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Nitrification and denitrification in a midwestern stream containing high nitrate: in situ assessment using tracers in dome-shaped incubation chambers

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Abstract The extent to which in-stream processes alter or remove nutrient loads in agriculturally impacted streams is critically important to watershed function and the delivery of those loads to coastal waters. In this study, patch-scale rates of in-stream benthic processes were determined using large volume, open-bottom benthic incubation chambers in a nitrate-rich, first to third order stream draining an area dominated by tile-drained row-crop fields. The chambers were fitted with sampling/mixing ports, a volume compensation bladder, and porewater samplers. Incubations were conducted with added tracers (NaBr and either $^{15}N[NO_3^-]$, $^{15}N[NO_2^-]$, or $^{15}N[NH_4^+]$) for 24–44 h intervals and reaction rates were determined from changes in concentrations and isotopic compositions of nitrate, nitrite, ammonium and nitrogen gas. Overall, nitrate loss rates (220–3,560 μmol N m^{-2} h^{-1}) greatly exceeded corresponding denitrification rates (34–212 μmol N m^{-2} h^{-1}) and both of these rates were correlated with nitrate concentrations (90–1,330 μM), which could be readily manipulated with addition experiments. Chamber estimates closely matched whole-stream rates of denitrification and nitrate loss using ^{15}N. Chamber incubations with acetylene indicated that coupled nitrification/denitrification was not a major source of N_2 production at ambient nitrate concentrations (175 μM), but acetylene was not effective for assessing denitrification at higher nitrate concentrations (1,330 μM). Ammonium uptake rates greatly exceeded nitrification rates, which were relatively low even with added ammonium (3.5 μmol N m^{-2} h^{-1}), though incubations with nitrite demonstrated that oxidation to nitrate exceeded reduction to nitrogen gas in the surface sediments by fivefold to tenfold. The chamber results confirmed earlier studies that denitrification was a substantial nitrate sink in this stream, but they also indicated that dissolved inorganic nitrogen (DIN) turnover rates greatly exceeded the rates of permanent nitrogen removal via denitrification.

Keywords Denitrification · Nitrification · Stream bed · Benthic chamber · Isotope tracer

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Introduction

The global nitrogen cycle has undergone acute anthropogenic alteration through industrial fixation of atmospheric nitrogen (Galloway et al. 2004; Schlesinger et al. 2006). Widespread, non-point source application of nitrogen fertilizer has led to severe ground water contamination, increased nitrogen loads

in rivers and streams, and subsequent eutrophication of many regional and coastal water bodies. In the Gulf of Mexico, for example, large loads of nitrogen delivered by the Mississippi River (Goolsby et al. 2001) contribute to repeated, seasonal formation of extensive areas of suboxic or hypoxic waters (Rabalais et al. 1994; Scavia et al. 2003). Although it is clear that in-stream nitrogen-cycling processes such as nitrification and denitrification must have an effect on nitrogen loads transported from agricultural fields to the ocean, unlike soils where the processes have been studied in greater detail, much less is known about them in aquatic environments. When and under what conditions do these processes have the greatest impact on stream channel nitrogen loads? What factors control the processes? And, how do they interact with each other and with the hydrologic regime?

Denitrification potentially has the greatest net impact on nitrogen loads in rivers and streams because it permanently removes fixed nitrogen via reduction of nitrogen oxides to nitrogen gas. The process is controlled primarily by oxygen concentration, available electron supply, and the amount of nitrate present (Knowles 1982). Most lotic waters are sufficiently oxic to restrict the process to zones with steep oxygen gradients, such as bottom sediments, hyporheic zones, and river banks. Thus, the significance of denitrification depends locally upon the flux and consumption of oxygen relative to the rate of water flow and re-aeration. A number of studies have documented the occurrence of denitrification in streams and stream sediments using a variety of techniques, including acetylene block assays (Schaller et al. 2004; Bartkow and Udy 2004; Groffman et al. 2005; Duff et al. 2008), ^{15}N tracers (Mulholland et al. 2004, 2008; Böhlke et al. 2004; Grimm et al. 2005), and changes in N_2/Ar ratios (Laursen and Seitzinger 2002; Pribyl et al. 2005; Smith et al. 2006a). The perspective has varied from potential assays conducted in laboratory flasks with sediment material and unlimited substrate concentrations (Groffman et al. 2005) to reach-scale, in-stream tracer tests at near-ambient concentrations (Mulholland et al. 2004, 2008; Böhlke et al. 2004). The approach taken dictates the specific relevancy of the results; some are more revealing about the nature and controls of the process itself, others more germane to the hydrology and mass flux within the particular environment.

Much less is known about in-stream nitrification and its effect on nitrogen loads, particularly in nitrogen-impacted streams. Nitrate is usually the dominant fixed nitrogen species, so nitrification may be overlooked as a potential contributor to nitrogen-cycling. Indeed, in pristine watersheds in-stream nitrification may be an important source of nitrate, but not necessarily a major sink for ammonium. For example, in a study of 16 headwater streams across the US, in-stream tests with $^{15}N[NH_4^+]$ demonstrated that nitrification rates were generally low, averaging only 20–30% of the total $^{15}N[NH_4^+]$ uptake rate (Peterson et al. 2001). However, ammonium concentrations can be controlled by cation exchange with sediments (Duff and Triska 2000), potentially masking the true significance of nitrification based solely on dissolved concentration. Nitrification can be closely coupled with rates of ammonium production (Starry et al. 2005) which can effectively maintain low ammonium loads while steadily producing nitrate. This could be particularly important if ammonium was entering a stream via ground water inputs. On the other hand, in watersheds with high groundwater nitrate concentrations, in-stream nitrification may not be important as a source of nitrate in the stream. Nitrification could have an effect on production and consumption of nitrite and nitrous oxide, intermediates of the denitrification pathway that may be present in nitrate-rich streams at low concentrations.

Multiple scales of hydrologic and process-level interactions are best examined using a variety of approaches. To accomplish this at a specific study site, a multidisciplinary team has been examining nitrogen loads, nitrogen speciation, and nitrogen-cycling processes within Sugar Creek and the Iroquois River in the Midwestern US. These are tributaries to the Illinois River; adjacent watersheds that are heavily impacted by fertilized, row-crop agriculture featuring tiled drainage. A patch-scale assessment of nitrification and denitrification is presented here using large volume, in-stream incubation chambers in Sugar Creek, the smaller of the 2 stream channels. These processes were quantified in the chambers using 3 techniques: (1) mass balance with and without added nutrients; (2) incubations in the presence of acetylene; and (3) tracking with ^{15}N tracers, with incubation times not exceeding 48 h. The purpose of this study was to examine in situ rates

of each process and the response to changes in nitrate, nitrite, and ammonium concentrations and to provide intermediate-scale results that link laboratory incubations using sediment cores with reach-scale in-stream tracer studies using ^{15}N-enriched nitrate. The chambers provided the opportunity to manipulate stream constituent concentrations in situ, an approach that is difficult to accomplish with reach-scale experiments and that may have limited extrapolative utility with sediment core incubations.

Methods

Study site

The study was conducted in Sugar Creek, a first to third order tributary of the Iroquois River, draining western Indiana and eastern Illinois (Fig. 1). The Iroquois River watershed is dominated by row-crop agriculture (corn and soybeans) that relies heavily upon tiled drainage. This region is a major contributor to Mississippi River nitrogen loads entering the Gulf of Mexico (Goolsby et al. 2001; Alexander et al. 2008). Nitrate concentrations in Sugar Creek ranged from 34 to 1,192 μM (mean 550 μM) during biweekly sample collection from February 2000 to June 2002 (Antweiler et al. 2005b). Nitrate concentrations are strongly correlated with discharge; peak values occur in late winter to early spring, lowest values occur during low base flow in late summer.

This study was part of a larger project to examine in-stream processes and their effect on dissolved inorganic nitrogen (DIN) transport in Sugar Creek and the Iroquois River (Böhlke et al. 2004, 2009; Antweiler et al. 2004, 2005a, b; Smith et al. 2006a). Sample site locations for this study were SC03 (near the Highway 57 bridge) and an up-stream first order reach (T2003) in which a reach-scale tracer test was conducted (Fig. 1). The in-stream tracer test involved a 12-h continuous injection in September 2003 of ^{15}N-enriched nitrate and sodium bromide (plus gas tracers), similar to one conducted in 2001 (T2001), as previously described (Böhlke et al. 2004).

In-stream incubations

Incubation chambers were constructed from 0.6-m-diameter (59 L), 0.6-cm-thick, clear acrylic, hemisphere domes (Fig. 2). Two support bars (1.3-cm-diameter PVC pipe) were attached (~15 cm from the bottom, at right angles to each other) inside

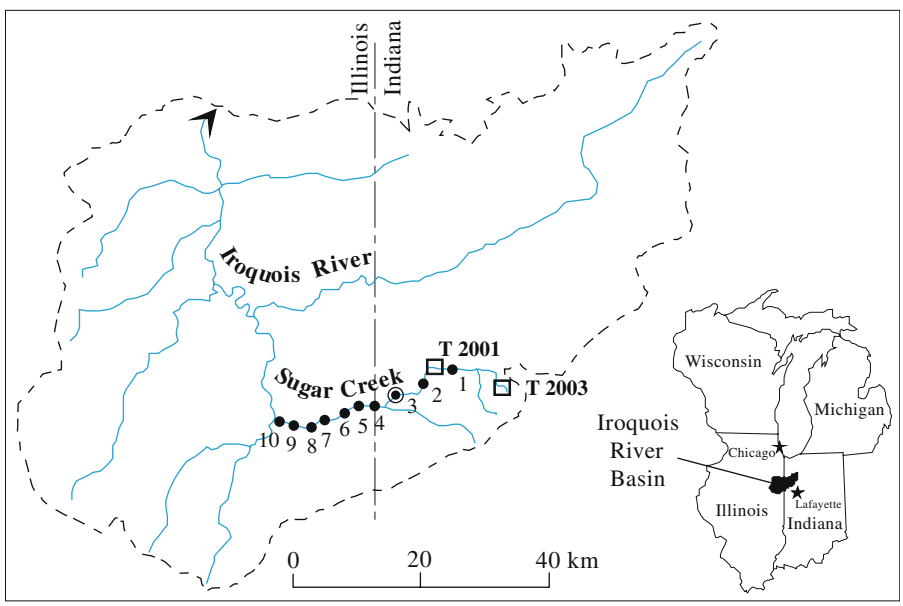

Fig. 1 Map of the Sugar Creek study site in the Iroquois River watershed in western Indiana. Location of fixed sampling stations are indicated and designated as SC01-SC10. The *boxes labeled "T"* are the reaches in which in-stream tracer tests with ^{15}N-nitrate were conducted in 2001 and 2003. Chamber incubations for this study were conducted at SC03 and T2003

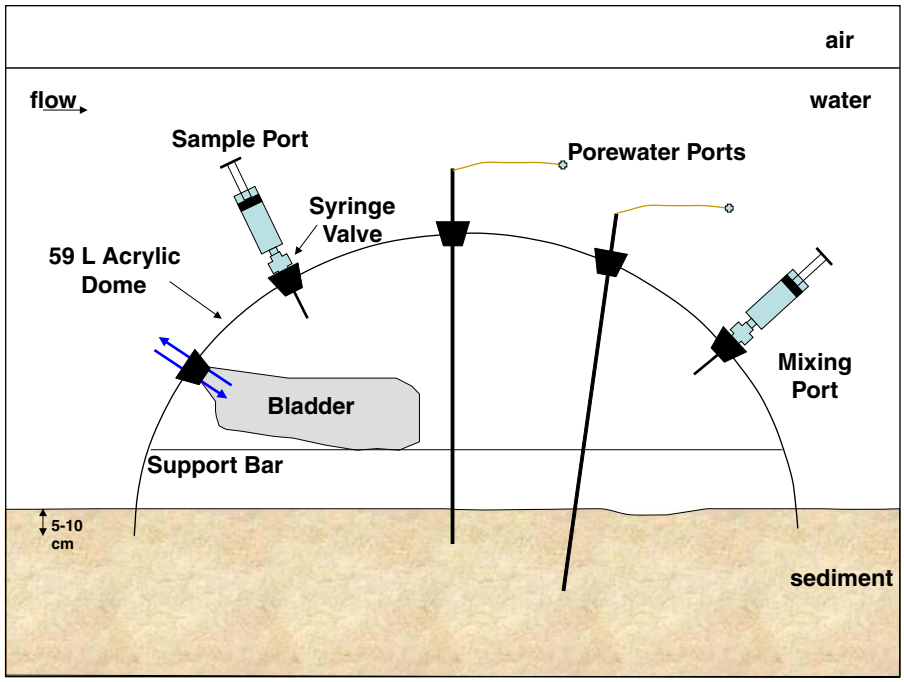

Fig. 2 Diagram of the dome-shaped incubation chambers used in this study

the domes to provide rigidity and to support the volume compensation bladder above the sediment-water interface. Five 1.3-cm-diameter holes were drilled through the acrylic. Two were fitted with rubber stoppers that were penetrated with 16 ga. syringe needles that were closed on the outside with 2-way syringe valves. These were used as the sampling and mixing ports. Two other holes were fitted with rubber stoppers that were penetrated with 1.6-mm-diameter porewater samplers constructed from stainless steel tubing (Duff et al. 1998). Norprene® tubing connected the open ends of the porewater samplers to 2-way syringe valves. The fifth hole was fitted with a rubber stopper penetrated with 6.3-mm-diameter copper tubing. On the inside of the dome, this stopper was placed into the opening of a 5-L, aluminum-coated, Mylar® bag. The bag served as a gas-impermeable volume compensation bladder open to stream water outside of the dome.

The incubation chambers were installed in the stream with the sampling ports and porewater samplers removed and ~1 L of water in the volume compensation bladder. The chambers were inserted 5–10 cm into the sediment. Then, the sampling ports and porewater samplers were put in place such that the porewater sampling screens were located under the center of the dome, one opposite the dome's bottom edge and the other 5 cm deeper. Concentrated tracer solutions were added by 60 mL syringe and mixed by withdrawing and reinjecting 60 mL aliquots through the sampling ports a minimum of 20 times. Tests with rhodamine and bromide indicated that this provided sufficient tracer mixing within the dome. Tracers used in various combinations were: NaBr, KNO_3, $^{15}N[KNO_3]$ (>98 atom %), KNO_2, $^{15}N[KNO_2]$ (99 atom %), $(NH_4)_2SO_4$, $^{15}N[(NH_4)_2SO_4]$ (98 atom %), rhodamine and acetylene. Acetylene was generated on-site from calcium carbide and water; 1.2 L of the resulting acetylene/air mixture was immediately added to each chamber.

At selected time intervals, for up to 44 h after tracer addition, the chamber water was mixed (as above) and sampled from the ports by syringe. Samples (15 mL) for anion analysis (nitrate, nitrite, chloride, bromide and sulfate) were filtered (0.45 μm) and frozen. Samples for ammonium analysis (20 mL) were filtered (0.45 μm) into glass vials and acidified with 50 μL of concentrated sulfuric acid. Samples for nitrous oxide, methane, and acetylene analysis (20 mL) were injected into a 30 mL

stoppered serum bottle that contained a helium headspace and 0.27 mL of 12.5 N potassium hydroxide. Samples for argon, oxygen, and nitrogen gas analysis (15 mL) were collected with a glass syringe and injected into either 5-mL test tubes that were filled to over-flowing and fitted with a ground glass stopper (in 2001) or into 20 mL stoppered serum bottles that contained a helium headspace and 0.4 mL of 18 N sulfuric acid (in 2003). Samples for stable isotope analysis were collected less frequently because of the larger volumes of water required. Water was collected from the chambers using a portable, variable-speed drill fitted with a peristaltic pump head and filtered through a 0.45 μm Gelman capsule filter cartridge. Isotope samples for nitrite (500 mL) were preserved with 2 pellets of potassium hydroxide in plastic bottles; for ammonium (125 mL) with 0.42 mL of concentrated sulfuric acid in glass bottles. Isotope samples for nitrate and nitrogen gas (20 mL) were collected with a gas-tight glass syringe and injected into 30 mL stoppered serum bottles containing a helium headspace and 0.27 mL of 12.5 N potassium hydroxide. Small volume samples (10 mL) were slowly collected ($\sim$5 mL min^{-1}) from the porewater samplers using a glass syringe and preserved for anion and ammonium analysis.

Analytical techniques

Anions and ammonium were analyzed by ion chromatography (Smith et al. 2006b). Nitrous oxide, methane and acetylene were analyzed by gas chromatography using a headspace equilibration technique (Brooks et al. 1992; Smith et al. 1993). Argon, oxygen and nitrogen gas were analyzed by membrane inlet mass spectrometry (MIMS) (Smith et al. 2006a) in 2001 and by gas chromatography (Smith et al. 2005) in 2003. Sediment carbon and nitrogen concentrations were determined on dried (50°C), sieved, ground, sediment grain-size fractions using an Exeter Analytical CHN analyzer (model CE440) at a combustion temperature of 980°C.

The N-isotopic compositions of nitrate, nitrite, ammonium and nitrogen gas were measured by modifications of procedures described previously (Böhlke et al. 2004, 2006, 2007; Smith et al. 2004, 2006a). Aliquots containing nitrate + nitrite were incubated with *Pseudomonas aureofaciens* to produce nitrous oxide, which was purged with helium, trapped with liquid nitrogen, and then released for analysis into a continuous-flow isotope-ratio mass spectrometer (CFIRMS) (Sigman et al. 2001; Casciotti et al. 2002; Coplen et al. 2004). Nitrite was separated for independent isotopic analysis by ion chromatography (Böhlke et al. 2004) or by incubation with *Stenotrophomonas nitritireducens* to produce nitrous oxide from nitrite alone (Böhlke et al. 2007). Ammonium was separated from water samples by sorption onto ammonium-selective zeolite, which was baked in sealed tubes with cuprous oxide + calcium oxide at 650°C to produce nitrogen gas for dual-inlet mass spectrometry (Böhlke et al. 2006). The isotopic content of nitrogen gas was measured in He headspace samples from 30-mL serum bottles containing approximately 15 mL of water. Headspace was pressurized, released into a calibrated loop, then flushed with He through a gas chromatograph into a CFIRMS (Smith et al. 2006a). All nitrogen isotope analyses were calibrated by analyzing synthetic solutions containing isotopic reference materials for each species by the same procedures as the samples. Data were normalized to δ^{15}N values of +0.7 ‰ for N_2 in air-saturated water, +0.43 ‰ for IAEA-N1, +4.7 ‰ for IAEA-N3, +180.0 ‰ for USGS32, and +4,731 ‰ for IAEA-311.

Calculations and numerical simulations

Rate calculations for chamber incubations conducted without using isotope tracers were based on linear regression of concentration changes with time. Nitrogen-cycling processes were assumed to be benthic; rates are expressed as fluxes across the sediment-water interface and have not been corrected for any water column activity that might have occurred within the chamber. For the purposes of this paper, the term "measured" indicates a rate calculated solely based on concentration change and not corrected for changes in the conservative tracer (bromide). If the latter adjustment has been made, the rate is termed "bromide-corrected" and the assumptions regarding the composition of the diluting water are given.

When ^{15}N was used during chamber incubations, rates of nitrogen cycling processes were determined using a numerical spreadsheet reaction model to simulate changes in concentration and isotopic composition of inorganic nitrogen species. The reaction

model calculates these parameters at each time step from 2 basic equations:

$$C_t = C_{t-\Delta t} - C_{\text{loss}} + C_{\text{gain}} \quad \text{with}$$
$$C_{\text{loss}} = \text{Rate}_{\text{loss}} \cdot \Delta t; \text{ and } C_{\text{gain}} = \text{Rate}_{\text{gain}} \cdot \Delta t \qquad (1)$$

$$x^{15}\text{N}_t = \left[\left(C_{t-\Delta t} \cdot x^{15}\text{N}_{t-\Delta t}\right) - \left(\text{Rate}_{\text{loss}} \cdot \Delta t \cdot x^{15}\text{N}_{t-\Delta t}\right) + \left(\text{Rate}_{\text{gain}} \cdot \Delta t \cdot x^{15}\text{N}_{\text{gain}}\right)\right] / C_t \qquad (2)$$

where C is the aqueous concentration in μmol N L^{-1}, t is time in hour, Δt is one time step (0.5–1 h), C_{loss} is the concentration decrease due to consumption inside the chamber plus transport out of the chamber, C_{gain} is the concentration increase due to production inside the chamber plus transport into the chamber, *Rate* for loss or gain is either a zero order rate constant (μmol N L^{-1} h^{-1}) or calculated from a first order rate constant (h^{-1})·C_t, and x^{15}N is the ^{15}N/(^{15}N + ^{14}N) mole fraction. Isotope fractionation was not considered to be significant for the interpretation of the isotope tracer results and was not included in the models. Equations 1 and 2 were modified to accommodate the individual nitrogen species relative to the particular ^{15}N tracer that was added. Models for chamber incubations with ^{15}N[NO_3^-] simulated nitrate using Eqs. 1 and 2 and nitrogen gas (expressed as N) from:

$$Ct = C_{t-\Delta t} + \left(\text{Rate}_{\text{NO}_3\text{ loss}} \cdot \Delta t \cdot f_{\text{NO}_3\text{ loss}}\right) + \left(\text{Rate}_{\text{N}_2\text{ trans}} \cdot \Delta t\right) \qquad (3)$$

$$x^{15}\text{N}_t = \left[\left(C_{t-\Delta t} \cdot x^{15}\text{N}_{t-\Delta t}\right) + \left(\text{Rate}_{\text{NO}_3\text{ loss}} \cdot \Delta t \cdot f_{\text{NO}_3\text{ loss}} \cdot x^{15}\text{N}_{\text{NO}_3,\, t-\Delta t}\right) + \left(\text{Rate}_{\text{N}_2\text{ trans}} \cdot \Delta t \cdot x^{15}\text{N}_{\text{N}_2\text{ trans}}\right)\right] / C_t \qquad (4)$$

where $f_{\text{NO}_3\text{ loss}}$ is the fraction of nitrate loss that is reduced to nitrogen gas and $\text{Rate}_{\text{N}_2\text{ trans}}$ is the rate of nitrogen gas transported into the chamber by water movement. For most simulations, $\text{Rate}_{\text{N}_2\text{ trans}}$ was not adjusted for bromide dilution, rather it was only adjusted to compensate for internal concentration changes not accounted for by denitrification. For nitrate, the ^{15}N mole fraction of the added N ($x^{15}\text{N}_{\text{gain}}$), which could be from nitrification or leakage of stream water into the chamber, was assigned to be 0.0037 (based on isotopic analyses of ambient nitrate, ammonium, and PON), except for the in-stream tracer test where $x^{15}\text{N}_{\text{NO}_3\text{ gain}}$ was 0.04 to account for the high ^{15}N[NO_3^-] present in both the chambers and the stream channel. For nitrogen gas, $x^{15}\text{N}_{\text{N}_2\text{ trans}}$ was assigned to be 0.00367, which is approximately equal to those of stream water and upwelling groundwater.

Simulations of the ^{15}N[NO_2^-] chamber incubations used Eqs. 1 and 2 for nitrite and Eqs. 3 and 4 for nitrogen gas but with $\text{Rate}_{\text{NO}_2\text{ loss}}$ and $f1_{\text{NO}_2\text{ loss}}$ (rate of nitrite loss and fraction of nitrite loss that is reduced to nitrogen gas) substituted for $\text{Rate}_{\text{NO}_3\text{ loss}}$ and $f_{\text{NO}_3\text{ loss}}$. Nitrate was simulated using equation 1 but substituting $C_{\text{gain}} = C_{\text{NO}_3\text{ prod}} + C_{\text{NO}_3\text{ trans}}$, where $C_{\text{NO}_3\text{ prod}}$ is nitrate production via oxidation of nitrite, $C_{\text{NO}_3\text{ trans}}$ is the concentration of nitrate transported into the chamber by water movement, and $C_{\text{NO}_3\text{ prod}} = \left(C_{\text{NO}_2\text{ loss}} \cdot f2_{\text{NO}_2\text{ loss}}\right)$, where $f2_{\text{NO}_2\text{ loss}}$ is the fraction of nitrite consumed that is oxidized to nitrate. The nitrate ^{15}N mole fraction of $C_{\text{NO}_3\text{ prod}}$ was equal to the $x^{15}\text{N}_{\text{NO}_2}$ at $t - \Delta t$ while the ^{15}N mole fraction of $C_{\text{NO}_3\text{ trans}}$ was assigned to be 0.0037.

For the chamber incubations with ^{15}N[NH_4^+], nitrate and nitrogen gas were simulated as described for the ^{15}N[NO_2^-] incubations. Ammonium was simulated using Eqs. 1 and 2 but substituting $\text{Rate}_{\text{NH}_4\text{ loss}}$ and $f_{\text{NH}_4\text{ loss}}$ (rate of ammonium loss and fraction of ammonium loss oxidized to nitrite) for $\text{Rate}_{\text{NO}_3\text{ loss}}$ and $f_{\text{NO}_3\text{ loss}}$. Nitrite concentrations were constant during this incubation (see Results). Hence, $\text{Rate}_{\text{NO}_2\text{ prod}}$ was set equal to $\text{Rate}_{\text{NO}_2\text{ loss}}$ and $f1_{\text{NO}_2\text{ loss}} + f2_{\text{NO}_2\text{ loss}} = 1$.

The variables in these reaction models were adjusted in a trial and error best-fit sequence using t_0 measured values of nitrogen species concentrations and isotopic compositions as initial values. Simulations were done by first fitting the added ^{15}N tracer and finishing with the most distant reaction product(s). Upper-limit simulations were obtained by including additional gain and loss terms for nitrogen gas assuming the bromide dilution rate represented water coming into the chamber at t_0 measured values of nitrogen gas concentration and isotopic composition, with an equal volume of water leaving the chamber at Δt with incremented values for nitrogen gas concentration and isotopic composition.

Results

Chamber incubations (14 total) were conducted in Sugar Creek at 2 locations to assess biogeochemical nitrogen transformations and sediment-water fluxes

in September 2001, June 2003, and September 2003, periods typified by moderate- to low-base-flow discharge, respectively (USGS 2008). Nitrate concentrations in Sugar Creek were 646 and 171 μM in June and September (2003), respectively, while for the same periods chloride concentrations ranged from 609 to 586 μM, and sulfate from 697 to 817 μM. Concentrations of dissolved species in sediment porewater varied considerably by site and date. In general, nitrate was highest in the stream and decreased with depth in the sediment porewater, while ammonium was lowest in the stream and increased with depth in the sediment (Fig. 3). Typically in the −5 to −10 cm interval the relative concentrations of nitrate and ammonium reversed, with ammonium being greater below and nitrate greater above. Ground water beneath the hyporheic zone generally has no measurable nitrate and large amounts of excess nitrogen gas (Böhlke et al. 2004). Chloride concentrations generally increased with depth in the sediments, but the shape of the chloride gradient varied considerably. In some cases the concentration increase was large and immediately below the sediment water interface, while in other cases, the gradient was less pronounced and deeper, below −10 cm (Fig. 3). Sulfate concentrations were generally less variable with depth; the shallow (0−10 cm) porewater sulfate concentration commonly was similar to the stream value.

Initial chamber deployments assessed changes in nitrate and dissolved gas concentrations in September 2001 (Fig. 4) during a 22 h incubation period. Companion incubations were conducted at ambient (76 μM) and added (720 μM) initial nitrate concentrations. The total loss rate for bromide, which was added as a conservative tracer, was 0.8 (±0.1) % h^{-1} and 0.6 (±0.1) % h^{-1} for the two experiments (2–22 h; $R^2 = 0.93$, 0.87, respectively). These bromide concentration decreases are interpreted as a result of either flux of bromide-free water into the chamber and (or) loss of bromide by diffusion into the sediment. For the ambient nitrate incubation, chloride and argon gas concentrations were unchanged after 22 h, while total nitrate and oxygen concentrations steadily decreased 1.4 (±0.1) and 3.3 (±0.2) % h^{-1}, respectively, and total nitrogen gas concentrations increased slightly 0.15 (±0.09) % h^{-1}. For the added nitrate incubation, total chloride and argon gas concentrations increased only slightly (<5% total), while total nitrogen gas concentrations increased steadily at 0.54 (±0.03) % h^{-1} and total nitrate and oxygen decreased steadily at 1.4 (±0.1) and 2.7 (±0.07) % h^{-1}, respectively. Oxygen concentrations in the two chambers were 158 and 92 μM after 22 h for the ambient and added nitrate incubations, respectively.

Determination of a bromide-corrected rate of change during the chamber incubations for any given nitrogen species is complicated because the source of the bromide-free dilution water is unknown. Hence, for nitrate and nitrogen gas, alternate calculations were made assuming the dilution was due to

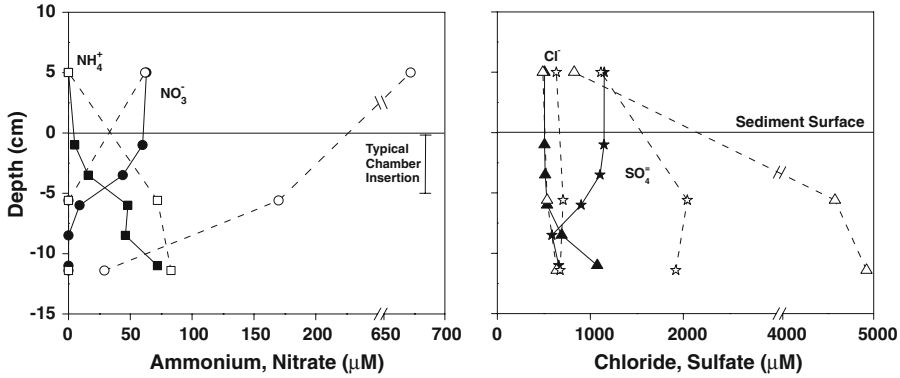

Fig. 3 Vertical profiles of dissolved nitrate (*circles*), ammonium (*squares*), sulfate (*stars*) and chloride (*triangles*) concentrations in the stream channel and sediment porewater at the Sugar Creek study site in September 2001. Concentration in the stream water is arbitrarily plotted at +5 cm. Solid symbols and lines are for samples collected with a USGS minipoint porewater sampler (Duff et al. 1998) outside of an incubation chamber. Open symbols and dashed lines depict highest and lowest concentrations found from porewater samples collected during the chamber incubations

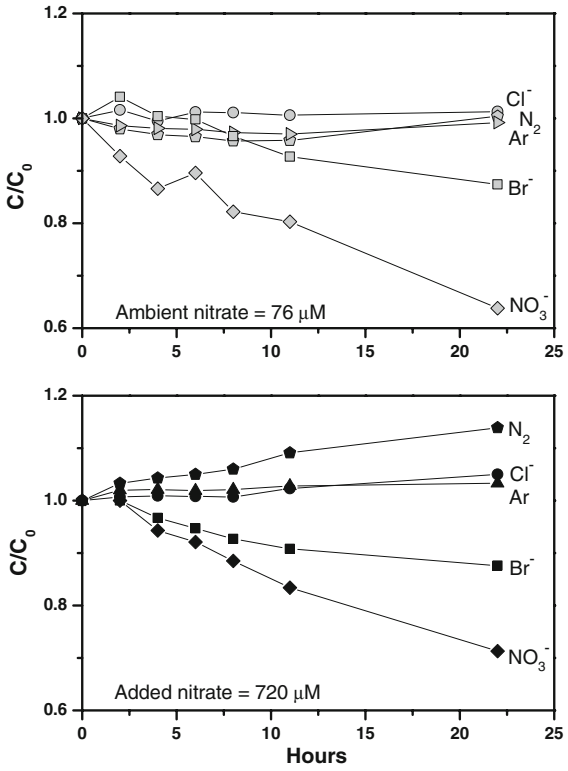

Fig. 4 Solute concentration changes, normalized to initial concentrations, during the course of chamber incubation experiments in Sugar Creek in September 2001 at ambient nitrate (*top*) and added nitrate (*bottom*) concentrations (initial nitrate concentrations as indicated). Sodium bromide was added as a conservative tracer for both tests

nitrate-containing stream water with air-saturated nitrogen gas or nitrate-free ground water that contained excess nitrogen gas (Table 1). These alternate dilution water sources are considered to represent end-member situations. As might be expected for the ambient nitrate experiment, dilution with stream water resulted in little difference between measured and bromide-corrected nitrate loss rates, but dilution with nitrate-free ground water resulted in a much lower bromide-corrected rate. When nitrate was added to the incubation chamber at a concentration much higher than ambient, it acted more like the bromide tracer; in that bromide-corrected loss rates were both substantially lower than the loss rate based solely on nitrate concentration change, regardless of the source of the dilution water. The true rate of nitrate consumption could have been higher for any of these incubations because concentration changes do not necessarily account for nitrate production within the chamber. An increase in nitrogen gas concentrations reasonably matched nitrate loss rates for both the ambient and the added nitrate incubations except when the assumption was made that ground water was the source of the diluting water (Table 1). In that case, the bromide-corrected rate of nitrogen gas increase was substantially smaller than either the measured rate of nitrogen gas increase or the bromide-corrected rate of nitrate loss, or even a negative value in the case of the ambient nitrate incubation. There was an increase in dissolved methane concentrations during both incubations (data not shown), clearly indicating that some ground water flux into the chambers from deeper zones did occur.

A similar set of chamber incubations was conducted at the same stream site in September 2003, this time using ^{15}N-enriched nitrate. During these

Table 1 Comparison of rate calculations for nitrate loss and nitrogen gas production during chamber incubations in September 2001 based on different assumptions for the composition of the diluting water

Incubation condition	Diluting water[a]	Rate of nitrate loss[b] (μmol N m^{-2} h^{-1})	Rate of N_2 gain[b] (μmol N m^{-2} hr^{-1})
Ambient nitrate (76 μM)	None	202 (21)	352 (226)
	GW	86	−231
	SW	204	354
Added nitrate (720 μM)	None	1,877 (140)	1,165 (69)
	GW	1,100	660
	SW	1,182	1,172

[a] Rate calculations based on linear regression of concentration change within the incubation chamber with no correction for bromide change (None), or assuming bromide dilution was due to input of groundwater (GW) with a composition of 0, 0, and 800 μM Br^-, NO_3^-, and N_2, respectively (Böhlke et al. 2004), or input of surface water (SW) with a composition of 0, 76, and 590 μM Br^-, NO_3^-, and N_2, respectively

[b] Brackets enclose standard error, $n = 6$

incubations, chloride concentrations increased while bromide concentrations decreased (Fig. 5), pointing to a ground water input, and there was again an increase in methane concentrations (data not shown). However, coincidentally the ^{15}N mole fraction in the nitrate pool decreased by >10% during the first 20 h of the ambient nitrate incubation (Fig. 6). This decrease must have been due to either nitrate production within the chamber or a flux of nitrate-containing water coming in from outside the chamber that was equivalent to 1.4 L h^{-1} of stream water. Overall, the indication from both sets of experiments (2001, 2003) is that a mixture of stream water and ground water, with a likely additional contribution from hyporheic water, results in dilution of the tracers in the chambers. This makes it difficult to distinguish nitrate movement into the chamber from nitrate production within the chamber during an incubation.

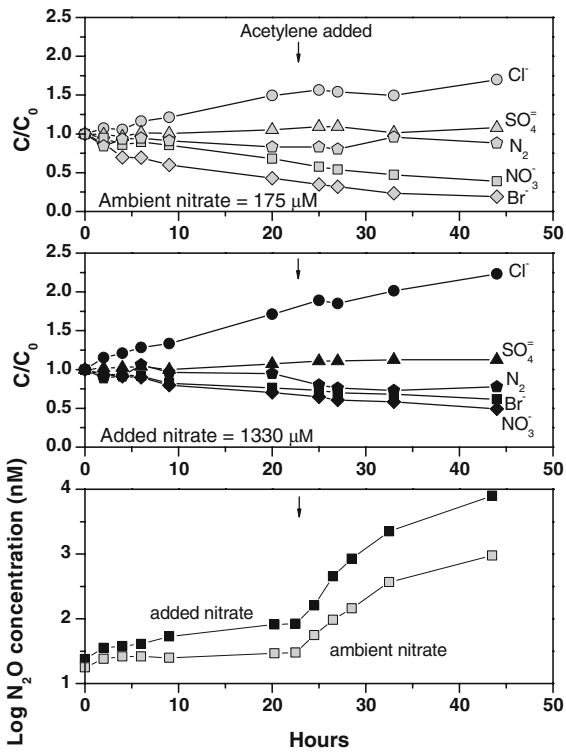

Fig. 5 Solute concentration changes, normalized to initial values, during the course of chamber incubation experiments in Sugar Creek in September 2003 at ambient nitrate (*top*) and added nitrate (*middle*) concentrations (initial nitrate concentrations are indicated) and log of the nitrous oxide concentration for each incubation (*bottom*). Bromide and $^{15}N[NO_3^-]$ were added as tracers; acetylene gas was added after 23 h

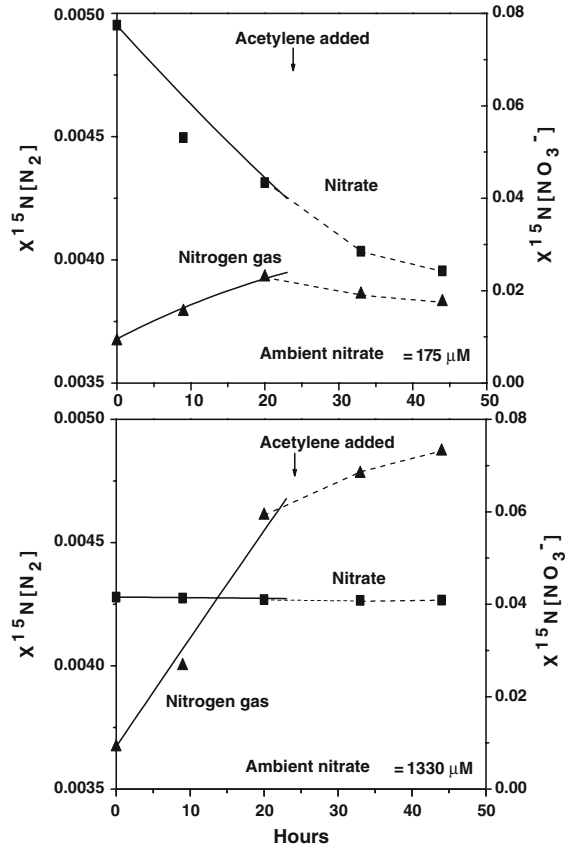

Fig. 6 ^{15}N mole fraction of nitrate and nitrogen gas during the chamber incubation experiments shown in Fig. 5 at ambient nitrate (*top*) and added nitrate (*bottom*) concentrations (initial nitrate concentrations are indicated). *Solid lines* are simulations using the reaction model rate parameters listed in Table 2 for the pre-acetylene time period. *Dashed lines* indicate trend after acetylene was added

Simulations of simultaneous nitrate loss and isotope dilution via nitrate gain in the September 2003 incubation chambers were used to determine the respective rates for each process. Best-fit solutions were 1,368 and 779 µmol N m^{-2} h^{-1} for rates of nitrate loss and gain, respectively, during the ambient nitrate incubation and 3,800 and 76 µmol N m^{-2} h^{-1} for the added nitrate incubation (Table 2; Fig. 6). In the latter case, the nitrate gain was too small to appreciably affect the ^{15}N-nitrate mole fraction even after 40 h. There was also recovery of the tracer ^{15}N in nitrogen gas (Fig. 6); more for the added nitrate incubation as compared to the ambient nitrate incubation. However, in both cases the amount of ^{15}N recovered in nitrogen gas was substantially smaller

Table 2 Parameter values from best-fit reaction model simulations for chamber incubation results with ^{15}N tracers in Sugar Creek in September 2003[a]

Incubation condition[b]	Nitrogen gas		Nitrate			Nitrite			Ammonium		
	$Rate_{N_2\,gain}$	$Rate_{^{15}N_2\,prod}$	$Rate_{NO_3\,loss}$	$f_{NO_3\,loss}$	$Rate_{NO_3\,gain}$	$Rate_{NO_2\,loss}$	$f1_{NO_2\,loss}/f2_{NO_2\,loss}$	$Rate_{NO_2\,gain}$	$Rate_{NH_4\,loss}$	$f_{NH_4\,loss}$	$Rate_{NH_4\,gain}$
$^{15}NO_3^-$ ambient	−1,258	34	1,368	0.025	779						
$^{15}NO_3^-$ added	−969	171	3,800	0.045	76						
$^{15}NO_3^-$ ambient w/in-stream test	113	213	532	0.4	380						
$^{15}NO_2^-$ added	1,758	49	1,140	nr[c]	380	485	0.18/0.1	26			
$^{15}NH_4^+$ added	1,520	0.6	779	nr[c]	0	3.5	0.2/0.18	3.5	955	0.0037	114

[a] All *Rate* parameters are μmol N m^{-2} h^{-1}; *f* is a unit-less fraction per hour; $f_{NO_3\,loss}$, fraction of nitrate loss that is reduced to nitrogen gas; $f1_{NO_2\,loss}$, fraction of nitrite consumed that is reduced to nitrogen gas; $f2_{NO_2\,loss}$, fraction of nitrite consumed that is oxidized to nitrate; $f_{NH_4\,loss}$, fraction of ammonium loss oxidized to nitrite. Gain is defined as the sum of production within the chamber and physical transport into the chamber; loss is defined as the sum of consumption within the chamber and physical transport out of the chamber

[b] Ambient refers to incubation at ambient concentration of ^{15}N tracer species. Added indicates substantial concentration change above ambient stream-water concentration

[c] nr = parameter not relevant for simulation; replaced with alternate parameters

than the ^{15}N-nitrate loss during the same time period (2.5 and 4.7% of the total). Rates of $^{15}N[N_2]$ production (based on increased ^{15}N mole fraction) were 34 and 171 μmol N m^{-2} h^{-1} for the incubations conducted at 175 and 1,330 μM nitrate, respectively, though in each case there was a net loss of nitrogen gas from the incubation chambers based on bulk concentration decreases (Table 2).

After 23 h, acetylene was added to the in-stream chambers and the incubations continued for an additional 21 h. Dissolved acetylene concentrations were ~700 μM and relatively constant with time. The presence of the acetylene had little effect on bromide, chloride, sulfate or nitrate concentration trends (Fig. 5). There was a slight decrease in nitrogen gas concentrations, presumably due to gas stripping into the acetylene gas headspace that was now present in the chambers. The presence of the acetylene completely stopped ^{15}N-labeled nitrogen gas production at the lower nitrate concentration. For the higher nitrate incubation, nitrogen gas production was inhibited, but still occurred at about 29% of the pre-acetylene rate (Fig. 6; Table 3). Prior to the acetylene addition, there were relatively linear but low rates of total nitrous oxide accumulation, 0.3 and 1.5 μmol N m^{-2} h^{-1} for the ambient and added nitrate concentrations, respectively (Table 3). Those rates increased markedly in the presence of acetylene to 20.3 and 183.7 μmol N m^{-2} h^{-1}. The nitrous oxide production rates after the acetylene addition were roughly similar (within a factor of 2) to the N_2 production rates derived from isotope data prior to the acetylene addition (Table 3).

Additional patch-scale comparisons of benthic denitrification rates at ambient nitrate concentrations were obtained by placing the incubation chambers in areas of the streambed with different grain size distribution and using ^{15}N tracer (Table 4). An area that was dominated with finer-grained material (mainly medium sand) had a higher rate of net nitrogen gas production (~2.9-fold) than did an area of coarser-grained material (mainly coarse sand and gravel). An area that appeared to be of mixed coarse- and fine-grained material had a rate similar to that of the coarse-grained site. These incubations were conducted in the main channel and did not include any rooted plants. The fine-grained sediment site had the lowest total solid carbon and nitrogen concentrations of any of the main channel sites tested

Table 3 Denitrification rates based on N_2O and $^{15}N[N_2]$ production in the presence and absence of acetylene for in-stream chamber incubations conducted in Sugar Creek in September 2003[a]

Incubation condition	N_2O production rate[b] (μmol N m^{-2} h^{-1})		$^{15}N[N_2]$ production rate[c] (μmol N m^{-2} h^{-1})	
	no C_2H_2	with C_2H_2	no C_2H_2	with C_2H_2
Ambient nitrate (175 μM)	0.3 (0.1)	20.3 (0.2)	34	0
Added nitrate (1,330 μM)	1.5 (0.3)	183.7 (13.8)	171	49

[a] Initial rates in the absence of acetylene determined for a 23 h incubation, after which acetylene was added to the chambers and the incubation continued for an additional 21 h

[b] Rates calculated from a linear regression of nitrous oxide concentration increases for 0–23 h (no acetylene) and 29–44 h (with acetylene) (see Fig. 5). Parentheses enclose standard error

[c] Simulated rates using the reaction model parameter values listed in Table 2

Table 4 Rates of $^{15}N[N_2]$ production and net N_2 concentration change for in-stream chamber incubations conducted in Sugar Creek in June 2003 in regions of visually differing sediment type

Sediment type at incubation site[a]	Sediment grain size range[b] (%)	Sediment carbon content[b] (%)	Sediment C/N[b]	Production rate[c] (μmol N m^{-2} h^{-1})	
				$^{15}N[N_2]$	Total N_2
Fine	2/44/53/1	0.07/0.62/1.34/0.02	22/72/78/40	157	1,145
Mixed	1/8/39/52	0.05/0.13/1.41/1.79	19/27/77/59	117	421
Coarse	1/14/32/53	0.04/0.19/1.06/1.91	23/49/76/91	78	390

[a] Chamber incubation sites chosen based on apparent visual differences in sediment surface texture when deploying the chambers

[b] Sediment analysis on samples collected from approximately 0–5 cm depth interval. Grain size fractions are: <0.125 mm, 0.125–0.5 mm, 0.5–2 mm, and >2 mm, respectively; percent by weight

[c] Simulated rates using the reaction model parameter values of 1,520 and 437 μmol N m^{-2} h^{-1} for $Rate_{NO_3\ loss}$ and $Rate_{NO_3\ gain}$, respectively, and 0.077 for $f_{NO_3\ loss}$ for 0 to 21 h incubations. Ambient nitrate concentration = 642 μM

(Table 4). Simulations accounting for isotope dilution indicated greater nitrogen gas gain than could be attributed to reduction of the ^{15}N-enriched nitrate. The source of the additional nitrogen gas could have been from ground water or hyporheic water influx into the chamber and (or) a nitrogen source other than nitrate.

The efficacy of the in-stream incubation chambers to quantify nitrogen-cycling processes was assessed by comparison with an in-stream, reach-scale tracer test using ^{15}N-enriched nitrate. The test was conducted at an up-stream location in Sugar Creek (T2003; Fig. 1) that was shallower ($z = 0.15$ m) and had lower nitrate concentrations (90 μM) than the site for the previous chamber incubations (SC03; Fig. 1). Tracers (bromide and $^{15}N[NO_3^-]$) were continuously added to the stream channel for 12 h (Böhlke et al. 2004, 2009). At a location 1,600 m down-gradient from the injection site, a series of four smaller volume (14.5 L) incubation chambers were simultaneously emplaced in the stream side-by-side, capturing the tracers that had been released into the stream at a fixed point in time. During a subsequent 8-h incubation, one chamber was sacrificed and sampled at each consecutive time point. The ^{15}N mole fraction of dissolved nitrogen gas and total tracer ^{15}N within the chambers increased with time, while the nitrate concentration decreased (Fig. 7). The consistency of the concentration and isotope trends during this in-stream test elucidates the reproducibility of replicate chamber incubations. During the incubation period the stream water nitrate ^{15}N mole fraction increased (the chambers were emplaced during the rising limb of the tracer injection). Hence, the within-chamber increase was likely due to a flux of ^{15}N-enriched stream water nitrate into the chamber during the course of the incubation. Incubation chamber rates of net nitrate loss and nitrogen gas production from ^{15}N-labelled nitrate were 532 and 213 μmol N m^{-2} h^{-1}, respectively (Table 5). Similar rates for these same processes, 486 and 154 μmol N m^{-2} h^{-1}, respectively,

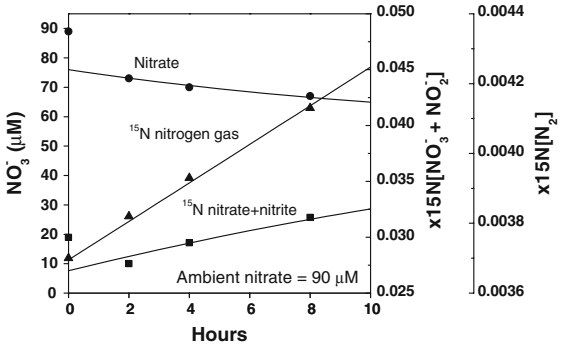

Fig. 7 Nitrate concentration and ^{15}N mole fraction of nitrate + nitrite and nitrogen gas recovered from chamber incubation experiments conducted in conjunction with an in-stream tracer test featuring a 12-h continuous injection of bromide and $^{15}N[NO_3^-]$ in September 2003. In-stream tracer was captured at a single location in replicate, 14.5-L domes. Each time point shown represents results from an individual chamber, which was sacrificed when sampled. *Solid lines* are simulations using the reaction model rate parameters listed in Table 2

Table 5 Comparison of rates of nitrate loss and $^{15}N[N_2]$ production for in-stream chamber incubations with an in-stream, reach-scale tracer test in Sugar Creek in September 2003

Process	Method of measurement	Rate (μmol N m^{-2} h^{-1})
Nitrate loss	Chamber[a]	532
	Reach[b]	486
^{15}N-labelled N_2 production	Chamber[a]	213
	Reach[b]	154

[a] Simulated rates using the reaction model parameter values listed in Table 2 for 0–8 h incubations

[b] Average simulated rates in a 1,250-m reach that included the chamber incubation site during a concurrent reach-scale tracer test with $^{15}N[NO_3^-]$ (T2003 in Fig. 1) (Böhlke et al. 2009). Ambient nitrate concentration = 90 μM

were determined from the concurrent reach-scale tracer test for the sub-reach containing the chambers (Table 5).

A composite comparison of all the chamber incubation results from SC03 is shown in Fig. 8, which includes ambient plus added nitrate incubation results and the results of the acetylene block incubations. There were strong correlations between the rate of measured nitrate loss and nitrate concentration and between ^{15}N-labelled nitrogen gas production and nitrate concentration. Using the slope of the correlation lines and the average water depth for the SC03

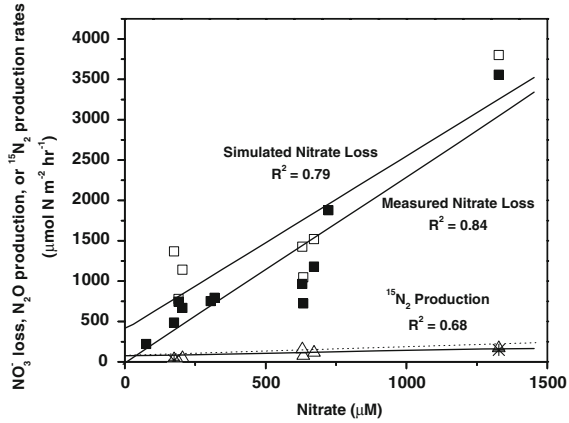

Fig. 8 Relation of rate of measured nitrate loss (*squares*), N_2O produced in the presence of acetylene (*cross*), or $^{15}N[N_2]$ produced (*triangles*) with nitrate concentration during the chamber incubation experiments at Sugar Creek site SC03. Data include incubations conducted from September 2001, June 2003, and September 2003. *Solid symbols* are rates measured from linear regression of concentration changes during incubation time course. *Open symbols* are rates that include isotope dilution from best-fit simulations of experiments conducted with ^{15}N. *Dashed line* shows upper limit effect on regression line for $^{15}N[N_2]$ when simulation includes transport of nitrogen gas into and out of chamber at rate of bromide concentration change

study site ($z = 0.38$ m), first order, in-stream rate constants for measured nitrate loss, simulated nitrate loss from the ^{15}N incubations, and ^{15}N-labelled nitrogen gas production (as N) were calculated to be 0.14, 0.15 and 0.007 day^{-1}, respectively. Including additional gain and loss factors for potential nitrogen gas transport into and out of the chamber, based on the bromide dilution rate, had a negligible effect on the simulated rate of $^{15}N[N_2]$ production (Fig. 8).

Incubations were also conducted using ^{15}N-enriched nitrite (Fig. 9) or ammonium (Fig. 10) to determine in situ nitrification potential. The chamber concentration for each of these solutes was higher than ambient in-stream concentration, which was <5 μM. Tracer ^{15}N appeared in both nitrate and nitrogen gas when added as either ammonium or nitrite. Production rates for both products were higher for the ^{15}N-enriched nitrite incubation. Nitrate and nitrogen gas production rates calculated from the best-fit simulations (0–21 h) were 87 and 49 μmol N m^{-2} h^{-1} from NO_2^- and 0.7 and 0.6 μmol N m^{-2} h^{-1} from NH_4^+, respectively. Only a small fraction (0.4%) of the simulated ammonium concentration

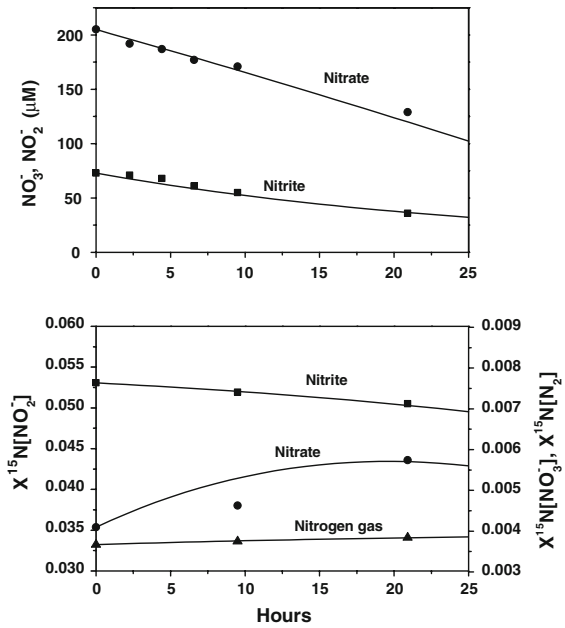

Fig. 9 Change in nitrate and nitrite concentration and recovery of tracer ^{15}N in nitrate, nitrite, and nitrogen gas during a chamber incubation experiment with added $^{15}N[NO_2^-]$ in Sugar Creek during September 2003. Final nitrite concentration was above ambient stream concentration. *Solid lines* are simulations using the t_0 measured values and the reaction model rate parameters listed in Table 2

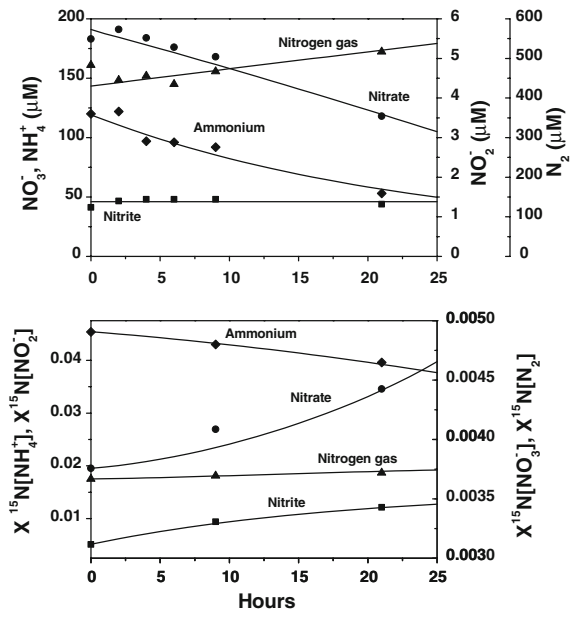

Fig. 10 Change in concentration and recovery of tracer ^{15}N in nitrate, nitrite, ammonium, and nitrogen gas during a chamber incubation experiment with added $^{15}N[NH_4^+]$ in Sugar Creek during September 2003. Final ammonium concentration was above ambient stream concentration. *Solid lines* are simulations using the t_0 measured values and the reaction model rate parameters listed in Table 2

decrease could be attributed to oxidization through nitrite, while 28% of the nitrite concentration decrease resulted in nitrate + nitrogen gas production.

Discussion

Increasingly, quantification of nitrogen-cycling processes in stream channels has taken an in situ approach. This has primarily involved addition of tracers into an open system coupled with downgradient sampling and analysis of conservative and reactive constituents (e.g., Tank et al. 2000; Wollheim et al. 2001; Peterson et al. 2001; Mulholland et al. 2004, 2008; Böhlke et al. 2004; Vidon and Hill 2004; Schade et al. 2005). This type of approach is important because it enables assessment of biogeochemical processes within the context of the physical, chemical, and biological factors that control and govern the function of the respective processes within the environment. However, a major limitation of working in an open system is the difficulty involved with performing experimental manipulations and isolating controlling variables. It is equally important to be able to quantify process-level responses to environmental perturbations and to do so with an in situ perspective. In-stream incubation chambers provide such an opportunity. A parcel of water can be captured, manipulated or altered, and the response in both the sediment bed and the water column assessed on short- to medium-length time scales. The results of such tests can be particularly informative if combined with in-stream, open channel tracer tests.

This study represents such a combined approach. In-stream incubations were conducted in large-volume chambers that provided sufficient volume to collect up to 1 L samples with time for isotopic and dissolved ionic and gaseous constituent analyses. The chambers provided the opportunity to alter the ambient nitrate or ammonium concentrations and add ^{15}N-enriched nitrate, nitrite, or ammonium, and they provided a sufficiently large footprint for examining the effect of the tracers on sediment porewater, examine patch scale variability, and simultaneously quantify fluxes of bulk and trace nitrogen species.

Nitrate flux and denitrification

All chamber incubations conducted in Sugar Creek resulted in a quantifiable decrease in nitrate concentration inside the dome. Even "water-only" incubations in which the sediment was covered with a plastic sheet resulted in a substantial nitrate decrease in 24 h (1.5% h^{-1}, data not shown). There was clearly a large potential for nitrate consumption in both the sediments and the water column of Sugar Creek. For example, when challenged with more than 1,300 μM nitrate, the measured nitrate concentration decrease was 3,560 μmol N m^{-2} h^{-1} (Fig. 8). This net decrease includes both assimilatory and dissimilatory nitrate-consuming processes. In addition, it could also include nitrate production via nitrification and influx of nitrate from outside the chamber. Because denitrification is the major permanent removal mechanism for fixed nitrogen from the system, it is particularly important to distinguish that process from any others that might contribute to nitrate flux. In a few cases when nitrate was added above ambient concentrations, an increase in the measured nitrogen gas concentration was roughly equivalent to the apparent nitrate loss (Table 1), whereas in most cases nitrate loss rates were larger than increases in nitrogen gas concentration. Collectively, these concentration changes provide broad limits for rates of denitrification, but the wide range that they represent is largely unsatisfactory for characterizing or simulating nitrogen cycling dynamics, particularly for incubations conducted at ambient nitrate concentrations. Two assay methods that were used in conjunction with the chamber incubations yielded improved estimates of denitrification rates. These were the acetylene block method and the use of ^{15}N-enriched nitrate.

Nitrous oxide production in the presence of acetylene has been used in a variety of environments to assess denitrification under in situ conditions (Knowles 1990). In some cases the approach was effective (Mosier et al. 1986; Joye et al. 1996; Holmes et al. 1996; Bernot et al. 2003), in others it was not (Kemp et al. 1990; Knowles 1990; Arah et al. 1993; Seitzinger et al. 1993; Bollmann and Conrad 1997; Watts and Seitzinger 2000; Smith et al. 2004). In Sugar Creek, at the ambient nitrate concentration (175 μM) acetylene completely stopped ^{15}N-labelled N_2 production and the rate of nitrous oxide production after the acetylene addition was slightly lower than the pre-acetylene ^{15}N-labelled nitrogen gas production rate (Table 3). Because acetylene also blocks nitrate production via nitrification (Knowles 1990), the latter result indicates that coupled nitrification-denitrification was not a major source of the Sugar Creek N_2 production during the incubation. However, at the high nitrate concentration (1,330 μM), the acetylene block was not completely effective (Fig. 6). Incomplete acetylene blockage of nitrous oxide reduction has been found when nitrate concentrations are relatively low or sulfide is present (Oremland et al. 1984; Knowles 1990). Yet neither situation was relevant for this incubation. It is not clear why the block was only partially effective, but the result suggests that if it is related to the nitrate concentration, then incubations with acetylene may not be appropriate for the full range of nitrate concentrations found in Sugar Creek or in similar streams. The total rate of N_2O + ^{15}N-labelled N_2 production during the high nitrate incubation in the presence of acetylene (232.7 μmol N m^{-2} h^{-1}) was comparable to the total prior to the acetylene addition (172.5 μmol N m^{-2} h^{-1}). For perspective, we note that the difference between the acetylene and $^{15}N[NO_3^-]$ incubation results in Sugar Creek were small in contrast to a large difference between the 2 methods (>20-fold) found using natural gradient tracer tests in a sand and gravel aquifer (Smith et al. 1996, 2004).

Although considerably more involved analytically, the use of ^{15}N-enriched nitrate has some distinct advantages over using the acetylene block method to assess nitrate consumption. These include minimal or no impact on nitrification (or any other process affected by acetylene), maintaining the appropriate electron stoichiometry (per nitrate-N reduced to N_2-N), and the ability to simultaneously assess dissimilatory nitrate reduction to ammonium. Perhaps the greatest advantage is the ability to quantify "new" nitrate gain via isotope dilution. This provides the capacity to determine the actual turnover of the nitrate pool. A similar situation occurs during the in-stream tracer tests (Böhlke et al. 2004). The "new" nitrate may be the result of either in-place nitrification or physical nitrate flux (by advection or diffusion) into the chamber. The differences in reaction rates computed by the different methods can be important. For example, in one chamber incubation

the rates of measured nitrate loss, bromide-corrected nitrate loss assuming dilution with nitrate-free water, and the simulated nitrate loss (which simultaneously fits the nitrate concentration, the nitrate ^{15}N mole fraction and the production of ^{15}N-labelled N_2) were 486, −365, and 1,368 μmol N m^{-2} h^{-1}. The differences are likely due to an influx of nitrate-containing water into the chamber during the course of the incubation, which can only be quantified by tracking the isotopic mole fraction of the ^{15}N tracer.

The ^{15}N incubations indicated that nitrate turnover in Sugar Creek was an active process. The estimated turnover time for the base flow nitrate pool at SC03 for June-September using the simulated ^{15}N incubations was 6.6 days (6.9 days when based on measured nitrate loss; calculated as reciprocal of the first order rate constants derived from Fig. 8). Even though denitrification rates were high, only a relatively small portion of the nitrate loss (~2%) was due to nitrate reduction to nitrogen gas (Fig. 8). The substantial decrease in nitrate concentrations during the course of the incubations (Figs. 4, 5, 6, 8) indicated that nitrate was being actively consumed; nitrate turnover was not simply movement of stream water into and out of the incubation chamber. Indeed, the 24-h nitrate concentration decrease in the closed-bottom "water-only" chamber was 63% of the nitrate concentration decrease in open-bottom chambers. Assimilatory nitrate uptake associated with primary and secondary productivity was clearly a major contribution to the apparent total nitrate loss. While assimilatory uptake may only represent a short-term DIN loss to the stream system, this rapid rate of uptake implies that a nearly equal and rapid rate of nitrate "return" must be functioning to keep the stream water nitrate concentrations from significantly decreasing or even disappearing with downstream transport. Thus, discerning the relative contributions of ground water input and in-stream mineralization of organic N to this nitrate "return" mechanism is an important next step, but difficult to do on the basis of $^{15}N[NO_3^-]$ tracer tests in gaining reaches like the Sugar Creek tracer study sites (Böhlke et al. 2004).

Evidence for nitrification

Nitrate was the predominant inorganic nitrogen species in Sugar Creek. Ammonium concentrations did not exceed 15 μM during a 2-year biweekly sampling period (Antweiler et al. 2005b) and generally were <5 μM during the chamber experiments. In contrast, porewater ammonium concentrations exceeded nitrate concentrations below about 5 cm beneath the sediment-water interface for many of the chamber deployment experiments at SC03 (Fig. 2). The total (sorbed + dissolved) ammonium pool represents a potentially large source of DIN that could be entering the stream as nitrate via nitrification, if it were occurring in the oxic surface sediments. Coupled nitrification/denitrification has been shown to be a major source of N_2 produced in some aquatic environments, particularly when dissolved nitrate concentrations are low (Jenkins and Kemp 1984; Seitzinger 1988; Kemp et al. 1990). As noted above, direct comparison of denitrification rates based on $^{15}N[N_2]$ production in the presence of acetylene in the incubation chambers (Fig. 6; Table 3) provided evidence that nitrification was not a major short-term N_2 source in Sugar Creek. Likewise, Smith et al. (2006a) concluded that coupled nitrification/denitrification was not the major source of N_2 production by comparing the results of two different analytical techniques (N_2/Ar measurements vs. ^{15}N-labelled N_2 production from $^{15}N[NO_3^-]$) during sediment core incubations to assess denitrification. These comparative approaches are capable of quantifying tightly coupled nitrification/denitrification, but they do not provide direct measurements of total nitrification independently of denitrification.

Ammonium consumption and nitrification were assessed independently by using $^{15}N[NH_4^+]$ and $^{15}N[NO_2^-]$ as tracers in the incubation chambers. The ammonium tracer results indicate that while nitrification was active in Sugar Creek, the rate of oxidation was relatively low compared to the overall decrease in ammonium concentration. Some of the apparent ammonium loss may have been due to sorption onto the shallow sediments, where background ammonium concentrations were relatively low. But it is also considered likely that some fraction of the ammonium loss was due to assimilation into biomass. For both the nitrite and ammonium incubations, $^{15}N[NO_3^-]$ production rate was higher than $^{15}N[N_2]$ production rate. This likely reflects greater contact of the tracer, which was added to the water column, with the oxic surface sediment layer as opposed to deeper anoxic, denitrifying sediment zones. Thus, the result does not necessarily indicate

that system-wide nitrification rates exceed denitrification rates, but rather that the most likely nitrite consumption mechanism in the top few centimeters of the sediments is nitrification. These results are consistent with a reach-scale isotope tracer experiment in Sugar Creek in which NO_2^- appeared to have a major source that was independent of denitrification (Böhlke et al. 2004). Interestingly, in the incubation to which ammonium was added, there was a substantial and simultaneous decrease in both the ambient nitrate and the added ammonium concentrations. Recent studies in marine and estuarine systems have demonstrated the importance of anaerobic ammonium oxidation coupled to nitrate/nitrite reduction, or anammox, in those environments (Dalsgaard et al. 2003; Trimmer et al. 2003, 2005; Arrigo 2005). While little is known about the potential for anammox in freshwater systems, the potential significance of that process in environments that might have mixing of oxic and anoxic waters (e.g., nitrate-containing stream water with ammonium-containing ground water) needs to be considered.

Rate comparisons

Denitrification rates in main channel sediments of Sugar Creek ranged from 34 to 212 μmol N m^{-2} h^{-1} for June and September based on simulations of $^{15}N[N_2]$ production in the chamber incubations. These rates were in the lower end of the range (50–3,936 μmol N m^{-2} h^{-1}) found by Smith et al. (2006a) for sediment cores collected from the same Sugar Creek location and incubated in the laboratory with re-circulated water. The highest rates reported by Smith et al. (2006a) were for cores containing rooted aquatic vegetation and related organic-rich surficial sediments, which were not present in the chambers during the current study. In the core incubations, diffusion is the main mechanism for solute movement across the sediment-water interface, whereas advection between the sediments and water column may be more prevalent within the in situ incubation chambers. The chamber denitrification rates agree reasonably well with those from in-stream, reach-scale tracer tests conducted in Sugar Creek with ^{15}N-enriched nitrate (213 vs. 154 μmol N m^{-2} h^{-1}; Böhlke et al. 2009; Table 5). The latter represents a reach-averaged rate and integrates the variety of sediment types and hyporheic flow situations found within the reach. Smith et al. (2006a) and Böhlke et al. (2004, 2009) have discussed Sugar Creek denitrification rates relative to those found in other systems. In general, the rates in Sugar Creek in June and September are high to very high and are presumed to reflect the high rates of primary production of reactive organic matter and large loads of nitrogen in this system.

In this study, denitrification in Sugar Creek was only weakly correlated with in-stream nitrate concentration but was higher in regions dominated by fine-grained material. In contrast, there was a strong correlation between the rate of nitrate loss and nitrate concentration (Fig. 8). This difference could reflect a limitation on the transport of nitrate to the deeper anoxic sediments where denitrification was occurring. High nitrate removal rates by assimilatory processes in shallow oxic sediments and hyporheic flow into the incubation chambers would limit the actual nitrate concentration present in the denitrifying zone. The combination of these two effects would tend to mute or mask a direct link between stream water nitrate concentration and benthic denitrification. Indeed, when these two factors are substantially reduced or removed, such as in core incubations, there then is a positive correlation between water column nitrate concentrations and denitrification (Smith et al. 2006a), though even in that situation the relation was still highly variable. This suggests that extrapolation of benthic denitrification rates based on changes in stream water nitrate concentrations and laboratory dose/response relations should be done with caution; changes in the in situ pathways or processes responsible for nitrate delivery to the denitrification zone could be equally important and perhaps even dominate the in situ response.

The nitrification rate indicated by the ammonium chamber experiment (3.5 μmol N m^{-2} h^{-1} of ammonium oxidized to nitrite) was much lower than typical denitrification rates measured in Sugar Creek. As these incubations were conducted with added ammonium, it is likely that nitrification rates at ambient ammonium concentrations would be even lower. In comparison, nitrification rates measured in other studies using both in-stream and microcosm incubations typically range from 0 to 300 μmol N m^{-2} h^{-1}. Most measurements using sediment incubations or from nitrogen-impacted streams fall in the range of 50–150 μmol N m^{-2} h^{-1} (Sheibley et al. 2003;

Starry et al. 2005; Duff et al. 2008), while rates in nutrient-limited, temperate headwater streams using reach-scale tracer experiments with $^{15}N[NH_4^+]$ are much lower at 0–5 μmol N m^{-2} h^{-1} (Tank et al. 2000; Peterson et al. 2001). In this study, when ammonium was added as a tracer, rates of nitrite oxidation to nitrate approximately equaled simultaneous rates of nitrite reduction to nitrogen gas. Thus, about 18% of the ammonium oxidation resulted in tightly coupled nitrification/denitrification (or possibly anammox), 20% resulted in nitrate production, and the remainder was consumed via other nitrite loss processes. It is important to note that the ammonium oxidation rate was substantially smaller than the simulated rates of nitrate addition from the $^{15}N[NO_3^-]$ incubation experiments (Table 2). When nitrite was added, all nitrite consumption processes were stimulated, but oxidation of nitrite to nitrate was stimulated to a greater extent than the others. Based on simulations that account for ammonium concentration decrease and isotope dilution, the net rate of ammonium loss in Sugar Creek was considerably higher (>250 times) than the rate of ammonium oxidation. Although some portion of the loss may have been due to sorption onto sediments, it does appear that in the short-term, ammonium assimilation is considerably greater areally than ammonium oxidation. Similar results have been reported for a variety of headwater streams using in-stream $^{15}N[NH_4^+]$ injection tests (Tank et al. 2000; Peterson et al. 2001). At the same time, dilution of the ammonium isotope tracer indicates that ammonium input (from ground water or sediment-water exchange) and (or) production were also important processes. The low ammonium concentrations in the sediment porewater suggest that production via mineralization in the sediments was the more significant contributing factor. The calculated ammonium addition rate was 114 μmol N m^{-2} h^{-1} (Table 2), or 12% of the loss rate and >30 times the oxidation rate for the added ammonium. If the tracer additions did not have a short-term effect upon ammonium production, then this addition rate would approximate the in situ rate of ammonium turnover within the surface sediments under the ambient concentrations present at the time of the incubation.

Chambers have been used many times to assess benthic metabolism and sediment-water fluxes; most commonly in lentic environments. In flowing or lotic waters, benthic oxygen respiration is often assessed in closed chambers with recirculation systems (Bott et al. 1978, 1997; Fellows et al. 2001). Another approach used in situ open-ended, flow-through chambers to include hyporheic contributions to whole-stream oxygen respiration (Uzarski et al. 2001, 2004). However, in situ chambers have rarely been used to assess nitrogen-cycling dynamics in streams and rivers. In general, it appears that the in-stream incubation chambers, with their relatively large sediment surface area (compared to cores) and ability to hold a parcel of water in place under in situ physical conditions are a smaller scale, cost-effective alternative to reach-scale tests. The chambers remove some types of uncertainty associated with reach-scale tests, such as the need to quantify gas-transfer velocities across the air–water interface (Böhlke et al. 2004). In this study many of the nitrogen cycling features assessed using the chamber incubations reflected what has been found with in-stream tracer and other studies. For example, the relative ratio of denitrification to nitrate uptake falls within the range reported from in-stream tracer tests conducted in a broad-based study (LINX II) encompassing 72 streams from a diverse range of biomes and land use types (Mulholland et al. 2008). Though the correlation in the latter was highly variable, the median nitrate removal via denitrification was 16%. The benthic denitrification rates assessed within the chambers fit well within the LINX II range of rates and within the range of rates assessed within Sugar Creek using a variety of assessment methods (Böhlke et al. 2009; Mulholland et al. 2008). Likewise, as noted above, the rate of nitrification matches relatively well with previously reported nitrification rates measured with in-stream tracers. However, the chambers are not without limitations. Though the design allows advection and diffusion across the sediment-water interface, total water movement could be either restricted or enhanced in areas of active hyporheic flow. Thus, rates could be affected by alteration of the residence time of nitrate, oxygen, or other species within the sediments.

In summary, in-stream incubations in large volume chambers were used to determine rates of nitrification and denitrification in a nitrate-rich stream that has been heavily impacted by agricultural drainage. The incubation chambers provided a patch- or intermediate-scale response to experimental alteration of

stream-water chemistry and provide a bridge between whole-stream tracer tests and laboratory incubations conducted with cores or sediment grab samples. The incubation results indicated high rates of denitrification and generally much lower rates of nitrification. Yet the down-stream inorganic nitrogen loads (primarily nitrate) did not decrease systematically (Antweiler et al. 2004). Assuming that nitrogen burial rates are small relative to these large loads, this implies that ground water inputs of nitrogen are needed to compensate for losses due to denitrification; a conclusion similar to one reached by Böhlke et al. (2004) based on in-stream tracer tests.

Even though denitrification rates in Sugar Creek were high, it is important to emphasize that the rates of net nitrate and ammonium consumption were even higher, up to several-fold higher. The relatively small changes in down-stream nitrogen loads do not mean that nitrogen was being transported conservatively. Clearly, biogeochemical nitrogen cycling was occurring rapidly within the stream channel relative to downstream transport. In particular, the dynamics of the organic nitrogen pool appear to be significant. The chamber incubations demonstrated simultaneous inorganic nitrogen consumption and addition, not all of which could be attributed to ground water inputs. Losses of inorganic nitrogen due to assimilation into organic forms must have been at least partially offset by concurrent organic nitrogen mineralization. The turnover and flux of organic nitrogen in Sugar Creek and other nitrate-impacted stream channels, the relation to productivity and other carbon cycling processes, as well as the role of hydrologic dynamics are topics that need further study. A clear understanding of all aspects and linkages of nitrogen-cycling processes in watersheds draining agricultural regions is needed to determine the role that these systems have in controlling nitrogen flux to sensitive coastal environments.

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References

Alexander RB, Smith RA, Schwarz GE, Boyer EW, Nolan JV, Brakebill JW (2008) Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River basin. Environ Sci Technol 42:822–830

Antweiler RC, Smith RL, Voytek MA, Böhlke JK, Richards KD (2004) Water-quality data from two agricultural drainage basins in northwestern Indiana and northeastern Illinois: I. Lagrangian data, 1999–2002. U S Geol Survey Open-File Report 2004-1317, Boulder

Antweiler RC, Smith RL, Voytek MA, Böhlke JK (2005a) Water-quality data from two agricultural drainage basins in northwestern Indiana and northeastern Illinois: II. Diel data, 1999–2001. U S Geol Survey Open-File Report 2005-1053, Boulder

Antweiler RC, Smith RL, Voytek MA, Böhlke JK, Dupre DH (2005b) Water-quality data from two agricultural drainage basins in northwestern Indiana and northeastern Illinois: III. Biweekly data, 2000–2002. U S Geol Survey Open-File Report 2005-1197, Boulder

Arah JRM, Crichton IJ, Smith KA (1993) Denitrification measured directly using a single-inlet mass spectrometer and by acetylene inhibition. Soil Biol Biochem 25:233–238

Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437:349–355

Bartkow ME, Udy JW (2004) Quantifying potential nitrogen removal by denitrification in stream sediments at a regional scale. Mar Freshw Res 55:309–315

Bernot MJ, Dodds WK, Gardner WS, McCarthy MJ, Sobolev D, Tank JL (2003) Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. Appl Environ Microbiol 69:5950–5956

Böhlke JK, Harvey JW, Voytek MA (2004) Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, mid-continent United States. Limnol Oceanogr 49:821–838

Böhlke JK, Smith RL, Miller DN (2006) Ammonium transport and reaction in contaminated groundwater: application of isotope tracers and isotope fractionation studies. Water Resour Res 42:W05411. doi:10.1029/2005WR004349

Böhlke JK, Smith RL, Hannon JE (2007) Isotopic analysis of N and O in nitrite and nitrate by sequential selective bacterial reduction to N_2O. Anal Chem 79:5888–5895

Böhlke JK, Antweiler RC, Harvey JW, Laursen AE, Smith LK, Smith RL, Voytek MA (2009) Multiscale measurements and modeling of denitrification in streams with varying flow and nitrate concentrations in the upper Mississippi River basin, USA. Biogeochemistry 93:117–141

Bollmann A, Conrad R (1997) Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. Soil Biol Biochem 29:1067–1077

Bott TL, Brock JT, Cushing CE, Gregory SV, King D, Petersen RC (1978) A comparison of methods for measuring primary productivity and community respiration in streams. Hydrobiologia 60:3–12

Bott TL, Brock JT, Baattrup-Pedersen A, Chambers PA, Dodds WK, Himbeault KT, Lawrence JR, Planas D, Snyder E,

Wolfaardt GM (1997) An evaluation of techniques for measuring periphyton metabolism in chambers. Can J Fisheries Aquat Sci 54:715–725

Brooks MH, Smith RL, Macalady DL (1992) Inhibition of existing denitrification enzyme activity by chloramphenicol. Appl Environ Microbiol 58:1746–1753

Casciotti KL, Sigman DM, Hastings GM, Böhlke JK, Hilkert A (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74:4905–4912

Coplen TB, Böhlke JK, Casciotti KL (2004) Using dual bacterial denitrification to improve $\delta^{15}N$ determinations of nitrates containing mass independent ^{17}O. Rapid Commun Mass Spectrom 18:245–250

Dalsgaard T, Canfield DE, Petersen J, Thamdrup B, Acuña-González J (2003) N_2 production by the anammox reaction in the anoxic water column of the Golf Dulce, Costa Rica. Nature 422:606–608

Duff JH, Triska FJ (2000) Nitrogen biogeochemistry and surface-subsurface exchange in streams. In: Jones JB, Mulholland PJ (eds) Streams and ground waters. Academic Press, New York

Duff JH, Murphy F, Fuller CC, Triska FJ, Harvey JW, Jackman AP (1998) A mini drivepoint sampler for measuring pore water solute concentrations in the hyporheic zone of sand-bottom streams. Limnol Oceanogr 43:1378–1383

Duff JH, Tesoriero AJ, Richardson WB, Strauss EA, Munn MD (2008) Whole-stream response to nitrate loading in three streams draining agricultural landscapes. J Environ Qual 37:1133–1144

Fellows CS, Valett HM, Dahm CN (2001) Whole-stream metabolism in two montane streams: contribution of the hyporheic zone. Limnol Oceanogr 46:523–531

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present and future. Biogeochemistry 70:153–226

Goolsby DA, Battaglin WA, Aulenbach BT, Hooper RP (2001) Nitrogen input to the Gulf of Mexico. J Environ Qual 30:329–336

Grimm NB, Sheibley RW, Crenshaw CL, Dahm CN, Roach WJ, Zeglin LH (2005) N retention and transformation in urban streams. J North Am Benthol Soc 24:626–642

Groffman PM, Dorsey AM, Mayer PM (2005) N processing within geomorphic structures in urban streams. J North Am Benthol Soc 24:613–625

Holmes RM, Jones JB Jr, Fisher SG, Grimm NB (1996) Denitrification in a nitrogen-limited stream ecosystem. Biogeochemistry 33:125–146

Jenkins MC, Kemp WM (1984) The coupling of nitrification and denitrification in two estuarine sediments. Limnol Oceanogr 29:609–619

Joye SB, Smith SV, Hollibaugh JT, Paerl HW (1996) Estimating denitrification rates in estuarine sediments: a comparison of stoichiometric and acetylene based methods. Biogeochemistry 33:197–215

Kemp WM, Sampou P, Caffrey J, Mayer M, Henriksen K, Boynton WR (1990) Ammonium recycling versus denitrification in Chesapeake Bay sediments. Limnol Oceanogr 35:1545–1563

Knowles R (1982) Denitrification. Microbiol Rev 46:43–70

Knowles R (1990) Acetylene inhibition technique: development, advantages, and potential problems. In: Revsbech NP, Sorensen J (eds) Denitrification in soil and sediment. Plenum Press, New York

Laursen AE, Seitzinger S (2002) Measurement of denitrification in rivers: an integrated whole reach approach. Hydrobiologia 485:67–81

Mosier AR, Guenzi WD, Schweizer EE (1986) Field denitrification estimation by nitrogen-15 and acetylene inhibition techniques. Soil Sci Soc Am J 50:831–833

Mulholland PJ, Valett HM, Webster JR, Thomas SA, Cooper LW, Hamilton SK (2004) Stream denitrification and total nitrate uptake rates measured using a field ^{15}N tracer addition approach. Limnol Oceanogr 49:809–820

Mulholland PJ, Helton AM, Poole GC, Hall RO Jr, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Neiderlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–206

Oremland RS, Umberger C, Culbertson CW, Smith RL (1984) Denitrification in San Francisco Bay intertidal sediments. Appl Environ Microbiol 47:1106–1112

Peterson BJ, Wolheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD (2001) Control of nitrogen export from watersheds by headwater streams. Science 292: 86–90

Pribyl AL, McCutchan JH, Lewis WM Jr, Saunders JF (2005) Whole-system estimation of denitrification in a plains river: a comparison of two methods. Biogeochemistry 73: 439–455

Rabalais NN, Wiseman WJ Jr, Turner RE (1994) Comparison of continuous records of near-bottom dissolved oxygen from the hypoxia zone along the Louisiana coast. Estuaries 14:850–861

Scavia D, Rabalais NN, Turner RE, Justic D, Wiseman WJ Jr (2003) Predicting the response of Gulf of Mexico hypoxia to variations in Mississippi River nitrogen load. Limnol Oceanogr 48:951–956

Schade JD, Welter JR, Marti E, Grimm NB (2005) Hydrologic exchange and N uptake by riparian vegetation in an aridland stream. J North Am Benthol Soc 24:19–28

Schaller JL, Royer TV, David MB (2004) Denitrification associated with plants and sediments in an agricultural stream. J North Am Benthol Soc 23:667–676

Schlesinger WH, Reckhow KH, Bernhardt ES (2006) Global change: the nitrogen cycle in rivers. Water Resour Res 42:W03S06. doi:10.1029/2005WR004300

Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr 33:702–724

Seitzinger SP, Nielsen LP, Caffrey J, Christensen PB (1993) Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry 23: 147–167

Sheibley RW, Duff JH, Jackman AP, Triska FJ (2003) Inorganic nitrogen transformations in the bed of the Shingobee River, Minnesota: integrating hydrologic and biological processes using sediment perfusion cores. Limnol Oceanogr 48: 1129–1140

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73:4145–4153

Smith RL, Miller LG, Howes BL (1993) The geochemistry of methane in Lake Fryxell, an amictic, permanently ice-covered, antarctic lake. Biogeochemistry 21:95–115

Smith RL, Garabedian SP, Brooks MH (1996) Comparison of denitrification activity measurements in groundwater using cores and natural-gradient tracer tests. Environ Sci Technol 30:3448–3456

Smith RL, Böhlke JK, Garabedian SP, Revesz KM, Yoshinari T (2004) Assessing denitrification in groundwater using natural gradient tracer tests with ^{15}N: In situ measurement of a sequential multistep reaction. Water Resour Res 40: W07101. doi:10.1029/2003WR002019

Smith RL, Buckwalter SP, Repert DA (2005) Small-scale, hydrogen-oxidizing-denitrifying bioreactor for treatment of nitrate-contaminated drinking water. Water Res 39: 2014–2023

Smith LK, Voytek MA, Böhlke JK, Harvey JW (2006a) Denitrification in nitrate-rich streams: application of N_2:Ar and ^{15}N-tracer methods in intact cores. Ecol Appl 16:2191–2207

Smith RL, Baumgartner LK, Miller DN, Repert DA, Böhlke JK (2006b) Assessment of nitrification potential in ground water using short term, single-well injection experiments. Microb Ecol 51:22–35

Starry OS, Valett HM, Schreiber ME (2005) Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply. J North Am Benthol Soc 24:753–768

Tank JL, Meyer JL, Sanzone DM, Mulholland PJ, Webster JR, Peterson BJ, Wollheim WM, Leonard NE (2000) Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N-tracer addition. Limnol Oceanogr 45:1013–1029

Trimmer M, Nicholls JC, Deflandre B (2003) Anaerobic ammonium oxidation measured in sediments along the Thames Estuary, United Kingdom. Appl Environ Microbiol 69:6447–6454

Trimmer M, Nicholls JC, Morley N, Davies CA, Aldridge J (2005) Biphasic behavior of anammox regulated by nitrite and nitrate in an estuarine sediment. Appl Environ Microbiol 71:1923–1930

USGS (2008) Stream-flow and water quality data. U S geological survey, national water information system. http://waterdata.usgs.gov/nwis, Reston

Uzarski DG, Burton TM, Stricker CA (2001) A new chamber design for measuring community metabolism in a Michigan stream. Hydrobiologia 455:137–155

Uzarski DG, Stricker CA, Burton TM, King DK, Steinman AD (2004) The importance of hyporheic sediment respiration in several mid-order Michigan rivers: comparison between methods in estimates of lotic metabolism. Hydrobiologia 518:47–57

Vidon P, Hill AR (2004) Denitrification and patterns of electron donors and acceptors in eight riparian zones with contrasting hydrogeology. Biogeochemistry 71:259–283

Watts SH, Seitzinger SP (2000) Denitrification rates in organic and mineral soils from riparian sites: a comparison of N_2 flux and acetylene inhibition methods. Soil Biol Biochem 32:1383–1392

Wollheim WM, Peterson BJ, Deegan LA, Hobbie JE, Hooker B, Bowden WB, Edwardson KJ, Arscott DB, Hershey AE, Finlay J (2001) Influence of stream size on ammonium and suspended particulate nitrogen processing. Limnol Oceanogr 46:1–13