



Nitrogen cycling and metabolism in the thalweg of a prairie river

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[1] Nutrient dynamics in rivers are central to global biogeochemistry. We measured ammonium (NH_4^+) uptake, metabolism, nitrification, and denitrification in the thalweg, the river region of greatest flow, of the Kansas River (discharge = 14,360 L/s). We estimated gross and net uptake with a depleted ^{15}N - NH_4^+ release, metabolism with diel O_2 measurements, and denitrification with dissolved N_2 measurements. Net ecosystem production was negative. Net NH_4^+ uptake length was 2.1 km when concentrations were elevated, and gross uptake length was 1.9 km at ambient concentrations. Gross uptake rate measurements were comparable to estimates made using extrapolations from data obtained from streams (systems with 1/10th or less the discharge). Calculated lengths were maximal because the isotope pulse was primarily confined to the thalweg and not the shallow side channels or backwaters. Denitrification and nitrification rates were below detection. In the Kansas River, rates of N cycling are driven by heterotrophic processes, and considerable processing of N, particularly NH_4^+ uptake, occurred over a few kilometers of river length, with net uptake rates of NH_4^+ increasing with greater NH_4^+ concentrations.

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1. Introduction

[2] Nitrogen (N) cycling and responses to human-caused N loading are central areas of research in ecosystem biogeochemical sciences. Nitrogen is a key limiting element in lotic waters [Dodds et al., 2002a; Tank and Dodds, 2003], and aquatic N pollution is a global concern [e.g., Rabalais, 2002; Smith, 2003]. Lotic waters connect land to downstream waters, so understanding the influence of these habitats on N retention and transformation is required to characterize large-scale transport of N [Bernot and Dodds, 2005].

[3] Efforts to quantify areas of the landscape responsible for N retention indicate that substantial N is retained by streams and small (<1 m mean depth) rivers [Darracq and

Destouni, 2005] before reaching large rivers, such as the Mississippi [Alexander et al., 2000]. Given the propensity of some biogeochemists to view rivers and streams as transport conduits, this unaccounted for N retention could be considered as “missing nitrogen.”

[4] Lotic biogeochemists have begun to characterize nutrient uptake and retention by using isotope tracer methods in small streams (mostly with discharge < 10 L/s) across drainage networks [Peterson et al., 2001; O'Brien et al., 2007; Mulholland et al., 2008]. Direct measures show small streams retain a substantial mass of N, transport length of inorganic N is a particularly important metric of retention, and ammonium (NH_4^+) is a central pool for N transformations [Peterson et al., 2001]. However, the largest of the lotic waters used by Peterson et al. [2001] had a maximum discharge of 800 L/s and a maximum N flux of 26 mg/s (Little Miami River). Tracer studies of NH_4^+ dynamics have not been published for greater fluxes or discharges.

[5] Mass balance models suggest that moderate sized rivers can also retain substantial amounts of N [Alexander et al., 2000; Darracq and Destouni, 2005; Wollheim et al., 2006], as do experimental pulsed increases in background concentration that can measure net, but not gross uptake [Tank et al., 2008]. In general mass balance models assume that areal N uptake rates in rivers and small streams are identical. This assumption is not based on a mechanistic understanding of nutrient uptake processes, but rather stems from the fact that N tracer studies have only been conducted in small streams and few data are available from rivers (except see Wollheim et al. [2001]). If systematic differences in areal N uptake exist between rivers and streams, then

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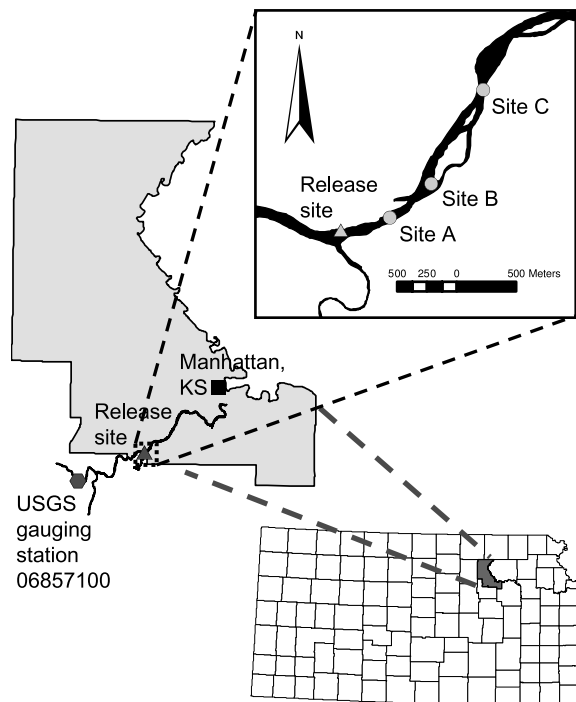


Figure 1. Location of study site in Kansas, United States, and map of locations of release and sampling sites on the Kansas River.

current models will not accurately represent patterns in N uptake across drainage networks.

[6] Differences in areal uptake rates among rivers and streams may result from numerous factors including differences in sediment composition and hyporheic zone development. Rivers tend to be of lower slope than headwater streams and consequently have smaller sized particles on the bed. Smaller particle size can translate to lower-sediment hydraulic conductivity, decreased subsurface flow, and a less developed hyporheic zone. Since hyporheic zones are well known biogeochemical hot spots [Mulholland *et al.*, 1997], reduced hyporheic development in rivers may result in lower areal N uptake rates. Furthermore, decreased exchange of surface and subsurface waters in sediments may promote the development of sediment anoxia and anoxic microbial metabolism with implications for areal uptake rates [Jones and Mulholland, 1998].

[7] Nutrient uptake processes in small streams are dominated by benthic metabolism because of the high ratio of benthic surface area to water column volume. However, this ratio is smaller in rivers and N processing by plankton within the water column may assume a greater importance. These processes could lead to elevated areal N uptake rates in rivers relative to small streams.

[8] Limited mechanistic information is available for biogeochemical characteristics of rivers, making it difficult to predict whether areal N uptake rates in rivers differ from small streams. Research on the ecology and biogeochemistry of rivers is scant because of technical difficulties associated with sampling large bodies of flowing water. A few whole river denitrification rates have been estimated using N budgeting approaches [Hill, 1979; Sjodin *et al.*, 1997], but this method requires the measurement of multiple

forms of N and estimates of nitrification and ammonification rates. The accumulation of small measurement errors can lead to poor denitrification estimates. Furthermore, the temporary storage of N by biological uptake can affect estimates of denitrification using a budget approach. Natural abundance of stable isotopes has been used to estimate nitrification and denitrification in the Seine River [Sebilo *et al.*, 2006]; however, this method does not detect benthic denitrification when the diffusion of nitrate from the water column into the benthos is the rate limiting step [Sebilo *et al.*, 2003; Lehmann *et al.*, 2003]. Some recent methodological advances allow for better characterization of river N cycling. Stable isotope experiments can assess in situ N cycling rates in aquatic habitats including entire streams [Webster *et al.*, 2003; Mulholland *et al.*, 2004], but these methods are prohibitively expensive to apply to rivers. Whole river denitrification rates can be estimated from patterns of dissolved dinitrogen (N_2) gases [e.g., Laursen and Seitzinger, 2004; Pribyl *et al.*, 2005], but no practical method has been published to estimate nitrification rates (water column and benthic) at the whole river scale.

[9] Our aim was to establish rates of biogeochemical N cycling in a central plains river using a novel $^{15}NH_4^+$ isotope depletion tracer technique to directly measure uptake and a whole system metabolism technique to estimate N demand. We explored NH_4^+ uptake by abiotic adsorption to particulates with a laboratory isotope tracer experiment. Measurements were made on a river with a discharge 16 times greater and an N flux 3.5 times greater than the largest published in prior studies on whole stream ambient (gross) NH_4^+ uptake rates in one publication [Peterson *et al.*, 2001]. One tracer study in the Arctic has been done on a river with about twice the discharge, but lower ammonium concentrations, so comparable ammonium flux rates [Wollheim *et al.*, 2001]. We present a novel technique of estimating N transformation rates (net and gross) that allows us to deal with the challenges of working in a system with substantially greater discharge than typically studied. Rivers may or may not lie within the considerable range of N transformation rates described for streams. Thus, we hypothesized that rates of cycling per unit area would fall within the range of measurements made in smaller streams (commonly 1–800 L/s discharge) with comparable water column chemistry, but that the greater discharge of the river as compared to the streams would lead to relatively longer processing distances.

2. Methods

2.1. Site Description, Isotope Release, and Sampling

[10] The research site was located on the Kansas River, Kansas, United States (Figure 1), a large prairie river. Discharge was at base flow (14,360 L/s) at the time of study. Average discharge at the site from 1966 to 2004 was 74,905 L/s. The river is mostly sand bottom and slightly braided over the study reach. Prior seasonal monitoring of this site indicated a mean of 0.5 g/L of suspended particulate material [Whiles and Dodds, 2002], relatively turbid compared to many other streams and rivers in the United States [Dodds and Whiles, 2004]. The average width was determined with 30 evenly spaced transects along the 2 km

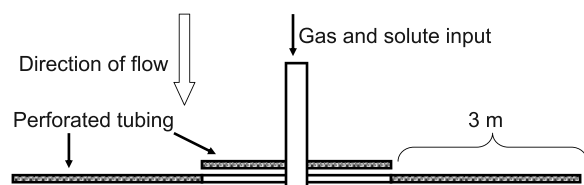


Figure 2. Schematic of pipe setup to release solutes and gasses into the river and allow even dispersion across the channel (top view).

study reach, with 10 depth measurements made at each width transect.

[11] The release point for our Lagrangian tracer release (slug injection) was 0.5 km above the first sampling point (Station A), 1 km above the second (Station B), and 2 km above the third (Station C; Figure 1). In order to maximize mixing of the release solution within the thalweg of the river, the release point was positioned where the channel was relatively narrow and confined.

[12] We used a ^{15}N depletion technique to characterize NH_4^+ dynamics. While this approach is somewhat less sensitive than the ^{15}N enrichment techniques and leads to a greater increase in NH_4^+ concentrations, it still allows for assessment of gross uptake at ambient concentrations. The method consists of releasing a plume of $(^{14}\text{NH}_4)_2\text{SO}_4$ and an inert dye and sampling as the plume moves down the river. The NH_4^+ is extracted from samples for isotopic composition and the samples are analyzed for inert dye concentrations. The dilution of the $^{14}\text{NH}_4^+$ by $^{15}\text{NH}_4^+$ over distance, in excess of that indicated by dilution of the inert dye, coupled with the change in total NH_4^+ concentration can be used to calculate the gross and net uptake (see section 2.4).

[13] The release solution was composed of 2 L of fluorescein (Ben Meadows FLT Yellow/Green) and 1.645 L of $(^{14}\text{NH}_4)_2\text{SO}_4$ (40 wt. % in H_2O , 99.99 atom % ^{14}N , Isotec/Aldrich). The solution was pumped into the river starting at 10:50 on 19 October 2005 at a rate of 0.54 L/min. All 3.645 L of solution were released over a 6.8 min period. The tracer gas, sulfur hexafluoride (SF_6), was simultaneously injected to estimate reaeration rates of O_2 [Wanninkhof *et al.*, 1990] and was released at 2 L/min by bubbling through two large airstones ($30 \times 3.5 \times 3.5$ cm each, S526–12 alumina air stones Keetonaqua, Wellington, Colorado, United States).

[14] Both the gas and the solution were released through a 10 cm inner diameter polyvinyl chloride tube-T with two extended 3.5 cm inner diameter cross pieces (Figure 2). The T was held in place approximately 0.30 m under water with metal fence posts driven into the river bottom. The tubing for the release solution (carrying dye and ^{15}N depleted NH_4^+) and both air stones were placed into the stem of the T. This release technique ensured that the gas and the tracer solutions were dispersed across the rapidly flowing section of the channel and released simultaneously (i.e., that the dye could be used to calculate dilution of the dissolved gas tracer).

[15] Sampling teams were positioned downstream at each station, and baseline samples were collected before the release solution reached the sampling stations. As the fluorescein dye approached, teams began taking alternating

samples of water (in 1 L acid washed containers) and gas (SF_6) approximately every minute at the top stations (A and B) and every two minutes at the lowermost station (C).

[16] The SF_6 samples were collected from below the surface using syringes. Care was taken not to include bubbles in the samples or cause degassing by taking the samples too rapidly. A needle was placed on the end of the syringe, and water was expelled to displace the gas in the needle. Then, 7 mL of river water were added to a 10 mL evacuated Exetainer (Labco Limited), and samples subsequently stored at 4°C upside down in 50 mL centrifuge tubes filled with water to minimize gas exchange with the atmosphere. This method has been shown to lead to minimal leakage over several months (S. K. Hamilton, personal communication, 2004).

[17] Water samples were filtered in the laboratory within 4 h of sampling with Whatman GF/F filters (glass fiber filters with $0.7 \mu\text{m}$ nominal retention). One set of subsamples was stored frozen until analysis of NH_4^+ , and a second set was stored at 4°C prior to determination of fluorescein concentration. Several sequential (2–4) samples were pooled to provide enough volume (about 2 L) for extraction of NH_4^+ for isotope analysis. We measured fluorescein in the pooled sample to correct for dilution specific to that entire sample.

[18] Water samples were collected from the river with a 60 mL syringe and transferred to glass tubes, with care taken not to include air bubbles, to assess N_2 mass by membrane inlet mass spectrometry (MIMS). Syringes were fit with a small extension tube to allow sample tubes to be filled from the bottom. Six replicate sample tubes (15 mL: Chemglass) were gently filled from the bottom and allowed to overflow with 3 volumes. Then 200 μL of 50% ZnCl_2 were added as a preservative, and the ground glass stopper was placed with care taken not to include any air bubbles. The samples were stored at 4°C (to avoid bubble degassing) and under water (for temperature stability and to minimize the potential for atmospheric contamination) until analysis. Any samples that contained bubbles were not analyzed. MIMS gas samples were taken at sites A and C at 14:33 on 18 October 2005 and at 8:00 and 13:30 on 19 October 2005. The last samples were taken immediately after the pulse of isotopically depleted $^{14}\text{NH}_4\text{SO}_4$ passed the lower station.

[19] Temperature and dissolved O_2 were measured every 5 min for the 24 h period bracketing the time of the experiment. The data were taken with duplicate sondes (one Yellow Springs Instruments and one Hydrolab (calibrated immediately before deployment)) positioned at station C to provide redundant data on determination of metabolism as calculated by the one-station metabolism method [Bott, 1996].

2.2. Sample Analyses

[20] Samples were analyzed for NH_4^+ concentration with the spectrophotometric phenol-hypochlorite method using a 5 cm cuvette, 0.095 $\mu\text{mol/L}$ detection limit [American Public Health Association, 1995]. Fluorometry was used to determine relative concentration of fluorescein as the signal of the peak at 440 nm excitation and 535 nm emission. $^{15}\text{N}/^{14}\text{N}$ analysis was done by applying the diffusion method [Holmes *et al.*, 1998], modified as described by O'Brien *et al.* [2007] to concentrate NH_4^+ from combined 2 L samples

Table 1. Variables and Units Used in This Paper

| Variable | Symbol | Units |
|--------------------------------------|--------------------|--|
| Time | T | min |
| Distance between sampling sites | X | m |
| Nitrogen concentration at time t | N_t | mmol m^{-3} |
| Nitrogen concentration at Distance x | N_x | mmol m^{-3} |
| Nitrogen in pulse | N_{pulse} | mmol m^{-3} |
| Background nitrogen | N_{bkd} | mmol m^{-3} |
| Dissolved oxygen concentration | O | mmol m^{-3} |
| Dissolved N_2 gas concentration | N_2 | mmol m^{-3} |
| Gas flux rate aeration | F_{aer} | $\text{mmol m}^{-2} \text{min}^{-1}$ |
| Gas flux rate concentration | F_{conc} | $\text{mmol m}^{-2} \text{min}^{-1}$ |
| Rate constant per distance | k_x | m^{-1} |
| Rate constant per time | k_t | min^{-1} |
| Dilution constant per time | $k_{t,d}$ | min^{-1} |
| Uptake constant per time | $k_{t,u}$ | min^{-1} |
| Inert solute concentration | S | Fluorescence units |
| ^{14}N concentration | ^{14}N | $\mu\text{mol L}^{-1}$ |
| ^{15}N concentration | ^{15}N | $\mu\text{mol L}^{-1}$ |
| Isotope atomic ratio | R | mole mol^{-1} |
| Gross uptake per unit area | U_{gross} | $\mu\text{mol m}^{-2} \text{min}^{-1}$ |
| Net uptake per unit area | U_{net} | $\mu\text{mol m}^{-2} \text{min}^{-1}$ |
| Mineralization, per volume | M_v | $\mu\text{mol m}^{-3} \text{min}^{-1}$ |
| Mineralization, per area | M_a | $\mu\text{mol m}^{-2} \text{min}^{-1}$ |
| Discharge | Q | $\text{m}^3 \text{min}^{-1}$ |
| Depth | D | m |
| Width | W | m |
| Velocity | V | m/min |
| Respiration of ecosystem | R_{eco} | $\text{mmol m}^{-2} \text{min}^{-1}$ |
| Gross primary production | GPP | $\text{mmol m}^{-2} \text{min}^{-1}$ |
| Net ecosystem production | NEP | $\text{mmol m}^{-2} \text{min}^{-1}$ |

onto a filter. In brief, the solution is made basic, an acidified filter encased in Teflon tape is added to the bottle, and it is sealed tightly. The basic environment converts the NH_4^+ in the river water to NH_3 , which then diffuses across the Teflon tape and is converted back to NH_4^+ on the acidified filter. This process was allowed to occur for 3 weeks on a shaker table, after which the filter pack was removed, dried and analyzed for $^{15}\text{N}/^{14}\text{N}$ on a continuous flow PDZ Europa “20-20” with a mass spectrometer coupled to an ANCA-SL elemental analyzer at the Woods Hole Stable Isotope Laboratory.

[21] We calculated nitrification rate by the rate of appearance of excess ^{14}N in the nitrate pool. Nitrate was isolated as described by Sigman *et al.* [1997]. Briefly, the NH_4^+ is removed by boiling the basic solution, and then NO_3^- is converted to NH_4^+ with Devarda’s alloy in sealed bottles at 60°C for 48 h. The NH_3 is then diffused onto acidic filters as described for analyses of NH_4^+ isotopes.

[22] Samples of SF_6 were analyzed on a gas chromatograph with an electron capture detector [Cole and Caraco, 1998] and SF_6 concentrations were corrected for dilution using the decline in fluorescein concentrations (see above). Distance between stations was used to calculate aeration. We converted k_{SF_6} to k_{O_2} on the basis of the ratio of their Schmidt numbers (1.4), following Wanninkhof *et al.* [1994].

[23] Membrane inlet mass spectrometry was used to determine concentrations of $^{28}\text{N}_2$, $^{29}\text{N}_2$, $^{30}\text{N}_2$ and $^{32}\text{O}_2$ [Kana *et al.*, 1994; An *et al.*, 2001]. Samples stored under water were shipped overnight to the University of Texas Marine Science Institute and analyzed immediately upon arrival. Three samples developed bubbles, and results from these samples were not used.

2.3. Abiotic Absorption Experiment

[24] We ran an experiment to calculate the relative contributions of abiotic and biotic rates of uptake of NH_4^+

associated with suspended river particles. Water was taken from the Kansas River on 21 September 2005 and returned to the laboratory. Replicate aliquots (60 mL in 125 mL Erlenmeyer flasks) of river water had either 2.4 mL of formalin solution adjusted to river pH (8.7) with borate or the same volume of deionized water added. We added 10 μL of 32 mg N L^{-1} $^{15}\text{NH}_4\text{Cl}$ solution to a target of 5,000 δ units, and flasks were shaken gently at 125 rpm. Replicate samples were removed at approximately 0, 2, 5, 10, 30, 60 and 120 min and filtered onto Whatman GF/F filters without washing (to avoid desorption of ions from the filtered particles). We used time zero samples to correct for isotope on the filter dissolved in the small amount of water retained on the moist filter, but not associated with particles. The time at which all visible water passed through the filter was recorded as the incubation stop time. Filters were dried and compressed into tin capsules, which were subsequently analyzed for ^{15}N content. Additional samples were also taken for analysis of suspended particle ash free dry mass [American Public Health Association, 1995].

2.4. Calculations and Statistics

[25] Fundamental relationships between stream uptake rates and physical characteristics of streams are described by Webster and Patten [1979]. The abbreviations and symbols used are given in Table 1 and were made following the equations used for calculations in Table 2. Standard equations for nutrient uptake and spiral lengths are taken from Stream Solute Workshop [1990] and Webster and Ehrman [1996]. The isotope calculations are specific to ^{15}N depletion experiments, but most are based upon existing isotopic enrichment calculations, and have been modified from the Blackburn-Caperon isotope dilution calculations [Blackburn, 1979; Caperon *et al.*, 1979] accounting for source averaging [Laws, 1984]. Gas flux calculations (metabolism and denitrification) are from Marzolf *et al.* [1994, 1998], Young and Huryn [1998], and Bott [1996].

[26] Hydrologic characteristics of the study reach were estimated by fitting a transient storage model [Bencala and Walters, 1983] to downstream observations of the released fluorescein using the OTIS-P software package [Runkel, 1998]. The OTIS-P model provided estimates of the dispersion coefficient (D), the cross-sectional area of the reach (A), the cross-sectional area of the storage zone (A_s), and the rate of exchange between the main channel and storage zone (α). From these parameters, A_s/A and F_{med} [Runkel, 2002], which describe the relative importance of the transient storage zone of the reach, were calculated. In addition, the reliability of the fitted parameters was assessed by calculating the experimental Damkohler number (Da) [Wagner and Harvey, 1997]. Finally, in order to calculate the amount of released tracer that was recovered at the downstream sites, the concentration time curves were integrated using the trapezoid method.

[27] Photosynthesis (GPP), aeration (k_t) and respiration (R_{eco}) were calculated by fitting the curve of dissolved O_2 over the 24 h prior to and encompassing the experiment. The model used light data from the nearby Konza Prairie Biological Station (<http://www.konza.ksu.edu/konza/datasets>) to fit R_{eco} , $k_{t,\text{oxygen}}$ and two parameters describing photosynthesis-irradiance relationships (maximum photo-

Table 2. Equations Used for Uptake and Gas Flux Calculations

| Equation | Comments |
|--|--|
| <i>Uptake Calculations</i> | |
| $k_t = v/k_x$ | relation between uptake per time and uptake constant per distance related to discharge |
| $U = k_t * d * C$ | calculation of uptake rate |
| $S_w = v/k_t$ | Spiral length |
| $dN/dx = (N_x - N_{x+1})/x$ | note that time can be related to distance with velocity |
| $U_{gross} = M + U_{net}$ | biological gross uptake combines net uptake and mineralization |
| $k_d = (\ln(S_{pulse,x} - S_{bkd}) - \ln(S_{pulse,x+1} - S_{bkd}))/x$ | calculate dilution constant from change in concentration of inert solute |
| $(N_{pulse \text{ from dilution},x+1} - N_{bkd}) = (N_{pulse,x} - N_{bkd}) e^{-(k_d * x)}$ | correct N in pulse for N lost to dilution |
| $k_u = (\ln(N_{pulse,x} - N_{bkd}) - \ln(N_{pulse,x+1} - N_{bkd} + N_{pulse \text{ from dilution}}))/x$ | calculate gross uptake constant from change in N pulse corrected for dilution |
| <i>Isotope Calculations</i> | |
| $R = {}^{15}N/{}^{14}N$ | isotopic molar ratio |
| $({}^{14}N_{pulse \text{ from dilution},x+1} - {}^{14}N_{bkd}) = ({}^{14}N_{pulse,x} - {}^{14}N_{bkd}) e^{-(k_d * x)}$ | correct ${}^{14}N$ concentration of pulse for dilution |
| $({}^{15}N_{pulse \text{ from dilution},x+1} - {}^{15}N_{bkd}) = ({}^{15}N_{pulse,x} - {}^{15}N_{bkd}) e^{-(k_d * x)}$ | correct ${}^{15}N$ concentration of pulse for dilution |
| $R_{x+1,dil \text{ corr}} = {}^{15}N/({}^{14}N_{pulse} + (({}^{14}N_{pulse,x} - {}^{14}N_{bkd}) e^{-(k_d * x)}))$ | correct expected isotope ratio for dilution (when pulse is ${}^{15}N$ depleted) |
| $M_v = \ln(R_{x+1,dil \text{ corr}}/R_x)/\ln(N_{x+1}/N_x) * (N_x - N_{x+1})/x * v * d$ | mineralization per volume based on change in dilution corrected isotope ratio (used for gross ammonium uptake) |
| $M_v = \ln(R_{x+1,dil \text{ corr}}/R_x) * (N_x)/t$ | mineralization per unit time based on change in isotope ratio with no change in base concentration (used to calculate nitrification) |
| <i>Gas Flux Calculations</i> | |
| $R_{aer} = SF_6/S$ | inert gas concentration scaled to inert solute concentration |
| $k_{x,gas} = (\ln(R_{aer \text{ station } 1}) - \ln(R_{aer \text{ station } 2}))/x$ | aeration rate corrected for dilution per unit distance |
| $F_{aer} = ((O_x - O_{sat}) e^{-(k_{x,oxygen})} - (O_x - O_{sat})) * v * d$ | oxygen or nitrogen flux per meter from aeration |
| $F_{conc} = (O_x - O_{x+1})/x * v * d$ | oxygen or nitrogen flux per meter from concentration change |
| $F = F_{aer} + F_{conc}$ | net gas flux rate for metabolism |

synthetic rate and the initial increase in photosynthetic rate, α) [Jassby and Platt, 1976; Dodds et al., 1999]. A similar modeling approach has been employed previously by Uehlinger et al. [2000]. In this model all rates are a function of temperature [Naegeli and Uehlinger, 1997]. The “solver” function in Microsoft Excel was used to vary α , maximum photosynthesis, R_{eco} and $k_{t,oxygen}$ to minimize the sum of squares of the difference between observed and modeled dissolved O_2 concentration over time.

[28] Differences among stations at baseline concentrations were tested using analysis of variance (ANOVA). Determination of which samples were above background levels was made by calculating the 95% confidence interval of all background samples.

3. Results

[29] The Kansas River is a broad, relatively shallow (Table 3) sandy bottom river typical of the Central Plains.

Over our study reach of 1.5 km (Figure 1), the travel time in the thalweg was estimated at 48 min, about twice the average velocity that was calculated from discharge and mean cross-sectional area (Table 3). Nighttime decreases in dissolved O_2 (Figure 3), indicating a diurnal pattern of metabolic activity, were accompanied by a significant increase in dissolved N_2 at the dawn sampling time as compared to the mid-day samples ($P < 0.001$). However, N_2 did not differ significantly between station A and station C ($P = 0.57$). These N_2 data suggested that denitrification rates were not great enough to lead to a significant difference in N_2 content between these stations.

[30] The two methods of determining aeration, modeling diurnal dissolved O_2 concentration and SF_6 loss from the water column, yielded estimates of reaeration (k_{O_2} at $20^\circ C$) of 11.9 and 7.6 d^{-1} respectively. These data suggest that measuring aeration with a gas tracer that was mostly confined to the thalweg was consistent with and applicable to metabolism calculations made with a single station

Table 3. Characteristics of Variables Describing the Research Site

| Parameter | Variable | Units | Mean | Max | Min | N |
|----------------------------|-----------|-----------------------------------|-------|-------|-------|------------------|
| Depth | d | m | 0.70 | 2.55 | 0 | 30 |
| Width | w | m | 75 | 120 | 49 | 300 |
| Discharge | Q | $m^3 \text{ min}^{-1}$ | 861.6 | | | Continuous |
| Velocity (mean calculated) | v | $m \text{ min}^{-1}$ | 16.4 | | | |
| Velocity (pulse measured) | v (pulse) | $m \text{ min}^{-1}$ | 31.3 | | | 1 |
| Ambient NH_4^+ | N | $mmol \text{ m}^{-3}$ | 0.463 | 0.543 | 0.347 | five per station |
| Ambient NO_3^- | NO3 | $mmol \text{ m}^{-3}$ | 10.4 | 11.1 | 9.6 | five per station |
| N_2 day | N2 | $mmol \text{ N}_2 \text{ m}^{-3}$ | 517 | 519 | 516 | six up, six down |
| N_2 night | | $mmol \text{ N}_2 \text{ m}^{-3}$ | 519 | 520 | 519 | six up, six down |
| Thalweg travel time a–b | t | min | 15 | | | 1 |
| Thalweg travel time a–c | t | min | 48 | | | 1 |
| Distance a–b | x | m | 500 | | | 1 |
| Distance a–c | x | m | 1500 | | | 1 |

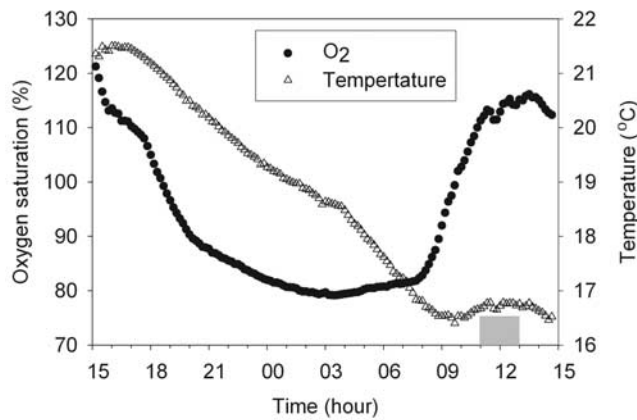


Figure 3. Dissolved O_2 concentrations and temperature at the downstream station (station C) for the day prior to and through the experimental period. Gray box indicates approximate time of the actual river isotope release.

diurnal dissolved O_2 concentration determined from measurements in the thalweg.

[31] Diurnal patterns of dissolved O_2 were evident and thus could be used to calculate respiration (R_{eco}) and GPP rates on the basis of the single station method. Respiration rates were about 1.4 times that of GPP, indicating a net heterotrophic system over the study period. These estimates were not corrected for groundwater dilution rates (and could not have been using our methods), so we could have overestimated R [Hall and Tank, 2005], but it is not likely the 2 km reach gained substantial discharge given the relatively homogenous nature of the substrata (sandy) and moderate change in elevation.

[32] Although the abiotic absorption experiments were done approximately 1 month before the release and at a temperature of about 9°C greater than occurred at the time of the main experiment, they yielded potentially relevant results. The gross NH_4^+ uptake rates were significantly greater in live than killed samples ($P < 0.035$). The concentration of NH_4^+ changed as the experiment proceeded, precluding the ability to measure the extent of label dilution given our methods for extracting NH_4^+ for isotope analyses; consequently, uptake rates were calculated over the first 5 min of incubation to minimize potential dilution effects [Laws, 1984]. The ratio of $^{15}NH_4^+$ uptake in live/killed samples was 5.0 over the first 5 min, which suggests that the majority of NH_4^+ uptake is driven by biotic processes, at least in the water column. The live (killed sample corrected) rate of gross NH_4^+ uptake was $0.034 \text{ mmol m}^{-3} \text{ min}^{-1}$.

[33] During the main river isotope release, we missed the central portion (laterally) of the peak at station B (Figure 4); we recovered 69% of the label at station B and 72% of the label at station C relative to station A as determined by trapezoidal integration. Since we obtained samples that were not in the central portion of the peak, they were relatively few and close to detection limits for isotope and differences in concentration of NH_4^+ , and we did not include station B in subsequent analyses.

[34] We modeled fluorescein response at station C using OTIS-P and determined As/A to be 0.15 and F_{med} to be 0.074. F_{med} is defined as the fraction of median travel time

solutes spend in the transient storage zone of the river reach; therefore, this value indicates that water spends about 7% of its time not in the main channel. The modeled cross-sectional area from the dye pulse was about half the measured area, suggesting that the thalweg covered approximately half the area of the stream width.

[35] We determined which data at stations A and C to use by analyzing the variance of samples collected for determination of dilution (fluorescein). We found that using data in the center 1/3 of the fluorescence-corrected gross NH_4^+ concentration peak at each site represents a balance between increasing the number of samples (statistical power) by including diluted samples that are close to the assays' detection limits and increasing variance by taking more samples on the tails of the peaks that are closer to limits of detection (Figure 5). Station C had a minimum coefficient of variation in values for NH_4^+ corrected for dilution at about 1/3 of the entire fluorescence peak. Standard error increased in Station C as more of the peak was sampled, but decreased

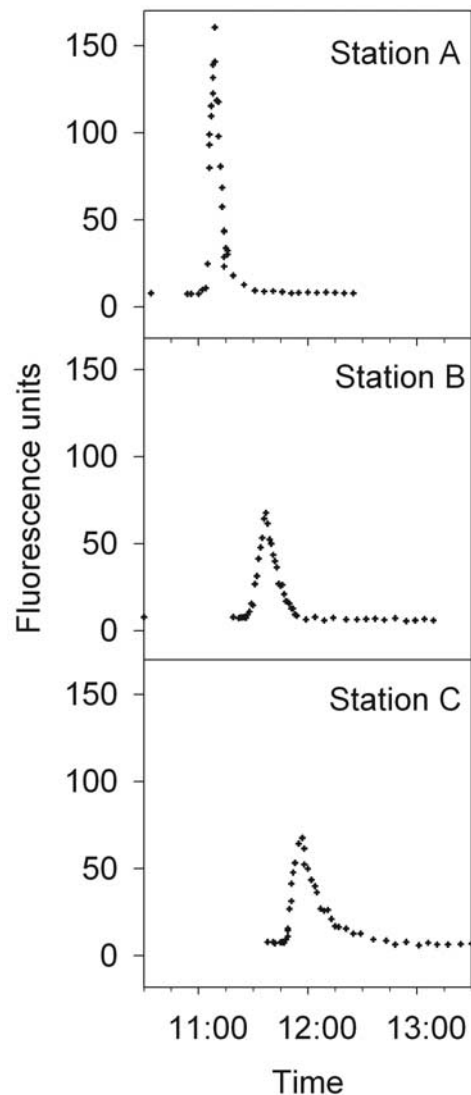


Figure 4. Fluorescence at each of the three sampling sites sampled in the Kansas River as a function of time following a pulsed release of fluorescein.

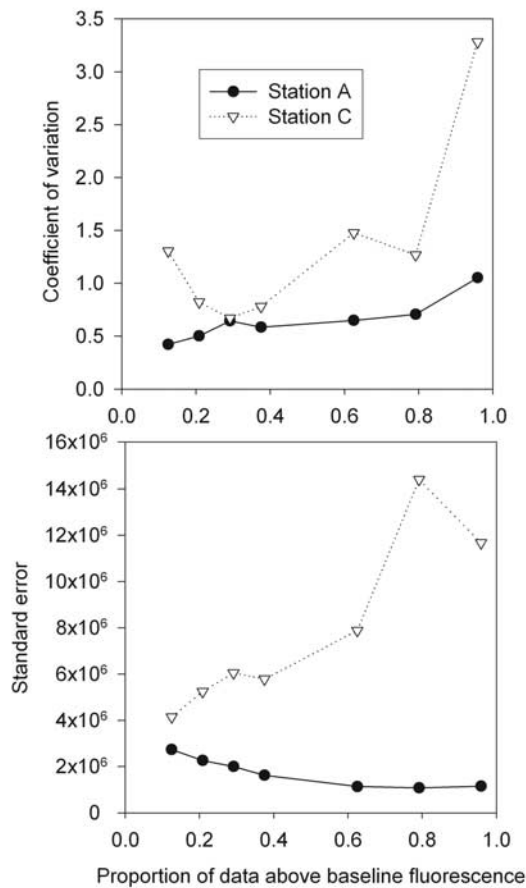


Figure 5. Coefficient of variation and standard error of the value of NH_4^+ corrected for dilution by fluorescent tracer concentration as a proportion of data points taken from the center of the peak. Only samples where fluorescence was greater than the 95% confidence interval of the background values were considered in the peak. The maximum fluorescence value was used to determine the center of the peak.

in Station A. Thus, we chose to use the center 1/3 of the peaks at stations A and C to maximize sample size and number while minimizing error associated with highly diluted samples at the leading and trailing edge of the pulse as it moved downstream.

[36] Ammonium concentration increased in the water column at station A 5.7 times over background as a result of the release pulse, but had declined to background concentrations by station C, which enabled us to calculate net NH_4^+ uptake between stations A and C (see Table 4 and the eighth uptake equation in Table 2). The net NH_4^+ spiral length, calculated from the net NH_4^+ uptake rate, was 2.1 km. The rate of net NH_4^+ uptake is comparable to the rate of gross uptake calculated during the abiotic adsorption experiment and scaled per unit area (Table 4). However, the abiotic adsorption experiments estimated uptake solely in the water column at 25°C, whereas in the river release experiment, uptake may have been affected by lower temperature (16°C during the river release experiment) or benthic processes.

[37] There was no significant difference in NH_4^+ concentration among stations immediately prior to the pulsed $^{14}\text{NH}_4^+$ addition ($n = 5$ per station, ANOVA, $p > 0.36$). A net uptake of zero leads to constant NH_4^+ concentrations and only isotopic tracers can be used to estimate gross uptake in this case [Dodds, 1993].

[38] Depletion of ^{15}N was detectable at stations A and C ($\delta^{15}\text{NH}_4^+$ had a mean of -46‰ at station A, -8.6‰ at station C, and 7.4‰ at background, Figure 6). Dilution of ^{14}N in the pulse between stations A and C was greater than could be accounted for by physical dilution as indicated by decrease in fluorescein and associated dilution by the relatively greater ^{15}N content in river water compared to that in the pulse. Given mass balance, excess loss must be accounted for by mineralization or input of NH_4^+ -rich groundwater. Assuming that mineralization and groundwater input rates did not change in response to the short-term (48 min) increase in water column NH_4^+ , we could calculate gross uptake rate at ambient concentrations (see the fifth uptake equation in Table 2). This rate of gross uptake at ambient NH_4^+ concentration was slightly greater than the calculated net uptake rate under enriched NH_4^+ concentration (Table 4). Gross uptake rate led to a calculated spiral length

Table 4. Rates of Uptake and Metabolism^a

| Value | Rate | Comment |
|------------------------|--------|---|
| R_{eco} | 0.263 | Rate of O_2 flux |
| GPP | 0.184 | Rate of O_2 flux |
| NEP | -0.079 | Rate of O_2 flux |
| U_{enrich} | 0.021 | Net uptake based on change in concentration of NH_4^+ |
| $M_{\text{a,amb}}$ | 0.035 | Mineralization + groundwater dilution based on dilution of $^{14}\text{NH}_4^+$ by $^{15}\text{NH}_4^+$ in excess of what could be accounted for by fluorescent label. This value is also equal to gross NH_4^+ uptake |
| $M_{\text{n,nitrate}}$ | 0.0008 | Maximum nitrification based on nitrate isotope dilution confidence interval |
| U_{partic} | 0.024 | Gross uptake of $^{15}\text{NH}_4^+$ to suspended particles based on experiment from the prior month at room temperature |
| U_{enrich} | 0.095 | Enriched uptake based on the work of Dodds et al. [2002b] regression method based on mean increase in ammonium due to pulse. This is a value calculated from ammonium concentration and rates from streams. |
| U_{amb} | 0.063 | Gross uptake based on the work of Dodds et al. [2002b] regression method based on mean background concentration. This is a value calculated from ammonium concentration and rates from streams. |
| U_{amb} | 0.009 | Gross uptake based on the work of Dodds et al. [2002b] extrapolation method. This is a value calculated from ammonium concentration and rates from streams. |
| U_{demand} | 0.060 | Gross uptake based on the work of Webster et al. [2003] method, that uses R and GPP to calculate an N demand. |

^aAll values in $\text{mmol m}^{-2} \text{min}^{-1}$.

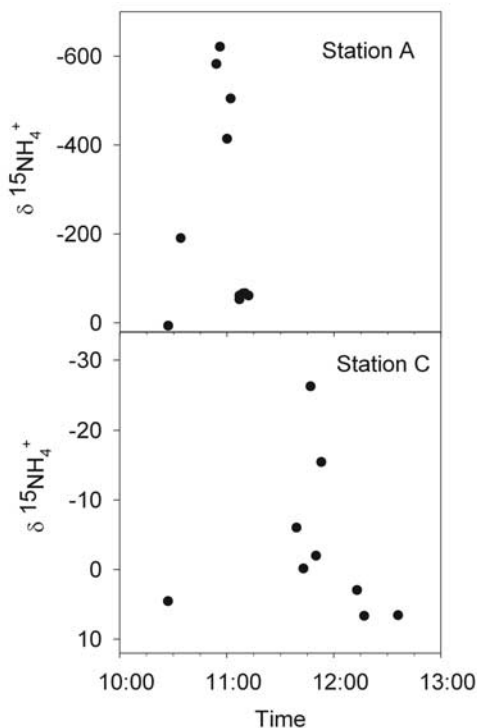


Figure 6. Isotope content of ammonium at station A and station C as a function of time.

of 1.9 km. Under elevated concentrations, gross uptake was approximately double the net uptake of NH_4^+ .

[39] Nitrification was not detectable as a dilution of $^{15}\text{N}\text{-NO}_3^-$ by ^{14}N because there was not a significant difference in the $\delta^{15}\text{N}\text{-NO}_3^-$ between stations A and C ($p > 0.05$, students T test). We calculated the maximum nitrification rate that would be detectable given the 95% confidence interval of the $\delta^{15}\text{N}\text{-NO}_3^-$ measured at station A (Table 4), and the rate was substantially less than the NH_4^+ uptake rates. Assuming that NO_3^- concentrations remain constant, then the maximum nitrification rates are bounded by the measured NH_4^+ uptake rates. Given ambient NO_3^- concentrations, the minimum spiral length for NO_3^- is 40 km in the thalweg assuming similar rates of processing downstream from the study reach.

[40] Denitrification was not detectable when measured as the change in N_2 concentration between the upstream and downstream stations. Given the measured aeration coefficients and the variance in the N_2 concentration measurements, we calculated the maximal rate of denitrification that would yield a detectable signal, which was $0.015 \text{ mmol N m}^{-2} \text{ min}^{-1}$. Also, the maximum flux of N nitrified and subsequently denitrified, calculated from the variance in the isotope ratios from the MIMS measurements of dissolved N_2 , was $34.7 \text{ nmol m}^{-2} \text{ min}^{-1}$. This maximum rate was about 1000 times less than gross NH_4^+ uptake rates.

4. Discussion

[41] A key question in lotic biogeochemistry is the degree to which medium-sized rivers sequester nutrients before water flows into larger river systems. While most nutrients that enter very large rivers may be transported to the ocean

(Darracq and Destouni [2005]; but see Alexander et al. [2000]), substantial N loss from the water column occurs in small streams [Peterson et al., 2001; Mulholland, 2004] and, according to mass balance models [Darracq and Destouni, 2005; Wollheim et al., 2006] and short-term pulse additions [Tank et al., 2008] also in medium-sized rivers. These losses could be due to assimilatory uptake or denitrification that leads to loss of N from the ecosystems to the atmosphere.

[42] More in situ measurements of N cycling rates in rivers are needed (see Tank et al. [2008], for meta-analysis). This includes both net and gross rates of NH_4^+ uptake because NH_4^+ is a key element in N turnover in many ecosystems, and a complete characterization of NH_4^+ cycling can be obtained if any two of gross uptake, mineralization + dilution, and net uptake are known. Some measurements have been made with river sediment cores [e.g., Strauss et al., 2006] but scaling to whole ecosystem rates requires huge numbers of samples or whole system methodologies to quantify uptake [e.g., Tank et al., 2008]. Large-scale budgeting approaches [Hill, 1979] do not characterize internal dynamics, only net fluxes. The cost and inherent technical challenges of applying in situ isotope labeling experiments to medium and large rivers, such as those done on small streams (e.g., Peterson et al. [2001], but see Wollheim et al. [2001]) have likely deterred researchers from this approach. One ingenious study, Sebilo et al. [2006] used natural abundance of ^{15}N and ^{18}O to trace source and processing rates of N from a sewage treatment plant through the Seine River and estuary. Still, there is a gap between modeling studies and the empirical measurements needed to provide parameters for mechanism-based models.

[43] We were not able to use the plateau isotope releases previously used in small streams [e.g., Webster et al., 2003; O'Brien et al., 2007] because they would require an extraordinarily large mass of isotope. Therefore, we developed a new pulsed (Lagrangian) release method that is useful in rivers and allows determination of gross uptake by isotopic dilution. Because of the necessity of sampling the primary pulse that was released, we sampled at constriction sites (which physically constrain solute into the thalweg of the river). A pulse that is released into the main channel of a river is confined to the thalweg because of modest lateral mixing after flowing through a constriction site. Our method yields a measurement of processes occurring in the thalweg of the river. We do not know how our rate estimates compare to those occurring in the side channels. Since water velocity is less, and assuming similar uptake rates per unit area, the equations for uptake length dictate that spiral lengths will be shorter than measured in the thalweg.

[44] Specifically, the velocity of the thalweg was about two times the average river velocity. Also, the cross-sectional area calculated for the release of dye from the OTIS-P fit was about half that measured in the field for the river. These data suggest that the calculated spiral lengths of approximately 2 km are approximately twice that of the whole river spiral lengths if biological activity is similar in thalweg and backwaters. It is likely that processing rates are even greater in backwaters than in the shifting sandy bottom thalweg, and that uptake lengths are even shorter than 1/2 those estimated from pulsed release in the thalweg.

[45] The single station, whole system metabolism estimates are probably influenced by the entire river, but little is known about how strongly backwater metabolism influences dissolved O_2 concentrations in the main stem of the river. Our metabolism estimates and NH_4^+ dynamic measurements, as indicated by the isotope release, may reflect processes occurring in different parts of the river. The pulsed uptake experiments reflected processing in the thalweg, whereas the whole system metabolism estimates are steady state numbers that reflect at least some influence of the backwaters and other slower flowing areas. The observation that the N demand calculated from metabolism measurements (Table 2) was close to the ambient NH_4^+ uptake rates indicates that the processing rates in the thalweg were not vastly different from those in the side channels.

[46] The NH_4^+ turnover rates were in the broad range for small streams and other aquatic habitats [Dodds et al., 2000]. We also used extrapolation methods based upon NH_4^+ uptake rates measured across 10 small streams to estimate what uptake would be at ambient and elevated uptake [Dodds et al., 2002b]. The predicted rates for the Kansas River derived from relationships developed using small streams were within an order of magnitude of the measured uptake rates. Interestingly, the uptake rates calculated from metabolism demand [Webster et al., 2003] were within twofold of the gross uptake rates suggesting that NH_4^+ accounts for a substantial proportion of metabolically driven N demand (Table 4). Finally, a recent meta-analysis suggests that our results from the Kansas River fall in line with those made in 297 previous measurements when expressed as an uptake velocity, including the Upper Snake River which is of a similar size but lower total N flux [Tank et al., 2008].

[47] The gross uptake rates calculated from the suspended particle experiment were very similar to those measured in the river, suggesting that uptake in the thalweg could be dominated by suspended particle dynamics, with little benthic uptake. Further experiments would be required to partition uptake between suspended particulates, benthic portions of the thalweg, and backwater habitats. Apparently little of the NH_4^+ uptake was related to nitrification. Amounts of $^{29}N_2$ and $^{30}N_2$ were not significantly different from background, indicating low levels of coupled nitrification-denitrification or anaerobic ammonium oxidation (anammox denitrification using NH_4^+ and nitrate) converting ^{15}N -depleted tracer to N_2 gas. Alternatively, the water residence time was insufficient to detect ^{15}N depletion of N_2 gas, and rates of coupled nitrification-denitrification were greater than our calculated minimum rates.

[48] Our estimates of upper limits of denitrification from the MIMS analyses of dissolved N_2 should be viewed with caution. Laursen and Seitzinger [2005] documented a number of potential problems with these measurements including wind and groundwater effects. We did not account for either of these in our measurements.

[49] We use a rarely adopted isotope depletion approach to estimate NH_4^+ dynamics. While this approach is less sensitive than the ^{15}N -enriched tracer, we could estimate gross uptake at ambient concentrations. In our particular case, the $^{14}NH_4^+$ was relatively inexpensive (\$41 per mol, about 80 times less expensive per mol than 60 atom

$\%^{15}NH_4^+$, but about 5 times less expensive to get detectable label) and we adopted the depletion approach in response. The drawback to the approach is that we needed to change the concentration of NH_4^+ to sense a response. In retrospect the $^{15}NH_4^+$ enrichment approach is probably superior.

[50] The exchange of gases across the air-water boundary in flowing waters influences processes ranging from the availability of oxygen to aquatic organisms to the emission of trace gases to the atmosphere [McMahon and Dennehy, 1999; Cole and Caraco, 2001; Richey et al., 2002; Reay et al., 2004]. Reaeration coefficients have been well studied in small streams [Genereux and Hemond, 1992; Marzolf et al., 1994; Mulholland et al., 2001] and large rivers [Devol et al., 1987; Clark et al., 1992; Marino and Howarth, 1993; Clark et al., 1996] but few measurements have been made in midsized rivers. Our two methods yielded estimates of a reaeration coefficient of 11.9 (modeling) and 7.6 per day (direct measurement), which is intermediate to the high values reported for small streams and the low values reported for large rivers. Our measurement compares well to reaeration coefficients measured in two Midwestern rivers of similar size to the Kansas River (1.06 and 7.58 per day; Laursen and Seitzinger [2002]).

[51] Metabolism expressed per unit area of the Kansas River falls within the range of metabolism rates reported previously for small streams [Dodds, 2006]. Respiration falls within the mean values reported for the River Glatt, which were 0.17 and 0.38 $mmol\ m^{-2}\ min^{-1}$ for winter and summer, respectively [Uehlinger et al., 2003]. Our methods could not estimate groundwater influence so metabolism rates should be viewed with caution [McCutchan et al., 2002].

[52] Our study suggests that rates of microbial metabolism per unit area are not substantially different in rivers compared to small streams, although there is a wide range of rates reported for streams. The greater depth of rivers may lead to longer uptake lengths relative to small streams, but uptake velocity (average movement rate of nutrients from the water column) is greater, leading to comparable uptake rates per unit area. Our results compare well to the two other rivers (Arctic and Rocky Mountain [Wollheim et al., 2001; Tank et al., 2008]) where comparable measurements have been made.

[53] Suspended particles may become more important in rivers than small streams because rivers have greater depth, increased turbulent energy that keeps particles suspended, and the propensity of many larger rivers to be turbid. Our data suggest that uptake associated with seston is potentially important in the total NH_4^+ uptake of this river, but we do not know the rate at which particulates enter and leave the benthic zone. Also, we do not know the importance of side channels and backwaters in this river with regard to N uptake and cycling.

[54] Our results do indicate active cycling, even in the most rapidly flowing portions of the river. As these are some of the first in situ NH_4^+ cycling rates for rivers we are aware of in the published literature, caution should be taken when extrapolating our results to other systems. The results do suggest that moderate sized rivers may not be simply transport conduits for N. In this prairie river, NH_4^+ was cycling actively. Active cycling is a prerequisite to retention. Retention of all forms of nitrogen, and subsequent

burial or denitrification are required for lowering rates of transport. Many rivers in areas with shallow river bed slopes are net depositional [Leopold, 1994], and could sequester substantial amounts of N. Such deposition requires net flux of N from the dissolved phase to the benthic particulate material, and NH_4^+ uptake indicates a substantial flux of N from dissolved to particulate phase.

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