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# Nitrate removal in a first-order stream: reconciling laboratory and field measurements

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**Abstract.** A combination of laboratory and field experiments were carried out to evaluate nitrate($NO_3^-$) removal during stream transport in a first-order agricultural drainage stream. Intact stream sediment cores overlain with stream and $NO_3^-$-amended stream water indicated $NO_3^-$ losses averaging 93 – 353 mg m$^{-2}$ day$^{-1}$, with $NO_3^-$ concentration exerting a primary control on loss rate. Isotopic data indicated enrichment of $NO_3^-$ - $\delta^{15}$N over time as $NO_3^-$ concentrations decreased, indicating a denitrification loss. Field experiments were designed to evaluate dilution of streamwater with low-$NO_3^-$ groundwater in addition to other $NO_3^-$ removal processes during transport. A series of bromide tracer and $NO_3^-$ - addition experiments were carried out in the field; groundwater dilution dominated the downstream $NO_3^-$ concentration trends, accounting for all observed decreases in $NO_3^-$ concentration. Isotopic data did not point to denitrification downstream as a major $NO_3^-$ removal process. This apparent disparity between simulated laboratory and *in-situ* stream removal rates appears to be a function of the hydrological processes controlling exchanges between stream bottom sediments and the overlying water. These results suggest that caution must be exercised in extrapolating potentials for $NO_3^-$ removal measured in laboratory experiments to the field, as these rates could be overestimated in some watersheds.

## Introduction

Nitrogen dynamics in first-order streams draining agricultural watersheds play an important role in subsequent downstream nitrogen transport, particularly nitrogen in the form of nitrate ($NO_3^-$). These agricultural drainage streams are widespread, large in number and typically transport high concentrations of dissolved nitrogen in close contact with stream bottom sediments which provide potential zones of removal (Seitzinger et al. 2002). Understanding and quantifying the extent to which $NO_3^-$ removal processes such as denitrification occurs is important in increasing current understanding of N-cycling processes and N-contamination in surface waters, as well as production of greenhouse gases in aquatic environments.

Evaluation of the processes responsible for downstream changes in $NO_3^-$ concentrations is complicated by the complexity of the nitrogen cycle and hydrological interactions. These include differences in hydraulic gradients and sediment conductivities in the vicinity of the stream, spatial variations in stream bottom sediments and water-sediment exchanges (Harvey and Bencala 1993; Triaka et al. 1993), denitrification in stream sediments (Kaushik and Robinson 1976; Hill 1979; Hill and Sanmugades 1985; Christensen et al. 1990; Bradley et al. 1995; Burns 1998), vegetative uptake (Cooper and Cooke 1984; Cooper 1990) and other in-situ production and reduction processes such as nitrification (Triaka et al. 1990; Kemp and Dodds 2002) and dissimilatory $NO_3^-$ reduction to ammonium (Hill and Sanmugadas 1985).

Experiments have pointed to microbial denitrification in bottom sediments as a major process leading to $NO_3^-$ removal in streams (Robinson et al. 1979; Hill 1983; Christensen and Sorensen 1988; Christensen et al. 1989; Kemp and Dodds 2002). This process can occur within millimeters of the sediment–water interface where conditions can become rapidly anoxic, and where in the absence of oxygen, $NO_3^-$ is used as an electron acceptor in the oxidation of organic carbon (Knowles 1982). The denitrification process occurs in anoxic sediments and requires a readily oxidizable carbon source. The concentration of dissolved organic carbon (DOC) is often used as an indicator of this availability (Faafeng and Roseth 1993). In laboratory-based experiments, which have been instrumental in identifying and isolating the denitrification process, sediment cores or bottom sediment samples are overlain or mixed with water containing $NO_3^-$ which is sampled over time to measure $NO_3^-$ losses (Chatarpaul et al. 1980; Hill and Sanmugdas 1985; Bradley et al. 1995). The acetylene block technique prohibits reduction of $N_2O$ to $N_2$ during denitrification. Accumulated $N_2O$ is measured to determine the denitrification potential of sediments (Christensen and Sorensen 1988; Bradley et al. 1995). This technique has been particularly useful in identifying denitrification as the major $NO_3^-$ removal process in waters in contact with these sediments.

However, in field settings, other processes could prevent observed laboratory-determined rates of removal from being realized. Groundwater dilution or vegetative uptake might alter apparent $NO_3^-$ removal rates and either masquerade as, or contribute to, $NO_3^-$ removal during stream transport. Consequently, the concurrent application of several techniques is needed, or estimates of the rate of removal in these situations could be seriously flawed.

Conservative tracers provide one means of differentiating between mixing and transformation processes. If concentrations of a conservative (i.e., non-reactive) chemical species differ between two waters contributing to flow along a pathway, the relative proportions contributed can be calculated using a simple two-component mixing model (Kendall 1998). Typical conservative tracers include chloride ($Cl^-$) (Burns and Nguyen 2002) and bromide ($Br^-$) (Bragan et al. 1997; Hill et al. 1998; Burns and Hauyen 2002) which can have concentrations that vary naturally in the waters of interest. Alternatively, ionic salts such as potassium bromide (KBr) can be used to artificially elevate concentrations in one of the contributing waters. This approach has been widely used in various hydrological and hydrogeological studies (e.g., (Hill et al. 1998; Hendriks et al. 1999; Burns and Nguyen 2002; Devlin et al. 2002).

Natural variations in stable isotopes of N-species can also provide information about biogeochemical N-transformations. The isotopic signature of nitrogen is expressed in units of permil (parts per thousand) as,

$$\delta^{15}\mathrm{N}(0/00) = \left[\frac{{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{sample}}}{{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{standard}}} - 1\right] \cdot 1000, \tag{1}$$

where $^{14}N$ and $^{15}N$ are the two stable isotopes of N (abundance of $^{14}N >>> ^{15}N$) and the standard is atmospheric $N_2$. Spatial and temporal changes in the natural *in-situ* abundances of $^{15}N/^{14}N$ of $NO_3^-$ along a flowpath allows identification of isotopic shifts consistent with microbial denitrification (Kendall 1998). As microbial denitrification is a fractionating process that effectively selectively removes $^{14}NO_3^-$, the residual $NO_3^-$ becomes increasingly enriched in $^{15}NO_3^-$ (i.e., $\delta^{15}N$ values increase) as $NO_3^-$ concentrations decrease. $NO_3^-$ N stable isotopes have been used in recent studies of stream and tile-drain discharges in a poorly drained agricultural watershed (Kellman and Hillaire-Marcel 2003), where $\delta^{15}N$ enrichment coupled with reductions in $NO_3^-$ concentrations showed patterns consistent with denitrification. Although useful in identifying this process in surface water environments, these evaluations have been primarily qualitative.

The challenges faced in making quantitative evaluations of $NO_3^-$ removal during stream transport suggest that a combination of laboratory and field methods might best allow the relative importance of these processes to be resolved in the field. This paper presents the results of a study using a combination of laboratory and field experiments to evaluate growing season $NO_3^-$ removal during stream transport in a first-order agricultural watershed possessing well-drained sandy soils.

In order to differentiate between the relative importance of the processes potentially contributing to changes in $NO_3^-$ concentrations during stream transport, denitrification, dilution with low-$NO_3^-$ groundwater, and vegetative uptake, several laboratory and field-based experiments were carried out. Stream conditions were simulated in the laboratory where dilution from groundwater was not a factor using sediment cores to observe net $NO_3^-$ losses, and to test for $NO_3^-$-N isotopic patterns consistent with such losses. Several sets of core experiments were carried out that were designed to monitor changes in and responses to some variations in the levels of DOC, DO and $NO_3^-$ in waters overlying cores. Responses to variations in DOC were necessary to monitor trends that might indicate limited availability or losses of a readily oxidizable carbon source. Monitoring and manipulation of DO levels in water overlying sediment cores was necessary to determine whether the simulation of stream conditions in the lab could alter DO of overlying core water, thereby altering $NO_3^-$ removal rates in sediments. Tests involving responses to elevated $NO_3^-$ concentrations provided a clearer indication of isotopic responses, and determined whether $NO_3^-$ removal processes were sensitive to stream $NO_3^-$ levels. In the field, a series of *in-situ* stream experiments were conducted using the addition of $NO_3^-$ and a bromide tracer to measure groundwater dilution rates. Finally, $NO_3^-$-N isotopic analyses of streamwater in both core experiments and *in-situ* field experiments were conducted to aid in identifying denitrification losses.

## Methods

### *Field experiments*

This study was conducted at the Bio-Environmental Engineering Research Center of the Nova Scotia Agricultural College (NSAC) near Truro, Nova Scotia, located in Atlantic Canada (45°22′N; 63°16′W). This is an experimental agricultural field station where relationships between water quality and cropping patterns and fertilizer applications are routinely conducted. The study was conducted in a first-order stream in tile drained fields which, during the growing season, were fed only by seepage from the fields and associated tile drains located above and below the segment of the stream studied. There was no evidence of flow from a roadside drainage ditch located several hundred meters further upstream. The stream level was approximately 1 m below the field soil surface level, with only a small grass buffer strip of approximately 1–2 m width on either side. The site is located 30 m above sea level on a coarse sandy loam unconsolidated material derived from Triassic sandstones. The soils are a well-drained sandy loam soil. Field plots were fertilized with inorganic NPK (nitrogen/phosphorus/potassium)at a rate of 70 kg/ha or liquid organic hog manure at a rate of 7 Tons/ha and cropped with wheat on one side and barley on the other.

In-stream water sampling was conducted at ten sampling sites located 20 m apart over a 180 m length of the stream. The stream sampling stations are labeled chronologically from site 1 (located at the upstream sampling point), to the own-stream site, site 10. A single tile drain that flowed until late-July was located between sites 1 and 2; tile drains outside of the stream segment of interest were not monitored. A v-notched weir was installed at site 10 to obtain manual stream discharge readings. The stream was less than 50 cm in width and averaged less than 5 cm in depth just upstream of the weir. A series of PVC drivepoint piezometers, which allowed chemical characterization of groundwater in the vicinity of the stream, were installed at 30 cm depth below the steam bottom at site 10, and at 30 and 90 cm depth at sites 7 and 3. Sediments underlying the stream were soft to a depth of approximately 25–35 cm; piezometer installation below this depth was made more difficult by the presence of a hardpan layer.

### *Stream addition experiments*

In order to examine groundwater dilution and downstream trends in $NO_3^-$ concentration and isotopic changes, a series of three stream addition trials were conducted over three weeks in August of 2000. A concentrated potassium bromide (KBr) and potassium nitrate ($KNO_3$) solution was added just upstream of site 2. Site 1 provided an initial signal of stream water unaltered by the addition. A peristaltic pump connected to a 12 V battery provided a constant flow of the solution into the stream over a period exceeding 3 h. Hydrological data suggested that during field trials, transport times between the addition site and the weir were on the order of 1.5 h, and that this allowed sufficient time for the added solution to achieve a steady state concentration in stream water. At the end of the addition, a set of downstream samples were collected.

### *Field sampling*

Stream water samples were collected midstream between May and September 2000. Downstream water samples were collected bi-weekly and groundwater samples approximately monthly. Stream addition experiments were conducted during 3 weeks in August, and laboratory experiments using stream sediment cores were carried out in August and September of the same year.

Hydrological data were collected by monitoring water levels behind the v-notched weir when stream samples were collected. Stream cross sectional measurements were made at each of the downstream sampling locations. These measurements facilitated measures of bottom sediment area, stream discharge, and quantification of groundwater dilution along the stream during addition experiments.

### *Water analysis*

During all field monitoring, temperature, dissolved oxygen (DO), conductivity and pH were tested in the field. All field samples collected for later analysis were stored on ice and transported to the laboratory where they were filtered and preserved for later analysis. Nitrate was analyzed on an Autoanalyzer using cadmium reduction, and DOC was analyzed on a Shimadzu TOC Analyzer. Samples were analyzed for bromide using ICP-MS at Memorial University in Newfoundland, and for $NO_3^-$ N isotopes at the Geotop Laboratory, University of Quebec in Montreal. Nitrate for isotopic analysis was collected on an anion exchange resin within 24 h of collection and refrigerated. The method used for extraction of $NO_3^-$-N for isotopic analysis from water samples was that of Silva et al. (2000). Samples were run in duplicate or triplicate on a continuous flow mass spectrometer.

### *Laboratory experiments*

Experiments conducted in the laboratory were designed to place stream bottom sediments in contact with stream water at both ambient and elevated $NO_3^-$ concentrations in order to measure $NO_3^-$ removal taking place when transport between stream water and underlying sediments was isolated, and diffusive transport of $NO_3^-$ into bottom sediments the dominant transport mechanism. Aeration of a subset of these experimental cores was conducted in order to introduce oxygen into and create turbulent conditions within the water column above the sediment, providing a contrasting set of conditions to non-aerated cores.

Intact bottom sediment cores approximately 10 cm in length were removed from the stream center using 5 cm in diameter clear plexi-glass tubes. The tubes were inserted into the sediment, capped and carefully withdrawn taking care to minimize disturbance of stream bottom sediment structure. This was facilitated by the soft nature of the sediment. Sediment cores were capped on the top and bottom, and transported in a cooler to the laboratory. Cores did not contain stream vegetation which tended to be present only at the sides of the stream. The cores and several liters of stream water taken from the weir were refrigerated until the start of the experiment. Laboratory experiments conducted using stream sediment cores were initiated within 24 h of removal from the field.

In the lab, stream sediment cores were uncapped on the top and stream water initially collected with the core was siphoned off, with care taken to minimize sediment losses. Streamwater collected in nalgene bottles in the field at the same time as the cores was poured onto the sediment cores in the laboratory experiment. Parafilm with small holes covered the tops of the tubes in order to minimize evaporative losses during the experiments, which could result in an underestimation of net $NO_3^-$ removal rates. Cores were kept at ambient room temperatures and exposed to indirect natural lighting.

Cores were overlain with water and this water sampled at 12–24 h intervals to monitor changes in $NO_3^-$ concentrations, DOC levels, DO concentrations and pH. Through the course of the laboratory experiments, a total of 16 cores were overlain with unaltered stream water (average concentration of approximately 2 mg/l N), and a total of nine cores were overlain with stream water amended with $NO_3^-$ to simulate responses to elevated stream $NO_3^-$ levels (average concentration of approximately 8 mg/l N). A subset of the cores in each set of experiments were either aerated using aquarium pumps to maintain DO levels as close to in-stream levels as possible or not aerated. Finally, a set of core experiments were designed to monitor isotopic shifts in stream water amended with $NO_3^-$ over time. To ensure sufficient $NO_3^-$ for isotopic analyses, elevated core water $NO_3^-$ levels were used, and one core sacrificed every 12 h (i.e., water overlying one of the cores was removed). This required the assumption that all cores behaved in an identical fashion in terms of the response to net $NO_3^-$ losses through the course of the experiment. Throughout the core experiments, differences in initial core water $NO_3^-$ concentrations in the various sets of core experiments were largely a function of differences in initial steam water $NO_3^-$ concentrations collected on different sampling dates.

## Results

### *Ambient field conditions*

Early in the growing season $NO_3^-$ concentrations in stream water varied little with distance from the tile drain. However, as the season progressed, increased concentrations were observed immediately downstream of the tile drain, and by the final sampling date, increased $NO_3^-$ concentrations were observed throughout the 180 m segment of the monitored stream (Figure 1). Nitrate concentrations discharged at the steam weir were relatively stable through the season, ranging from 1–2.5 mg/l N. Nitrate export from the steam weir ranged between 23 and 76 kg/day during the measurement period. Stream flow did not appear to be turbulent, but rather flowed along a soft smooth bottom sediment. Stream waters were well oxygenated, with oxygen levels consistently measured between 6 and 10 mg/l, showing no downstream trends. DOC did not indicate a limitation of available oxidizable organic carbon in stream waters, with concentrations ranging from approximately 5–25 mg/l over the measurement period. Shallow groundwater concentrations of $NO_3^-$ measured at 30 cm depth below the stream were variable but consistently lower than in the stream, averaging 0.55 mg/l. Background levels of bromide at the site estimated from several groundwater samples averaged 39 µg/l.

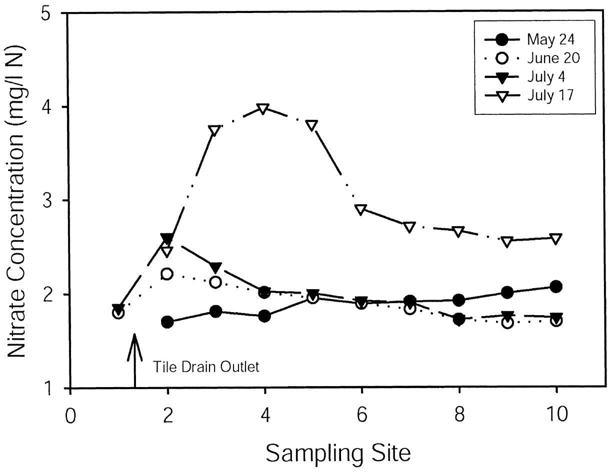

*Figure 1.* Nitrate concentrations of streamwater upgradient and downgradient of a tile drain outlet on four days during the 2000 growing season.

### *Sediment core experiments*

Temporal trends in $NO_3^-$ concentration and DO of water added to stream bottom sediment cores without $NO_3^-$ additions or aeration of overlying water are shown in Figure 2, while Figure 3 illustrates concentration and DO trends under aerated conditions. Aeration maintained core DO levels close to stream levels throughout the experiment (Figure 3(b)), while non-aerated samples showed a significant drop in DO, often to less than 2 mg/l, (Figure 2(b)) within the first 48 h. This is consistent with microbial oxygen consumption. Pumping of oxygen, while maintaining core oxygen levels similar to stream levels created greater turbulent mixing than observed in the streams, suspending some of the core sediment in the water column. In all core series, initial removal rates were high and decreased as core water $NO_3^-$ concentrations dropped; all cores, irrespective of core water oxygen levels showed similar patterns of net $NO_3^-$ losses with time. Nitrate removal rates, calculated from the first 24 h of data showed averaged losses of $94 \pm 18\,\mathrm{mg\,m^{-2}day^{-1}}$ (avg. ± SD) and $99 \pm 20\,\mathrm{mg\,m^{-2}day^{-1}}$ for these sediments under non-aerated and aerated conditions respectively. Figures 4 and 5 show the results of similar experiments, but with $NO_3^-$ concentrations elevated to approximately 8 mg/l N. Patterns of DO were similar to experiments without N additions, with high levels of DO maintained under aerated conditions (Figure 5(b)), and a large drop in DO under non-aerated conditions (Figure 4(b)). Nitrate concentration patterns were similar to cores without N-additions, although net $NO_3^-$ losses in the first 24 h were much greater, averaging $283 \pm 40\,\mathrm{mg\,m^{-2}day^{-1}}$ and $353 \pm 108\,\mathrm{mg\,m^{-2}day^{-1}}$ for the non-aerated and aerated cores respectively. The core sediment $NO_3^-$ removal rates are summarized in Figure 6. These removal rates are in the range of those found by Hill and Sanmugadas (1985) in core waters amended with 5 mg/l $NO_3^-$-N. Although in both

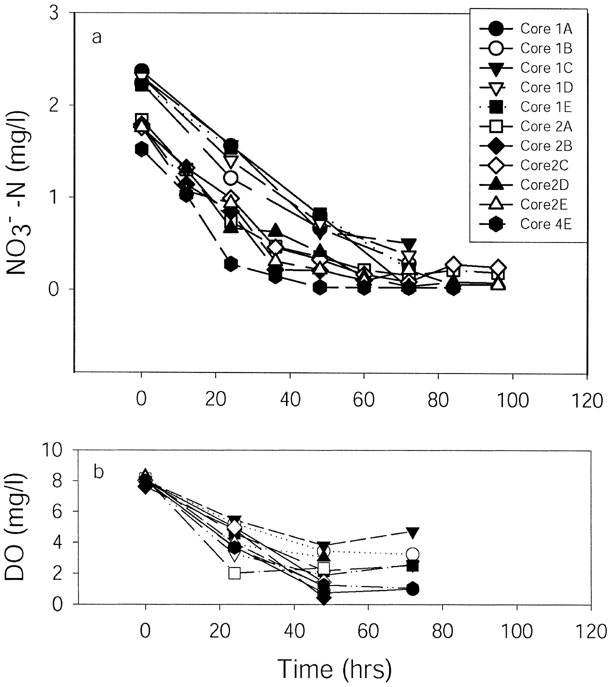

*Figure 2.* Laboratory core experiment results of temporal trends in $NO_3^-$ concentration (a) and DO (b) in non-aerated streamwater overlying intact stream sediment cores.

sets of experiments average $NO_3^-$ removal rates were greater when core water was aerated to maintain DO concentrations similar to stream levels, the errors suggest that the differences are not significant, and that as found in other studies, (Faafeng and Roseth 1993), core water DO level does not exert a major control on $NO_3^-$ removal rates. In contrast, $NO_3^-$ removal rates do appear to be sensitive to $NO_3^-$ concentrations, with much greater removal rates observed for high $NO_3^-$ core water. This has been observed in other studies (Christensen et al. 1990; Faafeng and Roseth 1993).

Figure 7 shows changes in $NO_3^-$ concentration and $NO_3^-$ isotopic signatures of water in the core experiments using the high N treatment. It demonstrates a clear inverse relationship between $NO_3^-$ concentration and $\delta^{15}N$ as $NO_3^-$ concentrations decrease over time (Figure 7(a)) and $NO_3^-$ in core waters shows a general pattern of $\delta^{15}N$ enrichment with time (Figure 7(b)). This is the pattern expected if denitrification were the primary process responsible for $NO_3^-$ removal from core waters.

### *Nitrate and bromide stream addition experiments*

Nitrate downstream concentration trends for the three addition experiments are illustrated in Figure 8. Initial stream $NO_3^-$ concentrations measured at site 1 were

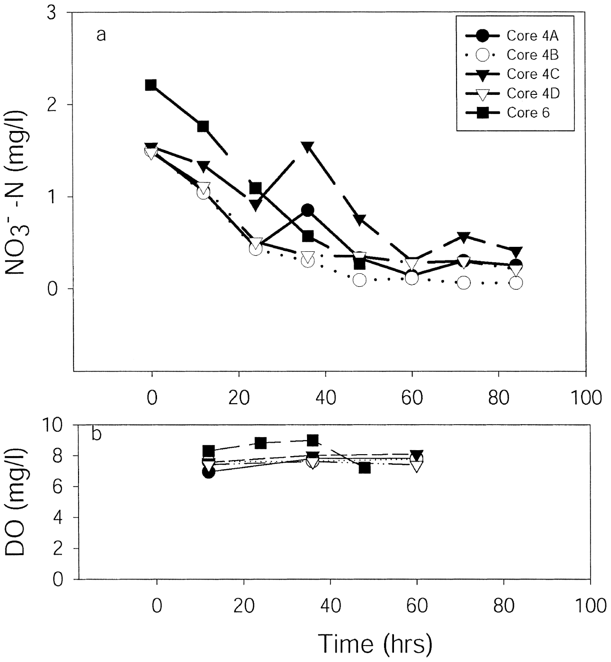

*Figure 3.* Laboratory core experiment results of temporal trends in $NO_3^-$ concentration (a) and DO (b) in aerated streamwater overlying intact stream sediment cores.

similar; measurements taken below the addition point were all elevated and declined rapidly downstream. Trials 1 and 2 showed consistent concentration drops downstream which initially appeared to be non-linear. Trial 3 also showed a concentration drop downstream, but an elevation in concentration at site 6. In all trials the dominant pattern was a rapid drop in stream $NO_3^-$ concentrations. Nitrate-N isotopic patterns, illustrated for the three trials in Figure 9 also showed no directional changes in $\delta^{15}N$ downstream from the point of addition. Added $NO_3^-$ was isotopically heavier than the streamwater $NO_3^-$, but no clear isotopic shifts occurred downstream. Delta-$^{15}N$ values for trials 1 and 2 were consistent throughout the stream length sampled but trial 3 showed both increases and decreases that are not easily interpreted. Overall trends show a large drop in $NO_3^-$ concentration downstream that might be a function of either removal or dilution, however the lack of isotopic enrichment downstream is not consistent with removal of a large pool of $NO_3^-$ due to denitrification.

Downstream concentrations of the bromide tracer are shown for the three trials in Figure 10. Initial stream concentrations were low, with an the upward shift to between 1000 and 2000 μg/l evident after the tracer addition point. In all three trials there was a consistent pattern of decreasing $Br^-$ concentration downstream. Sampling points above site 5 pointed to a non-linear drop in $Br^-$ concentrations

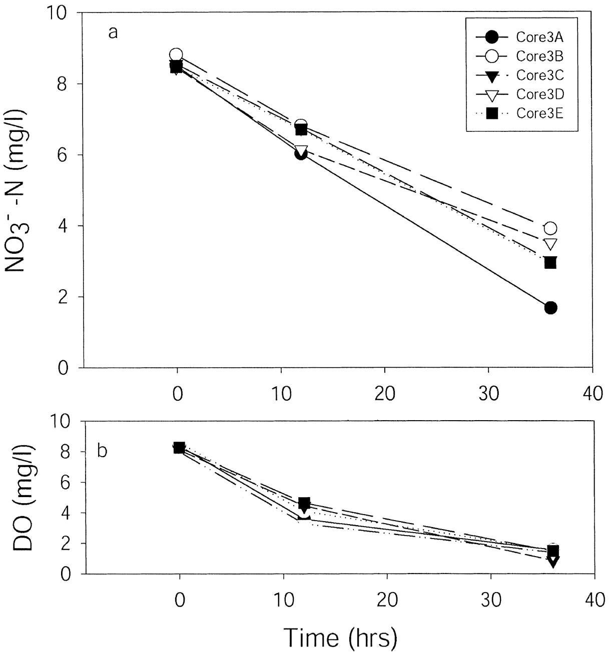

*Figure 4.* Laboratory core experiment results of temporal trends in $NO_3^-$ concentration (a) and DO (b) in non-aerated streamwater spiked with $NO_3^-$ overlying intact stream sediment cores.

that might have been due to insufficient mixing of the addition solution with surrounding stream water. In order to avoid errors associated with insufficient mixing of the solution with stream waters in the upper segment of the stream, groundwater dilution calculations were only made below site 5 where groundwater inputs appeared to be constant. The trial data were used to calculate total groundwater inputs along a stream segment between sites 6 and 10 using a simple two-component mixing model (Kendall and McDonnell 1998) with averaged groundwater $Br^-$ concentrations (of 39 μg/l) and streamwater $Br^-$ concentrations at site 6 used as endmembers. Using the groundwater dilution rates calculated from the bromide tracer data, $NO_3^-$ levels of water exiting at site 10 were estimated for a conservative transport of $NO_3^-$ between sites 6 and 10 (Table 1), and are compared to measured $NO_3^-$ concentrations at site 10. Groundwater inputs between sites 6 and 10 account for 22–35 percent of the water discharged at site 10. Combining this with the $NO_3^-$ concentrations, these data demonstrate that groundwater dilution can account for all decreases in $NO_3^-$ concentration along this stream segment. In fact, calculated $NO_3^-$ concentrations were slightly lower than measured $NO_3^-$ concentrations, suggesting that the estimates of groundwater $NO_3^-$ concentration used were low, and might need to be better resolved through more detailed groundwater sampling, or that $NO_3^-$ production via nitrification might be occurring.

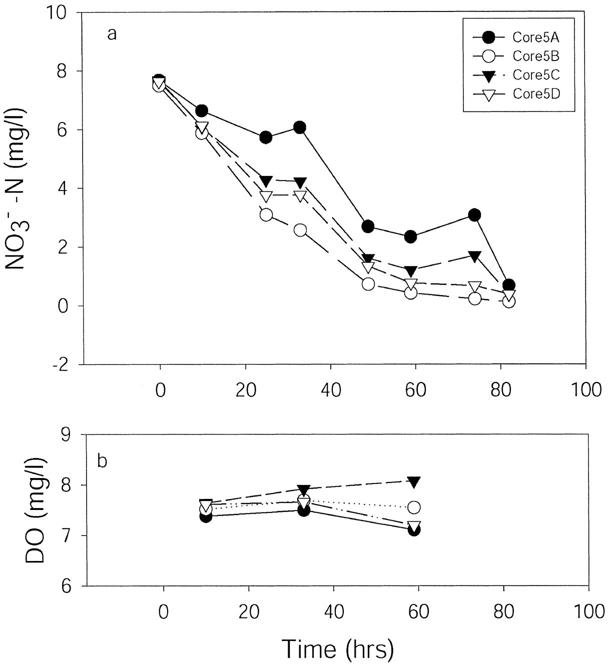

*Figure 5.* Laboratory core experiment results of temporal trends in $NO_3^-$ concentration (a) and DO (b) in aerated streamwater spiked with $NO_3^-$ overlying intact stream sediment cores.

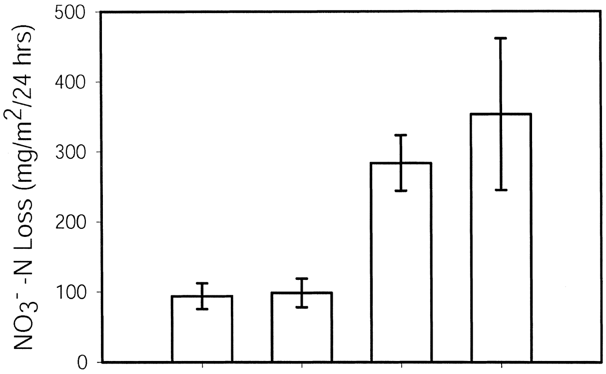

*Figure 6.* Twenty-four hour $NO_3^-$ removal rates (Avg. ± 1 SE) from water above cores, using streamwater (low N) and streamwater spiked with $NO_3^-$ (high N) aerated ($O_2$) and non-aerated (no $O_2$) core tests.

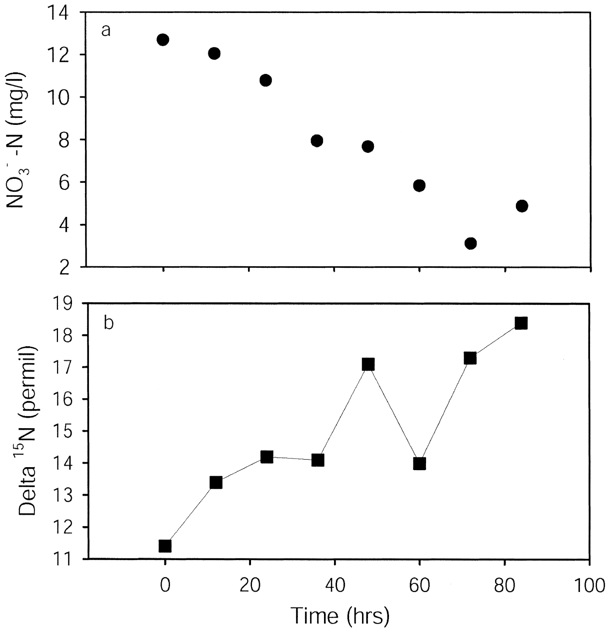

*Figure 7.* Laboratory core experiment results of temporal trends in $NO_3^-$ concentration (a) and $NO_3$-$\delta^{15}$N (b) in streamwaters spiked with $NO_3^-$. Each data point corresponds to water removed from a different core; all cores had the same initial conditions and were removed from the same area in the stream.

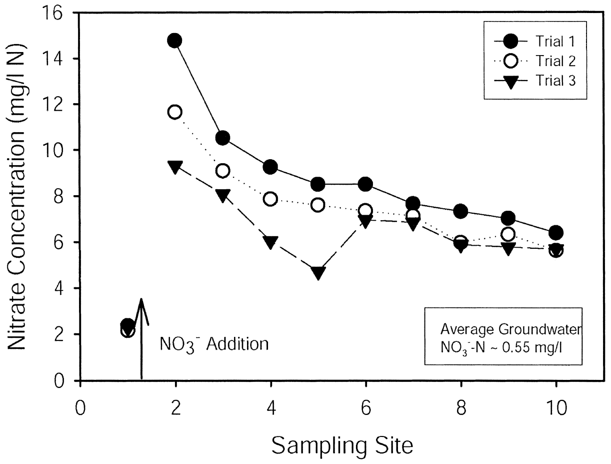

*Figure 8.* Nitrate concentrations in stream water between sampling sites 1 and 10 following $NO_3^-$ solution additions between sites 1 and 2 during three trials in August 2000.

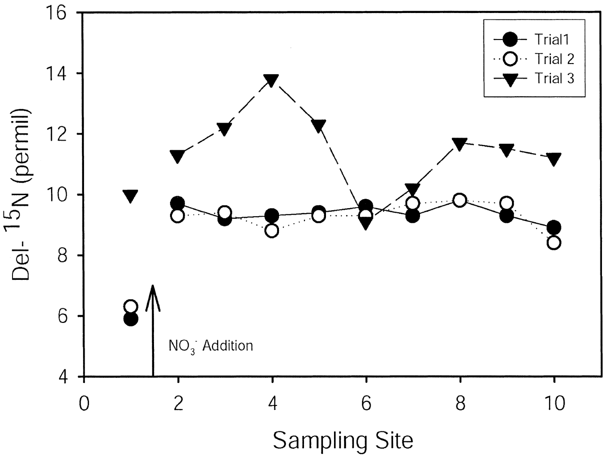

*Figure 9.* Nitrate $\delta^{15}$N trends in stream water between sampling sites 1 and 10 following $NO_3^-$ solution additions between sites 1 and 2 during three trials in August 2000.

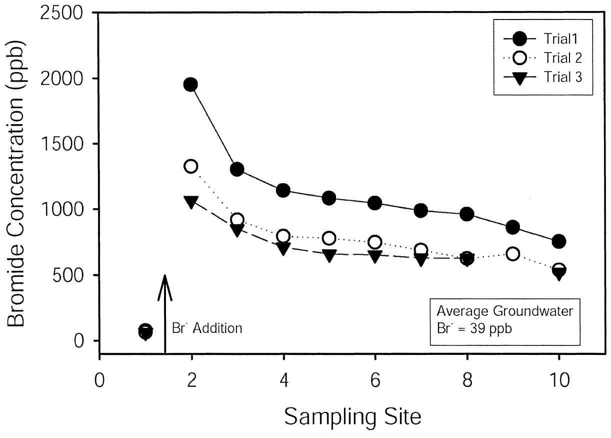

*Figure 10.* Concentrations the conservative tracer bromide in stream water between sampling sites 1 and 10 following bromide solution additions between sites 1 and 2 during three trials in August 2000.

*Table 1.* Calculated and measured ground water (GW), stream water (SW) $NO_3^-$ inputs between stream sampling sites 6 and 10 based on $Br^-$ tracer experiments.

| Trial | $Br^-$ Tracer (% GW) | $Br^-$ Tracer (% SW) | Measured $NO_3^-$ site 6 (mg/l N) | Calculated $NO_3^-$ site 10 (mg/l N) | Measured $NO_3^-$ site 10 (mg/l N) |
|---|---|---|---|---|---|
| 1 | 35 | 65 | 8.50 | 5.72 | 6.39 |
| 2 | 34 | 66 | 7.35 | 5.04 | 5.63 |
| 3 | 22 | 78 | 6.95 | 5.54 | 5.69 |

## Discussion

### *Potential of stream sediment to remove nitrate*

Laboratory experiments performed using intact sediment cores to simulate stream water–sediment interactions were consistent with findings of other studies. Net $NO_3^-$ removal occurs when stream water and stream water amended with $NO_3^-$ are in contact with stream sediments. One can infer from the removal rates and $\delta^{15}N$ enrichment over time that potential for $NO_3^-$ removal via denitrification is high in these sediments. This is consistent with the findings of many other studies (Chatarpaul et al. 1980; Faafeng and Roseth 1993; Bradley et al. 1995). The removal rates measured in this study (93 – 353 mg m$^{-2}$day$^{-1}$) are in the range of those found by Hill and Sanmugadas [1985] who amended stream sediments of various textures with 5 mg/l $NO_3^-$-N and measured removal rates of 37–412 mg m$^{-2}$day$^{-1}$, most of which was attributed to denitrification rather than other removal processes. Findings from a field study in a stream characterized by high $NO_3^-$ loading by van Kessell [1977] estimated removal rates on the order of 913 mg m$^{-2}$day$^{-1}$, but did not measure groundwater dilution.

The removal rates in the sediment core laboratory experiments observed in this study are sensitive to $NO_3^-$ concentrations in the contact water, with up to triple the removal found when initial core water $NO_3^-$-N concentrations were amended to bring them from 1–2 mg/l to approximately 8 mg/l. The removal rates observed in the laboratory experiments appear to be relatively insensitive to core water DO levels; since denitrification tends to occur primarily in sediments rather than the water column (Knowles 1982), this is not surprising. Sensitivities to temperature and light have been shown to affect removal rates in other studies (Sain et al. 1977; Nielsen et al. 1990), but were not investigated in this study. Therefore, the results are in the range of, and have produced trends that are typical of, other laboratory and field-based measurements of $NO_3^-$ removal through denitrification.

### *Reconciling laboratory and field measurements*

An estimate of potential $NO_3^-$ removal rates due to denitrification in stream sediments may be obtained from the laboratory experiments. Using the average $NO_3^-$ removal rate from the low-N experiment (Figure 6) and the stream bottom area between sites 1 and 10 at treatment 2, a removal rate of 58 percent of the mass of $NO_3^-$ exiting site 10 would be expected. However, this large removal potential was not realized in the field. Indeed, the results of field dilution trials (Table 1) showed no $NO_3^-$ loss from the stream once dilution by groundwater was taken into account. The absence of any field evidence for denitrification is especially surprising as the trials were conducted during low flow periods in the growing season when temperatures were most elevated, contact times between stream sediment and stream water were high, and therefore most conducive to removal processes.

However, isotopic $NO_3^-$-N measurements made during the field experiments support the absence of denitrification in stream sediments (Figure 9). The absence of any net $NO_3^-$ sinks during stream transport once groundwater dilution is considered (Table 1) also implies that vegetative uptake of $NO_3^-$ was not a significant factor at this site.

These results are in contrast to those found in an earlier study (Kellman and Hillaire-Marcel 1998) where *in-situ* variations in $\delta^{15}N$ and $NO_3^-$ concentration indicated that denitrification was actually occurring. The explanation for this difference appears to be in contrasting soil-sediment types and groundwater–stream interactions. The earlier study was conducted in the Prescott Basin where clay-rich stream bottom sediments probably inhibited rapid groundwater advection to the stream, allowing denitrification of $NO_3^-$ transported through streamwater-sediment diffusive transport to dominate the $NO_3^-$ concentration trends. In contrast, the stream in this study possessed sandy sediments that facilitated a more rapid groundwater advection into the stream which allowed dilution with low-$NO_3^-$ groundwater rather than denitrification in stream bottom sediments to dominate the stream $NO_3^-$ dynamics.

The results of this study question whether it is realistic to infer *in-situ* field processes and trends based upon laboratory experiments and points to the importance of evaluating the extent to which hydrological exchanges control chemical and biological streamwater–sediment interactions in the field. Many studies have relied upon the extrapolation of laboratory measures of removal processes such as denitrification to the field setting in order quantify N-cycling processes in these systems (van Kessel 1977; Bradley et al. 1995). As well, they are often the basis for inferring differences in N-cycling processes in the field, and are often used as the basis for modeling watershed N-cycling processes (Seitzinger et al. 2002). Although this study was conducted at a first-order stream where there is a large contact between stream sediments and overlying water, and the potential for $NO_3^-$ removal is high, soils and sediments are porous, and are therefore likely more conducive to denitrification than sites where groundwater dilution is inhibited by low hydraulic conductivities of sediments and low hydraulic gradients in the vicinity of the stream. These hydrological factors that influence $NO_3^-$ removal might be of greater importance when evaluating $NO_3^-$ removal in watersheds than measures of actual sediment potentials which may have little bearing in actual *in-situ* $NO_3^-$ removal and lead to overestimations in removal.

## Conclusions

The results of this study emphasize the importance of considering hydrological controls influencing $NO_3^-$ removal in streams. Unqualified extrapolation of laboratory-based estimates of $NO_3^-$ removal to watersheds could lead to severe overestimations of $NO_3^-$ removal rates. This problem is a function of general hydrological patterns and may not be characteristic of all sites. Overestimation is most likely to occur at sites characterized by coarser grained (i.e., sandy) sediments with high conductivities than finer grained (i.e., clayey) soils and sediments, and in areas with high hydraulic gradients in the vicinity of the stream.

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