Biogeochemistry (2005) 76: 85–111
DOI 10.1007/s10533-005-2199-6


# Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir

LAREINA G. WALL[1], JENNIFER L. TANK[1,*], TODD V. ROYER[2] and MELODY J. BERNOT[1]

*[1]Department of Biological Sciences, 192 Galvin Life Sciences, University of Notre Dame, Notre Dame, IN 46556; [2]Department of Biological Sciences, 256 Cunningham Hall, Kent State University, Kent, OH 44242; *Author for correspondence (e-mail: tank.1@nd.edu; phone: +1-574-631-3976; fax: +1-574-631-7413)*

Received 5 March 2004; accepted in revised form 14 February 2005

**Key words:** Agriculture, Denitrification, Nitrogen, Reservoir, River, Sediments

**Abstract.** Reservoirs are intrinsically linked to the rivers that feed them, creating a river–reservoir continuum in which water and sediment inputs are a function of the surrounding watershed land use. We examined the spatial and temporal variability of sediment denitrification rates by sampling longitudinally along an agriculturally influenced river–reservoir continuum monthly for 13 months. Sediment denitrification rates ranged from 0 to 63 μg $N_2O$ g ash free dry mass of sediments $(AFDM)^{-1}$ $h^{-1}$ or 0–2.7 μg $N_2O$ g dry mass of sediments $(DM)^{-1}$ $h^{-1}$ at reservoir sites, vs. 0–12 μg $N_2O$ g$AFDM^{-1}$ $h^{-1}$ or 0–0.27 μg $N_2O$ g$DM^{-1}$ $h^{-1}$ at riverine sites. Temporally, highest denitrification activity traveled through the reservoir from upper reservoir sites to the dam, following the load of high nitrate ($NO_3^-$-N) water associated with spring runoff. Annual mean sediment denitrification rates at different reservoir sites were consistently higher than at riverine sites, yet significant relationships among theses sites differed when denitrification rates were expressed per gDM vs. per gAFDM. There was a significant positive relationship between sediment denitrification rates and $NO_3^-$-N concentration up to a threshold of 0.88 mg $NO_3^-$-N $l^{-1}$, above which it appeared $NO_3^-$-N was no longer limiting. Denitrification assays were amended seasonally with $NO_3^-$-N and an organic carbon source (glucose) to determine nutrient limitation of sediment denitrification. While organic carbon never limited sediment denitrification, all sites were significantly limited by $NO_3^-$ -N during fall and winter when ambient $NO_3^-$-N was low.

**Abbreviations:** AFDM – ash free dry mass; ANOVA – analysis of variance; APHA – American Public Health Association; C – carbon; chl *a* – chlorophyll *a*; CV – coefficient of variation; DM – dry mass; DOC – dissolved organic carbon; Figure – figure; g – gram; He – helium; h – hour; km – kilometers; l – liter; LSMeans – least-squared means; LSV – Lake Shelbyville, Illinois; m – meter; mg – milligram; ml – milliliter; mM – millimolar; N – nitrogen; N + C – nitrogen plus carbon; $N_2$ – di-nitrogen gas; $N_2O$ – nitrous oxide; $NH_4^+$ – ammonium; $NO_3^-$ – nitrate; $O_2$ – oxygen; SE – standard error; TDN – total dissolved nitrogen; TN – total nitrogen; USEPA – United States Environmental Protection Agency; USGS – United Stated Geologic Survey; μg – microgram

## Introduction

Reservoirs represent a transition zone from lotic to lentic ecosystems and have been described as 'river–lake hybrids' given that they encompass intermediate

characteristics that define both lakes and rivers (Kimmel et al. 1990). Compared to natural lakes, reservoirs tend to have shorter water residence times and more complex hydrology due to the presence of one or more major water inlets, instead of multiple diffuse water sources characteristic of most natural lakes (Kennedy et al. 1985; Kennedy and Walker 1990). Reservoirs are intrinsically linked to the rivers that feed them (Baxter 1977), creating a river–reservoir continuum, in which water and sediment inputs are functions of the surrounding watershed land use (Kelly 2001). Little research has focused on the cycling and biogeochemical transformations of nitrogen (N) that occur within reservoirs and more importantly, how these processes change longitudinally within the river–reservoir continuum.

Anthropogenic activities, including fossil fuel burning, increased planting of N-fixing crops, and fertilizer production and application (David and Gentry 2000; Gentry et al. 2000) have nearly doubled the rate of N input to the terrestrial N cycle (Vitousek et al. 1997) and at least tripled the nitrate ($NO_3^-$-N) load carried by the Mississippi River (Goolsby et al. 2001; Rabalais et al. 2002). Fertilizer application, in combination with rapid water removal through tile drainage of fields, and channelization of streams in the agricultural Midwest, have lead to increased N loads carried by headwater streams, with $NO_3^-$-N concentrations often exceeding drinking water standards of 10 mg $l^{-1}$ (David et al. 1997; Townsend et al. 2003). These increased N loads are subsequently carried to the Mississippi River and contribute to a number of human health problems (Townsend et al. 2003), eutrophication of surface waters (Carpenter et al. 1998), and seasonal hypoxia in the Gulf of Mexico (Goolsby et al. 2001; Rabalais et al. 2002). Identifying factors that control N retention in upstream aquatic ecosystems is essential for determining the relative roles that wetlands, lakes, rivers, and reservoirs play in reducing high N loads.

Multiple processes contribute to $NO_3^-$ retention within aquatic ecosystems including biological assimilation, abiotic sedimentation, dissimilatory $NO_3^-$ reduction and denitrification (Schlesinger 1997; Saunders and Kalff 2001). Denitrification is the microbial reduction of $NO_3^-$ to N gases (dinitrogen, $N_2$ and nitrous oxide, $N_2O$) under anoxic conditions (Tiedje 1982). In aquatic systems, denitrification occurs readily in the top 2–5 cm of sediment when there is abundant $NO_3^-$, organic carbon, and anoxic microsites (Seitzinger 1988). Sediment denitrification is of particular interest in the context of N export because it represents a permanent removal of N from the aquatic ecosystem whereas other transformations (e.g. assimilatory uptake into biomass) yield only temporary storage of N. Mass-balance studies in reservoirs suggest sediment denitrification could be an important retention mechanism (Toetz 1973; Josette et al. 1999). Denitrification rates within river sediments can be limited by $NO_3^-$-N, organic carbon, temperature, and redox conditions (e.g., Garcia-Ruiz et al. 1998a, b; Pattinson et al. 1998). The effect of denitrification on N retention in riverine systems is determined by sediment–water interactions, including water residence time, water depth, and hydrological connectivity

between various aquatic habitats (e.g., Alexander et al. 2000; Saunders and Kalff 2001; Richardson et al. 2004).

We examined denitrification in a shallow, well-mixed reservoir in central Illinois that drains an agricultural watershed. Surface waters in the agricultural areas of Illinois experience $NO_3^-$-N concentrations that range annually from below detection to greater than 15 mg $NO_3^-$-N $l^{-1}$ (David et al. 1997), and denitrification can at times be nitrate limited (Royer et al. 2004). It is not known how the temporal variation in $NO_3^-$-N concentrations and the changes in physical (increased depth, decreased dissolved oxygen) and chemical parameters within the reservoir will affect the spatial and temporal variability of sediment denitrification rates along the river–reservoir continuum.

The objectives of this study were to measure sediment denitrification rates at different sites located longitudinally within a river–reservoir continuum and to determine factors influencing sediment denitrification at these sites. We predicted that: (1) sediment denitrification rates along a river–reservoir continuum would vary spatially and temporally in relation to longitudinal variation in factors limiting denitrification such as water column $NO_3^-$-N, organic matter availability, and oxygen concentration, (2) higher sediment denitrification rates would be associated with reservoir sites compared to riverine sites, and (3) sediments collected from different habitat types within the reservoir (e.g. littoral vs. profundal) would have different denitrification rates.

## Methods

### *Study site*

This study was conducted in Lake Shelbyville (39°24′22″ N, 88°47′00″ W), a reservoir located on the Kaskaskia River, which is a tributary of the Mississippi River in east-central Illinois (Figure 1). The Shelbyville Dam was constructed in 1970 for flood control and is located 19.3 km south of the West Okaw River confluence with the Kaskaskia River resulting in a Y-shaped reservoir. The Kaskaskia and West Okaw Rivers combined account for approximately 80% of the water inflow to Lake Shelbyville (LSV). These rivers are equipped with USGS gaging stations both upstream (Station numbers 591200 and 5591700) and at the LSV dam (Station 5592000), logging hourly stage readings (USGS 2003). LSV has a drainage basin of 2730 $km^2$ with land use of predominately row-crop agriculture ( > 80% corn and soybeans; Illinois Department of Agriculture 2003). The surface area of LSV is 44 $km^2$ under normal pool conditions and can expand up to 100 $km^2$ under flood conditions. The mean water depth was 4.7 m during our sampling period with greatest depths (maximum = 13 m) located close to the dam and more shallow depths (minimum = 0.1 m) located in upper reservoir sites. Water residence time in the reservoir from 1981 to 2003 has ranged from 2.5 to 10 months with a mean

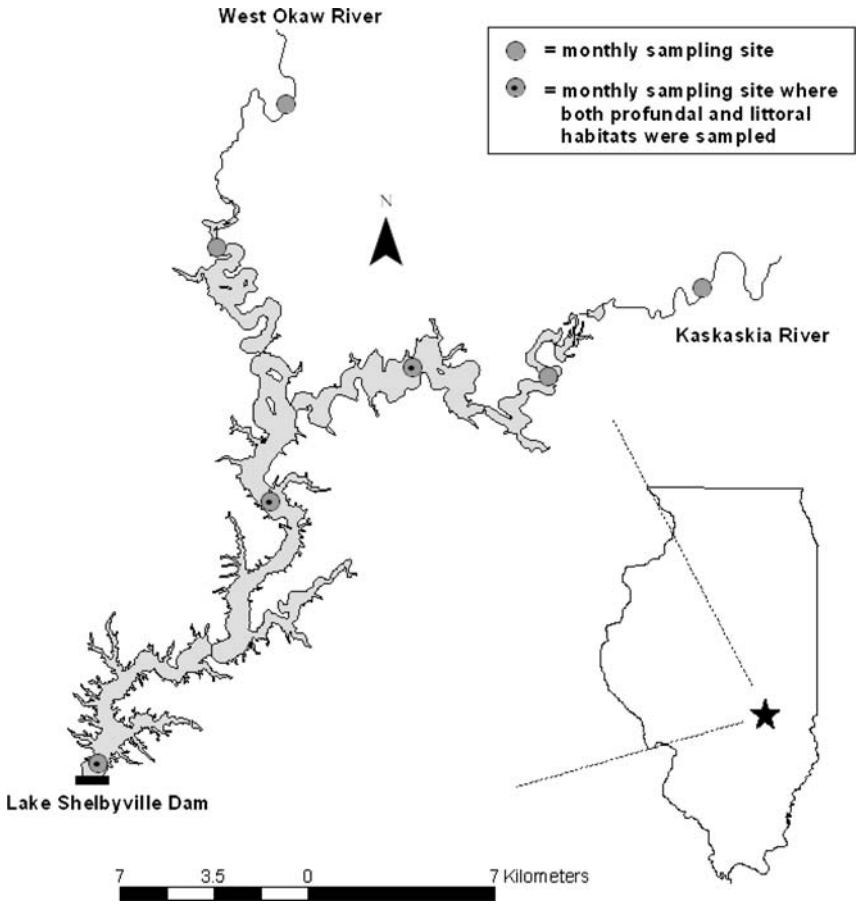


*Figure 1.* Locations of monthly sampling sites within the Lake Shelbyville, IL river–reservoir continuum. Dots mark the five sites sampled within the reservoir and the two riverine sampling sites in the West Okaw and Kaskaskia Rivers.

of 4.3 months (M. B. David, personal communication). In general, this is a shallow, well-mixed reservoir with long water residence time.

### *Field sampling regime*

Seven sites within the LSV river–reservoir continuum were sampled for sediments and water chemistry monthly from March 2002 to March 2003. Five of these sites were located within LSV and two were located upstream of the reservoir in the free-flowing Kaskaskia and West Okaw Rivers (Figure 1). Flooding occurred from May to July 2003. As a result, during August sampling, both riverine sites were relocated approximately 4 km upstream to avoid

reservoir water backflow as a result of high flows in the previous months. The five reservoir sampling sites were partitioned throughout the length of the reservoir to account for longitudinal variability typical of reservoir ecosystems (Kennedy et al. 1985). To account for variability within LSV, three distinct reservoir habitat types were encompassed within these sites including, (1) shallow upper reservoir, (2) profundal or deep water, and (3) shoreline or 'littoral' habitats. Habitat types were selected to account for variability in water depth, sediment quality, and submerged vegetation that could influence denitrification rates. Two upper shallow reservoir sites were sampled, one in the Kaskaskia reservoir branch and the other in the West Okaw reservoir branch, representing the transitional habitat from river to reservoir ecosystem described by Thorton et al. (1980). The other three reservoir sites were located further into LSV and sampled at both profundal and littoral habitats. LSV littoral habitat does not fit the classic definition of littoral habitat as described by Wetzel (2002) because of the continual erosion of the shoreline; therefore, we defined our littoral habitat as the area within 10–15 m of the shoreline regardless of depth or the presence of macrophytes.

*Field procedures*

Reservoir sediments were collected with an Ekman dredge, which sampled to a depth of ~3–6 cm depending on sediment texture at the particular site. The contents of three grabs were pooled, homogenized by gently stirring, and approximately 1 l of the composite sediment slurry was returned to the laboratory for denitrification assays. For the river sites, sediments were collected using a small coring device and spatula down to a depth of ~5 cm, pooled from multiple locations within each site and placed into a 1 l bottle. We were not able to collect river sediments for three months (May–July) due to high water. Unfiltered stream or reservoir water was collected 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. Preliminary experiments to determine sediment storage time confirmed that a 24 h storage period did not influence denitrification measurements; sediment denitrification rates from assays initiated immediately following collection were not significantly different from rates using sediments stored for 24 h (data not shown, one-way ANOVA, $p = 0.35$).

At each reservoir site, water for nutrient analyses was also collected within one meter of the benthic sediment surface using a Van Dorn sampler. Water at each river site was collected from the thalweg, except during flood conditions (May–July) when we collected samples in the inundated floodplain. Water samples were filtered (0.7 μm Whatman GF/F filters) in the field into acid-washed 60 ml Nalgene© bottles for dissolved nutrient analyses, except during winter months when samples were not filtered until we returned to the

laboratory. A separate 60 ml unfiltered water sample was collected for total nitrogen (TN) analysis. Water samples were placed on ice for transport to the laboratory and remained frozen until analyses were performed.

A known volume of epilimnetic water was filtered onto a GF/F filter for chlorophyll *a* (chl *a*) analysis. The filter was placed in a scintillation vial wrapped in foil and placed on ice until return to the laboratory, and kept frozen until analysis. Temperature and dissolved oxygen profiles of the reservoir water column were recorded using a Temperature/Dissolved Oxygen Meter 55 (YSI©, Yellow Springs, OH). To approximate the temperature and dissolved oxygen at the sediment–water interface, we allowed the probe to rest on the sediment surface and equilibrate.

### *Laboratory denitrification assays*

Sediment denitrification rates were estimated using a modified chloramphenicol-amended acetylene inhibition technique as originally described by Tiedje (1982). The acetylene inhibition technique blocks the reduction of $NO_3^-$ to $N_2$ at the $N_2O$ step so only $N_2O$ accumulates in the headspace of assay bottles. Approximately 25 ml of homogenized sediment slurry from each site was dispensed into four replicate 125 ml media bottles. Although sediments were disrupted to make a slurry, LSV sediments were naturally unconsolidated and regularly subjected to disturbance through wind turbulence and bottom-dwelling organisms. A chloramphenicol solution prepared with the unfiltered water from each site was added to the media bottles to obtain a final concentration of approximately 6 mM. Chloramphenicol was added to suppress *de novo* enzyme synthesis and yield measurements approximating *in situ* denitrification rates (Yoshinari and Knowles 1976; Smith and Tiedje 1979; Murray and Knowles 1999). The total volume in the bottles was brought to 75 ml with unfiltered site water. Media bottles were sealed with caps containing butyl septa, and the headspace was purged with ultra high purity He for 5 min, swirling periodically to ensure distribution. Following the He purge, 15 ml of acetylene gas was added to three of the assay bottles to obtain a 10% acetylene mixture in the headspace. The fourth assay bottle, the control, did not receive acetylene to quantify $N_2O$ accumulation in the absence of acetylene. The bottles were shaken to distribute the acetylene into the sediment slurry.

Based on our results and those of other studies (e.g., Rudolph et al. 1991; Bernot et al. 2003; Royer et al. 2004; Schaller et al. 2004), the chloramphenicol-amended acetylene inhibition technique is an accurate and cost-effective technique for estimating denitrification when replication and spatial scale would prohibit *in situ* methodology or coring methods (Bernot et al. 2003). We recognize that the acetylene inhibition technique can, in some situations, significantly underestimate denitrification rates (Seitzinger et al. 1993), particularly when used in sediment cores with low available $NO_3^-$-N, with high rates of coupled nitrification-denitrification (Rudolph

et al. 1991; Seitzinger et al. 1993). The present study was conducted in an agricultural reservoir that receives high inputs of $NO_3^-$-N from the watershed, and we believe nitrification to be a relatively minor source of $NO_3^-$-N for denitrification, relative to fertilizer contributions, in LSV. Additionally, the short duration of the assay incubations likely minimized potential errors caused by simultaneous nitrification inhibition (Bernot et al. 2003). If the experimentally induced anoxia combined with normally high *in situ* $NO_3^-$-N concentrations resulted in an increase in bacterial enzyme production, this would result in an exponential increase in $N_2O$ concentrations over time; however, in the presence of chloramphenicol, new microbial enzyme production was inhibited, and $N_2O$ production was linear.

Sediment denitrification assays were incubated at two temperatures: the mean *in situ* temperature at the sediment–water interface (ambient) and room temperature (22 °C). When the mean ambient temperature was ±4 °C of room temperature, only the room temperature incubation was conducted. Five 5-ml gas samples were drawn from the bottle headspace with a gas tight syringe approximately every hour over a 5-h period and each gas sample was transferred to a 3 ml vacutainer® (Becton-Dickinson). The sample volume was replaced with a 10% acetylene–He mixture to maintain constant pressure throughout the assay. Vacutainer® septa were sealed with a silicon bead and stored at room temperature until gas samples were analyzed for $N_2O$ on a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a Supelco® 80/100 HAYESEPQ 9′ 1/8″ column and an electron capture detector. Column and detector temperatures were 70 and 325 °C, respectively.

### *Nutrient-amended denitrification assays*

A separate set of denitrification assays were amended with nutrients ($NO_3^-$-N and glucose) in April (spring), July (summer), October (fall) and January (winter) to determine if there was seasonal nutrient limitation of denitrification within the river–reservoir continuum. We amended the sediment denitrification assays with either 6 mg $NO_3^-$-N $l^{-1}$ (N treatment), or 30 mg C $l^{-1}$ as glucose (C treatment), or both $NO_3^-$-N and C (N + C treatment). The N treatment and C treatment were conducted during all seasons; N + C treatment was conducted only in the fall and winter when ambient $NO_3^-$-N concentrations were low and there was a higher potential for co-limitation. All amended denitrification assays were conducted in the same fashion as the monthly denitrification assays and incubated at room temperature.

### *Nutrient and chlorophyll* a *analyses*

Filtered water samples were analyzed for $NO_3^-$-N, ammonium ($NH_4^+$-N), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC). A

separate unfiltered water sample was analyzed for TN. Nitrate concentrations were quantified using a DIONEX DX600 ion chromatograph (USEPA 1993). Ammonium concentrations were determined using the phenylhypochlorite technique (Solorzano 1969) and analyzed with a 10-cm cell on a Shimadzu UV 1601 spectrophotometer. TDN and TN were quantified using a modified version of the persulfate method 4500-$N_{org}$ (APHA 1995) and measured colorimetrically using a Lachat QuickChem®8000. Dissolved organic carbon was measured on acidified water samples using the total organic carbon method 5310 B (APHA 1995) and measured on a Shimadzu TOC-5000A. Chlorophyll *a* was extracted from the frozen filter with 90% ethanol, warmed in a 78 °C water bath, and allowed to stand over night at ~6 °C (Sartory and Grobbelaar 1984). Extracts were analyzed at room temperature with the modified fluorometric technique on a Turner10-AU fluorometer (USEPA 1997).

### *Sediment characterization*

From each assay bottle, subsamples of sediment were analyzed to quantify dry mass (DM), ash-free dry mass (AFDM), carbon content (%C) and nitrogen content (%N) as indicators of sediment quality. An aliquot of sediment slurry was added to a pre-ashed, pre-weighed tin, simultaneously as each denitrification assay bottle was filled. The sediments were then dried at 60 °C, weighed, ashed for at least 2 h at 550 °C, rewet, dried, and reweighed. An additional sub-sample from each site was dried, homogenized with a coffee grinder and/or mortar and pestle and analyzed for C and N on a Costech elemental combustion system.

Because there were inherent differences in sediment quality and texture, denitrification rates calculated from laboratory assays were expressed and analyzed both as μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ and as μg $N_2O$ $gDM^{-1}$ $h^{-1}$. In general, sediment denitrification rates are reported here as μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ unless results were significantly different when expressed as μg $N_2O$ $gDM^{-1}$ $h^{-1}$ and then results are reported both ways.

### *Statistical analyses*

Sediment denitrification rates were grouped by season to identify temporal trends (March–May = spring, June–August = summer, September–November = fall, and December–February = winter). Sediment denitrification rates from ambient temperature incubations were used for seasonal analyses. Room temperature incubations were used in analyses in which we wanted to eliminate seasonal effects on denitrification rates. In general, room temperature incubations resulted in higher denitrification rates than the ambient temperature assays.

We tested for habitat effects on mean annual denitrification rates using one-way analysis of variance (ANOVA). A post-hoc least-squares means test

(LSMeans) followed significant ANOVA result to determine differences among habitat types with $\alpha = 0.05$. A repeated measures ANOVA was performed using SAS 8.02 on sediment denitrification rates with ecosystem type (reservoir vs. river) and season as the fixed effects (SAS Institute 1991). If a significant reach * time interaction was found, a least-squares means test was used to obtain the effects of habitat and season. A Bonferroni test was used to obtain adjusted *p*-values and control cumulative Type I error. Non-normally distributed data were square root-transformed to meet the assumptions of parametric statistics.

Prior to conducting analyses on nutrient amendment results, the data were pooled based on the pre-treatment ambient $NO_3^-$-N concentration, with spring and summer as high $NO_3^-$ months and fall and winter as low $NO_3^-$ months. A two-factor ANOVA was used to determine whether denitrification was significantly affected by the N and C treatments (Tank and Dodds 2003). Single nutrient limitation was indicated when just one treatment (N or C) elicited a positive response (two-way ANOVA, $p < 0.05$) and the interaction term in the ANOVA was not significant. Denitrification was determined to be co-limited by N and C if an even greater response resulted when adding N and C together than when either was added alone.

We tested for seasonal differences in response to the nutrient amendments by sites with a one-way ANOVA performed on the ratio of the treatment (N, C or N + C treatment) to the control (denitrification at ambient nutrient concentrations; $p < 0.005$). If the ratio was undefined because the denitrification rate in the absence of nutrient amendment was equal to zero, a rate less than the lowest observed rate (0.001 μg $N_2O$ $gAFDM^{-1}$ $hr^{-1}$) was assigned to obtain a numerical value.

Simple linear regression and stepwise multiple regression analyses were used to examine relationships between sediment denitrification and measured independent variables (water column $NO_3^-$ concentration, DOC, dissolved oxygen, temperature and sediment nitrogen and organic content). We also calculated the annual coefficient of variation (CV) for sediment denitrification rates and independent variables and evaluated the relationship between the two with regression analysis. Because independent variables span such a broad range of values temporally, and the response of denitrification along this range can vary greatly, a two-dimensional Kolmogorov–Smirnov test was used to determine the threshold at which a nonrandom pattern between denitrification and the independent variable dissipates (Garvey et al. 1998).

## Results

### *Variation in physiochemical parameters within the river–reservoir continuum*

Sites differed in depth and sediment characteristics, yet were similar in parameters influenced by season (i.e., temperature and dissolved oxygen).

Profundal sites were significantly deeper than littoral and shallow upper reservoir sites (Table 1). Water temperature and dissolved oxygen at the sediment–water interface were not significantly different between sites with exceptions in spring when profundal sites had significantly lower dissolved $O_2$ (expressed as % $O_2$ saturation) and in winter when riverine water temperatures were significantly lower (Table 1). The sediment–water interface temperature was the highest during summer months at all sites, coinciding with the lowest dissolved $O_2$ concentrations.

The reservoir water column was not thermally stratified for most of the year, except periodically during summer. The difference between the mean temperatures of epilimnion and sediment–water interface in summer was highly influenced by wind-induced water column mixing and ranged from 10 °C on a calm day in July to 0.6 °C on a windy day in August. Dissolved $O_2$ in the reservoir exhibited a clinograde for most of the year. The difference in dissolved $O_2$ ranged from 11% saturation in February to 139% saturation in September and, like temperature, was influenced by wind, given the relatively shallow depths of LSV.

Sediment organic matter at profundal sites was significantly higher than littoral and riverine sites in spring and fall, but not during summer and winter months (Table 1). Sediment organic matter was not significantly different between the profundal and shallow upper reservoir sites. Nitrogen content of sediments was greatest at the profundal and shallow upper reservoir sites. In general, sediment characteristics at profundal and shallow upper reservoir sites were similar to each other and different from littoral and river sites.

*Spatial variability in sediment denitrification rates and $NO_3^-$-N within the river–reservoir continuum by season: results from incubations at ambient temperatures*

Sediment denitrification rates ranged from 0 to 63 μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ or 0–2.7 μg $N_2O$ $gDM^{-1}$ $h^{-1}$ for reservoir sites and 0–12 μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ or 0–0.27 μg $N_2O$ $gDM^{-1}$ $h^{-1}$ for riverine sites. We used the distance from the dam to quantify the position of each site within the reservoir relative to the other sites, and then evaluated the spatial variation in sediment denitrification rates and $NO_3^-$-N concentrations along the river–reservoir continuum. The distance from the dam also provides a relative measure of water residence time among the sites, although the actual residence time would vary seasonally as reservoir volume changed. It was not possible to calculate the residence time for individual sites within the reservoir.

In general, sediment denitrification rates within the reservoir, with respect to distance from the dam, changed with season (Figure 2a–d). All sites within the reservoir exhibited similar rates of sediment denitrification in spring regardless of distance from the dam (Figure 2a). During summer, sediment denitrification increased as one moved closer to the dam with the highest mean sediment denitrification rate (±1 SE) occurring approximately 27 km from the dam

*Table 1.* Mean seasonal physical parameters and sediment characteristics with coefficient of variation for each site within the LSV river–reservoir continuum.

| | | Profundal | | | | Littoral | | | | Shallow Upper Reservoir | | | | Riverine | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | ±1 SE | N | CV (%) | Mean | ±1 SE | N | CV (%) | Mean | ±1 SE | N | CV (%) | Mean | ±1 SE | N | CV (%) |
| Depth (m) | Spring | 9.33[a] | ±0.58 | 9 | 19 | 4.42[b] | ±1.08 | 11 | 81 | 1.97[c] | ±1.04 | 7 | 140 | 0.63[c] | ±0.07 | 6 | 29 |
| | Summer | 8.35[a] | ±0.94 | 8 | 32 | 4.56[b] | ±1.27 | 9 | 84 | 2.36[b] | ±0.67 | 5 | 64 | flooding | | | |
| | Fall | 6.42[a] | ±0.57 | 8 | 25 | 3.40[b] | ±0.66 | 9 | 58 | 1.39[c] | ±0.59 | 6 | 103 | 0.47[c] | ±0.02 | 6 | 11 |
| | Winter | 6.17[a] | ±1.48 | 3 | 42 | 2.83[b] | ±0.64 | 9 | 68 | 0.23[b,c] | ±0.08 | 5 | 78 | 0.51[c] | ±0.1 | 6 | 50 |
| Temperature (°C)** | Spring | 11.51[a,b] | ±1.23 | 9 | 32 | 9.94[a] | ±1.30 | 11 | 43 | 14.89[b] | ±2.26 | 7 | 40 | 10.65[a] | ±1.7 | 6 | 39 |
| | Summer | 20.59[a] | ±1.43 | 8 | 20 | 22.96[a] | ±1.60 | 9 | 21 | 23.62[a] | ±1.18 | 5 | 47 | 24.70[a] | ±0.2 | 2 | 1 |
| | Fall | 19.14[a] | ±2.32 | 8 | 34 | 18.79[a] | ±2.44 | 9 | 39 | 17.80[a] | ±3.42 | 6 | 11 | 17.52[a] | ±3.39 | 6 | 47 |
| | Winter | 2.77[a] | ±0.47 | 3 | 29 | 2.37[a] | ±0.34 | 9 | 43 | 1.18[a,b] | ±0.54 | 5 | 103 | 1.08[b] | ±0.46 | 6 | 104 |
| $O_2$ (% Saturation)** | Spring | 39.02[a] | ±10.90 | 9 | 125 | 78.10[b] | ±10.77 | 11 | 50 | 77.06[b] | ±14.67 | 7 | 50 | 96.97[b] | ±8.04 | 6 | 22 |
| | Summer | 25.14[a] | ±10.08 | 8 | 113 | 43.74[a,b] | ±10.11 | 9 | 69 | 18.82[a] | ±11.08 | 5 | 132 | 82.50[b] | ±5.5 | 2 | 9 |
| | Fall | 29.30[a] | ±13.00 | 8 | 84 | 59.77[b] | ±9.98 | 9 | 46 | 38.07[a,b] | ±9.54 | 6 | 61 | 70.70[b] | ±12.1 | 6 | 42 |
| | Winter | 54.80[a] | ±11.15 | 3 | 35 | 81.10[a] | ±11.81 | 9 | 44 | 93.63[a] | ±19.82 | 5 | 47 | 77.87[a] | ±16.3 | 6 | 51 |
| Sediment organic matter (%) | Spring | 4.17[a] | ±0.53 | 9 | 38 | 1.54[b] | ±0.09 | 11 | 20 | 5.58[a] | ±0.88 | 7 | 42 | 1.49[b] | ±0.29 | 6 | 48 |
| | Summer | 3.26[a,b] | ±0.71 | 8 | 62 | 2.44[a] | ±0.69 | 9 | 85 | 5.39[b] | ±1.37 | 5 | 57 | 1.54[a] | ±0.43 | 2 | 39 |
| | Fall | 3.47[a] | ±0.98 | 8 | 80 | 1.45[b] | ±0.36 | 9 | 74 | 4.96[a] | ±0.93 | 6 | 46 | 1.58[b] | ±0.43 | 6 | 67 |
| | Winter | 5.10[a,b] | ±0.77 | 3 | 26 | 2.55[b,c] | ±0.77 | 9 | 91 | 5.57[a] | ±0.98 | 5 | 39 | 1.36[c] | ±0.18 | 6 | 35 |
| Sediment nitrogen content (%) | Spring | 0.15[a] | ±0.02 | 9 | 44 | 0.04[b] | ±0.004 | 11 | 36 | 0.17[a] | ±0.04 | 7 | 57 | 0.06[b] | ±0.02 | 6 | 82 |
| | Summer | 0.14[a,b] | ±0.03 | 8 | 73 | 0.07[a] | ±0.03 | 9 | 129 | 0.19[b] | ±0.05 | 5 | 55 | 0.04[a,b] | ±0.02 | 2 | 77 |
| | Fall | 0.12[a] | ±0.03 | 8 | 65 | 0.05[b] | ±0.01 | 9 | 6 | 0.19[c] | ±0.02 | 6 | 31 | 0.05[b] | ±0.02 | 6 | 81 |
| | Winter | 0.20[a,b] | ±0.02 | 3 | 20 | 0.10[b] | ±0.03 | 9 | 94 | 0.21[a] | ±0.05 | 5 | 51 | 0.03[b] | ±0 | 6 | 22 |

**Indicates values measured at the sediment–water interface. Winter profundal values were only measured in December. Significant differences between habitat type means at the $\alpha = 0.05$ level are indicated by different superscript letters.

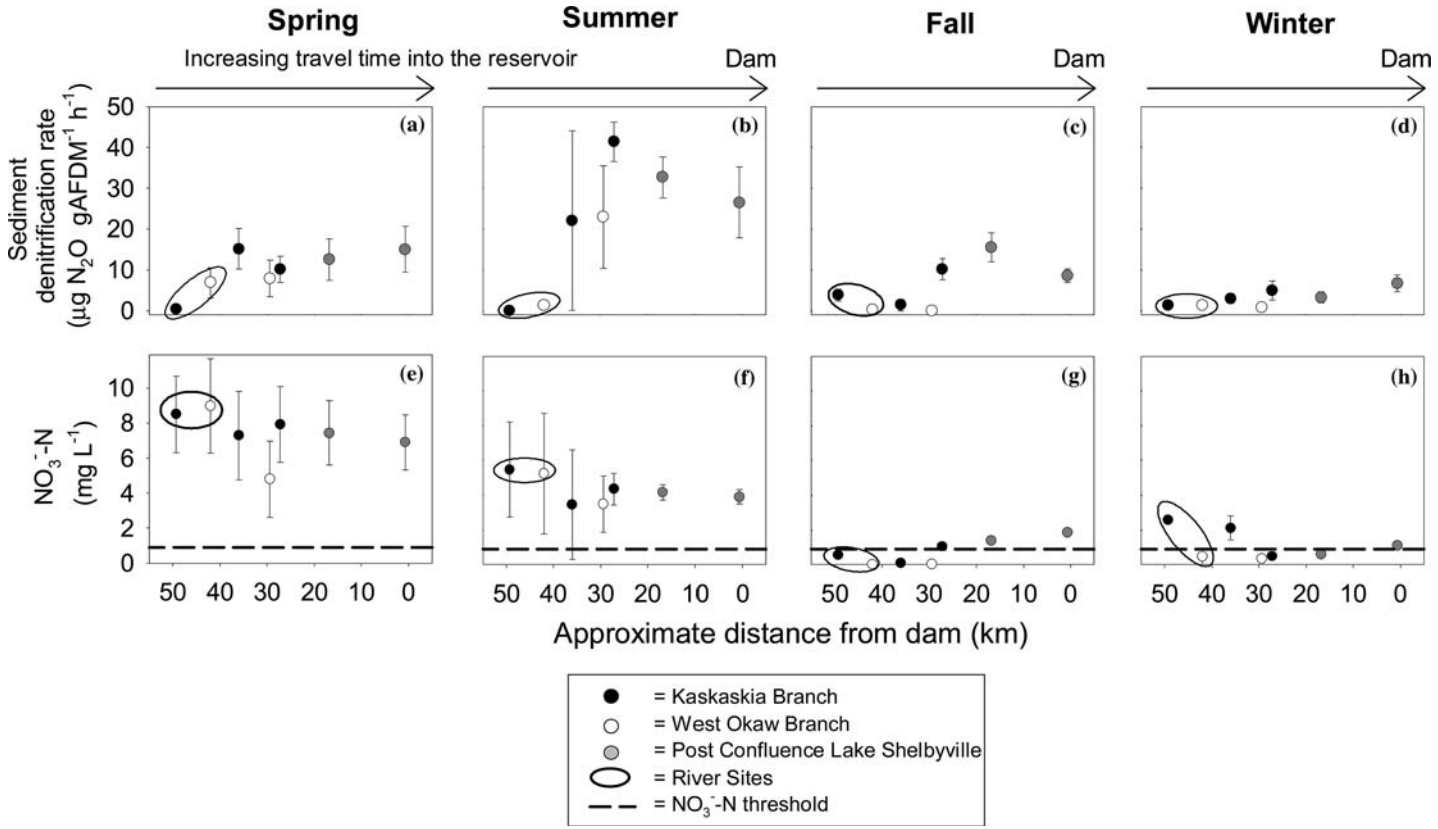
Spring
Summer
Fall
Winter
Increasing travel time into the reservoir
Dam
Dam
Dam
Sediment denitrification rate (µg $N_2O$ gAFDM$^{-1}$ h$^{-1}$)
$NO_3^-$-N (mg L$^{-1}$)
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
50 40 30 20 10 0
Approximate distance from dam (km)
= Kaskaskia Branch
= West Okaw Branch
= Post Confluence Lake Shelbyville
= River Sites
= $NO_3^-$-N threshold

*Figure 2.* Mean seasonal sediment denitrification rates (±1 SE) of assays incubated at ambient temperature (a–d) and $NO_3^-$-N concentration (e–h) within the Lake Shelbyville river–reservoir network plotted against the approximate travel distance from the dam (dam = 0). Spring = March–May, summer = June–August, fall = September–November and winter = December–February. Means include values of both the littoral and profundal habitat for sites where both habitats were sampled. River denitrification was sampled only once in the summer and therefore, no error bars are present on data points.

($41.3 \pm 4.8$ μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$; Figure 2b). Sediment denitrification within the reservoir was generally lower during fall, yet remained relatively high at the lower end of the reservoir when compared to upper reservoir sites (Figure 2c). Sediment denitrification rates were lowest during winter with the highest mean sediment denitrification rate (±1 SE) occurring at the dam site ($6.6 \pm 2.1$ μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$; Figure 2d). Although there were differences in denitrification rates between specific sites within the river–reservoir continuum on different sampling dates, in general, the mean reservoir sediment denitrification rate by season was significantly higher than the mean river sediment denitrification rate during spring and summer, and not significant different during fall and winter (repeated measures ANOVA, $p = 0.019$, $p < 0.001$, $p = 0.07$ and $p = 0.31$, respectively).

Nitrate concentrations in the river–reservoir continuum were highest in spring, decreased in summer and fall, and began to increase in riverine sites in winter (Figure 2e–h). Spring and summer months had high $NO_3^-$-N, with concentrations well above the calculated threshold $NO_3^-$-N concentration of 0.88 mg N $l^{-1}$ (two-dimensional Kolmogorov–Smirnov test, $D = 0.150$, $p = 0.0002$), shown as a horizontal dotted line in Figure 2e–h. During fall, $NO_3^-$-N concentrations at riverine and shallow upper reservoir sites dropped below the threshold value (Figure 2g). Sites with the highest sediment denitrification rates in the fall corresponded with $NO_3^-$-N concentrations at or above the threshold value. Nitrate concentrations at sites further into the reservoir dropped below the threshold value in the winter when riverine concentrations increased (Figure 2h).

### *Sediment denitrification rates associated with different habitat types*

We compared sediment denitrification rates of the four different habitat types sampled within the LSV river–reservoir continuum (riverine, shallow upper reservoir, profundal and littoral) using the mean annual sediment denitrification rates of assays incubated at room temperature. Profundal and littoral reservoir sites had significantly higher mean denitrification rate (20.7 and 19.6 μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ or 0.60 and 0.39 μg $N_2O$ $gDM^{-1}$ $h^{-1}$, respectively) compared to riverine sediments (3.95 μg $N_2O$ $gAFDM^{-1}$ $h^{-1}$ or 0.06 μg $N_2O$ $gDM^{-1}$ $h^{-1}$) regardless of how rates were expressed (Figure 3). Sediment denitrification was not significantly different between littoral and profundal

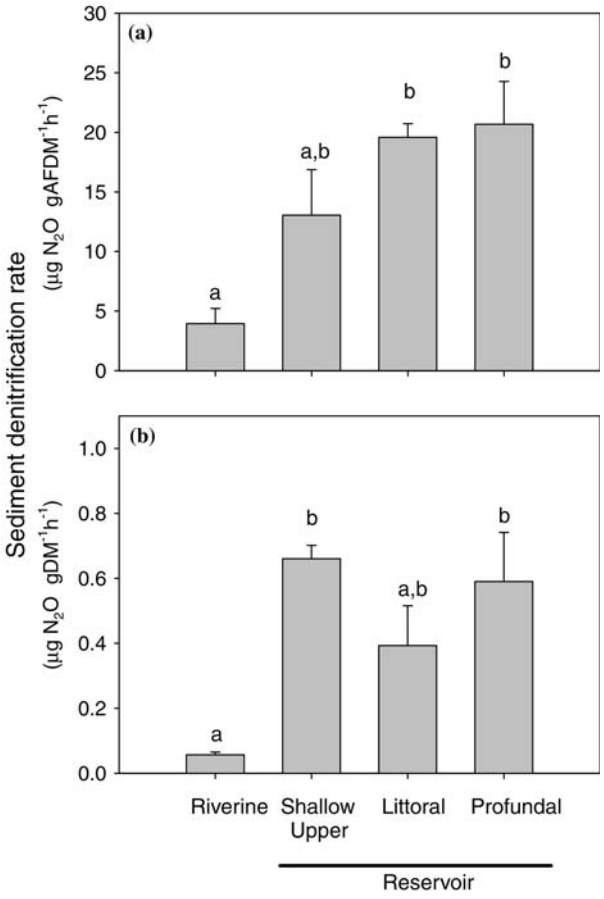


*Figure 3*. Mean annual sediment denitrification rates (± 1 SE) of different habitat types within the LSV river–reservoir continuum expressed as (a) µg $N_2O$ $gAFDM^{-1}$ $h^{-1}$, and (b) µg $N_2O$ $gDM^{-1}$ $h^{-1}$ . Different letters indicate significant differences between means (one-way ANOVA on annual means followed by LSMeans $p < 0.05$).

sites despite differences in water depth and sediment characteristics at those sites (Table 1). In contrast, mean sediment denitrification from shallow upper reservoir habitat was not significantly different from riverine habitat when expressed per gAFDM and significantly higher when expressed per gDM. Additionally, mean sediment denitrification rate from littoral habitat was significantly higher than mean sediment denitrification rate of riverine habitat when expressed as per gAFDM and not significantly different when expressed as per gDM (Figure 3). These contrasts are likely due to the high sediment organic matter and low DM at shallow upper reservoir sites and low organic matter and high DM at littoral sites relative to other sites. In general, sediment denitrification rates of reservoir sites were consistently higher than riverine sites.

*Physiochemical parameters at the sediment–water interface*

We examined the relationships between sediment denitrification and DOC, dissolved oxygen (%), temperature, and $NO_3^-$-N within the river–reservoir continuum. There was a significant inverse relationship between sediment denitrification and DOC concentration, but the model had little explanatory power (data not shown, $r^2 = 0.06$, $p = 0.01$). Sediment denitrification rates from ambient temperature incubations were positively related to temperature ($r^2 = 0.14$, $p < 0.001$; Figure 4a), and negatively related to dissolved oxygen ($r^2 = 0.11$, $p = 0.0006$; Figure 4b). There was greater variation in sediment denitrification rates at high temperatures and low dissolved oxygen concentrations, compared to the variation in denitrification at low temperatures and high dissolved oxygen concentrations.

There was a weak relationship between sediment denitrification rates and $NO_3^-$-N concentration (linear regression, $r^2 = 0.04$, $p = 0.03$) when examined across the entire range of $NO_3^-$-N values that occurred during the study (Figure 5a). However, a two-dimensional Kolomogorov–Smirnov test indicated a stronger relationship existed between these variables when $NO_3^-$-N concentration was less than 0.88 mg $NO_3^-$-N $l^{-1}$ ($r^2 = 0.14$, $p = 0.03$; Figure 5b). Above this $NO_3^-$-N threshold, factors such as temperature, carbon availability, scouring, and oxygen concentration may have controlled sediment denitrification rates. For example, in the spring (open circles, Figure 5) $NO_3^-$-N concentrations were high and unlikely limited denitrification. At that time discharge was also high and may have influenced carbon sources or redox conditions, which in turn could have limited denitrification. Finally, temperature was low throughout the river–reservoir continuum and could also have

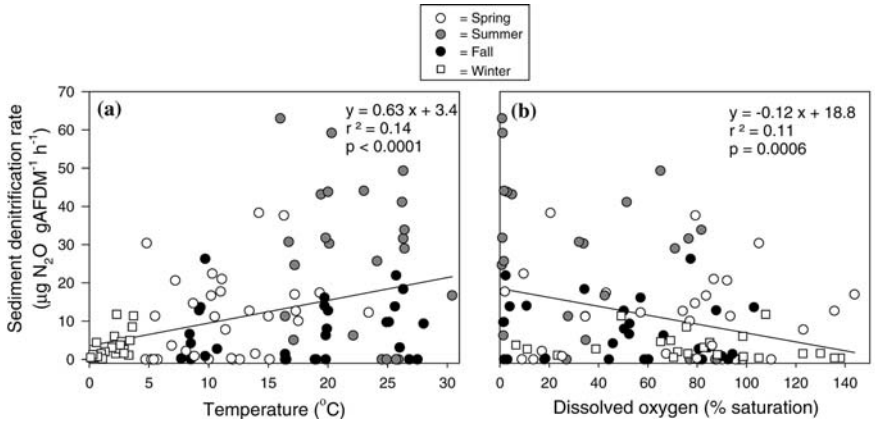


*Figure 4*. Sediment denitrification rates from assays incubated at ambient water column temperature plotted against (a) site temperature, and (b) dissolved oxygen (%) at the sediment–water interface.

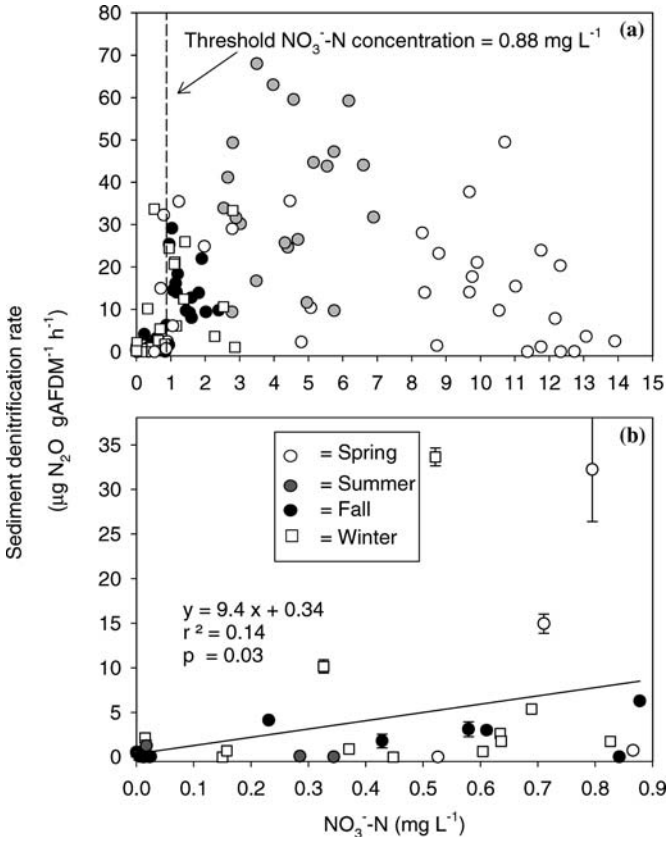


*Figure 5*. Sediment denitrification rates of sites within the river–reservoir continuum plotted against $NO_3^-$-N concentration for the (a) entire data set with the calculated threshold value from a two-dimensional Kolomogorov–Smirnov test marked by the vertical dashed line, and (b) only the rates (±1 SE) associated with $NO_3^-$-N concentration below the threshold value.

limited microbial activity. The coefficient of variation of sediment denitrification was positively related to the coefficient of variation for $NO_3^-$-N concentration within the river–reservoir continuum ($r^2 = 0.65$, $p = 0.005$), and variation in $NO_3^-$-N concentration and sediment denitrification exhibited an approximate 1:1 relationship. Riverine and upper shallow reservoir sites had the highest annual variation in $NO_3^-$-N concentrations and sediment denitrification rates (Figure 6). In contrast, the profundal and littoral sites were less variable in both $NO_3^-$-N concentrations and sediment denitrification rates and influenced the lower end of the trendline (Figure 6).

Because seasonal changes in nutrient concentrations may have influenced potential nutrient limitation of sediment denitrification rates, we conducted nutrient amendment assays seasonally. We evaluated the treatment response to added nutrients after log transforming the ratio of the treatment sediment

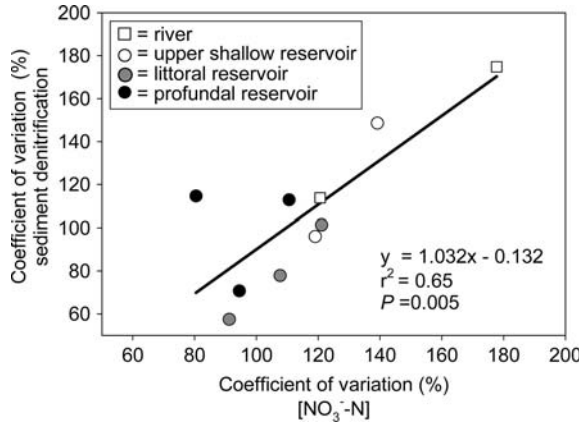


*Figure 6*. Relationship between annual variation in sediment denitrification (μg $N_2O$ gAFDM$^{-1}$ h$^{-1}$) and $NO_3^-$-N concentration within the LSV river–reservoir continuum.

denitrification rate to the control; a positive value indicated an increase in denitrification in response to the amendment and a negative value indicated a decrease in denitrification in response to the amendment. For nutrient amendments conducted when ambient $NO_3^-$-N concentrations were low (fall and winter), the N treatment had a significant positive affect on sediment denitrification rates at all sites (two-way ANOVA, $p < 0.005$), whereas the C treatment did not have a significant effect (Figure 7a). The N treatment alone and N + C treatment were not significantly different, yet rates of both were significantly higher than C treatment alone. For nutrient amendments conducted during spring and summer when $NO_3^-$-N concentrations were high, there were few significant responses to the N or C treatments (Figure 7b); the N treatment had a small but significant negative effect on sediment denitrification at the profundal sites and the C treatment had a significant negative effect on denitrification at the river sites (two-way ANOVA for both $p < 0.005$).

When comparing responses to nutrient amendments by habitat type, no significant differences were observed among sites (one way ANOVA, $p > 0.05$), except at riverine sites in spring and summer when there was a significant negative response to the C amendment (Figure 7b). In general, profundal and littoral sites responded similarly to all nutrient amendments and the shallow upper reservoir sites had the greatest positive response to all amendments during seasons exhibiting low $NO_3^-$-N concentrations (Figure 7a).

*Sediment characteristics*

There was no relationship between sediment denitrification and sediment characteristics of organic matter and N content when denitrification was expressed per gAFDM (data not shown, $p = 0.50$ and 0.93, respectively). In contrast, denitrification rates expressed per g DM were positively related to sediment organic matter and nitrogen content (both as %; Figure 8). Sediment

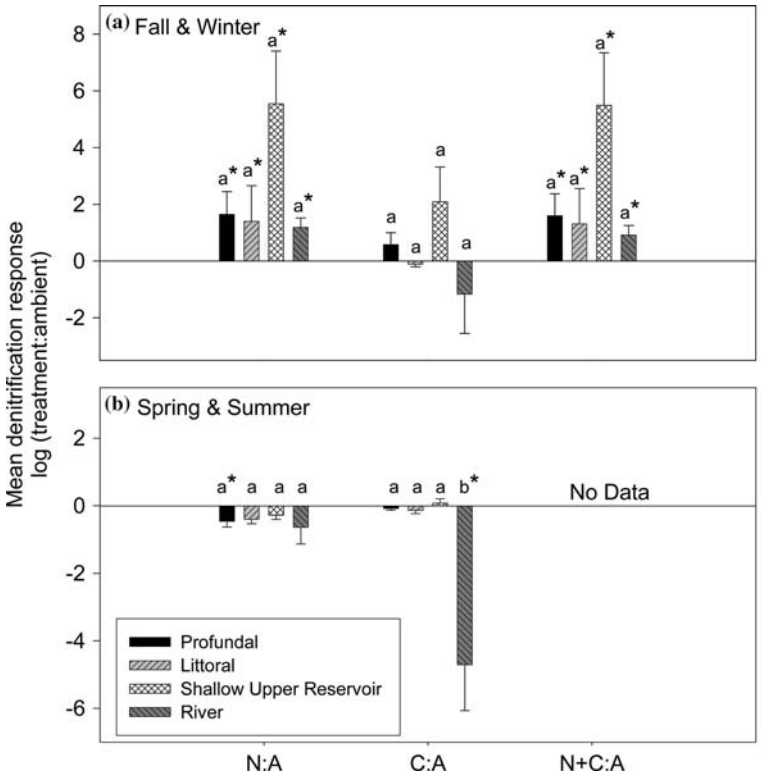


*Figure 7.* Mean sediment denitrification response to $NO_3^-$-N amendment (N), carbon amendment (C), and N + C treatments by habitat type for seasons (a) with low $NO_3^-$-N (fall and winter [$NO_3^-$-N] = 0.15–2.86 mg $l^{-1}$), and seasons (b) with high $NO_3^-$-N (spring and summer; [$NO_3^-$-N] = 3.49–13.07 mg $l^{-1}$). Nutrient amended denitrification rates are expressed relative to unamended, ambient denitrification rates (A). Spring, summer, fall, and winter were sampled April, July, October and January respectively. A positive bar indicates an increase in denitrification with nutrient amendment; a negative bar indicates an inhibitory effect on denitrification. Different letters indicate significant differences in response by habitat type (one-way ANOVA on log (treatment:ambient) data, $p < 0.05$) and a significant treatment effect is marked by an $*$ (results of a two-way ANOVA ran on log transformed data, $p < 0.005$).

organic matter and nitrogen content explained approximately equal amounts of the variation in sediment denitrification rates ($r^2$ = 0.18 and 0.15, respectively), likely because they were positively related to each other ($r^2$ = 0.78, $p < 0.001$). Sediments from profundal and shallow upper reservoir sites had organic matter, nitrogen content, and carbon content spanning the entire range of values measured. In contrast, the majority of the littoral and riverine sites had low organic matter and nitrogen content relative to the range of values measured, yet still had a large range in carbon content.

Using multiple stepwise regression, 39% of the variation in sediment denitrification rates from ambient temperature assays was explained by ambient

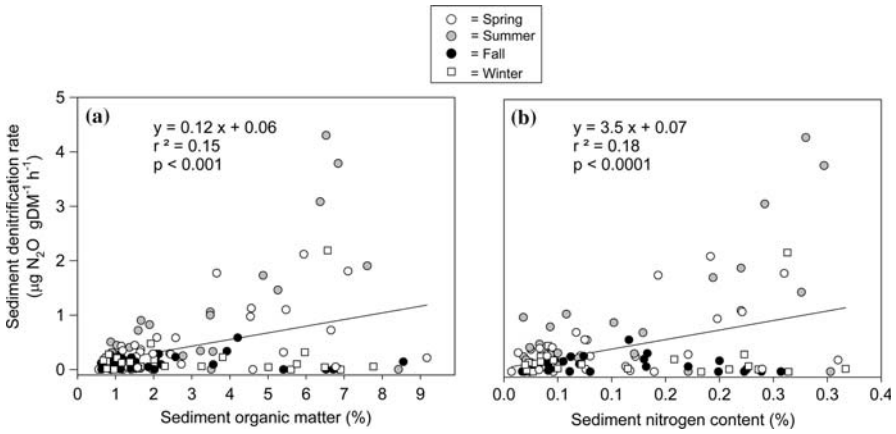


*Figure 8*. Sediment denitrification rates (µg $N_2O$ $gDM^{-1}$ $h^{-1}$) plotted against (a) sediment organic matter, and (b) sediment nitrogen content.

temperature, water depth, sediment nitrogen content, and dissolved $O_2$ ($r^2 = 0.39$, $p < 0.0001$). In contrast, ambient temperature, water depth, chl *a*, and sediment organic content were significant predictors of sediment denitrification rates from room temperature assays (multiple stepwise regression, $r^2 = 0.26$, $p < 0.0001$). Results of all regression analyses are summarized in Table 2.

## Discussion

### *Longitudinal variation in sediment denitrification along the river–reservoir continuum*

Peak sediment denitrification rates moved from upper reservoir sites through the reservoir closer to the dam, following the spring load of high $NO_3^-$ at the river inlets and seasonal increases in temperature and decreases in dissolved oxygen at the sediment–water interface (Table 1 and Figure 2a–d). High sediment denitrification rates measured within LSV in the summer were more similar to rates measured in created wetlands and small ponds (Fleischer et al. 1994; Xue et al. 1999), compared to rates reported for eutrophic lakes and rivers (Ahlgren et al. 1994; Garcia-Ruiz et al. 1998a,b; McMahon and Dennehy1999). Summer was also when we observed the greatest difference between reservoir and riverine sediment denitrification rates. But our measurements of riverine sediment denitrification in the summer may be an underestimate of the actual rates because we only sampled riverine sediments in August when the $NO_3^-$ concentrations were decreasing and we may have missed periods of high sediment denitrification at riverine sites during June

*Table 2.* Summary of sediment denitrification linear regression analyses.

| Parameter | Regression analysis | Incubation temperature/rate expression | $r^2$ | $p$ | Equation |
|---|---|---|---|---|---|
| $NO_3^-$-N (all data) | Linear | Room/ AFDM | 0.04 | 0.03 | $y = 0.86\ x + 12.7$ |
| $NO_3^-$-N (0.88 mg $l^{-1}$ and below) | Linear | Room/AFDM | 0.14 | 0.03 | $y = 9.4x + 0.34$ |
| DOC | Linear | Room/AFDM | NS | NS | NA |
| Temperature | Linear | Ambient/ AFDM | 0.15 | $<0.0001$ | $y = 0.63x + 3.4$ |
| Dissolved Oxygen (%) | Linear | Ambient/ AFDM | 0.11 | 0.0006 | $y = -0.12\ x + 18.8$ |
| Sediment N content (%) | Linear | Room/ DM | 0.18 | $<0.0001$ | $y = 3.5\ x + 0.07$ |
| Sediment organic content (%) | Linear | Room/ DM | 0.15 | $<0.0001$ | $y = 0.12\ x + 0.06$ |
| Temperature, water depth, Chl *a*, sediment organic content | Stepwise multiple linear | Room/ AFDM | 0.26 | $<0.0001$ | NA |
| Dissolved oxygen (%), temperature, water depth and sediment nitrogen content | Stepwise multiple linear | Ambient/ AFDM | 0.39 | $<0.0001$ | NA |

NS = not significant and NA = not applicable. Room temperature = 22 °C and ambient temperature = the mean water temperature at all sites sampled in a given month. All data pooled for analyses except for $NO_3^-$-N threshold regression.

and July flooding. However, high water velocities and sediment disturbance could counteract high $NO_3^-$ and keep river denitrification rates low.

During the fall, sediment denitrification within the reservoir generally decreased due to a combination of both lower $NO_3^-$ concentrations and higher dissolved oxygen throughout the river–reservoir continuum (Table 1 and Figure 2c). The general trend in $NO_3^-$-N concentrations throughout the continuum up until fall was a gradient of $NO_3^-$-N concentrations, with highest $NO_3^-$-N associated with riverine sites and lowest $NO_3^-$-N at the dam. The $NO_3^-$-N gradient inverted in the fall with lowest $NO_3^-$-N at riverine sites and highest $NO_3^-$-N at the dam, causing peak sediment denitrification rates within the river–reservoir continuum to shift closer to the dam where $NO_3^-$-N concentrations remained high (Figure 2c, g).

Seasonal trends in sediment denitrification due to changes in temperature have been documented in estuaries, with highest denitrification rates during the summer months and lowest denitrification rates in winter (Jørgensen 1989). Similar seasonal trends were observed in LSV. Our data suggest low sediment denitrification rates throughout the LSV river–reservoir continuum in winter were primarily the result of low temperatures, and secondarily the result of low $NO_3^-$ concentrations. Nitrate concentrations increased above the $NO_3^-$-N threshold at the Kaskaskia riverine and shallow upper reservoir sites in winter, yet sediment denitrification rates remained low Figure 2d, h). This suggests even if river inlets delivered high $NO_3^-$-N water to the reservoir in the winter, sediment denitrification will remain low as long as temperatures are low.

Although we found trends in sediment denitrification associated with both temperature and $NO_3^-$ concentration in the LSV river–reservoir continuum, these variables explained only a small portion of the variability in sediment denitrification (Figures 4a and 5a) compared to previous work in other aquatic ecosystems (Jørgensen 1989; Garcia-Ruiz et al. 1998a, b). In LSV, the lack of explanatory power by $NO_3^-$-N and temperature may be partly explained by the temporal and spatial discontinuity between $NO_3^-$-N and temperature oscillations throughout the river–reservoir continuum. For example, during spring when $NO_3^-$-N was high throughout the continuum, temperatures were low; during fall when temperatures were still relatively high, $NO_3^-$-N concentrations in the riverine and upper reservoir sites were low. Additionally, water column $NO_3^-$-N, which has been found to be the primary limiting substrate for sediment denitrification (Kaspar 1985; Holmes et al.1996; Mitchell and Baldwin 1999; Martin et al. 2001) was above the $NO_3^-$-N threshold value in LSV for the majority of the year. Our data suggested denitrifying bacteria are continually active under high $NO_3^-$-N conditions and can capitalize on other seasonal conditions that make denitrification more favorable (e.g. temperature). Therefore, multiple variables in combination, including $NO_3^-$-N concentration, dissolved oxygen, temperature, sediment organic matter and nitrogen content, and in some cases water depth and chl *a* (Table 1), influenced denitrification activity with

each variable explaining a small portion of the total variability in sediment denitrification rates within the LSV river–reservoir continuum.

### *LSV $NO_3^-$-N threshold*

Multiple studies have reported $NO_3^-$-N limitation of denitrification (Holmes et al. 1996; Mitchell and Baldwin 1999; Martin et al. 2001); however, none of these studies reported a threshold below which $NO_3^-$-N became a limiting factor for the denitrification process. The $NO_3^-$-N threshold of sediment denitrification within the LSV river–reservoir continuum was calculated to be 0.88 mg $l^{-1}$, and was higher than $NO_3^-$-N thresholds determined for headwater agricultural streams in Illinois (approx. 0.6 mg $l^{-1}$, T.V. Royer unpublished data) and Michigan (approx. 0.4 mg $l^{-1}$, Inwood et al. in press). Because the $NO_3^-$-N threshold for sediment denitrification within LSV was higher than the two stream studies, it suggests that in LSV sediment demand for $NO_3^-$-N associated with denitrification is high. Reservoir sediments are typically of higher organic content than stream sediments due to less frequent scouring and contributions from senescing phytoplankton, likely providing a readily available labile carbon source. Additionally, LSV was frequently stratified in the deeper regions with anoxic conditions within the first centimeter below the sediment–water interface (data not shown).

### *The relationships between DOC, sediment organic content and sediment denitrification*

Contrary to results from previous studies on the influence of DOC on denitrification (Stanford et al. 1975; Smith and Tiedje 1979; Hedin et al. 1998), variation in water column DOC did not influence sediment denitrification rates within LSV in the predicted fashion. This was further supported by the lack of positive response to the majority of C amended sediment denitrification assays (Figure 7). Potentially, a surplus of organic carbon was available to denitrifying bacteria, bulk DOC measurements do not reflect the available component, or DOC from the water column was not the primary electron donor for denitrification in LSV sediments. An alternative source of organic carbon for sediment denitrification is organic carbon associated with the sediments themselves (Garcia-Ruiz et al. 1998a, b). The positive relationship between sediment denitrification (expressed per g DM) in LSV and sediment organic matter (Figure 8a), suggests microbes within LSV sediments may utilize organic carbon from the sediments rather than the DOC from the water column.

*Comparing profundal and littoral sediment denitrification*

In this study we found that sediment denitrification rates within LSV did not differ between littoral and profundal sites (Figure 3), unlike previously published results from studies conducted in natural lakes (Kaspar 1985; Ahlgren et al. 1994). This difference, or lack of one, may be because profundal and littoral sites in LSV were not as physiochemically different compared to natural lakes (Kasper 1985) due to highly eroded and shifting shorelines. The greatest difference in $NO_3^-$ concentrations between a LSV site sampled at both profundal and littoral habitats was measured in July (profundal and littoral = 5.5 and 4.4 mg N $l^{-1}$, respectively); however both were well above limiting $NO_3^-$-N concentrations. Additionally, Ahlgren et al. (1994) suggested the difference in denitrification rates at profundal and littoral sites was due to a lower redox potential at the profundal sites that potentially induced competition with other chemical processes such as sulfate reduction and dissimilatory $NO_3^-$ reduction. Although profundal sites had significantly lower dissolved $O_2$ in spring and fall, dissolved $O_2$ concentrations were generally similar and unlikely to create such competition differences in LSV (Table 1).

*Nitrate limitation of sediment denitrification within the river–reservoir continuum*

Nitrate limitation of sediment denitrification coincided with seasonal precipitation patterns and crop growth. Little $NO_3^-$-N entered the LSV river–reservoir continuum when precipitation was low and crop biomass was high during late summer and early fall, resulting in lower sediment denitrification rates first in the riverine and later in upper reservoir sites. The shallow upper reservoir habitats had the highest degree of limitation as evidenced by the greatest positive response to the N amendment (Figure 7a). This suggests denitrifiers located in the shallow upper reservoir sites potentially store enzymes necessary for denitrification during periods of low $NO_3^-$-N concentrations, which enables a greater increase in rates when $NO_3^-$-N concentrations are non-limiting.

The negative response to the N and C amendments across all sites observed in spring and summer was unexpected. Potentially, adding $NO_3^-$-N to already high concentrations (end concentrations ranged from 15.7 to 19.1 mg $NO_3^-$-N $l^{-1}$ in the spring and 9.5–13.8 mg $NO_3^-$ -N $l^{-1}$ in the summer) resulted in concentrations that may inhibit denitrification activity directly or indirectly by stimulating competition with other anaerobic reductions such as dissimilatory nitrate reduction (DNR; Stanford et al. 1975). We did not measure DNR and did not find any previously published studies where both sediment denitrification and DNR were measured simultaneously in freshwater ecosystems; however, DNR has been found to occur simultaneously with denitrification in estuarine sediments (Koike and Hattori 1978; Jørgensen 1989). DNR was also suggested by Ahlgren et al. (1994) to be in competition with denitrification for $NO_3^-$-N, resulting in decreased denitrification rates. Increased competition for

$NO_3^-$-N via DNR may also explain lower sediment denitrification rates with the C amendment, as glucose has been found to stimulate DNR in soils (Standford et al. 1975). Additional research quantifying DNR simultaneously with denitrification at different $NO_3^-$-N and C concentrations for sediments from different freshwater ecosystems is needed to clarify the relative role of $NO_3^-$-N and C in controlling these biogeochemical transformations.

*Where is the highest sediment denitrification potential within the river–reservoir continuum?*

Regression analyses suggest that shallow upper reservoir sites will have the highest sediment denitrification potential along the river–reservoir continuum due to the high organic matter and nitrogen content of sediments and high $NO_3^-$-N concentrations in the water column. Sediment quality at shallow upper reservoir sites is the result of a chain of events that occur when water velocity declines, which increases the deposition of fine sediments that are generally high in organic matter and have high oxygen demand (Cole and Hannan 1990). Subsequent light penetration as a result of increased water clarity results in high primary production (Baxter 1977; Thorton et al. 1980), which further increases organic matter and nitrogen content of sediments, and oxygen demand through senescence of algae. Despite the potential for high sediment denitrification, shallow upper reservoir sites were the least buffered of the reservoir sites. Annual variation in $NO_3^-$-N concentration was generally higher (Figure 6) and dissolved oxygen concentrations spanned the largest range of values (Table 1). This variability resulted in more variable sediment denitrification rates when compared to reservoir sites closer to the dam (Figure 6). Sediment denitrification rates peaked in the shallow upper reservoir only when the conditions were favorable (i.e., high $NO_3^-$-N, low dissolved oxygen, and high temperature), and remained relatively low for a large portion of the year depending on the conditions of the inlet rivers.

In summary, longitudinal differences in sediment denitrification measured within the LSV river–reservoir continuum were related to differences in sediment quality and physical parameters associated with different habitat types along the continuum. Attempts to model watershed N retention (e.g., Seitzinger et al. 2002) may be greatly underestimating the significance of reservoirs in total N retention because the models neglect differences in sediment denitrification capacity along river–reservoir continua. Higher sediment denitrification rates along the LSV river–reservoir continuum are inevitably important to the overall N retention by LSV. If other reservoirs located in agricultural landscapes have equally high denitrification rates, these reservoirs combined may have a significant effect on decreasing export of $NO_3^-$-N to downstream aquatic ecosystems. Overall, downstream eutrophication of surface waters (Carpenter et al. 1998) and hypoxia in the Gulf of Mexico (Rabalais et al. 2002)

may be more extensive without denitrification associated with upstream reservoirs.

## Acknowledgements

We thank Sarah Inwood, Erin Hotchkiss, Jamie Schaller, Laura Taylor, Jon Loftus, and Jennifer Schaeffer for field and laboratory assistance; the USDA-CSREES National Research Initiative Competitive Grants Program for funding; Illinois Natural History Survey for use of their field station.

## References

Ahlgren I., Sörensson F., Waara T. and Vrede K. 1994. Nitrogen budget in relation to microbial transformations in lakes. Ambio 23: 367–377.

Alexander R.B., Smith R.A. and Schwarz G.E. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403: 758–761.

[APHA] American Public Health Association. 1995. Standard Methods for the Examination of Water and Waste. 19th ed. American Public Health Association., Washington DC, 20005, USA.

Baxter R.M. 1977. Environmental effects on dams and impoundments. Annu. Rev. Ecol. Syst. 8: 255–283.

Bernot M.J., Dodds W.K., Gardner W.S., McCarthy M.J., Sobolev D. and Tank J.L. 2003. Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. Appl. Environ. Microbiol. 69: 5950–5956.

Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. and Smith V.H. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecol. Appl. 8: 559–568.

Cole T.M. and Hannan H.H. 1990. Dissolved oxygen dynamic. In: Kimmel B.L., Payne F.E. and Thorton K.W. (eds), Reservoir Limnology: Ecological Perspectives. John Wiley and Sons, Inc, New York, pp. 71–107.

David M.B., Gentry L.E., Kovacic D.A. and Smith K.M. 1997. Nitrogen balance in and export from an agricultural watershed. J. Environ. Qual. 26: 1038–1048.

David M. and Gentry L.E. 2000. Anthropogenic inputs of nitrogen and phosphorous and riverine export for Illinois, USA. J. Environ. Qual. 29: 494–508.

Fleischer S., Gustafson A., Joelsson A., Pansar J. and Stibe L. 1994. Nitrogen removal in created ponds. Ambio 23: 349–357.

Garcia-Ruiz R., Pattinson S.N. and Whitton B.A. 1998a. Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biol. 39: 467–476.

Garcia-Ruiz R., Pattinson S.N. and Whitton B.A. 1998b. Denitrification and nitrous oxide production in sediments of the Wiske, a lowland eutrophic river. Sci. Tot. Environ. 210/211: 307–320.

Garvey J.E., Marschall E.A. and Wright R.A. 1998. From star charts to stoneflies: detecting relationships in continuous bivariate data. Ecology 79: 442–447.

Gentry L.E., David M.B., Smith-Starks K.M. and Kovacic D.A. 2000. Nitrogen fertilizer and herbicide transport from tile drained fields. J. Environ. Qual. 29: 232–240.

Goolsby D.A., Battaglin W.A., Aulenbach B.T. and Hooper R.P. 2001. Nitrogen input to the Gulf of Mexico. J. Environ. Qual. 30: 329–336.

Hedin L.O., von Fischer J.C., Ostrom N.E., Kennedy B.P., Brown M.G. and Robertson G.P. 1998. Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. Ecology 79: 684–703.

Holmes R.M., Jones J.B.Jr., Fisher S.G. and Grimm N.B. 1996. Denitrification in a nitrogen-limited stream ecosystem. Biogeochemistry 33: 125–146.

Illinois Department of Agriculture. 2003. Land Cover of Illinois, Available from http://www.agr.state.il.us/gis/landcover.html (verified 18 Aug. 2003).

Inwood S.E., Tank J.L. and Bernot M.J. 2005. Patterns of denitrification associated with land use in 9 midwestern headwater streams. J. N. Am. Benthol. Soc. 24: 227–245.

Jørgensen K.S. 1989. Annual pattern of denitrification and nitrate ammonification in estuarine sediment. Appl. Environ. Microbiol. 55: 1841–1847.

Jossette G., Leporcq B., Sanchez N. and Philippon 1999. Biogeochemical mass-balances (C, N, P, Si) in three large reservoirs of the Seine Basin (France). Biogeochemistry 47: 119–146.

Kaspar H.F. 1985. The denitrification capacity of sediment from a hypereutrophic lake. Freshwater Biol. 15: 449–453.

Kelly V.J. 2001. Influences of reservoirs on solute transport: a regional-scale approach. Hydrol. Processes 15: 1227–1249.

Kennedy R.H., Thorton K.W. and Ford D.E. 1985. Characterization of the reservoir ecosystem. In: Gunnisson D. (ed.), Microbial Processes in Reservoirs. Dr. W. Junk Publishers, Dordrecht, The Netherlands, pp 25–38.

Kennedy R.H. and Walker W.W. 1990. Reservoir nutrient dynamics. In: Kimmel B.L., Payne F.E. and Thorton K.W. (eds), Reservoir Limnology: Ecological Perspectives. John Wiley and Sons, Inc., New York, pp. 109–131.

Kimmel B.L., Lind O.T. and Paulson L.J. 1990. Reservoir primary production. In: Kimmel B.L., Payne F.E. and Thorton K.W. (eds), Reservoir Limnology: Ecological Perspectives. John Wiley and Sons Inc., New York, pp 133–191.

Koike I. and Hattori A. 1978. Denitrification and ammonia formation in anaerobic coastal sediments. Appl. Environ. Microbiol. 35: 278–282.

Martin L.A., Mulholland P.J., Webster J.R. and Valett H.M. 2001. Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. J. N. Am. Benthol. Soc. 20: 505–519.

McMahon P.B. and Dennehy K.F. 1999. $N_2O$ emissions from a nitrogen-enriched river. Environ. Sci. Technol. 33: 21–25.

Mitchell A.M. and Baldwin D.S. 1999. The effects of sediment desiccation on the potential for nitrification, denitrification, and methanogenesis in an Australian reservoir. Hydrobiologia 392: 3–11.

Murray R.E. and Knowles R. 1999. Chloramphenicol inhibition of denitrifying enzyme activity in two agricultural soils. Appl. Environ. Microbiol. 65: 3487–3492.

Pattinson S.N., Garcia-Ruiz R. and Whitton B.A. 1998. Spatial and seasonal variation in denitrification in the Swale-Ouse system, a river continuum. Sci. Tot. Environ. 210/211: 289–305.

Rabalais N.N., Turner R.E. and Wiseman W.J.Jr. 2002. Gulf of Mexico hypoxia, a.k.a. ''the dead zone''. Annu. Rev. Ecol. Syst. 33: 235–263.

Richardson W.B., Strauss E.A., Bartsch L.A., Monroe E.M., Cavanaugh J.C., Vingum L. and Soballe D.M. 2004. Denitrification in the upper Mississippi River: rates, controls, and contribution to nitrate flux. Can. J. Fish. Aquat. Sci. 61: 1102–1112.

Royer T.V., Tank J.L. and David M.B. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. J. Environ. Qual. 33: 1296–1304.

Rudolph J., Frenzel P. and Pfennig N. 1991. Acetylene inhibition technique underestimates in situ denitrification rates in intact cores of freshwater sediments. FEMS Microbiol. Ecol. 85: 101–106.

Sartory D.P. and Grobbelaar J.U. 1984. Extraction of chlorophyll *a* from freshwater plankton for spectrophotometric analysis. Hydrobiologia 114: 177–187.

SAS Institute. 1991. SAS/STAT User's Guide, 6.04. SAS Institute, Cary, North Carolina.

Saunders D.L. and Kalff J. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443: 205–212.

Schaller J.L., Royer T.V., David M.B. and Tank J.L. 2004. Denitrification associated with plants and sediments in an agricultural stream. J. N. Am. Benthol. Soc. 23: 667–676.

Schlesinger W.H.(ed.) 1997. The global cycles of nitrogen and phosphorous. In: Biogeochemistry: An Analysis of Global Change. 2nd ed. Academic Press, San Diego, California 92101, USA, pp. 383–400.

Seitzinger S.P. 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceanogr. 33(4 part 2): 702–724.

Seitzinger S.P., Nielsen L.P., Caffrey J. and Christensen P.B. 1993. Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry 23: 147–167.

Seitzinger S.P., Styles R.V., Boyer E.W., Alexander R.B., Billen G., Howarth R.W., Mayer B. and Van Breeman N. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern U.S.A. Biogeochemistry 57/58: 199–237.

Smith M.S. and Tiedje J.M. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol. Biochem. 11: 261–267.

Solorzano L. 1969. Determination of ammonia in natural water by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801.

Stanford G., Legg J.O., Dzienia S. and Simpson E.C.Jr. 1975. Denitrification and associated nitrogen transformations in soils. Soil Sci. 120: 147–152.

Tank J.L. and Dodds W.K. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biol. 48: 1031–1049.

Thorton K.W., Kennedy R.H., Carroll J.H., Walker W.W., Gunkel R.C. and Ashby S. 1980. Reservoir sedimentation and water quality – a heuristic model. In: Proceedings of the Symposium on Surface Water Impoundments. June 2–5, Minneapolis, MN, pp. 654–664.

Tiedje J. 1982. Denitrification. In: Page A.L. (ed.), Methods of Soil Analysis Part 2 Chemical and Microbiological Properties. ASA-SSSA, Madison WI, USA, pp. 1011–1026.

Toetz D.W. 1973. Nitrogen budget of the Great Plains impoundments. In: Ackerman W.C., White G.F. and Worthington E.B. (eds), Man Made Lakes: Their Problems and Environmental Effects. American Geophysical Union, Washington DC, pp. 567–571.

Townsend A.R., Howarth R.W., Bazzaz F.A., Booth M.S., Cleveland C.C., Collinge S.K., Dobson A.P., Epstein P.R., Holland E.A., Keeney D.R., Mallin M.A., Rogers C.A., Wayne P. and Wolfe A.H. 2003. Human health effects of a changing global nitrogen cycle. Front. Ecol. Environ. 1(5): 240–246.

[USEPA] United Stated Environmental Protection Agency. 1993. Method 300.0, Test Method for the Determination of Inorganic Anions in Water by Ion Chromatography. Environmental Monitoring Systems Laboratory, ORD, USEPA, Cincinnati, Ohio 45268.

[USEPA] United Stated Environmental Protection Agency. 1997. Method 445.0 rev1.2, In vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence. National Exposure Research Laboratory, ORD, USEPA, Cincinnati, Ohio 45268.

[USGS] United States Geological Survey. 2003. Daily Stream Flow Data [Online]. Available from http://www-il.usgs.gov/nwis-w/IL (verified 18 June 2003).

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. and Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7: 737–750.

Wetzel R.G. (ed.). 2002. Rivers and Lakes-their distribution, origins and forms. In: Limnology: Lake and River Ecosystems. 3rd ed. Academic Press, USA, pp 37–38.

Xue Y., Kovacic D.A., David M.B., Gentry L.E., Mulvaney R.L. and Lindau C.W. 1999. *In situ* measurements of denitrification in constructed wetlands. J. Environ. Qual. 28: 263–269.

Yoshinari T. and Knowles R. 1976. Acetylene inhibition of nitrous-oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Comm. 69: 705–710.