

Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale

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SUMMARY

1. Denitrification, net oxygen consumption and net nitrous oxide flux to the atmosphere were measured in three small rivers (discharge approximately 2–27 m³ s⁻¹) at the whole reach scale during Spring and Summer, 2002. Two of these rivers (Iroquois River and Sugar Creek in north-west Indiana – north-east Illinois, U.S.A.) drained agricultural catchments and the other (Millstone River in central New Jersey, U.S.A.) drained a mixed suburban–agricultural catchment.
2. Denitrification, oxygen consumption and N₂O flux were measured based on net changes in dissolved gas concentrations (N₂, O₂, and N₂O) during riverine transport, correcting for atmospheric exchange. On each date, measurements were made during both light and dark periods.
3. Denitrification rates in these rivers ranged from 0.31 to 15.91 mmol N m⁻² h⁻¹, and rates within each river reach were consistently higher during the day than during the night. This diurnal pattern could be related to cyclic patterns of nitrification driven by diurnal variations in water column pH and temperature.
4. Oxygen consumption ranged from 2.56 to 241 mmol O₂ m⁻² h⁻¹. In contrast to denitrification, net oxygen consumption was generally higher during the night than during the day.
5. River water was consistently supersaturated with N₂O, ranging from 102 to 209% saturated. Net flux of N₂O to the atmosphere ranged from 0.4 to 60 μmol N m⁻² h⁻¹. Net flux of N₂O was generally higher at night than during the day. The high flux of N₂O from these rivers strengthens the argument that rivers are an important contributor to anthropogenic emissions of this greenhouse gas.

Keywords: denitrification, membrane inlet mass spectrometry, nitrate, nitrous oxide, oxygen consumption

Introduction

Human activities in recent decades have dramatically increased nitrogen inputs to catchments (Vitousek *et al.*, 1997), much of which enters rivers, degrading

water quality. Denitrification, burial of organic matter in sediments, sediment sorption, and plant and microbial uptake can improve water quality by removing nitrogen from surface waters (Billen, Lancelot & Meybeck, 1991). However, denitrification is the only one of these processes representing permanent nitrogen removal; the end products (N₂ and N₂O) can be used by only a few organisms. Denitrification is a potentially important sink for nitrogen in rivers (Seitzinger, 1988; Sjodin, Lewis & Saunders, 1997).

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Advances in understanding the potential for denitrification to improve water quality in heavily impacted rivers and streams will require understanding factors that regulate denitrification in such systems.

Advances in membrane inlet mass spectrometry (MIMS) have allowed for very small changes in concentrations of dissolved gases to be measured rapidly and easily (Kana *et al.*, 1994), and MIMS has proved useful in measuring denitrification from the increase in dissolved N_2 in sediment core incubations (Kana *et al.*, 1994, 1998; Cornwell, Kemp & Kana, 1999). Recently, a new technique was introduced using MIMS to measure *in situ* production of molecular nitrogen during riverine transport (Laursen & Seitzinger, 2002a; McCutchan *et al.*, 2003). This technique has several significant advantages over previously used methods for measuring riverine denitrification. Denitrification is measured at the whole reach scale, and the difficulties inherent in extrapolating results from sediment cores to the field (e.g. reproduction of chemical and physical gradients in the laboratory, capturing patchiness in denitrifying activity) are avoided. The primary end-product of denitrification (N_2) is directly measured as opposed to inference based on a whole-reach nitrogen mass balance. The technique is easy to perform and is relatively inexpensive. Finally, the method measures denitrification at spatial and temporal scales appropriate for assessing its importance to nitrogen removal from rivers.

Anammox, the microbially mediated oxidation of NH_4^+ to N_2 with NO_2^- under anaerobic conditions (Strous *et al.*, 1997) is another potential sink for nitrogen in aquatic ecosystems, accounting for as much as 60% of N_2 production in some marine sediments (Thamdrup & Dalsgaard, 2002; Risgaard-Petersen *et al.*, 2003). However, current knowledge of anammox suggests that this process may be of limited importance in estuarine (Thamdrup & Dalsgaard, 2002) and freshwater systems (L.P. Nielsen, pers. comm.). Although open channel methods for measuring N_2 production in rivers (Laursen & Seitzinger, 2002a; McCutchan *et al.*, 2003) do not discriminate between denitrification and anammox, it is likely that total N_2 production in rivers approximates denitrification, particularly where the inorganic nitrogen pool is dominated by nitrate.

We are currently using this technique to measure denitrification in three rivers as part of a programme to increase understanding of factors that regulate

denitrification at the whole reach scale, and the role denitrification plays in nitrogen removal from rivers. Here we report on diurnal variations in riverine denitrification. Previous studies have shown diurnal patterns in sediments heavily colonised by benthic microalgae (e.g. Nielsen *et al.*, 1990b; Risgaard-Petersen *et al.*, 1994; Lorenzen *et al.*, 1998). In such sediments, denitrification is apparently regulated by oxygen dynamics related to photosynthesis by the microalgae. In deeper or more turbid rivers benthic microalgal production would not be expected to drive diurnal fluctuations in sediment oxygen penetration, and we might reasonably expect that denitrification would not show the same diurnal patterns. The primary objective of this study was to determine if denitrification does follow a diurnal pattern in three such rivers, to characterise the pattern and to elucidate its cause(s).

Secondarily, patterns of other metabolic activities manifested in net oxygen consumption and net fluxes of N_2O were investigated. Oxygen dynamics in an aquatic ecosystem are linked to denitrification through the inhibition of denitrification by oxygen, remineralisation of organic matter, and the subsequent oxidation of ammonium to nitrate. Thus, relationships between denitrification and oxygen consumption in these rivers were determined. Nitrous oxide is one of the gases contributing to global warming (Wang *et al.*, 1976), and plays a role in degradation of stratospheric ozone (Crutzen, 1970; Hahn & Crutzen, 1982). In recent decades anthropogenic emissions have increased atmospheric concentrations of N_2O (Khalil & Rasmussen, 1988, 1992). Model results indicate that a significant percentage of increased anthropogenic N_2O emissions comes from rivers, with increased inorganic nitrogen loading accelerating nitrogen cycling processes in aquatic ecosystems (Seitzinger & Kroeze, 1998). As N_2O is a byproduct of both denitrification (Smith & Zimmerman, 1981; Knowles, 1982; Firestone & Davidson, 1989) and ammonium oxidation (Yoshida & Alexander, 1970; Goreau *et al.*, 1980) we examined the relationships between nitrous oxide emissions from rivers and patterns of denitrification and oxygen consumption.

Methods

The Millstone River in central New Jersey drains a suburban–agricultural area in the Raritan River basin.

Our study reach was a 6-km section of the river near Griggstown, NJ with uniform channel morphology, no major tributaries and no known point sources of nitrogen (40°37'N, 87°43'W). Annual average discharge in this reach is typically approximately 11–14 m³ s⁻¹ (<http://waterdata.usgs.gov/nwis>) (station 01402000, Blackwells Mills, NJ, U.S.A.). The reach flows through an unglaciated piedmont region, minimising the influx of groundwater along the reach. Sugar Creek and the Iroquois River are located in the Illinois River Basin, draining agricultural land, primarily in corn and soybean cultivation. Our study reaches were a 5.5-km section of the Iroquois River near Foresman, IN (40°52'N, 87°18'W) and a 4.1-km section of Sugar Creek near Milford, IL, U.S.A. (40°37'N, 87°43'W). Annual average discharge in the Iroquois River reach is typically approximately 7–10 m³ s⁻¹ (<http://waterdata.usgs.gov/nwis>) (station 05524500, Foresman, IN, U.S.A.). We estimate that annual average discharge in the Sugar Creek reach is similar to that in the Iroquois reach based on measurements made in both rivers on the same dates.

Water samples for dissolved gas analyses (N₂, Ar, O₂, N₂O) were collected following a Lagrangian sampling scheme in the study reaches. In each study reach the same parcel of water was sampled at two or three locations as it moved downstream through time. Travel time of the parcel between sampling locations was estimated using mean current velocity. Samples were collected in a bucket from below the surface in the area of greatest discharge. Samples for nitrate analysis were filtered (Whatman GF/F) and frozen (-20 °C) prior to analysis. Nitrate was measured by ion chromatography (AG4A guard column, AS4A separator column; Dionex corporation, Sunnyvale, CA, U.S.A.) using a carbonate/bicarbonate eluent (1.8 mmol L⁻¹ Na₂CO₃/1.7 mmol L⁻¹ NaHCO₃). Samples for dissolved gases were transferred from the bucket to sample bottles by gravity filling with tygon tubing, allowing the equivalent of >three volumes to flush the sample bottle before removing the tube. Samples for N₂, Ar and O₂ were collected in 60-mL BOD bottles and samples for N₂O were collected in 60-mL serum bottles. Samples were preserved by pipetting a small volume of saturated HgCl₂ solution into each bottle (final concentration 0.25% vol vol⁻¹) immediately before inserting the ground glass stopper (N₂, Ar and O₂) or by adding a crystal of KOH to the serum bottle before sealing with

a butyl rubber stopper (N₂O) (stoppered underwater to prevent formation of a headspace). Samples for dissolved gas analyses were stored under water 1–2 °C below *in situ* temperature prior to analysis.

Production or consumption of N₂, O₂ and N₂O were estimated using the whole-reach approach described by Laursen & Seitzinger (2002a). Briefly, the production or consumption of a gas was modelled based on the measured change in concentration during downstream transport, corrected for change in concentration because of re-equilibration with the atmosphere. Dissolved N₂ and O₂ were measured using a MIMS system (Kana *et al.*, 1994; Laursen & Seitzinger, 2002b). Signals were measured for N₂, O₂ and Ar (m/z: 28, 32 and 40). Increases in N₂ were calculated from the change in N₂:Ar through time since replicate samples generally have a lower percentage standard deviation among N₂:Ar measurements (<0.05%) than among N₂ measurements (<0.10%). Changes in O₂ were similarly calculated. Nitrous oxide was measured by gas chromatography with electron capture detection (HP5890; Agilent Technologies Inc., Palo Alto, CA, U.S.A.; Porapak Q column; Supelco, Bellefonte, PA, U.S.A.) after dissolved gases were allowed to re-equilibrate with an introduced headspace (3-mL N₂ headspace). Nitrogen gases (N₂ and N₂O) and oxygen were assumed to change during transport as functions of metabolic activity and atmospheric re-equilibration. Argon concentration was assumed to behave conservatively, varying only as a function of a temperature corrected first-order gas transfer coefficient.

Routinely, we use volatile, non-reactive tracers to determine first-order transfer rates for N₂ and Ar (K_{N_2} and K_{Ar}). Because water temperature generally changes more rapidly than gases re-equilibrate in these small rivers, we regularly see Ar concentration anomalies (i.e. the concentration is not at equilibrium at a given water temperature). Previously, we demonstrated that our modelling approach, applying the experimentally determined K_{Ar} , could accurately reproduce these anomalies in dissolved Ar concentrations (Laursen & Seitzinger, 2002a). In this study, we used the measured change in Ar concentration during transport to solve for K_{Ar} by determining the rate of re-equilibration necessary to describe Ar behaviour. We then used these values to calculate first-order transfer rates for N₂, O₂ and N₂O as previously described (Laursen & Seitzinger, 2002a)

based on Schmidt numbers of these gases calculated for *in situ* water temperatures (Wanninkhof, 1992).

Wetted channel width and depth were measured at the upstream and downstream locations of each study reach. Depth was measured at a minimum of seven vertical transects, equally spaced across the width of the channel. Mean velocity was measured in Sugar Creek using a flow meter (Global Water, Gold River, CA, U.S.A.). Real-time discharge data for the Millstone River and the Iroquois River were obtained from the U.S. Geological Survey website (<http://waterdata.usgs.gov/nwis>). The cross-sectional surface area and discharge were used to calculate mean velocity in the Millstone and Iroquois Rivers. In previous studies, we have measured depth and width across eight to 15 lateral transects spaced evenly between upstream to downstream gas sampling locations along each study reach (unpublished data). Widths, depths and cross-sectional areas measured at each of the gas sampling locations were representative of reach averages ($\pm 10\%$), and measured stream velocity (Sugar Creek) or estimated velocity (Iroquois River and Millstone River) should provide a reasonable estimate of travel time between sampling locations.

For each site, we modelled the expected change in dissolved nitrogen gas due only to re-equilibration with the atmosphere and calculated the expected N_2 concentration at the downstream location. We calculated excess nitrogen as the difference between measured and expected N_2 concentrations at the downstream location. Excess nitrogen represents the amount of N_2 produced by denitrification during transport between the upstream and downstream locations. We then applied a range of denitrification rate constants to the model to determine the denitrification rate necessary to produce the measured excess value of dissolved N_2 . Net consumption of O_2 and net production of N_2O were modelled similarly.

Results

In the Iroquois River on 28 April 2002 measured concentrations of N_2 at downstream gas sampling locations exceeded predicted concentrations based solely on atmospheric re-equilibration, indicating N_2 production within this study reach (Fig. 1). Measured O_2 concentrations at downstream gas sampling locations were below predicted concentrations based on re-equilibration with the atmosphere, indicating O_2

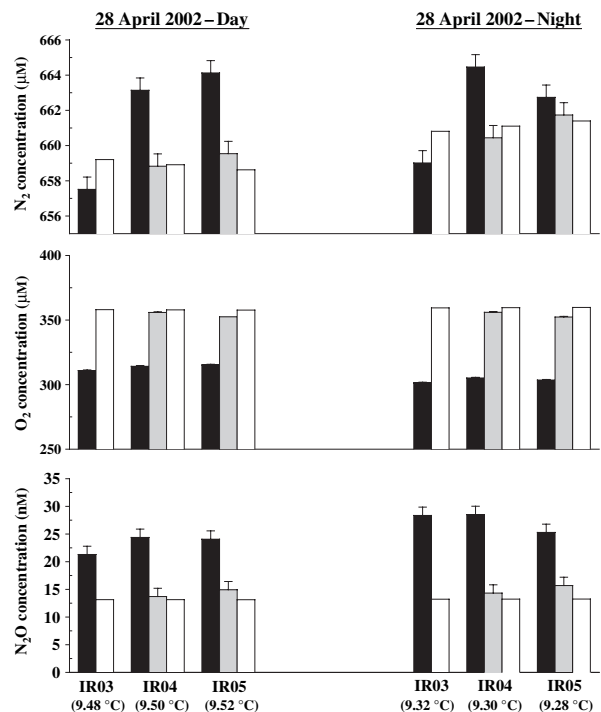


Fig. 1 Dissolved N_2 , O_2 , and N_2O in one study reach (Iroquois River). IR03, IR04, and IR05 indicate gas sampling locations. Black bars represent the measured concentrations of dissolved gases at each sampling location. White bars represent the theoretical equilibrium dissolved gas concentrations based on temperature at each sampling location. Grey bars represent the model predicted dissolved gas concentrations assuming no *in situ* production (of N_2 or N_2O) or consumption (O_2) (based on first-order transfer rates and change in temperature as the parcel of water moves downstream). The difference between the black bar and the grey bar at each sampling location represents the excess gases resulting from *in situ* production (N_2 and N_2O) or shortage resulting from *in situ* consumption (O_2).

consumption within the reach. Measured N_2O concentrations exceeded predicted concentrations based on re-equilibration, indicating N_2O production within the reach. The model was used to calculate N_2 and N_2O production rates and O_2 consumption rates required to solve for gas concentration anomalies (variations from predicted concentrations). Similarly gas concentration anomalies were measured for N_2 , N_2O and O_2 in the Iroquois River, Sugar Creek, and in the Millstone River on other sampling dates, and were used to calculate rates of gas production or consumption.

The rate of denitrification (range 0.31 – 15.91 $mmol\ N\ m^{-2}\ h^{-1}$; median 2.62 $mmol\ N\ m^{-2}\ h^{-1}$) was consistently higher during the day than at night, while net oxygen consumption (range 2.56 – 240 $mmol\ O_2\ m^{-2}\ h^{-1}$; median 7.82 $mmol\ O_2\ m^{-2}\ h^{-1}$) was

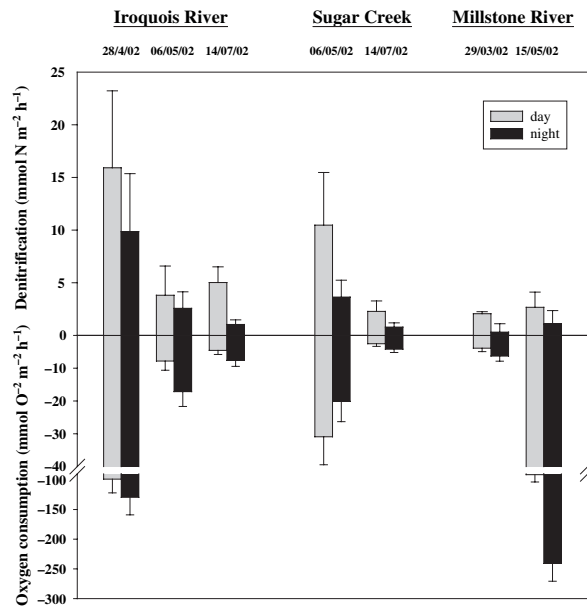


Fig. 2 Denitrification and net oxygen consumption in study reaches during day and night.

usually higher at night (Fig. 2). Higher rates of oxygen consumption at night generally decreased dissolved oxygen concentrations in the water column, slightly

reducing the percent saturation with oxygen (Table 1). Although these systems were net heterotrophic throughout a 24-h period, as indicated by O_2 saturation of $<100\%$, primary production in the water column presumably increased dissolved oxygen concentration during the day relative to that at night. Production of N_2O (range $0.4\text{--}60 \mu\text{mol N m}^{-2} \text{h}^{-1}$, median $6.9 \mu\text{mol N m}^{-2} \text{h}^{-1}$) was slightly higher at night than during the day. However, this was not a robust pattern and the percent saturation of nitrous oxide did not show a clear diurnal pattern (Table 1).

Nitrate concentrations were higher in the study reaches in the Illinois River Basin (Iroquois River and Sugar Creek) than in New Jersey's Millstone River (Table 1). Nitrate concentrations were consistently higher in rivers during the day than by night. Water temperature was generally higher during the day, although there were no large diurnal variations in water temperature.

Discussion

The rate of denitrification in these study reaches was higher than most literature values for riverine deni-

Table 1 Concentrations of oxygen, nitrous oxide and nitrate; nitrous oxide production; temperatures at sampling locations

Site, date, times (hours)	O_2 concentration ($\mu\text{mol O}_2 \text{ L}^{-1}$) (%)	N_2O production ($\mu\text{mol N m}^{-2} \text{h}^{-1}$)	N_2O concentration (nmol N L^{-1}) (%)	NO_3^- concentration ($\mu\text{mol N L}^{-1}$)	Temperature ($^\circ\text{C}$)
<i>Iroquois River</i>					
28 April 2002					
Day (12:30–18:49)	314 (88)	49 ± 24	23.54 (179)	761	9.5
Night [19:00–1:08 (29/4)]	304 (85)	60 ± 30	27.67 (209)	730	9.3
6 May 2002					
Day (12:30–16:07)	289 (95)	12 ± 12	13.65 (134)	907	16.3
Night [21:00–3:47 (7/5)]	278 (91)	9.2 ± 9.6	17.54 (172)	831	16.2
14 July 2002					
Day (14:00–20:16)	216 (85)	–	–	–	25.5
Night [22:06–4:46 (15/7)]	208 (79)	–	–	–	23.7
<i>Sugar Creek</i>					
6 May 2002					
Day (13:40–16:59)	287 (94)	0.4 ± 0.2	10.30 (102)	1019	16.3
Night [21:00–0:19 (7/5)]	302 (99)	4.4 ± 1.7	11.27 (110)	978	16.2
14 July 2002					
Day (12:50–19:13)	260 (99)	–	–	–	27.7
Night [21:55–4:18 (15/7)]	251 (93)	–	–	–	23.2
<i>Millstone River</i>					
28 March 2002					
Day (14:13–19:53)	317 (93)	1.4 ± 0.5	12.71 (105)	171	11.7
Night (19:30–23:53)	327 (93)	1.4 ± 0.6	13.30 (104)	157	10.3
15 May 2002					
Day (12:40–16:43)	251 (83)	6.9 ± 4.2	12.46 (123)	164	16.6
Night [23:15–1:56 (16/5)]	225 (71)	4.7 ± 2.9	11.45 (106)	91	14.8

trification in the literature. However, few measurements have been made in rivers draining areas with intensive arable agriculture. Böhlke, Harvey & Voytek (2004) recently used an $^{15}\text{NO}_3^-$ tracer technique to estimate denitrification in the headwaters of Sugar Creek (Indiana), reporting a rate of $240\ \mu\text{mol N m}^{-2}\ \text{h}^{-1}$ during September, 2001. This is similar to rates we have measured further downstream in Sugar Creek during late summer 2000 and 2001 based on the open channel N_2 production technique (unpublished data). Denitrification rates reported here are comparable to those measured in another agriculturally impacted river, the South Platte River (Colorado, U.S.A.) where nitrogen loss estimated over an annual cycle ranged between 0.14 and $7.14\ \text{mmol N m}^{-2}\ \text{h}^{-1}$, averaging $2.00\ \text{mmol N m}^{-2}\ \text{h}^{-1}$ (Sjodin *et al.*, 1997). McCutchan *et al.* (2003) measured an N_2 production rate in the South Platte River of $15.83\ \text{mmol N m}^{-2}\ \text{h}^{-1}$ during November, 1998.

Nitrous oxide fluxes were also comparable to those measured in the South Platte River over an annual cycle (range $0.26\text{--}97\ \mu\text{mol N m}^{-2}\ \text{h}^{-1}$, average $4.5\ \mu\text{mol N m}^{-2}\ \text{h}^{-1}$) (McMahon & Dennehy, 1999). Nitrous oxide emissions have been measured from only a few rivers and, among these rivers, emissions were highly variable ($0.2\text{--}8.0\ \mu\text{mol N m}^{-2}\ \text{h}^{-1}$) (Cole & Caraco, 2001 and references therein). There was a broad correlation between annual mean nitrate concentration and annual mean N_2O emissions among these rivers over a range of $4\text{--}400\ \mu\text{M NO}_3^-$ (Cole & Caraco, 2001). Although the correlation was weak, explaining only 36% of the variability in annual N_2O emissions, high nitrate concentration in our study reaches ($>700\ \mu\text{M}$ in Sugar Creek and the Iroquois River) could, in part, be responsible for the high fluxes of N_2O to the atmosphere.

Results of a model developed by Seitzinger & Kroeze (1998) to estimate N_2O emissions from surface waters indicate that global emissions of N_2O from rivers could contribute significantly to total anthropogenic emissions of this greenhouse gas from the land surface. McMahon & Dennehy (1999) calculate that annual emissions of N_2O from the South Platte River plus the Potomac River exceed emissions from all municipal sewage treatment plants in the continental United States. In contrast, Cole & Caraco (2001) found that the freshwater tidal Hudson River was a minor source of this greenhouse

gas relative to other sources in the Hudson Valley (particularly agricultural land). Our measured fluxes of N_2O provide further evidence that N_2O production in rivers could contribute significantly to the total anthropogenic emissions of this green house gas from the land surface.

Diurnal patterns of denitrification have been described in other aquatic ecosystems, including shallow streams or estuaries with good light penetration and sediments colonised by microphytes. In these systems, the response of benthic denitrification to light can be mediated by oxygen dynamics. Core incubation studies demonstrate that microalgal production in sediments during light cycles increases oxygen penetration, moving the denitrification zone further from the sediment surface (Nielsen *et al.*, 1990a,b; Lorenzen *et al.*, 1998; Laursen & Carlton, 1999). This displacement of the denitrification zone during the light cycle can effectively create a barrier to diffusion of nitrate from the overlying water to the denitrification zone (Nielsen *et al.*, 1990b; Rysgaard *et al.*, 1994), as nitrate may be consumed in the broadened oxic layer by benthic algae and heterotrophic bacteria. Consequently, denitrifying bacteria in the underlying anoxic layer face stronger competition for nitrate diffusing from the water column during the day. As a result, total denitrification rates may be lower during the light cycle in lake (Risgaard-Petersen *et al.*, 1994) and estuarine sediments (Andersen, Jensen & Sorensen, 1984; Risgaard-Petersen *et al.*, 1994), in biofilms (Dalsgaard & Revsbech, 1992), and stream sediments (Nielsen *et al.*, 1990b).

Diurnal patterns of riverine denitrification in this study are not similar to those described above. This could be because these systems are fundamentally different. Our rivers are turbid and the sediments are visible only near the very edge of each river where the depth is $<25\ \text{cm}$ (average depths in study reaches were $0.7\text{--}3.2\ \text{m}$). Light penetration would not be sufficient to support high rates of benthic algal production. Further, in some cores collected from the Millstone and Iroquois Rivers, the dark black colour indicative of iron bound sulphides extended to within the upper $2\text{--}3\ \text{mm}$ of the surface, indicating very limited oxygen penetration into the sediments. Therefore, it is unlikely that the observed denitrification patterns are related to sediment oxygen dynamics resulting from diurnal patterns of benthic photosynthesis.

Two recent studies of benthic denitrification demonstrated similar diurnal patterns to those presented here and may be relevant. An & Joye (2001), using benthic chambers, found that net N_2 flux from sediments was greater during the day than at night, while sediment oxygen consumption was greater at night than by day in subtidal estuarine sediments (Galveston Bay, TX, U.S.A.). Oxygen production during the day by benthic microalgae enhanced rates of, and coupling between, nitrification and denitrification. Enhancement of denitrification by coupled nitrification during the day was a factor of 2.1 (winter, high nitrate) to 22 (summer, low nitrate). These data suggest that coupled nitrification–denitrification was more significant than denitrification supported by diffusion from the water column. Similarly, Lorenzen *et al.* (1998) determined that, although denitrification occurred nearer the surface during dark incubations of stream sediment cores, total denitrification was greater during light incubations. Higher rates of nitrification in the light, enhanced by benthic microalgal O_2 production, increased denitrification by a factor of 1.3–2.5, with the greatest increase coming under conditions of low water column NO_3^- concentrations. These studies are relevant because they demonstrate that enhancement of denitrification by coupled nitrification in sediments can contribute strongly to diurnal patterns of denitrification. As noted above, benthic algal production in our turbid rivers was negligible and could not have enhanced nitrification near the sediment–water interface in our study reaches. However, nitrification could have been enhanced during the day by pH and temperature shifts in the water column, as discussed below, contributing to the diurnal pattern observed for denitrification.

We believe that the diurnal pattern in denitrification could be the result of that in nitrate concentration. Higher daytime concentrations of nitrate in the water columns of our study reaches (Table 1) suggest that whole system nitrification rates were greater during the day than at night, although we can not exclude the possibility of diurnal variations in nitrate loading. Increased nitrification might be expected as nitrification rates are strongly affected by pH within the range of values commonly measured in rivers (Wild, Sawyer & McMahon, 1971; Huang & Hopson, 1974), with an 80% reduction in nitrification rates when pH is lowered from 8.5 to 6.0 (Warwick, 1986). Although

we did not routinely measure pH during this study, we have previously measured pH in the Iroquois River and Sugar Creek over diurnal cycles in the spring and autumn and we have amassed diurnal pH data for 44 different locations with discharges comparable to our study reaches, by combining our previous observations with data from Warwick (1986) and data obtained from real-time monitoring stations throughout the continental United States maintained by the United States Geological Survey (<http://waterdata.usgs.gov/nwis>). In these locations, pH generally reaches a maximum during mid-afternoon and a minimum during the late night to early morning, consistent with our denitrification measurement intervals. Nitrification rates are also dependent on temperature (references in Warwick, 1986). In an aquatic system, warming of the water column and an increase in pH associated with primary production should result in higher nitrification rates during the day. Warwick (1986) found that in-stream nitrification rates could vary by a factor of four over a daily cycle with the highest rates in the late afternoon. It is, therefore, reasonable to expect that nitrification increased during the day in our study reaches.

Higher daytime nitrification in the river could increase total denitrification in two ways. First, it could increase the water column concentration of nitrate resulting in greater diffusional supply to anaerobic zones within the sediments or other surfaces (i.e. particulate matter, debris, biofilms, etc.). Secondly, it could increase coupled nitrification–denitrification if much of the nitrifying activity is associated with the sediment surface, biofilms or particulate matter, and if this nitrification occurs near an aerobic–anaerobic boundary (e.g. Lorenzen *et al.*, 1998; An & Joye, 2001). Coupled nitrification–denitrification can account for a significant fraction of total denitrification in freshwater sediments (Lorenzen *et al.*, 1998) and coastal marine sediments (calculated from Devol & Christensen, 1993; Devol, Codispoti & Christensen, 1997; Rysgaard *et al.*, 1998; An & Joye, 2001; Laursen & Seitzinger, 2002b). Presumably, it could also account for a significant fraction of whole system riverine denitrification.

The ability of these mechanisms (i.e. coupled nitrification–denitrification and NO_3^- flux) to explain diurnal denitrification patterns was assessed by first determining how increased daytime nitrate in the water column (resulting either from higher daytime

nitrification or higher daytime nitrate loading) would affect nitrate flux into the sediments. We used a simple reaction–diffusion model and based our calculations on the assumption that all denitrification at night was supported by NO_3^- diffusion from the water column to sediments. Therefore, flux of nitrate into sediments was set equal to night-time denitrification. Based on this assumption, we calculated a first-order reaction constant for denitrification at night according to:

$$J = C_o \sqrt{(D_s \cdot K_D)}$$

where J is flux of nitrate, C_o is the nitrate concentration in the overlying water, D_s is the nitrate diffusion coefficient for nitrate in freshwater (Li & Gregory, 1974) corrected for porosity, and K_D is the first-order reaction constant for nitrate consumption (Rasmussen & Jorgensen, 1992). Of course, we recognise that coupled nitrification–denitrification can contribute to total night-time denitrification (e.g. Risgaard-Petersen *et al.*, 1994), and our assumption that the flux of nitrate is equivalent to total denitrification may be false. However, this assumption allows us to make a reasonable estimate of K_D , and determining the absolute value of K_D is not critical for comparing night-time and daytime nitrate fluxes into sediments, as differences are based primarily on changes in the water column nitrate concentration and temperature. We assumed that the reaction constant for denitrification varied only as a function of temperature between day and night and that daytime increase in nitrate concentration exerted no kinetic control over denitrification rates, as an apparent K_m of $50 \mu\text{M}$ was calculated from saturation curves of denitrification in sediments from the Belgian coast (Billen, 1978). We applied a temperature quotient (Q_{10}) of two (a typical value for a wide variety of biological processes) to calculate daytime K_D values from night-time values for each pair of observations. We then used these temperature corrected values of K_D , temperature-corrected diffusion coefficients, and daytime water column nitrate concentrations to calculate the daytime flux of nitrate into sediments (Table 2).

Enhancement of nitrate flux into sediments during the day, caused by higher water column nitrate concentrations (and to a small degree by higher daytime temperatures), could contribute to the overall diurnal pattern of denitrification. In particular, increased flux of nitrate into sediments could explain most of the diurnal variation in denitrification in the

Table 2 Night time and calculated daytime nitrate fluxes into sediments, denitrification rates and percent of the measured daytime increase in denitrification supported by greater nitrate flux

Site	Nitrate flux (mmol $\text{N m}^{-2} \text{ h}^{-1}$)	Denitrification (mmol $\text{N m}^{-2} \text{ h}^{-1}$)	Percentage increase related to nitrate flux
<i>Iroquois River</i>			
28 April 2002			
Day	10.54	15.91	11
Night	9.85	9.85	
6 May 2002			
Day	2.84	3.81	22
Night	2.57	2.57	
<i>Sugar Creek</i>			
6 May 2002			
Day	3.84	10.47	3
Night	3.64	3.64	
<i>Millstone River</i>			
28 March 2002			
Day	0.40	2.06	5
Night	0.31	0.31	
15 May 2002			
Day	2.40	2.67	83
Night	1.09	1.09	

Millstone River on 15 May 2002 (Table 2). However, an increased nitrate flux into sediments does not account for most of the diurnal variation observed in the Iroquois River, Sugar Creek or the Millstone River in March. It is possible that the diurnal pattern at these sites is related to coupled nitrification–denitrification, and that much of the nitrification occurring in these systems is associated with surfaces in contact with the water column and located near aerobic–anaerobic boundaries. This is consistent with our expectation that little oxygen penetrates into the sediments and that the denitrification zone begins within 2–3 mm of the sediment surface. Within the upper 0–2 mm, nitrifying bacteria would be influenced by diurnal pH and temperature shifts in the water column, thus enhancing nitrification, and would also be closely associated with denitrifying bacteria below. Ideally, the argument that observed denitrification patterns were driven by diurnal patterns of nitrification would be strengthened by detailed studies of nitrifying activity in these rivers.

The export of large quantities of nitrogen from catchments to estuaries contributes to eutrophication of coastal marine ecosystems (National Academy of Sciences, 2000). As denitrification is the only mechanism for permanently removing nitrogen during trans-

port, it could provide a buffer by removing a portion of this nitrogen before it reaches the coast. Efforts to reduce nitrogen loading to coastal waters will require more detailed understanding of how nitrogen is processed during transport. Much that we know about riverine denitrification comes from laboratory studies, where it appears that factors such as nitrate concentration, organic carbon availability, sediment properties and pollutants can affect denitrification or nitrogen retention (e.g. Robinson *et al.*, 1979; Cooke & White, 1987; Christensen & Sorensen, 1988; Jansson, Leonardson & Fejes, 1994; Laursen & Carlton, 1999). It is important that we also understand what factors regulate denitrification and nitrogen removal *in situ* and at the reach scale, because policies aimed at reducing nitrogen export by rivers will be implemented at the reach to catchment scale. Recent studies suggest that physical characteristics of rivers (i.e. discharge, depth, depth / water residence time) may be related to the efficiency of nitrogen removal in rivers at the scale of reach to the whole river system and on an annual time scale (e.g. Howarth *et al.*, 1996; Smith, Schwarz & Alexander, 1997; Alexander, Smith & Schwarz, 2000; Seitzinger *et al.*, 2002). While these studies do not explicitly measure denitrification, annually integrated nitrogen removal should approximate annual nitrogen removal by denitrification. These studies suggest that the significance of denitrification as a sink for N, but not area specific denitrification rates, may decrease as the size of a river increases.

Although the efficiency of N removal may vary among reaches as a function of size, the efficiency of N removal by denitrification within a reach may depend on the reach's intrinsic factors, such as nitrate concentration, temperature, sediment characteristics, organic matter content, etc. These factors may fluctuate and on a much shorter time scale than 1 year. The majority of annual N transport can occur in some rivers over a short period of time (a few days to a few weeks) and the efficiency of N removal during this short window of time can be critical for determining how much N is exported by a river reach. It is therefore important to quantify denitrification and the associated proportion of N removed over a short time scale, preferably instantaneously. The method presented by Laursen & Seitzinger (2002a) permits the explicit measurement of denitrification in rivers at the reach scale and *in situ*, making it possible to study factors that will control denitrification, and therefore

nitrogen removal, within a reach. The results of the current study suggest that diurnal variability in denitrification can be important. However, the diurnal pattern of denitrification in these rivers is not driven by diurnal patterns of benthic microalgal production, and hence oxygen dynamics in sediments, as has been described in other systems. Rather, diurnal patterns of denitrification may be in response to nitrification driven by diurnal shifts in pH and temperature. If diurnal variability in nitrification does control the pattern of denitrification that was observed, nitrification and denitrification must have been tightly coupled in the upper few millimetres of sediments, because only modest increases in water column nitrate concentrations were observed during the day. Future denitrification measurements should be conducted during both light and dark periods to prevent biased estimates of riverine denitrification.

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