) than pool epilithon from both branch stream (Fig. 2). Significant differences were also found among the substrata-specific mineralization rates (ANOVA, $F_{1,8} = 9.40$, $P = 0.003$), with riffle epilithon tending to have higher mineralization rates than in the pool epilithon (Fig. 3). Mineralization rates were significantly greater than uptake rates in riffle epilithon at both south branch ($P < 0.001$) and NBS ($P = 0.002$). Uptake and mineralization were not significantly different from each other in the pool epilithon or filamentous algae.

Habitat-specific uptake

We used uptake rates from the chamber experiments to calculate habitat-specific uptake rates and uptake lengths. Riffle habitats had higher U_t (0.11 and 0.22 $\mu\text{g N m}^{-2} \text{s}^{-1}$ for north branch and SBS, respectively) than the habitat-specific U_t measured in pools (0.04 and 0.06 $\mu\text{g N m}^{-2} \text{s}^{-1}$ for north branch and SBS, respectively). The higher U_t in the riffles resulted in a shorter, habitat-specific S_w (24 and 27 m for north branch and SBS, respectively) than in pools (75 and 108 m, respectively) and the whole-stream average S_w (42.9 and 44.9 m, respectively).

Whole-stream NH_4^+ uptake kinetics

The short-term NH_4^+ addition experiments yielded plateau NH_4^+ concentrations of 5.6, 10.0, 22.3 and 63.6 $\mu\text{g L}^{-1}$ $\text{NH}_4^+\text{-N}$ at SBS and 10.0, 19.5, 35.5 and 39.4 $\mu\text{g L}^{-1}$ $\text{NH}_4^+\text{-N}$ at NBS. Uptake lengths ranged from 37 to 135 m at SBS and ranged from 54 to 171 m at NBS, and corresponded to the lowest and highest plateau NH_4^+ concentrations at each stream respectively. Uptake rates ranged from 0.36 to 1.15 $\mu\text{g N m}^{-2} \text{s}^{-1}$ at SBS and ranged from 0.65 to 1.18 $\mu\text{g N m}^{-2} \text{s}^{-1}$, and again corresponded to the lowest and highest plateau NH_4^+ concentrations at each stream respectively.

Given similarities between the two streams in terms of biological and physical characteristics, NH_4^+ retention data were pooled to establish a relationship between uptake and NH_4^+ concentration. There was a significant fit for the Michaelis–Menten model ($R^2 = 0.69$, $F_{1,7} = 69.6$, $P < 0.001$) (Fig. 4), with a V_{max} of

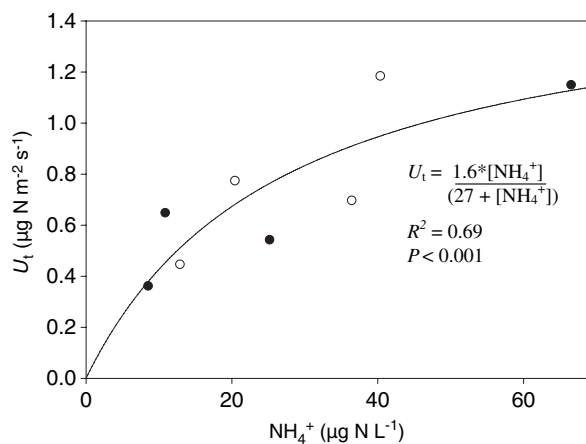


Fig. 4 Relationship between NH_4^+ concentration and N uptake rate (U_t) in north branch stream (\circ) and south branch stream (\bullet) from short-term NH_4^+ addition experiments approximates a Michaelis–Menten model.

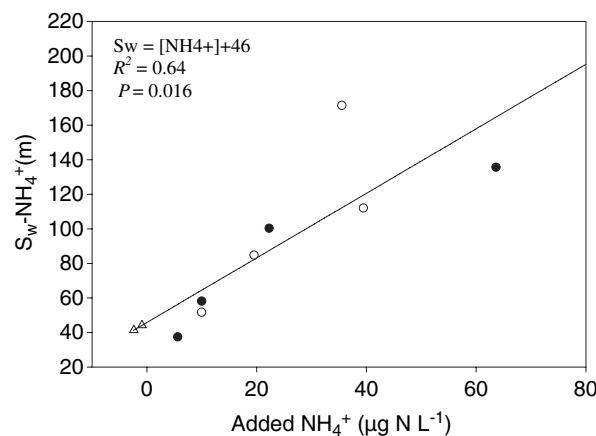


Fig. 5 Prediction of ambient NH_4^+ uptake length (S_w) from north branch stream (\triangle) and south branch stream (\blacktriangle), based on linear regression of short-term NH_4^+ addition experiments at both north branch (\circ) and south branch (\bullet) of Kings Creek.

1.56 $\mu\text{g N m}^{-2} \text{s}^{-1}$ and a K_s of 27.0 $\mu\text{g N L}^{-1}$. A significant linear regression was found between S_w and NH_4^+ concentration for the pooled north and south branches short-term addition experiments ($R^2 = 0.64$, $F_{1,7} = 10.89$, $P = 0.016$) (Fig. 5). According to the S_w projection method, ambient S_w was 41.4 m for SBS and 44.2 m for NBS.

Ambient NH_4^+ uptake rates

From the stepwise short-term NH_4^+ addition experiments, we calculated U_t at ambient concentration to

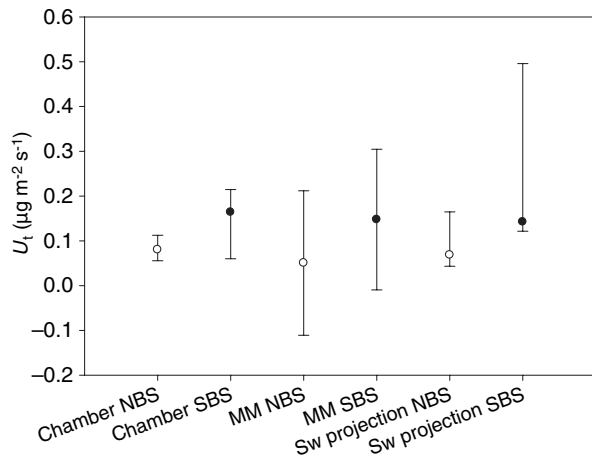


Fig. 6 A comparison of predicted ambient uptake rates from the results of the chamber experiments and from short-term nutrient addition experiments (Michaelis–Menten and S_w projection) for north branch stream (○) and south branch stream (●). Error bars indicate 95% CI, confidence intervals for S_w projection are of unequal variance due to the conversion between U_t and S_w .

be $0.15 \mu\text{g N m}^{-2} \text{s}^{-1}$ for south branch and $0.03 \mu\text{g N m}^{-2} \text{s}^{-1}$ for NBS, and similarly using the Michaelis–Menten model. Similarly, ambient U_t was $0.14 \mu\text{g N m}^{-2} \text{s}^{-1}$ for south branch and $0.04 \mu\text{g N m}^{-2} \text{s}^{-1}$ for NBS based on the S_w projections. When the scaled up to the entire reach, the substrata-specific ambient gross uptake rates were $0.14 \mu\text{g N m}^{-2} \text{s}^{-1}$ for south branch and $0.08 \mu\text{g N m}^{-2} \text{s}^{-1}$ for NBS. These data indicate that both approaches produce comparable results (Fig. 6).

Discussion

Rates of mineralization and uptake

The results of the substrata scale experiments indicate that mineralization rates were higher than uptake rates in stream conditions in all of the epilithic biofilms because base NH_4^+ concentrations in the chambers were higher than NH_4^+ concentrations in the stream. Uptake rates were greater than mineralization rates only in the filamentous green algae. Our data also indicate that rates of mineralization were greater for riffle epilithon than pool epilithon. These findings are counter to common assumptions that pools will have greater respiration and mineralization because of greater deposition of organic matter.

Rates of mineralization measured in this study are much higher than those estimated for Kings Creek by

Peterson *et al.* (2001). Ammonium regeneration rates of $0.0046 \mu\text{g m}^{-2} \text{s}^{-1}$ were considered maximal for Kings Creek, based on $^{15}\text{NH}_4^+$ leaving the stream after the conclusion of an extended ^{15}N enrichment experiment. However, Peterson *et al.* (2001) noted that their Kings Creek estimate could have been inaccurate because of a low level of isotopic enrichment found in the NH_4^+ pool. Our rates of mineralization, however, do fall within the range of rates presented by the Peterson *et al.* (2001) study in other streams where a more significant labelling signal was obtained.

The results of the substrata scale experiments suggest that a net increase in NH_4^+ concentrations should occur in the water of both stream reaches as it moved downstream. A net increase in NH_4^+ concentration was observed at NBS, but no such increase was observed at south branch (Fig. 7). Most of the net mineralization observed in the ambient NH_4^+ concentration at NBS (90% of the variation in NH_4^+ concentration) can be explained by differences in uptake and mineralization rates from the chamber experiments and by dilution from incoming ground water. Based on previous samples of hyporheic water and samples from nearby springs, NH_4^+ of the groundwater is assumed to be at or below the detection limit. Conversely, the difference between measured uptake and mineralization rates could not explain the variation in ambient NH_4^+ in SBS. Other processes must therefore be compensating for the additional NH_4^+ generated by the net mineralization of epilithic biofilms in this stream. A possible explanation is that the biota types may have accounted for hot-spots of mineralization (due to rapid N turnover within the biofilm) while missing biomass compartments, with similar rates of uptake to those in the chamber experiments but with lower rates of N turnover and mineralization [e.g. leaves or fine benthic organic matter (FBOM)] (Dodds *et al.*, 2004).

Habitat-specific uptake

We found that riffles were more active than pools in terms of both uptake and mineralization, resulting in shorter uptake lengths in this habitat type. This phenomenon is evident in several of the low-level NH_4^+ addition experiments at NBS because of a large pool in the centre of the reach (Fig. 7). Estimates of habitat-specific S_w , generated from the short-term addition experiment, indicate that the uptake length

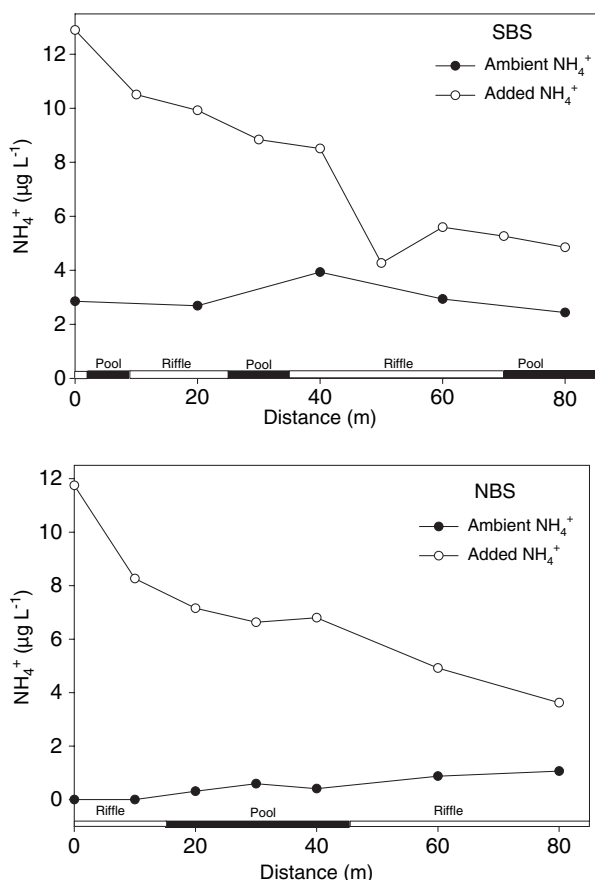


Fig. 7 Variation in NH_4^+ concentration along the stream reaches at south branch stream (SBS) and north branch stream (NBS) at ambient conditions (●) and at plateau conditions (○) during the short-term addition at constant rate. The ambient ammonium concentration at SBS did not show a net change with distance at ambient concentrations and showed no effects of pools in the experimental concentration. Ambient NH_4^+ concentrations at NBS demonstrate net mineralization with distance, and a reduction in net uptake in a large pool (15–45 m) during an experimental NH_4^+ addition.

in the pool segment (394 m) is 10× that in the riffles that precede (38 m) and follow it (40 m); however, there are too few points to statistically test a difference in slopes. The estimated pool and riffle-specific S_w are longer than those of the predicted by chamber experiments as a result of the effect of elevated NH_4^+ concentrations in the short-term addition experiment. The influence of the pools and riffles is obscured in the south branch reach, because of the lack of the spatial resolution in the sampling design as well as the relative size of the pools. The south branch reach lacked a pool large enough to encompass multiple sample points and demonstrate an effect of the pool environment.

The uptake length measured over a stream reach is dependent on the sizes and ratios of its pools and riffles. For expediency, researchers often avoid large pools when conducting whole-stream addition experiments, because they have very long travel and residence times. By avoiding pools, however, researchers may be missing an important part of the stream and biasing the results of their study when their estimates are scaled to the whole stream.

Uptake lengths of other nutrients, such as NO_3^- and PO_4^{3-} , can differ with stream habitats, with higher velocity habitats such as rock outcrops having shorter uptake lengths (Munn & Meyer, 1990). Riffles would be expected to be more active for uptake than pools for several reasons: (i) higher water velocity in riffles may increase transport across the diffusion boundary layer (Dodds & Biggs, 2002); (ii) algal photosynthesis may be stimulated by greater light penetration in shallower habitats and (iii) increased grazers in riffle habitats may increase uptake through maintenance of thin, metabolically active biofilms.

Whole-stream NH_4^+ uptake kinetics

Ammonium uptake rates demonstrated Michaelis-Menten saturation in response to acute NH_4^+ enrichment. The data suggest that organisms in Kings Creek were exhibiting N limitation, because ambient concentrations of NH_4^+ were much less than the value of K_s (Tilman, 1982). The degree of limitation can also be inferred from the ambient uptake relative to total potential uptake (V_{max}). In N-saturated systems, ambient U_t would be expected to be approximately equal to V_{max} . Ambient U_t averaged only 6% of V_{max} for NH_4^+ both streams. Thus, the capacity for N uptake is much greater than the actual rate of assimilation, indicating a strong degree of limitation. This N limitation is consistent with previous nutrient releasing bioassay data on this stream system (Tank & Dodds, 2003).

Relatively few studies have directly measured whole-stream uptake kinetics. Dodds *et al.* (2002) reported a half-saturation coefficient of 64 NH_4^+ -N $\mu\text{g L}^{-1}$ for a prairie reach of Kings Creek. Payn *et al.* (2005) reported half-saturation coefficients in forested streams of 6 $\mu\text{g NH}_4^+$ -N L^{-1} for Ball Creek, North Carolina, and 14 $\mu\text{g NH}_4^+$ -N L^{-1} for Walker Branch, Tennessee. Studies of uptake kinetics of N in benthic organisms yielded similar half saturation coefficients. Lohman & Priscu (1992) measured NH_4^+

uptake kinetics in *Cladophora*, finding half-saturation coefficients of 7.3–15.2 $\mu\text{g L}^{-1}$ for NO_3^- and 17.4–41.9 $\mu\text{g L}^{-1}$ for NH_4^+ . Kemp & Dodds (2002b) characterized uptake kinetics of different substratum types and, based on these, projected uptake kinetics for NH_4^+ ($V_{\text{max}} = 15.8 \mu\text{g m}^{-2} \text{s}^{-1}$ and $K_s = 6.7 \mu\text{g L}^{-1}$) for the whole stream. The half saturation coefficients for NH_4^+ reported in our study fall within the range of those reported in the literature.

Comparison of methods

The results of this study indicate that whole-stream ambient U_t predicted by the chamber experiments was not significantly different than projections made by short-term nutrient addition experiments. Additionally, an extended (4 week) $^{15}\text{NH}_4^+$ experiment on the SBS, conducted in the spring of 1998, found uptake rates ranging from 0.014 to 0.7 $\mu\text{g N m}^{-2} \text{s}^{-1}$ (Dodds *et al.*, 2000, 2002). These rates bracket the ambient uptake rates from the chamber and short-term NH_4^+ addition experiments in the current study.

There are inherent difficulties in scaling small-scale measurements, such as the results of the chamber experiments, to the whole aquatic ecosystem (Schindler, 1998). It seems, however, that our selection of biomass compartments included the most important or representative components in the stream. Cobbles collected from the stream represented filamentous algae, epilithic diatoms and a fraction of the FBOM components of the stream substrata, which were found to be the most active in terms of NH_4^+ uptake, by Kemp & Dodds (2002b). Epilithic biofilms and filamentous algae biota were in close physical contact with overlying stream water, were best positioned to have an immediate impact on stream NH_4^+ concentration, and most probably had the largest impact on short-term NH_4^+ releases.

The advantage of using whole substrata within recirculating chambers, as opposed to homogenized samples, is that they probably better mimic conditions in the stream. These chambers provide a better simulation of water flow and maintenance of diffusion boundary layers and turbulent mass transport than other methods. Bottle incubation methods, as used by Kemp & Dodds (2002b), involve the disruption of stream biota and a change in the availability of nutrients in stream water relative to the biota, which may alter results.

The disadvantage of the chamber method is that it relies on many of the same assumptions of single-nutrient addition experiment, by drastically altering the nutrient concentration and relying on a first order model to describe the resulting decline in concentration (rather than a Michaelis–Menten model). The chamber method may, therefore, produce uptake rates that are higher than those occurring in the stream at ambient concentrations (Steinman & Mulholland, 1996). However, the spike concentration used in this study was less than the K_s observed in the stream (and thus within the approximately linear portion of the curve), which may have mitigated this concern. This method will be most accurate when minimal spike concentrations are used.

The advantage of our experiments over isotopic methods is that they are easy to conduct and relatively inexpensive. The simplicity of these methods allows them to be available to a wider research community, as opposed to isotopic methods that are available to fewer institutions.

In summary, the results of the short-term NH_4^+ addition experiments indicate that uptake saturates in these streams in response increases in concentration, suggesting that stream biota are N limited at ambient concentrations. Mineralization associated with the epilithic biofilms was greater than the rate of uptake at ambient stream concentrations, which is probably because of the rapid turnover of N. These data also suggest that uptake in streams changes varies along the alternating pattern of pools and riffles, with riffles taking up NH_4^+ at a higher rate. Both the whole-stream addition experiments and chamber methods used in this study yielded comparable results for estimating the ambient rate of NH_4^+ uptake in these streams.

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