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Denitrification in Aquifer Soil and Nearshore Marine Sediments Influenced by Groundwater Nitrate

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We estimated rates of denitrification at various depths in sediments known to be affected by submarine discharge of groundwater, and also in the parent aquifer. Surface denitrification was only measured in the autumn; at 40-cm depth, where groundwater-imported nitrate has been measured, denitrification occurred consistently throughout the year, at rates from 0.14 to 2.8 ng-atom of N g^{-1} day^{-1}. Denitrification consistently occurred below the zone of sulfate reduction and was sometimes comparable to it in magnitude. Denitrification occurred deep (14 to 40 cm) in the sediments along 30 km of shoreline, with highest rates occurring where groundwater input was greatest. Denitrification rates decreased with distance offshore, as does groundwater influx. Added glucose greatly stimulated denitrification at depth, but added nitrate did not. High rates of denitrification were measured in the aquifer (17 ng-atom of N g^{-1} day^{-1}), and added nitrate did stimulate denitrification there. The denitrification measured was enough to remove 46% of the nitrate decrease observed between 40- and 14-cm depth in the sediment.

Groundwater discharge is known to be a source of nutrients to lakes in many parts of the world (3, 8, 34) and recently has been implicated as a source of nitrate to coastal marine sediments also (5, 14, 26). This groundwater influx may have an effect on phytoplankton productivity in the water column, which is often assumed to be nitrogen limited in marine systems (21, 25). However, groundwater-derived nitrate may also affect microbial activities in the sediments through which the groundwater effuses. In particular, this nitrate could promote denitrification, which would mitigate any effect on water-column productivity.

The groundwater on Long Island, New York is known to be heavily contaminated with nitrate (18). Submarine groundwater discharge through the sediments of Great South Bay, on the south shore of Long Island, has been well characterized (2), and its influence on sediment nitrate concentrations has been documented (5). We describe here our study of denitrification in these groundwater-influenced sediments and in the parent aquifer.

MATERIALS AND METHODS

Sites and sampling procedures. Great South Bay is a shallow (mean depth, 1.3 m) coastal lagoon between the south shore of Long Island and the barrier beach. It lies in sandy glacial outwash material containing the Upper Glacial water table aquifer (2). The primary site for this study was just east of the village of Patchogue, 15 m off the high-tide line; other sampling sites were spread west from there over a distance of 30 km. The aquifer sampling site was 5 km inland of the westernmost site.

Cores from the coastal sediments, at least 40 cm long, were taken in 6-cm-diameter polybutyrate core tubes and transported (time, <1 h) to the laboratory in ice and in the dark. The cores were sectioned rapidly. Two-centimeter sections at various depths of two or three cores were halved; one half was used for pore water analysis, and the other was used for denitrification incubations.

The soil sample from the aquifer was taken with a rotary drilling rig and a split-spoon sampler from a depth of 4 m. This sample was homogenized before use.

Pore water analysis. Sections of cores were centrifuged at 6,000 × *g* for 20 min in Delrin pore-water separating bottles (B. Brinkhuis, patent pending). Salinity profiles were determined with a Goldberg refractometer. Nutrients (NO_3^-, NO_2^-, and NH_4^+) were analyzed by standard methods (29) adapted for a Technicon Autoanalyser (35).

Denitrification assays. Sections of cores were incubated at in situ temperature in 125-ml Erlenmeyer flasks with black rubber stoppers, except for the aquifer soil, which was incubated in 15-ml crimp-seal serum bottles with butyl stoppers. The flasks were flushed during setup and for 2 min after sealing with a stream of O_2-free N_2.

Acetylene was added to the gas phase of each incubation to a concentration of 20% and denitrification was assessed by the accumulation of N_2O (1, 27, 36, 37). Measurements were generally made frequently during the first 12 h, and then at 24 h and 48 h. Nitrous oxide was measured on a Perkin-Elmer Sigma 2B gas chromatograph with a ^{63}Ni electron capture detector. The column was a 2-m-long, 3-mm-OD Porapak Q column; oven temperature was 60°C; detector temperature was 300°C; carrier gas was 1% CO_2 in nitrogen at a flow rate of 34 ml/min. The detector was calibrated with gas standards in concentrations from 4.1 pmol ml^{-1} to 4.1 nmol ml^{-1} at 25°C. Concentrations of N_2O in the gas phase were corrected for solubility. At the end of each experiment, gas-phase volumes were measured and wet and dry (at 105°C) weights of sediment were determined.

Loss on ignition was occasionally determined by first drying samples of sediments to constant weight at 105°C and then heating in a muffle furnace at 545°C for 6 h.

Where additions (e.g., of nitrate) were made to the sediment before incubation, 15 ml of filtered (0.45-μm Metricel) seawater was used to ensure adequate diffusion through the sediment (5 ml of distilled water for the aquifer samples).

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TABLE 1. Structure of cores and annual mean values of nitrate concentration and denitrification rate in different horizons from the primary site[a]

Core horizon	Width of band (cm)	Nitrate (μM)	Denitrification rate (ng-atom of N g^{-1} of dry sediment day^{-1})
Brown sand	0.1–4	20 ± 2.1	3.6 ± 2.8
Black sand	5–10	22 ± 2.7	0
White sand	10–15	24 ± 2.1	0.48 ± 0.11
Gray clay	4–8	21 ± 2.0	0.16 ± 0.040
Coarse sand/ shell fragments	>3	32 ± 2.5	0.69 ± 0.28

[a] Values are means ± SE; $n = 8$ for black sand layer, 9 for gray clay, 10 elsewhere. Denitrification rate was calculated from initial linear ($r^2 > 0.9$) rate.

The same volume of seawater or distilled water was also added to some of the control incubations.

Sulfate reduction. Sulfate concentrations were measured gravimetrically as barium sulfate. Rate of sulfate reduction was assessed by injection of $^{35}SO_4{}^{2-}$ into 5-cm core sections. After 8 h, the sample was acidified, and the released $H_2{}^{35}S$ was trapped in a 2% $CdCl_2$ solution, combined with Scintiverse, and counted as a gel on a Packard liquid scintillation counter with external channels ratio quench correction (15).

RESULTS

The same structure of the cores was always observed, though the width of each layer varied as shown (Table 1). Acetylene blockage appeared to work well in this sediment. Time courses showed that accumulation of nitrous oxide was linear ($r^2 > 0.9$) for at least 10 h and that nitrous oxide did not disappear from the incubations for up to 3 weeks (Fig. 1). A comparison experiment with ^{15}N gave denitrification rates within 13% of the acetylene blockage rates (data not shown); for this comparison, the surface sediment from two cores was homogenized and amended with 2 mM nitrate to provide detectable $^{15}N_2$. Coefficients of variation of two or three replicate sediment cores varied between zero and 34% at 24 h; the errors shown in Fig. 1 were among the largest ever obtained. Occasional denitrification determinations with two subsamples from the same core section varied by less than 10%. Nitrous oxide did not accumulate in any killed controls or in aerobic incubations. For the sediment incubations there was no significant difference (at $P \leq 0.05$) between the rates of N_2O accumulation in sections of whole cores and in cores with 15 ml of added seawater. However, for the aquifer soil, the initial rate in the replicates to which water was added was much slower than in the whole-core samples, so effects of added nitrate and glucose were assesssed in this case by comparison with the controls with added distilled water.

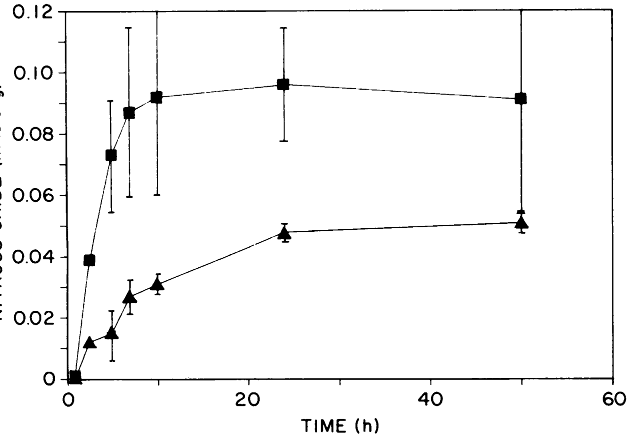

FIG. 1. Time course of nitrous oxide accumulation in the presence of acetylene. Values are means of two cores ± standard errors. Symbols: ■, white sand; ▲, sand/shell.

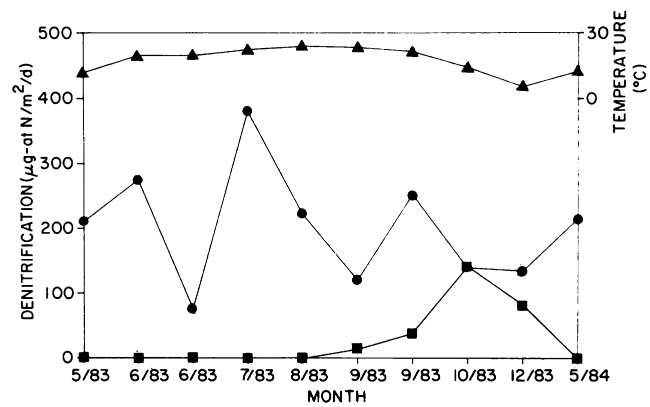

FIG. 2. Areal denitrification rates at the primary site from May 1983 to May 1984. Symbols: ■, surface; ●, Below 14 cm; ▲, temperature.

Depth profiles of denitrification. There was no denitrification in the surface oxidized layer in spring or summer. However, from September to December, denitrification in the surface was consistently measured at rates varying between 0.38 and 24 ng-atom of N g^{-1} day^{-1} (12 to 141 μmol of N m^{-2} day^{-1}) (Fig. 2). Deeper layers of the cores exhibited consistent patterns of denitrification throughout the investigation (Table 1). Denitrification was never observed in the black sand layer. Some denitrification was seen throughout the year in all three layers below this. Rates in the white sand layer and in the sand/shell layer were usually comparable with one another, ranging between 0.11 and 2.8 ng-atom of N g^{-1} day^{-1}, with lower rates being observed in the gray clay. The lowest values on a per-gram basis were obtained in the late summer, when the temperature was high (>25°C). However, when the results were converted to an

TABLE 2. East-west transect of Great South Bay[a]

Depth (cm) at:	Nitrate (μM)	Denitrification (ng-atom of N g^{-1} day^{-1})
Site 1		
0–3	12.4 ± 0.50	
35–38	17.6 ± 1.10	
Site 2		
0–3	10.8 ± 1.13	0
36–39	15.3 ± 2.70	0.044 ± 0.0031
Site 3		
0–3	11.7 ± 3.60	
36–39	20.7 ± 1.15	
Site 4		
0–3	16.4 ± 3.35	0
35–38	23.5 ± 1.55	0.45 ± 0.18

[a] Values are means of two cores ± standard error.

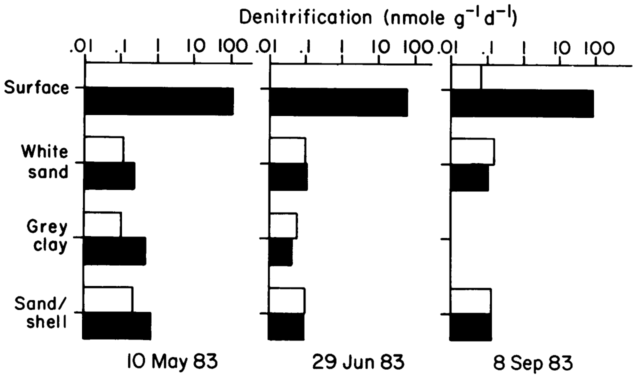

FIG. 3. Denitrification (nanomoles of NO_3^- denitrified per gram of dry sediment actually measured at 24 h) at different horizons in cores taken at the primary site on three dates. Open bars, endogenous production; solid bars, production with addition of 100 μM nitrate. Note log scale.

areal basis, there was no apparent temporal trend in denitrification below 14 cm (Fig. 2).

Total depth-integrated denitrification at the primary site varied between 76 and 380 μmol m^{-2} day^{-1} on individual sampling days. Table 1 gives the average values for nitrate concentration and denitrification in different layers of the cores over the year.

Offshore transects. Offshore transects on several occasions showed nitrate concentrations decreasing with distance from shore (5). Denitrification at 38-cm depth 5, 15, and 30 m offshore at this site was, respectively, 1.3 (± 0.038), 0.40 (± 0.040), and 0.10 (±0.044) ng-atom of N g^{-1} day^{-1}. Corresponding salinities were 3, 17, and 21‰.

East/west transect. Sites 1 through 4 were along 30 km of coastline from east to west, covering the area already studied (2) for its groundwater influx.

Nitrate concentrations at various depths and denitrification rates at 38-cm depth are shown in Table 2. At all sites, nitrate concentration was significantly higher ($P \leq 0.05$) at 38-cm depth than at 1-cm depth in the core. Nitrate concentrations at the two westernmost sites (3 and 4) were significantly higher than at the eastern ones (1 and 2) at each depth. Denitrification rate at site 4 was very much higher ($P \leq 0.01$) than at site 2.

Nitrate and glucose additions. Addition of nitrate to the lower layers of the sediments had no effect on denitrification in five experiments (data from three shown in Fig. 3). However, additions of 100 μM nitrate at the surface caused immediate, large stimulation even on days when no endogenous denitrification occurred.

The sediments at the primary site had low organic content (0.5% loss on ignition). Addition of glucose (a carbon source), while having no effect at the surface, stimulated denitrification in the lower layers by two to three times (Fig. 4a). Addition of glucose also stimulated denitrification at 40-cm depth at sites 2 (5 times) and 4 (2.6 times) (Fig. 4b).

Comparison with sulfate reduction. Sulfate reduction and denitrification were twice measured simultaneously at the primary site (Table 3).

On both occasions, nitrate concentration and denitrification showed the usual patterns. The rate of sulfate reduction decreased rapidly below 10 cm, although sulfate concentrations remained high down to 30 cm. On 30 November, the total areal rate of denitrification (205 nmol of NO_3^- denitrified cm^{-2} day^{-1}) was similar to that of sulfate reduction (340 nmol of SO_4^{2-} cm^{-2} day^{-1}). On 12 October, the areal rate of sulfate reduction was fourfold greater than that of denitrification, but in the lowest sand/shell layer the denitrification rate (0.69 nmol of NO_3^- denitrified cm^{-3} day^{-1}) was almost three times that of sulfate reduction (0.24 nmol cm^{-3} day^{-1}).

Denitrification in aquifer soil. In sections of aquifer soil from a depth of 4 m, in the saturated zone, denitrification rate was 17 ± 0.60 nmol of N g^{-1} day^{-1}. Pore-water nitrate concentration was 270 ± 10 μM.

For these samples, the initial rate of nitrous oxide production was very much slower in the replicates with added liquid than in the soil segments alone (Fig. 5), and the nitrous oxide concentration continued to increase for up to 98 h. At that time there were no significant differences among nitrous oxide concentrations in whole cores (17 ± 0.39 ng-atom of N g^{-1}), distilled-water controls (21 ± 2.2 ng-atom of N g^{-1}), and succinate treatments (29 ± 10 ng-atom of N g^{-1}). Production of nitrous oxide in the nitrate treatments, however, was significantly greater than in the distilled-water controls ($P \leq 0.01$) and continued to increase until the end of the experiment (195 h). It was then 3.8 times greater than production in the distilled-water controls.

DISCUSSION

The widespread submarine discharge of groundwater and the frequent contamination of aquifers by nitrate, particularly in urban areas, may have effects that are only beginning to be realized. The importance of nitrate input to coastal waters by groundwater discharge has been suggested in a

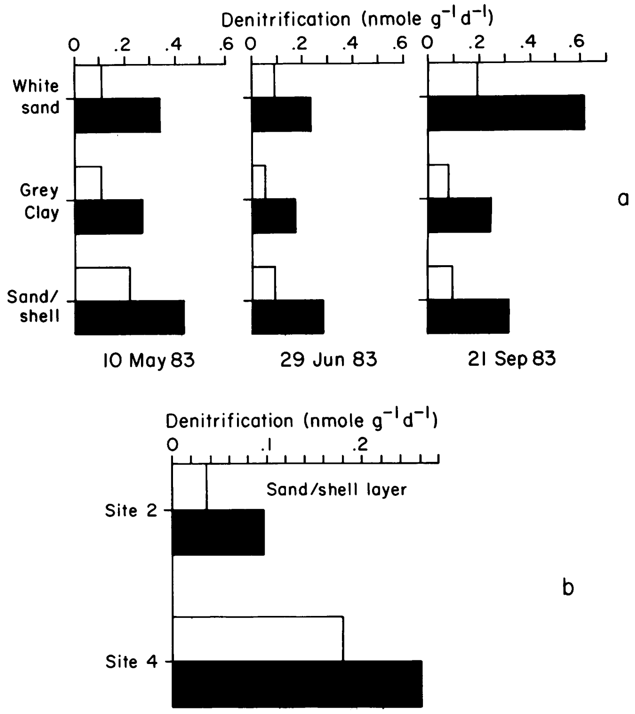

FIG. 4. Comparison of denitrification actually measured at 24 h (nanomoles of NO_3^- denitrified per gram of dry sediment per day) at different horizons. Open bars, without additions; solid bars, with 10 mM added glucose. Cores taken (a) at primary site on three dates and (b) at sites 2 and 4 on one date. No denitrification was observed in the surface sediment with or without added glucose.

TABLE 3. Comparison of rates of denitrification and sulfate reduction at primary site[a]

Date	Core layer	Nitrate (μM)	Denitrification (nmol of N cm^{-3} day^{-1})	Sulfate (μM)	Sulfate reduction (nmol cm^{-3} day^{-1})
12 October	Brown sand	16 ± 0.35	7.1 ± 1.5	20	230 ± 1.2
	Black sand	19 ± 1.9	0	20	24 ± 0.06
	White sand	17 ± 1.0	0.43 ± 0.0086	19	5.5 ± 0.57
	Gray clay	18 ± 0.51	0.18 ± 0.022	10	1.3 ± 0.05
	Sand/shell	22 ± 1.5	0.69 ± 0.10	3	0.24 ± 0.007
30 November	Brown sand	19 ± 0.65	47 ± 13.6	19	0.77
	Black sand	NA	0	21	62
	White sand	10 ± 0.78	0.80 ± 0.24	20	1.7
	Gray clay	13 ± 0.65	0.21 ± 0.0003	NA	NA
	Sand/shell	23 ± 1.8	0.52 ± 0.012	9.4	0.99

[a] Values are means of two cores except for sulfate reduction on 30 November and sulfate concentrations (one core each). NA, Not available.

Massachusetts salt marsh (30), in coastal areas near Perth, Western Australia (14), in the coral-reef ecosystem of Discovery Bay, Jamaica (6), and in these Great South Bay sediments (5).

Groundwater-derived nitrate discharged into nitrogen-limited coastal waters could affect phytoplankton production. A more subtle effect may occur in the sediments through which the groundwater effuses before discharge. Various nitrogen-transforming bacteria are active in shallow marine sediments and affect the ambient pools of nitrogen, and hence the exchange of N with overlying waters. In particular, denitrifying bacteria could intercept groundwater nitrate and reduce its concentration before it affects lakes or estuaries. There has been some previous speculation that denitrification might be the cause of the nitrate disappearance in groundwater flowing through high-organic soils around lakes (3, 17) and estuaries (26); the possible importance of denitrification in the disappearance of nitrate in groundwater flowing through coarse low-organic sands has been in dispute (19, 31, 32). Microbial activities in soils or sediments affected by groundwater-derived nitrate have not been measured in earlier studies, however.

Unusual patterns of denitrification for shallow marine sediments were noted in our studies and are presumably caused by groundwater input of nitrate. Denitrification rates in the lower layers of the core (the white sand, the gray clay, the sand/shell) were remarkably constant throughout the year, as was nitrate concentration (Table 1). This is unusually deep for high nitrate concentrations and denitrification in coastal marine sediments (10). Rates of denitrification below 14 cm were often greater than activity in the surface sediments. The total areal denitrification rates obtained are close to those measured in sediments of Danish fjords (22, 27), in New England coastal sediments (16), and in Japanese bays (20).

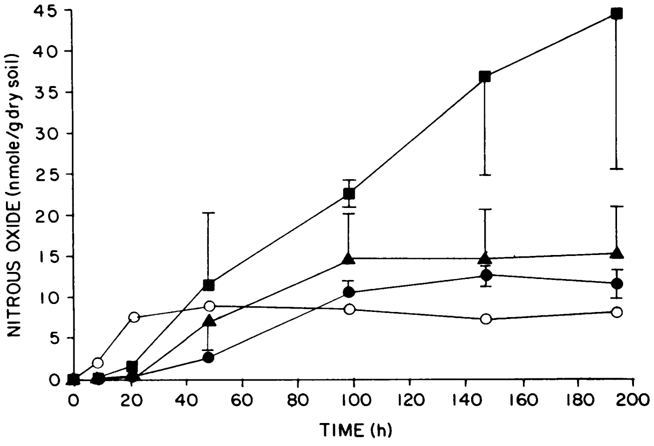

FIG. 5. Nitrous oxide accumulation in the presence of acetylene in anaerobic incubations of aquifer soil. Symbols: ○, whole sections of soil; ●, with added distilled water; ■, with 100 μM added nitrate; ▲, with 10 mM added succinate. Values are means of three replicates ± standard error.

Groundwater flow through the sediments is greatest nearshore, decreasing exponentially with distance offshore (2). Offshore transects at the experimental site on several occasions showed nitrate concentrations decreasing with distance from shore (5). The offshore transect in which we measured denitrification showed decreased groundwater influence (higher salinity) and decreased denitrification as distance from shore increased.

The experimental site is in a sparsely developed nonagricultural area; the major source of nitrate to Long Island groundwater is domestic (7), and nitrate concentrations increase westwards with increasing population (24). Four other sites along 30 km of coast were therefore investigated for comparison with the primary site (Table 2). The denitrification rates in the deepest core segments were low, in agreement with the low rates observed at the primary site in summer. The results indicate, however, that nitrate influx in submarine groundwater, and denitrification, occur over large areas of Great South Bay and are greatest in the developed western parts.

Groundwater-related denitrification at depth in the low-organic sediments (0.5% loss on ignition) of Great South Bay appears to be limited by carbon availability and not by nitrate (Fig. 3 and 4). Denitrification measured on the aquifer soil itself was apparently nitrate rather than carbon limited, even though nitrate concentration in the aquifer was very high and the carbon content was low. However, the lag before nitrous oxide began to accumulate in aquifer soil samples to which liquid had been added (Fig. 5) may suggest some adaptation or selection of the bacterial population in these incubations. No such lag was ever observed in the sediment experiments.

Denitrification measured on the unamended aquifer soil (for which the time course was the same as in sediments; see Fig. 5) was very much higher than in the estuarine sediments (17 ng-atom of N g^{-1} day^{-1} compared with an average of 0.69 in the lowest estuarine section). The field oxygen content of the pore water from this site was very low, between 0.05 and 0.10 mg liter^{-1} (data from Suffolk County Department of Health Services), suggesting that similar levels of denitrification may be occurring in situ. Other direct evidence for denitrification in aquifers is very limited. Large numbers (10^5 to 10^9 g^{-1}) of nitrate-reducing and ammonium-oxidizing bacteria were found in the Chalk aquifer in England (33); and denitrification has been directly measured in slurries from a sewage-contaminated aquifer in Massachusetts (R. L. Smith and J. H. Duff, Abstr. AGU/ASLO Winter Meeting, Eos Trans. Am. Geophys. Union **65**:879–880, 1984).

Nitrate concentration, and hence denitrification, at the interface and in the surface layers of the Great South Bay sediments must be influenced by physical and chemical conditions in the water column. Nitrification may also be an important source of substrate for denitrification in the surface layer, where oxygen is available (13). On at least one occasion, rates of nitrification and nitrate reduction at this site were closely coupled (12). The increased endogenous denitrification at the surface as the temperature falls and the depth of the surface oxidized layer increases (Fig. 2) suggests that nitrification rates may indeed be controlling denitrification there. The endogenous denitrification in this horizon is certainly limited by nitrate availability and apparently not by carbon (Fig. 3 and 4).

In marine sediments, although nitrate respiration is energetically preferable, sulfate reduction overwhelmingly predominates (28), and denitrification is usually limited to a narrow layer close to the surface. It is extremely unusual for nitrate respiration to become more important deeper in the sediment than sulfate reduction. However, in these sediments, denitrification appears to assume importance almost comparable with that of sulfate reduction, and this is particularly true at depth in the cores. There is clear vertical separation between sulfate reduction and denitrification in these sediments. Sulfate reduction occurs largely in the black sand, where no denitrification has ever been observed, and does not appear to be limited by sulfate concentration, at least between 10 and 30 cm. In contrast, depth of maximum numbers of sulfate-reducing bacteria at another estuarine site possibly influenced by freshwater intrusion fluctuated directly with sulfate concentration (11). As well as the high nitrate concentrations below 14 cm, some O_2 may be advected in by the groundwater. Sulfate reduction is more oxygen sensitive than nitrate respiration (4) and may be limited until the groundwater advecting through the sediments is deoxygenated.

The measurements made here suggest that denitrification occurring between 40- and 14-cm depth in the sediment can remove about 16% of the nitrate present at 40-cm depth and accounts for almost 50% of the nitrate decrease measured in our depth profiles between 40 and 14 cm (Table 1). Dissimilatory nitrate reduction to ammonium may account for the rest (10). Figure 6 indicates the apparent loss of nitrate in excess of simple mixing between aquifer water of salinity 0 and nitrate concentration 100 μM and pore water of the top 10 cm of the cores (salinity 24‰ and nitrate concentration 21 μM). The median nitrate concentration in the Upper Glacial Aquifer in Suffolk County in 1976 was 100 μM (24). Any higher value of nitrate concentration used for the aquifer end of the mixing line in Fig. 6 would increase the deficit, presumably due to biological activity. In fact, aquifer nitrate concentrations have probably increased since 1976; our own measurement of 270 μM 30 km west of this site bears this out.

Although sustained denitrification is occurring at depth in these sediments, our results indicate that the Upper Glacial Aquifer on Long Island still contains considerable nitrate as it approaches the sediment/water interface of Great South Bay. This is most likely the result of a limited amount of labile organic carbon. The average nitrate concentration we measured in the top 2 cm of sediment was 20 μM (Table 1). Assuming a total groundwater influx of 2×10^8 liters day^{-1} (2), nitrate input to the water column from the sediments may be as much as 4×10^9 μmol day^{-1}. This compares with an estimate of 10×10^9 μmol day^{-1} from river flow (9) and suggests that nitrate efflux from the sediments may be an important factor in the nitrogen cycle of the bay. We have not yet measured nitrate flux across the interface.

The effect of groundwater influx on microbial activities in sediments, though usually ignored, may be important. Groundwater flow, nitrate concentration, and also salinity were temporally variable in these sediments (2, 5). Some marine bacteria are known to have an obligate requirement for Na^+ (23). It is most likely that the bacteria of these strata have adapted to a varying Na^+ regime; however, it is possible that denitrifying activity is dependent to some extent on salinity, although we did not detect this in our data. Many other contaminants of groundwater are also of widespread concern (metals, pesticides, benzene derivatives), raising the possibility of the development of other specialized bacterial populations either tolerant of the toxin or, in the case of an organic pollutant, able to degrade it. It would be interesting to examine these possibilities experimentally.

Results of this study indicate that the actual effects of submarine nitrate influx in groundwater will depend primarily on the organic carbon content of the soils and sediments through which the water flows. In Great South Bay, some denitrification is supported by groundwater input, but the extent is limited by low availability of organic carbon. However, since carbon flow through the sediment system is altered and the energy flow is possibly increased by use of nitrate rather than sulfate as an electron acceptor, the effects on a system with higher organic content may be considerable. The results obtained in the aquifer itself, on the contrary, appear to suggest nitrate rather than carbon limitation there, in spite of the high nitrate concentration. We are now studying denitrification in this aquifer in detail. However, we have certainly established high levels of denitrifying activity in the unamended aquifer soil when

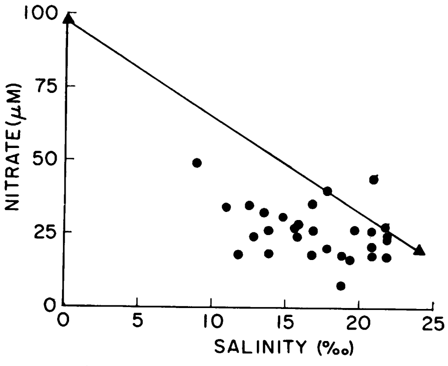

FIG. 6. Mean nitrate concentration plotted versus salinity in the 14- to 40-cm sections of cores taken at the primary site. The endpoints of the line drawn in the figure are the mean nitrate concentration and salinity in the Upper Glacial Aquifer itself and in the top 10 cm of the cores.

incubated anaerobically. The oxygen concentration of the Upper Glacial Aquifer varies considerably both spatially and temporally; around the sampling site described here, oxygen content of water from many observation wells is frequently below 5% saturation (Suffolk County Department of Health Services) and was below 0.5% saturation at the site itself. Thus it seems probable that there is a very active denitrifying population in situ in the aquifer.

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