Dissimilatory Nitrate Reduction and Nitrification in Estuarine Sediments

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Enumeration of populations of nitrate respiring bacteria in estuarine sediments of the River Tay, Scotland, showed that bacteria capable of dissimilating NO₃⁻ to NH₄⁺ predominated over those denitrifying NO₃⁻ to N₂. On the other hand, seasonal data and depth profile studies, using ^1⁵NO₃⁻, showed that denitrification was the principal route of dissimilatory NO₃⁻ reduction (78–90% of NO₃⁻ respired), with maximum rates of both processes occurring in the summer. Population densities of both populations of NO₃⁻ respiring bacteria were highest in the 0–2cm horizon in Tay estuary mud-flats where maximum rates of NO₃⁻ respiration were also recorded [28-56 µg N d⁻¹ (g dry wt sediment)⁻¹]. Autotrophic nitrification rates in Tay estuary sediments showed a distinct seasonality, highest rates [0-93 µg N d⁻¹ (g dry wt sediment)⁻¹] occurring during the summer. Nitrification rates declined rapidly with sediment depth and were not detectable below the oxidized zone (3 cm). Population densities of autotrophic NH₄⁺ and NO₃⁻ oxidizing bacteria followed a similar pattern of distribution. Heterotrophic nitrification appears to play an insignificant role in Tay estuary sediments.

INTRODUCTION

The respiratory reduction of NO₃⁻ to NH₄⁺ by bacteria in estuarine and inshore marine sediments has been considered by a number of workers to be potentially important (Koike & Hattori, 1978a; Sørensen, 1978; Herbert et al., 1980; Macfarlane & Herbert, 1982). The ecological significance of this process is that the NH₄⁺ produced as the end-product of nitrate respiration conserves nitrogen in a readily utilizable form, whereas the alternative process, denitrification, leads to the production of gaseous products (N₂O, N₂) from nitrate, and these are rapidly lost from the sediment.

The series of nitrogen intermediates involved in the anaerobic dissimilation of nitrate to ammonia are the reverse of those involved in nitrification, the aerobic oxidation of ammonia to nitrate by nitrifying bacteria. Sharp discontinuities in O₂ tension occur in marine and estuarine sediments over extremely small vertical distances or within micro-niches and so it is probable that both nitrification and nitrate respiration may proceed simultaneously. Experimental evidence to support this hypothesis has been provided by several workers. Koike & Hattori (1978b) using ^1⁵N-dilution techniques demonstrated the simultaneous processes of nitrification and nitrate respiration (denitrification and NO₃⁻ dissimilation to NH₄⁺) in Japanese marine sediments, whilst Grundmaris & Murray (1977) reported that nitrate produced by nitrification in oxidized sediments in Puget Sound was subsequently denitrified in the deeper anoxic zone.

The objectives of the present study were to investigate the relationships between populations of nitrate respiring (denitrifying bacteria and those respiring NO₃⁻ to NH₄⁺) and nitrifying bacteria in estuarine sediments in respect of the physico-chemical factors which influence their activities.

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Abbreviation: MPN, most probable number.
Methods

Physical characteristics of Kingoodie Bay sediments. Measurements of pH, $E_h$, temperature, salinity and dissolved $O_2$ tension were made in situ in 1 cm increments from the sediment surface to a depth of 5 cm, at monthly intervals at Kingoodie Bay, Tay estuary, NE Scotland. Depth profiles of pH and $E_h$ were determined using a Pye Model 293 pH meter and appropriate pH and $E_h$ electrodes (Russell pH, Auchtermuchty, Fife, Scotland) which had been previously calibrated against reference standards. Dissolved $O_2$ concentrations were measured using an EIL $O_2$ electrode coupled to an EIL model 15A dissolved oxygen meter.

Chemical characteristics of Kingoodie Bay sediments. Sediment samples were taken at monthly intervals from Kingoodie Bay using a sterile spargel core as previously described (Herbert, 1975) and returned to the laboratory within 30 min of collection. The sediment cores were aseptically sectioned into 1 cm segments, from the surface to a depth of 5 cm. NO$_3^-$, NO$_2^-$ and NH$_4^+$ in the sediment sections were extracted using 1 M-KCl, according to the method of Sørensen (1978). Total NH$_4^+$ (soluble, interstitial NH$_4^+$ and adsorbed exchangeable NH$_4^+$) was determined by the phenol/hypochlorite method (Solorzano, 1969), NO$_2^-$ according to the method of Barnes & Folkard (1951) and NO$_3^-$ by the method of Elliot & Porter (1971).

Enumeration of nitrate respiring and nitrifying bacteria in Kingoodie Bay sediments. Sediment samples were taken at monthly intervals and sectioned as previously described. From each sediment horizon, sub-samples were removed and serial ten-fold dilutions prepared in sterile one-quarter strength Ringer's solution.

Population densities of bacteria respiring nitrate to gaseous products (denitrification) and those dissimilating nitrate to NH$_4^+$ were determined using the most probable number (MPN) technique (Alexander, 1965). Nitrate nutrient broth (nutrient broth + 10 mM-KNO$_3$) was used to enumerate denitrifying bacteria and gas production was demonstrated by the inclusion of Durham tubes. Bacteria dissimilating NO$_3^-$ to NH$_4^+$ were enumerated using the medium of Jones et al. (1982); the tubes were incubated under anaerobic conditions for 7 d at 15 °C and NH$_4^+$ production was confirmed in the positive tubes with Nessler's reagent.

The micro-MPN method described by Rowe et al. (1977) was used to determine population densities of autotrophic NH$_4^+$ and NO$_2^-$ oxidizing bacteria and heterotrophic nitrifiers. Sediment samples were prepared as previously described and used to inoculate the wells of 8 x 12, sterile micro-titre plates (Corning, New York, USA). The NH$_4^+$/bicarbonate medium of Alexander & Clark (1965) was used to enumerate the NH$_4^+$ oxidizing populations, whilst the medium of Aalem & Alexander (1958) was used for the NO$_2^-$ oxidizers. The micro-titre plates were incubated at 30 °C for 28 d and to reduce desiccation were placed in plastic bags. Heterotrophic nitrifying bacteria were enumerated using the medium of Gode & Overbeck (1972) with acetate as C-source. N-serve (2-chloro-6-trichloromethyl pyridine) was added at a concentration of 10 mg l$^{-1}$ to inhibit autotrophic nitrifying bacteria and the plates were incubated for 14 d at 30 °C. Following incubation for the appropriate time period the MPN micro-titre plates were spot tested for NO$_2^-$ production/disappearance using Griess-Ilosvay's reagents (BDH). Production of NO$_2^-$ in autotrophic and heterotrophic micro-titre plate wells was taken as positive for NH$_4^+$ oxidation; conversely, the disappearance of NO$_2^-$ from the NO$_2^-$ oxidizer wells was taken as positive for autotrophic NO$_2^-$ oxidation. Uninoculated wells were included as controls.

Identification of the dominant nitrate respiring bacteria. The dominant nitrate respiring bacteria from Kingoodie Bay sediments were isolated and identified by methods described by Macfarlane & Herbert (1982). The end-products of nitrate respiration were determined in cultures grown anaerobically in the medium of Jones et al. (1982) under a gas atmosphere of 90% H$_2$/10% CO$_2$. Gas production was determined in Durham tubes whilst NO$_3^-$ and NH$_4^+$ in the spent media were determined using Griess-Ilosvay's and Nessler's reagents, respectively.

Determination of rates of nitrate respiration using $^{15}$NO$_3^-$. Sediment samples were obtained and sectioned as previously described. Sub-samples (about 5 g) from each horizon were transferred into a triplicate series of 15 ml serum bottles, closed with suba-seals and flushed with argon for 5 min. Finally, 100 µl Na$^{15}$NO$_3$ (99 atom % $^{15}$N) was injected into the bottles to give a NO$_3^-$ concentration of approximately 2 µmol (g sediment)$^{-1}$ and the contents were thoroughly mixed on a rotamixer prior to incubation in the dark at 15 °C. Samples treated with 1 ml 0·1 M-HgCl$_2$ immediately after the addition of $^{15}$NO$_3^-$ served as controls.

The rate of denitrification was determined by measuring the production of $^{15}$N gas. Using a gas-tight syringe, 100 µl samples from each bottle were injected into the inlet of a VG Micromass MM 601 mass spectrometer. To determine the rate of $^{15}$NH$_{4}+$ production the sediment slurries were extracted with $5$ ml 1·0 M-KCl as described by Sørensen (1978). NH$_4^+$ was separated and collected by steam distillation according to the method of Bremner & Keeney (1965). The NH$_4^+$ in the distillate was oxidized to N$_2$ by the Dumas method (Vose, 1980). The total quantity of NH$_4^+$ in the distillate was determined as described above.

Determination of nitrification rates in Kingoodie Bay sediments. Sediment samples were obtained and sectioned as described above. Nitrification rates were determined in the presence and absence of 5 mg N-serve l$^{-1}$ (final concentration), to discriminate between autotrophic and heterotrophic nitrification, using the $^{14}$C$/$bicarbonate dark uptake method described by Billen (1975).

Chemicals. N-serve was a gift from the Dow Chemical Co., and Na$^{15}$NO$_3$ was obtained from BOC Prochem, London, UK. All other chemicals were obtained from BDH.
Table 1. Typical physico-chemical profiles recorded in Kingoodie Bay sediments during February and July 1982

Results are mean values of three samples.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Month</th>
<th>pH value</th>
<th>$E_n$ value (mV)</th>
<th>$O_2$ concn (mg l$^{-1}$)</th>
<th>Conc (nmol ml$^{-1}$):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NH$_4^+$</td>
</tr>
<tr>
<td>0-1</td>
<td>Feb</td>
<td>6-9</td>
<td>+390</td>
<td>8.8</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>6-4</td>
<td>+320</td>
<td>8.7</td>
<td>50</td>
</tr>
<tr>
<td>1-2</td>
<td>Feb</td>
<td>7-0</td>
<td>+310</td>
<td>8.1</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>6-9</td>
<td>+200</td>
<td>7.0</td>
<td>460</td>
</tr>
<tr>
<td>2-3</td>
<td>Feb</td>
<td>7-2</td>
<td>+200</td>
<td>5.7</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>7-1</td>
<td>+50</td>
<td>4.7</td>
<td>510</td>
</tr>
<tr>
<td>3-4</td>
<td>Feb</td>
<td>7-4</td>
<td>+39</td>
<td>3.3</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>7-3</td>
<td>-100</td>
<td>1.1</td>
<td>516</td>
</tr>
<tr>
<td>4-5</td>
<td>Feb</td>
<td>7-5</td>
<td>-60</td>
<td>ND</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>7-4</td>
<td>-190</td>
<td>ND</td>
<td>530</td>
</tr>
</tbody>
</table>

ND, Not detected.

RESULTS

Physico-chemical characteristics of Kingoodie Bay sediments

The surface sediments in Kingoodie Bay were markedly more oxidized during the winter months due to a combination of lowered microbial activity, as a consequence of low temperatures (mean temperature 4.5°C), and increased resuspension and oxygenation of the sediments (Table 1). Below 4 cm the sediments were reduced and anoxic. The total NH$_4^+$ concentration was high compared with those for NO$_2^-$ and NO$_3^-$ and increased rapidly with depth. In contrast, NO$_3^-$ concentrations were highest at the surface and decreased with depth. These data indicate that in situ $O_2$ and NO$_3^-$ respiration were localized in the upper 0-4 cm horizon of these sediments, and this conclusion is substantiated by data on the spatial distribution of nitrifying and NO$_3^-$ respiring bacteria (Figs 1 and 2).

Spatial and seasonal distribution of NO$_3^-$ respiring and nitrifying bacteria

Cell population densities of autotrophic and heterotrophic nitrifying bacteria in Kingoodie Bay sediments showed a marked seasonality, with two distinct peaks in cell numbers of autotrophic organisms being recorded in early spring and July (Fig. 1). Both increases in cell populations were different in character. The increase in populations of NH$_4^+$ oxidizing bacteria in the spring was followed by a smaller increase in the NO$_2^-$ oxidizing population, suggesting that the oxidation of NH$_4^+$ to NO$_2^-$ stimulated the growth of these bacteria. However, in the summer, populations of both groups of bacteria increased concurrently with NO$_2^-$ oxidizers being present, if anything in greater numbers. These results indicate that a source of NO$_2^-$ other than that derived from autotrophic NH$_4^+$ oxidation may be involved. Depth profiles of autotrophic nitrifying bacteria showed that highest cell populations were present in the 0-2 cm horizon of the sediments and below this depth there was a rapid decrease in numbers. Population densities of heterotrophic nitrifying bacteria were always lower than those recorded for the autotrophs; this supports the view that these bacteria play an insignificant role in nitrification processes in estuarine sediments. This conclusion was further supported by the finding that the addition of 5 mg N-serve l$^{-1}$ almost totally inhibited nitrification irrespective of whether it was measured by the $[14C]$bicarbonate dark uptake method or NO$_3^-$ production.

Populations of those bacteria dissimilating NO$_3^-$ to NH$_4^+$ were always substantially greater (up to a factor of 10$^2$) than those denitrifying NO$_3^-$ to gaseous products (Fig. 2). In general, population densities of the nitrate respiring bacteria were more stable and less variable, on a seasonal basis, than those recorded for the autotrophic nitrifying bacteria. Depth profiles of nitrate dissimilating bacteria in Kingoodie Bay sediments showed that during the winter
months maximum cell populations were present in the 1–2 cm horizon but during the spring and summer months there was an apparent migration into the 0–1 cm horizon. These data are in agreement with the recorded changes of the redox \( (E_\text{h}) \) profiles of the sediments (Table 1) in which the surface layers became progressively more reduced during the summer.
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Fig. 3. Nitrifying activity in Kingoodie Bay sediments, December 1981 to October 1982. ●, 0–1; ■, 1–2; □, 2–3 cm depth.

Table 2. Generic distribution and products of NO₃⁻ reduction by dominant NO₃⁻ respiring bacteria isolated from Kingoodie Bay sediments

Bacterial groups were characterized according to the scheme of Dunn et al. (1980). One hundred colonies were tested.

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>No. of colonies</th>
<th>NH₄</th>
<th>NO₃</th>
<th>N₂ and/or N₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas/Vibrio</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Bacillus</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>21</td>
<td>0</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>

End-products produced by NO₃⁻ respiring bacteria

Sediment samples were diluted 10-fold in one-quarter Ringer's solution. Volumes (0·1 ml) were then plated out on the corresponding agar medium and from these, 100 isolates were selected at random. The dominant NO₃⁻ dissimilating bacteria (50% of the isolates) were identified, on the basis of limited morphological and biochemical tests, as belonging to the genera Aeromonas/Vibrio (Table 2). Ammonia was the principal product of dissimilatory NO₃⁻ reduction by these bacteria as with the eight isolates identified as members of the family Enterobacteriaceae. Fluorescent pseudomonads (15 isolates) and Alcaligenes spp. (21 isolates) were the dominant denitrifying bacteria isolated and they produced N₂ and/or N₂O as the end products of nitrate respiration. These results are in good agreement with those obtained by Dunn et al. (1980) and demonstrate the predominance of fermentative bacteria capable of respiring NO₃⁻ in these sediments.

Nitrification rates in Kingoodie Bay sediments

Depth profiles of nitrifying activity in Kingoodie Bay sediments, as measured by the indirect [¹⁴C]bicarbonate uptake method, showed that maximum rates occurred in the 0–1 cm horizon and decreased markedly with depth (Fig. 3). No significant nitrifying activity could be detected at depths greater than 3 cm and these results are in agreement with the bacterial counts (Fig. 1). Maximum rates of nitrification [0·93 µg N d⁻¹ (g dry wt sediment)⁻¹] occurred during the summer months when the sediments were warmest (19 °C), whereas lowest activities were recorded during the winter (mean temperature 5 °C). Maximum rates of nitrification occurred
Table 3. Rates of denitrification and NO\textsubscript{3}\textsuperscript{-} dissimilation to NH\textsubscript{4}\textsuperscript{+} in Kingoodie Bay sediments as determined using \textsuperscript{15}NO\textsubscript{3}\textsuperscript{-}

Rates are expressed as \(\mu g\) N d\textsuperscript{-1} (g dry wt sediment\textsuperscript{-1}) and are mean values of three samples.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N_2)</td>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>Total</td>
<td>(N_2)</td>
</tr>
<tr>
<td>0-1</td>
<td>6.2</td>
<td>1.1</td>
<td>7.3</td>
<td>12.5</td>
</tr>
<tr>
<td>1-2</td>
<td>9.7</td>
<td>2.1</td>
<td>11.8</td>
<td>16.1</td>
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<td>5.2</td>
<td>6.1</td>
<td>11.3</td>
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<td>1.3</td>
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<td>4-5</td>
<td>2.3</td>
<td>1.0</td>
<td>3.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

during the summer months and showed little correlation with seasonal maxima for autotrophic NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} oxidizing bacteria (Fig. 1), indicating that temperature probably exerts a greater influence on nitrifying activity than on cell numbers. The addition of 5 mg N-serve 1\textsuperscript{-1} totally inhibited nitrification, indicating that heterotrophic nitrification was not significant in the NH\textsubscript{4}\textsuperscript{+} oxidation processes in these sediments. These results are in keeping with the conclusions of Painter (1977), that heterotrophic nitrifiers are much less important than autotrophs in most ecosystems.

Nitrate respiration rates in Kingoodie Bay sediments

Initially, experiments were undertaken to determine whether \(N_2O\) was a major end-product of denitrification by incubating sediment samples in the presence and absence of 0.7\% acetylene, which blocks the reduction of \(N_2O\) to \(N_2\) (Yoshinari & Knowles, 1976). The rate of \(N_2O\) gas production in acetylene amended sediments was similar and paralleled the production of \textsuperscript{15}N\textsubscript{2} in control experiments. Total gas production at the end of these experiments was also similar, indicating that \(N_2O\) was not a major end-product of denitrification in these sediments. Denitrification rates were therefore determined solely on the production of \(N_2\) in the gas phase of the serum bottles.

Maximum rates of the combined activities of nitrate respiration [28.5 \(\mu g\) N d\textsuperscript{-1} (g dry wt sediment\textsuperscript{-1})] occurred at the 1–2 cm sediment horizon during July 1982 (Table 3). Whilst rates were lower in the 0–1 cm horizon, there was still substantial activity (82\% of that recorded at 1–2 cm depth in July). It is also evident that denitrification is the principal process of nitrate reduction in Kingoodie Bay sediments (83.2\% of NO\textsubscript{3} respired reduced to \(N_2\) at 0–2 cm in July 1982). Initially this would appear paradoxical since populations of denitrifying bacteria were substantially smaller than those capable of respiring NO\textsubscript{3} to NH\textsubscript{4}\textsuperscript{+} (Fig. 2). However, these results are similar to the findings of Sørensen (1978) for Danish coastal marine sediments and those of Koike & Hattori (1978a) for marine sediments in Japan. It was only with increasing depth (2–5 cm) that an increasing proportion of the NO\textsubscript{3} was reduced to NH\textsubscript{4}\textsuperscript{+}, but the total quantity of NO\textsubscript{3} respired was substantially less than that dissimilated in the surface sediments (0–2 cm). Thus, whilst denitrification is the predominant process in the surface sediments (0–2 cm), NO\textsubscript{3} reduction to NH\textsubscript{4}\textsuperscript{+} is not an inconsequential route of NO\textsubscript{3} dissimilation in Kingoodie Bay sediments.

DISCUSSION

The concentrations of interstitial NO\textsubscript{3}, NO\textsubscript{2} and NH\textsubscript{4}\textsuperscript{+} in vertical profiles of Kingoodie Bay sediments showed the generation of distinct gradients (Fig. 1). The principal species of inorganic-N present was NH\textsubscript{4}\textsuperscript{+} and concentrations increased with depth. Qualitatively, NH\textsubscript{4}\textsuperscript{+} profiles found in Tay estuary sediments were similar to those reported for other estuarine and inshore marine sediments which are produced as a consequence of ammonification (Focht & Verstraete, 1977; Sørensen, 1978; Stanley et al., 1981). Ammonium generated, in smaller quantities, as an end-product of NO\textsubscript{3} dissimilation (Table 3) supplemented that derived by ammonification. Conversely, NO\textsubscript{3} and NO\textsubscript{2} maxima as well as highest dissolved O\textsubscript{2}
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concentrations were recorded in the surface sediments (0–1 cm) and rapidly decreased with depth. Similar nitrogen profiles have been reported for sediments in the Limfjorden (Sørensen, 1978), the North Sea (Vanderborght & Billen, 1975) and Scottish west coast sea lochs (Stanley et al., 1981). Vanderborght & Billen (1975) considered that the NO₃⁻ profiles in the sluice dock sediments at Ostend resulted from nitrification in the upper layers of the sediment and denitrification in the lower horizons. Populations of autotrophic nitrifying bacteria (Fig. 1) and nitrification rates (Fig. 3) provide experimental evidence to substantiate the mathematical models of Vanderborght & Billen (1975). Further evidence to support this hypothesis has been obtained (G. T. Macfarlane & R. A. Herbert, unpublished data) using a modification of the gel stabilized system developed by Wimpenny et al. (1981). Gel systems established in the absence of the nitrification inhibitor N-serve generated concentration gradients of NO₃⁻, NO₂⁻ and NH₄⁺ which closely matched those in Kingoodie Bay sediments, whereas in the presence of N-serve (10 mg l⁻¹) these gradients failed to develop.

The dominant autotrophic NH₄⁺ and NO₂⁻ oxidizing bacteria were identified as strains of *Nitrosomonas* and *Nitrobacter* (Macfarlane & Herbert, 1984); these genera are the most commonly isolated representatives of autotrophic NH₄⁺ and NO₂⁻ oxidizing nitrifiers (Belser, 1979; Watson et al., 1981). Maximum nitrification rates [0.93–9 μg N d⁻¹ (g dry wt sediment)⁻¹] were recorded in the 0–1 cm horizon in summer, when sediment temperatures were highest (19°C). Nitrification rates decreased steadily with depth, however, and no activity was measured below 3 cm. The rates of nitrification recorded in Kingoodie Bay sediments are comparable to those reported by Christoff et al. (1979) for the Eden estuary [0.32 μg N d⁻¹ (g dry wt sediment)⁻¹], but are lower than those reported by Billen (1975) for North Sea sediments [6.72 μg N d⁻¹ (ml sediment)⁻¹]. Whilst heterotrophic nitrifying bacteria are present in Kingoodie Bay sediments, the experimental data obtained using 5 mg N-serve l⁻¹ to inhibit autotrophic nitrification indicate that they play an insignificant role in NH₄⁺ oxidation. These conclusions are consistent with those of Tate (1980) who demonstrated that large populations of heterotrophic nitrifying bacteria (10²–10³ times greater than those recorded in Kingoodie Bay sediments) did not contribute significantly to NH₄⁺ oxidation in soils.

Substantial populations of nitrate respiring bacteria (denitrifiers and bacteria dissimilating NO₃⁻ to NH₄⁺) were also present in the 0–2 cm horizon of Kingoodie Bay sediments and decreased with increased depth. The results in Table 3 show that denitrification was the principal process of NO₃⁻ reduction occurring in these sediments. These results are in agreement with those of Sørensen (1978) for the Limfjorden sediments and Koike & Hattori (1978a) for coastal marine sediments. Sørensen (1978) recorded a maximum rate of NO₃⁻ reduction of 22.6 μg N d⁻¹ (ml sediment)⁻¹ in the 0–3 cm horizon of Limfjorden sediments, and of this 10.6 μg N d⁻¹ (ml sediment)⁻¹ (46.8%) was in the form of NH₄⁺. NO₃⁻ dissimilation to NH₄⁺ in Kingoodie Bay sediments accounted for between 15.5 and 24.9% of the NO₃⁻ respired in the 0–2 cm horizon (Table 3), but, in a manner similar to that reported by Sørensen (1978), the proportion of NO₃⁻ dissimilated to NH₄⁺ increased with depth (27.6 to 47% at 4–5 cm depth) although the total quantity of NO₃⁻ reduced was less. Koike & Hattori (1978a) showed that the organic content of the sediments influenced the products of NO₃⁻ respiration. Organically rich sediments produced NH₄⁺ as the principal end-product of NO₃⁻ dissimilation, whereas the nutritionally poorer sediments produced only 16% and 7% NH₄⁺, respectively. Kingoodie Bay sediments have a low organic content (Herbert, 1975); the NO₃⁻ respiration rates and end-products produced reflect this and are similar to those recorded for Simoda Bay and Tokyo Bay sediments (Koike & Hattori, 1978a).

The experimental results presented in this paper show that not only are maximum populations of nitrifying and nitrate respiring bacteria present in the 0–2 cm horizon of these sediments but that this zone is also the site of maximum activity. While the rates of nitrate respiration are substantially greater than those recorded for nitrification, these results nonetheless indicate that both processes are occurring simultaneously in the oxidized surface sediments and corroborate and extend the field experiments of Sørensen (1978) and Koike & Hattori (1978b). Studies are now required to establish unequivocally the relationships between these physiologically distinct groups of bacteria which appear to operate an internal nitrogen cycle to their mutual advantage.


