Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the NO_3^- budget

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Abstract. We measured nitrification rates in sediment samples collected from a variety of aquatic habitats in Navigation Pool 8 of the Upper Mississippi River (UMR) 7 times between May 2000 and October 2001. We also conducted nutrient-enrichment experiments and analyzed vertical profiles of sediment to determine factors regulating nitrification. Nitrification rates were relatively high compared to other ecosystems (ranging from 0–8.25 µg N cm^{-2} h^{-1}) and exhibited significant temporal and spatial patterns. Nitrification rates were greatest during the summer and spring compared to autumn and winter (ANOVA, $p < 0.05$) and were greater in contiguous backwater and impounded habitats compared to main and side-channel habitats ($p < 0.05$). Regression analysis indicated that nitrification rates were weakly ($r^2 = 0.18$, $p < 0.0001$) related to temperature and exchangeable NH_4^+ of the sediment. However, nutrient-enrichment experiments showed that NH_4^+ availability did not limit nitrification in 3 sediment types with variable organic matter. Vertical profiles of sediment cores demonstrated that oxygen concentration and nitrification had similar patterns suggesting that nitrification may be limited by oxygen penetration into sediments. We conclude that temperature and sediment NH_4^+ can be useful for predicting broad-scale temporal and spatial nitrification patterns, respectively, but oxygen penetration into the sediments likely regulates nitrification rates in much of the UMR. Overall, we estimated that nitrification produces 6982 mt N/y of NO_3^- or 7% of the total annual NO_3^- budget.

Key words: nitrification, Upper Mississippi River, nitrogen cycle, nitrate budget.

Coastal eutrophication is an issue of global concern (Nixon 1995, Rabalais 2002). For example, in the northern Gulf of Mexico, excessive N loading and subsequent elevated microbial production have been singled out as the primary cause of seasonal hypoxia (Justic et al. 1993, Rabalais et al. 1998). Evidence suggests that much of this N originates from agricultural areas in the midwestern United States and enters the Gulf through the Mississippi River (Howarth et al. 1996). Alexander et al. (2000) estimated that 44% of the N load in the Mississippi River entering the Gulf of Mexico originates in the Upper Mississippi River (UMR) watershed.

Nitrogen concentrations in the main channel of the UMR are typically high; mean total N, NO_3^-, and NH_4^+ concentrations are 2.4, 1.6, and 0.1 mg N/L, respectively (Soballe et al. 2002). Upriver export and tributary discharges probably contribute much of the NO_3^- to the UMR reaches. Intrasystem NO_3^- production (i.e., nitrification, chemoautotrophic oxidation of NH_4^+ to NO_3^-) may also be a significant source, but this potential NO_3^- source has not been quantified. Nitrification is a ubiquitous N-cycle process and is the only means of NO_3^- production within a system. Many nitrification studies have been conducted in small streams (e.g., Jones et al. 1995, Bernhardt et al. 2002, Kemp and Dodds 2002, Strauss et al. 2002), but information on rates and patterns of nitrification in large riverine systems is scarce. A series of ^{15}N-tracer injections in 10 North American headwater streams as part of the LINX (Lotic Intersite Nitrogen eXperiment) study indicated that nitrification rates explain 50% of the variability observed in stream NO_3^- concentrations, suggesting the importance of nitrification to lotic NO_3^- budgets (Peterson et al.

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2001). However, the LINX streams were small and had relatively low inorganic-N concentrations (mean $NH_4^+ + NO_3^- = 77$ µg N/L). In an analysis of major world rivers, Caraco and Cole (1999) estimated that >80% of variability in NO_3^- export could be explained by point and nonpoint N loadings. This result implies that the contribution of nitrification to N budgets is likely small in large rivers; however, this prediction remains untested.

Nitrification can be limited by a variety of factors. Strauss et al. (2002) proposed a conceptual model that predicts certain physical and chemical variables (e.g., dissolved oxygen, pH, micronutrients) collectively constrain nitrification to some maximum rate. Within this constraint, regulation of nitrification depends on the ambient environmental C:N ratio. At low C:N ratios (C:N < 20), maximum nitrification is determined by NH_4^+ availability (positive effect), whereas at high C:N ratios (C:N > 20), nitrification rates are regulated by organic C quantity and quality (negative effect). Adequate information is lacking to predict what variable or variables may regulate nitrification in the UMR, but this model was useful for identifying potential regulating factors (i.e., NH_4^+ availability, organic C availability, and dissolved oxygen) for further examination.

Our goal was to quantify nitrification rates in navigation Pool 8 of the UMR, to identify factors regulating nitrification, and to estimate the contribution of nitrification to the overall NO_3^- budget within the system. To our knowledge, our study is the first attempt to quantify nitrification or intrasystem NO_3^- production in a large river, and it is certainly the first in the UMR.

Study Site

The UMR, as defined here, includes all reaches of the Mississippi River north of Cairo, Illinois, and the UMR watershed includes all watersheds and tributaries that deliver water to the UMR (Fig. 1A, B; Lubinski 1999). Most of the UMR (the portion upstream of St. Louis, Missouri) is partitioned into a series of impounded reaches or navigation pools by a sequence of locks and dams. Our study reach was navigation Pool 8, delineated by Lock and Dams 7 (upstream) and 8 (downstream), near La Crosse, Wisconsin (Fig. 1B, C). This 38-km reach is typical of many in the UMR, with a mean depth of

1.7 m, a median discharge of 815 m³/s, and 10,425 ha of wetted area under normal summer flows. This river ecosystem contains a diverse, interconnected network of aquatic habitat types including the main channel, side channels, impounded zones, and contiguous backwater lakes (Fig. 1C, Table 1). Impounded zones are large expanses of relatively slow-moving water created by the lock and dam system. This habitat composes the largest areal proportion of the aquatic habitats, followed by backwaters, side channels, and the main channel, respectively (Table 1). The system is highly modified for navigation and lateral flood control but, unlike the lower half of the Mississippi River or most large rivers of Europe, there is still active connection between flowing channels and off-channel floodplain lakes and marshes. During nonflood periods, most of the water flowing through the pool remains in the main channel and is aided by channel dredging and flow-directing structures (wing dams and side-channel closing dams). Backwater lakes are typically <1 m deep for most of the year, and have little exchange of water with the main channel (DMS, unpublished data). Only during flooding (typically spring and autumn), does much water flow out of the main channel and inundate the backwater lakes and the floodplain.

Methods

Seasonal measurements

Nitrification was measured in 375 samples between May 2000 and October 2001 from 69 sites located in Pool 8. A subset of the 69 sites was sampled during each of 7 sampling events: spring 2000 (Sp00, $n = 61$), summer 2000 (Su00, $n = 60$), autumn 2000 (A00, $n = 60$), winter 2001 (W01, $n = 15$), spring 2001 (Sp01, $n = 61$), summer 2001 (Su01, $n = 59$), and autumn 2001 (A01, $n = 59$). Sampling dates and physical/chemical characteristics of the river during sampling are presented in Table 2. The 12 months were equally distributed among the 4 seasons: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). Fewer samples were collected during W01 because of difficulty sampling through ice. Coordinates of sample sites were selected randomly among potential sites within each aquatic habitat (Fig. 1C, Table 1; Wilcox
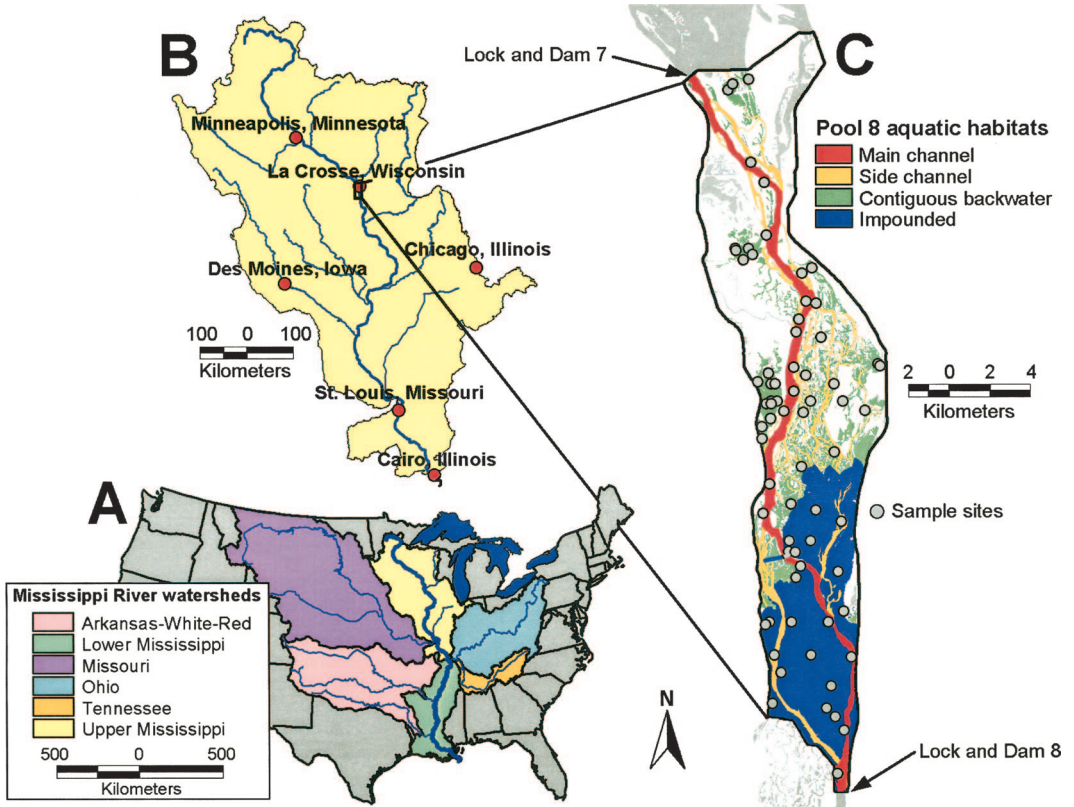
FIG. 1.   Study sites on the Upper Mississippi River. A.—Major Mississippi River watersheds. B.—Upper Mississippi River watershed. C.—Location of aquatic habitats and our sample sites in Navigation Pool 8 of the Upper Mississippi River, near La Crosse, Wisconsin.

1993). An equal number of sites were targeted for sampling within each aquatic habitat during each sampling event. However, deviations from this design were unavoidable because river conditions restricted access to some sites (e.g., ice was dangerously thin, site was too deep for sampling gear, site was too shallow for boat access, or site was dry). Nevertheless, the average deviation from this design was <7 sites per sampling event and, on average, 95% of the sites were sampled in consecutive events.

Intact sediment cores (5 cm deep x 2.54 cm diameter) and water-column samples were collected at each site, returned to the laboratory, and stored at 4°C. Water samples were collected at mid-depth when the site was shallower than 40 cm. For deeper sites, water samples were collected near the bottom if the site was stratified or near the surface if the site was not stratified. Sites were deemed stratified if surface and bottom water temperature differed by >2°C or dissolved oxygen (DO) differed by >1.0 mg/L. Nitrification measurements were initiated within 24 h. Gross nitrification rates were measured in the laboratory using a modification of the nitrapyrin method described by Strauss and Lamberti (2000). Nitrification from each site was measured as the difference in exchangeable NH_4^+ concentrations between incubations in two 125-mL Erlenmeyer flasks, one in which nitrification was allowed to occur and one in which nitrification was inhibited with nitrapyrin (2-chloro-6-[trichloromethyl]-pyridine, SIGMA Chemical Co., St. Louis, Missouri). The nitrification-inhibited flask received a final concentration of 10 mg/L nitrapyrin dissolved in dimethyl sulfoxide (DMSO). The other flask received DMSO only as a control. Each flask contained the sediment from an intact sediment core (25.3 cm^3) and 81 mL of unfiltered river water from

TABLE 1. Definition of aquatic habitats (modified from Wilcox 1993), mean (± SE) sediment total organic C (TOC) and total N content, and areal extent of aquatic habitats in Navigation Pool 8, Upper Mississippi River.

Aquatic habitat	Definition	Dry sediment characteristics		Total area in Pool 8 (km²)	Pool 8 aquatic habitat (% of total)
		TOC (g C/kg)	Total N (g N/kg)		
Impounded	Large, mostly open-water areas located in the downstream portions of the navigation pool. The downstream boundaries of impounded areas are the navigation dam and connecting dikes. Landward boundaries are the apparent shorelines or the boundaries of other aquatic habitats. Upstream boundaries are with islands and floodplain shallow aquatic zones. Riverward boundaries are channel border zones. Sediment is mostly a silt–clay mixture. Macrophytes are common in some areas.	10.8 (0.9)	1.00 (0.07)	36.9	45
Contiguous backwater	Areas that are beyond the banks of the main and secondary channels. Sediments are mostly organic and macrophyte coverage is extensive.	27.2 (4.3)	4.37 (0.26)	19.4	24
Side channel	Secondary and tertiary tributary channels often lateral to the main channel, containing a wide range of water velocities and depths. Sediment is mostly a sand–silt mixture. Macrophytes are occasionally located along channel margins.	5.7 (1.7)	0.39 (0.06)	13.2	16
Main channel	Areas including the designated navigation channel, sandbar, channel border, and tailwater areas. Within channel border areas are natural bank areas and areas associated with channel-training structures. Main channel contains most of the water volume passing through the pool and is dredged to ≥3.2 m depth. Sediment is exclusively sand with little to no macrophytes present.	2.2 (0.6)	0.18 (0.04)	12.6	15

TABLE 2. Sample dates and mean values of discharge, water temperature, water column NO_3^-, NH_4^+, and total N in Pool 8 of the Upper Mississippi River during sampling. Mean discharge is from Lock and Dam 7 (Fig. 1C). Remaining means were calculated using values from all sites sampled. Sampling events: spring 2000 (Sp00), summer 2000 (Su00), autumn 2000 (A00), winter 2001 (W01), spring 2001 (Sp01), summer 2001 (Su01), and autumn 2001 (A01). CV = coefficient of variation (%).

Event	Sampling dates	Discharge (m³/s)	Water temp. (°C) (CV)	NO_3^- (mg N/L) (CV)	NH_4^+ (mg N/L) (CV)	Total N (mg/L) (CV)
Sp00	2–16 May 2000	686	17.5 (15.4)	0.18 (197.5)	0.05 (74.5)	1.22 (43.7)
Su00	31 Jul–8 Aug 2000	625	24.1 (8.1)	1.67 (78.8)	0.13 (78.5)	2.46 (36.6)
A00	31 Oct–9 Nov 2000	648	11.1 (23.6)	0.57 (103.1)	0.09 (73.6)	1.88 (100.5)
W01	14–23 Feb 2001	488	0.2 (115.3)	0.01 (53.1)	0.28 (98.5)	1.88 (26.3)
Sp01	15–21 May 2001	2632	18.6 (9.4)	2.58 (24.7)	0.06 (68.7)	3.23 (16.7)
Su01	24 Jul–2 Aug 2001	844	27.2 (5.13)	0.72 (60.5)	0.12 (157.7)	1.72 (33.3)
A01	1–11 Oct 2001	448	14.9 (14.5)	0.73 (71.2)	0.05 (59.1)	1.27 (37.8)

that sampling site. The difference in organic C added between the nitrapyrin plus DMSO and the DMSO only amendments was small and likely not a problem with this method. Because nitrapyrin precipitates after it is added to the sample, the additional C contribution must be compared to the particulate organic C (POC) of the sample. This C amendment from nitrapyrin increases the POC of the samples from 0.02% for the backwater samples to 0.19% for the main-channel samples. The flasks were covered loosely with aluminum foil and incubated in the dark at ambient river temperature for 72 h on an orbital shaker (175 rpm). Initial and final exchangeable NH_4^+ concentrations were determined from centrifuged (8 min, 1000 g) 1N potassium chloride (KCl) extracts from each flask using the phenol hypochlorite method (Solorzano 1969). KCl extracts were made by adding 6 mL of 2N KCl to 6 mL of homogenized sediment slurry and incubated (with shaking) for 1 h prior to centrifugation. Gross nitrification rates over the incubation period were calculated by subtracting the observed change in NH_4^+ in the flasks containing only DMSO from the increase in NH_4^+ in the flasks that contained nitrapyrin plus DMSO. This method may overestimate nitrification to some extent because the laboratory incubations were conducted under ideal conditions (i.e., aerobic and well-mixed). However, these measurements should not be considered true *potentials* because the samples were not amended with NH_4^+. Two-way factorial analysis of variance (ANOVA) and the Least-Squares (LS) Means procedure (SAS statistical software, version 8.01, SAS Institute, Inc., Cary, North Carolina) were used to detect and identify significant differences in nitrification rates among the levels of the 2 factors (aquatic habitats and sampling events). The assumption of data normality was tested using the Shapiro–Wilk test and inspection of normal probability plots.

Physical and chemical characteristics of water and sediment (top 5 cm) at each site also were measured. Water temperature, specific conductivity, DO, and pH were measured in situ with a YSI 600XL multiparameter probe. Filtered (Whatman CA 0.45 μm) water samples were acidified (pH < 2 with H_2SO_4) and stored at 4°C until analysis for NH_4^+ and NO_3^-. Unfiltered samples were acidified (pH < 2 with H_2SO_4) and stored at 4°C until analysis for total N. All nutrient analyses were done on a Bran+Luebbe TrAAcs 800 Continuous Flow Analysis System according to standard methods (APHA 1998). Sediment temperature and pH were determined onsite with a Beckman Φ11 pH meter. Sediment was returned to the laboratory and analyzed for bulk density and % water content following Håkanson and Jansson (1983). Ash-free dry mass (AFDM) and exchangeable (KCl-extracted) NH_4^+ were also determined for each sediment sample. Sediment samples collected in 2001 also were analyzed for total organic C (TOC), total N, and C:N ratio on a Elementar VarioMax CN elemental analyzer. TOC of a sample was obtained by subtracting the total C of an ashed subsample (6 h, 500°C) from that of a dried subsample (24 h, 105°C). Stepwise regression was used to develop models that would best predict nitrification rates for each aquatic habitat using the chemical and physical variables measured.

Nutrient-enrichment experiments

Enrichment experiments were conducted to determine if NH_4^+ availability limited nitrification or if labile organic C (acetate) additions could suppress nitrification in 3 sediment types: 1) organic-rich silt sediment, 2) organic-poor sand sediment, and 3) moderately organic sand sediment (Table 3). Sediment and water samples were collected as described above. The experimental design consisted of 4 treatments: control (no additions), +N (2 mg N/L NH_4^+ added), +C (20 mg C/L acetate added), and +N+C (N and C added at above concentrations). Nitrogen and C were added as NH_4Cl and sodium acetate, respectively. Four replicates of each treatment were used and nitrification was measured as described above. Results from each sediment type were analyzed individually with 1-way ANOVA and significance of differences among treatment means was determined using the LS Means procedure.

Vertical profile study

Sampling was conducted in July 2000 at 2 sites (organic-rich silt sediment vs organic-poor sand sediment; Table 3) in Pool 8 to determine oxygen availability within the upper 5 cm of sediment and vertical stratification of nitrification potential. The 2 sites were the same locations where sediment was collected for the en-

TABLE 3. Total organic C (TOC), total N, exchangeable (Exch.) NH_4^+, and molar C:N ratio of the Upper Mississippi River sediments (dry) used in the enrichment experiment and the vertical profile study. Aquatic habitats: contiguous backwater (CB), side channel (SC).

Sediment	Aquatic habitat	TOC (g C/kg)	Total N (g N/kg)	Exch. NH_4^+ (mg N/L sed.)	Molar C:N
Organic-rich silt	CB	44.69	4.83	28.97	10.79
Organic-poor sand	SC	2.42	0.31	8.60	9.11
Moderately organic sand	CB	7.77	0.76	13.69	11.93

richment experiments. Sediment oxygen concentrations were determined onsite in an intact sediment core at 0.5 mm intervals using a micromanipulator and microfiberoptic oxygen sensor (MICROX1 and type A microoptrodes, Precision Sensing GmbH, Regensburg, Germany). Oxygen sensors were calibrated with oxygen-saturated and anoxic (0.5% Na_2SO_3) site water at ambient sediment temperatures.

Immediately after collection, separate sediment cores ($n = 5$, 7.62 cm diameter) for nitrification measurements were partitioned vertically into 1 cm sections and stored in sealed plastic bags. Upon arrival at the laboratory, nitrification rates were measured as described earlier. In our study, nitrification rates should be considered as *potentials* because samples were incubated under oxic conditions regardless of ambient oxygen availability. Differences in mean nitrification rates among the sites and depth profiles were determined using 2-way ANOVA and LS Means.

Intrasystem NO_3^- production

The seasonal nitrification measurements for each aquatic habitat were used to extrapolate estimates of seasonal and annual pool-wide NO_3^- production. First, seasonal aquatic habitat NO_3^- productions were calculated by multiplying mean seasonal nitrification rates for each aquatic habitat by the total surface area of the respective aquatic habitat and appropriate time conversions. Next, seasonal pool-wide NO_3^- productions were calculated as the sum of the seasonal aquatic habitat NO_3^- productions. Last, annual intrasystem NO_3^- production was estimated as the sum of the seasonal pool-wide estimates. Measurement error was extrapolated from initial estimates to pool-wide NO_3^- production values using standard error propagation formulas (Pitman 1993).

To quantify the importance of nitrification to the annual NO_3^- load in Pool 8 of the UMR, the annual pool-wide NO_3^- production estimate was compared to existing Pool 8 NO_3^- flux data (updated from Wasley 2000). Nitrate flux was estimated from rating-curve models using data collected in biweekly fixed-point sampling through the US Geological Survey administered Long Term Resource Monitoring Program (LTRMP) (Wasley 2000). Collective NO_3^- losses from the system (e.g., denitrification and NO_3^- uptake) were estimated as the difference between the sum of the NO_3^- inputs (Pool 8 inflow, tributary inflow, and nitrification) and the Pool 8 outflow.

Results

Seasonal and aquatic habitat nitrification

Our 17-mo study provided a wide range of river conditions for examining the effects of chemical and physical variables on nitrification. For example, mean discharge ranged from record flooding (2632 m^3/s in Sp01) to relatively low-flow conditions (448 m^3/s in A01; Table 2). Water temperature and N concentrations also varied among the sampling events, often being influenced by season or discharge (Table 2).

Variables identified as potential regulators of nitrification in the UMR were water temperature, exchangeable NH_4^+, sediment pH, and organic matter content as measured by AFDM (Table 4). Despite their high variability (Table 2), these independent factors were not particularly useful for elucidating what regulated nitrification. Linear regressions using these independent factors were relatively weak, explaining <24% of the variation observed in nitrification rates within each of the aquatic habitats and only 18% of the variation in the combined nitrification data set (Table 4).

TABLE 4. Regression models for predicting nitrification rates in the 4 aquatic habitats of Upper Mississippi River Navigation Pool 8. Regression models were determined using the stepwise selection technique and all parameter estimates in the model are significant at the 0.05 level. AFDM = ash-free dry mass, exch. = exchangeable.

Aquatic habitat	Regression model	r^2	Overall p value
Impounded	0.071(water temperature) + 0.16(AFDM) − 0.27	0.24	<0.0001
Contiguous backwater	0.079(water temperature) − 0.45	0.17	<0.0001
Side channel	−0.66(sediment pH) + 5.62	0.10	0.044
Main channel	0.032(exch. NH_4^+) + 0.16	0.24	0.011
All areas combined	0.057(temperature) + 0.030(exch. NH_4^+) − 0.30	0.18	<0.0001

Nitrification rates exhibited temporal and spatial patterns (Fig. 2A, B). A significant interaction between the sampling event and aquatic habitat factors ($p = 0.044$) was detected; however, the effects of the main factors on nitrification rates were stronger ($p < 0.0001$ for both factors). Nitrification rates were greatest during summer and spring and lowest during the autumn and winter (Fig. 2B). Spatially, nitrification rates were variable within and among aquatic habitats (nitrification range = 0–8.25 μg N cm^{-2} h^{-1}), but were lowest in the main (rate mean = 0.35 μg N cm^{-2} h^{-1}) and side channel (rate mean = 0.60 μg N cm^{-2} h^{-1}) habitats and greatest in the backwater (rate mean = 1.11 μg N cm^{-2} h^{-1}) and impounded (rate mean = 1.38 μg N cm^{-2} h^{-1}) habitats ($p < 0.05$) (Fig. 2A).

Nutrient-enrichment experiments

Nitrification rates were highest in the moderately organic sand sediment (mean rate ± SE in control = 1.72 ± 0.13 μg N cm^{-2} h^{-1}), followed by the organic-rich silt sediment (mean rate ± SE in control = 1.02 ± 0.01 μg N cm^{-2} h^{-1}) and organic-poor sand sediment (mean rate ± SE in control = 0.79 ± 0.09 μg N cm^{-2} h^{-1}), respectively (Fig. 3A, B, C). Nitrification rates in the moderately organic sand sediment and the organic-poor sand sediment did not respond significantly to the nutrient additions compared to the controls; however, the +N and the +N+C treatments in the organic-poor sand sediment had negative effects ($p < 0.05$) on nitrification.

Vertical profile study

The vertical profile study showed that nitrification potential was highest in the uppermost

1 cm of sediment (2.15 μg N cm^{-2} h^{-1}) and declined with depth to 3 cm in the organic-poor sediment (Fig. 4A). Below 3 cm nitrification did not change appreciably. Similarly, oxygen availability was greatest at the sediment–water interface (80% saturation) and declined to 0% saturation by 3 mm (Fig. 4B). In the organic-rich sediment, both nitrification and oxygen were relatively low and did not differ throughout the sediment profile.

Intrasystem NO_3^- production

The greatest NO_3^- contribution to annual NO_3^- production in UMR Pool 8 was estimated to occur in summer at 30.8 mt N/d, followed by spring (23.4 mt N/d), autumn (11.2 mt N/d), and winter (11.1 mt N/d) (Fig. 5). Within each of the 4 seasons, nitrification in the impounded and backwater areas collectively contributed ≥82% of the total intrasystem NO_3^- production. Conversely, little NO_3^- was produced in the main and side channel areas. Annual NO_3^- production (6982 mt N/y) within the Pool 8 system was equal to 7% of the total NO_3^- inputs (99,922 mt N/y) into the Pool 8 system (Fig. 6). Nitrate produced via nitrification was approximately equal to the NO_3^- load carried in tributary inflow (6912 mt N/y). Estimation of collective NO_3^- losses was 20,002 mt N/y.

Discussion

Patterns of nitrification

The UMR is a N-enriched system and the nitrification rates measured in our study reflect this condition. In comparison to rates reported for 42 streams in the northern United States
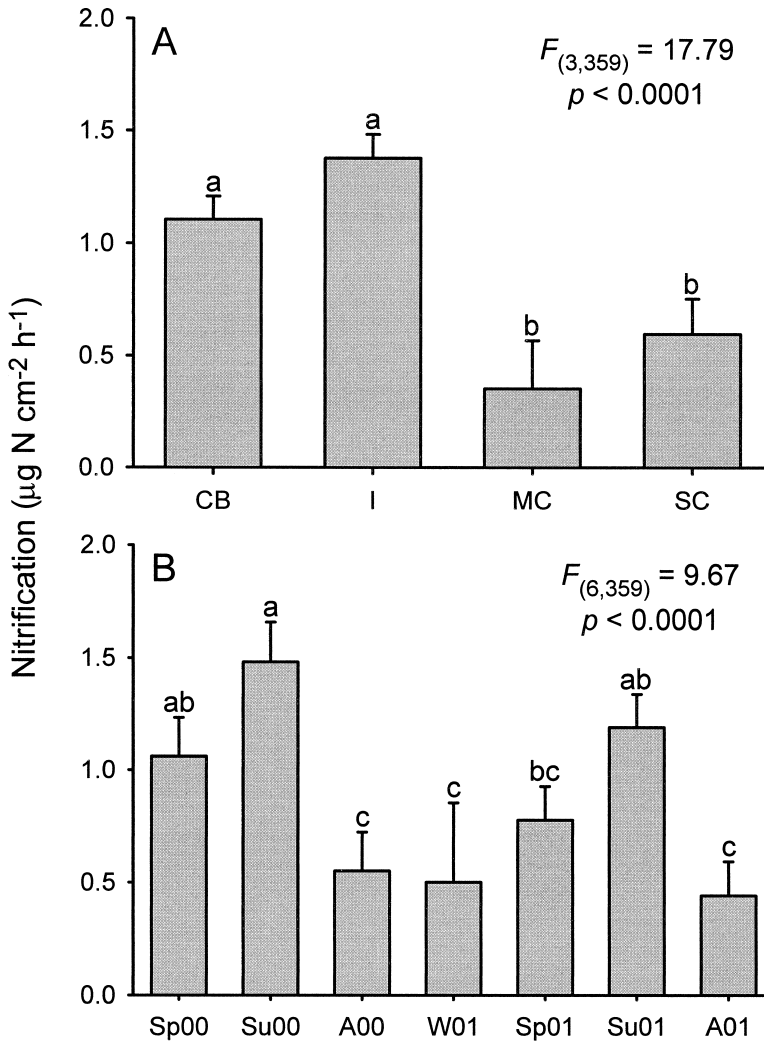
FIG. 2.   Nitrification rates measured in sediments of Upper Mississippi River Navigation Pool 8 from May 2000 to October 2001 categorized by aquatic habitats (A) and sampling event (B). Aquatic habitats: contiguous backwater (CB), impounded (I), main channel (MC), and side channel (SC). Sampling events: spring 2000 (Sp00), summer 2000 (Su00), autumn 2000 (A00), winter 2001 (W01), spring 2001 (Sp01), summer 2001 (Su01), and autumn 2001 (A01). Error bars are +1 SE. The aquatic habitat and sampling event F-statistics from the 2-way ANOVA are shown in panels A and B, respectively. Treatments with the same lower-case letter are not significantly different ($p > 0.05$, Least-Square Means procedure).

(Strauss 2000), the median rate was 7x greater in UMR Pool 8 (0.68 vs 0.097 μg N cm^{-2} h^{-1}) and the maximum rate was 10.6x greater (8.25 vs 0.78 μg N cm^{-2} h^{-1}). Rates also were relatively high compared to those measured in other lotic (Kemp and Dodds 2002), lentic (Hall 1986), and estuarine (Bianchi et al. 1999) ecosystems.

Nitrification in Pool 8 also exhibited distinct temporal and spatial patterns. Nitrification appeared to follow a cyclic seasonal pattern in which nitrification rates increase through the spring and summer months and decline during the autumn and winter. This pattern held regardless of aquatic habitat and is likely driven by river temperature. Growth and metabolism of nitrifying bacteria are positively correlated with temperature up to ~30°C (Watson et al.
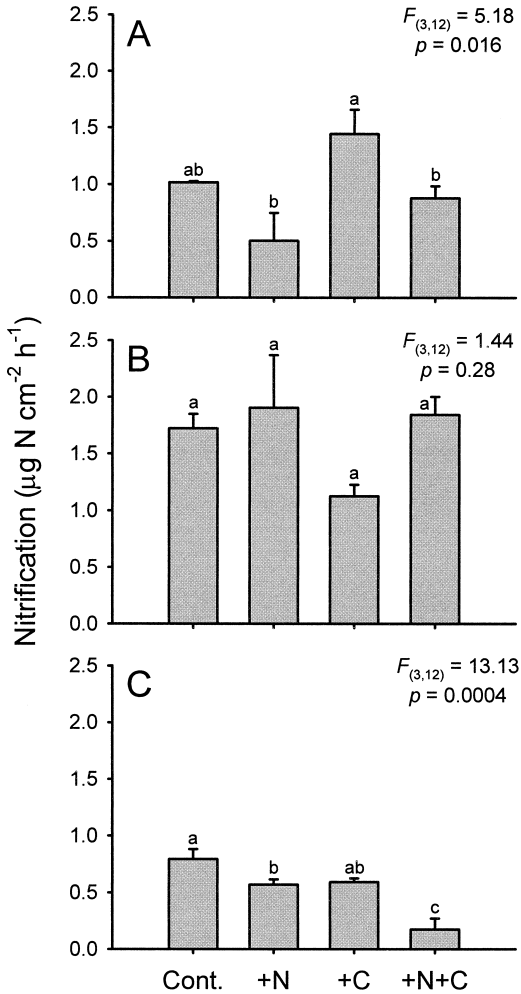
FIG. 3. Effect of acetate (+C, 20 mg C/L, as sodium acetate) and NH_4^+ (+N, 2 mg N/L, as NH_4Cl) additions on nitrification rates in organic-rich silt sediment (A), moderately organic sand sediment (B), and organic-poor sand sediment (C) collected from Upper Mississippi River Navigation Pool 8. Cont. = control (no C or N amendments). Error bars are +1 SE. The main effect (treatment) F-statistic from each 1-way ANOVA is shown in each panel. Treatments with the same lower-case letter were not significantly different ($p > 0.05$, Least-Square Means procedure).

1989), which could easily account for the elevated rates observed in the warmer months. Nitrification was highest in the impounded and backwater areas, perhaps because sediments in these areas also had higher concentrations of NH_4^+ (exchangeable and porewater) and total N compared to those in the main and side chan-

nels. The significant factors (temperature and exchangeable NH_4^+) present in the overall regression model (Table 4) further suggest that these 2 factors are important. However, other factors are likely also important in regulating nitrification in this system because the overall regression model ($r^2 = 0.18$) and the regression models developed for each of the aquatic habitats ($r^2 \leq 0.24$) explained relatively little of the variability observed in nitrification rates measured throughout the study.

Nutrient-enrichment experiments

Our enrichment experiments suggest factors besides temperature and NH_4^+ regulate nitrification. Increasing NH_4^+ availability did not elevate sediment nitrification rates in any of the sediment types. In fact, NH_4^+ additions suppressed nitrification in the organic-poor sand sediment. Furthermore, the lack of nitrification response to labile C additions (except for the +N+C treatment in the organic-poor sand sediment) suggests that the ambient C:N ratio favors nitrification (Strauss et al. 2002). Had labile C additions reduced nitrification (heterotrophic bacteria outcompeted nitrifiers for available NH_4^+), we could have assumed the ambient C:N ratio was high (>20) and nitrification was N limited.

Many studies have shown a direct relationship between nitrification and NH_4^+ availability (Triska et al. 1990, Jones et al. 1995, Strauss and Dodds 1997, Bianchi et al. 1999, Strauss et al. 2002). The diminished or slightly repressed NH_4^+/nitrification relationship observed in our study is probably not a typical occurrence in lotic ecosystems and may be restricted to systems (such as the UMR) that have experienced excessive N loading and accumulation over many years. For example, nitrification is often limited in NH_4^+-rich wastewater treatment wetlands because of low oxygen and high biochemical oxygen demand (Cronk 1996). High loading of N in aquatic systems is an increasing problem (Howarth et al. 1996), but the long-term subsequent effects on N cycling are not yet fully understood. In temperate forest ecosystems, nitrification appears to increase limitlessly with N loading (Aber et al. 1998). However, the oxic zone in sediments or soils (i.e., volume capable of supporting active nitrification) is typically much greater in terrestrial systems because oxygen in
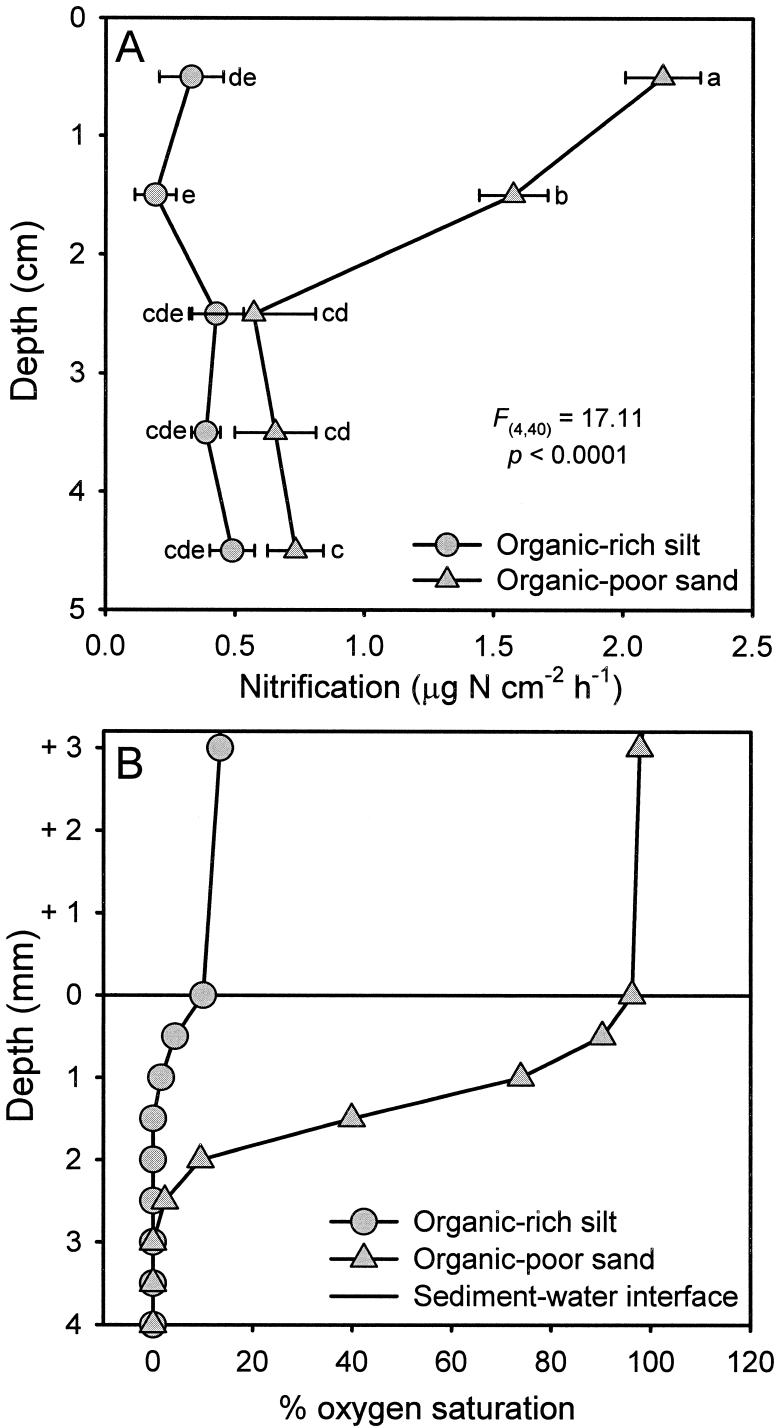
FIG. 4.   Vertical profiles of (A) nitrification and (B) % oxygen saturation in organic-rich silt and organic-poor sand sediments collected from Upper Mississippi River Navigation Pool 8. The error bars in panel A are ± 1 SE. The sediment-type/depth interaction F-statistic from the 2-way ANOVA is shown in panel A; means with the same lower-case letter were not significantly different ($p > 0.05$, Least-Square Means procedure).
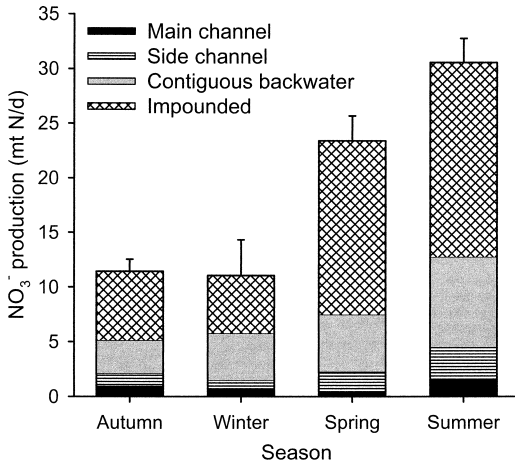
FIG. 5. Total NO_3^- production among aquatic habitats and seasons in Upper Mississippi River Navigation Pool 8. Error bars are +1 SE of mean seasonal NO_3^- production.

water-saturated sediments diffuses very slowly and is respired rapidly, particularly in highly organic sediment (Hargrave 1972).

Oxygen limitation

Limitation of nitrification in natural systems by lack of oxygen has been widely documented (Hall 1986, Triska et al. 1990, Kemp and Dodds 2001) and concentrations as high as 4.0 mg/L DO may still be limiting (Stenstrom and Poduska 1980). Our experimental results suggest that this may also be the situation in the UMR Pool 8 sediments. Vertical profiles demonstrated similar patterns (although at different spatial scales) between depth and oxygen and potential nitrification. In the organic-poor sediment, potential nitrification and oxygen were high near the sediment–water interface and declined with depth. The organic-rich sediment had low oxygen and potential nitrifica-
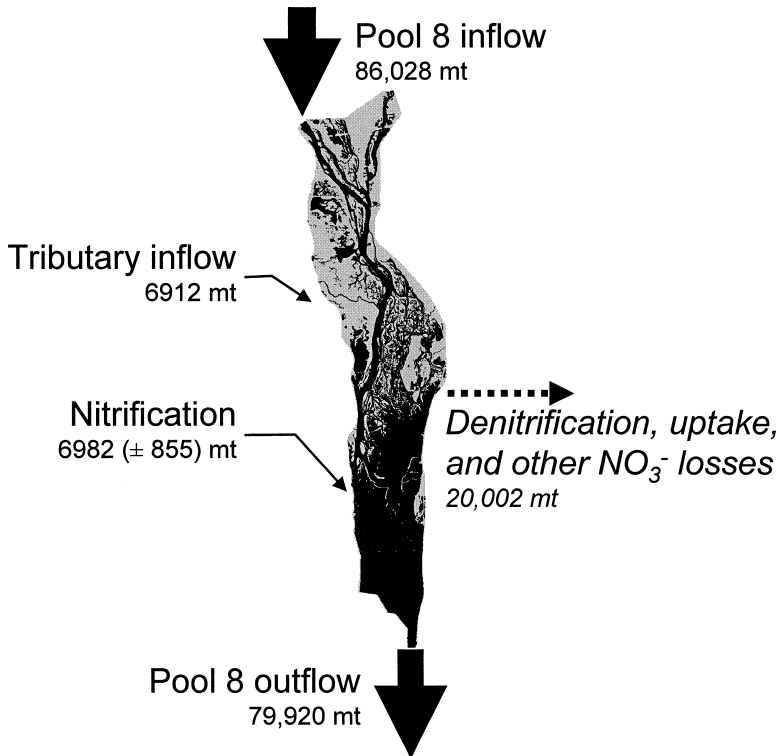

Pool 8 inflow
86,028 mt

Tributary inflow
6912 mt

Nitrification
6982 (± 855) mt

Denitrification, uptake, and other NO_3^- losses
20,002 mt

Pool 8 outflow
79,920 mt

FIG. 6. Annual NO_3^- budget of Upper Mississippi River (UMR) Navigation Pool 8. The annual nitrification estimate (±1 SE) was calculated from seasonal nitrification measurements for each aquatic habitat. Other NO_3^- inputs and NO_3^- losses through UMR Pool 8 outflow are from the US Geological Survey administered Long Term Resource Monitoring Program (Wasley 2000). Denitrification and other NO_3^- losses are estimated as the difference between the collective NO_3^- inputs and the NO_3^- loss through UMR Pool 8 outflow. Units are mt of NO_3^--N.
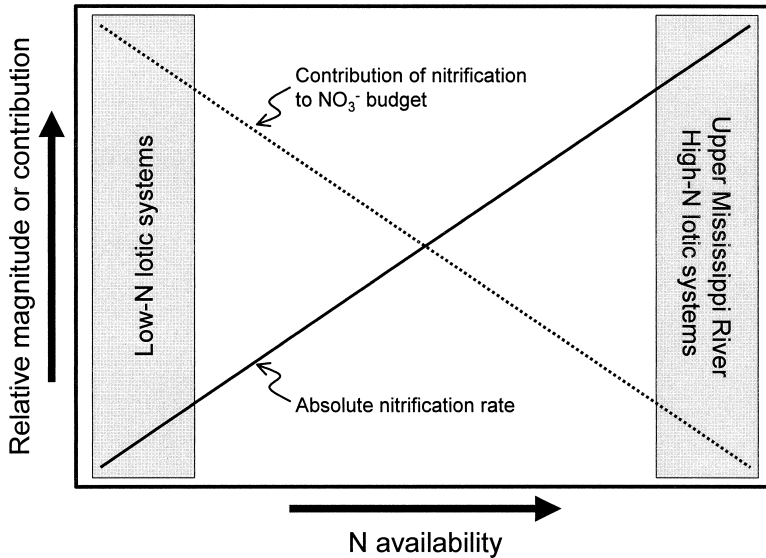
FIG. 7. Hypothesized relationship between N availability, nitrification rate, and contribution of nitrification to NO_3^- budget.

tion throughout the profile. It is important to note that we measured potential nitrification for all sediments under aerobic conditions (i.e., on a shaker table), so potential nitrification measured in the organic-poor sediment from below the measured presence of oxygen indicates an abundant population of nitrifying bacteria in the uppermost 2 cm of sediment. Nitrification potentials also showed that these microbes are readily activated when oxic conditions develop. This finding agrees with Bodelier et al. (1996), who contended that conservation of nitrifying capacity in anoxic conditions and the ability to respond quickly when oxic conditions develop are important traits of nitrifying bacteria in fluctuating oxic–anoxic environments. Such conditions may exist in oxygenated microsites, as are sometimes associated with the roots of macrophytes (Moorhead and Reddy 1988), or may be linked to transient events of oxygen penetration driven by hydrology or high primary productivity (Carlton and Wetzel 1988). Conversely, lower potential nitrification rates in the organic-rich sediment profile suggest few nitrifying bacteria were present, probably because of persistent anaerobic conditions. However, even in organic sediment, a substantial population of nitrifying bacteria may be present in a thin (1–2 mm) surficial layer that occasionally receives oxygen from overlying water and benthic primary productivity. In our measurements, the activity of

this layer could have easily been overwhelmed by inactivity of the underlying sediment and not detected with our 1-cm vertical resolution.

The results from our monitoring period and the vertical profile study suggest that temperature, sediment NH_4^+, and oxygen penetration may all be important variables influencing nitrification rates in this UMR reach. However, we would argue that sediment NH_4^+ availability is not a primary regulating factor in much of Pool 8, except for some parts of the main channel, because sediment NH_4^+ concentrations are generally high and the nutrient enrichment experiments failed to show NH_4^+ limited nitrification in any of the sediments tested. High sediment NH_4^+ availability, rather, may effectively identify areas where the potential for elevated nitrification is possible. Therefore, we contend that temperature and sediment NH_4^+ are useful in predicting broad-scale temporal and spatial nitrification patterns, respectively, but oxygen penetration into the sediments likely regulates nitrification rates in systems with high N loading like the UMR, especially in backwater and impounded areas where much of the nitrification occurs.

Intrasystem NO_3^- production

We estimated that nitrification supplies between 6.2 and 7.8% of the NO_3^- to the total

NO_3^- input in Pool 8. We are uncertain how this compares with other systems because we were unable to locate other studies that have done this calculation. We hypothesize, however, that this value is low compared to other systems (except other large rivers or other high-N systems) despite the high nitrification rates measured (Fig. 7). The large mass of NO_3^- (86,028 mt N/y) passing through this system from upriver sources simply overwhelms intrasystem NO_3^- production. Nitrification likely contributes a larger proportion of NO_3^- to the total NO_3^- budget in systems that have lower NO_3^- concentrations or transport (Fig. 7). For example, the low-N streams examined in the LINX study exhibited relatively low nitrification rates (<0.5 μg N cm^{-2} h^{-1}), yet nitrification rates explained 50% of the variability observed in stream NO_3^- concentrations, suggesting the importance of nitrification to NO_3^- budgets in small streams with relatively low N loads (Peterson et al. 2001).

Our finding that the largest proportion of pool-wide intrasystem NO_3^- production occurred in the impounded and backwater areas was not surprising because these areas also had the highest measured nitrification rates. The high NO_3^- contributions from impounded and backwater areas were aided by the fact that these areas also had the largest areal extent (45% and 24%, respectively, Table 1) among the classified aquatic habitats. Despite the high nitrification rates in backwater areas, water-column NO_3^- concentrations were generally low and often below detectable levels in these areas, implying that NO_3^- is rapidly used (via plant and microbial uptake or denitrification). If a significant proportion of this NO_3^- loss is through denitrification, the nitrification and denitrification processes are tightly coupled (especially in backwater areas) and this pathway may result in a significant loss of N from the system. Further analysis of denitrification and potential coupling between nitrification and denitrification in this system is warranted. It seems paradoxical that high production of NO_3^- in certain areas may lead to increased losses of NO_3^- and total N from the system, but this process may be true of UMR sediments.

Acknowledgements

Literature Cited

ABER, J. D., W. H. MCDOWELL, K. J. NADELHOFFER, A. MAGILL, G. BERNSTON, M. KAMAKEA, S. G. MCNULTY, W. CURRIE, L. RUSTAD, AND I. FERNANDEZ. 1998. Nitrogen saturation in temperate forest ecosystems: hypothesis revisited. BioScience 48: 921–934.

ALEXANDER, R. B., R. A. SMITH, AND G. E. SCHWARZ. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758–761.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1998. Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, Washington, DC.

BERNHARDT, E. S., R. O. HALL, AND G. E. LIKENS. 2002. Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. Ecosystems 5:419–430.

BIANCHI, M., FELIATRA, AND D. LEFEVRE. 1999. Regulation of nitrification in the land-ocean contact area of the Rhône River plume (NW Mediterranean). Aquatic Microbial Ecology 18:301–312.

BODELIER, P. L., J. A. LIBOCHANT, C. W. P. M. BLOM, AND H. J. LAANBROEK. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Applied and Environmental Microbiology 62:4100–4107.

CARACO, N. F., AND J. J. COLE. 1999. Human impact on nitrate export: an analysis using major world rivers. Ambio 28:167–170.

CARLTON, R. G., AND R. G. WETZEL. 1988. Phosphorus flux from lake sediments: effect of epipelic algal oxygen production. Limnology and Oceanography 33:562–570.

CRONK, J. K. 1996. Constructed wetlands to treat wastewater from dairy and swine operations: a review. Agriculture, Ecosystems, and Environment 58:97–114.

HÅKANSON, L., AND M. JANSSON. 1983. Principles of lake sedimentology. Springer-Verlag, New York.

HALL, G. H. 1986. Nitrification in lakes. Pages 127–156

in J. I. Prosser (editor). Nitrification. IRL Press, Oxford, UK.

HARGRAVE, B. T. 1972. Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Limnology and Oceanography 17:583–596.

HOWARTH, R. W., G. BILLEN, D. SWANEY, A. TOWNSEND, N. JAWORSKI, K. LAJTHA, J. A. DOWNING, R. ELMGREN, N. CARACO, T. JORDAN, F. BERENDSE, J. FRENEY, V. KUDEYAROV, P. MURDOCH, AND Z. L. ZHU. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry 35:75–139.

JONES, J. B., S. G. FISHER, AND N. B. GRIMM. 1995. Nitrification in the hyporheic zone of a desert stream ecosystem. Journal of the North American Benthological Society 14:249–258.

JUSTIC, D., N. N. RABALAIS, R. E. TURNER, AND W. J. WISEMAN. 1993. Seasonal coupling between riverborne nutrients, net productivity and hypoxia. Marine Pollution Bulletin 36:184–189.

KEMP, M. J., AND W. K. DODDS. 2001. Centimeter-scale patterns in dissolved oxygen and nitrification rates in a prairie stream. Journal of the North American Benthological Society 20:347–357.

KEMP, M. J., AND W. K. DODDS. 2002. Comparisons of nitrification and denitrification in prairie and agriculturally influenced streams. Ecological Applications 12:998–1009.

LUBINSKI, K. 1999. Floodplain river ecology and the concept of river ecological health. Pages (2)1–(2)12 *in* K. Lubinski (editor). Ecological status and trends of the Upper Mississippi River system 1998. Long Term Resource Monitoring Program Report. Upper Midwest Environmental Sciences Center, US Geological Survey, La Crosse, Wisconsin. (Available from: USGS-UMESC, 2630 Fanta Reed Rd, La Crosse, Wisconsin 54603 USA.)

MOORHEAD, K. K., AND K. R. REDDY. 1988. Oxygen transport through selected aquatic macrophytes. Journal of Environmental Quality 17:138–142.

NIXON, S. W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199–219.

PETERSON, B. J., W. M. WOLLHEIM, P. J. MULHOLLAND, J. R. WEBSTER, J. L. MEYER, J. L. TANK, E. MARTÍ, W. B. BOWDEN, H. M. VALETT, A. E. HERSHEY, W. H. MCDOWELL, W. K. DODDS, S. K. HAMILTON, S. GREGORY, AND D. D. MORRALL. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

PITMAN, J. 1993. Probability. Springer-Verlag, New York.

RABALAIS, N. N. 2002. Nitrogen in aquatic ecosystems. Ambio 31:102–112.

RABALAIS, N. N., R. E. TURNER, W. J. WISEMAN, AND Q. DORTCH. 1998. Consequences of the 1993 Mississippi River flood in the Gulf of Mexico. Regulated Rivers: Research and Management 14:161–177.

SOBALLE, D. M., J. R. FISCHER, L. A. HODGE-RICHARDSON, AND T. L. CLEMMENT. 2002. Limnological monitoring on the Upper Mississippi River system, 1993–1996: Long Term Resource Monitoring Program Onalaska Field Station. Upper Midwest Environmental Sciences Center, US Geological Survey, La Crosse, Wisconsin. (Available from: USGS-UMESC, 2630 Fanta Reed Rd, La Crosse, Wisconsin 54603 USA.)

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14:799–801.

STRAUSS, E. A. 2000. The effects of organic carbon and nitrogen availability on nitrification rates in stream sediments. PhD Thesis, University of Notre Dame, Notre Dame, Indiana.

STRAUSS, E. A., AND W. K. DODDS. 1997. Influence of protozoa and nutrient availability on nitrification rates in subsurface sediments. Microbial Ecology 34:155–165.

STRAUSS, E. A., AND G. A. LAMBERTI. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography 45:1854–1859.

STRAUSS, E. A., N. L. MITCHELL, AND G. A. LAMBERTI. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Canadian Journal of Fisheries and Aquatic Sciences 59:554–563.

STENSTROM, M. K., AND R. A. PODUSKA. 1980. The effect of dissolved oxygen concentration on nitrification. Water Research 14:643–649.

TRISKA, F. J., J. H. DUFF, AND R. J. AVANZINO. 1990. Influence of exchange flow between the channel and hyporheic zone on nitrate production in a small mountain stream. Canadian Journal of Fisheries and Aquatic Sciences 47:2099–2111.

WASLEY, D. M. 2000. Concentration and movement of nitrogen and other materials in selected reaches and tributaries of the Upper Mississippi River system. MS Thesis, University of Wisconsin-La Crosse, La Crosse, Wisconsin.

WATSON, S. W., E. BOCK, H. HARMS, H.-P. KOOPS, AND A. B. HOOPER. 1989. Nitrifying bacteria. Pages 1808–1834 *in* J. T. Stanley (editor). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Maryland.

WILCOX, D. B. 1993. An aquatic habitat classification system for the Upper Mississippi River system. LTRMP Technical Report No. 93-T003. US Geological Survey Environmental Management Technical Center, Onalaska, Wisconsin.