

## Estimates of Denitrification and Nitrification in Coastal and Estuarine Sediments

TAKASHI NISHIO,† ISAO KOIKE, AND AKIHIKO HATTORI\*

*Ocean Research Institute, University of Tokyo, Nakano ku, Tokyo 164, Japan*

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Denitrification and nitrification in sediments of Tama Estuary and Odawa Bay, Japan, were investigated by the combined use of a continuous-flow sediment-water system and a  $^{15}\text{N}$  tracer technique. At Odawa Bay, the nitrification rate was comparable to the nitrate reduction rate, and 70% of the  $\text{N}_2$  evolved originated from nitrogenous oxides (nitrate and nitrite) which were produced by the action of nitrifying bacteria in the sediments. At Tama Estuary, the nitrate reduction rate was 11 to 17 times higher than the nitrification rate, and nitrogenous oxides derived from ammonium accounted for only 6 to 9% of the  $\text{N}_2$  evolution by denitrification.

The distributions of nitrate and nitrite in sedimentary pore waters are controlled by their concentrations in the overlying water and their diffusivities in the sediments and by bacterial nitrification and denitrification activities in the sediments. In their model calculations, Vanderborgh and Billen (16), Vanderborgh et al. (17), and Billen (4) postulated that nitrification and denitrification take place in two separate layers, an upper oxic layer and a lower anoxic layer, respectively. However, Grundmanis and Murray (7) found that nitrification occurs in an intermediate sedimentary zone lying between the upper and lower denitrification zones when irrigation by burrowing benthic organisms is significant. According to Barnes et al. (1), excess  $\text{N}_2$  estimated from the  $\text{N}_2/\text{Ar}$  ratio in pore water of Santa Barbara Basin sediments exceeded what was expected from denitrification of interstitial nitrate. They suggested that  $\text{N}_2$  is formed from ammonium via intermediates or products of nitrification. Koike and Hattori (11) have demonstrated the co-occurrence of nitrification and nitrate reduction in a coastal sandy sediment by using a  $^{15}\text{N}$  isotope dilution technique. A possible occurrence of denitrification in oxic sediments of the eastern Atlantic has also been discussed by Wilson (20).

We (14) have previously developed a technique to determine in situ rates of nitrate reduction and denitrification in coastal sediments by the combined use of an experimental continuous-flow sediment-water system and a  $^{15}\text{N}$  tracer method. The present study extends this work

to nitrification in coastal and estuarine sediments and denitrification associated with this nitrification.

### MATERIALS AND METHODS

**Sampling.** Replicate sediment core samples were collected with Plexiglas tubes (inner diameter, 11.4 cm) on 21 December 1980 and 20 May 1981 at Tama Estuary, Japan, and on 8 May 1981 at Odawa Bay, Japan. A description of the sampling sites is given elsewhere (14). The Odawa Bay sediments consisted of fine sand rich in carbonate, and the Tama Estuary sediments consisted of muddy fine sand. Judging from the color, all of the sediments were anoxic except for a thin surface layer. There were many benthic organisms (polycheta, crustacea, etc.) at both locations. Sea grasses and seaweeds flourished at Odawa Bay, but there were few plants on Tama Estuary sediments. Special care was taken to preserve sediment structure during sampling and transportation. Overlying water samples were collected with Plexiglas bottles. Other sediment samples were collected with Plexiglas tubes (inner diameter, 6.0 cm) for analysis of the sedimentary pore waters. The sediment cores were sectioned into 1- to 3-cm-thick segments immediately after sampling and stored frozen until use. Characteristics of the sediments used in the experiments are described in Table 1.

**Experimental procedures.** The experimental setup and procedures used were essentially the same as described by Nishio et al. (14). The system consisted of (i) a rubber-stoppered Plexiglas tube containing a core of sediment 20 to 30 cm long with 4 to 5 cm of overlying water and (ii) a reservoir containing Whatman GF/C-filtered seawater supplemented with the indicated amount of [ $^{15}\text{N}$ ]ammonium or [ $^{15}\text{N}$ ]nitrate. The seawater flowed over the sediment at a fixed rate of 5 to 10 ml  $\text{min}^{-1}$ , and the water layer was mixed continuously with a magnetic stirrer. The turnover rate of the overlying water was 1 to 2 turnovers per h. At intervals, portions of the effluent were collected for chemical analysis. Two sets of experiments, one with

† Present address: Upland Farming Div., Hokkaido National Agricultural Experiment Station, Memuro, Kasai-gun, Hokkaido, 082, Japan.

TABLE 1. Characteristics of sediments used in the experiments

Location	Sampling date	Temp (°C)	Concn ( $\mu\text{M}$ ) in overlying waters			Concn ( $\mu\text{M}$ ) in sedimentary pore waters at indicated depth			
			$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NH}_4^+$	Depth (cm)	$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NH}_4^+$
Tama Estuary	21 December 1980	9.3	140.6 <sup>a</sup>		378	0-1	0.37	0.96	381
						1-3	0.58	0.58	316
						3-6	0.29	0.65	307
	20 May 1981	18.0	133.3	11.1	216	0-1	0.98	0.39	320
						1-2	0.27	0.76	401
						2-3	0.66	1.70	516
Odawa Bay	8 May 1981	22.9	5.27	0.83	4.0	0-1	0.73	0.44	97
						1-2	0.54	0.64	109
						2-3.5	0.59	0.58	105
						3.5-5.5	0.63	0.39	111

<sup>a</sup>  $\text{NO}_2^-$  was included.

[<sup>15</sup>N]nitrate and the other with [<sup>15</sup>N]ammonium, were run in parallel at in situ temperatures (Table 1).

**Analytical procedures.** The <sup>15</sup>N content in dissolved N<sub>2</sub> was determined as described by Koike et al. (12). Ammonium was separated by steam distillation and collected in 0.01 N HCl by a procedure described by Hattori et al. (8), and its <sup>15</sup>N content was determined by the method of Rittenberg (15). The method of Wada (18), as modified by Koike and Hattori (11), was used for determination of the <sup>15</sup>N content in nitrate plus nitrite. A Hitachi RMU-6 mass spectrometer fitted with a double collector and a dual inlet system for ratio-metry was used. The sensitivity of the <sup>15</sup>N determination by mass spectrometry was 0.0004 atom% <sup>15</sup>N.

Nitrous oxide was determined as described by Cohen (6) with a Shimadzu GC 4-CM gas chromatograph fitted with a <sup>63</sup>Ni electron capture detector. Nitrate, nitrite, and ammonium were determined with a Hirama autoanalyzer. The method of Wood et al. (21) for nitrate, the method of Bendschneider and Robinson (3) for nitrite, and the method of Head (9) for ammonium were applied with minor modifications.

## RESULTS

Figure 1 shows the changes with time in the concentrations of nitrate and nitrite during the experiments amended with [<sup>15</sup>N]ammonium. Nitrate concentrations in the effluent (overlying water) were invariably lower than those in the influent (source water). Since the overlying waters contained few microorganisms (14), this indicates that nitrate diffused from the overlying water into the sediment and was consumed there. Throughout the experiments, nitrate and nitrite concentrations in the overlying water remained practically constant, suggesting that the system was nearly in a steady state with respect to nitrate and nitrite. In the Tama Estuary sediment experiment, nitrite concentrations in the influent and effluent waters were identical, but the effluent nitrite concentration was at least

twice that of the influent in the Odawa Bay sediment experiment. The nitrite may be produced by nitrification in the sediment and diffused into the overlying water. The occurrence of nitrification was verified by the appearance of [<sup>15</sup>N]nitrate and [<sup>15</sup>N]nitrite from [<sup>15</sup>N]ammonium (Fig. 2).

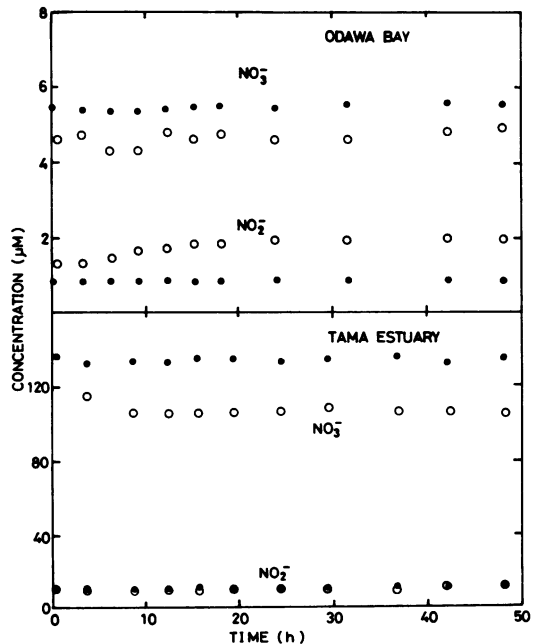


FIG. 1. Temporal profiles of nitrate and nitrite concentrations in the [<sup>15</sup>N]ammonium-amended continuous-flow sediment-water system. Symbols: ●, concentrations in influents; ○, concentrations in effluents. Concentrations of [<sup>15</sup>N]ammonium (99.1 atom% <sup>15</sup>N) added were 100  $\mu\text{M}$  in the 8 May 1981 experiment (Odawa Bay) and 25  $\mu\text{M}$  in the 20 May 1981 experiment (Tama Estuary).

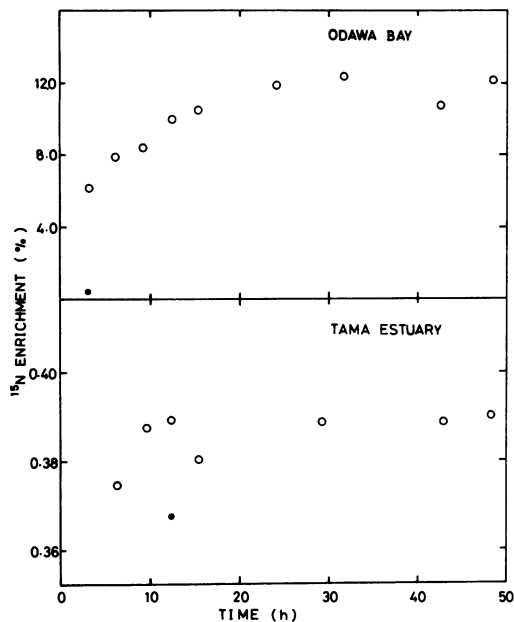


FIG. 2. Temporal profiles of  $^{15}\text{N}$  enrichment of nitrate plus nitrite in the  $^{15}\text{N}$ ammonium-amended continuous-flow sediment-water system. Symbols: ●,  $^{15}\text{N}$  enrichments in influents; ○,  $^{15}\text{N}$  enrichments in effluents. Data were obtained in the same experiments as in Fig. 1.

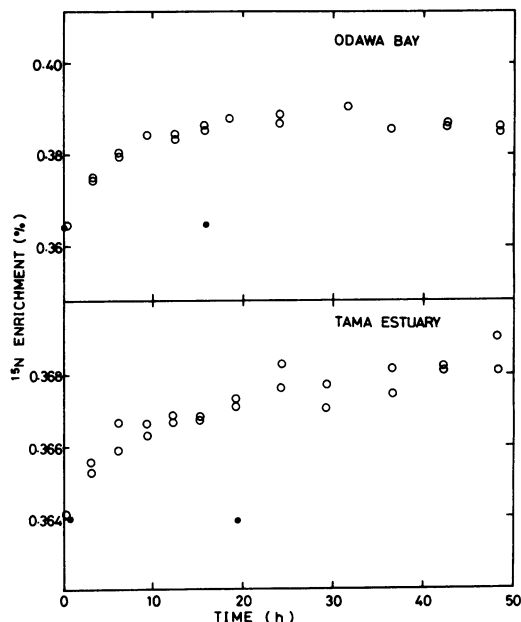


FIG. 3. Temporal profiles of  $^{15}\text{N}$  enrichment of  $\text{N}_2$  in the  $^{15}\text{N}$ ammonium-amended continuous-flow sediment-water system. Symbols: ●,  $^{15}\text{N}$  enrichments in influents; ○,  $^{15}\text{N}$  enrichments in effluents. Data were obtained in the same experiments as in Fig. 1.

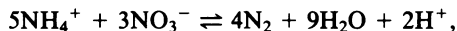
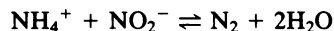
Figure 3 shows changes with time in the  $^{15}\text{N}$  enrichment of dissolved  $\text{N}_2$  in the overlying water in the  $^{15}\text{N}$ ammonium-amended experiment. The  $^{15}\text{N}$  enrichment of  $\text{N}_2$  increased and attained a steady level within 20 to 30 h; this is evidence that denitrifying bacteria in the sediment utilize nitrogenous oxides (nitrate and nitrite) produced from  $^{15}\text{N}$ ammonium by nitrification. The mean concentrations and  $^{15}\text{N}$  enrichments of nitrate plus nitrite in the overlying water during the steady state are given in Table 2 together with those of ammonium in the sedimentary pore water.

Temporal profiles of nitrate and nitrite in the  $^{15}\text{N}$ nitrate-amended experiments (Fig. 4) were similar to those in the  $^{15}\text{N}$ ammonium-amended experiments (Fig. 1), although nitrate concentrations in the effluent decreased later during the period of incubation in the Odawa Bay sediment experiment. Concentrations of evolved  $\text{N}_2$  attained steady levels within 10 h (Fig. 5), as observed in earlier experiments (14).

Figure 6 shows temporal changes in the  $\text{N}_2\text{O}$  concentration in the Tama Estuary sediment. In the Odawa Bay sediment experiment, the mean  $\text{N}_2\text{O}$  concentrations in the influent and effluent were 21.9 and 24.1 ng-atoms of N liter $^{-1}$ , respectively.

## DISCUSSION

Denitrification is the only identified biochemical process which releases free  $\text{N}_2$ . From a thermodynamic point of view, the following reactions could occur (2, 5):



but no organisms capable of catalyzing these reactions have ever been reported. Yoshida and Alexander (22) found that nitrifying bacteria produce  $\text{N}_2\text{O}$  from  $\text{NH}_3$ . According to Johnston (10),  $\text{N}_2$  can photochemically be produced from  $\text{N}_2\text{O}$ . However, the occurrence of this reaction in the present study is unlikely, because our system was kept in darkness. Biological nitrogen transformations in the overlying water were negligible (14). We conclude that the  $^{15}\text{N}_2$  in the  $^{15}\text{N}$ ammonium-amended experiments was produced by denitrifying bacteria from the nitrate and nitrite which resulted from bacterial nitrification in the sediments. The data presented in Fig. 2 clearly show  $^{15}\text{N}$ nitrate or  $^{15}\text{N}$ nitrite production from  $^{15}\text{N}$ ammonium.

As nitrification proceeds under oxic conditions and denitrification proceeds under anoxic

TABLE 2. Steady-state concentrations and  $^{15}\text{N}$  enrichments of nitrate plus nitrite and ammonium in the continuous-flow sediment-water system<sup>a</sup>

Water type	Nitrogen form	Tama Estuary				Odawa Bay (8 May 1981)	
		21 December 1980		20 May 1981		$\mu\text{M}$	atom% $^{15}\text{N}$
		$\mu\text{M}$	atom% $^{15}\text{N}$	$\mu\text{M}$	atom% $^{15}\text{N}$		
Influent	$\text{NH}_4^+$	459	17.7	237	9.08	98.7	95.1
	$\text{NO}_3^- + \text{NO}_2^-$	153	0.37	145	0.37	6.3	0.40
Effluent	$\text{NO}_3^- + \text{NO}_2^-$	145	0.42	116	0.39	6.6	11.9
Sedimentary pore <sup>b</sup>	$\text{NH}_4^+$	554	15.3	287	6.32	120	45.6

<sup>a</sup> The concentrations of [ $^{15}\text{N}$ ]ammonium (99.1 atom%  $^{15}\text{N}$ ) added were 100  $\mu\text{M}$  in the 21 December 1980 and 8 May 1981 experiments and 25  $\mu\text{M}$  in the 20 May 1981 experiment.

<sup>b</sup> The concentration and  $^{15}\text{N}$  enrichment of ammonium in sedimentary pore water in the surface layer (0 to 1 cm) at the end of the experiments are given.

conditions, dissolved substances diffusing into the sediment from the overlying water reach the active site of nitrification before they reach the denitrification site. When [ $^{15}\text{N}$ ]ammonium in the overlying water is allowed to diffuse into the sediments, its concentration at the active site of nitrification will reach a steady level within a relatively short time, and [ $^{15}\text{N}$ ]nitrate and [ $^{15}\text{N}$ ]nitrite will be produced there at a constant rate. If this [ $^{15}\text{N}$ ]nitrate and [ $^{15}\text{N}$ ]nitrite are

continuously supplied to the active denitrification site, a steady evolution of  $^{15}\text{N}_2$  will occur. The results depicted in Fig. 3 suggest that active sites of nitrification and denitrification of nitrate and nitrite originating from ammonium are confined to a shallow depth.

In our experimental sediment-water system,  $^{15}\text{N}$  enrichment of ammonium in the sedimentary pore waters was invariably less than in the overlying water (Table 2). It is evident that ammonium is produced by decomposition of organic matter in the sediments. The ammonium

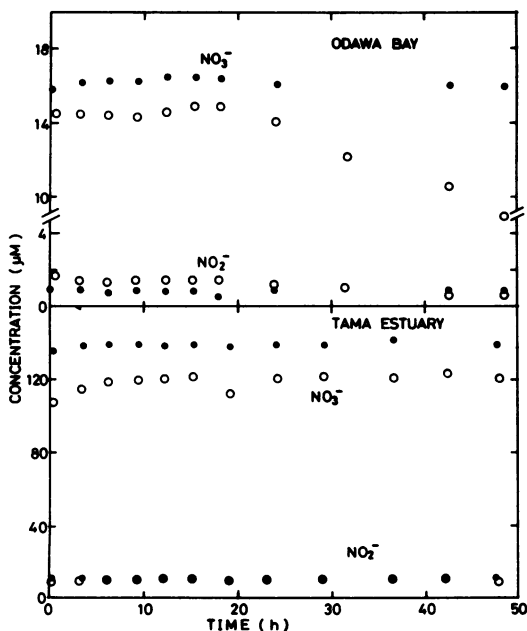


FIG. 4. Temporal profiles of nitrate and nitrite concentrations in the [ $^{15}\text{N}$ ]nitrate-amended continuous-flow sediment-water system. Symbols: ●, concentrations in influents; ○, concentrations in effluents. Concentrations of [ $^{15}\text{N}$ ]nitrate (99.6 atom%  $^{15}\text{N}$ ) added were 10  $\mu\text{M}$  in the 8 May 1981 experiment (Odawa Bay) and 5  $\mu\text{M}$  in the 20 May 1981 experiment (Tama Estuary).

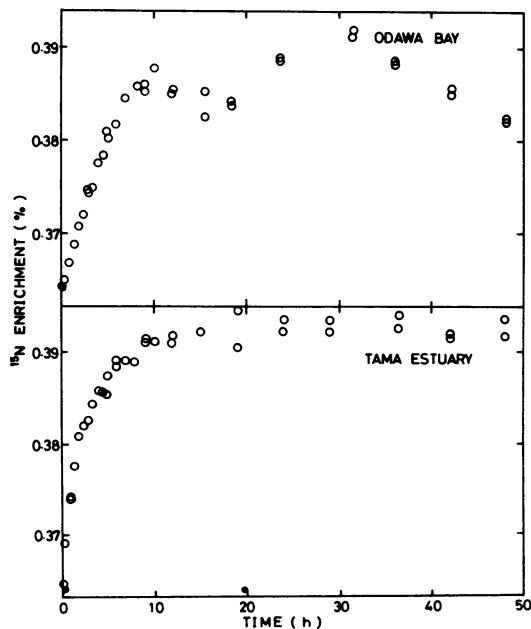


FIG. 5. Temporal profiles of  $^{15}\text{N}$  enrichment of  $\text{N}_2$  in the [ $^{15}\text{N}$ ]nitrate-amended continuous-flow sediment-water system. Symbols: ●,  $^{15}\text{N}$  enrichments in influents; ○,  $^{15}\text{N}$  enrichments in effluents. Data were obtained in the same experiments as in Fig. 4.

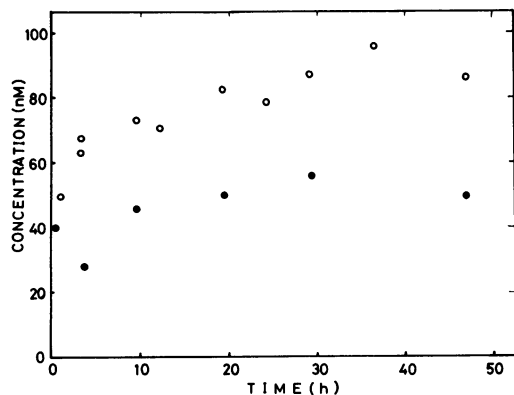


FIG. 6. Temporal profiles of  $N_2O$  concentration in the continuous-flow sediment-water system. Symbols: ●,  $N_2O$  in influents; ○,  $N_2O$  in effluents. Data were obtained in the same experiment (Tama Estuary) as in Fig. 4.

produced dilutes [ $^{15}N$ ]ammonium diffused from the overlying water and decreases the  $^{15}N$  enrichment of ammonium in the sedimentary pore waters.

Besides dissolved ammonium in pore water, there is ammonium which is adsorbed on mineral particles. This ammonium is quickly exchanged with the ammonium dissolved in the pore water, and an equilibrium between the two forms of ammonium is established within a few hours (H. Iizumi, doctoral dissertation, University of Tokyo, Japan, 1979). However, some effect of the adsorbed ammonium on the  $^{15}N$  enrichment in dissolved ammonium cannot be discounted during the initial period of the experiment. We therefore restrict our discussion to the events occurring during the steady state. The effect of ammonium fixed in the clay minerals can be safely disregarded in the time scale of our experiment.

The reported isotope discriminations associated with bacterial nitrification and denitrification are 2.1 to 3.5 and 2.3 to 2.9%, respectively (13, 19). If these isotope effects are disregarded, the  $^{15}N$  enrichment of the produced  $N_2$  should be equal to the  $^{15}N$  enrichment of ammonium at the active site of nitrification. Using the steady-state data on concentrations and  $^{15}N$  enrichment of  $N_2$  in the influent and the effluent water and on  $^{15}N$  enrichment of ammonium in the sedimentary pore waters in the [ $^{15}N$ ]ammonium-amended experiments, we can calculate the flux of  $N_2$  which results from denitrification coupled to nitrification (Table 3 [B]). On the other hand, denitrification which proceeds using nitrate and nitrite supplied from the overlying water can be independently estimated from the [ $^{15}N$ ]nitrate-amended experiment (Fig. 5), as described in our previous paper (14). Because of [ $^{15}N$ ]nitrate amendment, nitrate concentrations in the overlying water of our experimental system were much higher than in situ concentrations. This was especially the case in the Odawa Bay sediment experiment. Therefore, the values for the  $N_2$  fluxes (Table 3 [A]) which have been corrected for this concentration difference, assuming a linear relationship between denitrification rate and nitrate concentration (12), are used in the following calculation.

In the sediment, nitrate and nitrite are continuously produced from ammonium by nitrification and consumed by nitrate reduction. In a steady state, the net flux of nitrate plus nitrite across the sediment-water interface, or the difference between the downward and upward fluxes, should be equal to the disappearance of nitrate plus nitrite in the overlying water. This relation can be represented by the equation:

$$(I - E)V = (X - Y)S, \quad (1)$$

where  $I$  and  $E$  are the concentrations of nitrate

TABLE 3. Fluxes of  $N_2$ , nitrate plus nitrite, and  $N_2O$  across the sediment-water interface, and nitrification rates<sup>a</sup>

Location	Sampling date	Flux <sup>b</sup> of:				$N_2O$ (upward)	Nitrification ( $B + Y$ )
		$N_2$ (upward)		Nitrate + nitrite			
		A	B	X	Y		
Tama Estuary	21 December 1980	13.3	1.31	29.6	1.42	ND <sup>c</sup>	2.73
	20 May 1981	55.5	3.85	122	3.45	0.19	7.30
Odawa Bay	8 May 1981	1.02	2.67	9.49	7.30	<0.01	9.97

<sup>a</sup> All data are expressed as nanogram-atoms of N centimeters<sup>-2</sup> hours<sup>-1</sup>.

<sup>b</sup> A, Flux of  $N_2$  from denitrification using nitrate plus nitrite diffused from the overlying water. The values were estimated by the method of Nishio et al. (14) and corrected for the difference in nitrate concentrations between the in situ and experimental systems on the basis of an assumed linear relationship between denitrification rate and nitrate concentration. B, Flux of  $N_2$  from denitrification coupled to nitrification in sediments. X, Downward flux of nitrate plus nitrite. Y, Upward flux of nitrate plus nitrite.

<sup>c</sup> ND, Not determined.

plus nitrite in the influent and effluent (nanogram-atoms of N centimeters<sup>-3</sup>), respectively;  $X$  is the downward flux of nitrate plus nitrite from the overlying water (nanogram-atoms of N centimeters<sup>-2</sup> hours<sup>-1</sup>);  $Y$  is the upward flux of nitrate plus nitrite into the overlying water (nanogram-atoms of N centimeters<sup>-2</sup> hours<sup>-1</sup>);  $V$  is the flow rate (milliliters hours<sup>-1</sup>); and  $S$  is the surface area of the sediment core (centimeters<sup>2</sup>). We consider formally only the upward flux of nitrate plus nitrite produced by nitrification in the sediment; the upward flux of nitrate plus nitrite diffused into the sediment can be included in  $X$ . The mass balance relation with respect to <sup>15</sup>N in nitrate plus nitrite is given by the equation:

$$(Ii - Ee)V = (Xe - Yy)S, \quad (2)$$

where  $i$  and  $e$  are the <sup>15</sup>N enrichments of nitrate plus nitrite in the influent and effluent, respectively, and  $y$  is the <sup>15</sup>N enrichment of nitrate plus nitrite produced by nitrification in sediment. For the purpose of calculation, we assume that <sup>15</sup>N enrichment of nitrate plus nitrite produced by nitrification is equal to that of ammonium in the sedimentary pore water. The values for  $X$  and  $Y$  can thus be obtained by the solution of equations 1 and 2:

$$X = \left[ \frac{I(y - i)}{y - e} - E \right] \frac{V}{S}$$

and

$$Y = \frac{VI(e - i)}{S(y - e)}$$

The sum of  $Y$  and  $B$  represents the nitrification rate per unit area of sediment (Table 3).

In the above calculation, we used the average <sup>15</sup>N enrichment of ammonium in the surface layer (0 to 1 cm) of sediment as the <sup>15</sup>N enrichment of ammonium at the active site of nitrification. If nitrification occurs extensively within a couple of millimeters in the surface sediment, and if the <sup>15</sup>N enrichment of ammonium declines sharply with depth, then the values for  $Y$  and  $B$  obtained give overestimates. Unfortunately, vertical profiles of the <sup>15</sup>N enrichment of ammonium in the sediments are unknown. In the Odawa Bay experiment, the <sup>15</sup>N enrichment of ammonium in the overlying water (95.1%) was twice that in the sedimentary pore water (45.6%) (Table 2). The actual <sup>15</sup>N enrichment at the nitrification site is probably between these two values. Therefore, the estimate of the nitrification rate is correct at least within a factor of two. In the case of the Tama Estuary sediment, the

uncertainty in the estimate is much less.

To achieve sufficient <sup>15</sup>N enrichment of N<sub>2</sub>, the addition of a large amount of [<sup>15</sup>N]ammonium to the influent water of the experimental system was required. When the ambient concentration of ammonium is low, this may introduce another uncertainty in estimating the in situ rate of nitrification, because the increase in ammonium concentration may accelerate the nitrification rate. In the case of Odawa Bay, where the in situ concentration of ammonium in overlying water was 4 μM, the ammonium concentration in the experimental system (~100 μM) was nearly the same as that in the sedimentary pore water. However, nitrification in the overlying water was negligible in our system. The error caused by this concentration difference is, therefore, probably not very large, but data supporting this inference are needed.

A steady N<sub>2</sub>O flux of 0.19 ng-atom of N cm<sup>-2</sup> h<sup>-1</sup> across the sediment-water interface in Tama Estuary was calculated from the data shown in Fig. 6 and the flow rate of the overlying water (Table 3). The flux was 3 orders of magnitude lower than that of N<sub>2</sub> evolved by denitrification. The steady flux of N<sub>2</sub>O in the Odawa Bay sediment experiment (less than 0.01 ng-atom of N cm<sup>-2</sup> h<sup>-1</sup>) was negligible.

Disregarding the problems discussed above, we tentatively use the data given in Table 3 to illustrate the relative contributions of the individual processes in question to the nitrogen cycling in the coastal and estuarine sediments. In the Odawa Bay sediment, the rate of denitrification coupled to nitrification is 2.5 times higher than that of denitrification which proceeds using nitrate from the overlying water. The nitrate produced by nitrification acts as a major source for denitrification. Approximately 30% of the nitrate produced by nitrification is consumed by denitrification, and the rest diffuses into the overlying water. In the Tama Estuary sediment, the denitrification coupled to nitrification accounts for only 6 to 9% of the total denitrification, although almost one-half of the nitrate and nitrite produced by nitrification is utilized by denitrifiers in the sediments. High concentrations of nitrate in the overlying water are mainly responsible for the high denitrification activity at this site.

#### ACKNOWLEDGMENTS

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