Ecological Applications, 12(4), 2002, pp. 1027–1043

NITROGEN TRANSFORMATIONS AND NO_3^- REMOVAL AT A SOIL–STREAM INTERFACE: A STABLE ISOTOPE APPROACH

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Abstract. The natural removal of NO_3^- 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 NO_3^- and NH_4^+ 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 NO_3^- removal within a soil–stream interface. Within this region three water masses with unique biogeochemical characteristics intersect: a shallow flow rich in NH_4^+ and dissolved organic carbon (DOC), a deep groundwater mass rich in NO_3^- but depleted in DOC, and stream water low in NO_3^-, NH_4^+, and DOC. N isotope values for NO_3^- 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 NO_3^- via denitrification that was dependent upon a supply of DOC from shallow groundwater.

Quantitative estimates of the fraction of NO_3^- removed due to denitrification within the soil–stream interface were obtained from N isotope data using a modified Rayleigh equation. Conservative estimates of NO_3^- removal range from 0% to 86%. In conjunction with measurements of hydrological flows within the sampling wells we provide a novel estimate of NO_3^- removal based only on natural abundance isotope measurements. NO_3^- removal was found to vary from undetectable levels to 123 $mmol \cdot L^{-1} \cdot m^{-2} \cdot d^{-1}$ 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; nitrification; nitrogen isotopes; 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 remediate excess anthropogenic NO_3^- loadings (Lowrance et al. 1984, Cooper 1990, Triska et al. 1993*a*, *b*, Hanson et al. 1994, Jansson et al. 1994, Groffman et al. 1996, Hedin et al. 1998, Cey et al. 1999, Devito et al. 2000). While it is clearly recognized that denitrification within riparian zones can remove NO_3^- 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 NO_3^- 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 NO_3^- removal within soil–stream interfaces by evaluation of data on the concentration and isotopic composition of NO_3^- and NH_4^+ 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 (Hedin et al. 1998) by delineating in detail seasonal variation in NO_3^- removal and microbial controls on the isotopic composition of NO_3^- and NH_4^+. Furthermore, this study presents a new approach, based on natural abundance isotope data, for quantifying NO_3^- 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, Feigin et al. 1974, Kreitler and Jones 1975, Peters et al. 1978, Saino and Hattori 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 $\delta^{15}N$ signatures often behave nonconservatively within ecosystems as isotopic alteration can be imposed by mi-

Manuscript received 4 December 2000; revised 19 July 2001; accepted 17 September 2001; final version received 15 October 2001.

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crobial processes, plant utilization, and volatilization of NH_3 from soils (Hauck et al. 1972, Heaton 1986, Durka et al. 1994, Macko and Ostrom 1994, Nadelhoffer and Fry 1994, Ostrom et al. 1998). Because sources are often isotopically heterogeneous and N-isotope signatures in inorganic N tend to be nonconservative, evaluations of origins based on $\delta^{15}N$ are not likely to be better than semiquantitative (Heaton 1986, Durka et al. 1994, Hedin 1994, Macko and Ostrom 1994, Ostrom et al. 1998). Nonetheless, because enormous perturbations to N pools and cycles in nature result from anthropogenic activities, understanding origins and processes is imperative and the use of N isotopes is likely to continue despite these difficulties (Vitousek et al. 1997, Kendall 1998, Kendall and Aravena 1999).

Nonconservative behavior is due to several factors: (1) numerous N-cycling processes discriminate between N isotopes (e.g., denitrification, nitrification, ammonification, uptake, and volatilization), (2) the magnitude of isotopic segregation (as described by fractionation factors) during specific reactions tends to vary, (3) the manifestation of fractionation factors in natural environments often depends on local environmental 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 $\delta^{15}N$-signature of a N source is well characterized in space and time. Less appreciated, however, 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 behavior can provide insight into the predominant microbial and plant processes influencing N cycles in ecosystems. Within riparian environments, for example, marked increases in the isotopic composition of NO_3^- have provided clear evidence of NO_3^- removal by denitrification (Aravena and Robertson 1998, Hedin et al. 1998, Cey et al. 1999, Mengis et al. 1999).

An ideal environment to take advantage of nonconservative behavior of N isotopes is one in which signals imparted by different microbial processes are strong and spatially segregated. Here we document N transformations and quantify the role of denitrification in such an environment: a riparian wetland. The study was conducted along a highly spatially constrained well series (4 m horizontal range) in which the intersection of geochemically unique water masses results in variation in electron donors and acceptors from abundant to trace levels (Hedin et al. 1998). This fine spatial resolution enabled us to study processes occurring over short distances that may have been overlooked or undersampled in other studies which sampled greater areas. We use a theoretical approach to explicitly estimate how isotopic variation associated with microbial processes vary independently from differences arising from a mixture of sources. We carefully consider the causes of variation in fractionation factors during denitrification. Finally, we calculate the magnitude and spatial pattern of NO_3^- consumption by denitrification along integrated hydrologic flow paths.

Our results have considerable importance for the many $\delta^{15}N$ source–attribution studies in downstream aquatic ecosystems. First, we show that isotopic discrimination within soil–stream interfaces can be marked and can occur over short spatial distances. Furthermore, isotopic alteration may result from processes within the stream itself, such as nitrification and assimilation during photosynthesis, that may compromise downstream source apportionment. Finally, we illustrate that despite the considerable challenges in applying $\delta^{15}N$ techniques within natural ecosystems, it is possible to study and quantify processes, such as NO_3^- 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 within 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 77-km^2 watershed was located up gradient from the study site. The well fields were located within a riparian wetland which was vegetated predominantly with sedges and grasses and surrounded by an oak–hickory forest. 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 Hedin and Brown (1994) and Hedin 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 m^2 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 84 samples were collected for isotopic analysis, however, not all samples were analyzed for $\delta^{15}N$ 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 compromising hydrologic flow. Consequently, ~equal volumes of water from wells equidistant from the stream were pooled to provide sufficient sample for the concentration and isotopic data presented in this paper. This approach has been justified by the observation of

similar concentrations of NO_3^- and NH_4^+ in well series equidistant from the stream (Hedin et al. 1998). Slight variations in hydrologic flow across the well field, however, may result in small-scale differences in concentrations and isotopic abundances, therefore, the N isotope 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 filtered within 3 h of collection through prerinsed Gelman A/E glass fiber filters (Pall Gelman Laboratory, Ann Arbor, Michigan, USA), refrigerated at 4°C, and analyzed within 2 wk. We observed no significant change in concentration for ≤4 wk of storage using this protocol. Concentrations of NO_3^- were determined via ion chromatography using chemically suppressed conductivity detection and a Dionex AS4A column (Dionex, Sunnyvale, California, USA). Alpkem automated colorimetry was used to assess NH_4^+ concentrations (Alpkem 1992). Samples for determination of NO_3^- and NH_4^+ $\delta^{15}N$-values were similarly filtered and stored frozen (−20°C) until analyzed. Dissolved O_2 was quantified 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:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R is the abundance ratio of the heavy to light isotope and the internationally recognized standard for N is atmospheric N_2 gas. Extraction of NO_3^- for isotopic analysis was performed by standard steam-distillation procedures (Bremner and Keeney 1966, Velinsky et al. 1989, Ostrom et al. 1998). To convert NH_4^+ to volatile NH_3 gas the pH of the distillate was shifted to ≥10 by addition of 1 mL of NH_4^+-free 5-mol/L NaOH. Finely ground Devardas alloy (Fisher Scientific, Chicago, Illinois, USA), 0.3 g, was added to the distillate to reduce NO_3^- in the sample to NH_3 (Kreitler 1975). The condensate from the distillation was collected in a flask containing 20 mL of 0.03-mol/L HCl. NH_4^+ was bound by absorption onto 100 mg of a zeolite molecular sieve (Union Carbide Ionsiv W-85: UOP Molecular Services, Des Plaines, Illinois, USA; Velinsky et al. 1989). The sieve containing bound NH_4^+ was dried at 40°C and combusted in the presence of excess copper oxide and copper to obtain N_2 gas for isotopic analysis (Macko 1981). Evacuated samples were heated to 850°C and cooled gradually overnight to prevent the formation of carbon monoxide and N oxides. N gas was separated cryogenically from carbon dioxide and other combustion products on a vacuum line and analyzed for isotopic abundances on a Micromass Prism stable isotope ratio mass spectrometer (Micromass, Manchester, UK). The presence of background NH_4^+ from the distillation of NH_4^+-free water was undetectable. However, upon the addition of the Devardas alloy, 1.4 ± 0.6 μmol/L NH_4^+ was observed. Consequently, all NO_3^- samples were background corrected using a mass-balance equation (Ostrom et al. 1998). No correction was necessary for NH_4^+ $\delta^{15}N$. Because of NH_4^+ contamination from reagents, no samples were analyzed that had a concentration <7 μmol/L NO_3^-. Replicate samples deviated by 1‰ or less.

Results and Discussion

The dynamic nature of N cycling along the soil–stream interface of Smith Creek was illustrated by variations in the concentration and isotopic composition of NO_3^- and NH_4^+ on a spatial and seasonal basis (Fig. 1). Inorganic N varied from detection limits to nearly 270 μmol/L; such changes in concentrations often occurred over a distance of <1 m. The range in $\delta^{15}N$ 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_3^- ranged from a minimum of −7.7‰ to a maximum of 34.1‰. A narrower range was found for the $\delta^{15}N$ of NH_4^+ of 1.1–15.7‰. N utilization by plants is not likely to have such a control over the small scale of this study (4 m) and involves only a slight isotope effect (Nadelhoffer and Fry 1994). These changes in concentration and isotopic compositions within such a narrow region cannot be explained by contributions from sources alone but reflect isotopic fractionation associated with microbial processes. Large isotopic variation can complicate interpretations 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_3^- removed within the interface. Quantifying NO_3^- removal without disturbing the environment (such as the use of soil chambers and artificial substrates) is a new advance that potentially can be extrapolated to larger spatial scales.

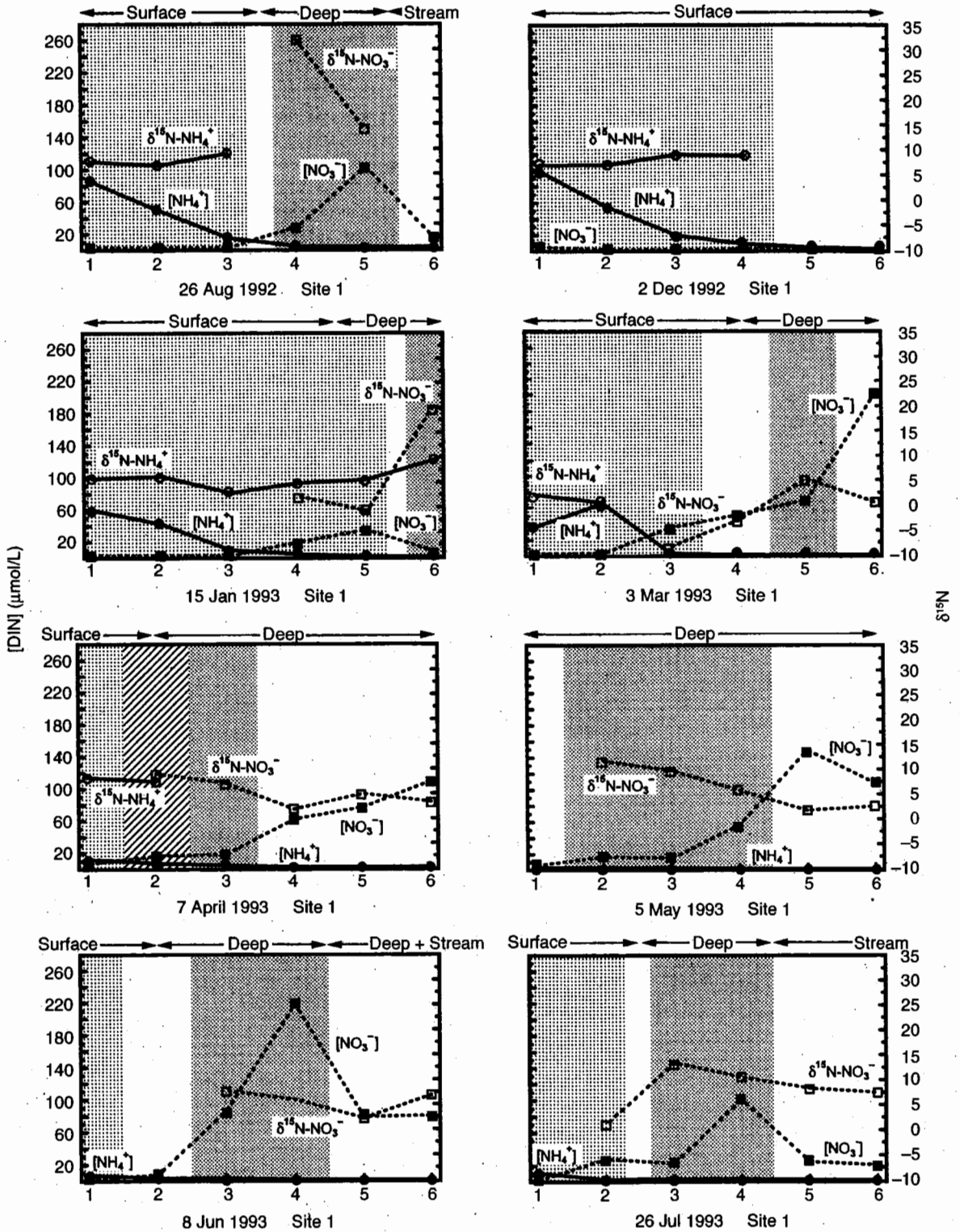

FIG. 1. The concentration of NO_3^- and NH_4^+ ([DIN], concentration of dissolved inorganic nitrogen) and isotopic composition in well series sampling the soil–stream interface at sites 1 and 2 on Augusta Creek, Michigan, USA. Well series 6 was within the stream itself; well series 1 was farthest inland. Analyses were determined 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 NO_3^- and NH_4^+. Zones of nitrification, dissimilatory NO_3^- reduction to NH_4^+ (DNRA), and denitrification were assessed based on unique shifts in the isotopic composition of NO_3^- and NH_4^+. Dotted lines connecting solid squares represent $[NO_3^-]$; dotted lines connecting open squares, $\delta^{15}N\text{-}NO_3^-$; solid lines connecting solid circles, $[N\text{-}NH_4^-]$; and solid lines connecting open circles, $\delta^{15}N\text{-}NH_4^-$.

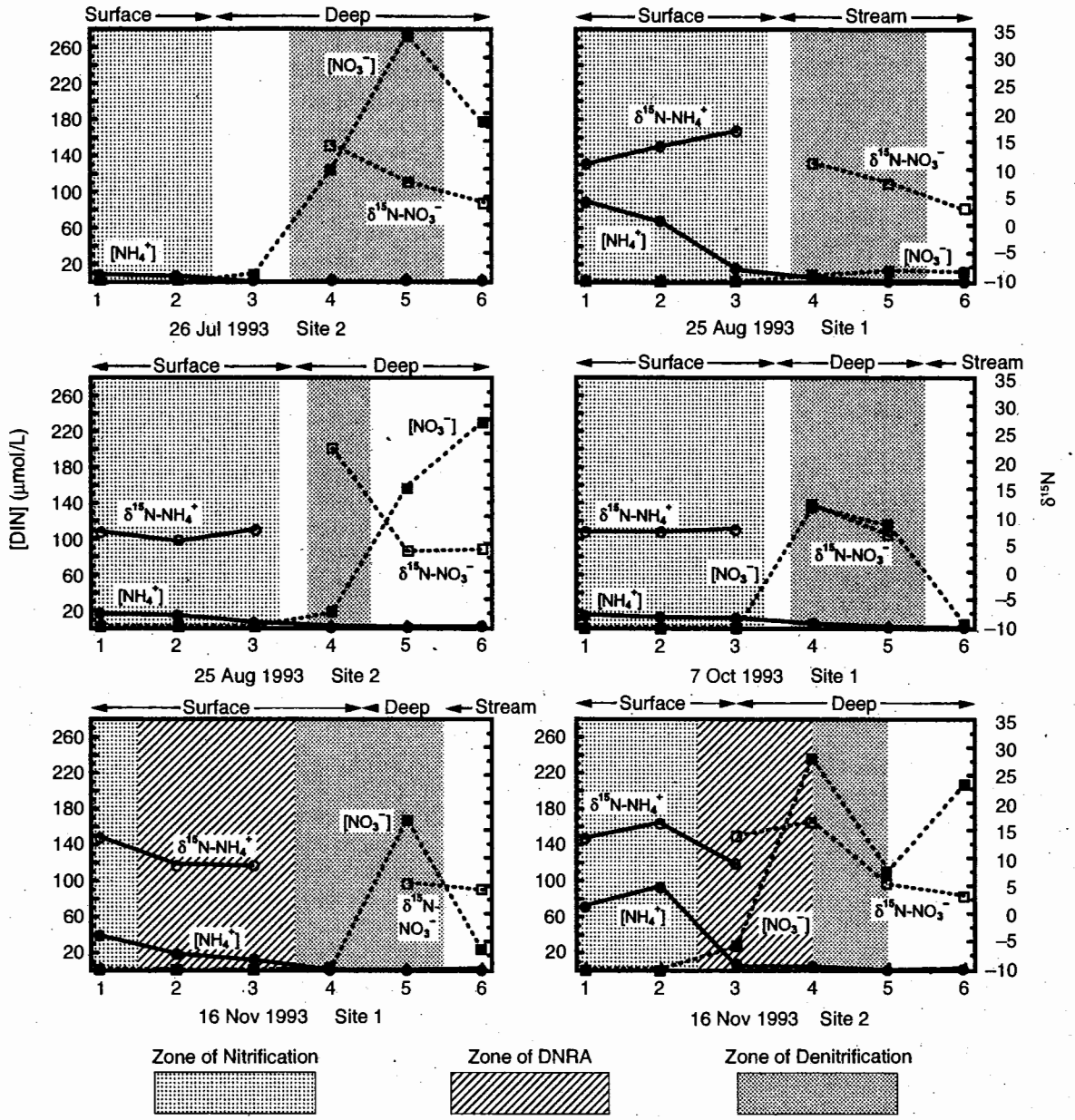

FIG. 1. Continued.

Sources of inorganic N

To characterize the $\delta^{15}N$ 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 NH_4^+ and NO_3^-, 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 NH_4^+ and NO_3^-, respectively (Hedin et al. 1998). With this understanding, the concentrations of NH_4^+ and NO_3^- 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 NH_4^+ and NO_3^- followed trends expected for nitrification or denitrification. Well series with the greatest concentrations of NO_3^- were generally characterized by low $\delta^{15}N$ values and likely reflect times of strong deep-groundwater input with minimal NO_3^- removal by denitrification. As denitrification causes increases in the $\delta^{15}N$ of NO_3^-, values >10‰ or well series that showed progressive ^{15}N enrichments were considered to have been altered. The mean $\delta^{15}N$

TABLE 1. Concentration and isotopic composition of NO_3^- and NH_4^+ in Smith Creek stream water.

Date	$[NH_4^-]$ (μmol/L)	$[NO_3]$ (μmol/L)	$\delta^{15}N$-NH_4^+ (‰)	$\delta^{15}N$-NO_3^- (‰)
26 August 1992	1.6	51.6	ND	−1.3
2 December 1992	7.9	53.6	11.5	−7.4
15 January 1993	5.7	57.1	14.2	0.1
3 March 1993	9.0	51.3	3.3	−3.1
7 April 1993	6.4	38.9	6.0	−2.3
5 May 1993	0.2	24.3	ND	3.4
8 June 1993	4.9	25.0	ND	5.6
26 July 1993	4.4	31.7	ND	3.5
25 August 1993	5.9	33.4	15.3	3.4
7 October 1993	3.6	34.3	ND	2.3
16 November 1993	4.4	35.1	9.9	6.1

Note: ND indicates that, owing to a low concentration, the $\delta^{15}N$ was not determined.

of NO_3^- in the remaining samples was 2.6 ± 1.8‰ (±SD, n = 18), consistent with groundwater derived from agricultural soils and/or fertilizers (−4–4‰; Macko and Ostrom 1994, Kendall 1998). The most upland well series was exclusively 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 ± 4.2‰, 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, Yoshida 1988, Brandes et al. 1996) or, during warmer periods, utilization of NH_4^+ during photosynthesis (Cifuentes 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 predominant 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 imparts 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.

Identification of water masses and microbial processes

Once the isotopic signature of NO_3^- and NH_4^+ within each of the three water masses is understood, characterization of concentration and isotope shifts permits identification of the predominant microbial processes at seasonal and spatial scales. The presence of each water mass can be recognized by unique inorganic N compositions: the shallow groundwater flow is rich in NH_4^+ but depleted in NO_3^-; the deep groundwater flow is rich in NO_3^- and deprived of NH_4^+; and stream water is depleted in both of these nutrients. Our interpretations of the location of each flow for each sampling date is shown in Fig. 1. Stream-water mass was rarely detected in the wells and only found in well series closest to the stream. Deep groundwater was generally present as an upwelling water mass in well series close to the stream, and shallow groundwater was present primarily in the inland well series (see also Hedin et al. 1998).

Unique concentration and isotope shifts in NO_3^- and NH_4^+ provide a basis to identify the predominance of a microbial process in the environment. Fractionation within the N cycle is almost exclusively controlled by kinetic rather than equilibrium isotope effects and, therefore, largely results from differences in the reaction rates of the heavy and light isotope (Macko and Ostrom 1994). Thus, reaction products tend to show depletions in ^{15}N relative to the initial substrate while the residual substrate is enriched. During nitrification, for example, NO_3^-, becomes depleted in ^{15}N relative to the $\delta^{15}N$ of the initial NH_4^+, while the residual NH_4^+ is characterized by progressively increasing values with time (Mariotti et al. 1981). The magnitude of isotopic fractionation may vary owing to differences in metabolic pathways, microbial communities involved, and substrate and electron donor concentrations (Mariotti et al. 1981, 1982*a*, *b*, Bryan et al. 1983, Shearer and Kohl 1988; Table 2). Nitrification exhibits a large degree of isotopic fractionation and NO_3^- produced by this process can be depleted in ^{15}N by as much as 35‰ (Mariotti et al. 1981, Yoshida 1988). Because NO_3^- is a substrate of denitrification the direction of isotopic fractionation is opposite of nitrification. Thus, increases or decreases in the $\delta^{15}N$ of NO_3^- can identify regions or times of denitrification or nitrification, respectively (Hedin et al. 1998, Ostrom et al. 1998).

Within the soil–stream interface, the observation that concentrations of NH_4^+ declined and concentrations of NO_3^- generally increased toward the stream suggests nitrification (Fig. 1). This trend, however, was only

TABLE 2. Isotopic enrichment factors for denitrification in groundwater, soils, seawater, laboratory cultures and inorganic or theoretical reactions.

Environment type	Isotopic enrichment factor, ε (‰)	Conditions	Reference
Ground water	−30 ± 6†	confined aquifer, western Kalahari Desert, South Africa	Vogel et al. (1981)
	−5.0−−4.7	chalk aquifer, northern France	Mariotti et al. (1988)
	−5.2−−4.0	shallow alluvial groundwater, southern France	Fustec et al. (1991)
	−15.9	unconfined sand and gravel aquifer, Germany	Böttcher et al. (1990)
	−14.6	unconfined sand, silt, and gravel aquifer, Germany	Grischek et al. (1998)
	−13.9	unconfined sand and gravel aquifer, Cape Cod, Massachusetts, USA	Smith et al. (1991)
	−27.6	riparian zone, Ontario, Canada	Mengis et al. (1999)
Soil	−19.1−−6.5	75 g dried soil, 125 mL H_2O, NO_3^--N	Chien et al. (1977)
	−11	75 g dried soil, 125 mL H_2O, NO_2^--N	Chien et al. (1977)
	0	75 g dried soil, 125 mL H_2O, NO_3^--N, 1% glucose	Chien et al. (1977)
	−4	75 g dried soil, 125 mL H_2O, NO_2^--N, 1% glucose	Chien et al. (1977)
	−23−−14	10 g soil, 6 mL H_2O, 10 mg NO_3^--N, 7.5 g glucose	Blackmer and Bremner (1977)
	−29.4−−24.6	100 g dried soil, N-serve, 100 mL H_2O, 20 mg NO_3^--N, acetylene	Mariotti et al. (1981)
	−33.2−−14.6	100 g soil, 100 mL, H_2O, 20 mg NO_2^--N or NO_3^--N	Mariotti et al. (1982*a*, *b*)
	−11.1	100 g soil, 100 mL, H_2O, 20 mg NO_2^--N, 0.5 g glucose	Mariotti et al. (1982*a*, *b*)
	−6.0−−5.6	in situ, forested soils, Japan	Koba et al. (1997)
Seawater	−20	incubation of a marine denitrifier. 1.53 g yeast, 2.36 g peptone, (19.78 g Na-NO_3, 500 mL seawater	Miyake and Wada (1971)
	−39	incubation of marine bacterium, *Serratia marinorubra*; 0.5% polypeptone, 0.1% yeast extract, 0.1% KNO_3, 3.55% NaCl, pH 7.5	Miyazaki et al. (1980)
	−40−−30	eastern tropical north Pacific	Cline and Kaplan (1975)
Laboratory cultures	−20	*Pseudomonas stutzeri*, 10–30 μmol/L NO_2^--N or NO_3^--N	Wellman et al. (1968)
	−20.8−−13.4	*Pseudomonas denitrificans*	Delwiche and Steyn (1970)
	−20–0	assimilatory nitrate reduction by pearl millet	Mariotti et al. (1982*c*)
	−15.8	*Pseudomonas stutzeri*, 0.86 mmol/L NO_2^-	Bryan et al. (1983)
	−6.9	*Pseudomonas stutzeri*, 0.14 mmol/L NO_2^-	Bryan et al. (1983)
	−23−−5	*Pseudomonas stutzeri*, 25 mmol/L succinate, 0.07–2.2 mmol/L NO_2^-	Bryan et al. (1983)
	−22−−6	cell-free extracts of *P. stutzeri*, 25 mmol/L succinate, 0.07–2.2 mmol/L NO_2^-	Bryan et al. (1983)
	−18–0	*Pseudomonas stutzeri*, 0.29 mmol/L NO_2^-, 0.01–25 mmol/L succinate	Bryan et al. (1983)
	−33−−10	cell-free extracts of *P. stutzeri*, 2.1 mmol/L NO_2^-, 0.75–30 mmol/L phenazine methosulfate	Bryan et al. (1983)
	−11	*Pseudomonas stutzeri*, 0.107 mmol/L NO_2^-, 25 mmol/L succinate	Shearer and Kohl (1988)
Inorganic/ theoretical	−65.9	calculated equilibrium isotope exchange between ^{15}N-O and ^{14}N-O bonds	Urey (1947)
	−75	alkaline reduction with Fe(II)	Brown and Drury (1967)
	−34	alkaline reduction with Fe(II)	Brown and Drury (1967)
	−8.0	diffusion of NO_3^-	calculated
	−6.2	diffusion of NO_3^- hydrated with one water molecule	calculated

† Mean ± 1 SD.

occasionally accompanied by marked increases and decreases in the $\delta^{15}N$ of NH_4^+ and NO_3^-, respectively, that characterize nitrification (Mariotti et al. 1981, Yoshida et al. 1988). Although increases in $\delta^{15}N$ associated with declines in NH_4^+ concentration were observed occasionally, the magnitude of these shifts were small relative to those expected for nitrification. Nitrification most certainly occurs within the soil–stream interface but our data indicate that it is not a significant process at the depths sampled by the wells. Most likely nitrification occurs at shallower depths, in the vicinity of the saturated–unsaturated interface, where O_2 is more readily available. Furthermore, O_2 was not detected in the wells and this further suggests that conditions were not suitable for nitrification (although ideal for denitrification) at the depths that the wells sampled. More variable and higher $\delta^{15}N$ values for NH_4^+ in well series closer to the stream (series 3 and 4) suggests that these well series sampled water nearer to a region of nitrification. Consequently, we conclude that the observed decline in NH_4^+ and increase in NO_3^- toward the stream is not primarily controlled by nitri-

fication but a consequence of the intersection of two groundwater flows, the shallow flow rich in NH_4^+ and the deep flow present near the stream that is rich in NO_3^-. Secondarily, microbial processes impart changes in the concentration and isotopic composition of inorganic N. The decline in NH_4^+ concentrations toward the stream could be interpreted as representing nitrification along a single flow path, however, this is clearly not the case; each well series samples a unique flow path with a unique history. The predominant control on geochemical trends within the interface is the physical mixing of two distinct groundwater flows. The intersection and mixing of the two flows provides the necessary substrates to enhance microbial activity which imparts a secondary control on the abundances and $\delta^{15}N$ values of inorganic N.

The concentration of NO_3^- 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 NO_3^- 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 NO_3^- concentrations. Higher NO_3^- 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 flood-plain deposits. Highest $\delta^{15}N$ values tended to occur in the well series just inland of the peak in NO_3^- concentration and lowest $\delta^{15}N$ values were found within the region of maximum NO_3^- concentrations (Fig. 1). Such trends are consistent with bank storage of stream water following high-discharge events in the well series closest to the stream (Fetter 2001), upwelling of NO_3^--rich deep groundwater near the stream, and denitrification in the inland well series. Consequently, we interpret a trend between well series with marked declines in NO_3^- abundance and high $\delta^{15}N$ values in an upland direction as a zone of denitrification (Fig. 1).

On two occasions, site 1 on 7 April 1993 and site 2 on 16 November 1993, a decline in the $\delta^{15}N$ of NH_4^+ toward the stream was concomitant with moderate NO_3^- concentrations (Fig. 1). A similar decline in NH_4^+ $\delta^{15}N$ was observed at site 1 on the same day in November 1993, however, much lower concentrations of NO_3^-(<1 μmol/L) were observed. The decline in the $\delta^{15}N$ of NH_4^+ is opposite the direction of fractionation for nitrification and may best be explained as resulting from dissimilatory NO_3^- reduction to NH_4^+ (DNRA). Within low-O_2 environments, denitrification may produce the gaseous products N_2 and N_2O or, alternatively, yield NH_4^+ 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 Annadotter 1989). Thus N_2 production is most efficient within electron-donor-limited environments (low DOC) and NH_4^+ production is likely favored when NO_3^- is limiting and electron donors are abundant (high DOC). 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 $\delta^{15}N$ of NH_4^+ is consistent with the direction of fractionation expected for this process (McCready 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 NO_3^- in any sample is a function of the concentration in and 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 NO_3^- (Q_m) can be described as a mixture of two flows:

$$Q_m = Q_a + Q_b \tag{2}$$

in which Q is defined as the concentration of NO_3^- 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 NO_3^- in any sample (δ_m) resulting from the mixture of two water masses

$$\delta_m Q_m = \delta_a Q_a + \delta_b Q_b. \tag{3}$$

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

$$\delta_m = Q_a(\delta_a - \delta_b)/Q_m + \delta_b. \tag{4}$$

Within shallow groundwater (well series 1–3) NO_3^-, if detected, was a small fraction, $<2\%$, of that in the near-stream well series. Consequently, the mass of NO_3^-, Q_a, in the shallow flow is approximated as zero. Because shallow groundwater essentially lacks NO_3^-, Eq. 4 indicates that regardless of the degree of mixing of shallow and deep groundwater the isotopic composition of NO_3^- is unchanged and equal to the deep flow, δ_b. The $\delta^{15}N$ of NO_3^-, 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

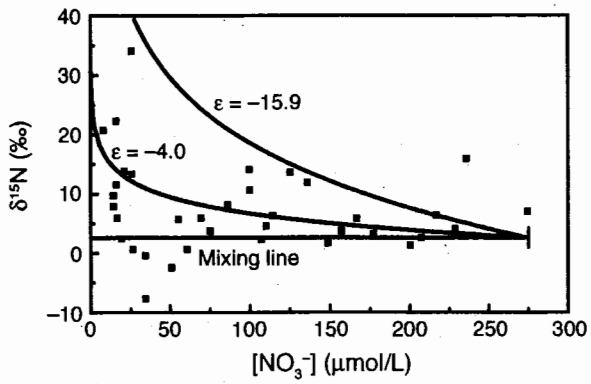

FIG. 2. The concentration and $\delta^{15}N$ of NO_3^- within the soil–stream interface for those samples not influenced by stream water. Lines represent the relationship of $\delta^{15}N$ to NO_3^- concentration for denitrification of deep groundwater for isotopic enrichment factors of −15.9 (Böttcher et al. 1990) and −4.0 (Fustec et al. 1991) and mixing of the deep and shallow groundwater masses. The error bar (at the right-hand end of lines) indicates 1 SD around the estimate of the $\delta^{15}N$ of NO_3^- within deep groundwater.

(δ_s) is related to that of the initial substrate (δ_{s0}), an isotopic enrichment factor (ε), and the ratio of the observed to initial substrate concentration (C/C_0; Mariotti et al. 1981):

$$\delta_s = \delta_{s0} + \varepsilon \ln(C/C_0). \quad (5)$$

Fractionation during reactions controlled by the masses of molecules are described by the fractionation factor, α, or the isotopic enrichment factor, ε. In this paper, we follow the convention of Mariotti et al. (1981) by defining these as

$$\alpha = k_2/k_1 \quad (6)$$

where k_1 and k_2 are the reaction rates for the light and heavy isotopically substituted compounds, respectively (although some authors use the inverse of this ratio), and

$$\varepsilon = (\alpha - 1) \times 1000. \quad (7)$$

Eq. 5 describes a relationship by which the isotopic composition of NO_3^- is linear with respect to the natural log of its concentration. In this manner, mixing of water masses yields a linear relationship between $\delta^{15}N$ and $1/C_0$ whereas denitrification produces a relationship in which $\delta^{15}N$ is linear with respect to $\ln(C_0)$ (Mariotti et al. 1988). A distinction of mixing from denitrification, however, becomes difficult if both processes occur simultaneously (Böhlke and Denver 1995, McMahon and Böhlke 1996). Within the soil–stream interface of Smith Creek, this distinction is simplified since mixing has no effect on the isotopic composition of NO_3^- while NO_3^- concentrations are affected by both mixing and denitrification. Because fractionation during denitrification can be characterized by determination of the $\delta^{15}N$ of the sample, the $\delta^{15}N$ of the initial source of NO_3^-, and the isotopic enrichment factor, the fraction of NO_3^- removed due to denitrification, f_{NR}, can be determined independent of a concentration measurement:

$$f_{NR} = 1 - e^{(\delta_s - \delta_{s0})/\varepsilon} \quad (8)$$

where f_{NR} 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 Drury 1967) or calculated based on bond-strength differences between the trace 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_2^- or NO_3^- or inversely with the concentration of organic reductant (Kohl and Shearer 1978, Mariotti et al. 1982*a*, 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:

$$\underset{\text{out}}{NO_3^-} \rightleftharpoons \underset{\text{in}}{NO_3^-} \xrightarrow{AH_{NR}H} NO_2^- \rightarrow \text{reduced N}$$

This latter process is dependent upon NO_3^- reductase (NR), and a supply of electron donors (AH). In the natural environment, NO_2^- 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. 1982*c*, 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. 1982*c*). 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

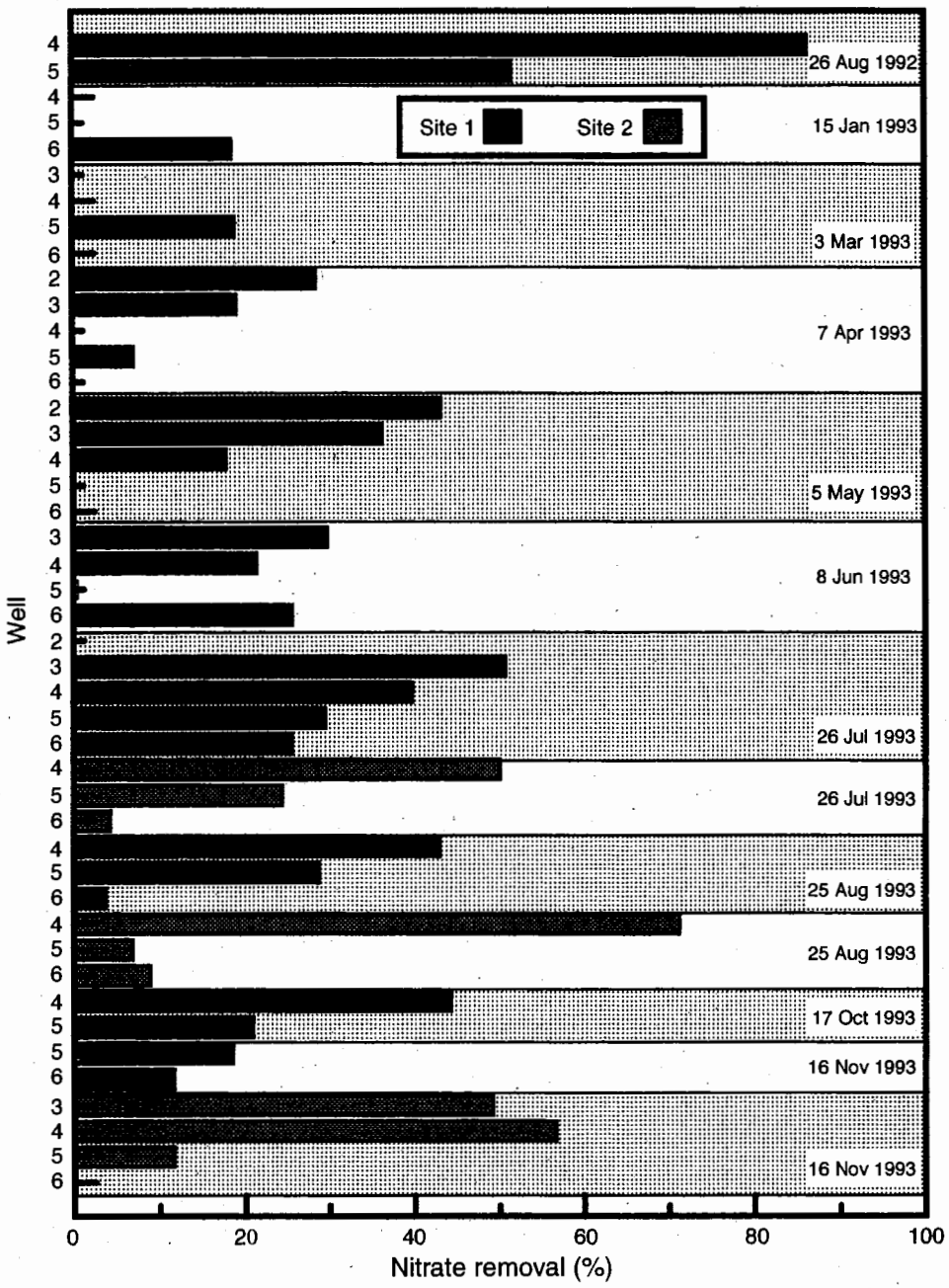

FIG. 3. Seasonal and spatial variation in NO_3^- removal within the soil–stream interface as determined by Eq. 8 and based on an isotopic enrichment factor of −15.9. Well series with no values shown indicate that there was sufficient NO_3^- for an isotopic determination to be made but removal was not detected.

allows expression of the isotope effect during reduction within the cell. In contrast, when the supply of NO_3^- to the cell is less than the rate of reduction, little if any NO_3^- escapes the cell to express the isotope effect. Hence, the observed isotope effect during reduction is proportional to the external concentration of NO_3^- (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, Koba et al. 1997). Indeed, addition of glucose to soils has been

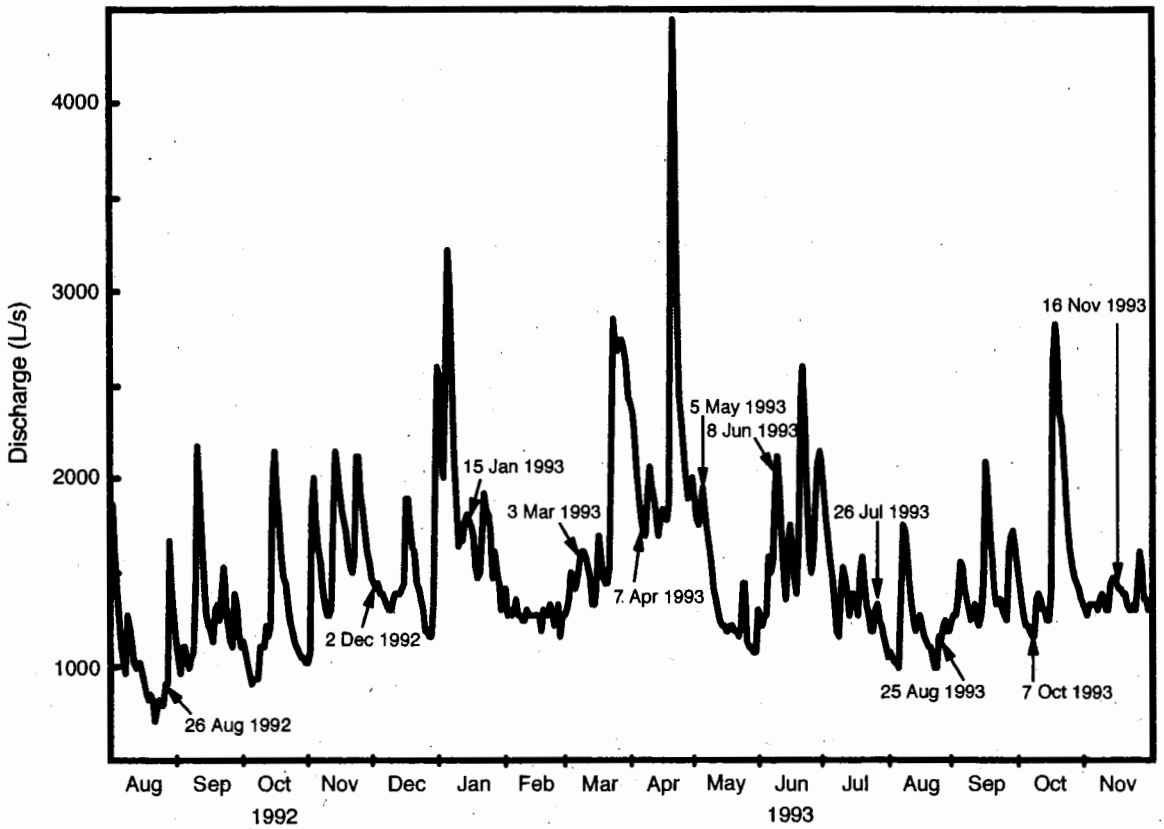

FIG. 4. Stream discharge data for the period of August 1992–December 1993 at the United States Geological Survey gauging station on Augusta Creek. Arrows indicate discharge on dates for which samples from the well series in Fig. 1 were obtained for concentration and isotopic determination.

shown to reduce the expression of fractionation (Blackmer and Bremner 1977, Chien et al. 1977, Mariotti et al. 1982*a*, *b*). Furthermore, a limitation of DOC might explain why isotopic fractionation is greater in ancient groundwaters (Vogel et al. 1981) and is smaller in DOC-rich groundwater receiving sewage effluent (Smith et al. 1991).

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 ε. We estimated that deep groundwater has an initial $\delta^{15}N$ value of 2.6 ± 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 ε 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 ε 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, Grischek et al. 1998). In Fig. 2, we present the concentration and $\delta^{15}N$ values for NO_3^- in the soil–stream interface for samples not influenced by stream water in relation to the concentration and $\delta^{15}N$ of NO_3^- in deep groundwater and the solution of Eqs. 4 and 5 using values of ε of −15.9‰ and −4.0‰.

The majority of NO_3^- $\delta^{15}N$ values lie between a line produced by Eq. 5 using an ε 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 $\delta^{15}N$. Most of the points outside the mixing and fractionation lines are within 1 SD of the estimated $\delta^{15}N$ value for the deep groundwater endmember (2.6 ± 1.8) and likely represent uncertainty in characterizing the concentration and isotopic composition of NO_3^- of this endmember. In addition, nitrification and DNRA 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 ε value of −4‰ is a poor predictor

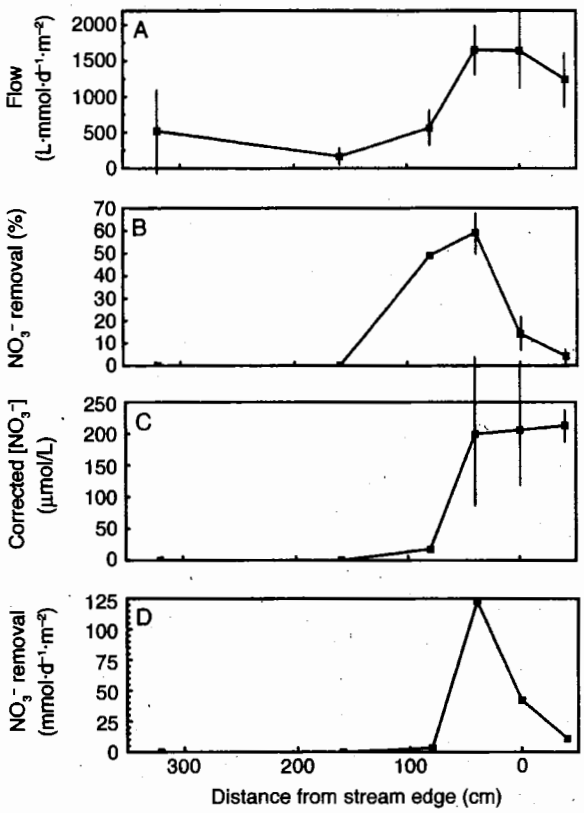

FIG. 5. (A) Hydrologic flow, (B) percentage of NO_3^- removal, (C) corrected NO_3^- concentrations, and (D) quantity of NO_3^- removal for site 2. The corrected concentration of NO_3^- is the sum of the observed concentration and losses due to denitrification. Error bars indicate ±1 SD around the mean of four measurements of hydrologic data and three measurements of NO_3^- concentration. NO_3^- removal in well series 3 (80 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‰ denitrification line, either mixing of the shallow and deep groundwater was substantial and/or ε varied between −15.9‰ and −4.0‰. Variation in the denitrification fractionation factor by the soil–stream interface microbial community is quite likely given that the availability of DOC changes with the relative predominance of the two groundwater flows. The use of −15.9‰ for ε (Eq. 8) describes the data well and results in low estimates of NO_3^- removal relative to a value of −4‰.

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 δ_{s0} and $\delta^{15}N$ values for each well series on a particular sampling date as δ_s in Eq. 8. Mixing of water masses would normally invalidate the use of a Rayleigh model, however, because the contribution of NO_3^- in shallow groundwater is negligible and concentration measurements are not required, 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 (81%; Hedin et al. 1998). The use of −15.9‰ for ε provides lower estimates of NO_3^- removal than the use of −4‰; hence our results are conservative. NO_3^- removal varied between well series and sampling dates, however, two important trends are evident. First, on sampling dates with sufficient data, NO_3^- removal tended to occur to a greater extent in the inland well series than in the near stream well series, most notably on 7 April, 26 July, 25 August, 17 October, and 16 November 1993. This observation is consistent with the need for DOC from the shallow groundwater to fuel denitrification in deep groundwater. Concentrations of DOC in well 5 were generally <0.5 mg C/L and substantially lower than those in the upland well which had concentrations of DOC in excess of 10 mg C/L (Hedin et al. 1998). Furthermore, changes in DOC between adjacent well series were indeed consistent with consumption of DOC during denitrification. For example, at site 1 on 25 August 1993 we observed a reduction in NO_3^- of 141 µmol/L between well series 5 and 4, which, based upon a DOC:NO_3^- stochiometry of 106 to 94.4 for denitrification (Von Gunten et al. 1991), corresponds to a decrease in DOC of 1.9 mg/L. This reduction is similar to the difference in DOC observed between these well series on 30 August 1993 of ~1 mg/L (Hedin et al. 1998). The slight difference in DOC consumption between predicted and observed may reflect heterogeneity of hydrologic flows between the sampling dates, some degree of mixing of the shallow and deeper flows, variation in the stochiometric relationship between DOC and NO_3^-, and the fact that measured DOC concentrations represent the residual substrate of denitrification (Fig. 3). Secondly, sampling dates dominated by deep groundwater and high stream

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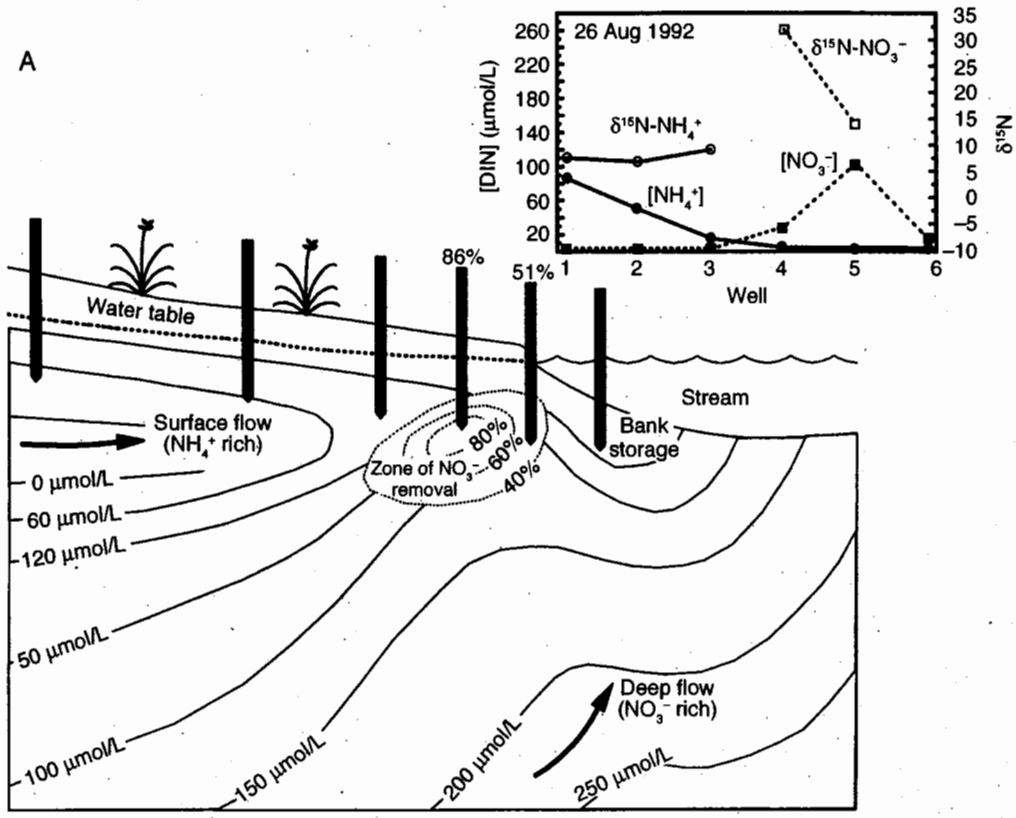
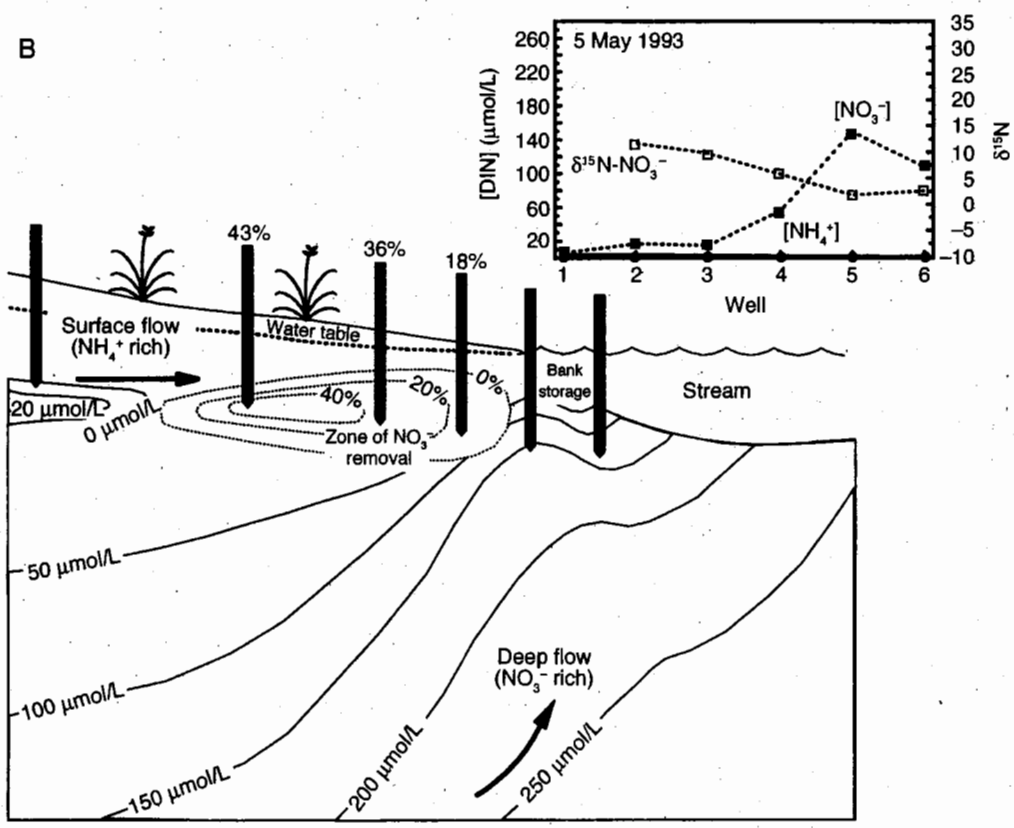
FIG. 6. Conceptual diagram of changes in the concentration and isotopic abundances of NO_3^- and NH_4^+ along the soil–stream interface of Augusta Creek in relation to hydrologic flows for (A) 26 August 1992, a period of low discharge, and (B) 5 May 1993, a period of high discharge. Insets show the measured data on NO_3^- and NH_4^+ for the respective dates. The percentage of NO_3^- removal is shown above each well series for which values were >0. The well transect spans 4 m, and the 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 NH_4^+ concentrations and the zone of denitrification are shown. The intersections of three flows are evident: a shallow NH_4^+-rich flow, a deep NO_3^--rich flow, and stream water as bank storage.


A
26 Aug 1992
[DIN] (μmol/L)
δ15N
δ15N-NO3−
δ15N-NH4+
[NO3−]
[NH4+]
Well
86%
51%
Water table
Stream
Surface flow
(NH4+ rich)
Bank
storage
80%
60%
40%
Zone of NO3− removal
0 μmol/L
60 μmol/L
120 μmol/L
50 μmol/L
100 μmol/L
150 μmol/L
200 μmol/L
250 μmol/L
Deep flow
(NO3− rich)


B
5 May 1993
[DIN] (μmol/L)
δ15N
δ15N-NO3−
[NO3−]
[NH4+]
Well
43%
36%
18%
Surface flow
(NH4+ rich)
Water table
Bank
storage
Stream
40%
20%
0%
20 μmol/L
0 μmol/L
Zone of NO3− removal
50 μmol/L
100 μmol/L
150 μmol/L
200 μmol/L
250 μmol/L
Deep flow
(NO3− rich)

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

$$NR = F(C_i - C) \quad (9)$$

in which the concentration of NO_3^- prior to denitrification, C_i, is equivalent to $C(1 + f_{NR})$.

On four occasions during the twelve months following September of 1993 we quantified hydrologic flow in each of the well series at site 2 (Hedin 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 isotopic measurements we determined that along the 4-m well-series transect NO_3^- removal varied from undetectable levels to as much as 123 $\mu mol \cdot L^{-1} \cdot m^{-2} \cdot d^{-1}$ or 1.73 g $N \cdot m^{-2} \cdot d^{-1}$ (Fig. 5). Our estimate of NO_3^- removal is substantially greater than the range of 2.6–10.1 $\mu mol \cdot L^{-1} \cdot m^{-2} \cdot d^{-1}$ reported for the biogeochemically 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 noncontemporaneous 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 this environment. Natural-abundance isotope studies have marked advantages over incubation studies that invoke 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 interfaces

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 a 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 isotopic fractionation associated with this process. Consequently, we believe that nitrification is active at shallower depths than sampled by the wells and most likely occurs in the vicinity of the saturated–unsaturated 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 an increased predominance of deep groundwater at the depths sampled by the wells. Concentrations of NH_4^+ 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 NH_4^+-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 inland 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 $\delta^{15}N$ values and appreciable NO_3^- removal as determined from Eq. 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 all 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 $\delta^{15}N$ 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 on 5 May 1993. Under these conditions, nitrification was not evident within the study site, the shallow flow was likely shifted to an upland and shallower 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 presences 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 changes in hydrology occurred at a much shorter intervals 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 of soil–stream interfaces. Our study illustrates that substantial quantities of NO_3^- can be removed prior to impacting the stream environment and more distal ecosystems. The proper functioning of the soil–stream interface in NO_3^- removal, however, depends on a supply of DOC to support denitrification in deep NO_3^--rich groundwater upwelling at the stream edge. This supply of DOC can be derived from organic-rich soils or subsurface deposits (Hedin et al. 1998, Devito et al. 2000). NO_3^- removal will not be fostered by intensive agriculture on the flood plain since agricultural environments tend to leach NO_3^--rich and DOC-poor groundwater (Delprat 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 DOC in soil leachate (Hedin et al. 1995, Delprat et al. 1997). In addition, if our 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 to inducing changes in discharge from dams as a mechanism for producing habitats beneficial to recreation and fish reproduction (Rubin et al. 1998). An additional benefit of regulation of hydraulic discharge may be in optimizing the removal of NO_3^- entering streams and rivers.

Acknowledgments

We gratefully appreciate the efforts of Emily Bell, Keith Knoke, Michael G. Brown, and Brian P. Kennedy for assistance in the collection or analysis of samples. Many thoughtful comments have been provided by Grahame J. Larson and two anonymous reviewers. This work was supported by a grant from the National Science Foundation (BSR92-08395).

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