

## **Sediment-Water Nitrogen Fluxes in a Backwater System of the Upper Mississippi River**

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**PURPOSE:** This research quantifies nitrogen (N) fluxes and transformations at the sediment-water interface in relation to N loading and uptake for a backwater system of the Upper Mississippi River. Goals of this work were to provide a better understanding of the roles that backwater sediments play in uptake and removal of N for purposes of determining the management potential of increasing hydrological connectivity between large rivers and adjacent backwaters, wetlands, and the floodplain to enhance overall N removal efficiency.

**BACKGROUND:** Accelerated nitrogen (N) loading (primarily in the form of nitrate) from agriculturally dominated watersheds has been linked to water quality impairment of N-sensitive coastal and estuarine habitats (Rabalais et al. 1994, Nixon 1995, Vitousek et al. 1997). In addition to reducing N runoff through best management practices, there is a need to promote in-stream retention of N via biological uptake, transformation, and denitrification in order to reduce N eutrophication (Mitsch et al. 2001, Seitzinger et al. 2002). While the floodplain, wetlands, and backwaters of large rivers offer the potential for increasing in-stream processing of N, regulation of pool elevation has led to decreased hydrological connectivity and load routing between the main channel and adjacent backwater areas (Richardson et al. 2004). Unfortunately, N uptake efficiency is typically low in the main channel of large rivers due to higher velocities and lack of suitable substrate and habitat for N processing (Seitzinger 1988, Richardson et al. 2004). However, it can increase to greater than 40 percent in connected backwaters (James et al. 2006a), wetland complexes (Phipps and Crumpton 1994, Spieles and Mitsch 2000), and diversion structures (Lane et al. 2004). Thus, increasing flow and N loading to these aquatic habitats in large river systems may be an effective management strategy in combination with watershed BMPs for reducing overall N transport to coastal ecosystems.

More information is needed regarding inorganic N processing in backwaters in order to develop sound engineering strategies for optimizing N retention capacity and efficiency in larger river systems. In particular, the capacity for sediment and water column biological uptake needs to be evaluated in relation to N delivery and residence time in backwater systems. The importance of bacterial nitrification, which results in additional internal inputs of nitrate to the system, and denitrification, which acts as a permanent sink for N, needs to be placed within a budgetary framework that includes inflow-outflow analysis and diffusive fluxes into and out of the sediment for improved understanding of the importance of N flux at the sediment-water interface in N cycling and retention in backwaters. The objectives of this study were to expand earlier

findings (James et al. 2006a) with greater attention to quantification of diffusive exchanges of nitrate nitrite-N and ammonium-N between the sediment and water column in relation to N delivery and uptake for a shallow backwater lake on the Upper Mississippi River that receives controlled flows via a gated culvert.

## METHODS

**Study site.** Third Lake is a part of the interconnected Finger Lakes backwater system (Clear, Lower Peterson, Schmokers, Third, Second, and First Lakes) located in navigation pool 5, immediately downstream of the Lock and Dam 4 dike on the Upper Mississippi River (Figure 1; Johnson et al. 1998). It is shallow (mean depth = 0.6 m and maximum depth = 2.0 m at the nominal pool elevation of 201.2 m MSL) with a surface area of 11 ha and a volume of 67,500 m<sup>3</sup>. Dense stands of submersed and emergent aquatic plants, dominated by *Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Nymphaea odorata*, cover ~60 percent of the lake's surface area (James et al. 2006a). Controlled flows into the lake are via a 0.9-m-diam culvert fitted with an adjustable vertical slide gate. Culvert flow can be controlled between 0 and 1.6 m<sup>3</sup>·s<sup>-1</sup>. In 2004, culvert flow was maintained at ~0.6 m<sup>3</sup>·s<sup>-1</sup>, which resulted in a mean theoretical water residence time of ~1.7 days.

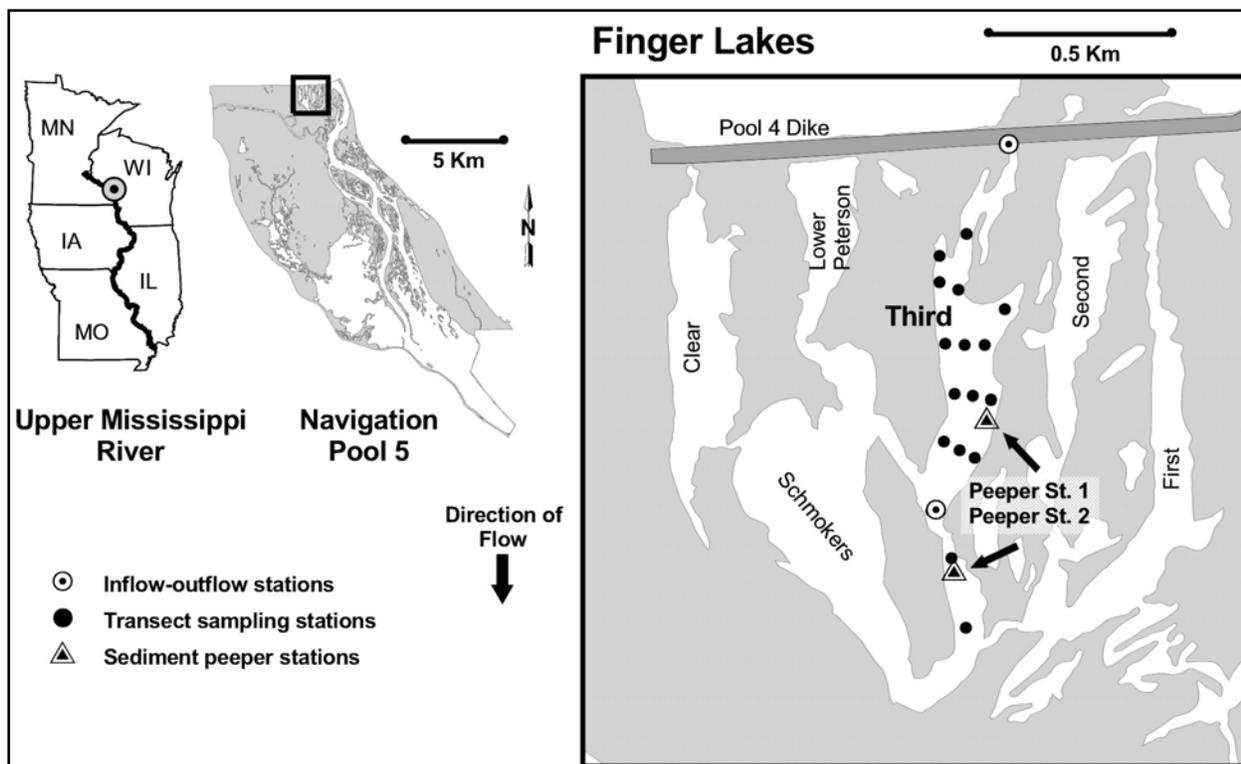


Figure 1. Map of Third Lake showing sampling station locations.

Constituent loads to the lake are regulated to a large extent by source water concentrations from the Mississippi River when culvert flow is held at a constant rate. Nitrogen species entering the lake are dominated by nitrate-nitrite-N (>70 percent of the total N). The lake also exhibits high

chlorophyll and phosphorus concentrations (chlorophyll =  $55 \text{ mg}\cdot\text{m}^{-3}$ ; total P =  $0.082 \text{ mg}\cdot\text{L}^{-1}$ ; soluble reactive P =  $0.041 \text{ mg}\cdot\text{L}^{-1}$ ).

**Hydrology and N loading.** Flow and loading determinations are detailed in James et al. (2006a). Water samples were collected at the culvert inflow and the outflow at weekly to biweekly intervals between May and October 2004 for determination of N species (Figure 1; see *Chemical Analyses* below). Culvert flow was measured using a Flo-Mate model 2000 velocity meter (Marsh-McBirney Inc., Fredrick, MD). For inflow-outflow budgetary analysis, culvert loading of total and soluble constituents was calculated as the product of flow and concentration. Outflow constituent discharge was estimated as the product of culvert flow (i.e., culvert inflow ~ water discharge from the lake) and concentration measured at the outflow station. Uptake capacity ( $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ; positive rate) or export (negative rate) of constituent loads was calculated as inflow load minus outflow discharge. Uptake efficiency (%) was calculated as net uptake capacity divided by culvert load and multiplied by 100.

**Close-interval water column sampling.** Additional sampling stations were established near the middle of the lake and immediately downstream of the outflow for examination of water column N gradients (Figure 1). Nominal water column depth to the sediment interface was ~0.7 m and 0.4 m at stations 1 and 2, respectively. At monthly intervals between June and October, water samples were collected at 0.1-m depth intervals in the water column at each station for determination of N species. The samples were collected with a peristaltic pump and small diameter weighted tubing. The sample was pumped directly into a syringe and filtered through a  $0.45\text{-}\mu\text{m}$  syringe filter for later analysis of soluble constituents. Temperature, dissolved oxygen, pH, and specific conductivity were measured in situ at the same depth intervals using a Hydrolab Quanta water quality meter (Hach Co., Loveland, CO) that was precalibrated against known standards.

**Continuous and spatial in situ monitoring.** Water temperature, dissolved oxygen, and pH were monitored in situ approximately 5 cm above the sediment interface at station 2 between May and October 2004 using recording water quality monitors (YSI Model 6600; Yellow Springs Instruments, Yellow Springs, OH). The monitor was deployed by fastening it to a fixed brace on a 1.5-in.-diam PVC pipe that had a cap glued to the top end (Figure 2). The PVC pipe and attached monitor could then be slipped over a 0.75-in.-diam anchoring iron pipe to position the dissolved oxygen probe exactly 5 cm above the sediment interface. A hole was drilled into the end cap for air displacement as the PVC pipe was slipped over the anchoring pipe. At biweekly intervals the deployed monitor was removed for servicing and replaced with a precalibrated monitor. Monitors were post-calibrated and cleaned in the laboratory. The dissolved oxygen membrane and electrolyte solution were replaced and the pH probe was cleaned before calibration and redeployment.

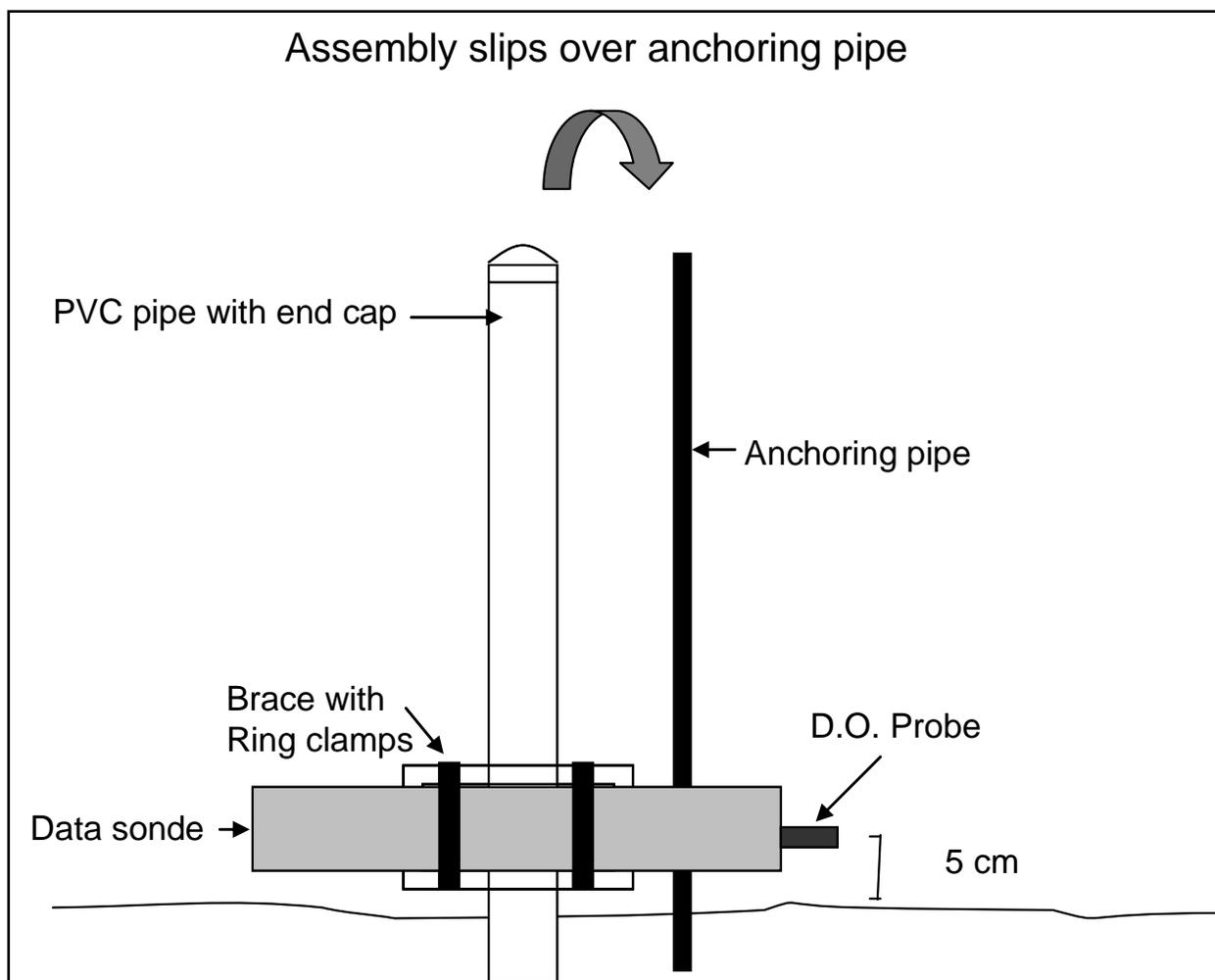


Figure 2. Methods of positioning continuously recording data sondes above the sediment interface.

As part of another study (James et al. 2006a), discrete in situ measurements of temperature, dissolved oxygen, pH, and specific conductance were collected at biweekly intervals between May and October. Measurements were made at the lake surface and 0.1 m above the sediment surface at the station locations shown in Figure 1. Spatial analysis of variations in bottom water dissolved oxygen concentration was performed using kriging statistical methods (SURFER version 8.0, Golden Software, Golden, CO).

**Sediment oxygen demand.** Nine intact 3.81-cm-diam sediment cores were collected at station 2 in September 2004 for determination of sediment oxygen demand (SOD) using a modification of methods described in Plumb (1981). In the laboratory, the upper 2 cm of the sediment core were placed on a trivet inside a 1-liter glass beaker (10-cm diam by 20-cm height). The trivet held the sediment core 3 cm above a magnetic stir bar that was placed in the bottom of the beaker to provide circulation during SOD experiments. Very small magnetic stir bars (10-mm length by 3-mm diam) were used to create gentle circulation in each system to minimize enhanced dissolved oxygen diffusion into the sediment. Individual beakers containing a sediment dish, trivet, and magnetic stir bar were slowly filled with 0.75 L of filtered (Gelman A/E glass

fiber; nominal pore size = 2  $\mu\text{m}$ ) lake water that had been pre-equilibrated with the atmosphere and placed on a magnetic stir motor in an environmental chamber (Figure 3). Dissolved oxygen concentration at the start of each SOD experiment was between 8.0 and 8.5  $\text{mg}\cdot\text{L}^{-1}$ .



Figure 3. Methods for measuring sediment oxygen demand.

A YSI Model 6600 data sonde, fitted with a temperature and dissolved oxygen probe, was inserted into each beaker so that no airspace was left in the system, and was secured in the environmental chamber with a stand and clamp (Figure 3). The junction between the sonde and beaker was sealed with silicon grease and a rubber gasket to prevent atmospheric reaeration during experiments. The dissolved oxygen probe was positioned 5 cm above the sediment interface for all SOD determinations. Prior to each experiment, dissolved oxygen probes were pre-calibrated against known Winkler titrations. Temperature and dissolved oxygen were monitored at 10-minute intervals in each sediment system for a period of 1000 to 3000 minutes (i.e., 0.7 to 2.0 days).

Rates of dissolved oxygen depletion were calculated as the change in dissolved oxygen mass over the linear range of depletion, divided by time and the area of the sediment dish ( $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). Control systems that did not contain sediment were also run in order to account for oxygen demand in the water column. SOD was calculated as the rate of dissolved oxygen depletion in the sediment systems minus the rate of dissolved oxygen depletion in the control systems.

**In situ sediment porewater dynamics.** N gradients in the sediment porewater were examined in situ at stations 1 and 2 in Third Lake using sediment peeper dialysis techniques. The peepers were deployed in triplicate at each station in June, July, August, September, and October 2004. Each peeper consisted of 12 water-filled chambers spaced at 2-cm intervals that were covered with a dialysis membrane (2.0- $\mu\text{m}$  pore size). Prior to deployment, each chamber was filled with nitrogen-purged distilled water and the assembled peepers were placed in a nitrogen-purged water bath to maintain anoxic conditions during transport to Third Lake. The peepers were gently pushed into the sediment so that at least six chambers were exposed to porewater; they were allowed to equilibrate for a minimum of 14 days. Upon retrieval, samples were immediately collected from each chamber, filtered through a 0.45- $\mu\text{m}$  membrane filter in the field, and stored on ice for transport back to the laboratory. Samples were analyzed for ammonium-N and nitrate nitrite-N. Fickian diffusional fluxes ( $J$ ) for ammonium and nitrate across the sediment interface were estimated as  $J = -\Phi\cdot D\cdot\theta^2\cdot(dC/dx)$  where  $\Phi$  is the porosity (0.89) of the sediment,  $D$  is the areal sediment diffusion coefficient ( $1.9 \times 10^{-5} \text{ cm}^2\cdot\text{s}^{-1}$  for nitrate and  $1.98 \times 10^{-5} \text{ cm}^2\cdot\text{s}^{-1}$  for ammonia; Li and Gregory 1974),  $\theta^2$  is the sediment tortuosity (Ullman and Aller 1982, Sweerts et al. 1991), and  $dC/dx$  is the N gradient across the sediment-water interface over distance  $x$ . The 2-cm distance between chambers was used as the diffusional path distance for  $J_{\text{NH}_3}$  determination. For  $J_{\text{NO}_3}$ , sharp nitrate gradients generally occur over  $< 2$  mm distance (Jensen et al. 1993), versus the 2-cm vertical resolution of the peepers. Thus, the nitrate concentration in the overlying water immediately above the sediment-water interface was used with a 2-mm diffusional path distance to calculate  $J_{\text{NO}_3}$ .

**Sediment nitrate and ammonium nitrogen fluxes.** Twenty sediment cores were collected in the vicinity of station 2 in September for determination of rates of nitrate nitrite-N and ammonium-N flux under oxic and anoxic conditions and as a function of various initial nitrate concentrations. Acrylic core liners (6.5-cm inner diameter and 50-cm length) were gently pushed into the sediment to collect intact sediment cores (undisturbed). The core liners, containing both

sediment and overlying water, were sealed with caps and stored in a protective box until analysis the next day. Additional lake water was collected for later incubation with the collected sediment.

In the laboratory, the cores were carefully drained of overlying water and the upper 10 cm of sediment was immediately transferred intact to a smaller acrylic core liner (6.5-cm diam and 20-cm height) using a core remover tool. Three hundred mL of filtered lake water (Gelman A-E glass fiber filter) was siphoned onto the sediment in the smaller acrylic core liner without causing sediment resuspension. Sediment incubation systems consisted of the upper 10 cm of sediment and filtered overlying water contained in acrylic core liners that were sealed with rubber stoppers. They were placed in a darkened environmental chamber and incubated at a constant temperature of 20 °C. The oxidation-reduction environment in each system was controlled by gently bubbling either air (oxic; 10 replicates) or nitrogen (anoxic-10 replicates) through an air stone placed just above the sediment surface. Bubbling action ensured complete mixing of the water column but did not disrupt the sediment interface.

The same 20 sediment incubation systems were used to determine rates of nitrate nitrite-N and ammonium-N flux as a function of the following initial nitrate ( $\text{KNO}_3$ ) concentrations in the overlying water; 0.1, 1, 2, 4, and 8  $\text{mg}\cdot\text{L}^{-1}$ . All systems were spiked with nitrate standard to achieve a target initial nitrate concentration. James et al. (2006a) found that organic carbon supply was not limiting nitrification-denitrification in sediments of Third Lake, so supplemental organic carbon was not added to the incubation systems. They were then sampled at 0.5, 4, 20, 28, 44, and 68 hours by removing 7 mL from the center of each system and immediately filtering the sample through a 0.45- $\mu\text{m}$  syringe filter (Nalge). The water volume removed from each system during sampling was replaced by addition of filtered lake water preadjusted to the proper oxidation-reduction condition. The systems were then carefully drained and refilled with fresh filtered water for the next experiment. Experiments were run consecutively over an 18-day period. Rates of nitrate nitrite-N and ammonium-N flux from sediments ( $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) were calculated as the linear change in concentration in the overlying water divided by time and the area of the incubation core liner. Differences in diffusive flux under oxic and anoxic conditions were interpreted to estimate rates of nitrification and denitrification (see *Results*).

**Chemical and statistical analyses.** Samples for total and total soluble N determination were digested with potassium persulfate according to Ameal et al. (1993) prior to analysis. Chemical analyses for total N, nitrate nitrite-N, and ammonium-N were performed on a Lachat QuikChem A/E using standard procedures (American Public Health Association (APHA) 1998). Particulate organic N (PON) was calculated as the difference between total and total soluble N. Dissolved organic N was calculated as total soluble N minus the sum of nitrate nitrite-N and ammonium-N. Statistical regression and t-test analyses were performed using the software package Statistical Analysis System (SAS) (SAS 1994).

## RESULTS

**Loading patterns and water column N gradients.** During the summer, N loading varied as a function of seasonal variations in source water N concentration because culvert flows were held constant to maintain a theoretical water residence time of ~ 1.7 days (Figure 4). Total N was low in May and exhibited concentration peaks in June and late July, which were associated with watershed runoff from the Upper Mississippi River system. Nitrate nitrite-N was the dominant N species in the inflow, accounting for 70 percent of the mean summer total N. In contrast, ammonium-N concentrations in the inflow were low. PON and DON represented 15 percent each of the total N (not shown), while ammonium concentrations in the inflow were negligible. Third Lake was a sink for total N, PON, and nitrate nitrite-N and a source for ammonium-N and DON (Table 1). Nitrate nitrite-N uptake efficiency was 41 percent, while the equivalent of over four times the inflow ammonium-N load was exported from the lake.

Nitrate nitrite-N was also the dominant N form in the water column at stations 1 and 2 in Third Lake and it fluctuated seasonally in relation to both inflow concentration and distance from the culvert (Figure 5). Periods of higher inflow nitrate nitrite-N concentration occurred in June and October in conjunction with higher loading. As loading decreased in July through September, water column concentrations declined at both stations. Gradients of decreasing water column concentration from inflow > station 1 > station 2 were observed throughout the study period, indicating net uptake of nitrate nitrite-N. Vertical gradients in nitrate nitrite-N in the water column were usually minor at both stations.

Ammonium-N concentrations accounted for a minor portion of the total N in the water column. They were low to negligible in the inflow and there was a trend of increasing concentration at stations 1 and 2 as flows moved through the system. Concentration maxima occurred at each station in September and October. Vertical water column gradients of increasing concentration toward the sediment interface were also observed during several months of the summer, suggesting some net flux out of the sediment. DON represented an intermediate portion of the total N concentration and it was nearly constant vertically in the water column. PON often exhibited peaks near the lake surface and was seasonally variable at both stations. PON generally declined in concentration, while DON increased from inflow > station 1 > station 2 > outflow.

**Dissolved oxygen dynamics and sediment oxygen demand.** Anoxia (defined as dissolved oxygen concentrations less than  $1 \text{ mg}\cdot\text{L}^{-1}$  at a depth of 0.1 m above the sediment interface) developed in several areas of Third Lake between late June and mid-September (Figure 6). Anoxic conditions developed in the eastern embayment of the lake in late June and persisted until September. Bottom water anoxia developed in the middle portion of the lake and accounted for ~ 30 percent of the lake surface area in late June and late July through early August.

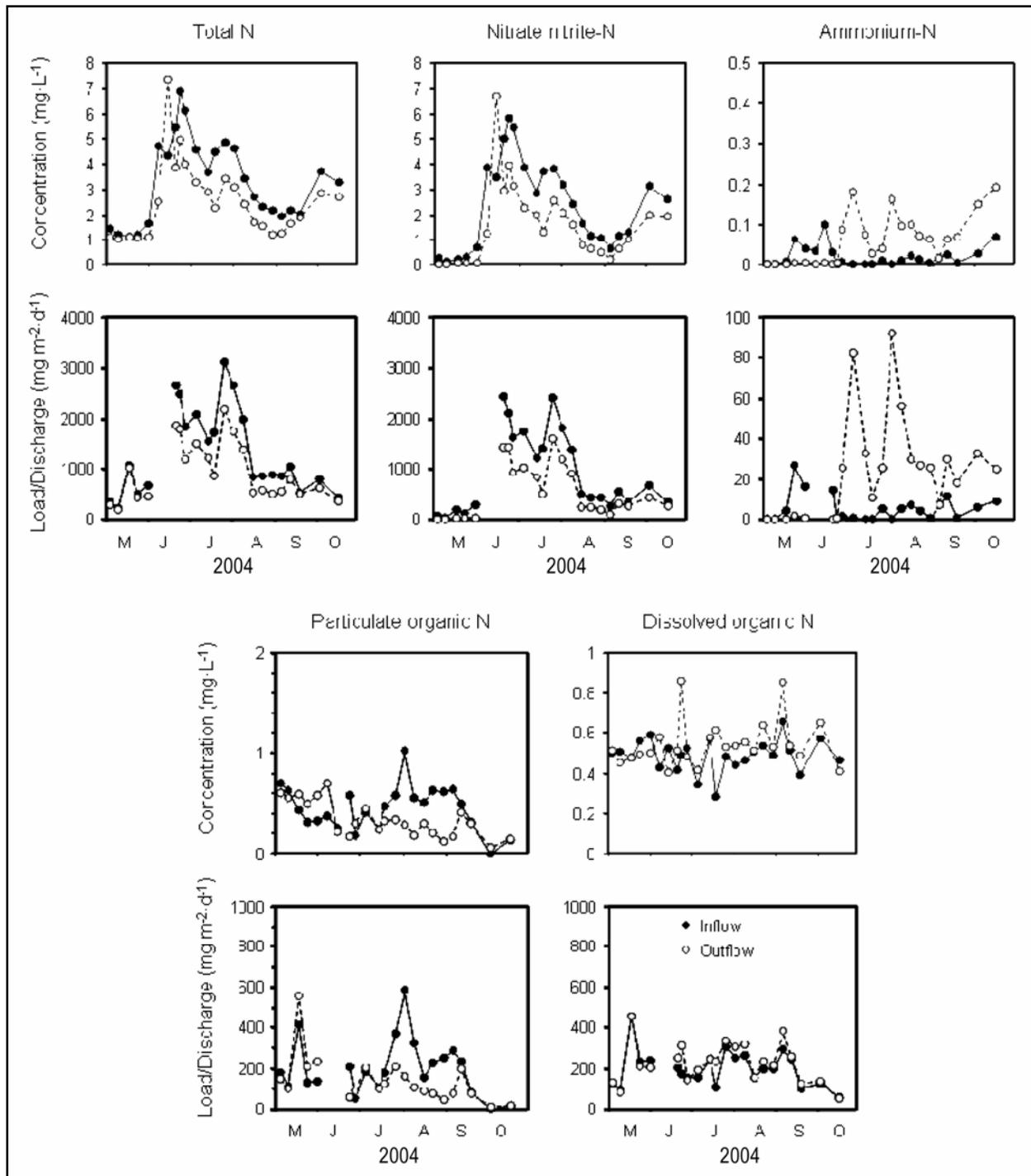


Figure 4. Seasonal variations in concentration (upper panels) and loading or discharge (lower panels) in Third Lake for different nitrogen (N) species.

<b>Table 1</b> <b>Loading and discharge fluxes of various nitrogen (N) species to Third Lake during the period May through October 2004. NO<sub>3</sub>NO<sub>2</sub>-N = nitrate-nitrite-N. NH<sub>4</sub>-N = ammonium-N, PON = particulate organic N, DON = dissolved organic nitrogen.</b>				
<b>Variable</b>	<b>Culvert Loading (mg·m<sup>-2</sup>·d<sup>-1</sup>)</b>	<b>Discharge (mg·m<sup>-2</sup>·d<sup>-1</sup>)</b>	<b>Net uptake/export (mg·m<sup>-2</sup>·d<sup>-1</sup>)</b>	<b>Net uptake/export (%)</b>
Total N	1325	936	389	29.4
NO <sub>3</sub> NO <sub>2</sub> -N	927	544	383	41.3
NH <sub>4</sub> -N	5.5	23.9	-18.4	-434.5
PON	201	112	89	44.3
DON	200	227	-27	-113.5

Dissolved oxygen concentrations above the sediment interface fluctuated seasonally and on a diel basis at station 2 (Figure 7). During July and early August, concentrations declined below 1 mg·L<sup>-1</sup> at night on several dates, indicating anoxic conditions under the macrophyte canopy. The macrophyte canopy had reached the lake surface in July (aerial surface coverage near 90 to 100 percent in most areas) and mean biomass was 58 g·m<sup>-2</sup> (± 11.2 S.E.; James et al. 2006a). In mid- to late August, dissolved oxygen concentrations remained below 1 mg·L<sup>-1</sup> for a period of ~ two weeks. Concentrations increased in September through October in conjunction with the onset of macrophyte senescence and breakup of the canopy.

Dissolved oxygen depletion in sediment incubation systems exhibited a biphasic pattern (Figure 8). Depletion was approximately linear between 8 and 4 mg·L<sup>-1</sup> and declined in a nonlinear pattern as the dissolved oxygen concentration fell below 4 mg·L<sup>-1</sup>. The mean sediment oxygen demand (corrected for water column demand) was 0.861 g m<sup>-2</sup> d<sup>-1</sup> (±0.078 S.E.).

**Porewater N dynamics.** At both stations, strong gradients in ammonium-N concentrations, measured in situ using sediment peepers, occurred at the sediment-water interface on all dates (Figure 9). Mean concentrations were lowest in the water column above the interface. However, seasonal increases in ammonium-N were observed immediately above the interface in August through October at both stations, indicating diffusive flux out of the sediment. Mean concentrations increased by one to two orders of magnitude in the sediment porewater at a depth of 1 cm below the interface. Peaks in mean porewater concentration typically occurred 3-5 cm below the sediment interface and they declined in a linear pattern below these depths. Mean porewater concentration peaks ranged between ~3 and 4.5 mg·L<sup>-1</sup> and tended to be lowest in June with higher concentrations occurring in July through October. When integrated over the upper 12-cm sediment depth (i.e., mg NH<sub>4</sub>-N porewater·m<sup>-2</sup> over the upper 12 cm), porewater ammonium-N exhibited a net increase of 1.2 and 1.7 mg·m<sup>-2</sup>·d<sup>-1</sup> between June and August for stations 1 and 2, respectively.

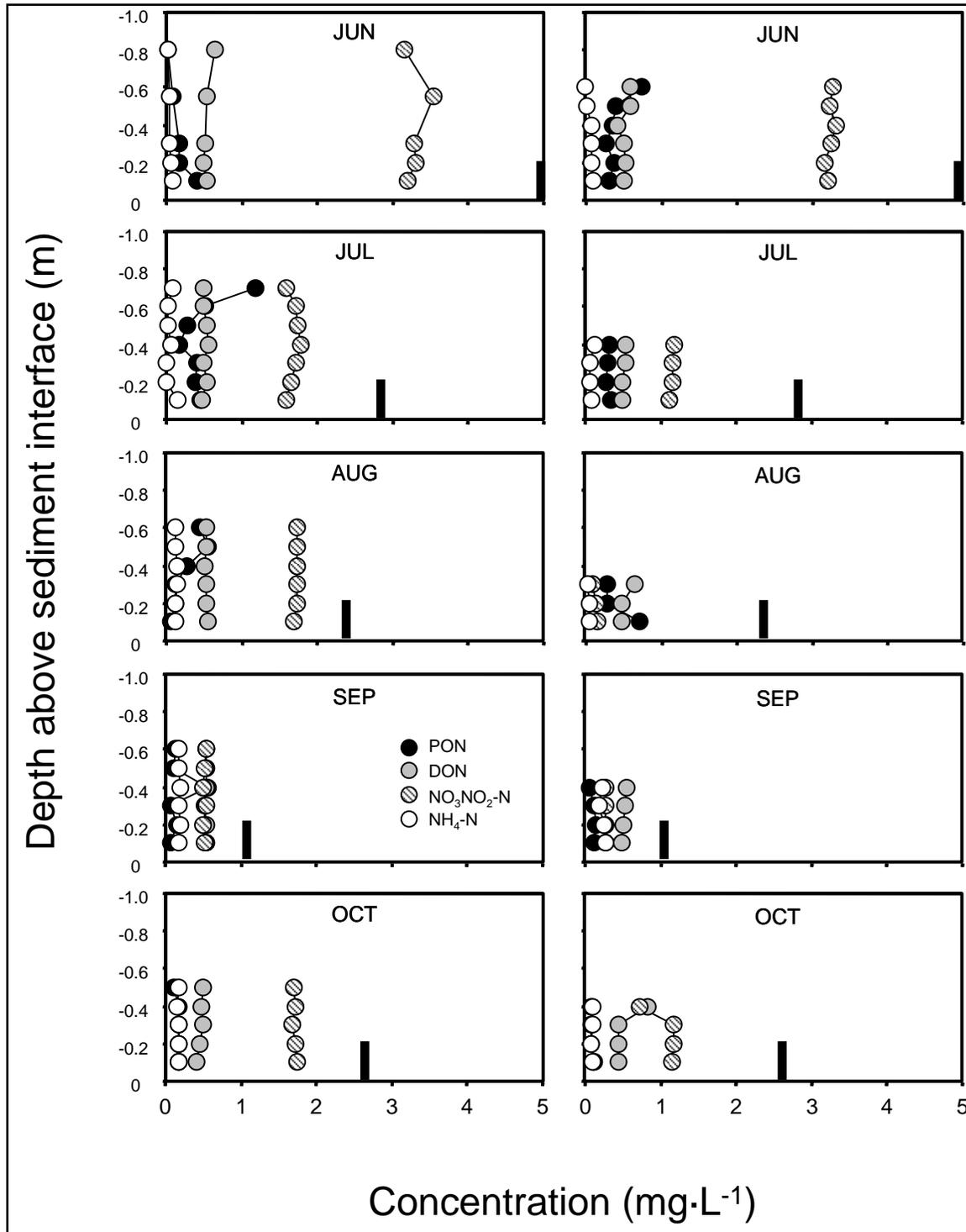


Figure 5. Seasonal and vertical variations in particulate organic nitrogen (PON), dissolved organic nitrogen (DON), nitrate nitrite nitrogen (NO<sub>3</sub>NO<sub>2</sub>-N), and ammonium nitrogen (NH<sub>4</sub>-N) at Stations 1 and 2. Vertical bar denotes the nitrate nitrite nitrogen concentration in the inflow to the lake.

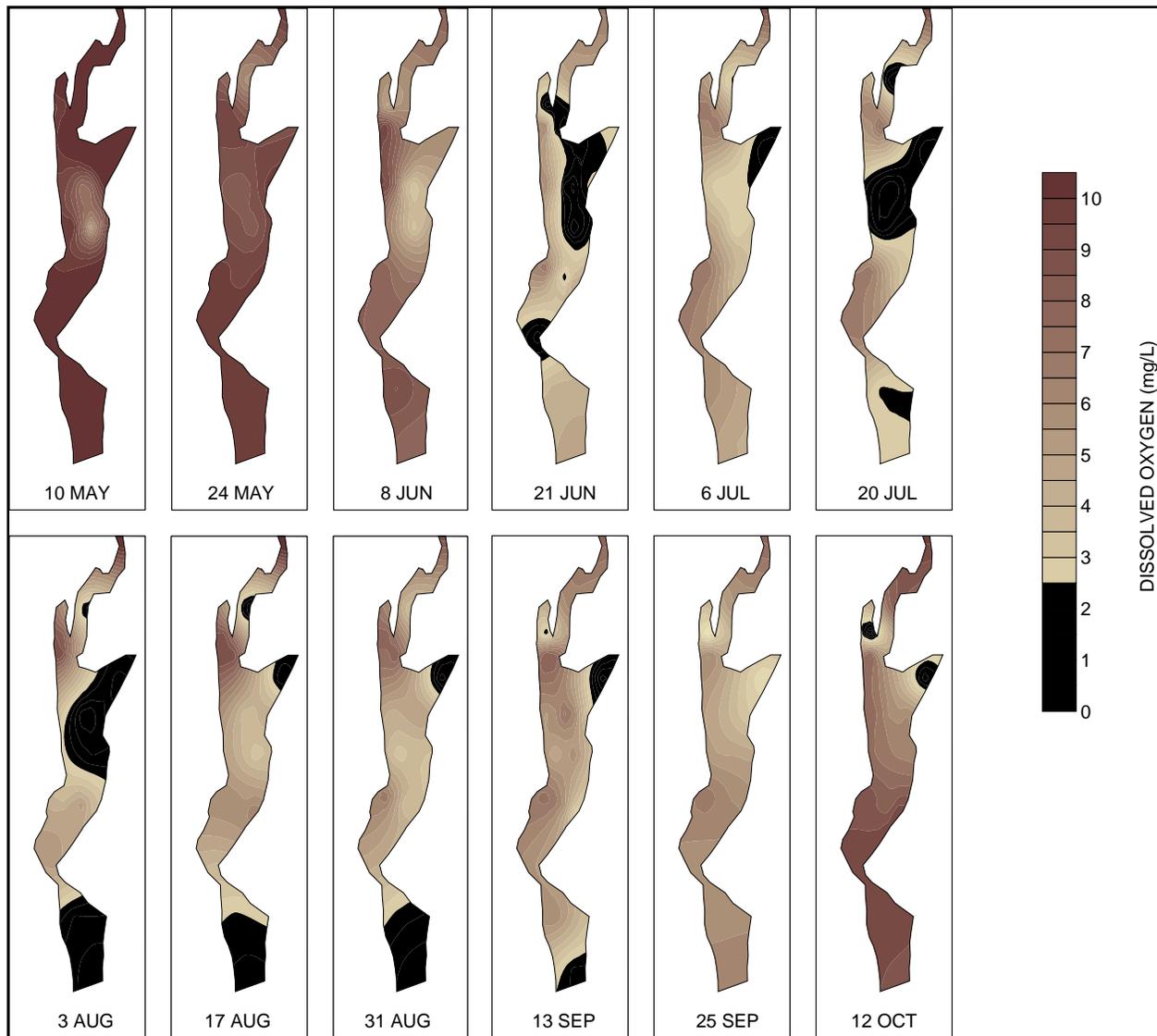


Figure 6. Spatial variations in bottom water dissolved oxygen contours for Third Lake. Black areas represent regions in the lake where dissolved oxygen concentrations were less than 2 mg·L<sup>-1</sup>.

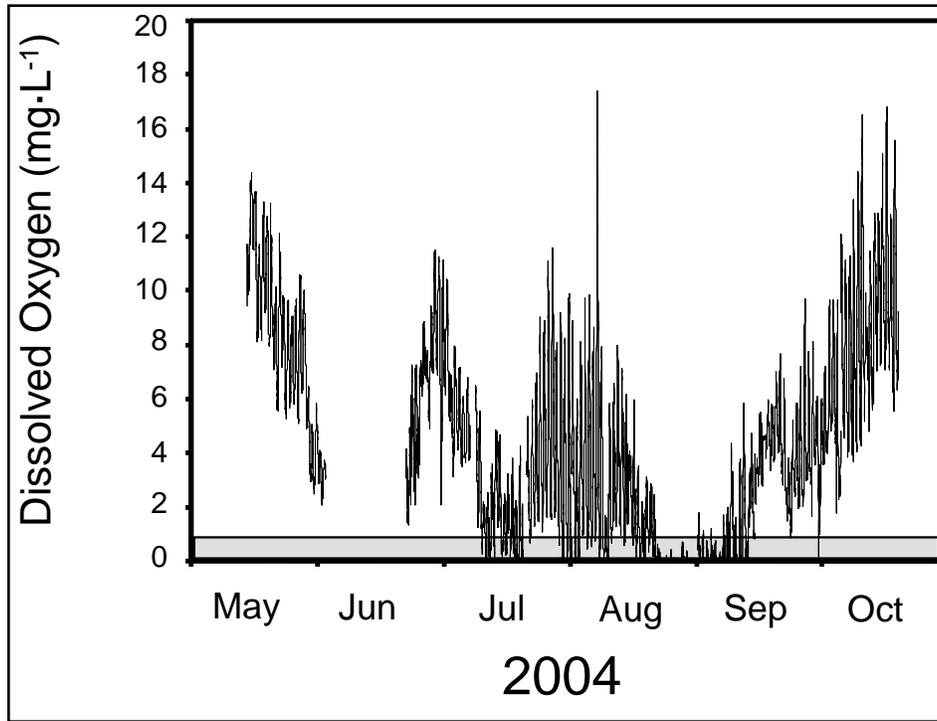


Figure 7. Seasonal and diel variations in dissolved oxygen 5 cm above the sediment surface at Station 2. The grey horizontal bar in panel (b) represents dissolved oxygen concentrations less than 1 mg·L<sup>-1</sup>.

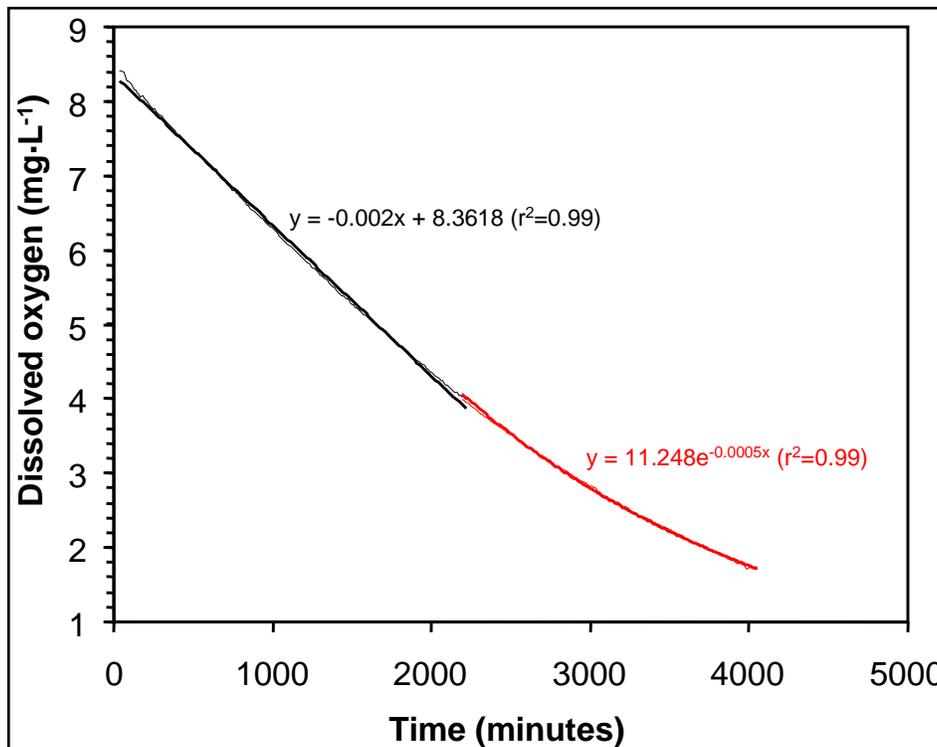


Figure 8. Changes in dissolved oxygen versus time in a sediment oxygen demand chamber containing sediment from Third Lake Station 2. The rate of dissolved oxygen depletion was estimated over the linear (black line) portion of the graph.

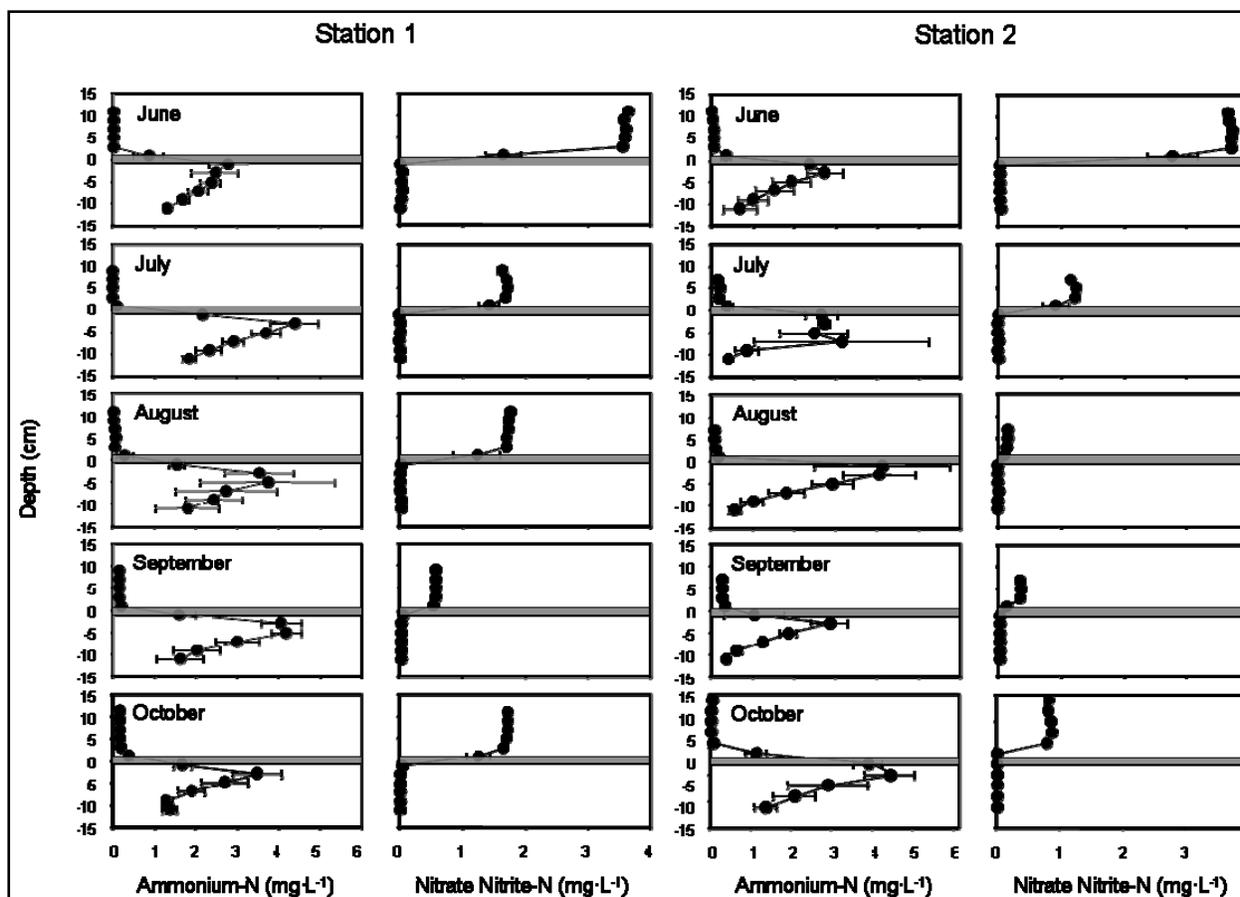


Figure 9. Seasonal and vertical variations in mean (n=3; horizontal bars represent 1 standard error) ammonium-N and nitrate nitrite-N concentrations in the overlying water column and sediment porewater at Stations 1 and 2 (grey horizontal bar represents the location of the sediment-water interface).

Nitrate nitrite-N exhibited the opposite pattern at both stations, with greatest mean concentrations in the water column, strong negative gradients at the sediment interface, and negligible concentrations in the sediment porewater (Figure 9). Unlike ammonium-N, variations in concentration gradients were a function of inflow sources of nitrate nitrite-N and uptake along the longitudinal axis of Third Lake. Mean concentrations were greatest in the water column immediately above the sediment interface in conjunction with periods of higher loading in June and October at both stations. As loading decreased in July through September, mean concentrations declined above the sediment interface and concentration gradients diminished at the sediment-water interface due to diffusive flux into the sediment and biological processing. Distance from inflow loads had an impact on concentration gradients during this period as they were lower at station 2, versus station 1. This pattern indicated that diffusive flux and biological uptake of nitrate nitrite-N at upstream locations were leading to limitation of nitrate delivery to sediments as loads moved downstream. Mean nitrate Fickian flux into sediments was greatest at station 1 due to its close proximity to inflow loads and much lower at peeper station 2 (Table 2). In contrast, mean ammonium Fickian flux out of sediments was greatest at peeper station 2, coincident with longer periods of anoxia in this region.

<b>Table 2</b> <b>Mean (n=30) Fickian diffusional fluxes of nitrate into and ammonium out of the sediment between June and October 2004.<sup>1</sup></b>		
<b>(a)</b> <b>Station</b>	<b>(b)</b> <b>Nitrate (mg·m<sup>-2</sup>·d<sup>-1</sup>)</b>	<b>(c)</b> <b>Ammonium (mg·m<sup>-2</sup>·d<sup>-1</sup>)</b>
1	94.8 (12.8)*	10.8 (1.0)*
2	56.9 (18.8)*	17.3 (2.6)*
Both	75.9 (11.7)	17.0 (1.5)

<sup>1</sup> Fluxes in column (c) represent nitrate nitrite-N diffusional fluxes calculated over a 1-mm diffusional gradient (see *Discussion*). Asterisks indicate significant differences ( $p < 0.05$ ) in fluxes between stations 1 and 2 (t-test; SAS 1994).

**Sediment core N release experiments.** Mean rates of nitrate nitrite-N diffusive flux into the sediment (i.e., negative flux) increased as a function of increasing initial nitrate nitrite-N concentration in the overlying water (Figure 10). The relationship between these variables was linear over the 0-4 mg·L<sup>-1</sup> initial nitrate nitrite-N concentration range. At the highest initial concentration, the mean diffusive rate leveled off in a nonlinear pattern. Mean diffusive rates were also greater under anoxic than under oxic conditions, particularly at initial nitrate nitrite-N concentrations above 0.1 mg·L<sup>-1</sup>. In contrast to nitrate nitrite-N diffusive flux, mean rates of ammonium-N diffusive flux out of the sediment (i.e., positive flux) were constant throughout the range of initial nitrate nitrite-N concentrations. In addition, mean ammonium-N diffusive flux was greatest under anoxic conditions.

Because anoxic conditions inhibited bacterial nitrification in these experiments, the difference between the anoxic and oxic ammonium-N diffusive flux was assumed to approximate the rate of bacterial nitrification. The mean ammonium-N diffusive flux over all treatments was 16.9 mg·m<sup>-2</sup>·d<sup>-1</sup> ( $\pm 3.1$  SE;  $n = 38$ ) and 55.4 mg·m<sup>-2</sup>·d<sup>-1</sup> ( $\pm 3.3$  SE;  $n = 38$ ) under oxic and anoxic conditions, respectively, indicating a nitrification rate of 38.5 ~ mg·m<sup>-2</sup>·d<sup>-1</sup> ( $\pm 6.4$  S.E.). This rate was equivalent to ~25 percent of the laboratory-derived sediment oxygen demand (0.861 g·m<sup>-2</sup>·d<sup>-1</sup>  $\pm 0.078$  S.E.), assuming that 4.3 g of oxygen are required to oxidize one gram of ammonium-N via bacterial nitrification (Kadlec and Knight 1996).

It was assumed that the rate of nitrate nitrite-N diffusive flux into the sediment under anoxic conditions approximated the gross denitrification rate of external nitrate sources in the absence of internal nitrate inputs derived from nitrification of sediment ammonium-N (i.e., no coupled nitrification-denitrification). The rate of nitrate nitrite-N diffusive flux under oxic conditions approximated the net denitrification rate and was lower than the gross denitrification rate due to the input of additional nitrate nitrite-N sources from bacterial nitrification. Lakewide average gross and net denitrification rates were estimated from laboratory-derived relationships between sediment core rates versus initial nitrate nitrite-N concentration (Figure 10) and inflow concentrations of nitrate nitrite-N to Third Lake (Figure 4). The mean summer gross and net denitrification rates were 122.0 ( $\pm 12.3$  S.E.) and 63.4 mg·m<sup>-2</sup>·d<sup>-1</sup> ( $\pm 8.3$  S.E.), respectively.

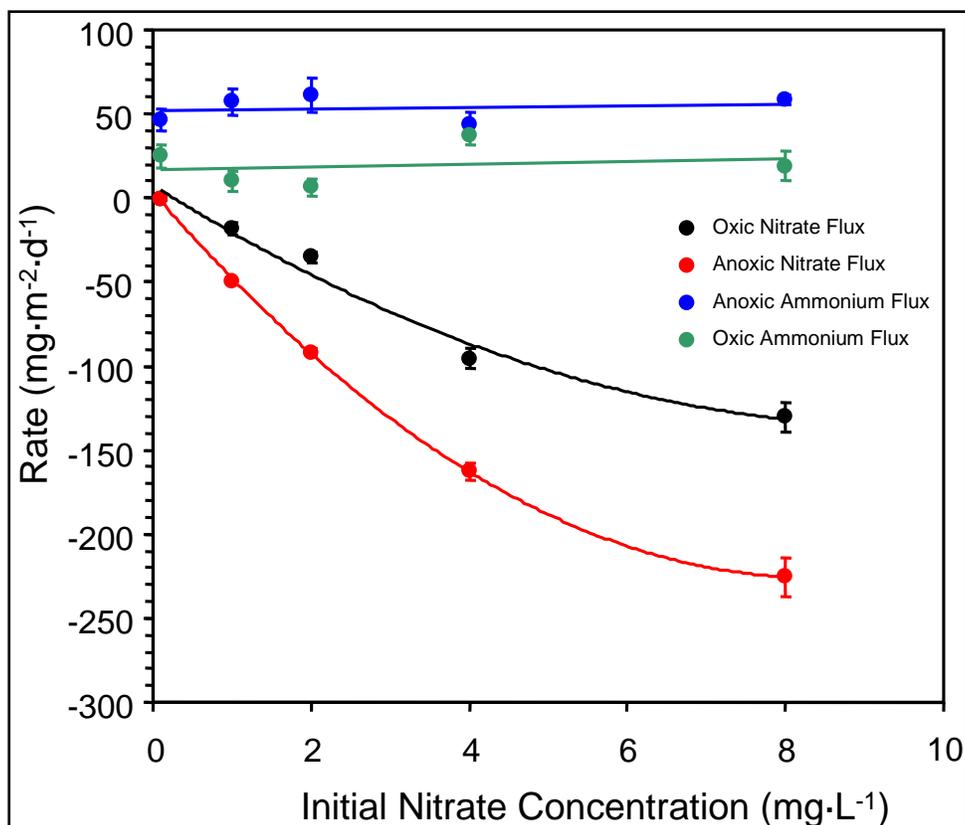


Figure 10. Variations in nitrate nitrite-N and ammonium-N flux under oxic and anoxic conditions (measured using sediment core incubation systems) as a function of initial nitrate concentration in the overlying water.

Nitrate diffusive flux under anoxic conditions would approximate gross denitrification of external nitrate in the absence of coupled nitrification-denitrification. Under oxic conditions, nitrate diffusive flux would approximate net denitrification and be lower than the gross denitrification rate due to a longer diffusional path for nitrate created at the sediment-water interface by oxygen penetration and input via bacterial nitrification (Jensen et al. 1994, Rysgaard et al. 1994). Average gross and net denitrification rates estimated from laboratory-derived flux experiments (Figure 10) and nitrate concentrations measured in the backwater (Figure 4) were  $122.0 (\pm 12.3 \text{ S.E.})$  and  $63.4 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1} (\pm 8.3 \text{ S.E.})$ , respectively.

**Integration of N fluxes into the inflow-outflow budget of Third Lake.** Figure 11 is a flowchart of lakewide inorganic N fluxes and transformations for Third Lake. Development of anoxia in bottom water under macrophyte canopies would have inhibited nitrification in underlying sediment, resulting in elevated ammonium efflux as observed in the sediment core incubation studies. It was assumed that anoxic conditions occurred at the sediment-water interface in areas of the lake where in situ dissolved oxygen was  $< 1 \text{ mg}\cdot\text{L}^{-1}$  in the overlying water column. Ammonium efflux and nitrification were weighted with respect to both the average area of anoxic zones and their duration during summer ( $13.4\% \text{ anoxic area}\cdot\text{summer}^{-1}$ ) to estimate average lakewide rates of  $22.0$  and  $33.4 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , respectively (Figure 11). The summer  $J_{\text{NH}_3}$  of

17.0  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  (Table 2) was similar to that estimated independently from sediment core incubations. The sediment core incubation studies indicated that up to 60 percent of the sediment ammonium efflux was nitrified (Figure 11). The remainder entered the water column becoming a potential N source for biological uptake and nitrification by microbial communities attached to macrophytes. Overall, net ammonium efflux into the water column was four times greater than influx, resulting in net export of 18.4  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  (5.5 - 23.9  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). Net ammonium efflux exceeded net export, suggesting retention via biological uptake.

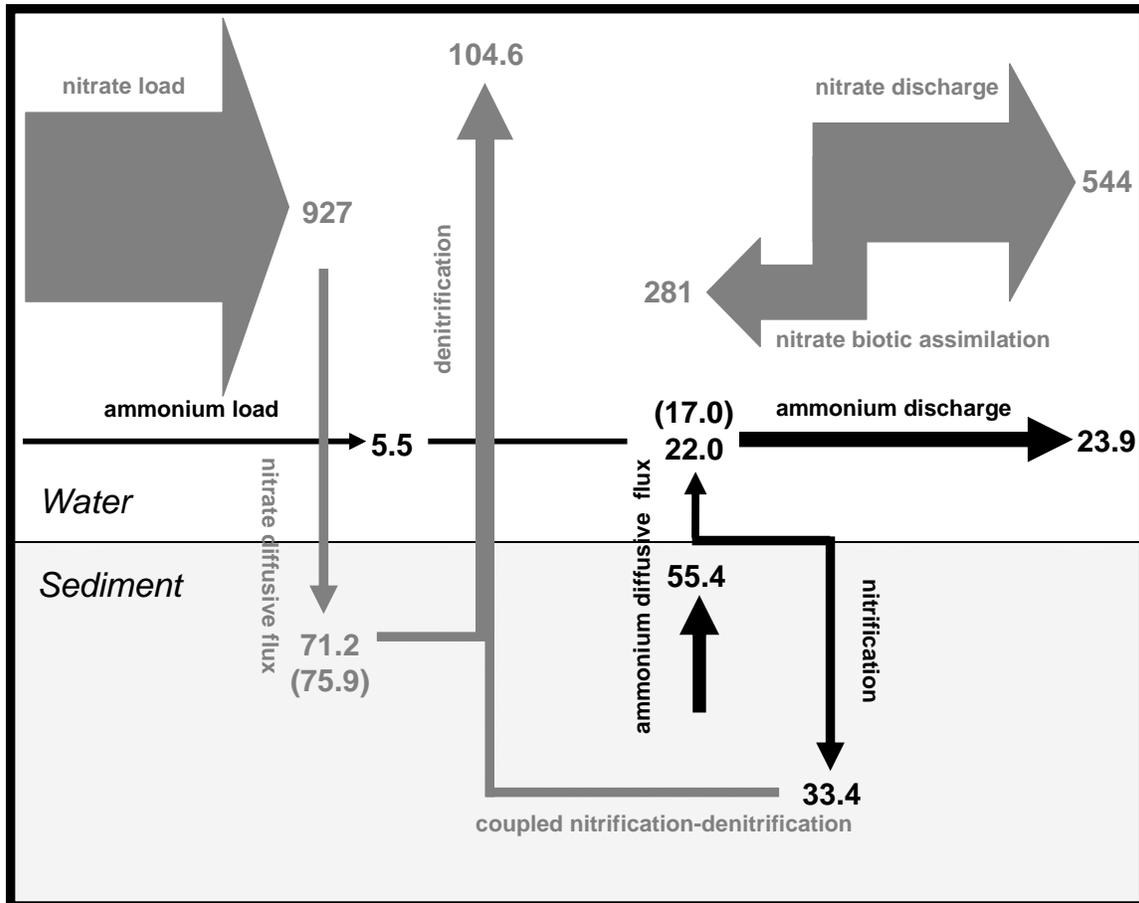


Figure 11. Budgetary flow chart analysis of major summer fluxes and transformations of nitrate nitrite-N (grey) and ammonium-N (black) in Third Lake.

Total summer nitrate inputs from external sources and internal sediment nitrification were  $960 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  ( $927 + 33.4 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) while output was  $544 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . Total nitrate uptake in the system was  $416 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  (net nitrate uptake =  $383 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). Lakewide diffusive flux of nitrate into sediment, adjusted for area and duration of anoxia in the backwater, represented 68 percent of the denitrification rate and coupled nitrification-denitrification 32 percent (Figure 11). Lakewide, the estimated denitrification rate ( $104.6 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) was only 27 percent of the nitrate uptake. Thus, uptake by algae, macrophytes, and biofilms represented 73 percent of the nitrate budget.

**DISCUSSION:** The occurrence of porewater ammonium accumulation during the summer indicated that inputs via organic N mineralization were exceeding outputs and transformations due to macrophyte root uptake, bacterial nitrification, equilibrium exchanges between the porewater and adsorbed ammonium, and diffusive fluxes out of the sediment. Concentration maxima observed within the first 5 cm of the sediment profile also suggested the occurrence of an active zone of porewater ammonium accumulation immediately below the sediment-water interface, which fueled diffusive fluxes out of the sediment. Porewater accumulation rates of 1 to 2  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  were modest compared to diffusive fluxes and rates of nitrification. However, James et al. (2006a) observed significant increases in sediment-adsorbed ammonium between May and July, which translated into a much higher accumulation rate of 29.1  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , assuming that most of the ammonium mineralized from organic N became adsorbed to the sediment. The summer organic N mineralization rate of 87  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , calculated as the sum of the net sediment exchangeable ammonium accumulation rate, nitrification rate, diffusive ammonium flux, and 2  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  net macrophyte root uptake from sediment (James et al. 2006a), was very high compared to annual ammonification rates observed for bogs and wetlands (up to 35  $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ; Kadlec and Knight 1996) and, thus, represented an important N transformation in relation to the buildup of ammonium concentrations in the sediment during the summer.

Ammonium export from the backwater could be entirely accounted for by diffusive flux out of the sediments. In fact, diffusive flux exceeded the export rate, indicating that a portion of the flux was taken up by pelagic biota and/or transformed to nitrate by epiphytic nitrifiers attached to macrophytes (Eriksson and Weisner 1999) during transport to the outflow. Longitudinal patterns of concentration maxima in the mid-lake area with declining values toward the outflow supported this contention. However, most of the internally derived ammonium load was being flushed from the system without transformation as export accounted for the majority (81 percent) of the diffusive flux. Reasons for the lack of greater uptake are not clear since it is energetically more efficient to assimilate ammonium than nitrate and the presence of ammonium can also inhibit assimilation of nitrate (Nichols and Keeney 1976). Diffusive flux could have been underestimated; however, independently determined  $J_{\text{NH}_3}$  was similar to the rate derived from sediment core determinations. Other possible factors include 1) a very high ratio of nitrate to ammonium in the system that precluded preferential uptake of ammonium over nitrate (Mickle and Wetzel 1978) and 2) preferential uptake of ammonium from deeper sediment layers versus the water column by rooted submersed macrophytes (Barko et al. 1991).

The nonlinear pattern between nitrate diffusive flux and nitrate concentration observed in the core incubation experiments was likely limited by two factors; diffusion and saturated enzyme activity. Over the lower nitrate concentration range (0-4  $\text{mg}\cdot\text{L}^{-1}$ ), denitrification rates were regulated by diffusive flux as indicated by the strong linear relationship. Thus, bacterial nitrate demand exceeded diffusion (i.e., no porewater nitrate buildup at the sediment-water interface). This diffusional constraint of denitrification probably occurred throughout most of the summer as water column nitrate concentrations were < 4  $\text{mg}\cdot\text{L}^{-1}$ . There is strong evidence that nitrate gradients between the overlying water and first few millimeters of sediment resulting from diffusion are rate-limiting for denitrification (Reddy et al. 1978). Bacterial enzyme activity could

have become saturated at higher nitrate additions ( $> 4 \text{ mg}\cdot\text{L}^{-1}$ ), leading to some buildup of pore-water nitrate at the sediment-water interface and decreased concentration gradients to drive nitrate flux.

While sediment core incubations were useful to determine nitrate and ammonium diffusive fluxes, this technique may sometimes underestimate bacterially driven N transformations. Unlike  $^{15}\text{N}$  isotopic pairing and dilution analysis (Rysgaard et al. 1994), depletion of nitrate in overlying water does not distinguish between denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Denitrification represents permanent N removal while DNRA transforms nitrate into a biologically available form (Tiedje 1988). Denitrification may be generally more important in freshwater versus marine systems, but more evidence is needed to confirm this hypothesis (Burgin and Hamilton 2007). Denitrification was assumed to be the dominant pathway in the sediment core incubations based on high nitrate concentration and low C:N ratios (Tiedje 1988). Nitrate depletion experiments can also underestimate the potential role of coupled nitrification-denitrification (Steingruber et al. 2001). An accurate approximation of coupled nitrification-denitrification was derived from the evaluation of nitrate depletion and ammonium effluxes over multi-level nitrate additions. Unlike sediment slurry techniques, the cores preserved the integrity of diffusional pathways that play an important role in regulating bacterial transformation. Thus, rate determinations from sediment core incubations reasonably approximate diffusive flux, denitrification, nitrification, and coupled nitrification-denitrification in Third Lake.

Denitrification rates estimated for Third Lake sediments were within commonly reported ranges for streams, wetlands, and lakes (Seitzinger 1988, Steingruber et al. 2001, Royer et al. 2004). Although denitrification represented a permanent removal pathway for nitrate, it accounted for only 27 percent of the summer uptake in the backwater system. Denitrification by microbial biofilms attached to macrophytes in the water column (Caffrey and Kemp 1992; Eriksson and Weisner 1996, 1997, 1999; Bastviken et al. 2003; Toet et al. 2003) was not measured but may have been an important additional component to the budget, particularly since anoxia developed under macrophyte canopies throughout the summer. Coupled nitrification-denitrification did not appear to be an important flux for fueling denitrification in relation to much greater external nitrate loading. In contrast, coupled nitrification-denitrification is probably the primary nitrate flux pathway for isolated backwaters of the Upper Mississippi River that receive very limited nitrate delivery due to low connectivity with nitrate sources from the main channel (Richardson et al. 2004, Strauss et al. 2004). Generally, higher nitrate uptake efficiencies associated with streams, wetlands, and backwaters are assumed to be linked to enhanced (but often unmeasured) denitrification. However, denitrification was probably not the dominant uptake mechanism in Third Lake. Rather, the majority of the unmeasured nitrate uptake was likely incorporated as biomass and, therefore, represented a labile pool for later transformation, transport, and burial. Phytoplankton and epiphytic algae attached to macrophytes can assimilate nitrate for growth when supplies of ammonium are limiting (Chan and Campbell 1978). Evidence also indicates that filamentous green algae such as *Spirogyra* sp. and submersed rooted macrophytes can utilize nitrate (Mickle and Wetzel 1978, Irfanullah and Moss 2004). Submersed and floating-leaved macrophytes with rudimentary root structure such as *Ceratophyllum* (which is a dominant

species in Third Lake) and *Lemna* sp. rely on nutrient supplies from the water column and can remove nitrate for growth (Toetz 1971, Goulder and Boatman 1971, Cedergreen and Madsen 2002). Competition for nitrate uptake by these macrophyte species (mean macrophyte biomass =  $58 \text{ g} \cdot \text{m}^{-2} \pm 11.2 \text{ S.E}$  in July; James et al. 2006a) in the backwater could have been largely responsible for limiting nitrate delivery to the sediment, explaining the apparently limited role played by sediment denitrification in overall nitrate uptake. If so, nitrate-derived senescent biomass could also be responsible for fueling the high organic N mineralization that contributes to the buildup of porewater ammonium and export from the system in the summer.

Overall uptake efficiency of nitrate nitrite-N loads by Third Lake during the study period was ~41 percent, which was high relative to removal efficiencies determined for main channel reaches of larger river systems. Results suggest that up to one third of this load can be removed via denitrification. Uptake efficiency of backwater systems like Third Lake can be increased to a certain extent by considering the optimum residence time distribution and contact time required for maximum uptake (James et al. 2006b). However, source water concentrations entering backwaters cannot be manipulated to the extent that they can be in engineered wetlands. Thus, uptake efficiency cannot be optimized by increasing both source water concentrations and residence time. Nevertheless, results from this study suggest that increasing hydrological connectivity with backwaters may be a viable option for improving in-stream nitrate uptake and processing for large river systems.

**SUMMARY:** Inflow was the dominant nitrate source (>95 percent) versus nitrification and varied as a function of source water concentration since flow was constant. Nitrate uptake length increased linearly, while uptake velocity decreased linearly, with increasing inflow concentration to  $2 \text{ mg} \cdot \text{L}^{-1}$ , indicating limitation of N uptake by loading. N saturation at higher inflow concentration coincided with maximum uptake capacity, 40 percent uptake efficiency, and an uptake length two times greater than the length of the backwater. Nitrate diffusion and denitrification in sediment accounted for 27 percent of the backwater nitrate retention, indicating that assimilation by other biota and denitrification on other substrates were the dominant uptake mechanisms. Ammonium export from the backwater was driven by diffusive efflux from the sediment. Ammonium increased from near zero at the inflow to a maximum mid-lake, then declined slightly toward the outflow due to uptake during transport. Ammonium export was small compared to nitrate retention.

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