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Nitrate cycling in streams: using natural abundances of NO_3^--δ^{15}N to measure *in-situ* denitrification

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Abstract. Contamination of surface- and groundwaters as a result of anthropogenic nitrate loading is of concern in regions subjected to intense agricultural activities. The capacity of watersheds to absorb, process or release nitrate to outflow drainage waters, however, is poorly constrained.

An investigation of in-stream denitrification was conducted in a small stream draining a heavily fertilized agricultural watershed by analyzing natural isotopic abundances of nitrate-nitrogen. Using δ^{15}N isotopic signatures, we show that denitrification plays a large role in reducing nitrate levels during stream transport over a relatively short distance. We found *in-situ* nitrate losses of up to 50% and a corresponding shift in NO_3^--δ^{15}N values of 10‰ over a 600 m distance downstream consistent with denitrification. Our results suggest that in-stream nitrate losses must be considered when examining nitrate cycling and contamination in watersheds. Not only should attempts to identify nitrate contamination sources using NO_3^--δ^{15}N signatures be carried out with caution (as nitrate-N isotopic values can be altered during stream transport such that they no longer reflect the original nitrate source), but in-stream measures of nitrate concentrations aimed at monitoring contamination levels may underestimate nitrate inputs to surface waters due to denitrification during transport.

Introduction

The loading of nitrogen in terrestrial ecosystems has greatly altered how nitrogen is cycled in waters draining many regions (Kinzig & Socolow 1994; Jaffe 1992). This problem is most evident in the mobile form of nitrate (NO_3^-), known to cause health problems in humans (Rajagopal & Tobin 1989) and to contribute to the eutrophication of surface waters (Cooper 1993). In addition, shifting the balance of nitrogen within ecosystems has significant long-term implications, including soil acidification and the release of nitrogen-related greenhouse gases (Olof-Tamm 1991; Paul & Clark 1996). Global projections are that the extent of agricultural lands and the use of nitrogen fertilizers will increase substantially in future decades, particularly in developing countries (Duxbury et al. 1993; Matthews 1994). Current as well as projected alterations in the nitrogen balance in watersheds makes it important that we understand how nitrogen is cycled, how nitrogen loading alters this cycle in contaminated environments, and to what extent *in-situ* denitrification occurs. On a global scale, natural ecosystems balance NO_3^- production at some stage during its transport, most often through the process of denitrification, whereby in oxygen limiting environments, and in the presence of organic carbon and denitrifying bacteria, nitrate can be used as an electron acceptor and reduced to N_2O and N_2 (Knowles 1982). Watersheds have the capacity to absorb part of their NO_3^- load through biomass accumulation and nitrate reduction in groundwater and riparian zones (Lowrance et al. 1984; Warwick & Hill 1988; Fustec et al. 1989; Cooper 1990; Jordan et al. 1993; Lowrance et al. 1995). Often overlooked in NO_3^- cycling studies, however, are losses resulting from denitrification during stream transport.

Denitrifying bacteria are present in stream waters; however, relatively high O_2 levels tend to inhibit denitrification which instead occurs in stream sediments due to diffusion of NO_3^- into the sediments from the overlying water (Knowles 1982). Microsensor techniques have clearly illustrated the denitrification process in aquatic sediments (Christensen et al. 1989; Nielsen et al. 1990). Measurements of *in-situ* losses of NO_3^- in streams have thus far been based on downstream changes in NO_3^- concentrations (Kaushik & Robinson 1976; van Kessel 1977; Black et al. 1993; Faafeng & Roseth 1993). Due to the complexity of the N cycle, it is difficult to say with certainty whether denitrification is responsible for the changes in NO_3^- concentration downstream. This link has generally been made through laboratory analysis of sediment samples collected from stream bottoms (Duff & Triska 1990). In such studies, it has been established that denitrification can and does occur in aquatic sediments, and that the *potential* for denitrification in aquatic sediments can be quite high (Hill 1979; Swank & Caskey 1982; Bradley et al. 1995; Holmes et al. 1996).

Laboratory measurements were initially made by measuring reductions in levels of NO_3^- amended to solutions overlying cores (e.g. Sain et al. 1977). Several other methods have since been develped which use acetylene inhibition, ^{15}N tracers, and N_2 fluxes to estimate denitrification. There are problems associated with all of these methods. In particular, acetylene inhibits denitrification and does not always block all N_2O reduction to N_2. As a result, these core measurements can be problematic, and as has been illustrated by Seitzinger et al. (1993) in a comparative study of these techniques using lake sediment cores, different techniques can yield vastly different results. Although these core measurement techniques have been useful in establishing the *potential* for denitrification in stream sediments, they do not provide a means whereby *in-situ* denitrification can be measured in streams beyond inferences based upon changes in concentration.

Large *in-situ* decreases in NO_3^- concentrations along the length of a stream have been observed in numerous studies (Vincent & Downes 1980; Cooper & Cooke 1984; Cooper 1990). Cooper (1990) observed decreases in NO_3^- concentrations along stream lengths and based upon acetylene inhibition of extracted sediment cores concluded that although denitrification played a small role in reducing NO_3^- concentrations during transport, most of the loss was a result of plant uptake. Vincent and Downes (1980) also conducted a study in which they estimated that the majority of inorganic N removal during stream transport occured as a result of plant uptake.

Denitrification in stream sediments is controlled by a complex set of exchanges and interactions between the overlying water and underlying sediments, factors including pool and riffle sequences along the stream bottom, and stream bottom sediment characteristics (Triska et al. 1993a, b). These authors and others (including Harvey & Bencala 1993; Hendricks & White 1990; Hill 1983) show that the spatial variations in stream sediment/water exchanges and resulting biogeochemical transformations along a stream bottom can be great.

Temperatures can affect denitrification both directly and indirectly, with the net result that increased temperatures tend to increase denitrifier activity and accentuate O_2 limited conditions (Kaushik et al. 1981). The time of day measurements are made can have an effect on the NO_3^- losses as increased photosynthetic activity mid-day may increase biological NO_3^- assimilation (Cooper & Cooke 1984). However, in their mass balance study of Swifts Brook, Kaushik and Robinson (1976) found no significant NO_3^- removal with increased photosynthetic activity.

Changes in NO_3^- concentrations along the length of a stream may be due to a number of factors. Firstly, NO_3^- concentrations may drop along the stream length if stream water is being diluted by groundwater with low NO_3^- concentrations. This water may have low NO_3^- concentrations as a result of denitrification prior to movement into streams, or may have reduced concentrations due to movement into anoxic stream sediment zones where denitrification subsequently occurs. Secondly, NO_3^- can be assimilated by plants during transport along the stream length, manifested as a constant decrease in the stream NO_3^- load. Thirdly, NO_3^- may be denitrified by denitrifying bacteria in stream sediments during transport (Kaushik et al. 1981; Duff et al. 1984; Fustec et al. 1989). Isolating the various mechanisms responsible for observed NO_3^- concentration decreases downstream are complicated by a lack of suitable techniques for separating and monitoring N-transformations in watersheds.

Use of the stable isotopes of nitrogen to examine nitrogen cycling is becoming more widespread, with improvements in techniques and the availability of analytical equipment (Blackburn & Knowles 1993). The isotopic signature of nitrogen is expressed as δ^{15}N, where:

$$\delta^{15}\mathrm{N}\ (‰) = ((^{15}\mathrm{N}/^{14}\mathrm{N}\ \text{sample})/(^{15}\mathrm{N}/^{14}\mathrm{N}\ \text{standard}) - 1) \times 1000$$

The standard is atmospheric N_2. Nitrate-δ^{15}N isotopic signatures have been widely used in groundwater analyses of nitrate sources (Heaton 1986; Aravena et al. 1993; Wilson et al. 1994) and denitrification zones (Mariotti 1986; Mariotti et al. 1988; Böttcher et al. 1990; Smith et al. 1991). In source identification, it is assumed that no isotopic changes have taken place, and that NO_3^--δ^{15}N values reflect those of the sources. In environments contaminated by NO_3^- the sources of greatest interest are animal wastes, and mineral nitrogen fertilizers. The earliest attempts to identify NO_3^- sources conducted using N isotopic abundances (Kohl et al. 1971) were very controversial (e.g. Hauck et al. 1972) due in large part to the oversimplification of the problem, and to a lack of recognition of potential fractionation processes during transport. The effect of denitrification on the isotopic composition of NO_3^- has been studied extensively, and it has been well established that denitrification results in isotopic changes of the NO_3^- pool, as bacteria preferentially reduce ^{14} NO_3^- over ^{15} NO_3^-, leaving an enriched pool of $^{15}NO_3^-$ (Chien et al. 1977; Blackmer and Bremner 1977; Mariotti et al. 1981; Mariotti et al. 1982). Thus if denitrification is occurring, NO_3^- concentrations will decrease along a flowpath, and the remaining NO_3^- will become increasingly enriched in ^{15}N. Several factors can affect the extent of this fractionation, including temperature, water content of soils, and concentrations of substrate (Mariotti et al. 1982).

Here we present the results of a study in which we examine the role in-stream denitrification plays in altering NO_3^- concentrations and δ^{15}N signatures during stream transport in a small agricultural basin located within the St. Lawrence Lowlands of Quebec. Nitrate concentrations and δ^{15}N values monitored over a growing season at the outlet of a stream within the basin (Kellman 1997) have shown a clear relationship between the NO_3^- concentrations and corresponding δ^{15}N values (Figure 1). Most importantly, the plot of the NO_3^- concentration versus the corresponding δ^{15}N values showed that the high δ^{15}N values were always associated with the low end of the NO_3^- concentrations, and the high NO_3^- concentrations with the low end of the δ^{15}N values. The trend observed in the NO_3^- concentration versus δ^{15}N plot suggests that the nitrate pool may be undergoing a fractionation consistent with denitrification.

A decrease in NO_3^- concentrations along the length of the stream may reflect dilution by low NO_3^- groundwater, biological assimilation during

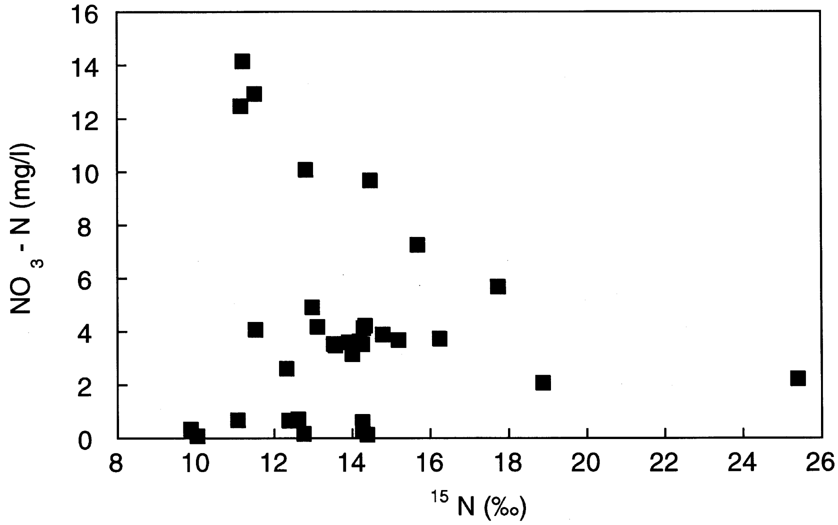

Figure 1. Nitrate-N concentration versus δ^{15}N data at the mouth of the studied stream in the Prescott Basin during the summer of 1995.

stream transport, denitrification during transport, or some combination of these mechanisms. As biological assimilation is a non-fractionating process (Mariotti et al. 1988), a decrease in NO_3^- concentrations will not be accompanied by a change in the corresponding δ^{15}N values during transport if assimilation alone is the primary mechanism reponsible for the reduced concentrations. If NO_3^- in stream water is not being removed, but merely diluted with groundwater with low NO_3^- concentrations and high δ^{15}N values, then we would expect to see a decrease in NO_3^- concentrations and an increase in corresponding δ^{15}N values during transport. Similarly, if denitrification alone is responsible for NO_3^- losses downstream, we would expect to observe the theoretical relationship predicted by Mariotti (1988; i.e. an exponential increase in δ^{15}N values as NO_3^- concentrations decrease). Information provided by groundwater samples (i.e., NO_3^- data and information from other chemical species) may differentiate between the latter two mechanisms.

The objective of this study is to use measurements of the natural abundances of ^{15}N of nitrate in a stream to determine the fate of nitrate downstream. By measuring downstream changes in NO_3^- concentration and natural abundances of δ^{15}N, an attempt will be made to determine a) whether evidence exists that NO_3^- does not retain the isotopic signature it had upon entering the stream, and b) whether by sampling stream water along a downstream transect and measuring downstream changes in concentration and δ^{15}N values, it is possible to measure *in-situ* denitrification, and if so, to establish during what seasons denitrification is an important in-stream NO_3^- removal process in the agricultural areas of the St. Lawrence Lowlands represented by this study site.

Study site

The present study was carried out in a heavily fertilized agricultural watershed in the St. Lawrence Lowlands of Quebec, Canada. In this flat and fertile region, fields are often tile drained and many streams have been artificially created to increase drainage. The Prescott Basin is a 500 hectare basin made up of sandy clay loam soils which are underlain by clay and are tile drained. Tile drains provide point sources of high NO_3^- water to the streams in this basin (Kellman 1997). The stream in which this particular study was conducted has a vegetative buildup through the growing season which peaked from late July through early September. During this period, water levels are also reduced. Nitrate concentrations in the streams draining this watershed are elevated due to fertilization of fields with pig manure and mineral fertilizers. As these two N sources are thought to be responsible for the elevated concentrations of NO_3^- in the streams, initially an attempt to weigh their relative contributions using $\delta^{15}N$ measurements was made. Preliminary results from the mouth of one of the streams in this watershed, however, showed $\delta^{15}N$ values higher than those of the most enriched source (pig manure organic N; see Table 1 for the $\delta^{15}N$ signatures of nitrates from different sources in this watershed). Although the pig manure organic N-$\delta^{15}N$ signature does not necessarily represent the maximum NO_3^--$\delta^{15}N$ value of this source (see discussion for more information), this fact, in addition to the observation that elevated $\delta^{15}N$ values were associated with low concentrations of nitrate suggested that denitrification might be responsible. We therefore decided to undertake a more detailed survey aimed at examining whether in-stream denitrification could provide a partial explanation for this ^{15}N enrichment by collecting samples during the growing season along a transect in this drainage stream.

Sampling and methods

Sampling was conducted between the summer of 1995 and the fall of 1996. Measurements were taken from seven stations (S1 through S7) located 100 m downstream of each other (see Figure 2). At each location 2 liters of water was collected to ensure there was sufficient nitrate for isotopic analysis. Samples were stored in ice immediately after collection, and were filtered

Table 1. Nitrate isotopic source signatures in the Prescott Basin.

NO_3^--N source	$\delta\ ^{15}N$ Signature (‰)
Rain water	$\sim$ + 0.5 (Prescott Basin)
Chemical fertilizers	$\sim$ + 1.5 (Prescott Basin)
Soil organic matter	$\sim$ + 4 to + 9 (Heaton 1986)
Pig manure organic N	$\sim$ + 12.5 (Prescott Basin)

(0.45 μm) within hours of sampling in the lab. Additional samples were removed and preserved with sulfuric acid or frozen for analysis of NO_3^- and dissolved organic carbon (DOC), and other major ions (including Na, K, Mg, Ca, Cl, and SO_4). Nitrate concentrations were analyzed on an Autoanalyzer using the cadmium reduction method (EPA Method, N87-0065), and DOC concentrations by oxidation with potassium persulfate using a Dohrman samper (Annual Book of ASTM Standards 1988). Further details of analyses conducted and procedures used can be obtained in Kellman (1997).

The relatively low concentrations of NO_3^- often found in surface water environments (even 'contaminated' surface water evironments when compared to groundwater environments can be low) make extraction of NO_3^- a very laborious and complicated procedure using the traditional methods (e.g. steam distillation; Bremner & Edwards 1965; Bremner 1965). A new procedure, developed by Silva et al. (1997, manuscript in review; other studies published using this technique include Kendall et al. (1995) and Wassenaar (1995)) allows for the extraction of nitrates using anion exchange resins, and the further concentration by freeze-drying samples. This technique has several advantages: it eliminates the need for preserving samples (with, for example, $HgCl_2$) as the NO_3^- extraction is carried out immediately upon return to the laboratory, problems of isotopic fractionation and cross-contamination are avoided (Mulvaney 1986) as is the time consuming nature of the steam distillation procedure. Within 24 hours of collection, filtered samples were passed through anion exchange columns to extract the NO_3^-. These columns were stored at 4 °C until receipt of the NO_3^- concentration data, whereupon it was determined how much NO_3^- was available for the analysis. In order to minimize measurement errors, attempts were made to ensure that each sample contained approximately 2 mg of N, although in some instances, low NO_3^- concentrations dictated that less N was available for the analysis. Samples were then analyzed on a V.G. PRISM dual inlet, triple collector mass spectrometer. Instrumental precision is ±0.02‰. The overall analytical uncertainty as determined using KNO_3^--ammended spring water

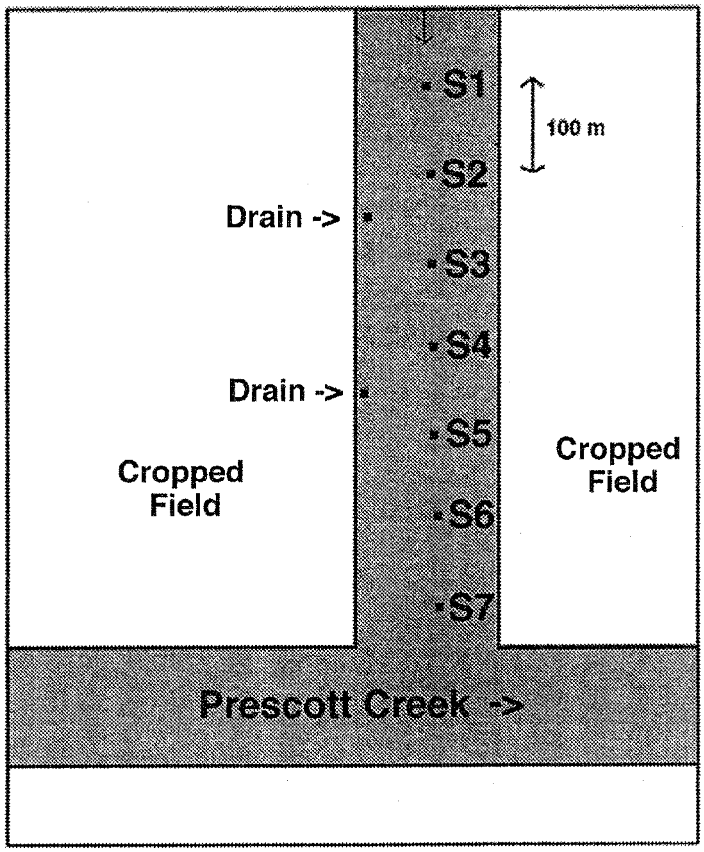

Figure 2. Location of sampling points and tile drainage outlets at the study site. Note that the stream width is exaggerated. Actual stream width is 1–2 meters.

with similar specific ion concentrations as sample waters was ±0.2‰. Blanks were run to ensure there was no contamination during sample processing.

Results

Nitrate concentrations in the stream were generally high within the stream over the survey period (typically on the order of 2–10 mg/l; Kellman 1997) with the lowest concentrations observed during late summer when the basin was drier and water levels low. Dissolved organic carbon was measured in the stream over a full growing season (1995), with values generally exceeding 5 mg/l. Dissolved oxygen levels in the stream were variable, but generally on the order of 5–10 mg/l.

Table 2. Groundwater and downstream chloride concentration data. Groundwater chloride data is an average of 64 samples taken in May-July 1995. Downstream samples were taken on August 1, 1995.

Sample location	Chloride concentration (mg/l)
S1	20
S2	21.2
S3	17.2
S4	16.2
S5	20.6
S6	16.4
S7	17
Groundwater (64 samples)	3.7 (±0.7)

Shallow groundwater and streamside soil water samples showed that NO_3^- concentrations (particularly in groundwater) were very low (typically well below 0.2 mg/l NO_3^--N), suggesting that any water entering the stream from this source had NO_3^- concentrations which were low or close to zero (Kellman 1997). In 1995 shallow groundwater samples were analyzed for Cl^- concentrations, as Cl^- is conservative and can therefore aid in discriminating between downstream changes in NO_3^- concentrations due to denitrification and downstream changes due to dilution with incoming groundwater. Table 2 lists downstream Cl^- data for the downstream series taken on August 1 1995, and includes the streamside groundwater Cl^- concentrations obtained from May through July of the same year for comparison. Groundwater Cl^- concentrations were less than 4 mg/l on average, and there was no clear decrease in Cl^- concentration downstream.

It is expected that if denitrification is occurring, NO_3^- concentrations will decrease downstream, $\delta^{15}N$ values will increase downstream, and the natural logarithm of the NO_3^- concentration will have an inverse and linear correlation with the $\delta^{15}N$ value, similar to that which has been observed in groundwater denitrification zones (Mariotti 1988; Smith et al. 1991).

Tile drainage outlets discharging water with different nitrate and $\delta^{15}N$ values were located along the stream transect (Figure 2). These point drainage inputs were sampled when present and the measured inputs of nitrate (concentrations and $\delta^{15}N$ signatures) are indicated on the Figures.

Three situations were observed, and illustrative sets of measurements are presented. They correspond respectively to: (1) situations in which downstream measurements *are not* consistent with denitrification, (2) situations in which downstream measurements *are* consistent with denitrification (2 examples), and (3), situations in which subsurface drainage inputs of nitrate have overprinted downstream denitrification.

Case 1: Example of downstream measurements *not* consistent with denitrification: May 5, 1996

This set of measurements was made in late spring prior to planting in the basin. Figure 3a shows that downstream changes in NO_3^- concentration are somewhat variable with values on the order of 9–10 mg/l. Point drainage inputs of NO_3^--rich water do not produce any clear downstream changes in concentration. This was also the case with the downstream $\delta^{15}N$ measurements, again with point inputs of low NO_3^--$\delta^{15}N$ drainage water having no clear effects on the downstream values (Figure 3b). The absence of an inverse linear correlation between the $\delta^{15}N$ value and the ln $[NO_3^-\text{-N}]$ (Figure 3c) indicates that denitrification is not a dominant process.

Case 2a: Example of downstream measurements consistent with denitrification: August 1, 1995

This set of measurements was made 1–2 weeks following a significant precipitation within the basin. Tile drains were dry (i.e. no point discharge), and in the stream, concentrations dropped close to 50% over the span of 600 m, from 5.4 to 2.2 mg/l (Figure 4a). Nitrate-$\delta^{15}N$ values increased over the same distance by almost +10‰, from +16‰ to +25‰ (Figure 4b). The linear inverse relationship produced by the $\delta^{15}N$ vs ln $[NO_3^-\text{-N}]$ ($R^2 = 0.96$) is consistent with a loss of nitrate resulting from denitrification (Figure 4c).

Case 2b: Example of downstream measurements consistent with denitrification: July 29, 1996

In this case the same pattern is observed, in the mid-to-late growing season following a rainfall, and with no point discharges from tile drains. Nitrate losses of 2.2 mg/l over 400 m are observed (Figure 5a; last 2 data points are missing due to a storage problem), with a corresponding increase in $\delta^{15}N$ over the 600 meters (Figure 5b). Figure 5c shows a clear inverse relationship between the $\delta^{15}N$ value and the ln $[NO_3^-\text{-N}]$ ($R^2 = 0.97$).

Case 3: Example of subsurface drainage inputs of NO_3^- overprinting downstream denitrification: September 20, 1996

This series of measurements presents a more complicated situation, as tile-drainage inputs have overprinted the NO_3^- concentration and $\delta^{15}N$ downstream series. Up- and downstream of each of the two tile drainage outlets,

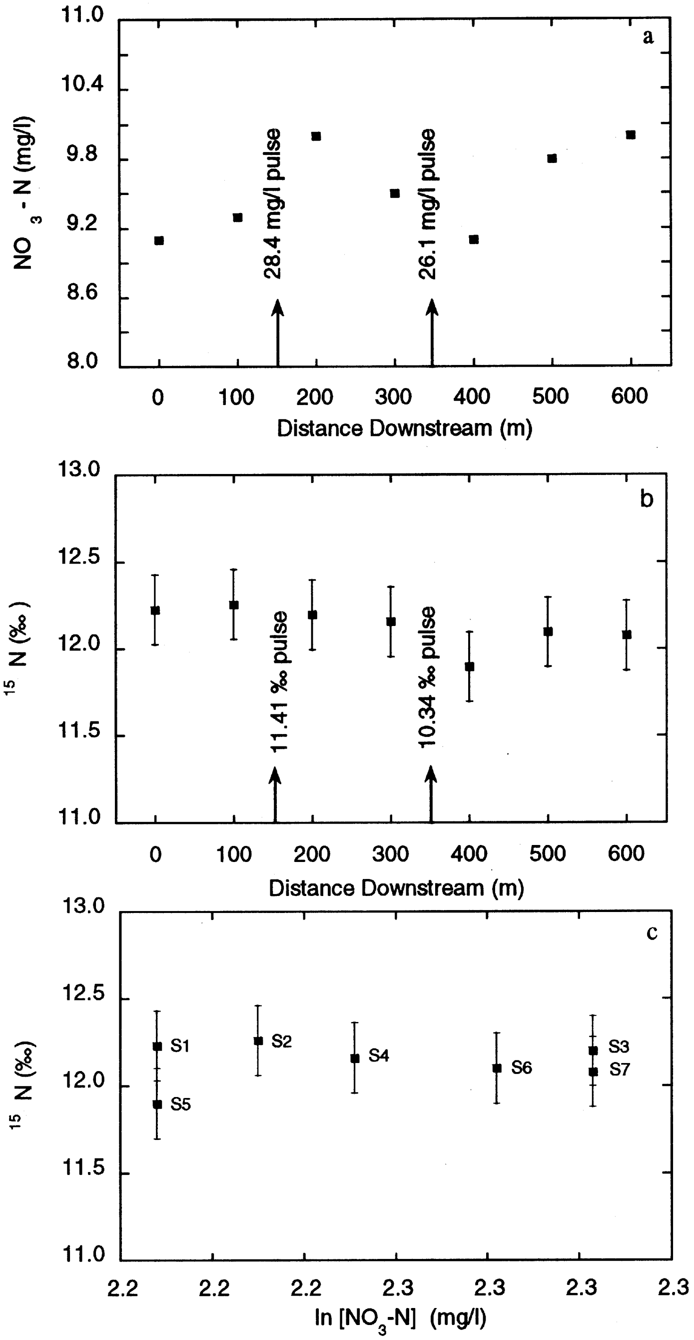

Figure 3. Observations on May 5 1996 of: a) Nitrate concentration changes with increased distance downstream (arrows indicate point inputs from tile drainage outlets), b) Nitrate-δ^{15}N value changes downstream, and c) Nitrate-δ^{15}N values versus the natural logarithm of the NO_3^--N concentration for downstream stations.

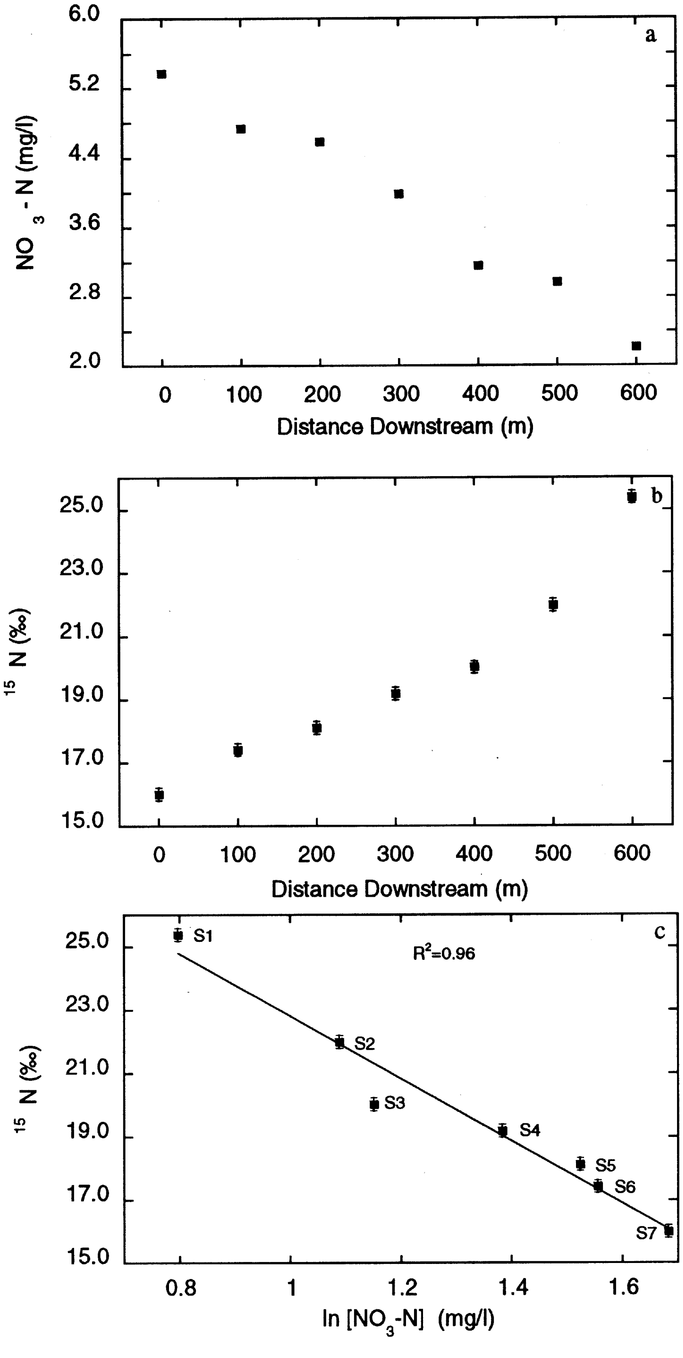

Figure 4. Observations on August 1 1995 of: a) Nitrate concentration changes with increased distance, b) Nitrate-δ^{15}N value changes downstream, and c) Nitrate-δ^{15}N values versus the natural logarithm of the NO_3^--N concentration for downstream stations.

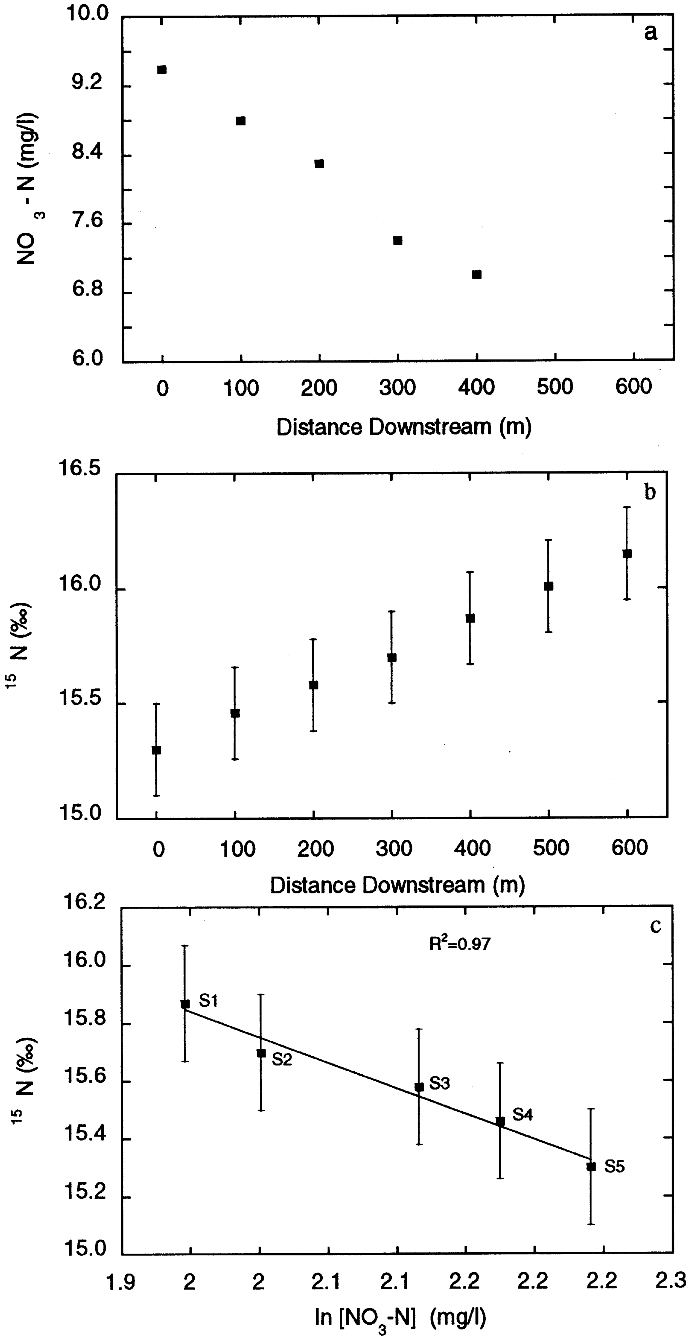

Figure 5. Observations on July 29 1996 of: a) Nitrate concentration changes with increased distance downstream, b) Nitrate-δ^{15}N value changes downstream, and c) Nitrate-δ^{15}N values versus the natural logarithm of the NO_3^--N concentration for downstream stations.

decreases in NO_3^- concentrations are observed; Figure 6a shows a nitrate concentration decrease of 1.3 mg/l over the first 100 meters, and a decrease of over 2 mg/l over the final 200 meters. Although the point discharge δ^{15}N values from tile drains have produced shifts in the in-stream δ^{15}N values, these in-stream values clearly increase over the first 100 m and over the final 200 meters (Figure 6b). Points S3 and S4 are less clear, and may be related to seepage from an old drainage tile. Although these drainage inputs have fragmented the downstream series, the plot of δ^{15}N versus ln [NO_3^--N] (Figure 6c) points to inverse and linear relationships up- and downstream, (points S3 and S4 are less clear) with similar slopes, again suggesting that the same fractionation processes are involved along the length of the stream, despite these point influxes of NO_3^- with different δ^{15}N signatures.

Discussion

Examining the role denitrification might play in reducing NO_3^- loads in streams of the Prescott Basin became of interest when it was observed a) that high δ^{15}N values of NO_3^--N in the drainage waters of this watershed often exceeded the most enriched source of NO_3^-, that of pig manure organic N, and b) that very elevated δ^{15}N values were always associated with low NO_3^- concentrations and vice versa. Although a measure of the pig manure organic matter N-δ^{15}N values does not necessarily signify the upper δ^{15}N source signature for NO_3^--δ^{15}N in the basin, as ammonia volatilization after transformation of pig manure organic N to ammonium can be further enriched due to volatilization of ^{15}N-depleted ammonia (Heaton 1986), the fact that the NO_3^--δ^{15}N values were consistently elevated in drainage waters nevertheless lead to further exploration of this question. As ammonium concentrations in draining waters within the watershed were close to zero (Kellman 1997), it is very likely that this occurred shortly following spring and fall application of fertilizers. Low ammonium concentrations in streams within the basin and tile drains also suggests that the NO_3^--δ^{15}N fractionations observed are not likely due to N-transformations taking place during transport.

The data presented show that during certain periods (typically late summer periods when water levels are low in the stream) nitrate concentrations can drop rapidly over relatively short distances, as was observed in cases 2a, 2b and 3. Concentration decreases along the length of a stream alone can be explained by several different mechanisms; dilution by low NO_3^- groundwater, assimilation by vegetation, or denitrification. Using concentrations alone, it is difficult to distinguish between *in-situ* losses due to these various factors. However, analysis of the natural abundances of NO_3^--δ^{15}N during transport can reduce this uncertainty, as denitrification results in a fractiona-

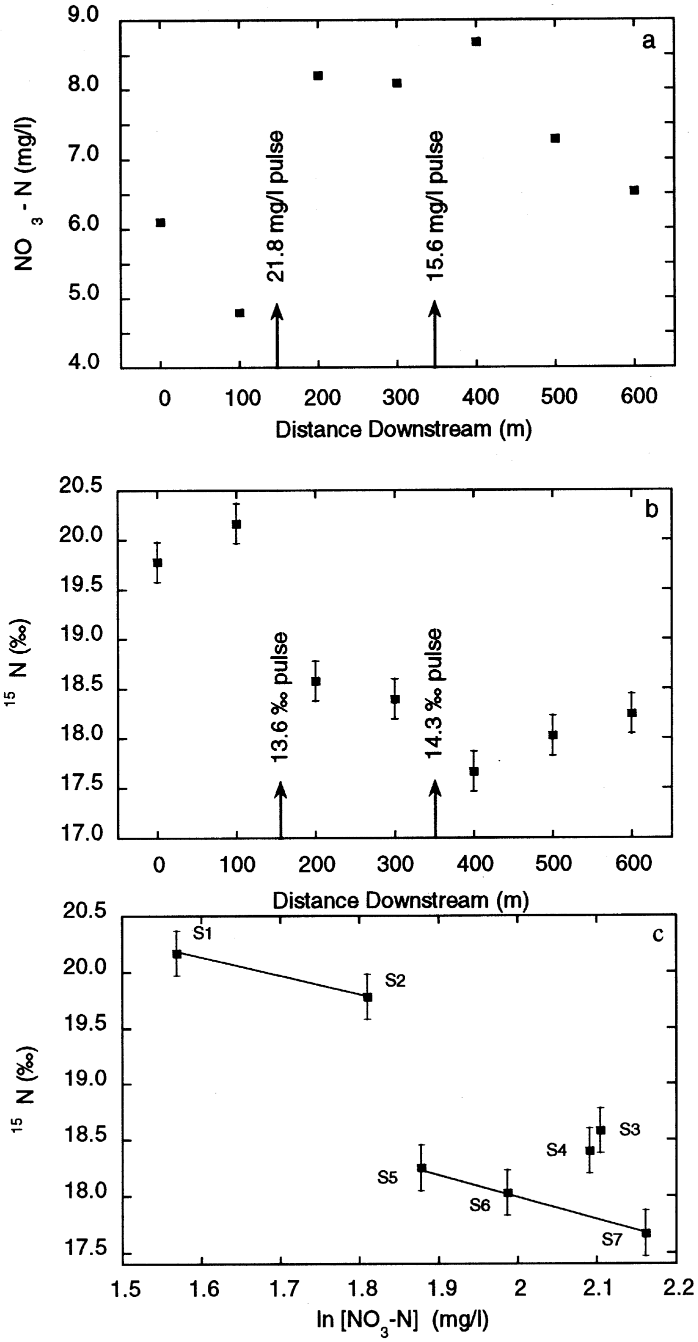

Figure 6. Observations on September 20 1996 of: a) Nitrate concentration changes with increased distance downstream (arrows indicate point inputs from tile drainage outlets), b) Nitrate-δ^{15}N value changes downstream, and c) Nitrate-δ^{15}N values versus the natural logarithm of the NO_3^--N concentration for downstream stations.

tion of the NO_3^- pool as it is reduced (Blackmer & Bremner 1977; Mariotti 1981). The downstream changes in NO_3^- concentration and $\delta^{15}N$ presented in cases 2a, 2b and 3 could be explained by a mixing of stream water with low NO_3^- and high NO_3^--$\delta^{15}N$ groundwater. As $\delta^{15}N$ analyses of groundwater were never conducted due to the very low NO_3^- concentrations (and hence insufficient $\delta^{15}N$ for analysis; Kellman 1997), this question must be addressed using other data. Chloride data shows that groundwater and stream waters have very different Cl^- concentrations (Table 2), and that the observed changes in concentration cannot be explained by a mixing of these two waters. If NO_3^- concentration changes were a result of assimilation by vegetation, on the other hand, there would not be a fractionation of the nitrate pool downstream as assimilation by vegetation is a non-fractionating process (Mariotti et al. 1988). The data in cases 2a, 2b and 3, show that there is a clear fractionation during transport, and that this fractionation is consistent with that expected if denitrification were the primary NO_3^- removal mechanism. Although the NO_3^- concentration decreases and corresponding $\delta^{15}N$ value changes observed in these cases clearly show that denitrification plays a key role in reducing NO_3^- loads, further study is needed in order to determine to what extent NO_3^- removal by plant uptake plays a role, and whether superimposed upon these downstream NO_3^- losses due to denitrification, is also a downstream loss due to vegetative uptake.

The data presented in this study suggests that denitrification can play an important role in reducing NO_3^- levels during stream transport. Downstream changes in nitrate concentration and $\delta^{15}N$ values consistent with denitrification were observed during late summer and early fall periods when water levels are reduced and contact time between stream waters and stream sediments is increased. During these drier periods it is clear that NO_3^--$\delta^{15}N$ values no longer reflect NO_3^- sources, but reflect primarily denitrification processes. More complicated situations are observed during higher streamflow periods due to incoming pulses of NO_3^- from tile drains located downstream (case 3). Such cases only serve to reinforce the contention that denitrification is responsible for NO_3^- losses downstream as they conform to theoretical expectations. Although concentration and $\delta^{15}N$ data does not point to denitrification as a dominant NO_3^--removal process during higher flow periods, the question remains as to whether during these periods, discrete denitrification continues, and that due to the short residence time of water in the stream and/or due to a larger pool of NO_3^-, the isotopic signatures are not significantly altered.

In this study it appears that the *potential* for denitrification is high and that contact time between stream waters and sediments limits NO_3^- reduction. Understanding controls on denitrification in watersheds, particularly contaminated watersheds, is critical to implementing management strategies which can aid in reducing contamination levels. Understanding NO_3^- cycling in watersheds is essential if we are to fully understand how loading of nitrogen, alters these environments. Advances in the technical abilities to extract and measure NO_3^--$\delta^{15}N$ values may lead to attempts to use $\delta^{15}N$ signatures to identify NO_3^- sources and cycling processes in streams and lakes. We caution that a proper understanding of potential fractionation processes is necessary when designing these types of studies in both disturbed and undisturbed watersheds. If fractionation of nitrate during transport is significant, this could alter the $\delta^{15}N$ signatures of the remaining nitrate, such that it no longer reflects the original nitrate sources.

If significant amounts of nitrate are denitrified during stream transport, the loading of nitrate in many streams of contaminated environments may be underestimated. Studies designed to monitor nitrate contamination levels in surface waters must take this into account.

In future studies of this type we suggest that the above *in-situ* $\delta^{15}N$ approach be complimented with other core-based and mass balance N studies in streams, with a greater emphasis placed on quantifying NO_3^- losses due to denitrification during stream transport. More recently, $\delta^{18}O$ of nitrate has also been used to constrain the problem of identifying NO_3^- sources (Durka et al. 1994; Kendall et al. 1995; Wassenaar 1995), and may aid in further constraining the problem of identifying sources versus denitrification of NO_3^- along flow paths.

Acknowledgements

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