

Denitrification, nitrate reduction to ammonium, and inorganic nitrogen pools in intertidal sediments

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Abstract

Dissimilatory nitrate reductions and inorganic nitrogen pools in intertidal sediments of Delaware Inlet near Nelson, New Zealand, were studied between March 1981 and January 1982. The acetylene block technique was used in sediment cores to estimate rates of *in situ* denitrification. In the top 10.5 cm of sediment the denitrification rates were 1.2 to 11.0 mg N m⁻²d⁻¹ (*Juncus maritimus* marsh), 1.6 to 7.9 mg N m⁻²d⁻¹ (sand-flat, colonised by *Euglena* spp. and *Oscillatoria* spp.), and 1.2 to 6.0 mg N m⁻²d⁻¹ (*Zostera novazelandica* bed). These rates were similar to those obtained in an earlier denitrification study of a Delaware Inlet mudflat. The highest *in situ* activity varied between the top 1.5 cm (eelgrass bed) and the 6 to 9 cm layer (sand-flat). Denitrification capacities as determined by the acetylene block technique in nitrate-saturated sediment slurries generally decreased with depth and were 1 943, 163, and 136 mg N m⁻²d⁻¹, respectively, in the top 10.5 cm of marsh, sandflat and eelgrass bed. A comparison with another study suggested net denitrification, except in the sand-flat during blooms of N-fixing benthic microalgae when these areas are sources of bound N. A combination of the acetylene block technique and ¹⁵N techniques in both sediment slurries and intact cores showed that 70 to 95% of the reduced nitrate was denitrified, with the rest being reduced to ammonium. The inorganic N pool in the top 10.5 cm of the mud-flat ranged from 130 mg N m⁻² in autumn to 420 mg N m⁻² in spring, and in the sand-flat it ranged from 100 to 287 mg N m⁻². Generally, ammonium was the dominant component of this pool. In spring, nitrate concentrations increased, and in the sand-flat it transiently became the major inorganic N species. The ammonium and nitrate profiles suggest an export of inorganic nitrogen from the intertidal sediments.

Introduction

Primary production in coastal marine ecosystems is generally thought to be limited by the availability of bound

nitrogen (Ryther and Dunstan, 1971). The importance of sediments in the supply of the limiting nutrient to the overlying water has been recognised (Billen, 1978). The amounts of ammonium and nitrate entering the water column from the sediment can be similar to those needed for primary production (Harrison, 1978). The flux of ammonium and nitrate from the sediment depends on physical and chemical conditions (e.g. ion exchange capacity) and the biological processes mineralisation, immobilisation, nitrification, denitrification, and dissimilatory nitrate reduction to ammonium. Among these processes, denitrification causes the only real loss of bound nitrogen from the ecosystem comprising sediment and coastal water. Dissimilatory nitrate reduction to ammonium competes with denitrification for nitrate and could, therefore, prevent the loss of bound nitrogen. Denitrification in coastal, estuarine and intertidal sediments has been observed (Knowles, 1982), and dissimilatory nitrate reduction has been reported to occur in coastal and estuarine deposits (Koike and Hattori, 1978; Sørensen, 1978 a; Buresh and Patrick, 1981). However, little information is available on the quantitative importance of these microbial processes in the overall N cycle of coastal systems (Billen, 1978; Henriksen *et al.*, 1981).

This study presents estimates of denitrification and dissimilatory nitrate reduction to ammonium in 4 distinct sediments of an unpolluted tidal inlet. Although *in situ* denitrification rates were low, a comparison with an N fixation study suggested net denitrification in most sediments. Ammonium production from nitrate accounted for only a small part of total dissimilatory nitrate reduction. Ammonium and nitrate profiles in the sediments indicated a flux of inorganic nitrogen from the intertidal sediments into the coastal water.

Materials and methods

Study site and sampling

The study was carried out between March 1981 and January 1982 in Delaware Inlet near Nelson, New Zea-

Table 1. Characterisation of study sites in Delaware Inlet, New Zealand. Values for moisture and volatile solids were determined in May 1981

Site	Description	% Moisture ^a		% Volatile solids ^b	
		0–1.5 cm	9–10.5 cm	0–1.5 cm	9–10.5 cm
Mud ^c	Slurry-like mud on top, dense clay below 6 cm. Reduced matrix, oxidised top layer and cavity walls. Mud snail (<i>Amphibola crenata</i>), mud crab (<i>Helice crassa</i>). Flooded for 3–4 h per tidal cycle	30.1	21.0	3.2	2.1
Marsh	Sediment similar to mud flat, but not as dense in deeper layers, densely rooted. Mainly reduced, but oxidised along roots. <i>Juncus maritimus</i> present. Flooded only at spring high tides	45.9	57.4	7.5	13.5
Sand	Fine/medium sand. Oxidised surface, reduced underneath. <i>Euglena</i> spp. and <i>Oscillatoria</i> spp. present. Flooded 3–4 h per tidal cycle	21.2	21.6	2.0	1.9
Eelgrass bed	Sediment similar to sand flat containing some cockle shells, moderately rooted. Thin oxidised surface layer, heavily reduced underneath. <i>Zostera novazelandica</i> and some <i>Chione stutchburyi</i> present. Flooded 10–12 h per tidal cycle	17.8	17.8	2.2	2.0

^a 105 °C for 16 h

^b 550 °C for 2 h

^c Further details about this site are given elsewhere (Kaspar, 1982)

land. The 3.1 km² basin, containing several distinct intertidal communities, has been described elsewhere (Stanton *et al.*, 1977). Four sites, representing most of the microbial activity in Delaware Inlet sediments were chosen, and their characteristics are summarised in Table 1.

All samples were taken with disposable plastic syringe tubes (50 ml, 12 cm long, 2.65 cm inner diam) from which the tips had been cut off. After pushing the tubes into the sediment the cores were dug out. Those samples which were not visibly disrupted were stoppered at the bottom and brought to the laboratory within 30 min. Processing of cores for chemical analyses was started immediately after arrival in the laboratory, and the experiments were done within a few hours.

Denitrification

For the estimate of the *in situ* denitrification rate, the cores were cut into seven 1.5 cm segments, and each segment was incubated for 4 h at room temperature in the presence of acetylene. In order to obtain a meaningful *in situ* denitrification estimate, the disruption of the physical structure of the samples was kept at a minimum. The denitrification rate was determined as the rate of nitrous oxide accumulation in the presence of acetylene, and a correction was made to allow for the incomplete inhibition of N₂O reduction. A detailed description of the method including error estimates is given elsewhere (Kaspar, 1982).

The denitrification capacity of the sediments was determined as the rate of N₂O production by nitrate-saturated

slurries in the presence of acetylene. Details of the assay system and of the procedure have been described (Kaspar and Tiedje, 1980; Kaspar, 1982). The experiments were carried out in duplicate slurries at room temperature.

In situ estimates as well as capacity measurements were done in March and April, 1981.

Partitioning of dissimilatory nitrate reduction

A combination of acetylene-block and ¹⁵N techniques was used to determine the partitioning of nitrate reduction between denitrification and dissimilatory nitrate reduction to ammonium. A preliminary experiment showed that 10 kPa acetylene in a slurry of the top 3 cm from the mud-flat had no significant effect on the end-product partitioning between nitrogen gas and ammonium. It was assumed that the N₂:NH₄⁺ ratio in the other sediments was similarly unaffected by acetylene.

Preliminary experiments showed that where nitrate was added to sediment samples, ammonium consuming processes were insignificant compared with ammonium production by nitrate reduction. Therefore, the measured ammonium accumulation from added nitrate was assumed to be the actual amount of ammonium produced by dissimilatory nitrate reduction. The partitioning experiments were carried out between October and December, 1981.

Experiments with slurries

Five cores from each site were cut into 3 cm segments. Segments from the same depth were pooled and mixed

with the same weight of seawater. Portions (10 ml) of the evenly mixed slurry were dispensed into anaerobe culture tubes (Bellco, Vineland, New Jersey) which were then stoppered and flushed with $N_2:CO_2$ (19:1) until the air was replaced. After an overnight pre-incubation of the samples at room temperature (to allow complete reduction of naturally present nitrate) 2 ml of acetylene were added and equilibrated between the gaseous and aqueous phase by shaking the tubes horizontally on a rotary shaker (10 min, 200 rpm). Then, 0.3 ml of a 1 mM sodium nitrate solution (99.7 atom % ^{15}N , B.O.C. Ltd., London) was added, and the slurries were incubated on the rotary shaker at room temperature. After 4 to 7 h the incubation was stopped by adding 0.2 ml $HgCl_2$ solution (6%) and 0.1 ml chloroform. N_2O in the tubes was determined by gas chromatography using an electron capture detector (Kaspar and Tiedje, 1980; Kaspar, 1982). The slurries were extracted for 1 h with 2.5 ml 4 M KCl and then steam-distilled (Bremner, 1965). Ammonium in the distillates was determined colorimetrically (Weatherburn, 1967), and the content of ^{15}N in the ammonium was measured by UV emission spectrometry (Statron, Leipzig, Model NOI-5). Preliminary experiments showed a complete recovery of N_2O and NH_4^+ .

Experiments with intact sediment

Segments of the top 1.5 cm were prepared in the same way as for the *in situ* denitrification estimate. Immediately after the acetylene injections, 1 ml of a 1 mM sodium nitrate solution (99.7 atom % ^{15}N , B.O.C. Ltd., London) was injected in 10 equal portions evenly spread over the core surface. Following an 18 h incubation at room temperature, 8 ml $HgCl_2$ solution (6%) was added, and the samples were shaken for 10 min to equilibrate nitrous oxide between the gaseous and aqueous phase. After the measurement of N_2O in the gas phase, the slurries were extracted for 1 h

with 4 ml 4 M KCl and then steam-distilled. Ammonium and ^{15}N determinations were carried out as described above.

Sediment analyses

The inorganic nitrogen pools of the mud-flat and sand-flat were measured at intervals of 2 mo between March 1981 and January 1982. Cores (8 to 10 per site) were sectioned at 0.5, 1.5, 3.5, 6.5, and 10.5 cm depths, and slices of the same depths were mixed. Subsamples were then extracted with 1 M KCl and assayed for nitrate (Strickland and Parsons, 1968) and ammonium (Solórzano, 1969).

Results

Denitrification

Denitrification rates in individual 1.5 cm core segments ranged from nil to $4 \text{ mg N m}^{-2} \text{ d}^{-1}$ (detection limit: 0.1 to $0.2 \text{ mg N m}^{-2} \text{ d}^{-1}$), and only in one series did all 10 replicates show a measurable activity. Highest activities were from 3 to 7.5 cm depth in the marsh, 6 to 9 cm in the sand-flat, and in the top 1.5 cm of the eelgrass bed (Table 2). The mean *in situ* denitrification rates in the top 10.5 cm were 4.1 , 2.9 , and $2.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ in marsh, sand-flat, and eelgrass, respectively. With the previously described error estimate (Kaspar, 1982), the corresponding ranges were 1.2 to 11.0, 1.6 to 7.9, and 1.2 to $6.0 \text{ mg N m}^{-2} \text{ d}^{-1}$. Data for the mud flat sediment have previously been published in more detail (Kaspar, 1982).

The denitrification capacities (Table 3) were markedly higher than their corresponding *in situ* rates. The highest capacities of individual 1.5 cm layers were $582 \text{ mg N m}^{-2} \text{ d}^{-1}$, and in all sediments the capacity decreased with depth. The top 10.5 cm of marsh, sand-flat and eelgrass bed showed denitrification capacities of 1943, 163, and $136 \text{ mg N m}^{-2} \text{ d}^{-1}$, respectively.

Table 2. *In situ* denitrification estimates ($\text{mg N m}^{-2} \text{ d}^{-1}$) in Delaware Inlet sediments, based on the measurement of N_2O accumulation during 4 h incubation of undisturbed core segments in the presence of acetylene. Experiments were done in March and April, 1981. Values below detection limits (0.19 , 0.11 and $0.11 \text{ mg N m}^{-2} \text{ d}^{-1}$ for marsh, sand and eelgrass, respectively) are given as 0. Data for mud-flat are given elsewhere (Kaspar, 1982)

Sediment depth (cm)	Marsh		Sand		Eelgrass	
	Range	Mean	Range	Mean	Range	Mean
0 - 1.5	0 - 0.90	0.33	0 - 0.19	0.03	0 - 3.95	1.23
1.5 - 3.0	0 - 0.30	0.11	0 - 0.19	0.03	0 - 0.55	0.19
3.0 - 4.5	0.49 - 2.27	0.93	0 - 1.40	0.30	0 - 1.51	0.36
4.5 - 6.0	0 - 2.44	0.68	0 - 2.90	0.60	0 - 1.01	0.19
6.0 - 7.5	0 - 2.66	0.85	0 - 2.41	0.66	0 - 0.30	0.11
7.5 - 9.0	0 - 1.84	0.44	0 - 2.30	0.74	0 - 0.49	0.11
9.0 - 10.5	0 - 1.56	0.74	0 - 1.56	0.58	0 - 0.11	0.03
0 - 10.5	1.23 - 10.96 ^a	4.08	1.64 - 7.92 ^a	2.94	1.23 - 6.00 ^a	2.22

^a Range based on mean from 10 replicate samples and error estimates as described elsewhere (Kaspar, 1982)

Table 3. Denitrification capacities ($\text{mg N m}^{-2} \text{d}^{-1}$) in Delaware Inlet sediments measured as rate of N_2O accumulation in nitrate-saturated anaerobic slurries in presence of acetylene. Values are means of duplicate assays. Data for mud-flat are given elsewhere (Kaspar, 1982)

Sediment depth (cm)	Marsh	Sand	Eelgrass
0 - 1.5	464	51	33
1.5 - 3.0	582	44	27
3.0 - 4.5	452	27	20
4.5 - 6.0	168	27	8
6.0 - 7.5	122	9	22
7.5 - 9.0	93	5	26
9.0 - 10.5	62		
0 - 10.5	1 943	163	136

Partitioning of dissimilatory nitrate reduction

Triplicate samples of sediment slurries from all sites and depths were assayed, but only the marsh sediment produced enough ammonium for a ^{15}N analysis of all samples (Table 4).

The recovery of added nitrate-N as N_2O -N plus ammonium-N was between 39 and 96%. The fraction of nitrate denitrified varied between 75 and 95%, and no marked differences were found between sediment types and depths (Table 4). In top segments of undisturbed cores (Table 5), 35 to 40% of the added nitrate-N was recovered as N_2O -N plus ammonium-N and between 70% (sand) and 85% (marsh) of the recovered N was denitrified.

Table 4. Partitioning of dissimilatory nitrate reduction in anaerobic slurries of Delaware inlet sediments amended with ^{15}N -nitrate. The experiments were done between October and December, 1981

Site + depth (cm)	Replicates for $^{15}\text{NH}_4^+$ analysis	Recovery of NO_3^- -N (%)			Denitrification (% of total NO_3^- reduction)
		N_2O^a	NH_4^+	Total	
Mud					
0-3	2	41.4	10.4	51.8	79.9
3-6	1	34.2	6.0	40.2	85.1
6-9	0	35.9	-	-	-
Marsh					
0-3	3	77.4	10.4	87.8	88.2
3-6	3	81.2	14.5	95.7	84.9
6-9	3	56.1	19.2	75.3	74.5
Sand					
0-3	1	33.6	5.7	39.3	85.5
3-6	0	67.0	-	-	-
6-9	1	41.0	2.1	43.1	95.1
Eelgrass					
0-3	2	49.0	12.4	61.4	79.8
3-6	2	38.2	6.5	44.7	85.5
6-9	2	37.9	7.2	45.1	84.0

^a Mean of 3 replicates

Inorganic nitrogen pools

In the mud-flat, the dissolved plus exchangeable ammonium concentrations were lowest in early autumn and reached a peak in early spring (Fig. 1A). In the cold seasons the deeper sediment layers had higher ammonium pools than the surface sediment, but during spring and summer more ammonium was found in the top layers. Nitrate concentrations were markedly lower than ammonium concentrations. Generally, there was more nitrate in surface sediments than in deeper layers. A slight build-up of nitrate occurred in spring (Fig. 1B).

The dissolved plus exchangeable ammonium pool in the sand-flat was generally smaller than in the mud-flat, but it showed similar seasonal dynamics (Fig. 2A). Ammonium slowly built up during autumn and winter to reach a maximum in early spring. After a sudden decrease in early summer, the ammonium concentration reached another peak in late summer. The surface sediment

Table 5. Partitioning of dissimilatory nitrate reductions in top 1.5 cm of undisturbed sediment cores from Delaware Inlet (means of triplicates). The experiments were done between October and December, 1981

Site	Recovery of NO_3^- -N (%)			Denitrification (% of total NO_3^- reduction)
	N_2O	NH_4^+	Total	
Mud	28.9	7.6	36.5	79.2
Marsh	30.1	5.2	35.3	85.3
Sand	26.9	11.4	38.3	70.2
Eelgrass	32.4	6.9	39.3	82.4

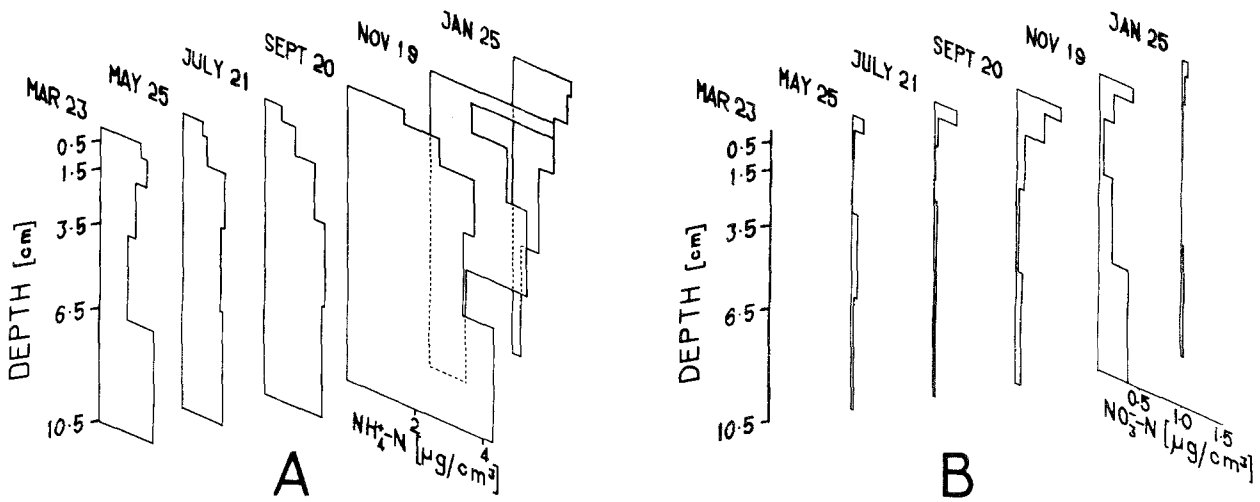


Fig. 1. Inorganic N concentration profiles in Delaware Inlet mud-flat between March 1981 and January 1982 ($\mu\text{g N cm}^{-3}$ wet sediment). (A) Dissolved plus KCl-extractable ammonium; (B) nitrate

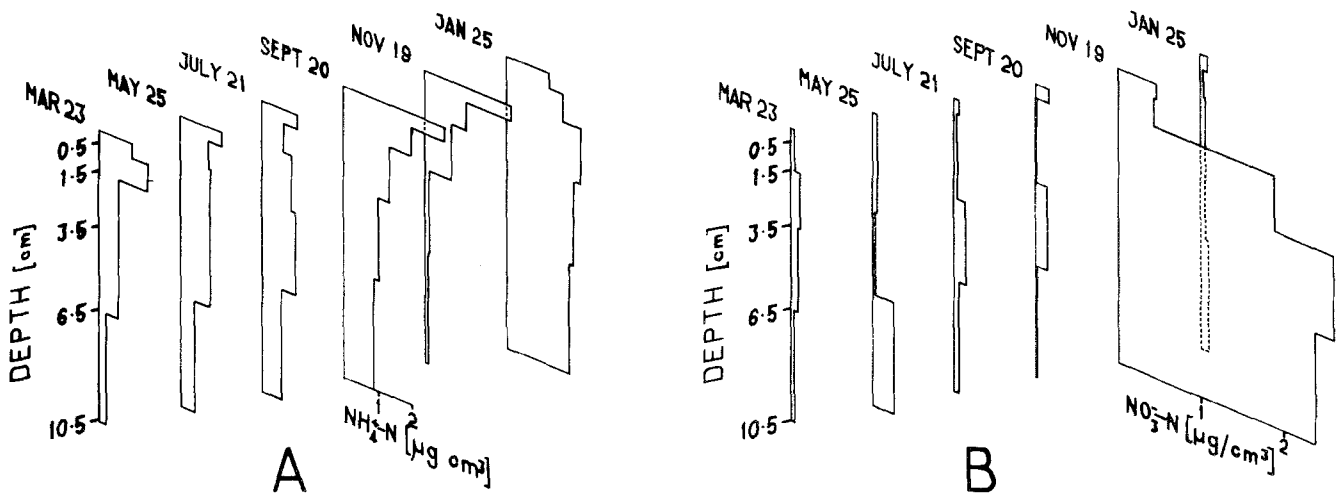


Fig. 2. Inorganic N concentration profiles in Delaware Inlet sand-flat between March 1981 and January 1982 ($\mu\text{g N cm}^{-3}$ wet sediment). (A) Dissolved plus KCl-extractable ammonium; (B) nitrate

Table 6. Seasonal dynamics of inorganic N pools in top 10.5 cm of Delaware Inlet mud-flat and sand-flat. Values are mg N m^{-2}

Date	Mud-flat			Sand-flat		
	NH_4^+-N	NO_3^--N	Total inorganic N	NH_4^+-N	NO_3^--N	Total inorganic N
Mar. 23, 1981	152	<1	<153	93	7	100
May 25, 1981	124	6	130	112	11	123
July 21, 1981	161	<7	<168	105	8	113
Sep. 20, 1981	397	23	420	212	<7	<219
Nov. 19, 1981	276	24	300	123	164	287
Jan. 25, 1982	142	3	145	232	7	239

generally contained more dissolved plus exchangeable ammonium than deeper layers. Nitrate concentrations were low except for a short period in early summer. At that time the surface sediment contained less nitrate than the deeper layers (Fig. 2 B).

In the top 10.5 cm of the mud-flat the total extractable inorganic nitrogen ranged from 130 to 420 mg N m⁻², and nitrate made the highest contribution in early summer with 8% of the total pool. In the sand-flat, the total inorganic N pool ranged from 100 to 287 mg N m⁻² (Table 6). There, the proportion of nitrate was generally higher than in the mud-flat and reached 57% in early summer.

Discussion

In situ denitrification rates were estimated at 1.2 to 11 mg N m⁻² d⁻¹. Similar rates have been estimated for similar sediments in other studies emphasising the conservation of the physical and chemical conditions in the core samples (Sørensen, 1978 b; Kaplan *et al.*, 1979; Seitzinger *et al.*, 1980; Kaspar, 1982; Nishio *et al.*, 1982). The denitrification capacities were 50 to 500 times higher than the *in situ* estimates, thus confirming the magnitude of the difference between the maximum potential rate and the actual rate (Kaspar, 1982). At all sites, the denitrification capacity decreased with depth, suggesting a correlation with the heterotrophic activity (P. A. Gillespie, personal communication). In contrast, the layers with highest *in situ* activities were found to vary with the sediment type, thus illustrating the dependence of denitrification in natural environments on the availability of nitrate which, in turn, is strongly influenced by physical, chemical, and macrobiological factors (Vanderborght and Billen, 1975; Grundmanis and Murray, 1977; Phillips *et al.*, 1978; Reddy *et al.*, 1978; Sherr and Payne, 1978).

The denitrification estimates presented here and in an earlier study (Kaspar, 1982) were up to 20 times higher than epibenthic N fixation rates (Bohlool and Wiebe, 1978) for the mud-flat, marsh, and eelgrass bed, but in the sand-flat, which was colonised by *Euglena* spp. and *Oscillatoria* spp. more nitrogen was fixed than lost through denitrification. The total N fixation rates in this comparison (particularly those for the marsh and eelgrass bed) may have been higher due to heterotrophic N fixation below 1 cm sediment depth (Patriquin, 1972), but the data suggest that the net flux of molecular nitrogen is generally directed from tidal flat sediments into the atmosphere and that this flux can be reversed, e.g. by large populations of N-fixing benthic microalgae.

The partitioning of dissimilatory nitrate reduction between the two end-products N₂ and NH₄⁺ was assayed in slurries and intact sediment cores. Denitrification accounted for 70 to 95% of the dissimilatory nitrate reduction, regardless of assay method, sediment type and depth. This may be an over-estimate since some of the labelled

ammonium was probably immobilised during the experiment (Buresh and Patrick, 1981). Comparable literature values are 38, 83 and 90% in three Japanese coastal sediments with great differences in organic matter content (Koike and Hattori, 1978), and 32 to 54% in a highly reduced Danish sediment (Sørensen, 1978 a). In naturally occurring slurries with low redox potential and high organic matter content, the denitrification capacity is smaller than the capacity for dissimilatory NH₄⁺ production (Kaspar *et al.*, 1981; Kaspar and Tiedje, 1981), and lowering the redox potential in slurries of an estuarine sediment decreases the fraction of nitrate which is denitrified (Buresh and Patrick, 1981). However, in undisturbed sediment, nitrate generally diffuses from aerobic zones into anaerobic zones with a relatively high redox potential where denitrification is favoured, and only a small part of the nitrate is expected to penetrate into the highly reduced areas which favour ammonium production. The results presented here suggest that in these sediments the partitioning of dissimilatory nitrate reduction was not markedly affected by the sediment type.

The total KCl-extractable inorganic N pool in the top 10.5 cm of mud-flat and sand-flat sediment ranged from 100 to 420 mg N m⁻². Similar values have been reported for sediments of shallow coastal waters (Henriksen *et al.*, 1981), *Spartina alterniflora* marshes (Haines *et al.*, 1976), and *Thalassia testudinum* beds (Patriquin, 1972). The inorganic N pools were lowest in late summer and highest in spring, showing a pattern which has also been reported in *S. alterniflora* marshes (Haines *et al.*, 1976). As expected for marine sediments, ammonium was generally the dominant fraction of the inorganic N pool. In the mud-flat, its concentration generally increased with sediment depth, suggesting that mineralisation and upward diffusion into the water column were the major processes controlling the ammonium pool. In contrast, the highest ammonium concentrations in the sand-flat were generally found at the sediment surface, indicating that ammonium was diffusing both into the overlying water and into deeper sediment layers from where it was presumably lost through leaching at low tide (3.4 cm h⁻¹ infiltration rate; P. A. Gillespie, unpublished data).

Nitrate was present in the mud-flat only in small amounts, and a slight increase occurred in late spring when the ammonium pool was decreasing. In the sand-flat the same pattern was observed, but in spring the nitrate pool transiently became larger than the ammonium pool. The presence of nitrate in both sediments indicated continuous nitrification, but only in spring were the conditions favourable for nitrate accumulation. This accumulation was very pronounced in the sandy sediment, suggesting that solute movement (diffusion and percolation) within the sediment was an important factor influencing the interactions between nitrate-producing and nitrate-consuming mechanisms. Although the nitrate concentrations were low even in the surface sediments, comparisons between interstitial and overlying water (P. A. Gillespie, T. H. Blackburn and H. F. Kaspar, unpublished

data) suggest that generally there was a flux of nitrate from the sediments into the water column.

The net fluxes of molecular nitrogen, ammonium, and nitrate from the intertidal sediments have to be compensated by an import of organic nitrogen which is subsequently mineralised. Gillespie and MacKenzie (1981) presented evidence for this and suggested a two-way exchange of nutrients between the largely heterotrophic microbial populations of the tidal flats and the benthic primary producers in the channels which collect the nutrient-rich run-off at low tide. Similarly, an exchange of bound nitrogen between the tidal flats and the coastal water may link the microbial mineralisation in intertidal sediments with the planktonic primary production. Despite the loss through denitrification, intertidal sediments appear to supply bound nitrogen to the coastal water, thus increasing its productivity.

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