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# Dissolved inorganic nitrogen cycling in Banzu intertidal sand-flat, Japan

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**Abstract**

The sediment-water exchange of nitrogen, nitrification, denitrification and sedimentary oxygen production were simultaneously measured in the Banzu intertidal sand-flat, Tokyo Bay, Japan. The exchange flux across the sediment-water interface showed a sink of dissolved inorganic nitrogen (DIN) in the light. However, in the dark, the intertidal sediment acted as a source. The diffusive flux obtained by the ammonium profile explained only 22% of the source flux directly measured in the dark, suggesting that bioturbation or excretion by macrofauna greatly contributed to the exchange flux. The total microphytobenthic uptake of DIN estimated from $O_2$ productivity and the Redfield ratio was $573.4 \pm 64.4$ $\mu$mol N $m^{-2}$ $h^{-1}$, 31% ($175.9 \pm 64.4$ $\mu$mol N $m^{-2}$ $h^{-1}$) of which was assumed to be derived from the overlying water. The release rate of DIN from the sediment to the water column (1.1 mmol N $m^{-2}$ $day^{-1}$) was balanced with the removal rate of DIN from the water column by benthic microalgae on a diurnal basis. This result suggests that DIN was recycled within the sediment, and the microalgae on the sediment surface played a significant role in suppressing the release of mineralized DIN from the sediment. The measured denitrification rate using an acetylene inhibition technique was $99.6 \pm 23.5$ $\mu$mol N $m^{-2}$ $h^{-1}$. Since the direct supply of nitrate or nitrite from the water column only accounted for 27% of the total denitrification at the highest estimate, nitrogenous oxide in the sediment pool was the major contributor to sedimentary denitrification.

## Introduction

High nutrient loading in coastal ecosystems has recently caused serious eutrophication problems. In a eutrophic shallow environment, oxygen-depleted water is occasionally generated at the bottom of the water column due to the accumulation of organic matter (Ochi and Takeoka, 1986; Kemp et al., 1992) and can cause the death of benthic macrofauna (Rosenberg and Loo, 1988). Autochthonous nitrogen from sediments as well as allochthonous nitrogen from rivers can both contribute to eutrophication. Benthic mineralization is considered as the important nitrogen pathway in shallow ecosystems (e.g., Blackburn and Henriksen, 1983; Kemp et al., 1992). Recycled dissolved inorganic nitrogen (DIN) is released from the sediment to the overlying water through sediment-water exchange processes and can be taken up by phytoplankton.

Several processes have been shown to regulate the sediment-water exchange of nutrients: (1) molecular diffusion, caused by a nutrient gradient at the sediment-water interface (Sweerts et al., 1991), (2) faunal activity, such as ventilation or excretion (Blackburn and Henriksen, 1983; Rutgers van der Loeff et al., 1984; Kristensen, 1985; 1988), and (3) benthic algal uptake of nutrients (Sundbäck et al., 1991; Rizzo et al., 1992; Rysgaard et al., 1995). An intertidal flat, where the sediment is regularly exposed and sufficient light penetrates to the sediment, has characteristically high levels of benthic microalgal biomass and productivity (Colijn and de Jonge, 1984; Varela and Penas, 1985). Photosynthetic processes can result in large diurnal changes and affect the nutrient cycle near the sediment surface due to algal demand. Therefore, the flux of DIN at the sediment-water interface can be controlled by benthic algae in the intertidal flat ecosystems.

Denitrification is also known to be a significant sink in the coastal ecosystem by the formation of gaseous nitrogen (e.g., Kaplan et al., 1977; Koike and Hattori, 1978; Nedwell and Trimmer, 1996). The

sedimentary denitrification rate is affected by bacterial processes associated with DIN cycling in marine estuaries in two ways: (1) ammonium oxidation by nitrification in the sediment is strongly coupled with denitrification (Jenkins and Kemp, 1984; Rysgaard et al., 1995; Ogilvie et al., 1997), and thus nitrification itself indirectly removes nitrogen through these coupled processes, and (2) dissimilatory nitrate reduction to ammonium competes with denitrification for nitrate as the terminal electron acceptor for respiratory electron transport (Herbert and Nedwell, 1990). The competition between the denitrifier and ammonifier under anaerobic conditions consequently affects the removal of nitrogen by sedimentary denitrification.

The importance of intertidal sediments around Japan in contribution to nitrogen circulation in ambient seawater has been discussed (Kurihara, 1988; Sasaki, 1989), however, the quantitative significance of the sediments associated with coastal nitrogen cycling is not well established. Since the sediment-water flux measurements were mainly conducted in subtidal areas (e.g., Sundbäck et al., 1991; Rizzo et al., 1992; Cowan et al., 1996), the flux data in the eutrophic intertidal area are very few (Falcão and Vale, 1990; Asmus et al., 1998). In this study, we simultaneously measured the rates of sediment-water exchange of nitrogen, nitrification, denitrification and sedimentary oxygen production in the Banzu intertidal flat which is located in an eutrophic shallow bay. These data were then used for examination of the relative importance of processes which affect the sediment-water exchange and denitrification in the tidal sand-flats, characterized by high benthic microalgal production.

## Materials and methods

### *Study site and sampling*

This study was carried out at the Banzu intertidal flat (7.6 $km^2$), located in the east coast of Tokyo Bay, Japan (Figure 1) on September 18, 1997. The geomorphological slope is ca. 1/1000 in an on-offshore direction, and the tidal range is 1.6 m (spring tide) and 0.5 m (neap tide). The sediment is characterized by well-sorted fine sand with a mean grain size of 180 $\mu$m. A preliminary study revealed that seawater advected by tidal movement is the major source of nitrogen although there is an adjacent river mouth. There is no vegetation except that the macroalga *Gracilaria verrucosa* can be found at times. Pennate diatoms dominate the epibenthic microalgal flora.

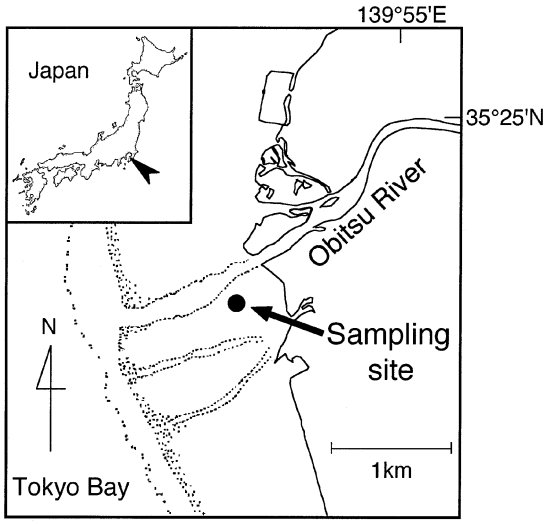


*Figure 1.* Location of the study site in Tokyo Bay, Japan.

The sampling site was located 20 cm above mean sea level and was therefore exposed and submerged by each tidal phase. All of the sediment samples were taken when the sediment was exposed on the ebb tide. A water sample was taken in cubitainers at a lower site, which was submerged at the time of sampling.

### *Sediment and water column characterization*

Sediment temperature, redox potential and nutrient profiles in the pore water were measured. Temperature was measured every 10 min with a thermistor-type thermometer (Tabai Espec, RT-10) inserted into the sediment. The redox potential was measured in situ with a pH/mV meter (TOA, HM-14P) equipped with combined a platinum and reference electrode.

Triplicate 20-cm sediment cores (4.5 cm inner diameter) were cut into 0–1 cm, 1–2 cm, 2–3 cm, 3–4 cm, 4–5 cm, 9–10 cm, 14–15 cm and 19–20 cm segments for pore water extraction. Slices of sediment were centrifuged for 10 min at 2000 rpm (580 $\times$ *g*) at ambient temperature. Extracted water was filtered through membrane disposable filters (Millipore HA) and frozen for the later analysis of ammonium ($NH_4^+$), nitrite ($NO_2^-$) and nitrate ($NO_3^-$) by an analyzer (Bran+Luebbe, TRAACS-800).

The overlying water obtained at the lower site was filtered and analyzed in the same way.

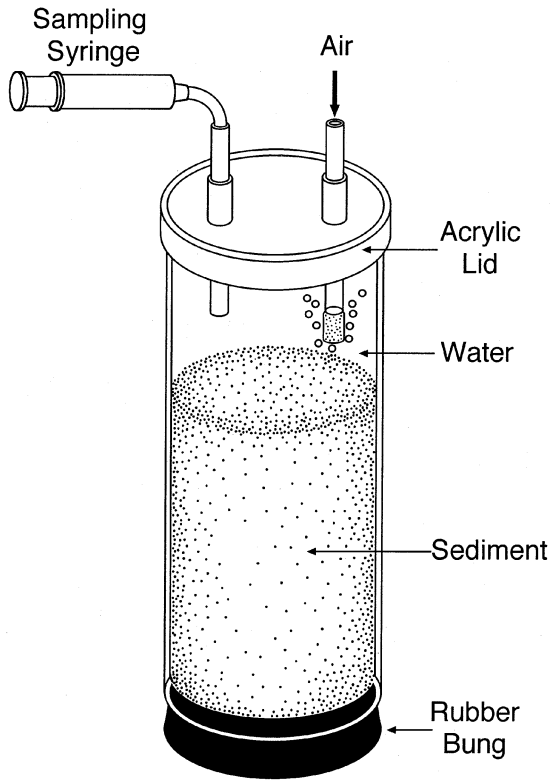


*Figure 2.* Schematic diagram of the incubation core used in the experiment of sediment-water exchange of nutrients.

*Calculation of diffusive fluxes across sediment-water interface*

The diffusive efflux of nutrient from the sediment can be calculated from Fick's first law described by Berner (1980):

$$J = -\Phi Ds(\mathrm{d}C/\mathrm{d}X)$$

where $J$ is the rate of efflux, $\Phi$ is the sediment porosity, $Ds$ is the diffusion coefficient and $\mathrm{d}C/\mathrm{d}X$ is the concentration gradient across the sediment-water interface. The diffusion coefficient in particle-free water was corrected for temperature (Li and Gregory, 1974) and tortuosity using the measured porosity and porosity-tortuosity relationships reported by Sweerts et al. (1991).

*Sediment-water exchange of nutrients*

At the site, eight replicate cores of intact undisturbed sediment were taken to a depth of 20 cm using acrylic core tubes (8.6 cm internal diameter × 30 cm length), each sealed at the bottom with a rubber bung. Water collected in the cubitainers was used to fill the top 10 cm of each core tube, taking care not to disturb the surface of the sediment. Another eight replicate cores filled with only water were used as the controls. The top of each core tube was capped by an acrylic lid equipped with two ports for sampling and aeration (Figure 2). The cores were left one night with aeration in a water bath held at in situ temperature to allow sediment to re-occupy the artifactual micro gaps between the tube and the sediment.

Next morning, the water in each of the 16 core tubes was replaced with water from the cubitainer and left to reequilibrate for 1 h prior to the experiment. The water columns were aerated in order to ensure a continuous oxygen supply and stirring during the experiment. The cores were then incubated in the water bath maintained at ambient temperature with the four sediment cores and four control cores being exposed to the sunlight, and the rest darkened. The photon flux of photosynthetically available radiation (PAR) was continuously measured during the experiment using a Biospherical quantum sensor.

Dissolved inorganic nitrogen samples were taken four times during a 7-h period. The exchange rate of nutrients was estimated from the rate of linear change in concentration calculated by a regression analysis during the incubation period. If no significant regression was found ($p > 0.05$), the rate was considered to be zero. The rates of change in the water column control cores were then subtracted from the rates of change in the sediment cores.

After the experiment, the sediment in each core was sieved (1 mm mesh) to retain macrofauna. Macrofauna were preserved in 10% formalin-seawater solution buffered with sodium borate and stored for later counting.

*Nitrification*

Nitrification rates were obtained by measuring the changes in the $NO_2^-$ concentration in the sediment samples containing allylthiourea (ATU) or sodium chlorate ($NaClO_3$) to inhibit the oxidation of $NH_4^+$ and $NO_2^-$, respectively (Bianchi et al., 1994; Gilbert et al., 1997). The collected water was filled into six sets of triplicate flasks (200 ml). Two triplicate sets of flasks received 10 mg $l^{-1}$ ATU (final conc.), two received 10 mM $NaClO_3$ while the others served as controls. The upper 5 cm of the sediment was divided into subsamples (6 g wet) and added to one to two sets of the control, ATU and $NaClO_3$ flasks. The sub-

*Table 1.* Characteristics of water column and sediment on sampling day.

| | |
|---|---|
| Water characteristics | |
| High tide level (cm) | 77–81 |
| Nutrients concentration ($\mu$M) | |
| $NH_4^+$ | 5.3 |
| $NO_2^-$ | 2.1 |
| $NO_3^-$ | 19.4 |
| DIN | 26.8 |
| Sediment charateristics | |
| Wet density (g $cm^{-3}$ $\pm$ SE) | $1.86 \pm 0.01$ |
| Porosity ($\pm$ SE) | $0.46 \pm 0.01$ |
| Chlorophyll *a* ($\mu$g $g^{-1}$ wet $\pm$ SE) | $0.94 \pm 0.26$ |
| Dominated macrofauna ($\times 10^3$ ind. $m^{-2}$ $\pm$ SE) | |
| *Armandia* sp. | $32.9 \pm 8.2$ |
| *Ruditapes philippinarum* | $21.1 \pm 2.5$ |

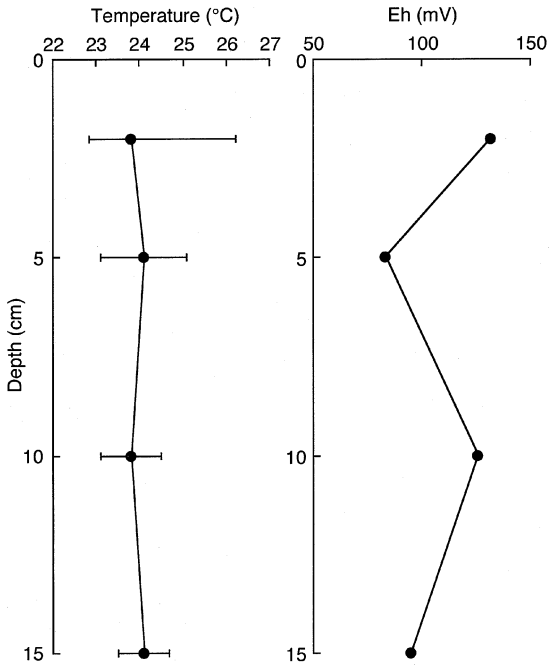


*Figure 3.* Profiles of temperature (°C) and redox potential (Eh). Bars indicate minimum and maximum values.

samples were thoroughly mixed with the water and incubated at the in situ temperature.

The concentration of $NO_2^-$ was measured every 3 h from zero time to 9 h. At the time of sampling, the flasks were vigorously shaken and 5 ml samples were taken and analyzed. The rate of nitrification was calculated from the data obtained during the linear $NO_2^-$ production or consumption phases. If no significant correlation was found ($p > 0.05$), the rate was considered to be zero. The rate was expressed in $\mu$mol N $m^{-2}$ $h^{-1}$ (range of sediment depth: 0-5 cm) using the wet density of the sediment samples. The experiment was performed within 24 h of sampling.

### *Denitrification*

An acetylene ($C_2H_2$) inhibition technique was used to assay for denitrification. This method measures the accumulation of nitrous oxide ($N_2O$) since $C_2H_2$ inhibits the reduction of $N_2O$ to $N_2$ (Balderston et al., 1976; Koch et al., 1992; Joye and Paerl, 1993). A water sample (30 ml) saturated with $C_2H_2$ was placed in each of the five replicate 60 ml gas-tight vials. The sediment subsamples (5 g) were added to the vials. The vials were placed on a shaker for 10 min, then incubated at ambient temperature. $N_2O$ concentrations of head space gas were determined by gas chromatography (Shimadzu, GC-14B) with an electron capture detector at time zero and after 6 h of incubation. The rate of denitrification was estimated by calculating the rate of change in the concentration of $N_2O$ during incubation. The experiment was performed within 24 h of sampling.

### *Oxygen production*

At the site, triplicate sediment cores were taken to a depth of 20 cm using acrylic core tubes (4.5 cm internal diameter $\times$ 30 cm length). Core samples were left in the laboratory in the same way as in the nutrient exchange experiment previously described. Next morning, the water in the cores was replaced with the water from the cubitainer and left to reequilibrate. The top of each core was completely capped to exclude air bubbles, and the cores were incubated in the water bath. The concentration of dissolved $O_2$ (DO) in the water was measured with an $O_2$ electrode (YSI No. 5730) in each core. The water in the cores was stirred by the electrode itself. Data were monitored every 5 min for 1 h. The rates of change in DO concentration were statistically analyzed for linearity with respect to time.

At the end of the experiment, the upper 1 cm of the sediment in each core was used for chlorophyll *a* analysis. Chlorophyll *a* was extracted using 90% acetone solution and extinction was measured in a spectrophotometer (Hitachi, U-3200).

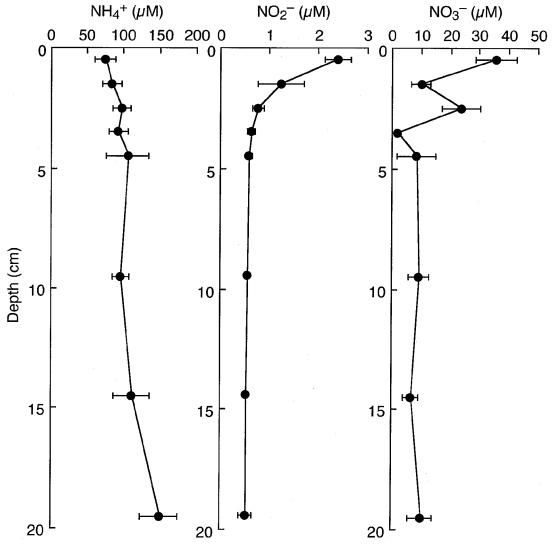


*Figure 4.* Pore water profiles of nutrient ($\mu$M). Bars indicate SE.

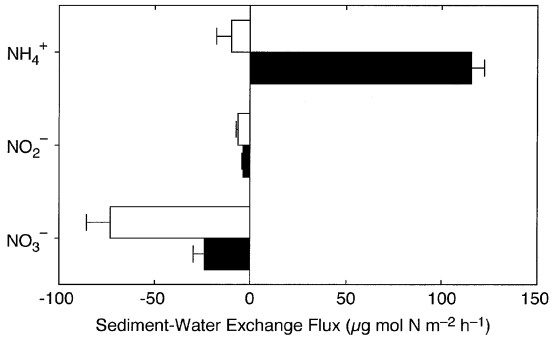


*Figure 5.* Fluxes of $NH_4^+$, $NO_2^-$ and $NO_3^-$ across the sediment-water interface. Bars indicate SE.

## Results

### *Characteristics of water column and sediment*

Sediment temperature ranged from 22.6 to 26.2 °C (Figure 3). The diurnal change in temperature was large in the upper levels, but less at depth. The redox potential (Eh) within the top 15 cm displayed positive values which ranged from +80 to +130 mV. The Eh showed no gradient with depth. The chlorophyll *a* content in the sediment was 0.94 ± 0.26 $\mu$g $g^{-1}$ wet (mean ± SE), which corresponded to 1.70 ± 0.47 $\mu$g $cm^{-2}$ (mean ± SE). The polychaete *Armandia* sp. and the bivalve *Ruditapes philippinarum* dominated the macrofauna (Table 1). Other indices are shown in Table 1.

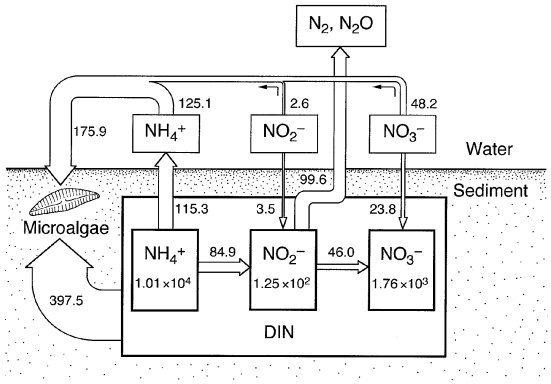


*Figure 6.* Dissolved inorganic nitrogen cycling during the submerged and lightened period in the Banzu intertidal flat. Values are for a depth of 5 cm on a $m^2$ basis. Fluxes are in $\mu$mol N $m^{-2}$ $h^{-1}$. Standing stocks are in $\mu$mol N $m^{-2}$.

### *Nutrient concentration profiles and diffusive fluxes*

Pore water nutrient profiles showed significant concentration gradients with depth (Figure 4). The ammonium profile exhibited a gradual increase in concentration from the interface. In contrast, concentrations of $NO_2^-$ and $NO_3^-$ sharply declined until a depth of 4 cm. The percents of $NH_4^+$ and $NO_3^-$ concentrations in the sediment DIN pool within the top 5 cm were 84.8% and 14.1%, respectively, while $NO_2^-$ was a minor component (1.1%).

The diffusion coefficients of $NH_4^+$, $NO_2^-$ and $NO_3^-$ were 3.96, 3.82 and 3.80 × $10^{-6}$ $cm^2$ $s^{-1}$, respectively. The diffusive effluxes of $NH_4^+$, $NO_2^-$ and $NO_3^-$ were then estimated as 25.1, 0.1 and 5.7 $\mu$mol N $m^{-2}$ $h^{-1}$, respectively.

### *Sediment-Water exchange of nutrients*

The measured mean PAR was 383 $\mu$mol $m^{-2}$ $s^{-1}$ during the incubation period. The direction of all the fluxes tended to be sink (Figure 5) in the presence of sunlight. Nitrite and $NO_3^-$ uptake fluxes were significantly higher in the sunlight than in the dark (One-way ANOVA, $NO_2^-$: $0.01 < p < 0.05$; $NO_3^-$: $0.001 < p < 0.01$). The flux of $NH_4^+$ in the dark showed release from the sediment and was significantly different from the flux in the light ($p < 0.001$). The exchange rate of DIN was −88.9 ± 18.6 $\mu$mol N $m^{-2}$ $h^{-1}$ (mean ± SE) in the light, therefore, the sediment showed a sink of DIN in the daytime. However, in the dark, the exchange rate of DIN was 88.0 ± 9.9 $\mu$mol N $m^{-2}$ $h^{-1}$ (mean ± SE), indicating that the sediment acted as a source of DIN at night. The uptake rate of $NO_3^-$ by the sediment was significantly higher ($p < 0.001$)

*Table 2.* Range of sediment-water fluxes of $NH_4^+$ and $NO_3^-$ measured with core or bell jar incubation in very shallow area.

| Study area | Depth (m) | Sediments | $NH_4^+$ flux ($\mu$mol N $m^{-2}$ $h^{-1}$) | $NO_3^-$ flux ($\mu$mol N $m^{-2}$ $h^{-1}$) | Authors |
|---|---|---|---|---|---|
| Ria Formosa, Portugal | Intertidal | sand, mud | 0–24 | | Falcão and Vale, 1990 |
| Laholm Bay, Kattegat | 15 | sand, mud | −10–160 | −45–30 | Sundbäck et al., 1991 |
| Chesapeake Bay | 9–18 | | 163–734 | | Kemp et al., 1992 |
| Neuse River estuary, North Carolina | 4.5 | sand | −12–22 | −6–10 | Rizzo et al., 1992 |
| Kertinge Nor, Denmark | 0.5 | sand | −10–750 | −130–30 | Rysgaard et al., 1995 |
| Fourleague Bay, Louisiana | 1.5 | mud | 12–182 | −21–42 | Miller-Way and Twilley, 1996 |
| Puck Bay, Baltic Sea | 2–50 | sand, mud | 0–60 | | Bolalek and Graca, 1996 |
| Mobile Bay, Alabama | 3 | | −22–181 | −14–67 | Cowan et al., 1996 |
| Konigshafen, North Sea | Intertidal | sand, mud | −242–1141 | −492–173 | Asmus et al., 1998 |
| Banzu intertidal flat, Tokyo Bay | Intertidal | sand | −10–115 | −73–−24 | This study |

than $NH_4^+$ both in the light and in the dark. The fluxes of $NH_4^+$ and $NO_3^-$ in this study were in the range of that measured in other very shallow areas (Table 2).

### *$O_2$ productivity by benthic microalgae*

The measured sedimentary $O_2$ consumption was 62.4 ± 2.1 mg $O_2$ $m^{-2}$ $h^{-1}$ (mean ± SE) in the dark. In the light, the measured sedimentary $O_2$ production was 89.5 ± 15.9 mg $O_2$ $m^{-2}$ $h^{-1}$ (mean ± SE). Thus microphytobenthic gross primary production was estimated to be 151.9 ± 17.1 mg $O_2$ $m^{-2}$ $h^{-1}$. The uptake of DIN by benthic microalgae, calculated from the gross $O_2$ productivity assuming a Redfield C:N ratio of 106:16 and a photosynthetic quotient of 1.25 (Williams et al., 1979), was estimated to be 573.4 ± 64.4 $\mu$mol N $m^{-2}$ $h^{-1}$.

### *Nitrification and denitrificaiton*

The rates of measured $NH_4^+$ and $NO_2^-$ oxidation were 84.9 ± 4.1 and 46.0 ± 3.8 $\mu$mol N $m^{-2}$ $h^{-1}$ (mean ± SE), respectively. The rates of $NH_4^+$ oxidation were significantly higher ($p < 0.001$) than the rate of $NO_2^-$ oxidation in the top 5 cm of sediment. The measured denitrification rate was 99.6 ± 23.5 $\mu$mol N $m^{-2}$ $h^{-1}$ (mean ± SE), and was not significantly different from the $NH_4^+$ oxidation rate ($p > 0.05$).

## Discussion

### *Comparison of calculated diffusive fluxes and measured fluxes*

The diffusive fluxes calculated from the concentration gradient of pore water showed much lower values than those directly measured. Dense macrofaunal populations at the sampling site (Table 1) indicate that porosity may be underestimated due to bioturbation. However, if the upper limit of porosity ($\Phi$ = 1) is used for calculating the diffusive flux, the results give only 2.2 times larger fluxes than those directly measured, suggesting that the molecular diffusion process is a minor contributor to the exchange rates of nutrient across the sediment-water interface. The nutrient flux measured in situ with a benthic chamber has been shown to be 1 to 10 times larger than the flux calculated from pore water profiles in the Potomac River estuary (Callender and Hammond, 1982), and 2 to 10 times larger in Gullmarsfjorden (Rutger van der Loeff et al., 1984). The difference between the calculated diffusive flux and directly measured flux in the dark may be caused by excretion the bivalves (Blackburn and Henriksen, 1983) or ventilation between pore water and the water column by the polychaetes (Kristensen, 1985). Kristensen (1988) reported that the populations of burrow systems (macrofaunal density: 20-6000 ind. $m^{-2}$) generate 17 to 90% of the total $NH_4^+$ flux in coastal sediments. In this study, a larger proportion (78%) of biogenic flux may be due to the higher densities of macrofauna in the sampling site. Furthermore, macrofauna may promote mixing of pore water with the overlying water. This is supported

by the $NO_3^-$ and $NO_2^-$ concentration in the pore water in the top 0–2 cm, where it is most bioturbated, were similar in concentration to the overlying water and sharply decreased with depth.

*Nutrient uptake by microalgae*

The measurements of the sediment-water exchange fluxes showed that light conditions significantly affected the sediment-water flux of nutrients. This indicates a strong uptake of nutrients by benthic microalgae in the presence of sunlight, as has been reported in the Neuse river estuary (Rizzo et al., 1992) and in the Kertinge Nor estuary (Rysgaard et al., 1995). Assuming that the difference between the fluxes of nutrients in the light and in the dark are explained by algal uptake processes, then the uptake rate of $NH_4^+$, $NO_2^-$ and $NO_3^-$ from the overlying water can be calculated by subtracting the sediment-water flux in the dark from that in the light (Figure 5), being 125.1, 2.6 and 48.2 $\mu$mol N $m^{-2}$ $h^{-1}$, respectively (Figure 6). It is likely that these rates are underestimated due to the possible uptake of DIN by benthic microalgae in the dark, which has been observed in several phytoplankton species (Malone et al., 1975). Therefore, our estimated algal uptake rate of DIN from the overlying water may represent a minimum. The gross algal uptake of DIN estimated from the $O_2$ productivity and Redfield ratio was 573.4 $\mu$mol N $m^{-2}$ $h^{-1}$, and consequently, at least 31% (175.9 $\mu$mol N $m^{-2}$ $h^{-1}$) of the total algal uptake of DIN was derived from the overlying water, and the remainder (397.5 $\mu$mol N $m^{-2}$ $h^{-1}$) from the pore water.

On a diurnal basis, the submerged and lightened time was 6 h on the sampling day, and during this period, benthic microalgae would use DIN directly from the water column, accounting for 1.1 mmol N $m^{-2}$ $day^{-1}$. The sediment-water efflux of DIN during the submerged period (12 h) is calculated to be 1.1 mmol N $m^{-2}$ $day^{-1}$, which is balanced with the algal uptake rate of DIN from the overlying water in the submerged and lightened period. This indicates that DIN was recycled within the sediment, and that the microalgae on the sediment surface has a significant role in suppressing the release of mineralized DIN from the sediment to the overlying water.

*Major processes to denitrification*

The denitrifying bacteria utilize $NO_3^-$ and $NO_2^-$ as the terminal electron acceptor for the respiratory process (Nishio et al., 1982). The ambient $NO_3^-$ concentration is widely reported as being the parameter most closely related to denitrification (e.g., Ogilvie et al., 1997). In the denitrification experiment, the $NO_3^-$ concentration in the overlying water added to the sediment was slightly higher than the mean $NO_3^-$ concentration in the top 5 cm of the in situ sediment. Therefore, there is a possibility that the denitrification rate was overestimated.

The sediment was a sink for $NO_3^-$ and $NO_2^-$ in the present study. This indicates that the consumption of $NO_3^-$ was larger than the production in the sediment. However, the rate of ammonium oxidation, which results in the production of $NO_2^-$ and $NO_3^-$, was similar to that of denitrification, a consumption process of $NO_3^-$ and $NO_2^-$. This suggests that another substantial consuming process, perhaps dissimilatory $NH_4^+$ production, was present in the sediment. This is supported by the Eh profile, which was rather anaerobic in the summer. Denitrifiers and ammonifiers in sediments compete for $NO_2^-$ and $NO_3^-$, and the contribution of denitrification to total $NO_3^-$ reduction has been reported to be 93% in Marlborough Sounds, New Zealand (Kasper et al., 1985), and 27 to 57% in the Tama estuary, Tokyo Bay (Nishio et al., 1982).

A strong coupling of nitrification and denitrification has been shown in several estuarine sediments (e.g., Jenkins and Kemp, 1984; Ogilvie et al., 1997), and $NO_3^-$ produced by nitrification can be a major contributor to sedimentary denitrification compared to $NO_3^-$ derived from the overlying water. In this study, even if all of the supplying fluxes of $NO_3^-$ and $NO_2^-$ from the water column are used for denitrification, they only account for 27% of the total denitrification. This suggests that the $NO_3^-$ and $NO_2^-$ pool in the sediment was the major contributor to sedimentary denitrification compared to nitrogenous oxide derived from the overlying water (Figure 6). The nitrification-denitrification coupling process in sediments via degradation of organic nitrogen into $NH_4^+$ was more important in removing nutrients from the overlying water in the Banzu intertidal flat than direct removal of $NO_3^-$ and $NO_2^-$ by denitrification.

## Conclusion

The intertidal sand-flat is a sink of DIN during the lightened and submerged period. Microalgae on the sediment surface plays a significant role in removing DIN from the overlying water by its photosynthetic

process and in suppressing the release of mineralized DIN from the sediment. However, in the dark, in the absence of the uptake of DIN by the benthic microalgae, the intertidal sediment acts as a source of DIN. Bioturbation or excretion by macrofauna greatly contributes to the exchange of DIN at the sediment-water interface, while molecular diffusion is minor. Denitrification is also important for eliminating $NO_3^-$ and $NO_2^-$ from the intertidal flat ecosystem, including the sediment and overlying water. Denitrifiers use $NO_3^-$ and $NO_2^-$ derived from the sediment more than from the overlying water.

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