NITROGEN TRANSFORMATIONS AND NO₃⁻ REMOVAL AT A
SOIL-STREAM INTERFACE: A STABLE ISOTOPE APPROACH

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Abstract. The natural removal of NO₃⁻ by denitrification within riparian zones of streams and rivers is an area of considerable interest owing to its potential to minimize the impacts of excess anthropogenic loadings. In this study we utilize natural variations in stable N isotopic compositions of NO₃⁻ and NH₄⁺ within a transect of shallow wells extending 4 m inland from Smith Creek, a southwestern Michigan stream, to provide insight into microbial processes and the extent of NO₃⁻ removal within a soil-stream interface. Within this region three water masses with unique biogeochemical characteristics intersect: a shallow flow rich in NH₄⁺ and dissolved organic carbon (DOC), a deep groundwater mass rich in NO₃⁻ but depleted in DOC, and stream water low in NO₃⁻, NH₄⁺, and DOC. N isotope values for NO₃⁻ within the well transect were highly variable (~7.7–34.1‰) and reflected intense microbial activity within this narrow region. Isotopic variation was primarily controlled by upwelling of deep groundwater near the stream and partial loss of NO₃⁻ via denitrification that was dependent upon a supply of DOC from shallow groundwater.

Quantitative estimates of the fraction of NO₃⁻ removal due to denitrification within the soil-stream interface were obtained from N isotope data using a modified Rayleigh equation. Conservative estimates of NO₃⁻ removal range from 0% to 86%. In conjunction with measurements of hydrological flows within the sampling wells we provide a novel estimate of NO₃⁻ removal based on natural abundance isotope measurements. NO₃⁻ removal was found to vary from undetectable levels to 123 mmol L⁻¹ on a d⁻¹⁻¹ for the narrow region of the soil-stream interface in which deep and shallow groundwater intersect.

Key words: ammonium; denitrification; hydrologic flow paths; Michigan; nitrate removal; nitritification; nitrogen; isotope; riparian zone; soil-stream interface.

INTRODUCTION

The biogeochemistry of riparian zones has been an area of considerable interest owing to the potential for microbial processes occurring within these environments to control stream chemistry and mediate excess anthropogenic N₂O₃ loadings (Lowrance et al. 1984, Cooper 1990, Triatia et al. 1993a, b; Hanson et al. 1994, Jansson et al. 1994, Greifman et al. 1996, Hedlin et al. 1998, Cey et al. 1999, Devito et al. 2000). While it is clearly recognized that denitrification within riparian zones can remove NO₃⁻ carried into streams in surface or groundwater flows, a detailed understanding of the physical and chemical factors that control various microbial processes and the extent of NO₃⁻ removal near groundwater-soil-stream interfaces is still emerging.

In this study, we focus on understanding the dynamic relationship between changes in hydrology and microbial processes and their effect on NO₃⁻ removal within soil-stream interfaces by evaluation of data on the concentration and isotopic composition of NO₃⁻ and NH₄⁺ within the soil-stream interface of Smith Creek in southwestern Michigan. This work expands upon our earlier research on the thermodynamic constraints on N cycling within the soil-stream interface (Hedlin et al. 1998) by delineating in detail seasonal variation in NO₃⁻ removal and microbial controls on the isotopic composition of NO₃⁻ and NH₄⁺. Furthermore, this study presents a new approach, based on natural abundance isotope data, for quantifying NO₃⁻ removal within these environments.

Natural-abundance stable N isotopes have been used as a tracer of the origins and cycling of N in both marine and terrestrial ecosystems since the early 1970s (Kohl et al. 1971, Feigie et al. 1971, Kreitzler and Jones 1975, Peters et al. 1978, Saino and Hatton 1980). Early on researchers recognized, however, that source apportionment can be compromised by potential overlap in the isotopic composition of sources and variation within a single source. A further challenge is that δ¹⁵N signatures often behave nonconservatively within ecosystems as isotopic alteration can be imposed by mi-

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Nonconservative behavior is due to several factors: (1) numerous N-cycling processes discriminate be- tween N isotopes (e.g., denitrification, nitrification, am- monification, uptake, and volatilization), (2) the mag- nitude of isotopic segregation (as described by frac- tionation factors) during specific reactions tends to vary, (3) the manifestation of fractionation factors in natural environments often depends on local environ- mental conditions (e.g., substrate concentration and the completeness of a reaction), and (4) interpretations about transformation processes require, to at least some degree, that the δ¹⁵N-signature of a N source is well characterized in space and time. Less appreciated, how- ever, is the fact that the nonconservative behavior of N isotopes can, in fact, contain important information about ecosystem function. Although compromising studies of source apportionment, nonconservative be- havior can provide insight into the predominant mi- crobial and plant processes influencing N cycles in eco- systems. Within riparian environments, for example, marked increases in the isotopic composition of NO₃⁻ have provided clear evidence of NO₃⁻ removal by de- nitrification (Aravena and Robertson 1998, Hedlin et al. 1998, Coy et al. 1999, Mengis et al. 1999).

An ideal environment to take advantage of noncon- servative behavior of N isotopes is one in which signals imparted by different microbial processes are strong and spatially segregated. Here we document N trans- formations and quantify the role of denitrification in such an environment: a riparian wetland. The study was conducted along a highly spatially constrained well se- ries (4 m horizontal range) in which the intersection of geochemically unique water masses results in var- iation in electron donors and acceptors from abundant surface soils (Hedlin et al. 1998). Thin film spatial resolution enabled us to study processes occurring over short distances that may have been overlooked or un- dersampled in other studies which sampled greater ar- eas. We use a theoretical approach to explicitly estimate how isotopic variation associated with microbial pro- cesses vary independently from differences arising from a mixture of sources. We carefully consider the causes of variation in fractionation factors during de- nitrification. Finally, we calculate the magnitude and spatial pattern of NO₃⁻ consumption by denitrification along integrated hydrologic flow paths.

Our results have considerable importance for the many δ¹⁵N source-attribute studies in downstream aquatic ecosystems. First, we show that isotopic dis- crimination within soil-stream interfaces can be marked and can occur over short spatial distances. Fur- thermore, isotopic alteration may result from processes within the stream itself, such as nitrification and as- similation during photosynthesis, that may compromise downstream source apportionment. Finally, we illus- trate that despite the considerable challenges in apply- ing δ¹⁵N techniques within natural ecosystems, it is possible to study and quantify processes, such as NO₃⁻ removal, that are otherwise difficult to understand in undisturbed natural environments. In this manner, the obstacles imposed by the nonconservative nature of N isotopes become an asset instead of a liability.

METHODS

Study area and sampling approach

Our study site consisted of two well fields along the riparian zone of Smith Creek, a first-order stream with- in the Augusta Creek drainage basin of southwestern Michigan, in which the prevailing hydrologic flow was from the soil into the stream. Approximately 60% of the 75 km² watershed was located up gradient from the study site. The well fields were located within a riparian wetland which was vegetated predominantly with sedg- es and grasses and surrounded by an oak-hickory for- est. Houghton-muck soils predominate along the creek and are underlain by thick deposits of glacial till and outwash. Detailed descriptions of the soils, geology, and watershed characteristics can be found in Hedlin and Brown (1994) and Hedlin et al. (1998). Each field consisted of four or five parallel transects of six wells spanning a distance of 4 m perpendicular to the stream and encompassing ~8–12 m² of soil area. Site 2 was located 14-m upstream and on the opposite bank from site 1. Samples for isotopic analysis were collected—monthly from August 1992 to November 1993. A total of 94 samples were collected for isotopic analysis, however, not all samples were analyzed for δ¹⁵N because concentrations were not sufficiently in excess of analytical background.

Wells were constructed of 2.5 cm inner diameter PVC pipe, screened at the bottom and placed 40 cm beneath the soil surface. Owing to the need for 250 mL of fluid for isotopic analysis, single wells were not capable of providing sufficient fluid without compro- mising hydrologic flow. Consequently, equal vol- umes of water from wells equidistant from the stream were pooled to provide sufficient sample for the con- centration and isotopic data presented in this paper. This approach has been justified by the observation of

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similar concentrations of NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} in well series equidistant from the stream (Hedin et al. 1998). Slight variations in hydrologic flow across the well field, how-
ever, may result in small-scale differences in concen-
trations and isotopic abundances, therefore, for the N iso-
topic values should be considered volume-weighted trends along a transect perpendicular to the soil–stream interface.

We calculated water flow through the soil–stream interface using standard graphical flow net construction (Fetter 2001). Hydraulic potential was measured at the sample wells that served as piezometers. Based on the precise well locations and water potential at each well, we plotted equipotential lines as a function of depth and distance from the stream and determined hydraulic gradients from the plot. Subsequently, we calculated water flow through areas of the soil–stream interface using Darcy’s Law and hydraulic conductivity from Hedin et al. (1998).

Analytical techniques

For concentration measurements, samples were fil-
tered within 3% of collection through preirradiated Gelman A/E glass filter filters (Fall Gelman Laboratory, Ann Arbor, Michigan, USA), refrigerated at 4°C, and ana-
lyzed within 2 weeks. We observed no significant change in concentration for >4 wk of storage using this pro-
tocol. Concentrations of NO\textsubscript{3}\textsuperscript{-} were determined via ion chromatography using chemically suppressed conduc-
tivity detection and a Dionex AS4A column (Dionex, Sunnyvale, California USA). Alkphs automated col-
umetry was used to assess NH\textsubscript{4}\textsuperscript{+} concentrations (Alp-
ken Kem 1992). Samples for determination of NO\textsubscript{2}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} 23Na values were similarly filtered and stored fro-
zen (−20°C) until analyzed. Dissolved O\textsubscript{2} was quan-
tified on several dates based on the method of Carpenter (1965) but never found to be above detection limits in any of the wells. N stable-isotope ratios are expressed in per mil (%) notation:

\[ \delta^{15}N = \left[ \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right] \times 1000 \]  

(1)

where \( R \) is the abundance ratio of the heavy to light isotope and the internationally recognized standard for N is atmospheric N\textsubscript{2} gas. Extraction of NO\textsubscript{3}\textsuperscript{-} for iso-
topic analysis was performed by standard steam-distil-
tillation procedures (Bremner and Kenney 1966, Ve-
linsky et al. 1989, Ostrom et al. 1998). To convert NH\textsubscript{4}\textsuperscript{+} to volatile NH\textsubscript{3} gas, the pH of the distillate was shifted to \( \geq 10 \) by addition of 1 mL of NH\textsubscript{3}·2H\textsubscript{2}O. 

RESULTS AND DISCUSSION

The dynamic nature of N cycling along the soil–stream interface of Smith Creek was illustrated by var-
iations in the concentration and isotopic composition of NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} on a spatial and seasonal basis (Fig. 1). Inorganic N varied from detection limits to nearly 270 \text{μmol/L}; such changes in concentrations often oc-
curred over a distance of <1 m. The range in \( 8^{15}N \) values for inorganic N along our transect of 4 m at the soil–stream interface approaches that reported for soil or groundwater in the literature (Macko and Ostrom 1994). N isotope values for NO\textsubscript{3}\textsuperscript{-} ranged from a min-
imum of ~7.7% to a maximum of 34.1%. A narrower range was found for the \( 8^{15}N \) of NH\textsubscript{4}\textsuperscript{+} of 1.1–15.7%. N utilization by plants is not likely to have a control over the small isotope of this study (4 m) and involves only a small isotope effect (Nedeffelger and Fry 1994). These changes in concentration and isotopic compositions within such a narrow region cannot be explained by contributions from sources alone but re-
flect isotopic fractionation associated with microbial processes. Large isotopic variation can complicate in-
terpretations of inorganic N sources and processes. Consequently, our interpretation of the microbial and isotopic dynamics is presented within two frameworks. The first involves traditional qualitative assessment of N sources to the interface and the predominance of distinct microbial processes based upon changes in the concentration and isotopic composition of inorganic N. The second applies hydrologic and isotopic modeling to quantify NO\textsubscript{3}\textsuperscript{-} removal within the interface. Quan-
tifying NO\textsubscript{3}\textsuperscript{-} removal without disturbing the environ-
ment (such as the use of soil chambers and artificial substrates) is a new advance that potentially can be extrapolated to larger spatial scales.
FIG. 1. The concentration of $\text{NO}_3^-$ and $\text{NH}_4^+$ (TDN), concentration of dissolved inorganic nitrogen) and isotopic composition in well series 6 sampling the soil-stream interface at sites 1 and 2 on Augusta Creek, Michigan, USA. Well series 6 was within the stream itself, well series 1 was farthest inland. Analyses were conducted on pooled samples collected from wells equidistant from the stream (well series). Shallow-, deep-, and stream-water masses were identified on the basis of unique abundances of $\text{NO}_3^-$ and $\text{NH}_4^+$. Zones of nitrification, dissimilatory NO$_3^-$ reduction to $\text{NH}_4^+$ (DNRA), and denitrification were assessed based on unique shifts in the isotopic composition of $\text{NO}_3^-$ and $\text{NH}_4^+$. Dotted lines connecting solid squares represent (NO$_3^-$); dotted lines connecting solid circles, $\delta^{15}$N-$\text{NO}_3^-$; and solid lines connecting open circles, $\delta^{15}$N-$\text{NH}_4^+$. 
Sources of inorganic N

To characterize the δ15N of sources of inorganic N to the soil-stream interface we first established the location of each of the three flows based on concentrations of NH₄⁺ and NO₃⁻. and, secondly, identified which samples had been affected by the fractionation during nitrification and denitrification (Fig. 1). Our previous work has demonstrated that the shallow and deep groundwater flows are characterized by high concentrations of NH₄⁺ and NO₃⁻, respectively (Hedin et al. 1998). With this understanding, the concentrations of NH₄⁺ and NO₃⁻ provided a basis to conclude that upland well series (series 1, 2, and 3) were heavily influenced by the shallow groundwater flow whereas well series 4 and 5 were dominated by flow from deep groundwater. We then excluded data from wells and times in which the changes in the concentration and isotopic abundance of NH₄⁺ and NO₃⁻ followed trends expected for nitrification or denitrification. Well series with the greatest concentrations of NO₃⁻ were generally characterized by low δ15N values and likely reflect times of strong deep-groundwater input with minimal NO₃⁻ removal by denitrification. As denitrification causes increases in the δ15N of NO₃⁻, values >10% or well series that showed progressive δ15N enrichments were considered to have been altered. The mean δ15N
of NO$_3^-$ in the remaining samples was 2.6 ± 1.8% (23%, n = 18), consistent with groundwater derived from agricultural soils and/or fertilizers (~4-46%, Macko and Ostrom 1994, Kendall 1998). The most upland well series was 'excessively shallow groundwater' (Hedin et al. 1998) and had the least isotopic variation, indicating little influence from microbial activity. We, therefore, use the $\delta^{15}N$ value for NH$_4^+$ from this well series, 7.8 ± 3.3% (n = 8), to define the shallow groundwater endmember. This value is similar to that of soil organic matter collected at the site, 7.0 ± 3.2% (n = 13), and indicates NH$_4^+$ derived from the mineralization. Thus, inorganic N in the shallow and deep groundwater had unique origins that could be readily distinguished by $\delta^{15}N$.

Within stream water, the $\delta^{15}N$ of NH$_4^+$ and NO$_3^-$ were highly variable seasonally (Table 1). The mean $\delta^{15}N$ of NO$_3^-$ in stream water, 0.9 ± 2.4%, was indicative of an agricultural origin. NH$_4^+$ in stream water appeared to have a unique origin from NO$_3^-$ as revealed by a mean $\delta^{15}N$ of 10.0 ± 4.7%, similar to the isotopic composition of NH$_4^+$ in shallow groundwater. At each sampling of stream water the $\delta^{15}N$ of NO$_3^-$ was less than that of NH$_4^+$ and consistent with the direction of fractionation expected for nitrification (Mariotti et al. 1981, Yodaishi 1983, Brandes et al. 1996) or, during warmer periods, utilization of NH$_4^+$ during photosynthesis (Cliffrones et al. 1988). In Smith Creek, it is not entirely clear if the isotopic character of NH$_4^+$ and NO$_3^-$ is controlled to a greater extent by in-stream processes or by inputs of inorganic N from groundwater. It is likely that both are influential. The observation of low $\delta^{15}N$ values for NO$_3^-$ both in the stream and in wells closest to the stream suggests that the predominate flow paths from deep groundwater to the stream are least affected by denitrification. Thus NO$_3^-$ entering the stream largely escapes denitrification at the soil-stream interface. Within the stream, nitrification impacts a secondary isotopic signal on inorganic N as indicated by high and low N isotope values for NH$_4^+$ and NO$_3^-$, respectively. Despite the dynamic nature of stream inorganic N, the combination of unique concentrations and isotopic compositions of NO$_3^-$ and NH$_4^+$ in the stream provides a basis to distinguish this source of water to the sampling wells from shallow and deep groundwater.

### Table 1: Concentration and isotopic composition of NO$_3^-$ and NH$_4^+$ in Smith Creek stream water.

<table>
<thead>
<tr>
<th>Date</th>
<th>NO$_3^-$ (μM/L)</th>
<th>NH$_4^+$ (μM/L)</th>
<th>$\delta^{15}N$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 August 1992</td>
<td>5.6</td>
<td>51.6</td>
<td>-1.3</td>
</tr>
<tr>
<td>2 December 1992</td>
<td>7.9</td>
<td>53.6</td>
<td>11.5</td>
</tr>
<tr>
<td>15 January 1993</td>
<td>5.7</td>
<td>54.2</td>
<td>2.0</td>
</tr>
<tr>
<td>3 March 1993</td>
<td>9.0</td>
<td>51.3</td>
<td>3.1</td>
</tr>
<tr>
<td>7 April 1993</td>
<td>6.4</td>
<td>58.9</td>
<td>6.0</td>
</tr>
<tr>
<td>5 May 1993</td>
<td>0.2</td>
<td>24.3</td>
<td>ND</td>
</tr>
<tr>
<td>6 June 1993</td>
<td>4.9</td>
<td>25.0</td>
<td>ND</td>
</tr>
<tr>
<td>26 July 1993</td>
<td>4.4</td>
<td>31.7</td>
<td>ND</td>
</tr>
<tr>
<td>25 August 1993</td>
<td>5.9</td>
<td>33.4</td>
<td>13.3</td>
</tr>
<tr>
<td>7 October 1993</td>
<td>3.6</td>
<td>34.3</td>
<td>ND</td>
</tr>
<tr>
<td>16 November 1993</td>
<td>4.4</td>
<td>35.1</td>
<td>9.9</td>
</tr>
</tbody>
</table>

*Note: ND indicates that, owing to low concentration, the $\delta^{15}N$ was not determined.*
Table 2. Isotopic enrichment factors for denitrification in groundwater, soils, seawater, laboratory cultures and inorganic or theoretical reactions.

<table>
<thead>
<tr>
<th>Environment type</th>
<th>Isotopic enrichment factor, ε (%)</th>
<th>Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground water</td>
<td>-30 ± 69</td>
<td>confined aquifer, western Karahari Desert, South Africa</td>
<td>Vogel et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>-3.0 ± 4.7</td>
<td>chalk aquifer, northern France</td>
<td>Mariotti et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>-5.3 ± 4.0</td>
<td>shallow alluvial groundwater, southern France</td>
<td>Furst et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>-15.9</td>
<td>unconfined sand and gravel aquifer, Germany</td>
<td>Böttcher et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>-14.6</td>
<td>unconfined sand and silt, sou aquifer, Germany</td>
<td>Glisic et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>-13.9</td>
<td>unconfined sand and gravel aquifer, Cape Cod, Massachusetts, USA</td>
<td>Smith et al. (1961)</td>
</tr>
<tr>
<td></td>
<td>-27.6</td>
<td>riparian zone, Ontario, Canada</td>
<td>Menges et al. (1999)</td>
</tr>
<tr>
<td>Soil</td>
<td>-19.1 ± 6.5</td>
<td>75 g dried soil, 125 ml HNO₃, 0.01 M NaCl,</td>
<td>Chien et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>-11</td>
<td>75 g dried soil, 125 ml HNO₃, 0.01 M NaCl, 1% glucose</td>
<td>Chien et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>75 g dried soil, 125 ml HNO₃, 0.01 M NaCl</td>
<td>Chien et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>-4</td>
<td>10 g soil, 6 ml H₂O, 10 mg NO₂-N, 7.5 M glucose</td>
<td>Blackmer and Brenner (1977)</td>
</tr>
<tr>
<td></td>
<td>-29.4 ± 24.6</td>
<td>100 g dried soil, N-service, 100 ml HNO₃, 20 mg</td>
<td>Mariotti et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>-33.3 ± 14.6</td>
<td>NO₂-N, acetylene</td>
<td>Mariotti et al. (1982b)</td>
</tr>
<tr>
<td></td>
<td>-11.1</td>
<td>100 g soil, 100 ml H₂O, 20 mg NO₂-N or NO₃-N</td>
<td>Mariotti et al. (1982b)</td>
</tr>
<tr>
<td></td>
<td>-6.0 ± 5.6</td>
<td>in situ, forested soils, Japan</td>
<td>Koba et al. (1997)</td>
</tr>
<tr>
<td>Seawater</td>
<td>-20</td>
<td>incubation of a marine diatom, 1.53 g yeast, 2.56 g peptone, (1578 g NaNO₃, 500 ml seawater</td>
<td>Miyake and Wada (1971)</td>
</tr>
<tr>
<td></td>
<td>-39</td>
<td>incubation of marine bacterium, Seriata marinna rubra, 0.5% polyethylene, 0.1% yeast extract, 0.1% KNO₃, 3.556 NaCl, pH 7.5</td>
<td>Miyazaki et al. (1980)</td>
</tr>
<tr>
<td></td>
<td>-40 ± 30</td>
<td>eastern tropical north Pacific</td>
<td>Cline and Kaplan (1975)</td>
</tr>
<tr>
<td>Laboratory cultures</td>
<td>-20</td>
<td>Pseudomonas stutzeri, 10-30 mmol/L NO₂-N or NO₃-N</td>
<td>Wellman et al. (1968)</td>
</tr>
<tr>
<td></td>
<td>-20.8 ± 13.4</td>
<td>pseudomonas demineralizes</td>
<td>Delwiche and Stryj (1970)</td>
</tr>
<tr>
<td></td>
<td>-20.0</td>
<td>assimilatory nitrate reduction by pearl milt</td>
<td>Mariotti et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>-15.8</td>
<td>Pseudomonas stutzeri, 0.86 mmol/L NO₂-N</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-6.9</td>
<td>Pseudomonas stutzeri, 0.14 mmol/L NO₂-N</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-23.5</td>
<td>Pseudomonas stutzeri, 25 mmol/L succinate, 0.07-2.2 mmol/L NO₃-N</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-22.6</td>
<td>cell-free extracts of P. stutzeri, 25 mmol/L succinate, 0.07-2.2 mmol/L NO₃-N</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-18.0</td>
<td>Pseudomonas stutzeri, 0.29 mmol/L NO₃-N, 0.01-25 mmol/L succinate</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-33.3 ± 10</td>
<td>cell-free extracts of P. stutzeri, 2.1 mmol/L NO₃-N, 0.75-30 mmol/L phenazine methosulfate</td>
<td>Bryan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>-11</td>
<td>Pseudomonas stutzeri, 0.107 mmol/L NO₃-N, 25 mmol/L succinate</td>
<td>Shearer and Kohle (1988)</td>
</tr>
<tr>
<td>Inorganic/ theoretical</td>
<td>-65.9</td>
<td>calculated equilibrium isotope exchange between</td>
<td>Urey (1947)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH₃ and NO bonds</td>
<td>Brown and Drury (1967)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alkaline reduction with Fe(OH)</td>
<td>Brown and Drury (1967)</td>
</tr>
<tr>
<td></td>
<td>-75</td>
<td>alkaline reduction with Fe(OH)</td>
<td>Brown and Drury (1967)</td>
</tr>
<tr>
<td></td>
<td>-34</td>
<td>diffusion of NO₃ in aqueous solution</td>
<td>calculated</td>
</tr>
<tr>
<td></td>
<td>-6.2</td>
<td>diffusion of NO₃ hydrated with one water molecule</td>
<td>calculated</td>
</tr>
</tbody>
</table>

1 Mean ± 1 σ
fication but a consequence of the interaction of two groundwater flows, the shallow flow rich in NH₄⁺ and the deep flow present near the stream that is rich in NO₃⁻. Secondly, microbial processes impart changes in the concentration and isotopic composition of inorganic N. The decline in NH₄⁺ concentrations toward the stream could be interpreted as representing nitrification of a single flow path, however, this is clearly not the case, as each well series samples a unique flow path with a unique history. The predominance of nitrate on geochemical trends within the interface is the physical mixing of two distinct groundwater flows. The interaction and mixing of the two flows provide the necessary substrates to enhance microbial activity which impacts a secondary control on the abundances and 8/15N values of inorganic N.

The concentration of NO₃⁻ within the soil-stream interface is a balance between supply from stream and deep groundwater and loss from denitrification. The presence of deep groundwater was evident by the observation of maximum NO₃⁻ concentrations for most sampling periods, in well series (4 and 5) located just inland from the stream well series (Fig. 1). The pattern of variation in concentration and isotopic abundances within both sites were similar, however, site 2 generally had greater NO₃⁻ concentrations. Higher NO₃⁻ at site 2 suggests that this site was more influenced by deep groundwater and differences between the sites is likely a consequence of heterogeneities within floodplain deposits. Highest 8/15N values tended to occur in the well series just inland of the peak in NO₃⁻ concentration and lowest 8/15N values were found within the region of maximum NO₃⁻ concentrations (Fig. 1). Such trends are consistent with bank storage of streamwater following high-discharge events in the well series closest to the stream (McCredy et al. 2001). The high content of NO₃⁻ and deep groundwater near the stream, and denitrification in the inland well series. Consequently, we interpret a streamwell with shallow water series with marked declines in NO₃⁻ abundance and high 8/15N values as an upland direction as a zone of denitrification (Fig. 1).

On two occasions, site 1 on April 1993 and site 2 on November 1993, a decline in the 8/15N of NH₄⁻ toward the stream was concomitant with moderate NO₃⁻ concentrations (Fig. 1). A similar decline in NH₄⁺/8/15N was observed at site 1 on the same day in November 1993, however, much lower concentrations of NO₃⁻ (<1 µM/l) were observed. The decline in the 8/15N of NH₄⁺ is opposite the direction of fractionation for nitrification and may be explained as resulting from dissimilatory NO₃⁻ reduction to NH₃⁺ (DNRA). Within low-8/15O environments, denitrification may produce the gaseous products N₂ and N₂O, or, alternatively, yield NH₃⁺ via the less common pathway of DNRA (Korom 1992). Denitrification has been hypothesized to prevail when the dissolved organic carbon (DOC) to electron acceptor ratio is low and DNRA favored as this ratio increases (Tiedje et al. 1982, Bengtsson and Annadotte 1989). Thus, N₂ production is most efficient within electron-donor-limited environments (low DOC) and NH₄⁺ production is likely favored when NO₃⁻ is limiting and electron donors are abundant (high DGC). Both conditions occur at the intersection of the shallow and deep groundwater flows since it is within this unique region that electron donors and acceptors range from trace to abundant levels (see Hedin et al. 1998). Although we are not aware of published fractionation factors for DNRA, the decrease in the 8/15N of NH₄⁺ is consistent with the direction of fractionation expected for this process (McCredy et al. 1983). Evidence for DNRA was only present during two sampling dates (7 April 1993 and 16 November 1993), indicating that the area over which this process occurs is very restricted spatially and may be limited by its unique geochemical requirements.

Assessment of water-mass mixing and quantifying denitrification

The quantity of NO₃⁻ in any sample is a function of the concentration in soil relative contribution of water masses contributing to that sample. The influence of stream water on the sampling wells was only occasionally evident and readily distinguished from the shallow and deep groundwater masses, consequently the mass of NO₃⁻ (Q) can be described as a mixture of two flows:

\[ Q = Q_v + Q_s \]

(2)

in which Q is defined as the concentration of NO₃⁻ weighted by the volume of water. In our example, we denote the shallow and deep groundwater flows by the subscripts a and b, respectively. By consideration of isotopic mass balance the following relation can be written that describes the isotopic composition of NO₃⁻ in any sample (b), resulting from the mixture of two water masses:

\[ bQ_b = aQ_a + bQ_s \]

(3)

Eqs. 2 and 3 can be combined to obtain (Mariotti et al. 1988):

\[ bQ_b = aQ_a + bQ_s \]

(4)

Within shallow groundwater (well series 1–3) NO₃⁻, if detected, was a small fraction, <2%, of that in the near-stream well series. Consequently, the mass of NO₃⁻, Q, in the shallow flow is approximated as zero. Because shallow groundwater essentially lacks NO₃⁻, Eq. 4 indicates that regardless of the degree of mixing of shallow and deep groundwater the isotopic composition of NO₃⁻ is unchanged and equal to the deep flow, b. The 8/15N of NO₃⁻, however, is both a function of mixing and denitrification.

Isotopic fractionation during many microbial reactions, including denitrification, has been described using a Rayleigh distillation equation in which the isotopic composition of the residual substrate of a reaction
The fraction of NO_3^- removed due to denitrification, f_d, can be determined independently of a concentration measurement:

\[ f_d = 1 - \rho_d \]  

(8)

where \( f_d \) is equal to 1 - C/C_0.

A fundamental limitation to the use of Eqs. 5 and 8 to describe denitrification in the natural environment is the wide range of isotopic enrichment factors, ~-75-0%, reported for this process (Table 2). The greatest degree of fractionation during rupture of N=O bonds has been documented for inorganic reactions (Brown and Druy 1967) or calculated based on bond-strength differences between the trac and abundant isotopes (Urey 1947). The lack of observed fractionation of this magnitude during denitrification in laboratory and field studies is an indication that additional factors minimize the observed fractionation. Isotopic enrichment factors can be determined from field studies if the fundamental assumptions inherent in the use of the Rayleigh equation, mainly a unidirectional reaction within a closed system, are not severely compromised. In many groundwater environments these assumptions are reasonably valid.

Isotopic fractionation during denitrification is largely controlled by the velocity of microbial reduction and varies proportionally with the initial concentration of NO_3^- or NO_2^- or inversely with the concentration of organic reductant (Kohl and Shearer 1977, Mariotti et al. 1982a, Bryan et al. 1983, Shearer and Kohl 1988). The isotope effect during denitrification is described by the following pathway in which discrimination varies as a function of NO_3^- movement into or out of the cell and the reduction step:

\[ AH + H \rightarrow AN \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow \text{reduced N} \]

This latter process is dependent upon NO_3^- reductase (NR), and a supply of electron donors (AH). In the natural environment, NO_3^- is rarely observed, owing to its efficient reduction. Consequently, observation of an isotope effect during this step is unlikely. Fractionation during the movement of NO_3^- into or out of cells is small to negligible (Mariotti et al. 1982c, Bryan et al. 1983). Therefore, isotopic fractionation during denitrification is largely a consequence of the initial reduction of NO_3^- within the cell that is subsequently expressed upon egress. With this understanding the magnitude of fractionation during NO_3^- reduction depends on the relative rates of NO_3^- uptake, reduction of intracellular NO_3^-, and transport out of the cell. The relative importance of these rates depends on the relative concentrations of NO_3^- within and external to the cell, and abundance of NR and reductant within the cell (Mariotti et al. 1982c). When NO_3^- is abundant outside the cell, the supply of NO_3^- to the cell is high and the reduction step is not substrate limited. This
allows expression of the isotope effect during reduction within the cell. In contrast, when the supply of NO\textsubscript{3}\textsuperscript{-} to the cell is less than the rate of reduction, little if any NO\textsubscript{3}\textsuperscript{-} escapes the cell to express the isotope effect. Hence, the observed isotope effect during reduction is proportional to the external concentration of NO\textsubscript{3}\textsuperscript{-} (Bryan et al. 1983). Similarly, the availability of the electron donor can minimize expression of fractionation if abundant or maximize expression if limiting.

This argument emphasizes the importance of DOC as an electron donor in the natural environment as a control on the magnitude of isotope fractionation during denitrification. Availability of DOC may, in part, explain why fractionation tends to be greater in agricultural soils which are often depleted in DOC, relative to forest soils where DOC tends to be more abundant (Mariotti et al. 1981, Delprat et al. 1997, Kubo et al. 1997). Indeed, addition of glucose to soils has been
shown to reduce the expression of fractionation (Blackmer and Bormer 1977, Chien et al. 1977, Micotti et al. 1982a, b). Furthermore, a limitation of DOC might explain why isotopic fractionation is greater in arsenic-limited groundwater (Vogel et al. 1981) and is smaller in DOC-rich groundwater receiving sewage effluent (Smith et al. 1993).

To assess the relative importance of mixing and denitrification as controls on the abundance of NO$_3^-$ in the soil–stream interface it is essential to characterize the initial concentration and isotopic composition of NO$_3^-$ in deep groundwater and the $e$. We estimated that deep groundwater has an initial $^{15}$N value of 2.6 $\pm$ 1.8‰. Since dilution and denitrification reduce NO$_3^-$ concentrations within groundwater, we assume the initial concentration in the deep groundwater is equivalent to the maximum observed concentration of 274.4 μmol/L. Because $e$ was not determined within the soil–stream interface and may vary with DOC supply, we have chosen a range of values (from $-15.9$ to $-4.0$‰) from studies within similar environments and in which assumptions inherent in the use of the Rayleigh equation are reasonably valid (Table 2). These include values for $e$ determined within shallow flood-plain groundwater (Fustec et al. 1991), forest soils (Koba et al. 1997), and unconfined aquifers in Cape Cod, Massachusetts, USA (Smith et al. 1991) and Germany (Böttcher et al. 1990, Gischke et al. 1998). In Fig. 2, we present the concentration and $^{15}$N values for NO$_3^-$ in the soil–stream interface for samples not influenced by stream water in relation to the concentration and $^{15}$N of NO$_3^-$ in deep groundwater and the solution of Eqs. 4 and 5 using values of $e$ of $-15.9$‰ and $-4.0$‰. The majority of NO$_3^-$ $^{15}$N values lie between a line produced by Eq. 5 using an $e$ value of $-15.9$‰, and a mixing line based on Eq. 4 (Fig. 2). The mixing line has a slope of 0, assuming shallow groundwater lacks NO$_3^-$, and hence mixing has no effect on $^{15}$N. Most of the points outside the mixing and fractionation lines are within 1 s.d. of the estimated $^{15}$N value for the deep groundwater endmember (2.6 $\pm$ 1.8‰) and likely represent uncertainty in characterizing the concentration and isotopic composition of NO$_3^-$ of the endmember. In addition, nitratation and DNRX may have also resulted in shifting some values outside the region between mixing and fractionation lines (Fig. 2). With these few exceptions, the model presented in Fig. 2 successfully describes the concentration and isotopic composition of NO$_3^-$ within the soil–stream interface of Smith Creek. An $e$ value of $-4$‰ is a poor predictor.
To assess the fraction of NO$_3^-$ reduced by denitrification within the soil-stream interface we applied the isotopic composition of NO$_3^-$ in the deep groundwater, 2.6%, as $\delta_{15}N$ and $\delta^{18}N$ values for each well series on a particular sampling date at $\delta_{15}N$ in Eq. 8. Mixing of water around would normally be utilized using a Rayleigh model, however, because the contribution of NO$_3^-$ in shallow groundwater is negligible and concentration measurements were not requested, Eq. 8 can be used to quantify NO$_3^-$ removal. Based on an isotopic enrichment factor of $-15.9\%$, the percentage of NO$_3^-$ removal varied from nondetectable levels to $86\%$ (Fig. 3). Our highest estimate of removal is remarkably similar to what we obtained previously upon addition of DOC (31%; Hedlin et al. 1998). The use of $-15.9\%$ for $\epsilon$ provides lower estimates of NO$_3^-$ removal than the use of $-57\%$; hence our results are conservative.

Fig. 5. (A) Hydrologic flow. (B) percentage of NO$_3^-$ removal. (C) corrected NO$_3^-$ concentrations, and (D) quantity of NO$_3^-$ removed for site 2. The corrected concentration of NO$_3^-$ is the sum of the observed concentration and losses due to denitrification. Error bars indicate $\pm 2$ standard deviations of the mean. The measurements of hydrologic data and flow measurements of NO$_3^-$ concentration, NO$_3^-$ removal in well series 3 (390 cm) consisted of only one measurement, and in well series 1 (320 cm) and 2 (160 cm) NO$_3^-$ removal was not detected.

of the data, hence we believe that $-15.9\%$ is more appropriate. Since most of the data presented in Fig. 2 lie to the left of the $-15.9\%$ deuterofication line, either mixing of the shallow and deep groundwater was sub-

stantially and/or $\epsilon$ varied between $-15.9\%$ and $-4.0\%$. Variations in the deuterofication fractionation factor by the soil-stream interface microbial community is quite likely given that the availability of DOC changes with the relative dominance of the two groundwater flows. The use of $-15.9\%$ for $\epsilon$ (Eq. 8) describes the data well and results in low estimates of NO$_3^-$ removal relative to a value of $-45\%$.

Fig. 6. Conceptual diagram of changes in the concentration and isotopic abundances of NO$_3^-$ and NR$_x$ along the soil-stream interface of Augusta Creek in relation to hydrologic and flow. A) 28 August 1992, a period of low discharge. B) 7 May 1993, a period of high discharge. Inset shows the measured data on NO$_3^-$ and NR$_x$ for the respective dates. The percentage of NO$_3^-$ removal is shown above each well series for which values were $>0$. The well nearest to the stream 4 m, and this depth of penetration by each well is 40 cm. Hence, the trends shown below the wells are largely conjectural. Hypothesized gradients in NO$_3^-$ and NR$_x$ concentrations and the trend of denitrification are shown. The intersections of three flows are evident: a shallow NR$_x$-rich flow, a deep NO$_3^-$-rich flow, and stream water in back storage.
discharge, 7 April to 8 June 1993 (Fig. 4), had lower NO$_3^-$ removal than most other periods. While more detailed sampling is needed to verify if this trend is consistent, our preliminary results suggest that a reduction in NO$_3^-$ removal within the soil stream interface may result from periods of high stream discharge.

Upon assessment of the hydrologic flow ($F$) within each water mass, the concentration of NO$_3^-$, and estimates of the fraction of NO$_3^-$ removal, the magnitude of NO$_3^-$ loss due to denitrification (NR) at the soil-stream interface can be obtained

$$NR = FIC - C$$

(9)

in which the concentration of NO$_3^-$ prior to denitrification, C, is equivalent to $C(1 + \epsilon$). On four occasions during the twelve months following September of 1993 we quantified hydrologic flow in each of the well series at site 2 (Heddle et al. 1998). Within this same time period, we determined the fraction of NO$_3^-$ removal on three different occasions (Fig. 5). Based on the mean hydrologic and isotope measurements we determined that along the 4-m well-series transient NO$_3^-$ removal varied from undetectable levels to as much as 123 $\mu$mol L$^{-1}$ m$^{-1}$ d$^{-1}$ or 1.73 g m$^{-1}$ d$^{-1}$ (Fig. 5). Our estimate of NO$_3^-$ removal is substantially greater than the range of 2.6-10.1 $\mu$mol L$^{-1}$ m$^{-1}$ d$^{-1}$ reported for the biologically active riparian zone by Lowrance et al. (1995). The spatial coverage of these two studies, however, is markedly different and the contrast in NO$_3^-$ removal is likely the result of our emphasis on a small zone of high microbial activity (4 m) that is not readily sampled by studies focusing on much larger scales. Seasonal variation in hydrologic flows and denitrification activity and the fact that our hydrologic and isotopic measurements were means of nonconcomitant measurements may all contribute uncertainty to our results. We present our results as an illustration of the use of natural abundance isotope data in this regard and a preliminary estimate of NO$_3^-$ loss due to denitrification within the environment. Natural-abundance isotope studies have several advantages over incubation studies that involve artificial conditions on the microbial community and our approach illustrates that these sampling strategies provide a new measure of NO$_3^-$ removal.

Summary of geochemical and hydrological changes at soil-stream interface

In Fig. 6 we present a conceptual model of N-cycling dynamics within the soil-stream interface. This figure shows a comparison of our interpretations of changes in inorganic N for two sampling periods: the time of lowest stream discharge (26 August 1992) and a period of strong groundwater flow at the interface and high stream discharge (5 May 1993). As mentioned, the soil-stream interface consists of the intersection of three flows, a shallow flow rich in NH$_4^+$ and DOC, a deep flow rich in NO$_3^-$, and depleted in DOC, and stream flow depleted in NO$_3^-$, NH$_4^+$, and DOC. The two predominant processes affecting inorganic N within the soil-stream interface are nitrification associated with the shallow flow and denitrification occurring at the intersection of the shallow and deep groundwater flows. Although nitrification acted to reduce NH$_4^+$ concentrations, there was only occasional evidence of iso- tactic fractionation associated with this process. Consequently, we believe that nitrification is active at shallow depths than sampled by the wells and most likely occurs in the vicinity of the saturated-un saturated interface where supplies of O$_2$ for this process are available (the water-table boundary in Fig. 6). Hence, consistent reductions in NH$_4^+$ concentrations toward the stream reflect nitrification and as increased predominance of deep groundwater at the depths sampled by the wells. Concentrations of NO$_3^-$ on 5 May 1993 are much lower than those present on 26 August 1992, and we interpret this distinction as a consequence of a stronger deep-groundwater flow in May that displaced the shallow groundwater to shallower depths and more inland. In addition, the lower NH$_4^+$ concentrations in May could result from dilution of the shallow flow with NO$_3^-$-depleted rainwater.

Denitrification within the soil-stream interface is restricted to the region in which DOC can be supplied from shallow groundwater and NO$_3^-$ from deep groundwater. This zone occurs from the stream edge and as a result NO$_3^-$ in deep groundwater is able to enter the stream minimally affected by denitrification even though denitrification within the interface is substantial. We define a zone of denitrification that is indicated by elevated $^{15}$N values and appreciable NO$_3^-$ removal as determined from Fig. 8. The exact boundaries of the zone of denitrification are uncertain. The contours in Fig. 6 are based on values of NO$_3^-$ removal and expected boundaries for the intersection of the shallow DOC-rich groundwater and the NO$_3^-$-rich deep groundwater. Removal of NO$_3^-$ on 26 August 1992 was greater than that on 5 May 1993, however, the number of well series sampling the zone of denitrification was reduced. The data indicate that the zone of denitrification is variable in strength and location as a consequence of changes in hydrology. Although variations in discharge occurred at a higher frequency than our sampling interval, a relationship of changes in the relative predominance of the stream, shallow, and deep groundwater, and microbial processes to changes in hydrology is evident. The presence of at least three flows or approximately equal predominance of shallow and deep groundwater was characteristic of low-discharge or base-flow conditions (Figs. 1 and 4). Base flow may represent as close to a steady-state condition as the soil-stream interface obtains. During base flow, high $^{15}$N values indicated that denitrification was active in removing NO$_3^-$ within the interface and declines in NH$_4^+$ concentrations to undetectable levels toward the stream were indicative of nitrification or
dilution. Discharge between April and June of 1993 was generally high and characterized by several strong flow events. During this interval the soil-stream interface was characterized by a strong presence of the deep groundwater, even to the exclusion of the other water masses in May 1993. Under these conditions, nitratification was not evident within the study area, the shallow flow was likely shifted to an upland and shallow location, and NO$_3^-$ removal was low relative to base flow conditions. On 2 December 1992 and 15 January 1993, the soil-stream interface was characterized by a strong influence of the shallow groundwater and the presence of NH$_4^+$ in nearly all well series. Both of these sampling events occurred approximately 8–10 d following a period of maximum discharge. We interpret the predominance of shallow groundwater at these times to reflect a transition period between high groundwater discharge to base-flow conditions. The exact geochemical conditions and presence of the different water masses characteristic of high-flow, transition-flow, and base-flow conditions are not clear in this study because specific hydrologic events, such as a high rainfall period, were often measured directly by only one sampling period and no changes in hydrology occurred at a much shorter interval than we could sample. Consequently, our interpretations of the changes in N-geochemical dynamics of the soil-stream interface in relation to changes in hydrology require more detailed studies with higher sampling frequencies and use of conservative tracers to better distinguish water masses.

NO$_3^-$ removal from groundwater entering a stream is a desirable attribute of natural ecosystem function. Our study illustrates that substantial quantities of NO$_3^-$ can be removed prior to impacting the stream environment and may distal ecosystems. The proper functioning of the soil-stream interface in NO$_3^-$ removal, however, depends on a supply of DO to support denitrification in deep NO$_3^-$ rich groundwater upwelling at the stream edge. The supply of DO can be derived from organic-rich soils or surface- or sub-surface riparian systems (Hedin et al. 1998, DeBreo et al. 2000). NO$_3^-$ removal will not be fostered by intensive agriculture or the flood plain since agricultural environments tend to leach NO$_3^-$ rich and DOC-poor ground water (DePrat et al. 1997). Proper management of stream environments for NO$_3^-$ removal, therefore, should encourage the growth of forests on the flood plain that are characterized by the presence of high levels of DO in soil leachate (Hedin et al. 1995, DePrat et al. 1997). In addition, preliminary data are supported by subsequent studies, maintenance of hydraulic discharge in streams and rivers may have an effect on the quantity of NO$_3^-$ removed at soil-stream interfaces. Recent studies have shown benefits in inducing changes in discharge from dams as a mechanism for producing habitats beneficial to the recreation and fish reproduction (Rubis et al. 1998). An additional benefit of regulation of hydraulic discharge may be in optimizing the removal of NO$_3^-$ entering streams and rivers.

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