

Short Research Note

## Limitations to measuring riverine denitrification at the whole reach scale: effects of channel geometry, wind velocity, sampling interval, and temperature inputs of N<sub>2</sub>-enriched groundwater

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### Abstract

A model-based approach was recently introduced for measuring riverine denitrification based on measured changes in dissolved N<sub>2</sub> concentration during riverine transport (Laursen & Seitzinger, 2002a). Inputs to the model, including water temperature, channel depth, wind velocity, and time-of-travel between sampling locations, vary greatly among natural systems. Simulations were run by varying the values of these inputs and determining rates of N<sub>2</sub> accumulation in river water and the detection limits for measuring denitrification using this method. Dinitrogen was found to accumulate most rapidly in streams that were shallow, particularly under conditions of low wind velocity. Dissolved N<sub>2</sub> concentrations, modeled in rivers with a diurnal temperature variation of 5 °C and under conditions of no denitrification or 1 mmol N m<sup>-2</sup> h<sup>-1</sup>, showed that sensitivity of the method can vary as temperatures change. Under low wind conditions and in rivers < 1m in depth, this method is capable of detecting denitrification rates as low as 30–100 μmol N m<sup>-2</sup> h<sup>-1</sup>. This limit of detection should be adequate to measure *in situ* rates in many North American streams, particularly in agricultural watersheds. In deeper rivers N<sub>2</sub> accumulated more slowly and the method became less sensitive. The results of this study should guide decisions regarding the application of this method based on the specific characteristics of a study reach (channel geometry) and the physical conditions (i.e. wind velocity and water temperature) under which measurements are to be made. The input of N<sub>2</sub>-enriched groundwater along a study reach can result in N<sub>2</sub> accumulation that could be misinterpreted as denitrification. Some knowledge of the inputs of groundwater along a reach should also guide decisions regarding the application of this method.

Recent alterations in land use have significantly altered the global nitrogen cycle, increasing nitrogen inputs to watersheds (Vitousek et al., 1997), and consequently to aquatic ecosystems. Nitrate pollution of rivers is a pervasive environmental problem in North American rivers (Beaulac & Reckhow, 1982; David et al., 1997), and the transport of nitrogen to estuarine and coastal

ecosystems threatens these systems with impairment of function (National Academy of Sciences, 2000). Riverine denitrification is potentially an important sink for nitrogen (Seitzinger, 1988b; Sjodin et al., 1997) and could help reduce loading of nitrogen to coastal waters.

Although the importance of denitrification in rivers is generally recognized, the tools to measure

this process *in situ* have only recently become available. Previously *in situ* denitrification has been estimated based on nitrogen mass balance as the net loss in total nitrogen or change in nitrate concentration (Swank & Caskey, 1982; Hill, 1983; Billen et al., 1985; Jacobs & Gilliam, 1985; Chesterikoff et al., 1992; Mulholland, 1992; Jansson et al., 1994; Sjodin et al., 1997). However, this approach is an indirect measure of denitrification as the endproduct of denitrification ( $N_2$ ) is not measured. Further, temporary storage of nitrogen in a reach by autotrophic uptake or physical retention can affect estimates of denitrification based on mass balance, and a complete budget of nitrogen inputs and outflows can be difficult to obtain.

Recently, the isotope-pairing technique (Nielsen, 1992) has been adapted for use in open systems to quantify denitrification in small streams (Böhlke et al., 2004; Mulholland et al., 2004). Widespread application of this technique will greatly enhance our understanding of denitrification in small streams draining a variety of land-use categories in different North American biomes (LINX II, <http://sparc.ecology.uga.edu/webdocs/linx/>). While this is a powerful approach to quantifying denitrification *in situ*, the costs associated with the addition of  $^{15}N-NO_3^-$  tracer and the isotope analyses currently limit its application to relatively small streams.

Advances in the use of membrane inlet mass spectrometry (Kana et al., 1994) have made possible the quantification of very small changes in gas concentrations rapidly and easily, and this technology has been successfully applied to measuring denitrification in aquatic systems based on changes in dissolved  $N_2:Ar$  (Kana et al., 1998; Cornwell et al., 1999; Laursen & Seitzinger, 2002b). This technology recently has been adapted for use in open channels (Laursen & Seitzinger, 2002a, 2004; McCutchan et al., 2003) with denitrification measured based on small changes in dissolved  $N_2$ , corrected for atmospheric re-equilibration. This method has an advantage over isotope-pairing techniques in that it is far less expensive and can, therefore, be applied to larger rivers. To date, this method has been applied to rivers draining agricultural watersheds and a mixed suburban – agricultural watershed, ranging in size from 3rd through 7th order, and with high nitrate concen-

trations and associated high rates of denitrification (Laursen & Seitzinger, 2002a, 2004; McCutchan et al., 2003). While this method performs well in these rivers, its application in other rivers could be limited by the specific physical conditions of those reaches. Presented here are model generated data based on variations in channel depth, gas exchange rates, time of travel, and temperature. Variations in these parameters affect the sensitivity of this method, and under certain conditions the sensitivity may be too low to measure the *in situ* rates of denitrification with current technology. These data could be useful in selecting reaches where application of this method is appropriate, and in determining if a different approach (e.g. isotope-pairing) is more appropriate.

Inputs to the recently described model-based approach to measuring denitrification *in situ* include  $K_{N_2}$  (first-order exchange rate of gases), depth, width, temperature, and  $\Delta$  time (time elapsed between collection of samples) (Laursen & Seitzinger, 2002a) (Table 1). Velocity is also included as a model input, but its effect in the model is to determine time-of-travel between two fixed sampling points as a parcel of water moves downstream.

First-order exchange rates determine how rapidly dissolved  $N_2$  will re-equilibrate with the atmosphere when the concentration is out of equilibrium. In previous studies volatile, non-reactive tracers (propane and isobutane) (Laursen & Seitzinger, 2002a) or Ar anomalies (i.e. Ar concentrations deviating from equilibrium at a given water temperature) (Laursen & Seitzinger, 2004) have been used to determine first-order transfer rates for  $N_2$ . These previously measured first-order exchange rates were normalized to piston velocities, multiplying by depth and correcting for differences in temperature according to  $k_{600} = K_{N_2} \times (Sc_{N_2}/600)^{-2/3}$  (equation 1), where  $Sc_{N_2}$  is the Schmidt number of nitrogen at a given temperature (Wanninkhof, 1992). These normalized piston velocities were combined with published piston velocities from other rivers, lakes, and ponds to develop an empirical relationship between atmospheric exchange and wind velocity (Fig. 1). This relationship was used to calculate  $K_{N_2}$  as a model input to determine the effects of wind velocity on  $N_2$  accumulation and limits of detection for denitrification.

Table 1. Components and calculation procedures of model used to quantify denitrification from dissolved N<sub>2</sub> concentrations

Model inputs	
Measured	Derived
Channel depth (m)	$\frac{d}{dt}$ Equilibrium N <sub>2</sub> concentration (mmol N <sub>2</sub> m <sup>-3</sup> h <sup>-1</sup> )
Channel width (m)	
Velocity (m min <sup>-1</sup> )	
Δ Time (h)	
Initial N <sub>2</sub> concentration (mmol N <sub>2</sub> m <sup>-3</sup> )	$\frac{d}{dt}$ Temperature (°C h <sup>-1</sup> )
Initial Equilibrium N <sub>2</sub> concentration (mmol N <sub>2</sub> m <sup>-3</sup> )	
Initial temperature (upstream) (°C)	
Final temperature (downstream) (°C)	
Initial K <sub>N2</sub> (h <sup>-1</sup> )	
Denitrification rate (mmole N <sub>2</sub> m <sup>-3</sup> min <sup>-1</sup> )	

Calculations. Total N<sub>2</sub> in the parcel of water (mmol N<sub>2</sub>) = N<sub>2</sub> concentration (mmol N<sub>2</sub> m<sup>-3</sup>) \* width (m) \* distance traveled in 1 min (m). At each time step, total N<sub>2</sub> is recalculated as N<sub>2</sub>' = (N<sub>2</sub> + N<sub>2</sub> prod) - N<sub>2</sub> loss where, N<sub>2</sub>' = total N<sub>2</sub> in parcel at previous time step (mmol N<sub>2</sub>) N<sub>2</sub> prod is the total produced (mmol N<sub>2</sub>) within the parcel during the 1 min time step, calculated as denitrification rate (mmol N<sub>2</sub> m<sup>-3</sup> min<sup>-1</sup>) \* 1 min \* width (m) \* depth (m) \* distance (m) traveled in 1 min. N<sub>2</sub> loss (mmol N<sub>2</sub>) = N<sub>2</sub> flux (mmol N<sub>2</sub> m<sup>-2</sup> min<sup>-1</sup>) \* 1 min \* width (m) \* distance (m) traveled in 1 min N<sub>2</sub> flux (mmol N<sub>2</sub> m<sup>-2</sup> min<sup>-1</sup>) = depth (m) \* (1 h/60 min) \* K<sub>N2</sub>' (h<sup>-1</sup>) \* [N<sub>2</sub>measured (mmol N<sub>2</sub> m<sup>-3</sup>) - N<sub>2</sub>'<sub>equil</sub> (mmol N<sub>2</sub> m<sup>-3</sup>)]. K<sub>N2</sub>' (h<sup>-1</sup>) = first-order transfer rate of N<sub>2</sub> given the current temperature (K<sub>N2</sub>' varies through time as the water temperature changes). N<sub>2</sub>'<sub>equil</sub> (mmol N<sub>2</sub> m<sup>-3</sup>) = equilibrium N<sub>2</sub> concentration in the parcel at current time step given the current temperature (N<sub>2</sub>'<sub>equil</sub> varies through time as the water temperature changes) Dissolved N<sub>2</sub> (mmol N<sub>2</sub> m<sup>-3</sup>) is then recalculated from total N<sub>2</sub> after each 1 min time step

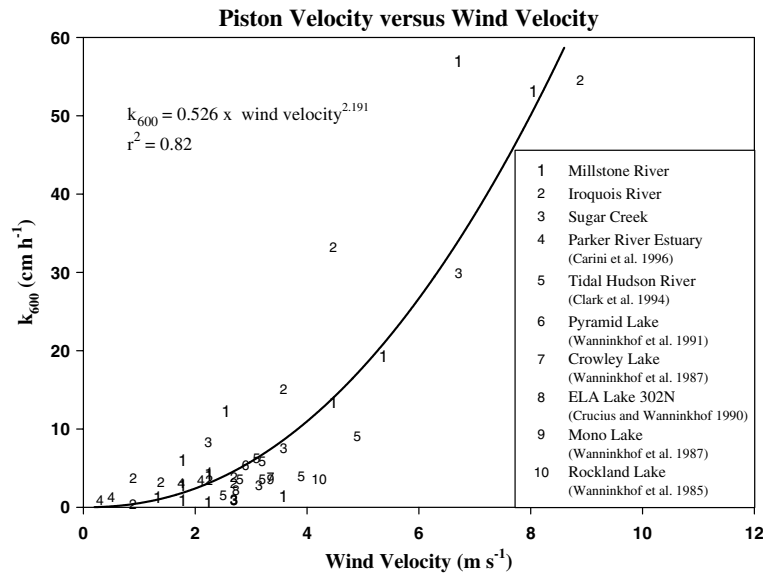


Figure 1. Relationship between wind velocity and piston velocity.

Channel depth affects the extent of interaction between the water column and the sediments. In a shallow river there is little water relative to the sediment surface area and the flux of N<sub>2</sub> from denitrifying sediments can greatly affect the N<sub>2</sub>

concentrations in the water column. In deep rivers there is greater volume relative to sediment surface area. Hence, in deep rivers a higher rate of N<sub>2</sub> flux from sediments is required to observe a change in water column N<sub>2</sub> concentration in the short term.

Depth, as a model input, was varied to determine its effects on  $N_2$  accumulation and limits of detection for denitrification.

While wind can affect the temperature corrected first-order exchange rates of  $N_2$  (Fig. 1), temperature can also affect atmospheric exchange. Diffusion is a temperature dependent process and as temperature increases diffusion, and hence the first-order exchange rate, increases. Temperature dependent first-order exchange rates were calculated from piston velocities by rearrangement of equation 1. The effects of temperature on accumulation of  $N_2$  and limits of detection for denitrification were determined under conditions of constant temperature, modeled at 5, 10, 15, 20, and 25 °C.

Sampling a parcel of water as it moves downstream through time will generally require convenient fixed sampling locations, such as bridges. As stream velocity increases, the time of travel between these fixed points ( $\Delta$  time) decreases. Measuring denitrification requires a measurable increase in dissolved  $N_2$  concentration over time. As  $\Delta$  time increases, this effort becomes easier and the method more sensitive. Time of travel ( $\Delta$  time) as a model input was varied to determine the effect on  $N_2$  accumulation and limits of detection for denitrification.

In model simulations of  $N_2$  accumulation a constant rate of denitrification ( $1 \text{ mmol N m}^{-2} \text{ h}^{-1}$ ) was applied while varying the model inputs (as described above) one at a time. This value of denitrification exceeds rates expected in many streams, particularly those draining relatively pristine watersheds. However it is a reasonable value for many large rivers, or rivers draining agricultural watersheds (Table 2). In the first series of simulations a water temperature of 20 °C was used. As the model was developed in a study reach (Millstone River, central New Jersey, USA) with an approximate channel width of 30 m and depth of 1 m, these values were used in initial simulations, and wind velocity was allowed to vary between 1 and 5  $\text{m s}^{-1}$  (Table 3). In the second series of simulations, water temperature was set to 20 °C, wind velocity was constant at 2  $\text{m s}^{-1}$ , and depth was varied between 0.1 and 10 m. In the third series of simulations wind velocity was constant at 2  $\text{m s}^{-1}$ , depth was 1 m, and water temperature varied between 5 and 25 °C (as above). All simulations began with dissolved  $N_2$  concen-

tration equal to the theoretical equilibrium concentration and the accumulation of  $N_2$  was modeled over 24 h.

River water temperature can vary over a diurnal cycle, particularly in shallow streams. As water temperature varies, dissolved  $N_2$  concentrations will track, but rarely equal, a theoretical equilibrium  $N_2$  concentration. In a fourth simulation, dissolved  $N_2$  concentrations were modeled with wind velocity constant at 2  $\text{m s}^{-1}$ , depth of 1 m, and water temperature varying between 15 and 20 °C over a diurnal cycle. Temperature changed at a constant rate over the 24 h period. Dissolved  $N_2$  was modeled with denitrification inputs of 0 or 1  $\text{mmol N m}^{-2} \text{ h}^{-1}$ , assuming initial  $N_2$  concentrations were in balance (production = flux to atmosphere) resulting in an initial  $N_2$  concentration in the 1  $\text{mmol N m}^{-2} \text{ h}^{-1}$  simulation that exceeded equilibrium  $N_2$  concentration.

The sensitivity of this approach for measuring denitrification was calculated by determining the lower limit of detection (modeled minimum rate of denitrification required for  $N_2$  accumulation) while varying depth, wind velocity, temperature, and  $\Delta$  time. In freshwater, the equilibrium  $N_2$  concentration ranges between 448.99 and 821.98  $\mu\text{mol l}^{-1}$  (for 30 and 0 °C, respectively) (based on calculations from Weiss, 1970). As variation in  $N_2$ :Ar measured in replicate standards is typically <0.05% (Laursen & Seitzinger, 2002a), analytical error in measuring  $N_2$  at equilibrium is 0.22–0.41  $\mu\text{mol l}^{-1}$ . In calculating the sensitivity of this method for measuring denitrification in rivers, a conservative analytical uncertainty of 1  $\mu\text{mol l}^{-1}$  was applied; modeled  $N_2$  must exceed the theoretical equilibrium  $N_2$  by at least this amount for denitrification to be considered measurable.

The accumulation of nitrogen was also modeled based on inputs of  $N_2$ -rich groundwater. The model was modified to allow increases in river discharge (from 1 to 20%) due to groundwater inputs, with incremental increases in depth during riverine transport, and to allow for increases in total dissolved  $N_2$  in the water column as  $N_2$ -enriched groundwater (1–10  $\mu\text{M}$  in excess of river water) mixed with river water. Accumulation of  $N_2$  (apparent denitrification) was modeled with a wind velocity of 2  $\text{m s}^{-1}$ , depth of 1 m, width of 30 m, temperature of 20 °C,  $\Delta$  time of 6 h, and no denitrification.

Table 2. Area specific denitrification rates from a variety of river systems

Location	Denitrification rate ( $\mu\text{mol N m}^{-2} \text{h}^{-1}$ )	Reference
<b>Small streams in pristine watersheds (<math>Q &lt; 1 \text{ m}^3 \text{ s}^{-1}</math>)</b>		
San Francisquito Creek and little lost man Creek, CA, USA	0–27 <sup>1</sup>	Duff et al. (1984)
West Fork, Walker Branch, TN, USA	10–70 (avg 33) <sup>2</sup>	Mulholland (1992)
East Fork, Walker Branch, TN, USA	24 <sup>3</sup>	Mulholland et al. (2004)
Neversink River, NY, USA	145–3050 <sup>4</sup>	Burns (1998)
<b>Small nutrient rich streams (<math>Q &lt; 1 \text{ m}^3 \text{ s}^{-1}</math>)</b>		
Gelbaek and Rabis Baek, Denmark	4–58 <sup>1</sup>	Sorensen et al. (1988)
Gelbaek and Rabis Baek, Denmark	42–460 <sup>5</sup>	Christensen & Sorensen (1988); Nielsen et al. (1990b)
Gelbaek, Denmark	230–460 <sup>5</sup>	Nielsen et al. (1990b)
Dode A, Denmark	8900 <sup>5</sup>	Nielsen et al. (1990a)
Lowland stream, Denmark	340–1240 <sup>5</sup>	Christensen et al. (1989)
Lowland stream, Denmark	0–710 <sup>6</sup>	Pind et al. (1997)
River Wiske, United Kingdom	99–2586 <sup>7</sup>	Garcia-Ruiz et al. (1998)
West Duffin Creek, ON, Canada	100–125 (avg) <sup>2</sup>	calculated from Hill (1988)
Various streams, ON, Canada	580 <sup>2</sup>	Wyer & Hill (1984)
Carnagigue, Canada	150–270 <sup>8</sup>	Chatarpaul et al. (1980)
Juday Creek, IN, USA	964 <sup>9</sup>	Laursen & Carlton (1999)
Sugar Creek, IN, USA	240 <sup>3</sup>	Böhlke et al. (2004)
<b>Midsized Rivers with agricultural influences (<math>1 &lt; Q &lt; 10 \text{ m}^3 \text{ s}^{-1}</math>)</b>		
River Raan, Sweden	1–3286 (avg 628) <sup>1</sup>	Jansson et al. (1994)
River Raan, Sweden	4000–8000 <sup>2</sup>	Jansson et al. (1994)
River Dorn, United Kingdom	141–221 <sup>10</sup>	Calculated from Cooke & White (1987)
Swale – Ouse River system, United Kingdom	20–740 <sup>1</sup>	Pattinson et al. (1998)
Ouse – Trent River system, United Kingdom	100–3800 <sup>2</sup>	Owens et al. (1972)
Swift Brook, ON, Canada	1300 <sup>2</sup>	Kaushik & Robinson (1976)
Swift Brook, ON, Canada	121–302 <sup>4</sup>	Robinson et al. (1979)
Nottawasaga River, ON, Canada	200–600 <sup>4</sup>	Hill (1983)
Various rivers in ON, Canada	110–1230 <sup>8</sup>	Hill & Sanmugadas (1985)
South Platte River, CO, USA	140–7140 (avg 2000) <sup>4</sup>	Sjodin et al. (1997)
South Platte River, CO, USA	15830 <sup>11</sup>	McCutchan et al. (2003)
Iroquois River, IN, USA	2157–15910 <sup>11</sup>	Laursen & Seitzinger (2002a), (2004)
Sugar Creek, IL, USA	270–10470 <sup>11</sup>	Laursen & Seitzinger (2002a), 2004
<b>Other rivers</b>		
Oise River, France	1600 <sup>2</sup>	Billen et al. (1989)
Charente River, France	70–1670 (avg 460) <sup>12</sup>	Torre et al. (1992)
Canal receiving municipal effluents, Netherlands	2718 <sup>4</sup>	van Kessel (1977)
Ivel and Gade River, location unspecified, USA	300–4300 <sup>8</sup>	Edwards & Rolley (1965)
Millstone River, NJ, USA	310–15810 (avg 4790) <sup>11</sup>	Laursen & Seitzinger (2002a), (2004)

Table 2. (Continued)

Location	Denitrification rate ( $\mu\text{mol N m}^{-2} \text{h}^{-1}$ )	Reference
<b>Large rivers</b>		
Delaware River	166–345 <sup>13</sup>	Seitzinger (1988a)
Potomac River	210–235 <sup>13</sup>	Seitzinger (1988b)
Seine	500–3000 <sup>2</sup>	Chesterikoff et al. (1992)
Thames River Estuary	8209–11407 <sup>1,8</sup>	Trimmer et al. (2000)
Chiangjiang	2820, 5740 (avg 4280) <sup>11</sup>	Yan et al. (2004)

<sup>1</sup> acetylene block method, <sup>2</sup> nitrogen mass balance, <sup>3</sup> tracer (<sup>15</sup>N) method in open channel, <sup>4</sup> nitrate mass balance, <sup>5</sup> acetylene block with N<sub>2</sub>O microsensors, <sup>6</sup> isotope pairing, <sup>7</sup> combination of acetylene block and <sup>15</sup>N tracer, <sup>8</sup> nitrate depletion in cores, <sup>9</sup> NO<sub>3</sub><sup>-</sup> microsensor, <sup>10</sup> calculated from nitrate profiles using reaction-diffusion model, <sup>11</sup> N<sub>2</sub>:Ar approach in open channels, <sup>12</sup> acetylene inhibition with benthic chambers, <sup>13</sup> Direct N<sub>2</sub> flux.

Table 3. Model inputs for simulation series

Simulation series	Denitrification rate ( $\text{mmol N m}^{-2} \text{h}^{-1}$ )	Water temperature ( $^{\circ}\text{C}$ )	Depth (m)	Wind velocity ( $\text{m s}^{-1}$ )
1	1	20	1	1–5
2	1	20	0.1–10	2
3	1	5–25 (constant)	1	2
4	0 or 1	15–20 (fluctuating)	1	2

The rate of N<sub>2</sub> accumulation in river water decreased as a function of wind velocity (Fig. 2a). Modeled N<sub>2</sub> concentration exceeded equilibrium (+ analytical error) after 1.5 h for all wind velocity  $e_s \leq 5 \text{ m s}^{-1}$ . The concentration of N<sub>2</sub> reached a maximum of 531.3  $\mu\text{M}$  (at 5  $\text{m s}^{-1}$ ) after 10 h, while at a wind velocity of 1  $\text{m s}^{-1}$  it did not reach a plateau, exceeding 539  $\mu\text{M}$  after 24 h. Accumulation of N<sub>2</sub> was strongly dependent on channel geometry. Dinitrogen accumulated very rapidly when the modeled reach was shallow (0.2 m) with concentration exceeding 540  $\mu\text{M}$  in less than 3 h (Fig. 2b). In a deep model reach (10 m), N<sub>2</sub> accumulated slowly, reaching only 529.5  $\mu\text{M}$  after 24 h. Temperature in the range of 5–25  $^{\circ}\text{C}$  had a minimal effect on N<sub>2</sub> accumulation (here expressed as excess N<sub>2</sub> since equilibrium N<sub>2</sub> is temperature dependent) during the first 10–12 h (Fig. 2c). After 24 h, excess N<sub>2</sub> was 1.5  $\mu\text{M}$  higher in a simulation at 5  $^{\circ}\text{C}$  than at 25  $^{\circ}\text{C}$ .

As temperature fluctuated between 15 and 20  $^{\circ}\text{C}$  over a daily cycle, the rate of change in modeled N<sub>2</sub> concentrations (no denitrification and 1  $\text{mmol N m}^{-2} \text{h}^{-1}$ ) lagged the rate of change in equilibrium N<sub>2</sub> (Fig. 3). As the rates of

re-equilibration in the simulations were slower than the rate of temperature change, the river water continued to be out of equilibrium even after a steady-state was reached (consistent maximum and minimum predicted N<sub>2</sub> concentrations from day to day) (data not shown). Over a 24 h period, modeled N<sub>2</sub> (no denitrification) differed from equilibrium N<sub>2</sub> by as much as  $\pm 3\%$  ( $\sim 15 \mu\text{M}$ ). This could be easily misinterpreted from a snapshot of N<sub>2</sub> concentration as exceptionally high rates of denitrification or as nitrogen fixation. Over a daily cycle, modeled N<sub>2</sub> (both simulations) changed by  $\sim 40 \mu\text{M}$  as gases re-equilibrated to changing temperatures. This magnitude of change was much larger than the signal generated by denitrification, as the maximum difference between simulations with no denitrification and 1  $\text{mmol N m}^{-2} \text{h}^{-1}$  was 2.39  $\mu\text{M}$ . This demonstrates the importance of knowing not only gas exchange rates, but also the rate at which water temperature is changing. Although these model simulations are based strictly on predicted behavior of N<sub>2</sub> gas, in practice N<sub>2</sub> concentrations are calculated from N<sub>2</sub>:Ar, assuming Ar behaves conservatively (no produc-

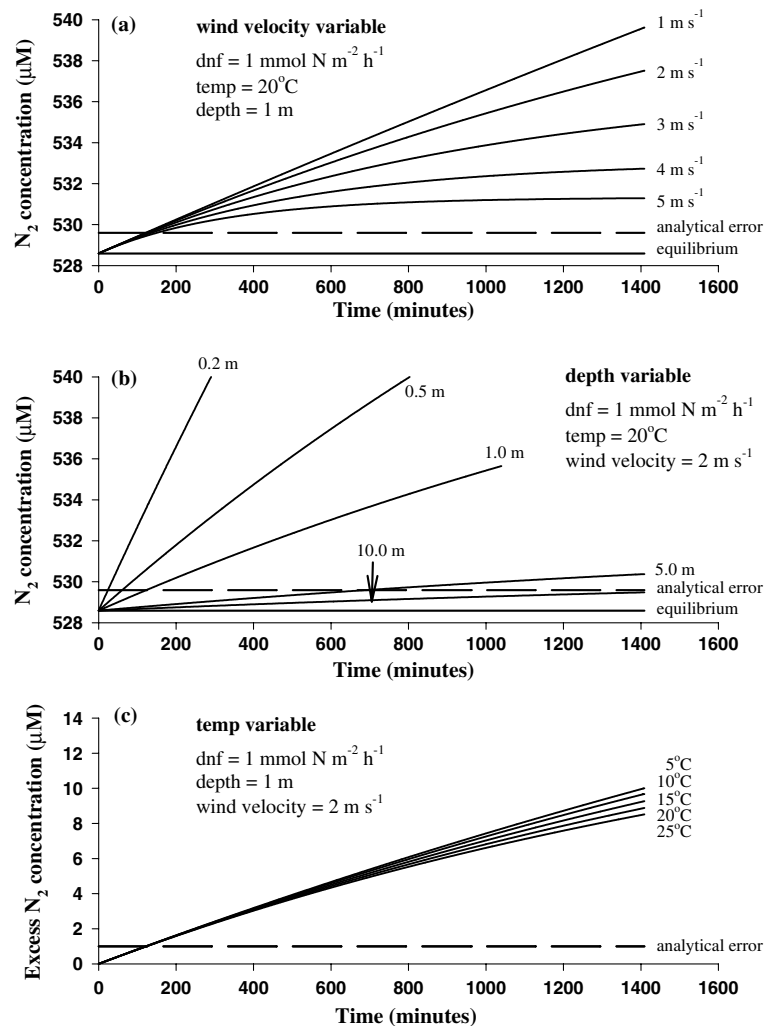


Figure 2. Effects of (a) wind velocity, (b) channel geometry, and (c) temperature on  $N_2$  accumulation during transport given a constant rate of denitrification. Dnf = denitrification.

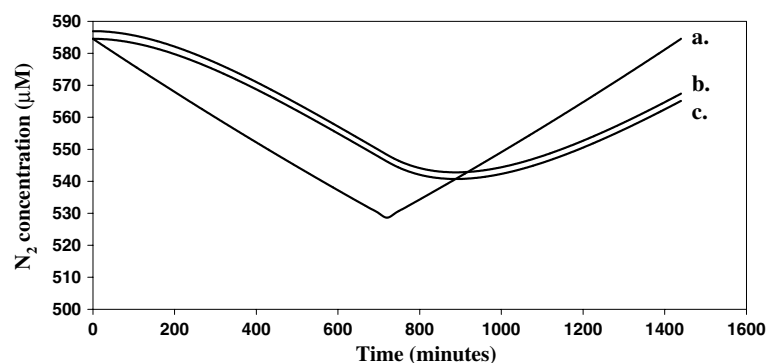


Figure 3. Response of modeled nitrogen concentrations under conditions of fluctuating temperatures. Temperature increases from  $15^\circ\text{C}$  to  $20^\circ\text{C}$ , then returns to  $15^\circ\text{C}$  over a 24 h period. (a) equilibrium  $N_2$  concentration over this interval, (b) modeled  $N_2$  concentration with a denitrification rate of  $1 \text{ mmol N m}^{-2} \text{ h}^{-1}$ , (c) modeled  $N_2$  concentration with no denitrification.

tion or consumption). When water temperature is constant the concentration of Ar does not change through time. However, when temperature changes Ar will re-equilibrate. Uncertainty associated with Ar behavior adds a source of error in estimating denitrification under conditions of changing temperature (Laursen & Seitzinger, 2002a).

The limits of detection for denitrification rate were most sensitive to channel depth and  $\Delta$  time, and to a lesser extent to wind velocity and temperature. In shallow reaches (0.2 m) at wind velocity of  $1 \text{ m s}^{-1}$  and temperature of  $5^\circ\text{C}$ , the

detection limit ranged from  $134 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 3 h travel time to  $27 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 15 h (Fig. 4a). When wind velocity increased to  $5 \text{ m s}^{-1}$  the detection limit ranged between  $154 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 3 h and  $52 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 15 h. In these same reaches at a wind velocity of  $1 \text{ m s}^{-1}$  and temperature of  $25^\circ\text{C}$ , the detection limit ranged between  $135 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 3 h travel time and  $28 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 15 h (Fig. 4b). At a wind velocity of  $5 \text{ m s}^{-1}$  the detection limit ranged from  $181 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 3 h to  $89 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  after 15 h. Limits of detection for

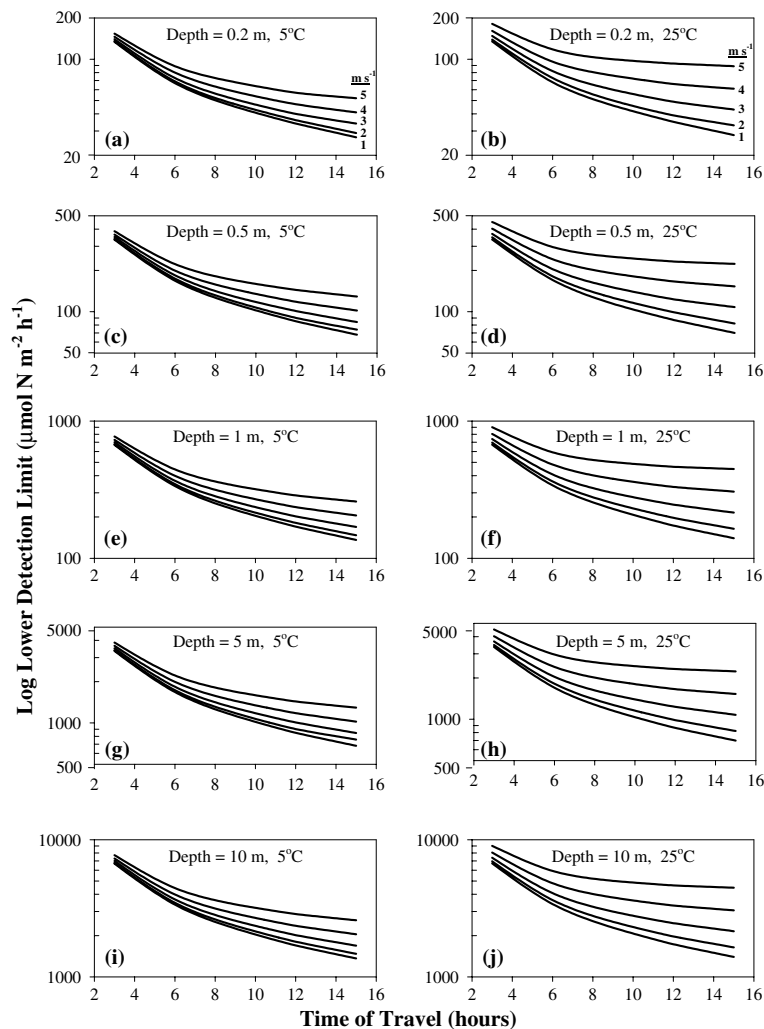


Figure 4. Lower limits of detection for denitrification as a function of travel time for river reaches differing in channel depth at 5 and  $25^\circ\text{C}$ . Isopleths are for detection limits under different wind conditions (1, 2, 3, 4, and  $5 \text{ m s}^{-1}$ ; lower limits of detection consistently correspond to lower wind velocities).

denitrification scaled directly to depth, with a doubling of depth resulting in a doubling in detection limit.

Groundwater can contribute a significant amount of  $N_2$  to river water. When discharge increased by only 1% over a reach, excess  $N_2$  in groundwater had very little effect on water column  $N_2$  concentration, even with an excess concentration of up to  $10 \mu\text{M}$  (Fig 5). However, when discharge increased by a greater amount groundwater contributed a significant amount of  $N_2$  to the water column, which can be misinterpreted as denitrification (apparent denitrification). Given an increase in discharge of 20% over a reach and an excess  $N_2$  concentration in groundwater of  $10 \mu\text{M}$ , apparent denitrification was  $789 \mu\text{mol N m}^{-2} \text{h}^{-1}$ . When excess  $N_2$  concentration in groundwater was low ( $1 \mu\text{M}$ ), apparent denitrification was low ( $39 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ) even with an increase in discharge of 20% over the reach. Although not modeled, groundwater containing less dissolved  $N_2$  than river water could result in underestimating riverine denitrification. Actual differences in groundwater and river water  $N_2$  can vary. The concentration of  $N_2$  in river water is primarily determined by current temperature, and  $N_2$  concentration in groundwater is determined by the temperature of the water at the time it became entrained in groundwater and by mixing with other

groundwater plumes. In the Millstone River, measured excess  $N_2$  in groundwater has ranged between  $-25$  and  $60 \mu\text{M}$  over an annual cycle, with typical values of  $5$ – $10 \mu\text{M}$  excess  $N_2$  (Paul Probo-sco, undergraduate research thesis), it is not possible to suggest a normal range for this difference.

The results of this modeling exercise suggest that this method can be very effective when applied under appropriate conditions. In shallow rivers and streams sensitivity can be excellent (detection limits  $< 50 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ), provided wind velocity is low and sampling stations are located far enough apart to permit a long  $\Delta$  time ( $> 6$  h). This method does appear to be sensitive enough to measure denitrification in many North American rivers that are impacted by agricultural runoff and that may be expected to have high denitrification rates (Table 2). Under less optimal conditions, such as deep water, high winds, or short  $\Delta$  time, this method may not be appropriate to measure *in situ* denitrification rates. In very large rivers, sensitivity of the method may be adequate if steady-state conditions are assumed (i.e. no temperature change and  $N_2$  flux =  $N_2$  production; e.g. Yan et al., 2004). Rough stream beds in shallow water may result in turbulent mixing with rapid atmospheric gas exchange, limiting application of this method in such streams. This method may also be inappropriate in systems that are little

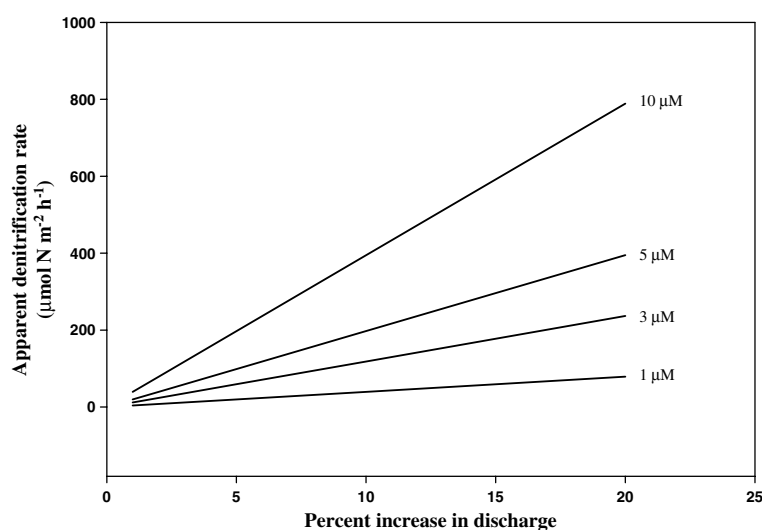


Figure 5. Apparent denitrification resulting from groundwater inputs with excess  $N_2$  (travel time = 6 h, wind velocity =  $2 \text{ m s}^{-1}$ , depth = 1 m, temp =  $20^\circ\text{C}$ ).

impacted by anthropogenic sources of nitrogen as rates of denitrification may fall below detection limits (Table 2). Studies that are designed to use this method over an annual cycle must also consider sampling locations carefully, as discharge, velocity, channel width, and channel depth vary greatly over an annual cycle. We have found that time of travel between two fixed sampling points may be <3 h during the spring and >24 h during late summer in one study reach. This can greatly affect the sensitivity of this method over the annual cycle.

Efforts to reduce nitrogen export to coastal waters require a better understanding of the role denitrification plays in nitrogen removal during riverine transport. The approach based on measuring *in situ* N<sub>2</sub> production (Laursen & Seitzinger, 2002a; McCutchan et al., 2003) is relatively simple and inexpensive, and is more direct than nitrogen mass balance approaches as the endproduct of denitrification (N<sub>2</sub>) is directly measured. Further, the method can be adapted to estimate *in situ* production of other biogenic gases simultaneously. Advancements in analytical instrumentation to increase the sensitivity of N<sub>2</sub> and Ar measurements in the laboratory and in the field would extend the range of environments to which this method can be applied. It is our hope that this analysis will help researchers judiciously apply this method where appropriate, or choose an alternate method where necessary.

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### References

- Beaulac, M. N. & K. H. Reckhow, 1982. An examination of land use – nutrient export relationships. *Water Resources Bulletin* 18: 1013–1024.
- Billen, G., S. Dessery, C. Lancelot & M. Meybeck, 1989. Seasonal and year-to-year variations of nitrogen diagenesis in the sediments of a recently impounded basin. *Biogeochemistry* 8: 73–100.
- Billen, G., M. Somville, E. De Becker & P. Servais, 1985. A nitrogen budget of the Scheldt hydrographical basin. *Netherlands Journal of Sea Research* 19: 223–230.
- Böhlke, J. K., J. W. Harvey & M. A. Voytek, 2004. Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, mid-continent USA. *Limnology and Oceanography* 49: 821–838.
- Burns, D. A., 1998. Retention of NO<sub>3</sub><sup>-</sup> in an upland stream environment: a mass balance approach. *Biogeochemistry* 40: 73–96.
- Chatarpaul, L., J. B. Robinson & N. K. Kaushik, 1980. Effects of tubificid worms on denitrification and nitrification in stream sediment. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 656–663.
- Chesterikoff, A., B. Garban, G. Billen & M. Poulin, 1992. Inorganic nitrogen dynamics in the River Seine downstream from Paris (France). *Biogeochemistry* 17: 147–164.
- Christensen, P. B., L. P. Nielsen, N. P. Revsbech & J. Sorensen, 1989. Microzonation of denitrification activity in stream sediments as studied with a combined oxygen and nitrous oxide microsensor. *Applied and Environmental Microbiology* 55: 1234–1241.
- Christensen, P. B. & J. Sorensen, 1988. Denitrification in sediment of lowland streams: regional and seasonal variation in Gelbaek and Rabis Baek, Denmark. *FEMS Microbiology Ecology* 53: 335–344.
- Cooke, J. G. & R. E. White, 1987. The effect of nitrate in stream water on the relationship between denitrification and nitrification in a stream-sediment microcosm. *Freshwater Biology* 18: 213–226.
- Cornwell, J. C., W. M. Kemp & T. M. Kana, 1999. Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. *Aquatic Ecology* 33: 41–54.
- David, M. B., L. E. Gentry, D. A. Kovacic & K. M. Smith, 1997. Nitrogen balance in and export from an agricultural watershed. *Journal of Environmental Quality* 26: 1038–1048.
- Duff, J. H., F. J. Triska & R. S. Oremland, 1984. Denitrification associated with stream periphyton: chamber estimates from undisturbed communities. *Journal of Environmental Quality* 13: 514–518.
- Edwards, R. W. & H. L. J. Rolley, 1965. Oxygen consumption of river muds. *The Journal of Ecology* 53: 1–19.
- Garcia-Ruiz, R., S. N. Pattinson & B. A. Whitton, 1998. Denitrification and nitrous oxide production in sediments of the Wiske, a lowland eutrophic river. *Science of the Total Environment* 210/211: 307–320.
- Hill, A. R., 1988. Factors influencing nitrate depletion in a rural stream. *Hydrobiologia* 160: 111–122.

- Hill, A. R., 1983. Nitrate-nitrogen mass balances for two Ontario rivers. In Fontaine, T. D. & S. M. Bartell (eds) *Dynamics of lotic ecosystems*. Ann Arbor Science, Ann Arbor, MI: 457–477.
- Hill, A. & K. Sanmugadas, 1985. Denitrification rates in relation to stream sediment characteristics. *Water Research* 19: 1579–1586.
- Jacobs, T. C. & J. W. Gilliam, 1985. Headwater stream losses of nitrogen from two coastal plain watersheds. *Journal of Environmental Quality* 14: 467–472.
- Jansson, M., L. Leonardson & J. Fejes, 1994. Denitrification and Nitrogen Retention in a Farmland Stream in Southern Sweden. *Ambio* 23: 326–331.
- Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham G. E. Bennett & J. C. Cornwell, 1994. A membrane mass spectrometer for rapid high precision determination of N<sub>2</sub>, O<sub>2</sub>, and Ar in environmental water samples. *Analytical Chemistry* 66: 4166–4170.
- Kana, T. M., M. B. Sullivan, J. C. Cornwell, K. M. Groszkowski & M. K., 1998. Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. *Limnology and Oceanography* 43: 334–339.
- Kaushik, N. K. & J. B. Robinson, 1976. Preliminary observations on nitrogen transport during summer in a small spring-fed Ontario stream. *Hydrobiologia* 49: 59–63.
- Laursen, A. E. & R. G. Carlton, 1999. Responses to atrazine of respiration, nitrification, and denitrification in stream sediments measured with oxygen and nitrate microelectrodes. *FEMS Microbiology Ecology* 29: 229–240.
- Laursen, A. E. & S. P. Seitzinger, 2002a. Measurement of denitrification in rivers: an integrated, whole reach approach. *Hydrobiologia* 485: 67–81.
- Laursen, A. E. & S. P. Seitzinger, 2002b. The role of denitrification in nitrogen removal and carbon mineralization in Mid-Atlantic Bight sediments. *Continental Shelf Research* 22: 1397–1416.
- Laursen, A. E. & S. P. Seitzinger, 2004. Diurnal patterns of denitrification, oxygen consumption, and nitrous oxide production in rivers measured at the whole-reach scale. *Freshwater Biology* 49: 1448–1458.
- McCutchan, J. H. Jr., J. F. Saunders III, A. L. Pribyl & W. M. Lewis Jr., 2003. Open-channel estimation of denitrification. *Limnology and Oceanography Methods* 1: 74–81.
- Mulholland, P. J., 1992. Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, riparian, and instream processes. *Limnology and Oceanography* 37: 1512–1526.
- Mulholland, P. J., H. M. Valett, J. R. Webster, S. A. Thomas, L. W. Cooper, S. K. Hamilton & B. J. Petersen, 2004. Stream denitrification and total nitrate uptake rates measured using a field <sup>15</sup>N tracer addition approach. *Limnology and Oceanography* 49: 809–820.
- National Academy of Sciences, 2000. Clean coastal waters: understanding and reducing the effects of nutrient pollution. Committee on the Causes and Management of Eutrophication, Ocean Studies Board, Water Science and Technology Board, National Research Council. 428 p.
- Nielsen, L. P., 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Ecology* 86: 357–362.
- Nielsen, L. P., P. B. Christensen, N. P. Revsbech & J. Sorensen, 1990a. Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxide and oxygen. *Microbial Ecology* 19: 63–72.
- Nielsen, L. P., P. B. Christensen, N. P. Revsbech & J. Sorensen, 1990b. Denitrification and photosynthesis in stream sediment studied with microsensor and whole-core techniques. *Limnology and Oceanography* 35: 1135–1144.
- Owens, M., J. H. N. Garland, I. C. Hart & G. Wood, 1972. Nutrient budgets in rivers. Symposium of the Zoological Society of London 29: 21–40.
- Pattinson, S. N., R. Garcia-Ruiz & B. A. Whitton, 1998. Spatial and seasonal variation in denitrification in the Swale-Ouse system, a river continuum. *The Science of the Total Environment* 210/211: 289–305.
- Pind, A., N. Risgaard-Petersen & N. P. Revsbech, 1997. Denitrification and microphytobenthic NO<sub>3</sub><sup>-</sup> consumption in a Danish lowland stream: diurnal and seasonal variation. *Aquatic Microbial Ecology* 12: 275–284.
- Robinson, J. B., H. R. Whiteley, W. Stammers, N. K. Kaushik & P. Sain, 1979. The fate of nitrate in small streams and its management implications. In Lord, R. C. (ed). *Best Management Practices for Agriculture & Silviculture*. Ann Arbor Science Publications, Ann Arbor: 247–259.
- Seitzinger, S. P. 1988a. Benthic nutrient cycling and oxygen consumption in the Delaware estuary. In Majumdar, S. K., E. W. Miller & L. E. Sage (eds), *The Ecology and Restoration of the Delaware River Basin*. Pennsylvania Academy of Sciences 132–147.
- Seitzinger, S. P., 1988b. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnology and Oceanography* 33: 702–724.
- Sjodin, A. L., W. M. Lewis Jr. & J. F. Saunders III, 1997. Denitrification as a component of the nitrogen budget for a large plains river. *Biogeochemistry* 39: 327–342.
- Sorensen, J., T. Jorgensen & S. Brandt, 1988. Denitrification in stream epilithon: seasonal variation in Gelbaek and Rabis Baek, Denmark. *FEMS Microbiology Ecology* 53: 345–353.
- Swank, W. T. & W. H. Caskey, 1982. Nitrate depletion in a second-order mountain stream. *Journal of Environmental Quality* 11: 581–584.
- Torre, M., J. P. Rebillard, H. Ayphassorho, L. Labroue C. Helmer, 1992. In situ assessment of denitrification in running waters: example of the Charente river. *Annales des Limnologie* 28: 263–271.
- Trimmer, M., D. B. Nedwell, D. B. Sivyer & S. J. Malcolm, 2000. Seasonal benthic organic matter mineralisation measured by oxygen uptake and denitrification along a transect of the inner and outer River Thames estuary. *Marine Ecology Progress Series* 197: 103–119.
- Van Kessel, J. F., 1977. Removal of nitrate from effluent following discharge on surface water. *Water Research* 11: 533–537.
- Vitousek, P. M., J. D. Aber, R. H. Howarth, G. E. Likens P. A. Matson, D. W. Schindler, W. H. Schlesinger D. G. Tilman, 1997. Human alteration of the global nitrogen

- cycle: source and consequences. *Ecological Applications* 7: 737–750.
- Wanninkhof, R., 1992. Relationship between wind speed and gas exchange over the ocean. *Journal of Geophysical Research* 97: 7373–7382.
- Weiss, R. F., 1970. The solubility of nitrogen, oxygen, and argon in water and seawater. *Deep Sea Research* 17: 721–735.
- Wyer, M. D. & A. R. Hill, 1984. Nitrate transformation in southern Ontario stream sediment. *Water Resources Bulletin* 20: 581–584.
- Yan, W., A. E. Laursen, F. Wang, P. Sun & S. P. Seitzinger, 2004. Measurement of denitrification in the Chiangjiang River. *Environmental Chemistry* 1: 95–98.