Dissimilatory Nitrate Reduction in Anaerobic Sediments Leading to River Nitrite Accumulation

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Recent studies on Northern Ireland rivers have shown that summer nitrite (NO_2^-) concentrations greatly exceed the European Union guideline of $3 \mu\text{g}$ of N liter⁻¹ for rivers supporting salmonid fisheries. In fast-flowing aerobic small streams, NO_2^- is thought to originate from nitrification, due to the retardation of *Nitrobacter* strains by the presence of free ammonia. Multiple regression analyses of NO_2^- concentrations against water quality variables of the six major rivers of the Lough Neagh catchment in Northern Ireland, however, suggested that the high NO_2^- concentrations found in the summer under warm, slow-flow conditions may result from the reduction of NO_3^- . This hypothesis was supported by field observations of weekly changes in N species. Here, reduction of NO_3^- was observed to occur simultaneously with elevation of NO_2^- levels and subsequently NH_4^+ levels, indicating that dissimilatory NO_3^- reduction to NH_4^+ (DNRA) performed by fermentative bacteria (e.g., *Aeromonas* and *Vibrio* spp.) is responsible for NO_2^- accumulation in these large rivers. Mechanistic studies in which ¹⁵N-labelled NO_3^- in sediment extracts was used provided further support for this hypothesis. Maximal concentrations of NO_2^- accumulation (up to 1.4 mg of N liter⁻¹) were found in sediments deeper than 6 cm associated with a high concentration of metabolizable carbon and anaerobic conditions. The ¹⁵N enrichment of the NO_2^- was comparable to that of the NO_3^- pool, indicating that the NO_2^- was predominantly NO_3^- derived. There is evidence which suggests that the high NO_2^- concentrations observed arose from the inhibition of the DNRA NO_2^- reductase system by NO_3^- .

It is widely assumed that nitrite (NO_2^-) concentrations in freshwaters are negligible (36, 49), and the worldwide average NO_2^- concentration has been estimated to be $1 \mu\text{g}$ of N liter⁻¹ (31). Recent studies in Germany and Northern Ireland, however, have reported river concentrations of nitrite of 100 to 200 μg of N liter⁻¹ (46, 55), values which greatly exceed the European Union NO_2^- guideline of $3 \mu\text{g}$ of N liter⁻¹ for rivers supporting salmonid fisheries (11). The toxicity of NO_2^- to fish was originally thought to arise from its conversion of oxyhemoglobin to methemoglobin, a form incapable of oxygen transport (27). Some authors (10, 14, 43) have claimed that this mechanism may not be the prime cause of death; instead, death is thought to occur through toxic action on essential enzymes (2).

Reports of NO_2^- accumulation appear to be linked to large inputs of agriculturally derived nitrogen (N) substrates into the aquatic environment. However, only 40% of the NO_2^- occurring in Northern Ireland rivers is believed to originate directly from land drainage, with the remainder originating from sediment-water interface transformations of nitrogenous substrates from point source pollution (46). Indeed, it has been suggested that there has been a shift in focus of the N cycle, with more transformations occurring in the aquatic system (54).

Nitrite occurs as an intermediate in several oxidative and reductive pathways (Fig. 1). Previous studies in our laboratory have shown that ammonium (NH_4^+) oxidation via nitrification is responsible for the major proportion of the elevated NO_2^- levels observed in river sediment-water systems incubated un-

der aerobic conditions. Confirmation that this process occurred in situ was provided by multiple regression analysis of water quality data from 28 streams (47). However, correlations between NO_2^- and nitrate (NO_3^-) were also evident from this study. Denitrification in anaerobic sediments may play a major role in removing NO_3^- from river water (24), but dissimilatory NO_3^- reduction to NH_4^+ (DNRA) may be in direct competition (15, 38), especially in anoxic water-logged sediments (33). The ratio between available electron donors (i.e., carbon) and electron acceptors (i.e., NO_3^-) is important not only in influencing which pathway is followed (53) but also in determining what end products are produced (45). DNRA is favored when NO_3^- is limiting, while denitrification is favored when carbon is in limited supply.

For NO_2^- accumulation to occur in the denitrification and DNRA processes, NO_3^- reduction must occur at a greater rate than that of NO_2^- reduction. Similarly, in the nitrification pathway, elevation of NO_2^- concentrations can occur only if the oxidation of NH_4^+ is greater than that of NO_2^- . Both of these scenarios have been reported to occur in the aquatic environment (17, 47). *Nitrobacter* spp., which are responsible for the oxidation of NO_2^- in the nitrification process, may be partially inhibited by free ammonia (NH_3) (1, 47), leading to NO_2^- accumulation. Likewise, NO_2^- accumulation is a common trait of DNRA (7) due to either inhibitory effects of NO_3^- on the NO_2^- reductase (45) or repression of this enzyme (37). The organisms possessing this pathway are fermentative bacteria (e.g., *Aeromonas* and *Vibrio* spp.) (28). In contrast, denitrification is performed by respiratory organisms (30, 53) (e.g., *Pseudomonas* and *Bacillus* species) (28, 36) and normally proceeds from NO_3^- through to the formation of gaseous nitrogen (N_2), without accumulation of NO_2^- (40).

The aim of the present study was to investigate if NO_2^- accumulation observed in the major rivers in the Lough Neagh

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FIG. 1. Nitrogen transformation processes that involve NO_2^- production and consumption. 1, denitrification; 2, DNRA; 3, nitrification.

system could be ascribed to DNRA. Our previous study (47), in which fast-flowing streams were examined, demonstrated that NH_4^+ was a NO_2^- precursor. Sediments were incubated under aerobic conditions, which prevail only in the top 1.5 to 5.5 mm of sediment (16). In the present study anaerobic conditions were provided for sediment incubation experiments in order to deduce what NO_2^- production pathways are dominant through the sediment profile by employing ^{15}N -labelled NO_3^- .

MATERIALS AND METHODS

Lough Neagh study area. The catchment area of Lough Neagh is situated centrally in Northern Ireland ($54^\circ30'30''\text{N}$, $6^\circ20'30''\text{E}$) and covers 4,453 km^2 , which is approximately one-third of the total land area of the country (13,480 km^2) (Fig. 2). There are six main rivers flowing into Lough Neagh, but only one outflow via the Lower Bann River, which runs north to the Atlantic Ocean. The inflowing rivers drain 88% of the catchment area of Lough Neagh. The average daily rainfall is 2.31 mm (1994–1995 average), and the mean daily air temperature is 9.32°C (1994–1995 average). The geology of the catchment has been described by Wilson (56), and the climate has been described by Betts (4). Over the study period, the major land use was grassland and rough grazing, which together accounted for 85 to 87% of the area; an additional 7 to 9% was under

FIG. 2. Major rivers of the Lough Neagh system and the location of this system in Northern Ireland.

arable cultivation. This is very similar to the data for Northern Ireland as a whole (18).

Field sampling. The seven major rivers entering and leaving Lough Neagh were sampled at weekly intervals from January 1994 until December 1995. The river sampling locations were at convenient locations as close to Lough Neagh as possible, but any backwash from the lough was avoided. Field measurements of temperature and dissolved oxygen were taken with a portable oxygen meter. Approximately 5 liters of surface water was taken from the midstream section of each river, stored in polyethylene bottles, and returned within 3 h to the laboratory for chemical analysis.

Chemical analyses. The samples were stored at 4°C prior to analysis, which took place within 24 h of sampling. The water samples were filtered (pore size, $0.45\text{ }\mu\text{m}$; Whatman), and the pH was measured with an Orion expandable ion analyzer (model EA940). Measurements of NO_3^- were made by employing the hydrazine reaction (9) with a Technicon Instruments model TRAACS 800 continuous-flow analyzer. The concentrations of NO_2^- and NH_4^+ were determined spectrophotometrically with a Hitachi model V-2000 spectrophotometer by using the sulfanilamide-naphthylenediamine (39) and Berthelot (42) methods, respectively. Carbon and nitrogen contents of sediments were analyzed by using a Carlo Erba model NA 1500 elemental analyzer coupled to a stable-isotope mass spectrometer (Europa Scientific Tracermass). The amounts of free ammonia present were calculated from the NH_4^+ concentration with the following equation by employing the formula of Barnabe (3): $\text{free NH}_3 = \text{NH}_4^+ / [1 + 10^{(10.068 - 0.0337 \cdot \text{pH})}]$, where T is the water temperature (in degrees Celsius). The accuracy of the methods was monitored by participation in the United Kingdom Water Research Centre's AQUACHECK program.

Experimental design. Approximately 25 liters of surface water was collected from the middle of Lough Neagh, stored in polyethylene bottles, and returned immediately to the laboratory. The water was filtered through GF/C filters (pore size, $0.45\text{ }\mu\text{m}$; Whatman) and refrigerated at 4°C . Sediment was collected in November 1996 from a sampling station (Irish Grid Reference J192 429, corresponding to station UB7 of Foy and Kirk [12]) on the Upper Bann River, which drains an agricultural catchment in Counties Armagh and Down. Eleven sediment cores (5 cm in diameter by 15 cm deep) were collected by pushing Plexiglas tubes into areas of sediment accumulation that were more than 20 cm deep. The top end of each tube was closed with a rubber stopper, and then the tube was pulled out with the intact core. The stopper was removed, and a plunger was inserted into the bottom end to push the core upward. The sediment was divided into sections (0 to 5, 6 to 10, and 11 to 15 cm), and the sections from the same depth were pooled, thoroughly mixed, and left at room temperature for 48 h. One-third of a core from the appropriate depth was added to 180 ml of Lough Neagh water in 93 Kilner jars. Then 10 ml of a solution containing 2 mg N liter⁻¹ as NH_4Cl was added to boost the NH_4^+ concentration to approximately 1 mg of N liter⁻¹, the minimum concentration for NH_4^+ detection by mass spectrometry. In addition to the three different layers of sediment, two carbon treatments in 10-ml aliquots were used to give a low glucose concentration of 0.1 g of CH_2O liter⁻¹ and a high concentration of 1.0 g of CH_2O liter⁻¹. NO_3^- was added to both treatments by adding 10 ml of a solution containing 7 mg of N liter⁻¹ as K^{15}NO_3 , at 40 atom% excess, to obtain a NO_3^- concentration of approximately 13 mg of N liter⁻¹. Any $^{15}\text{NO}_2^-$ impurity was removed from the labelled NO_3^- by oxidizing the NO_2^- impurity to NO_3^- with UV light in the presence of titanium dioxide (29). During incubation there was approximately 2 cm of water above the sediment. Immediately after addition of the NO_3^- substrate, a nylon lid with a gas-sampling septum was fitted to each jar with an O ring to form a gas-tight seal. Each treatment was replicated three times, and the replicates were randomly distributed in incubators maintained at 23°C . Analyses were made initially, and subsequent destructive sampling was carried out after 1, 2, 3, 4, and 5 days.

Gas analyses. At each sampling time prior to destructive sampling, two 12-ml gas samples were removed through the gas-sampling septum by using a 20-ml gas-tight syringe with a push button valve and then injected into evacuated ($<100\text{ Pa}$) septum-capped Exetainers (Europa Scientific, Crewe, United Kingdom). The gas was analyzed to determine the ^{15}N contents of N_2O and N_2 and the concentration of N_2O by continuous-flow isotope ratio mass spectrometry. Analyses were performed with a Europa Scientific model ANCA-NT 20-20 stable-isotope analyzer interfaced to a Europa Scientific trace gas preparation system with a Gilson autosampler. The valve switching was automated so that the ^{15}N contents of N_2 and N_2O could be determined from the same sample. The ion currents (I) at m/z 44, 45, and 46 allowed us to calculate ^{45}R ($^{45}\text{I}/^{44}\text{I}$) and ^{46}R ($^{46}\text{I}/^{44}\text{I}$) for N_2O . The ^{15}N content of the N_2O was calculated either from ^{45}R by using equations 5 and 7 of Stevens et al. (50) or from ^{46}R by using equations 6 and 7 of Stevens et al. (50). For N_2 , the I values at m/z 28, 29, and 30 were measured by isotope ratio mass spectrometry (50). The differences between ^{29}R ($^{29}\text{I}/^{28}\text{I}$) and ^{30}R ($^{30}\text{I}/^{28}\text{I}$) for enriched and normal atmospheres allowed us to calculate the flux of N_2 (32). The concentration of N_2O was calculated as described by Stevens et al. (50) from measurements of ^{44}I , ^{45}I , and ^{46}I . The flux of N_2O was calculated from the change in N_2O concentration with time. It was assumed that the concentration of N_2O at the start of each flux measurement was 310 ppb.

Analysis of nitrogen fractions. Immediately after centrifugation at 2,000 rpm for 10 min (MSE mistral 3000i centrifuge), the supernatant was filtered sequentially through GF/C and GF/F filters (Whatman International Ltd., Kent, United

FIG. 3. Field observations of changes in N species in the Six Mile Water, one of the major rivers draining into Lough Neagh.

Kingdom). To determine the ^{15}N contents of NH_4^+ , NO_2^- , and NO_3^- , a sufficient amount of KCl was added to the filtrate to produce a 2 M KCl solution. Concentrations of NO_2^- were determined spectrophotometrically with the Hitachi model V-2000 spectrophotometer by using the sulfanilamide-naphthyl-enediamine reaction (39). Concentrations of NO_3^- and NH_4^+ in the KCl solutions were determined by segmented flow analysis with the Technicon Type 800+ Random Access Automated Chemistry System (5). The amount of particulate organic nitrogen (PON) was calculated by the method of Koike and Hattori (25), as follows: $\text{PON} = (\text{total } ^{15}\text{N added originally}) - (^{15}\text{N in } \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^- + \text{N}_2\text{O} + \text{N}_2)$.

The ^{15}N contents of NO_2^- , NO_3^- , and NH_4^+ were analyzed by methods based on the conversion of these ions to N_2O (26, 51).

Statistical analyses. Multiple regression analyses were performed for individual rivers, and the choice of variables was based on a backward selection procedure (6) to determine if any significant correlations between NO_2^- concentrations and other independent physical and chemical parameters (flow, conductivity, pH, temperature, dissolved oxygen, and NO_3^- , NO_2^- , NH_4^+ , and NH_3) were evident. The logarithms of concentration data from the sediment studies were subjected to an analysis of variance by using Genstat (13) to determine the significance of the effects of the two carbon treatments and three sediment depths on N transformations.

RESULTS

River studies. The relationships among NO_2^- , NO_3^- , and NH_4^+ were determined by examining the weekly plots for these ions for several of the rivers of the Lough Neagh system; one example, for the Six Mile Water, is shown in Fig. 3. Concentrations of NO_2^- were maximal in the summer months between April and early September 1995, with the peak level, $0.093 \text{ mg of N liter}^{-1}$, recorded in June. The rise in NO_2^- concentrations coincided with a decline in NO_3^- concentrations from $3.5 \text{ mg of N liter}^{-1}$ at the beginning of April to $1 \text{ mg of N liter}^{-1}$ by mid-August. The lowest NO_3^- concentration was observed at the same time as the maximum NH_4^+ concentration; the NH_4^+ concentration increased from basal values of 0 to $0.1 \text{ mg of N liter}^{-1}$ during the period from January to June to a maximum of $1.3 \text{ mg of N liter}^{-1}$ in August. During the winter months, the maximum NO_3^- concentrations were observed when both NO_2^- and NH_4^+ concentrations were at basal values. Summer data (data for April to September) for the Lough Neagh rivers were analyzed by using stepwise multiple regression procedures to determine if the variation in NO_2^- concentrations observed in these months could be linked to changes in water quality variables (Table 1). The

NO_3^- concentrations from three of the six rivers examined showed a correlation with NO_2^- concentrations, and the coefficients were significant at $P < 0.05$. The accumulation of NO_2^- was also shown to be temperature dependent in five of the six rivers, with a level of significance of $P < 0.01$. There was no correlation between NO_2^- and flow or dissolved oxygen data, while NH_4^+ and free NH_3 data showed only isolated correlations.

Interstitial NO_3^- concentrations during incubations. The rate and mechanism of NO_3^- utilization were shown to be dependent on the depth of the sediment incubated. Mean particle size (ϕ) and C/N ratio analyses showed little variation with depth, with the mean particle size ranging from 0.03 to 0.33 and the C/N ratio stable at 12. Supplements of NO_3^- were depleted in an exponential manner in all treatments with time ($P < 0.001$), and between 63 and 93% of the substrate was removed within 5 days. Utilization of NO_3^- was most marked in the 11- to 15-cm layer (Fig. 4). The presence of a metabolizable carbon energy source significantly enhanced the rate of NO_3^- depletion ($P < 0.001$).

Accumulation of NO_2^- . Although the rates of utilization of NO_3^- were broadly similar in the 0- to 5-cm layer and the 11- to 15-cm layer, the fate of NO_3^- and the fate of the NO_2^- produced were very different. Figure 5 shows the changes in

TABLE 1. Regression models for NO_2^- concentrations as the dependent variable in the major rivers entering Lough Neagh

River	Independent variable ^a						R^2
	NO_3^-	NH_4^+	Free NH_3	Conductivity	pH	Temp	
Ballinderry		0.10***				2.62**	0.39
Blackwater					-30.13***	4.67***	0.43
Main	0.03**					3.14***	0.29
Moyola	0.01*						0.10
Six Mile Water	0.02**					3.60**	0.37
Upper Bann			-1.02*	0.41*		6.35**	0.51

^a ***, $P < 0.001$; **, $0.001 < P < 0.01$; *, $0.01 < P < 0.05$.

FIG. 4. Utilization of NO_3^- in sediment-water incubations in which the 11- to 15-cm layer was used. Error bars indicate the standard errors of the means ($n = 3$).

NO_2^- concentrations with time for the three sediment depths and two carbon treatments. Following the addition of a NO_3^- substrate, NO_2^- accumulated, with the concentration peaking at day 2 for all treatments, and then declined. For the low-glucose treatment, the NO_2^- concentrations in the three sediment layers were similar and reached maxima of 186 to 348 μg of N liter⁻¹, which represented only 1.1 to 2.1% of the ^{15}N in the original NO_3^- pool (Table 2). For the high-glucose treatment, the levels of $^{15}\text{NO}_3^-$ recovered as $^{15}\text{NO}_2^-$ were 2.24, 9.62, and 8.97% for the 0- to 5-cm layer, the 6- to 10-cm layer, and the 11- to 15-cm layer, respectively. There was a significant difference between the maximum NO_2^- concentrations for the two carbon treatments for the 0- to 5-cm layer ($P < 0.001$). However, the highest concentration was approximately only one-quarter the concentrations in the two deeper layers. Figure 6 illustrates the enrichment of the NO_2^- pools in the high-glucose treatment incubated with sediment from the three different depths. The ^{15}N enrichment of NO_2^- at day 1 was very similar to that of NO_3^- for sediment from all three depths, suggesting that at this point in the incubations all of the NO_2^- was NO_3^- derived. After day 1, the enrichment of the NO_2^- in the deeper sediments was still similar to that of NO_3^- . However, the reduction in ^{15}N enrichment of the NO_2^- pool derived from the 0- to 5-cm layer subsequent to day 1 suggests that this NO_2^- pool was being diluted by NO_2^- arising from nitrification of NH_4^+ .

Gaseous products. Accumulation of N_2O in the headspace was rapid under all conditions, and approximately 400 μg of N liter⁻¹ was produced by sediment layers below 6 cm, which was more than twice the level produced in the 0- to 5-cm layer. Production of N_2O peaked at day 4 in incubations with the 0- to 10-cm layers and then declined, probably due to transformations to N_2 . The enrichment of N_2O was very similar to that of the initial $^{15}\text{NO}_2^-$ pool, implying that reduction of NO_2^- was the main biochemical pathway for its formation. Initially (day 1), the mole fraction of N_2O , i.e., $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$, was as high as 0.75 for the 6- to 10-cm layer incubated under low-glucose conditions. By day 5, the mole fraction of N_2O was greatly reduced under all test conditions; e.g., it was 0.02 in the incubation with sediment from 0 to 5 cm under high-glucose conditions and 0.18 in the 11- to 15-cm layer under low-glucose conditions.

Interstitial NH_4^+ concentrations. For all treatments the NH_4^+ pool remained much less enriched than either the NO_2^- or N_2O pools due to the high initial concentrations of native NH_4^+ , which diluted the ^{15}N label. The changes in $^{15}\text{NH}_4^+$ enrichment with time were difficult to interpret because of the significant variation in NH_4^+ concentrations through the sediment profile and because of oscillating rates of utilization and production. To overcome this problem, the percentages of ^{15}N label recovered in the different N fractions during the incubations were calculated (Table 2). The presence of an easily degradable carbon substrate did make a significant difference in the proportion of NH_4^+ originating from NO_3^- ($P < 0.001$), and approximately twice the concentration of ^{15}N label was found in NH_4^+ formed under additional-carbon conditions. Although the pathway of NO_3^- reduction to NH_4^+ was successfully traced, a large proportion (between 61 and 85.6%) of the label was still unaccounted for, and the loss was greatest with sediment from the 11- to 15-cm layer. A small proportion of the label that was unaccounted for was assumed to be due to a combination of experimental error and conversion to other minor nitrogenous gaseous products (e.g., nitric oxide) (25), which were not measured. However, the main sink for the ^{15}N that was unaccounted for was probably uptake of NH_4^+ to support bacterial growth leading to the accumulation of PON.

FIG. 5. Concentrations of NO_2^- occurring at three depths of sediment previously supplemented with low (0.1 g liter^{-1}) and high (1.0 g liter^{-1}) levels of glucose. (a) Sediment taken from 0 to 5 cm below the surface. (b) Sediment taken from 6 to 10 cm below the surface. (c) Sediment taken from 11 to 15 cm below the surface. Error bars indicate the standard errors of the means ($n = 3$).

TABLE 2. Percentages of ^{15}N label recovered in nitrogen fractions during incubation with sediment from different depths and with different carbon treatments

Sediment depth (cm)	Glucose concn (g liter ⁻¹)	Nitrogen fraction	% of ^{15}N recovered					
			Zero time	Day 1	Day 2	Day 3	Day 4	Day 5
0–5	0.1	NO_3^-	100.00	64.36	50.19	39.8	30.17	30.34
		NH_4^+	0.00	0.20	0.36	0.24	0.15	0.41
		NO_2^-	0.00	0.70	1.19	0.89	0.45	0.03
		N_2O	0.00	0.11	0.28	0.31	0.50	0.37
		N_2	0.00	0.34	1.28	2.61	4.47	4.54
		$\Sigma \text{ D.P.}^a$	0.00	1.35	3.11	4.05	5.57	5.35
	1.0	NO_3^-	100.00	58.71	45.91	21.34	17.33	11.6
		NH_4^+	0.00	0.4	0.29	0.46	0.3	0.5
		NO_2^-	0.00	2.06	2.24	0.45	0.2	0.17
		N_2O	0.00	0.23	0.19	0.09	0.13	0.14
		N_2	0.00	0.55	1.99	4.62	6.91	6.93
		$\Sigma \text{ D.P.}$	0.00	3.24	4.71	5.62	7.54	7.74
6–10	0.1	NO_3^-	100.00	71.83	58.08	45.95	44.42	33.93
		NH_4^+	0.00	0.09	0.22	0.33	0.26	0.34
		NO_2^-	0.00	0.94	1.15	0.75	0.27	0.14
		N_2O	0.00	0.17	0.16	0.87	1.02	0.78
		N_2	0.00	0.06	1.46	1.46	2.32	3.77
		$\Sigma \text{ D.P.}$	0.00	1.26	2.99	3.41	3.87	5.03
	1.0	NO_3^-	100.00	69.18	46.13	34.4	31.27	24.62
		NH_4^+	0.00	0.15	0.59	0.65	0.58	0.56
		NO_2^-	0.00	2.95	9.62	4.23	1.67	0.75
		N_2O	0.00	0.23	0.18	0.56	0.82	0.67
		N_2	0.00	0.30	1.25	3.06	3.65	4.65
		$\Sigma \text{ D.P.}$	0.00	3.63	11.64	8.5	6.72	6.63
11–15	0.1	NO_3^-	100.00	66.78	35.77	39.44	17.96	12.33
		NH_4^+	0.00	0.2	0.73	0.35	0.64	0.41
		NO_2^-	0.00	1.99	2.16	0.91	0.92	0.24
		N_2O	0.00	0.05	0.38	0.67	0.80	1.10
		N_2	0.00	0.05	1.42	1.39	3.54	4.83
		$\Sigma \text{ D.P.}$	0.00	2.29	4.69	3.32	5.9	6.58
	1.0	NO_3^-	100.00	62.17	30.03	16.79	11.57	6.4
		NH_4^+	0.00	0.57	0.56	0.81	0.71	0.61
		NO_2^-	0.00	5.84	8.97	2.5	1.98	0.79
		N_2O	0.00	0.24	0.31	0.16	0.39	0.54
		N_2	0.00	1.24	2.83	3.27	5.59	6.04
		$\Sigma \text{ D.P.}$	0.00	7.89	12.67	6.74	8.67	7.98

^a $\Sigma \text{ D.P.}$, total percentage of ^{15}N recovered in (N_2 , N_2O , NH_4^+ , and NO_2^-).

DISCUSSION

Potential mechanisms of NO_2^- formation. Nitrite is a common intermediate in at least three different biochemical pathways that occur in freshwater sediments: nitrification, denitrification, and DNRA (Fig. 1). These processes do not occur in isolation but are integrated, and products of one process may act as substrates for another. Although there is evidence from the present study which suggests that all of these processes operate within river sediments, their relative rates are dependent on environmental conditions. The nitrification process is oxygen (O_2) dependent (8), and normally is restricted to an O_2 diffusion zone 1.5 to 5.5 mm from the surface (16). Evidence that nitrification is restricted to the surface 0 to 5 cm of sediment is presented in Fig. 6, which shows that there is a substantial reduction in the $^{15}\text{NO}_2^-$ pool due to dilution from $^{14}\text{NO}_2^-$ production via nitrification. The route taken for NO_3^- reduction in sediments is largely dependent on the bacterial populations present, which have been shaped by environmental conditions. Tiedje (54) reported that continuously anoxic

habitats (e.g., deep sediments where oxygen demand from chemical and biological processes exceeds the supply from overlying water [15]) would be expected to select for vigorous fermenters and obligate anaerobes, characteristics of some DNRA reducers, but not of denitrifiers. Alternatively, the more oxygen-rich environments (e.g., the upper few millimeters of sediment) are expected to support bacterial populations that are most efficient and competitive at using the carbon supply under respiratory conditions, such as denitrifiers.

Environmental evidence for DNRA. Support for the hypothesis that the majority of the river-derived NO_2^- observed in the present study was formed from the reduction of NO_3^- comes from the multiple regression analysis of field data for NO_2^- versus other water quality variables (Table 1). The results show that there is a positive correlation between NO_3^- and NO_2^- that appears to be temperature dependent (Fig. 3). These results do not provide evidence to suggest which NO_3^- reduction mechanism is responsible. However, examination of weekly plots of N species in the rivers (Fig. 3) provided field

FIG. 6. Enrichment of NO_2^- (^{15}N atom% excess) under elevated-carbon conditions. Error bars indicate the standard errors of the means ($n = 3$). Symbols: ◆, sediment from 0 to 5 cm below the surface; ■, sediment from 6 to 10 cm below the surface; ▲, sediment from 11 to 15 cm below the surface.

data which suggest that DNRA is the predominant NO_3^- reduction pathway. The decline in NO_3^- concentration coincided with an elevation in NO_2^- levels, which was followed by a substantial increase in NH_4^+ concentration when those of NO_3^- were minimal. These observations suggest that NO_2^- accumulation results from inhibition of the DNRA bacterial NO_2^- reductase system by NO_3^- . The samples were collected from the lower reaches of rivers, where the flow rate is low and is accompanied by a reduction in dissolved oxygen values in sediments, which provides ideal conditions for DNRA. In addition, the sediments from algal blooms observed in these sections of the rivers provide elevated carbon concentrations as an energy source, while at the same time the release of hydroxyl ions resulting from photosynthesis elevates the pH. Nommik (34) showed that DNRA was favored by strictly anaerobic conditions with large quantities of readily oxidizable organic matter and at a high pH. The possibility that DNRA is favored at high pH has recently been supported by soil data obtained by Stevens et al. (52).

Experimental evidence for DNRA from sediment-water incubations. Further evidence for our hypothesis that DNRA may be the major NO_3^- reduction mechanism was obtained from the results of the sediment-water incubations. Denitrification reportedly occurs only in the top 0 to 5 cm of sediment (15, 21, 28, 35, 41), and a rapid decrease in denitrification capacity accompanies increasing sediment depth (22, 23, 48). The proportion of NO_3^- reduced to NH_4^+ , however, increases down the sediment profile (28) due to larger NH_4^+ -producing bacterial populations (20), reflecting the role of O_2 regulation (54). Hence, we would expect to find more DNRA in sediment at greater depths. Larger accumulations of NO_2^- observed at depths of greater than 5 cm in our present study (Fig. 5) are indicative of the fermentative DNRA pathway (7), where NO_2^- concentrations must increase to a certain threshold before the NO_2^- reduction pathway is completely derepressed. In contrast, denitrification normally proceeds to completion

without the accumulation of NO_2^- or any other intermediate (40). The products of dissimilatory NO_3^- reduction vary depending on the bacterial species (15), but reduction beyond the NO_2^- formation step is believed to be limited more by the environmental conditions than by the genetics of the organism (44). A variety of bacteria perform NO_3^- respiration in which NO_3^- is reduced in a dissimilatory pathway to NO_2^- alone (36, 38). Alternatively, the DNRA pathway may be repressed at the NO_2^- formation stage due to the inhibitory effects of NO_3^- (present throughout this investigation) on the NO_2^- reductase (45). Although the NO_2^- reductase system may have been inhibited, a capacity for some DNRA was shown by ^{15}N analysis of the $^{15}\text{NH}_4^+$ pool, in which between 0.3 and 0.6% of the ^{15}N label was recovered (Table 2). This suggests that the remainder of the ^{15}N label (as $^{15}\text{NH}_4^+$) was taken up immediately by bacteria to maintain high growth rates, thus reflecting the conservatory nature of DNRA. DNRA is favored under high available C/N ratio conditions at the expense of denitrification (19), while lower carbon concentrations support denitrifiers. Under the elevated carbon concentrations employed in the present study, maximum NO_2^- concentrations of 1,380 to 1,480 μg of N liter $^{-1}$ in incubations with sediment from 6 to 15 cm below the surface were observed, compared to 390 μg of N liter $^{-1}$ in the surface 5 cm. Hence, there is evidence which suggests that the high NO_2^- concentrations observed in the present study may have originated predominantly from DNRA under high-carbon conditions at sediment depths greater than 5 cm.

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