DENITRIFICATION AND THE NITROGEN BUDGET OF A RESERVOIR IN AN AGRICULTURAL LANDSCAPE

MARK B. DAVID,[1,4] LAREINA G. WALL,[2] TODD V. ROYER,[3] AND JENNIFER L. TANK[2]

[1]*University of Illinois, Department of Natural Resources and Environmental Sciences, W-503 Turner Hall, 1102 S. Goodwin Avenue, Urbana, Illinois 61801 USA*
[2]*University of Notre Dame, Department of Biological Sciences, 192 Galvin Life Sciences, Notre Dame, Indiana 46556 USA*
[3]*Indiana University, School of Public and Environmental Affairs, 1315 East 10th Street, Bloomington, Indiana 47405 USA*

Abstract. Denitrification is an important process in aquatic sediments, but its role has not been assessed in the N mass balance of upper-Midwestern (USA) reservoirs that receive large agricultural riverine N inputs. We used a 4400-ha reservoir to determine the role of denitrification in the N mass balance and effectiveness in reducing downstream transport of NO_3-N. Sediment denitrification was (1) measured monthly (March 2002–March 2003) at eight sites in the Lake Shelbyville reservoir in central Illinois using the acetylene inhibition, chloramphenicol technique, (2) scaled to the overall reservoir and compared to N not accounted for in a mass balance, and (3) estimated indirectly using long-term (1981–2003) mass balances of N in the reservoir. Denitrification rates in the reservoir were high during spring and early summer of 2002, when maximum NO_3-N concentrations were measured (10–14 mg NO_3-N/L). We estimated that denitrification for the year was between 2580 and 5150 Mg N. Missing N from the mass balance was 3004 Mg N, suggesting that sediment denitrification was the sink. Areal rates of sediment denitrification in the reservoir ranged from 62 to 225 g $N \cdot m^{-2} \cdot yr^{-1}$, with rates a function of both denitrification intensity (μg $N \cdot g$ dry $mass \cdot h^{-1}$) and the overall mass of sediment present. From 1981 to 2003 the average NO_3-N inlet flux was 8900 Mg N/yr. About 58% of the total NO_3-N input was removed, and annual NO_3-N removed as a percentage of inputs was significantly related to reservoir retention time (average = 0.36 yr for the 23 years, range = 0.21–0.84 yr). By scaling denitrification in Lake Shelbyville to other reservoirs in Illinois, we estimated a sink of 48 900 Mg N/yr. When combined with estimated in-stream denitrification, 60 900 Mg N/yr was estimated to be removed by sediment denitrification. This reduces riverine export from Illinois to the Gulf of Mexico, where the flux during the 1990s was about 244 000 Mg N/yr, and illustrates the importance of reservoir denitrification as an N sink in Midwestern agricultural landscapes.

Key words: agricultural watersheds; denitrification; mass balance; nitrate; nitrogen removal; reservoirs; retention time; sediment; stream export.

INTRODUCTION

Modern agricultural practices of fertilizing fields and planting crops that fix atmospheric N_2 combined with fossil fuel combustion have more than doubled the rate of reactive N inputs to the global N cycle (Vitousek et al. 1997, Galloway et al. 2003). In the Mississippi River basin, this has tripled NO_3-N export to the Gulf of Mexico via the Mississippi River, primarily due to agricultural N losses (Goolsby et al. 2001, Rabalais et al. 2002b). A large portion of this N originates from agricultural states in the Midwest including Iowa, Illinois, Indiana, and Ohio (David and Gentry 2000, Goolsby et al. 2001). Seasonal incidents of hypoxia in

the Gulf of Mexico are thought to have occurred since the 1930s, but only recently did evaluations of sediment cores suggest that the severity and size of the hypoxic zone correlates with agriculturally induced changes in Mississippi River N loads (Rabalais et al. 2001). Concerns about hypoxia have heightened interest in quantifying upstream N retention mechanisms that may decrease delivery of N to the Gulf of Mexico (Mitsch et al. 2001, Saunders and Kalff 2001, Seitzinger et al. 2002) and identifying factors that influence N retention (Alexander et al. 2000, Peterson et al. 2001).

Mass balance studies have shown that reservoirs may retain a large portion of their N inputs (Toetz 1973, Kennedy and Walker 1990, Garnier et al. 1999, Kelly 2001). However, little is known about the relative importance of different N retention mechanisms within reservoirs, including biological assimilation, abiotic sedimentation, dissimilatory NO_3 reduction (DNR) and denitrification (Saunders and Kalff 2001). Of these

processes, assimilation, sedimentation, and DNR retain N within the reservoir ecosystem in various forms. In contrast, denitrification permanently removes N from the reservoir via the microbial reduction of NO_3 to N_2 and N_2O under anoxic conditions. In aquatic ecosystems, denitrification occurs readily in the top 2–5 cm of sediment when there is abundant NO_3, organic carbon, and anoxic conditions (Seitzinger 1988).

In an analysis of previously published denitrification rates and N loading for various rivers, lakes, and wetlands, sediment denitrification accounted for a significant portion of the total N retained (Saunders and Kalff 2001). Because reservoirs have characteristics of both lakes and rivers (Kimmel et al. 1990), we predicted that denitrification could be an important N sink in reservoirs in the Midwestern United States that often receive river inputs with high NO_3-N concentrations (>5–10 mg N/L). Previous work in three reservoirs in France that received NO_3-N concentrations of about 5 mg N/L removed about 40% of the N load (Garnier et al. 1999). Reservoirs in the agricultural regions of the Midwest are of particular interest because the watersheds in which they occur contribute among the highest N loads to the Mississippi River and Gulf of Mexico (Goolsby et al. 1999, Alexander et al. 2000). Additionally, the relatively shallow depths and high sediment organic matter in many Midwestern reservoirs may result in high rates of denitrification, making some reservoirs potentially important N sinks that mitigate the effects of agricultural runoff on downstream water bodies.

We measured sediment denitrification rates in Lake Shelbyville, Illinois and determined the effect of denitrification on the N budget of the reservoir. Lake Shelbyville is an impoundment of the Kaskaskia River (a tributary to the Mississippi River) and provided an opportunity to examine the role of a large reservoir on riverine N cycling in an agricultural landscape. Much of this tile-drained area of Illinois has riverine N loads dominated by high concentrations of NO_3-N, ranging from 10 to 20 mg N/L, and with only small concentrations of NH_4-N or organic N (Royer et al. 2004). Previous mass balance work (David and Gentry 2000) indicated that the Kaskaskia River had lower N exports compared to other rivers in the state of Illinois, despite draining intensive agricultural watersheds. Lake Shelbyville provided an opportunity to evaluate the role of impoundments on N losses in an area with high riverine NO_3-N concentrations. Additionally, long-term data on N inputs and outputs to Lake Shelbyville were available and allowed us to examine the role of denitrification on the N budget during a 23-year period. Our objectives were to: (1) construct a detailed one-year N budget for Lake Shelbyville, estimating annual N retention in the reservoir via both mass balance and monthly denitrification measurements; (2) use N mass balances to examine long-term N losses in the reservoir; and (3)

examine the large-scale factors controlling N retention in the reservoir.

METHODS

Study site description

Lake Shelbyville (39°24′22″ N, 88°47′00″ W) is an impoundment on the Kaskaskia River, a tributary of the Mississippi River in east-central Illinois. The Shelbyville dam (height = 33 m and width = 922 m) was completed and the gates closed in 1970; it is a multipurpose facility with a primary function of flood control. The dam is located ~20 km south of the confluence of the West Okaw and Kaskaskia Rivers resulting in a Y-shaped reservoir (Fig. 1). During March 2002 through March 2003, mean water depth was 5.52 m with deepest depths located near the dam (maximum = 13 m) and shallowest depths in reservoir inlets and dendritic coves (minimum = 0.1 m). Reservoir surface area and storage volume fluctuate depending on annual precipitation patterns. Land use in the 273 000-ha watershed is >80 % row-crop agriculture, mainly corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr] (data *available online*).[5] Nitrogen inputs to the watershed are a result of corn fertilization and soybean N_2 fixation, with outputs dominated by grain harvest and riverine losses (David et al. 1997, David and Gentry 2000). Much of the watershed is artificially drained with subterranean tiles, which facilitates NO_3 loss from fields at rates of 5–50 kg $N·ha^{-1}·yr^{-1}$ (David et al. 1997, Gentry et al. 2000, Mitchell et al. 2000, McIsaac and Hu 2004).

Sampling regime

Sediments for denitrification assays were sampled from eight sites within the Lake Shelbyville river–reservoir continuum on a monthly basis from March 2002 to March 2003 (see Wall et al. 2005 for further details). The sites were partitioned throughout the length of the reservoir to account for longitudinal and habitat variability typical of reservoir ecosystems (Kennedy et al. 1985). Sites were sampled at both profundal (deep water) and littoral habitats when possible. Reservoir sediments were collected with an Ekman grab sampler, which sampled to a depth of ~2–5 cm depending on sediment texture at the particular site. The contents of three grabs were pooled, homogenized by gently stirring, and ~1 L of the composite sediment slurry was returned to the laboratory for denitrification assays. Unfiltered stream or reservoir water was collected from within one meter of the benthos using a Van Dorn sampler and placed in an acid-washed 1-L bottle for use during the denitrification assays. Water and sediment samples were stored on ice packs for transport to the laboratory and were stored for <24 h (~6°C) until denitrification assays were initiated.

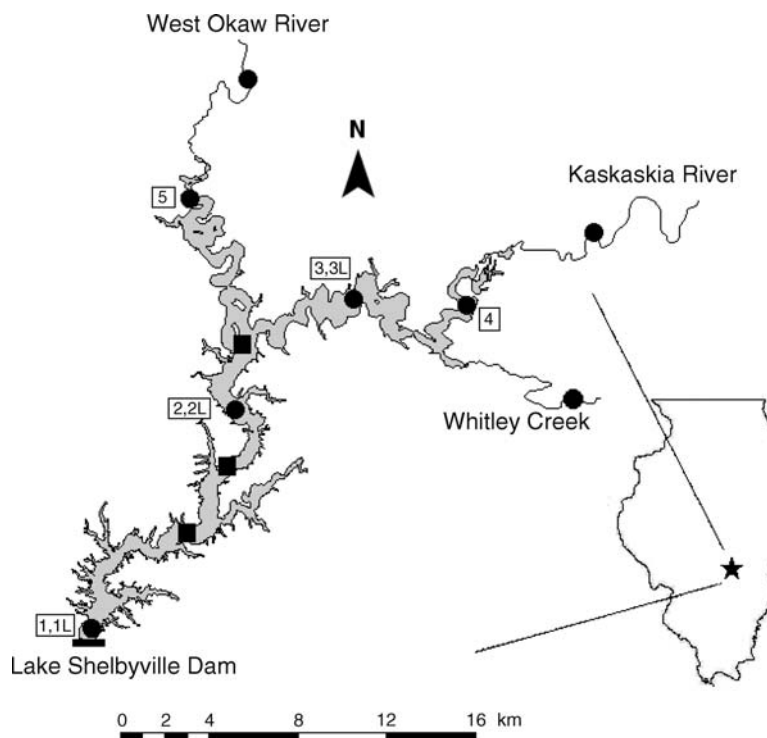[5] ⟨http://www.agr.state.il.us/gis/landcover.html⟩

FIG. 1. Map showing the Lake Shelbyville (Illinois, USA) reservoir and inlet rivers with locations of sampling sites. Circles show monthly sampling sites; squares show additional coring sites.

Water samples for NO_3-N, NH_4-N, and total N were also collected monthly from each reservoir site at the same time as the sediment sampling and analyzed at the University of Notre Dame. These samples were filtered with a 0.7-μm filter (Whatman GF/F) and analyzed for NO_3-N by ion chromatography and NH_4-N by the phenylhypochlorite technique (Solorzano 1969) using a Shimadzu UV 1601 spectrophotometer (Shimadzu, Kyoto, Japan). Total N was determined on unfiltered water using a modified version of the persulfate method 4500-N_{org} (American Public Health Association 1998) with colorimetric detection using a Lachat QuickChem 8000 (Hach Company, Loveland, Colorado, USA). Additional water samples were collected for NO_3-N, NH_4-N, and total N at the West Okaw River, Kaskaskia River, Whitley Creek, and the reservoir tail water (dam outlet) every one to two weeks during high discharge events and analyzed at the University of Illinois using standard methods (American Public Health Association 1998). Following 0.45-μm filtration, NO_3-N was determined by ion chromatography, NH_4-N using a Lachat QuickChem 8000, and total N following Kjeldahl digestion with detection of NH_4-N using the Lachat. Illinois Environmental Protection Agency also collected water samples at these same sites every six weeks and all N species were measured using standard methods (American Public Health Association 1998). For all samples, organic N was determined as the difference between total N and NO_3-N plus NH_4-N. All inlet and outlet data were used for flux calculations, with a total of ~35 samples collected for each site from March 2002 to March 2003.

Laboratory sediment denitrification assays

Sediment denitrification rates were measured in the laboratory using the acetylene inhibition technique as described by Smith and Tiedje (1979) and Tiedje (1982) and modified by Royer et al. (2004). Approximately 25 mL of sediment slurry from each site was dispensed into three replicate 125-mL media bottles. Unfiltered water from each site was added to bring the final slurry volume in the bottles to 75 mL. To reduce bottle effects (e.g., new enzyme production induced by anoxia), chloramphenicol was added to the assay bottles to reach an end concentration of ~6 mmol/L. Using chloramphenicol results in linear N_2O production during incubations (Royer et al. 2004, Inwood et al. 2005, Wall et al. 2005) and the method compares favorably to more sophisticated techniques, such as membrane-inlet mass spectrometry, for estimating denitrification rates (Bernot et al. 2003). Assay bottles were sealed with caps containing butyl septa and the headspace was purged with He to create anoxic conditions. Pure acetylene was added to the headspace of each bottle to achieve a final gas mixture of 10% C_2H_2 and 90% He. The bottles were shaken to insure complete infusion of the C_2H_2 and incubated at the mean ambient water column temperature at the time of collection. Gas samples were drawn

from the headspace approximately every hour during a 4-h incubation period. Gas samples were analyzed for N_2O on a Hewlett Packard 5890 Series II Gas Chromatograph (Hewlett Packard, Palo Alto, California, USA) equipped with a Supelco 80/100 HAYESEPQ 3 m column (Supelco, Bellefonte, Pennsylvania, USA) with electron capture detector. Column and detector temperatures were 70°C and 325°C, respectively. Sediment denitrification rates were calculated from the change through time in headspace N_2O concentration, corrected with the appropriate Bunsen coefficient (Tiedje 1982). A subsample of sediment from each replicate bottle was analyzed for ash-free dry mass (AFDM) and dry mass (DM), which were used to express denitrification rates (μg $N_2O \cdot g$ $AFDM^{-1} \cdot h^{-1}$ or μg $N_2O \cdot g$ $DM^{-1} \cdot h^{-1}$).

Sediment core survey

To estimate the DM and AFDM of sediment per unit surface area within the reservoir, sediment cores of a known surface area were collected in March 2003 from each of the monthly sampling sites and three additional locations within the reservoir. Sediment cores were collected from profundal sites with a gravity corer whereas a handheld coring device was used at wadeable sites. The upper 5 cm of each core was placed in a plastic container and stored in a cooler for transport to the laboratory. Individual cores were dried and weighed to determine areal g DM, and then homogenized, subsampled, ashed in a muffle furnace at 550°C for at least four hours and reweighed to determine areal AFDM. We determined that the organic matter content of sediment cores was not significantly different from the respective mean organic matter content of sediments used for the denitrification assay at seven of the eight sites sampled within Lake Shelbyville (two-sample t test, $P > 0.05$; data not shown). Therefore, areal estimates of DM (g DM/m) determined from the single coring survey that sampled all of Lake Shelbyville were used to scale-up denitrification rates from the monthly assays (μg $N \cdot g$ $DM^{-1} \cdot h^{-1}$) to an areal denitrification rate (μg $N \cdot m^{-2} \cdot h^{-1}$). Samples collected in June 2002 from profundal site 2 and littoral site 4 had higher organic matter content compared to the 11 other monthly sampling dates. Windy conditions on the reservoir that day limited our ability to collect sediments using the Eckman and resulted in a sample of only the upper 1–2 cm of organic rich sediments, which may have influenced the high denitrification rates measured that month.

Estimating NO_3-N demand due to denitrification

Uptake velocity (V_f), also known as a mass transfer coefficient, has been used frequently to describe biological nutrient demand, relative to concentration, within river ecosystems (Stream Solute Workshop 1990, Davis and Minshall 1999, Hall and Tank 2003). Using a modification of nutrient spiraling theory, we calculated a NO_3-N uptake velocity for sediment denitrification

($V_{f,dn}$) to measure the relative demand for NO_3-N due solely to denitrification within reservoir sediments (Royer et al. 2004). For each sampling site within Lake Shelbyville, $V_{f,dn}$ (mm/min) was calculated as

$$V_{f,dn} = U/N_a \quad (1)$$

where U (μg $N \cdot m^{-2} \cdot min^{-1}$) is the aerial uptake of NO_3-N due to sediment denitrification and N_a (μg N/L) is the ambient NO_3-N concentration of water sampled within 1 m of the benthos at a given site within the reservoir.

NO_3-N budget calculations

We quantified riverine NO_3-N inputs and outputs for Lake Shelbyville and estimated NO_3-N deposition (wet and dry) to create a NO_3-N mass balance from March 2002 through March 2003 (hereafter referred to as the 2002–2003 sampling). Total inputs were considered as riverine inputs plus deposition on the surface of the reservoir and the release from the dam was considered the only output in the model. Total NO_3-N retained during the 2002–2003 sampling period (N_r, in Mg) was calculated as

$$N_r = \left[\left(\sum I_r \right) / 0.588 \right] - \sum O_d \sum D_{wd} + \Delta W + \Delta S \quad (2)$$

where I_r represents the daily riverine NO_3-N inputs, O_d represents the daily output from the dam, D_{wd} equals the total (wet and dry) atmospheric NO_3-N deposition, ΔW equals the change in transitory storage of NO_3-N within the water column from the beginning to the end of the sampling period, and ΔS equals the amount of N retained by sedimentation of particles onto the lake bed.

Daily U.S. Geological Survey flow data for the Kaskaskia River, West Okaw River, and Whitley Creek, combined with NO_3-N concentrations of water samples, were used to determine I_r (Eq. 2) (flow data *available online*).[6] The U.S. Geological Survey stations included 58.8% of the total Lake Shelbyville watershed (Table 1), and we assumed that the unaccounted portion of the watershed contributed proportional NO_3-N loads. Daily N output from the reservoir (O_d) was calculated as mean daily discharge through the dam multiplied by the NO_3-N concentration of water sampled below the dam. Because data on the daily fluctuations in NO_3-N concentrations were not available, daily input and output NO_3-N concentrations were estimated by linear interpolation.

Atmospheric deposition was estimated using 2002–2003 seasonal wet deposition data at Bondville, Illinois, the closest National Atmospheric Deposition Program/National Trends Network site to Lake Shelbyville (data *available online*).[7] Dry deposition of HNO_3^- and NO_3-N

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[6] ⟨http://waterdata.usgs.gov/il/nwis/sw⟩
[7] ⟨http://nadp.sws.uiuc.edu/nadpdata/multsite.asp?state=IL⟩

Table 1. U.S. Geological Survey gaging stations upstream and at the dam of the Lake Shelbyville reservoir, Illinois, USA.

River	Flow	Gage location	Station number	Drainage area (km^2)	Fraction of watershed (%)
Kaskaskia River	inlet	Cooks Mills	5591200	1225	44.9
West Okaw River	inlet	Lovington	5591700	290	10.6
Whitley Creek	inlet	Allenville	5591550	90	3.3
Kaskaskia River	outlet	Shelbyville	5592000	2730	100

was calculated as 40% of wet deposition, a conservative estimate as dry deposition in aquatic ecosystems has not yet been thoroughly evaluated. Additionally, the water column represents a transitory storage pool of NO_3-N within the reservoir. Differences in annual precipitation and snow melt between the start and end of the 2002–2003 sampling period resulted in different water column storage pools of NO_3-N within the reservoir (ΔW). This resulted in additional NO_3-N retention and made it necessary to incorporate ΔW into Eq. 2.

We did not estimate ΔS for NO_3-N that was incorporated into algal biomass and removed through sedimentation, and assumed it was small compared to the other terms in the mass balance. Inputs and outputs of particulate organic N were included in our measurements of total N; allochthonous inputs along the lake shore were not measured but likely were small relative to riverine inputs.

To determine total NO_3-N removal due to sediment denitrification in Lake Shelbyville, the reservoir was longitudinally partitioned into denitrification regions which were determined from similarities in water depth and sediment characteristics. The proportion of the reservoir surface area that each region represented was determined using a Geographic Information System for normal pool conditions. Littoral area within each denitrification region was calculated as the area within 15 m from the shoreline multiplied by the relative proportion of the total shoreline distance. Total NO_3-N removal from each denitrification region included sediment denitrification rates from both littoral and profundal habitat sites when applicable. We assumed the proportional area of each region remained constant when the surface area of the reservoir was below or at normal pool. When the reservoir volume was above normal pool conditions, we kept surface areas constant except for the upper regions of each arm of the reservoir (Fig. 1), where floodplain inundation occurred. Deeper areas lower in the reservoir appeared not to experience large changes in surface area as volume increased above normal pool conditions. Summing the monthly NO_3-N removal from each region during the 2002–2003 sampling period provided an estimate of the total NO_3-N removal from the reservoir due to sediment denitrification (N_d). We made two estimates, one using a sediment depth of 5 cm, and another using 2.5 cm, to provide a range in N_d that might have occurred throughout the reservoir.

Long-term reservoir calculations for N removal

We used U.S. Army Corps of Engineers (USACE) data on Lake Shelbyville volume and surface area from the 1984 sedimentation report (Simons 1985) in combination with daily stage height at the dam to determine daily changes in storage volume and surface area for the period of 1 October 1980 through 30 September 2003. A conversion factor was generated from maximum (flooded) and normal pool conditions and the corresponding stage heights (maximum stage height reported by USACE in 1974) for both surface area and storage volume. We assumed the maximum surface area of the reservoir was constant over time; however, maximum storage volume was corrected for a decrease in storage due to sedimentation (0.37% per yr) within the reservoir using data from Simons (1985). We calculated N inputs, change in storage, and output for the 1981 through 2003 water years using N concentration data collected by the Illinois Environmental Protection Agency, U.S. Geological Survey daily flow data, and our own N concentration data where available from our intensive sampling. Retained or removed N (N_r, Eq. 2) was calculated for each water year using these data, and compared with annual water residence time, estimated as total annual outflow divided by average volume. Percent N removal and retention time data from the 23-year record at Lake Shelbyville were combined with an average value for headwater streams (Royer et al. 2004) and river and reservoir data from Seitzinger et al. (2002) to develop an empirical relationship between N removed (as a percentage of inputs) and depth divided by time of travel (water residence time) using nonlinear regression (SAS 2001).

RESULTS

Water and nitrogen fluxes 2002–2003

Late April and May of 2002 were wet months with high rainfall (36.3 cm of rainfall from 20 April through 13 May 2002) and corresponding large water inflows to the reservoir, with peak discharge during 12–14 May (Fig. 2a). For example, the Kaskaskia River at Cooks Mills had discharges of 303 and 289 m^3/s on 13 and 14 of May 2002, the highest recorded during our 23-year study period. Nearly all the inflow to the reservoir occurred during these two months for the year of our intensive study, with lesser amounts in April, and relatively little inflow after June. Outflow was buffered by the impoundment, although large water releases occurred throughout
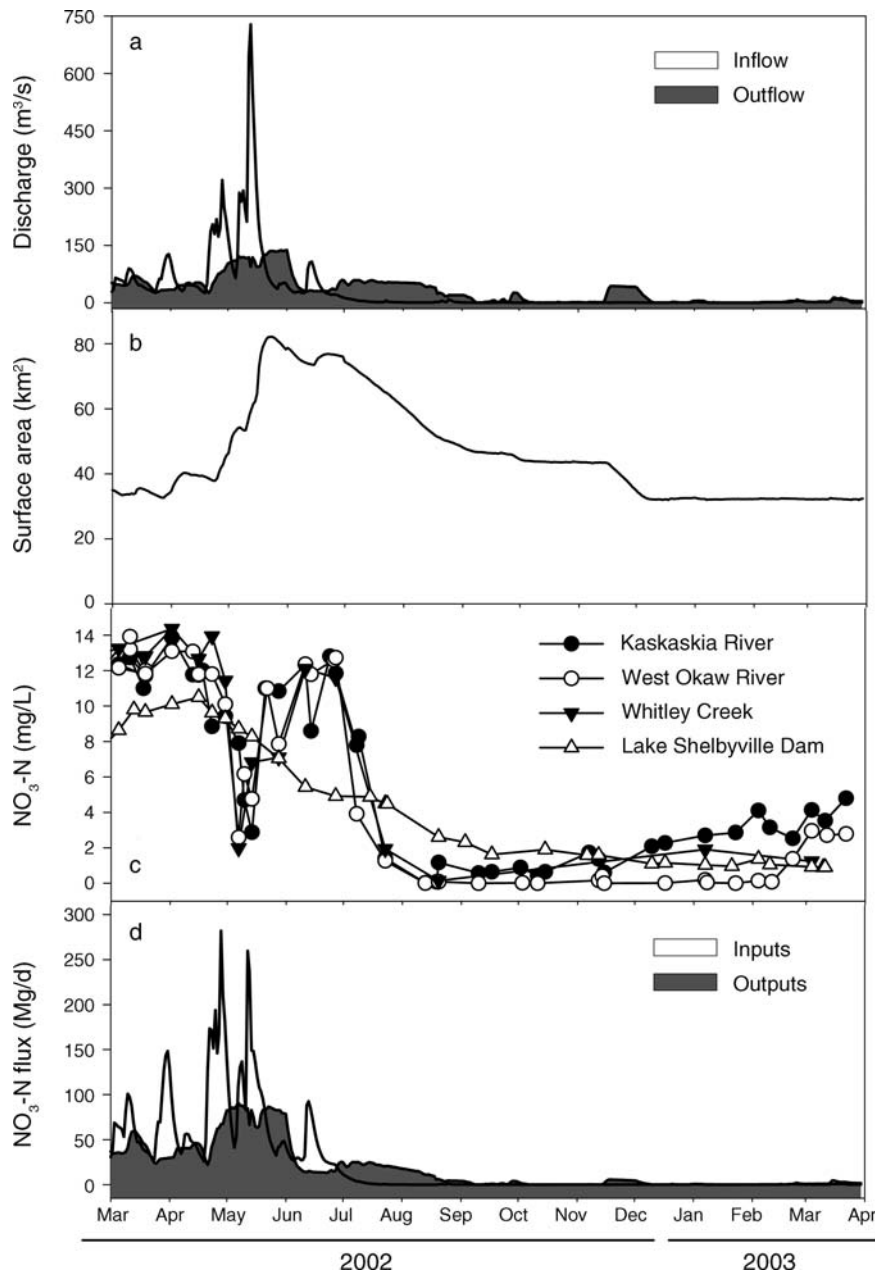
FIG. 2. Daily (a) discharge of inlets and reservoir outlet, (b) reservoir surface area, (c) stream water NO_3-N concentrations, and (d) input and output NO_3-N fluxes for March 2002–March 2003 for the Lake Shelbyville reservoir.

June and early July. This caused surface area to peak in late May at about 80 km^2, about twice the normal surface area (Fig. 2b). Nitrate concentrations in all inlet streams were ~12–14 mg N/L in March through June, except when overland flow during storms in mid-May diluted concentrations to ~3 mg N/L (Fig. 2c). Concentrations of streamwater NO_3-N then declined to 0.5 to 4 mg N/L for the rest of the study year and discharges were below normal. Concentrations of NO_3-N in water leaving the reservoir at the dam were somewhat buffered and lagged behind input concen-

trations, reaching 10 mg N/L in May and declining to ~1.5 mg N/L by the next winter. The NO_3-N input/ output fluxes were similar to discharge, with most of the NO_3-N flux into the reservoir during May and June, with NO_3-N flux highest throughout the spring, and then declining to only small amounts from September through the rest of the study year (Fig. 2d).

Sediment denitrification

Denitrification rates were greatest in all sediments during late spring to early summer when both water

temperatures and NO_3-N concentrations were highest (Table 2). Sediment denitrification rates in the upper reservoir quickly decreased to below detection by August of 2002, due to low NO_3-N concentrations in the inlet streams. When averaged for the entire year by sampling site, rates varied from 0.11 to 0.41 µg N·g $DM^{-1}·h^{-1}$ (Table 3). When averaged by habitat, highest rates were in the profundal sediments, followed by the upper reservoir and then the littoral areas (Table 3).

Mean NO_3-N uptake velocity due to sediment denitrification ($V_{f,dn}$; Eq. 1) generally increased longitudinally through the reservoir (Fig. 3), yet the littoral site located at the dam had a lower mean $V_{f,dn}$ compared to the profundal site at the dam. Without the littoral site at the dam, mean $V_{f,dn}$ within the reservoir was significantly related to distance from dam (linear regression, $r^2 = 0.77$, $P = 0.01$). Mean $V_{f,dn}$ at profundal and littoral habitats within a given site were not significantly different from each other (two-sample t test for means, $P > 0.05$) and there was no consistent relationship between $V_{f,dn}$ at profundal and littoral habitats among sites (Fig. 3).

Overall, annual areal denitrification rates ranged from 62 to 225 g N·m^{-2}·yr^{-1} throughout the reservoir, but were not related to habitat type (Table 3). Profundal areas of the reservoir accounted for most of the sediment denitrification because they made up a large fraction of the reservoir surface area. The upper reservoir site 5 also had high denitrification, which was due to the large surface area it represented during the particularly wet spring. Some of the variation in areal denitrification rates can be explained by variability in sediment dry mass (Table 3). Much of the reservoir had dense, compact sediments, particularly in the littoral regions. However, profundal site 3 in the upper end of the reservoir, had a much lower sediment standing stock resulting in lower denitrification rates per unit area.

Lake Shelbyville nitrogen mass balance and denitrification estimate

We constructed a detailed mass balance of N for the period 11 March 2002 through 10 March 2003 (Fig. 4). Riverine inputs were dominated by NO_3-N with 8400 Mg NO_3-N added to the reservoir, representing 93% of the total N inputs. Atmospheric deposition added small amounts of N (36 Mg) through wet and dry deposition. Large amounts of NO_3-N were exported from the reservoir through the dam outlet (6500 Mg NO_3-N), along with relatively small amounts of NH_4-N and organic N. There was an important change in the storage pool of NO_3-N in the reservoir from the beginning to end of the study year, which was a net loss of 1300 Mg N. Summing the inputs plus change in storage, and subtracting the outputs indicated that 3004 Mg N were unaccounted for in the mass balance. Depending on assumptions about microbially active sediment depth, our independent sediment denitrification estimates ranged from 2580 to 5150 Mg N for the

year indicating that the unaccounted for N in the mass balance could be explained by sediment denitrification or storage in sediments.

Long-term nitrogen mass balances

We were able to calculate total N and NO_3-N inputs and outputs for a 23-year period for the reservoir. Because NO_3-N dominated all N species as shown above in the one-year intensive study, we focus on this flux. Volume weighted NO_3-N concentrations varied from year to year, but were typically ~10 mg N/L for the streams entering the reservoir (Fig. 5). Volume weighted outlet NO_3-N concentrations were always lower than the inlet, and during some years were as low as ~0.5 mg N/L. During the 23 years, the average NO_3-N inlet flux was 8900 Mg N/yr, and change in storage varied from −864 to 713 Mg N/yr. Outlet NO_3-N flux averaged 4046 mg N/yr for the reservoir, so, over the long term, ~58% of NO_3-N inputs were removed in the reservoir.

Nitrate-N removed as a percentage of inputs was significantly related to reservoir water retention time (Fig. 6a). Retention time averaged 0.36 yr for the 23 years, but varied from 0.21 to 0.84 yr and was highly related to NO_3-N removal ($r^2 = 0.83$, $P < 0.0001$). The absolute amount of NO_3-N removed in the reservoir was not related to retention time (Fig. 6b), but was related to volume weighted inlet NO_3-N concentrations or inlet loads (Fig. 7a, b). Years with high NO_3-N concentrations and loads had correspondingly greater losses in the reservoir, ranging from 1600 to as much as 7400 Mg N/yr. Overall during the 23-year study period, 205 000 Mg N entered the reservoir through riverine transport, and 93 000 Mg N left via stream flow below the dam. Therefore, 112 000 Mg N were removed by the reservoir during this time period, an average of 4900 Mg N/yr. The average surface area of the reservoir during this time period was 45 km^2, leading to an average NO_3-N removal of 108 g N·m^{-2}·yr^{-1}.

DISCUSSION

The role of denitrification in the reservoir N mass balance

A large amount of N was removed from the water column of the reservoir during the 23-year period as well as in our one-year intensive study. Based on our direct denitrification measurements, that pathway seems to be able to explain the unaccounted for N from our mass balance. Nitrate-N concentrations can often limit denitrification in aquatic systems (e.g., Garcia-Ruiz et al. 1998, Kemp and Dodds 2002). However, in Lake Shelbyville, NO_3-N concentrations in the water column were likely well above concentrations that would be limiting to denitrification, particularly closer to the dam. In fact, for the majority of the 2002–2003 year, experimental amendments showed that NO_3-N concentrations in Lake Shelbyville were above concentrations that would limit sediment denitrification (Wall et al. 2005). Sites closer to the dam were not subject to the

TABLE 2. Sediment denitrification rates (all values are μg N·g DM^{-1}·h^{-1}, mean ± SE) for each site and sampling date by habitat type within the Lake Shelbyville reservoir from March 2002 through March 2003.

Date	Profundal			Littoral	
	1	2	3	1L	2L
2002					
11 Mar				0.25 ± 0.04	<0.01 ± <0.01
13 Apr	0.03 ± 0.02	0.53 ± 0.05	0.49 ± 0.04	0.09 ± 0.02	0.01 ± 0.01
10 May	0.65 ± 0.21	0.46 ± 0.05	0.36 ± 0.17	0.01 ± <0.01	0.17 ± 0.03
14 Jun	0.11 ± 0.01	1.41 ± 0.09		0.42 ± 0.04	0.10 ± 0.01
8 Jul	0.57 ± 0.01	0.46 ± 0.05	0.32 ± 0.03	0.11 ± 0.02	0.26 ± 0.07
13 Aug	0.21 ± 0.01	0.67 ± 0.04	0.93 ± 0.16	0.14 ± 0.04	0.64 ± 0.28
10 Sep	0.21 ± 0.07	0.13 ± 0.02	0.13 ± 0.03	0.05 ± 0.02	0.37 ± 0.04
11 Oct	0.15 ± 0.04	0.18 ± 0.02	<0.01 ± <0.01	0.07 ± 0.01	0.14 ± 0.01
15 Nov		0.12 ± 0.02	0.69 ± 0.04	0.02 ± 0.01	<0.01 ± <0.01
17 Dec	0.29 ± 0.08	0.06 ± <0.01	0.09 ± 0.01	0.04 ± 0.02	0.13 ± 0.04
2003					
23 Jan				0.01 ± 0.01	<0.01 ± <0.01
22 Feb				0.02 ± <0.01	0.03 ± <0.01
22 Mar	0.15 ± 0.06	0.02 ± 0.02	0.40 ± 0.02	0.21 ± 0.02	0.19 ± 0.01

Note: Missing values reflect times when weather conditions or water levels within the reservoir prevented access to some locations.

large fluctuations in riverine NO$_3$-N concentrations that occurred in the upper reservoir sites, resulting in more temporally consistent denitrification rates near the dam. Overall, sites closer to the dam potentially had a larger influence on reducing water column NO$_3$-N concentrations compared to upper reservoir sites, as represented by the higher $V_{f,dn}$ (Fig. 3).

Our mass balance for 2002–2003 resulted in 3004 Mg N unaccounted for, and our independent annual estimate of sediment denitrification ranged from 2580 to 5150 Mg N, indicating that this was a potential pathway for the missing NO$_3$-N. Because we used homogenized sediment for the denitrification assays, we do not know to what depth NO$_3$-N from the water column was diffusing into the sediments, and available for denitrification. Therefore we calculated the mass of denitrified N using a range of sediment depths (2.5–5

cm). Many studies have shown a rapid decrease in sediment pore water NO$_3$-N with depth. For example, Mozeto et al. (2001) found that NO$_3$-N decreased from about 9 mg N/L in the overlying water to <1 mg N/L in the pore water in just the top 1–2 cm of sediment in the Guarapiranga reservoir in Brazil.

This study is one of the few examples where mass balance estimates of denitrification are compared to direct measurement of denitrification rates. Recently, Groffman et al. (2006) reviewed denitrification methods, and discussed the problems in not being able to close mass balances because denitrification rates for large ecosystems are not known. Here, we show that independent methods for obtaining denitrification losses gave similar estimates.

Our results are consistent with a study in France where sediment denitrification, estimated using benthic

TABLE 3. Fraction of reservoir surface area each habitat and site represents at normal pool, sediment dry mass for the upper 5 cm (mean ± SE), and annual sediment denitrification within the Lake Shelbyville reservoir from March 2002 through March 2003.

Habitat and site	Fraction of reservoir surface (%)	Sediment dry mass (g DM/m)	Sediment denitrification		
			Mean rate per mass (μg N·g DM^{-1}·h^{-1})	Mean rate per area (g N·m^{-2}·yr^{-1})	Mean rate (Mg N/yr)
Profundal					
1	18.8	51 800 ± 800	0.26	124	939
2	32.4	43 000 ± 9000	0.41	109	1390
3	19.1	20 300 ± 1700	0.38	62	468
Littoral					
1L	1.9	70 200 ± 3200	0.11	64	51
2L	3.3	57 700 ± 6100	0.15	87	104
3L	2.0	70 700 ± 3300	0.31	225	179
Upper reservoir					
4	9.6	32 400 ± 3500	0.21	82	587
5	12.9	31 800 ± 1600	0.31	152	1430

Note: Values shown are mean rates per gram dry mass, as well as per unit area and mass amounts for each region of the reservoir, calculated using a 5-cm sediment depth.

TABLE 2. Extended.

Littoral	Upper reservoir	
3L	4	5
$<0.01 \pm <0.01$		0.02 ± 0.02
$<0.01 \pm <0.01$	0.35 ± 0.07	$0.03 \pm <0.01$
	0.56 ± 0.08	0.23 ± 0.04
1.74 ± 0.08		1.70 ± 0.16
1.10 ± 0.03	0.28 ± 0.02	1.21 ± 0.10
0.20 ± 0.05	$<0.01 \pm <0.01$	$<0.01 \pm <0.01$
0.07 ± 0.01	$<0.01 \pm <0.01$	$<0.01 \pm <0.01$
0.09 ± 0.04	$<0.01 \pm <0.01$	$<0.01 \pm <0.01$
0.10 ± 0.02	$0.06 \pm <0.01$	$<0.01 \pm <0.01$
0.10 ± 0.03		$0.06 \pm <0.01$
0.07 ± 0.01	0.02 ± 0.01	$0.01 \pm <0.01$
0.14 ± 0.03	0.18 ± 0.01	$0.03 \pm <0.01$
$0.15 \pm <0.01$	0.63 ± 0.02	0.75 ± 0.02

FIG. 4. Nitrogen budget for the Lake Shelbyville reservoir, with the box representing the reservoir pool, from 11 March 2002 through 10 March 2003. Fluxes are proportional to thickness of the lines. Denitrification is estimated using sediment depths of 2.5 and 5 cm. Units are Mg N/yr.

bell jars, represented 83–100% of the total NO_3-N retention in three off-channel reservoirs (Garnier et al. 1999). Nitrate inputs to the French reservoirs were also a result of agricultural activities, yet they were not as high as NO_3-N inputs to Lake Shelbyville. Our results combined with previous work suggest that high NO_3-N retention may be common in reservoirs with large NO_3-N inputs because of relatively long retention times and generally unlimited NO_3-N concentrations driving high denitrification rates.

Our analysis of long-term input and outputs to the Lake Shelbyville reservoir are consistent with our one-year intensive study, and demonstrate that large amounts of N are retained by the reservoir and returned
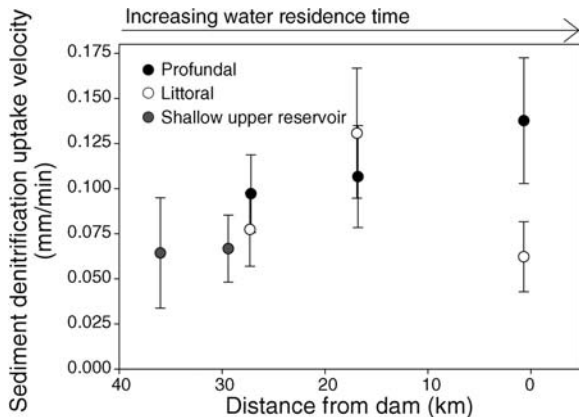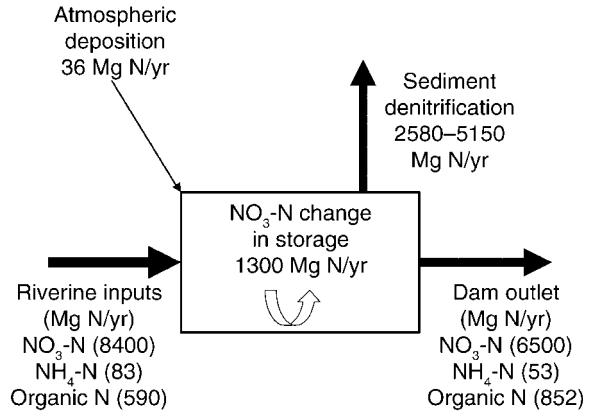

FIG. 3. The relationship between sediment denitrification NO_3-N uptake velocity (mean $\pm$ SE) of the Lake Shelbyville reservoir sites and location of sediments within the reservoir. Approximate distance from dam was used as a surrogate for water residence time, with water located at the dam (dam = 0 km) having the longest water residence time.

to the atmosphere via denitrification. Retention time was clearly a primary control in NO_3-N removal, as shown by the strong relationship of removal as a percent of inputs versus retention time (Fig. 6a). During an average year, retention time was ~4.3 months and 58% of NO_3-N inputs were removed. Drier years with smaller inputs had removal of nearly 90%, and resulted in NO_3-N concentrations at the dam <1 mg N/L.

We applied the RivR-N model (Seitzinger et al. 2002) to our one year intensive N budget of Lake Shelbyville (Fig. 4), using the observed parameters of an average depth of 5.52 m and retention time of 0.26 yr. The RivR-N model predicted 29% of N inputs removed, and our mass balance estimate was also 29%, suggesting that the model relationships held for Lake Shelbyville. However, we then applied the model to our 23-year record, and it predicted an average of 32% of N inputs removed (range 26–48% of N inputs), whereas we obtained 58% removal (range 31–91% of N inputs) (Fig. 8). This suggests that for Lake Shelbyville the relationship between water residence time (m/yr) and percent N removal is steeper at shorter water residence compared to the lakes and few reservoirs that were used in developing the relationship presented by Seitzinger et al. (2002). Seitzinger et al. (2002) used mainly low N concentration lakes in addition to the reservoirs from Garnier et al. (1999) in developing their equation, and notably, the reservoir values were outliers from their fitted equation. In contrast, our data generally overlapped the reservoir data from Garnier et al. (1999), and when we combined these with an average value taken from Royer et al. (2004) for agricultural streams, we found that the empirical relationship between N removal and water residence time showed a much steeper response as water retention time increased (Fig. 8). One possible explanation for this difference is that denitrification in the low
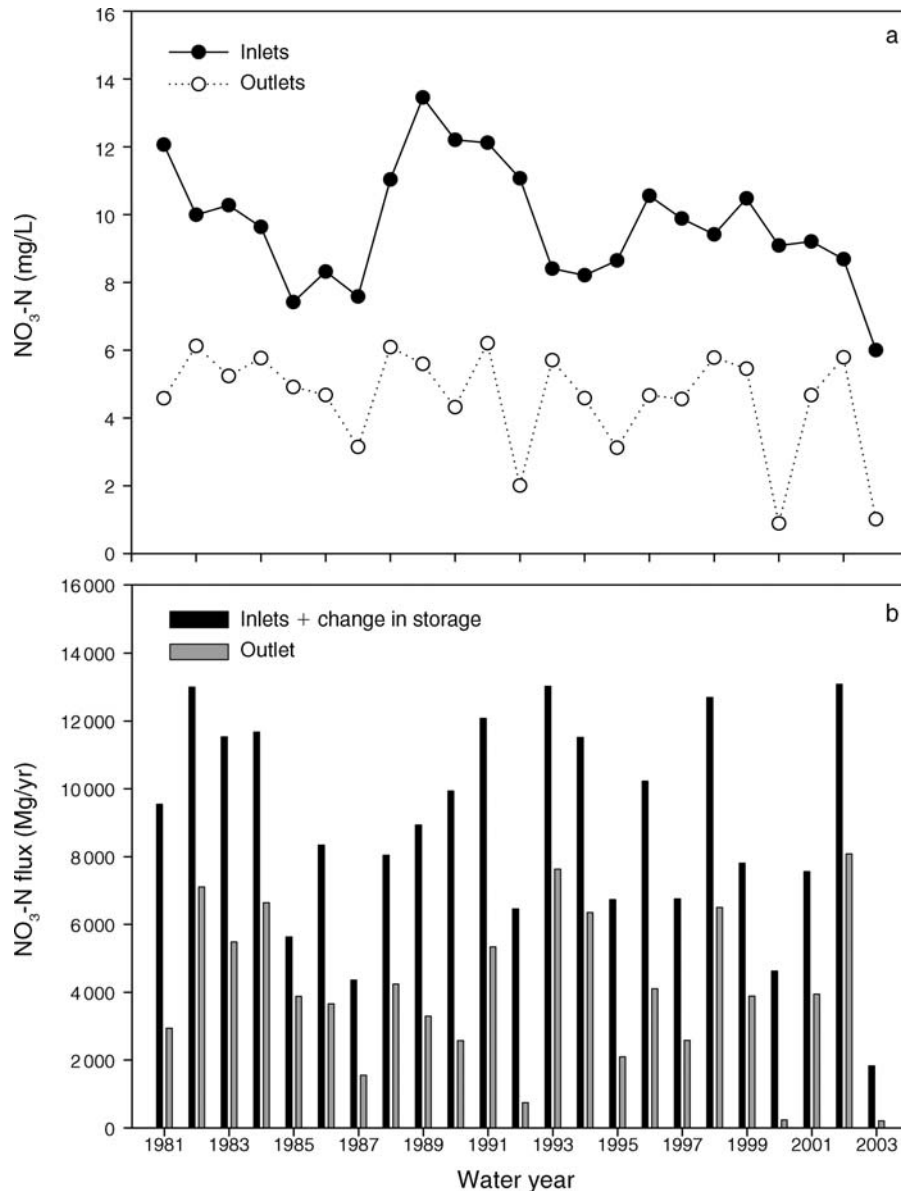
FIG. 5. (a) Volume-weighted annual inlet and outlet NO_3-N concentrations and (b) flux for the 1981 through 2003 water years for the Lake Shelbyville reservoir.

NO_3-N lakes used by Seitzinger et al. (2002) could have been limited by NO_3-N availability which could have reduced overall N removal.

The absolute amount of NO_3-N removed was not related to retention time (Fig. 6b), but was related to inlet NO_3-N concentrations and loads (Fig. 7a, b). As more NO_3-N entered the reservoir during wetter years, retention time decreased but the higher NO_3-N likely allowed consistently higher denitrification rates. For example, wet years had absolute NO_3-N removal as great as 7400 Mg N/yr, whereas removal in drier years was only 1600 to 1700 Mg N/yr. However, the end result was that removal as a percentage of inputs decreased

with shorter retention times, because denitrification removed a smaller proportion of the incoming N.

Aquatic denitrification and the mass balance of N in Illinois

David and Gentry (2000) constructed a mass balance of N for Illinois for 1980 through 1997, with an estimated riverine export for the state of 244 000 Mg N/yr and in-stream denitrification sink of 132 000 Mg N/yr. This denitrification sink was calculated by assuming 35% of the riverine flux was denitrified, a value obtained from Howarth et al. (1996) from both the stream and reservoir denitrification summary in that work. More
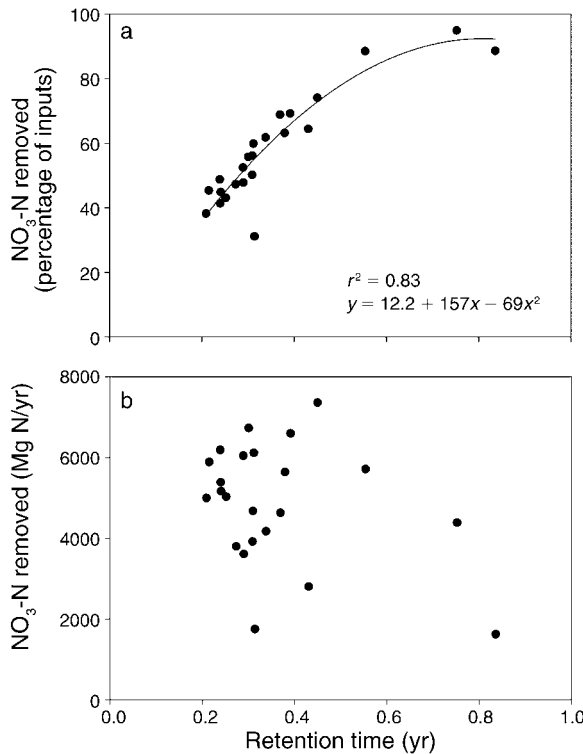
FIG. 6. Annual NO_3-N removed, calculated as the difference of stream water inputs + change in storage and dam output, as (a) a percentage of inputs and (b) a mass amount, plotted against retention time for the 1981–2003 water years for the Lake Shelbyville reservoir.

recent studies (Alexander et al. 2000, Peterson et al. 2001) have suggested that small streams are important sinks for denitrification. However, Royer et al. (2004) and Schaller et al. (2004) showed that denitrification was a small sink for NO_3-N in agricultural streams in Illinois, primarily due to decreased water retention times. The depth/time of travel value calculated from Royer et al. (2004) and used in Fig. 8 was 30 400 m/yr for streams. Although denitrification rates were high, because of high NO_3-N concentrations and limited retention time, uptake velocities were generally low in these streams (<0.0095 mm/min; Royer et al. 2004). Given these results for streams, reservoirs are potentially much larger sinks for riverine NO_3-N loads due to their longer retention times and higher uptake velocities ranging from 0.06 ± 0.02 mm/min to 0.14 ± 0.04 mm/min (mean ± SE).

Our estimates for Lake Shelbyville suggest an average denitrification loss of 4900 Mg N/yr. Using data from the National Inventory of Dams, we summed the surface areas of impoundments north of 39.5° latitude in Illinois (data *available online*).[8] We chose this latitude because this is approximately the demarcation across the state

between the tile-drained regions to the north and non-tile-drained areas to the south (based on soils). Non-tile-drained regions of Illinois have much lower stream water NO_3-N concentrations than do tile-drained areas (McIsaac and Hu 2004). There are 345 impoundments in Illinois above 39.5° latitude, with a combined surface area of 41 000 ha. Applying the Lake Shelbyville average denitrification rate of 108 g $N\cdot m^{-2}\cdot yr^{-1}$ to the combined surface area would give an additional 44 000 mg N/yr, or 48 900 when including Lake Shelbyville. Although there are another 125 000 ha of impounded waters on the Illinois River, these reservoirs have short retention times of just several days and the main channels are dredged, with little mixing of the flowing water with the extensive shallow (<25 cm) areas where sediment denitrification might be high. This is similar to the backwater lake on the Mississippi River studied by Richardson et al. (2004), where they found hydrologic connectivity was a major limitation to denitrification (along with NO_3-N concentrations). Therefore, these Illinois River impoundments probably function as the main river channel does, with limited reduction in N load due to denitrification. Based on data from Royer et al. (2004) and Schaller et al. (2004) it is likely that in-stream denitrification (including the Illinois River impound-
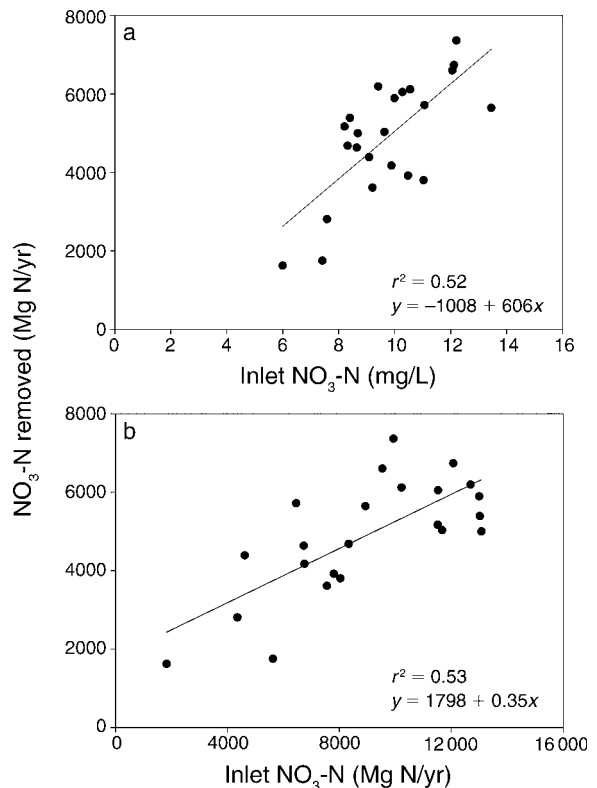

FIG. 7. Annual NO_3-N removed, calculated as the difference of stream water inputs + change in storage and dam output, plotted against (a) inlet NO_3-N concentration or (b) flux for the 1981–2003 water years for the Lake Shelbyville reservoir.
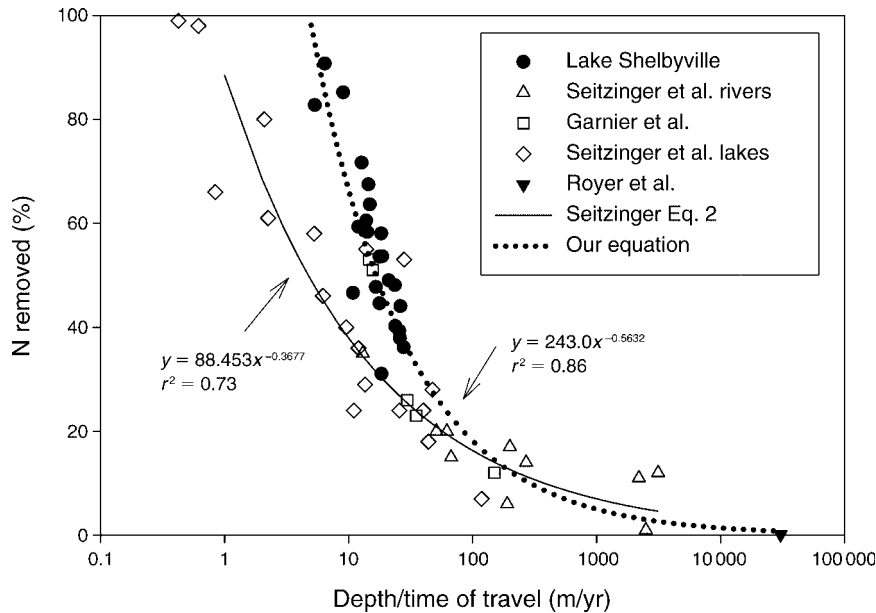
FIG. 8. Relationship between N removed (as a percentage of inputs) in a study area and depth divided by time of travel (water retention time for lakes and reservoirs). Values shown are NO_3-N data for 23 water years at Lake Shelbyville, average NO_3-N stream value from Royer et al. (2004), river and lake data and equation from Seitzinger et al. (2002), Garnier et al. (1999) NO_3-N reservoir data, and a new equation using all data except those from lakes.

ments) represents no more than 5% of the riverine flux. When combined with the riverine flux from David and Gentry (2000), we estimate maximum in-stream losses within Illinois to be about 12 000 Mg N/yr. The sum of the reservoir and in-stream losses is 60 900 Mg N/yr, about half the 132 000 Mg N/yr estimate of the in-stream denitrification sink from David and Gentry (2000). From this portion of the N mass balance we conclude that (1) overall, aquatic sediment denitrification occurs primarily in the reservoirs of the state; (2) in-stream processing is a relatively small N sink; and (3) without the aquatic sediment denitrification sink, Illinois rivers would export about a 25% larger load of NO_3-N to the Mississippi river and Gulf of Mexico.

Role of reservoirs and wetlands in reducing riverine N export

Illinois, typical of the corn belt states in the Midwest, is one of the major contributors of NO_3-N to the Gulf of Mexico (David and Gentry 2000, Goolsby et al. 2001). Denitrification in the surface waters of Illinois has been directly assessed in streams (Royer et al. 2004, Schaller et al. 2004), in small wetlands receiving tile flow (Xue et al. 1999, Kovacic et al. 2000), and in a large reservoir (this study). Denitrification rates per gram of sediment have been high in all ecosystems, but the overall effectiveness of denitrification as a sink for NO_3-N is primarily a function of water retention time. Lake Shelbyville removed on average ~58% of the N inputs to the reservoir, with an estimated rate of 108 g $N \cdot m^{-2} \cdot yr^{-1}$. For small wetlands in Illinois, Kovacic et

al. (2000) reported an overall removal of 37% of N inputs, with a rate of 33.3 g $N \cdot m^{-2} \cdot yr^{-1}$, about one-third the Lake Shelbyville rate. Retention times in the Kovacic et al. (2000) wetlands ranged between 5 and 32 d, much less than the average Lake Shelbyville retention time of 131 d.

To reduce N loading to the Mississippi River, Mitsch et al. (2001) proposed large scale reconnection of terrestrial ecosystems with floodplains in the upper Midwest, using wetlands and riparian zones. However, because of the large flow volumes that occur during only a portion of the year (typically March through June), and because drainage tiles would circumvent riparian zones, creating wetlands that could effectively reduce riverine NO_3-N transport will be difficult to achieve. Creating new, large reservoirs is not feasible because of land values and removal of highly productive crop lands, as well as the many detrimental effects of dams on riverine ecosystems (Baxter 1977, Benke 1990, Dynesius and Nilsson 1994). Alternatively, it could be effective to incorporate best management practices that would maximize N-removal in reservoirs located in watersheds with large riverine NO_3-N by maximizing water retention time in late spring and early summer when the largest NO_3-N loads enter the reservoir. However, Garnier et al. (2000) found that altering retention times within realistic values had little influence on the absolute amount of NO_3-N denitrified. In addition, a management scheme that might maximize NO_3-N retention should be applied cautiously, because it may alter natural flow regimes that could detrimentally affect

downstream biota. The reconnection of rivers to flood-plains (Junk et al. 1989) at an intermediate scale might be the best approach. This would be larger than a wetland on an individual tile (Kovacic et al. 2000), but smaller than large reservoirs like Lake Shelbyville. The key is slowing down the water enough when NO_3-N loads are high to allow sediment denitrification to be effective. Maximizing denitrification in reservoirs and other aquatic ecosystems (without large scale changes) within the Mississippi watershed will likely not remove enough N to solve seasonal hypoxia problems in the Gulf of Mexico (Richardson et al. 2004), but may partially mitigate the effects of high NO_3-N loads until larger watershed policies designed to reduce the source of the problem are initiated (Tilman et al. 2001, Rabalais et al. 2002a).

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