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RELATIONSHIPS BETWEEN DENITRIFICATION ACTIVITY AND ENVIRONMENTAL FACTORS IN THE TIDAL SEDIMENT OF THE INNER AREA OF THE ARIAKE BAY

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SYNOPSIS

The relationships between denitrification activity and environmental factors in the tidal sediments of the inner area of the Ariake Bay were investigated based on in situ and laboratory data. Statistically significant differences were found between the mean values of the number of denitrifiers in the muddy sediments, and in the mud-sandy sediments and in the sandy sediments (*t* test, $P<0.05$). In the muddy sediments, a significant correlation was found between denitrification rate in the sediment and $NO_3^-+NO_2^-$-N flux from the overlying sea water into the tidal sediment ($r^2=0.25$, $P<0.01$). This study concluded that 47 % of $NO_3^-+NO_2^-$-N intake from the overlying water into the tidal sediment was consumed by denitrification, and that the remainder was used as a substrate for assimilation of NO_3^- reduction. The denitrification activity in the muddy sediments was influenced strongly by C/N ratio and $NO_3^-+NO_2^-$-N in the sea water.

INTRODUCTION

In coastal zones, detritus of land and sea origins are accumulated and concentrated near the sediment-water interface (benthic boundary layer), and most of it is decomposed and mineralized by benthos and microorganisms (1)–(3). The nutrient release from sediments plays an important role in the nutrient requirements of primary producers in the shallow coastal waters (4), (5). Thus, the fluxes of materials such as nutrients in the benthic boundary layer are important factors in the aquatic environment and ecosystem of coastal zones (6). However, the effects of physical and biochemical factors in the benthic boundary layer on the metabolism of microorganisms and the process of nutrient release have not been clearly determined. In addition, the type and the activity of microorganisms related to decomposition and mineralization of organic matter in the benthic boundary layer are not yet clearly understood.

The Ariake Bay located in the western coast of Kyushu, Japan, has a length of about 90 km, a width of about 20 km, an average depth of about 20 m and a total area of almost 1700 km^2. The Ariake Bay is a semi-closed bay with tidal flats of about 200 km^2 and a maximum tidal range of about 6 m in spring tide. The high concentration of suspended mud layer is mainly observed in the inner area of the Ariake Bay (*IAB*), because sedimentation and resuspension of large amounts of fine sediment particles and detritus occur due to periodic tidal currents and waves, etc. (7), (8). Recently, environmental degradation and ecosystem deterioration, such as frequent occurrences of red tide and hypoxic water and rapid decreases in bivalve production, have become serious problems in the *IAB* (9)–(11). A great deal of research has been conducted on the investigation into the causes of environmental deterioration and restoration of the ocean environments (12)–(14). The environmental purification function of tidal flats, including the removal of nitrogen and phosphorus from the sea, plays an important role in improving of the aquatic environment. Denitrification is a key process of nitrogen recycling in the Ariake Bay. Yanagi and Abe (15) found that denitrification accounts for about 20 % of the total amount of nitrogen elimination in the Ariake Bay. However, little information is available concerning the seasonal variations of the in situ rate of denitrification in the muddy tidal flats that extended to the *IAB*. In addition, the relationships between denitrification activity and environmental factors in the tidal sediment are not clearly understood yet.

In previous studies, we clarified the spatial distributions of the number of denitrifiers in the tidal sediments of the *IAB*, and the seasonal variations of number of denitrifiers and denitrification rate in the muddy sediments in the *IAB* (16). In this study, 14 sampling sites were selected on the tidal sediments in the *IAB*, and the number of denitrifiers and the physical sediment properties were investigated at all sampling sites in four seasons from 2006 to 2007. Here, we focus on the relationship between the spatial distribution and seasonal changes in the number of denitrifiers and the sediment characteristics. Next, a fixed station was set on the typical muddy sediment in the *IAB*, and the physical sediment properties, the inorganic nitrogen concentration in sediment pore water, the number of denitrifiers, and the in situ denitrification rate were investigated biweekly each month in 2006–2008. Here, we focus on the effects of environmental factors on the seasonal changes in denitrification rate in the muddy sediment by multiple regression analysis.

MATERIALS AND METHODS

Study sites and sample collection

Figure 1 shows the positions of sediment sampling sites (S1–S14) and the in situ investigation site (S9) for this study. The sampling sites were located on the muddy, mud-sandy, and sandy tidal flats in the coastal zone of the *IAB*. The sampling sites (A–C) and (D–G) were located on the tidal flats and in the shallow sea of the *IAB*, respectively. To examine the relationship between the spatial distributions of number of denitrifiers and the tidal sediment characteristics in the *IAB*, sediment samples were collected at the sampling sites (S1–S14) during the ebb tides in May, August, November, and December 2006 and 2007 using clear acrylic cores (83 cm in diameter, 21 cm in length). The sediment cores were closed with rubber stoppers and transferred to the laboratory within a few hours. Measurements of the number of denitrifiers and analyses of physical sediment properties were conducted in a laboratory using the sediment samples from the sediment surface to a depth of 4 cm. To investigate the relationship between the seasonal changes in the number of denitrifiers and the sediment characteristics on muddy tidal flats, five undisturbed sediment samples were collected biweekly at sampling site S9 using clear acrylic cores (83 mm in diameter, 21 cm in length) each month from

April 2006 to March 2008. One of the undisturbed sediment samples was divided into subsamples at intervals of 2 cm from the sediment surface to a depth of 10 cm. Parts of the subsamples were used for measuring the number of denitrifiers, and for analyzing pore water geochemistry and physical sediment properties, and the remaining parts were used for measuring the denitrification rate and sediment-water flux of inorganic nitrogen. For a comparison between the distribution of number of denitrifiers in the tidal flats that undergo periodic submersion and emersion and in the shallow sea that is within the infralittoral zone, two undisturbed sediment samples were collected monthly by a diver at sampling sites (A–G) from August 2007 to July 2008 using clear acrylic cores (50 mm in diameter, 30 cm in length). The samples obtained near the undisturbed sediment surface (0–2 cm in depth) were used for measuring the number of denitrifiers and for analyzing physical sediment properties.

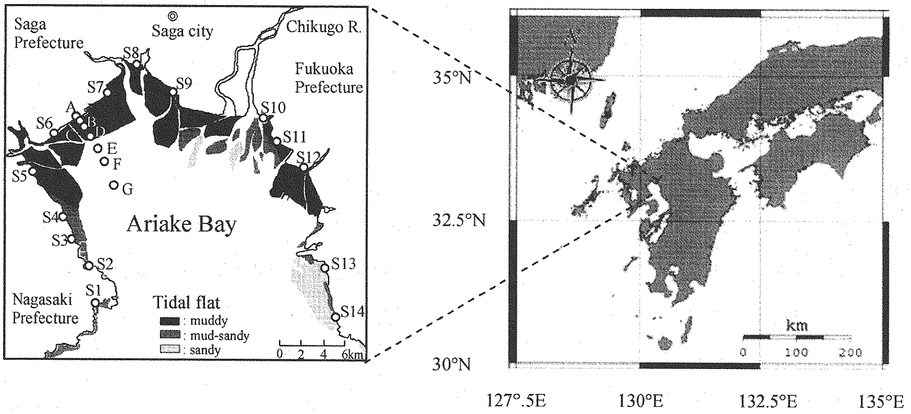

Fig. 1 Locations of sediment sampling sites (S1–S14 and A–G) and investigation site (S9)

Physical sediment properties and sediment pore water

The oxidation-reduction potential (Eh), sediment temperature, the mud (silt and clay) content, the total organic carbon (TOC), and the total nitrogen (TN) were measured to determine the geochemical sediment properties. Eh was measured by an ORP meter (RM-20p, TOA) inserted directly into the sediment sample and was corrected with sediment temperature, and sediment temperature was measured in the field using a digital thermometer (SK-1100, Sato). Eh value was calculated as $\mathrm{Eh}=E\mathrm{c}+206-0.7\times(ST-25)$, where Ec is the observed ORP and ST is the sediment temperature. TOC and TN contents of the sediment were analyzed using a CHN analyzer (JM-10, J-ScienceLAB). Before analysis, sediment samples were treated with 1 N HCl for 24 h to remove carbonates, and subsequently dried at 60 °C under vacuum to eliminate HCl vapor. Sediment pore water was collected by centrifuging sediment subsamples and filtered using a 0.45 μm membrane filter. The pore water samples were frozen and stored at –20 °C until analyses were carried out. NH_4^+-N and $NO_3^-+NO_2^-$ concentrations in pore water were analyzed using an auto analyzer (SWAAT, BLTEC).

Enumeration of denitrifiers

The Most Probable Number (MPN) method was used to count denitrifiers in the sediments taken in acrylic cores at the locations. Samples of 10 g of the sediments were dispersed in 100 ml of sterile distilled water. After sonication of the suspension for two minutes, the supernatant was used as an inoculum source. Tenfold serial dilutions of the inoculum source were prepared, and then 1-ml aliquots were transferred to five replicate test tubes. The test tubes containing Giltay medium (pH 7.0–7.2) and the Durham tubes had been previously sterilized by autoclaving. The inoculated tubes were incubated at 30 °C for one week. The color change from yellow to blue in the inoculated tubes and nitrogen gas production in the Durham tubes were checked to confirm the occurrence of denitrification(17).

Denitrification rate

Denitrification rate was measured by the Acetylene Inhibition Technique in laboratory incubation. This is a simple, quick, sensitive, and low-cost method to measure denitrification rate (18)–(20). Denitrification rate measurements were conducted in the laboratory immediately after sampling of sea water (2l) and undisturbed sediment cores (8.3 cm in diameter, 21 cm in length) at sampling site S9 as follows: some of the sea water (100 ml), that was filtered using a 0.45μm membrane filter, dissolved 10 % of C_2H_2 was injected into 50 points of the undisturbed sediment cores by 1 ml using a microsyringe and the remaining part was poured into the surface of the sediment core slowly. The headspace of the sediment core was filled with nitrogen gas, and the sediment cores were capped with a rubber stopper and then incubated in the dark at in situ sediment temperature (Table 1) for 24 h. At 2, 4, 6, 8, and 24 h from the beginning of incubation, 2 ml headspace samples in the sediment cores were collected using a microsyringe, and N_2O concentration of the gas phase was measured by gas chromatography (G3810, Yanako). The denitrification rate, R_d (μ mol-N m^{-2} d^{-1}), was calculated by Eq.(1) based on the rate of N_2O production in the sediment core during incubation. R_d was considered as the in situ denitrification rate, because the denitrification rate measurements were conducted at the in situ sediment temperature using the field sea water. The sediment temperature and the inorganic nitrogen concentration of the overlying sea water in the denitrification rate measurements are listed in Table 1.

Table 1 Sediment temperature and inorganic nitrogen concentration of the overlying sea water in the denitrification rate measurements

Sampling period	Sediment temperature(°C)*	NH_4^+-N (μ mol l^{-1})**	NO_3^--N (μ mol l^{-1})**	NO_2^--N (μ mol l^{-1})**
Spring (Mar–May 2006)	13.9–20.4	146.4–205.0	2.9–7.1	1.4–2.9
Summer (Jun–Aug 2006)	24.7–28.0	32.1–189.3	0.0–0.7	0.0–6.4
Fall (Sep–Nov 2006)	16.4–28.7	14.3–96.4	0.0–1.4	0.7–8.6
Winter (Dec 2006–Feb 2007)	10.9–16.9	0.7–95.0	0.0–7.1	0.0–2.9
Spring (Mar–May 2007)	10.3–25.4	5.7–147.1	0.7–7.1	0.0–6.4
Summer (Jun–Aug 2007)	24.9–29.4	42.1–245.0	2.9–12.9	2.9–10.7
Fall (Sep–Nov 2007)	14.2–28.4	27.9–105.7	0.7–7.9	0.7–7.9
Winter (Dec 2007–Feb 2008)	6.1–12.8	35.0–112.9	0.0–4.3	1.4–4.3
Spring (Mar 2008)	14.1–18.6	14.3–31.4	1.4–11.4	0.7–2.1

* Mean value from the surface to a depth of 10 cm in the undisturbed sediment core.

** NH_4^+-N, NO_3^--N, and NO_2^--N concentrations of the overlying sea water in the undisturbed sediment core.

$$R_d = \frac{89.29C\left(V_g - \alpha V_l\right)}{A\,T} \tag{1}$$

where C is N_2O concentration (ml l^{-1}) of gas phase in the sediment core, V_g and V_l are the volumes (l) of gas and liquid phases in the sediment core, respectively, A is the cross-sectional area (m^2) of sediment core, T is the unit time (d), α is the Ostwald solubility coefficient (e.g., α =0.530 ml ml^{-1} at 30 °C), and 89.29 is the mass of N_2O-N in the ideal gas (μ mol l^{-1}).

Inorganic nitrogen flux between sediment and overlying sea water

Inorganic nitrogen flux experiments were conducted in the laboratory immediately after collecting sea water (2 l) and undisturbed sediment cores (8.3 cm in diameter, 21 cm in length) at sampling site S9. 400 ml of sea water filtered using a 0.45μm membrane filter was gently and slowly flushed onto the sediment surface so that disturbance and resuspension of the sediment were minimized. The sediment cores were settled in a thermostated water bath at the in situ sediment temperature and in the dark. During incubation, the overlying sea water was continuously stirred with an electric propeller about 10 cm above the sediment surface. Samples of 10 ml of the overlying sea water were collected at 2, 4, 6, 8, and 24 h after commencement of incubation. Water samples were filtered and frozen for analysis of NH_4^+, NO_3^-, and NO_2^- concentrations. NH_4^+-N flux (F_{NH4}) and NO_3^-+NO_2^--N flux ($F_{NO3+NO2}$) from the sediment into the overlying sea water (μ mol-N m^{-2} d^{-1}) were calculated using Eq.(2) based on the temporal changes in NH_4^+-N and NO_3^-+NO_2^--N concentrations in the overlying sea water.

$$F_{NH4} \text{ or } F_{NO3+NO2} = \frac{(C_0 - C_T)V}{AT} \qquad (2)$$

where C_0 and C_T are the NH_4^+-N or NO_3^-+NO_2^--N concentrations (μ mol l^{-1}) in the overlying sea water at times (d) 0 and T, respectively, and V is the volume (l) of overlying sea water.

RESULTS AND DISCUSSION

Relationships between the seasonal variations of number of denitrifiers and the sediment physical properties

Figure 2 shows the mud contents of tidal sediment at sampling sites (S1–S14) in 2006. Mud contents were 22 %–47 % in the mud-sandy sediments at sampling sites S1–S4, 77 %–99 % in the muddy sediments at sampling sites S5–S12, and 6 %–11 % in the sandy sediments at sampling site S13 and S14. Most of the sediments in the *IAB* were muddy sediment. The circulation patterns of residual current in the bay head of the Ariake Bay have important effects on the formation of the muddy tidal flat in the *IAB* (21).

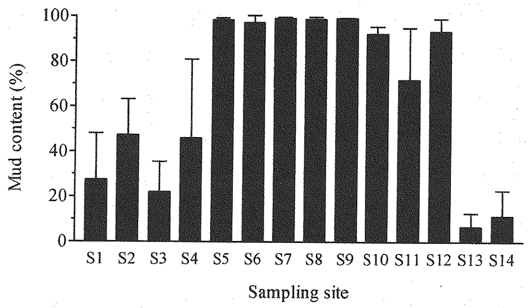

Fig. 2 Mud (silt and clay) contents (mean and standard deviation) of sediment at the sampling sites S1–S14 in 2006 (n=4)

Table 2 shows the number of denitrifiers in each sediment type for the four seasons in 2006 and 2007. Except for spring 2007, statically significant differences were found between the mean values of number of denitrifiers in the muddy sediment, and in the mud-sandy sediment and in the sandy sediment in each season (*t* test, $P<0.05$). The mean number of denitrifiers in the muddy sediments was higher than those in the mud-sandy sediments and the sandy sediments. The mean numbers of denitrifiers for the two years were about 5.8×10^{2} cells g-dry^{-1} in the sandy sediment, about 1.1×10^{3} cells g-dry^{-1} in the mud-sandy sediment, and about 5.0×10^{4} cells g-dry^{-1} in the muddy sediment (Table 2).

Table 2 Number of denitrifiers in each sediment type of every the season in 2006 and 2007

Site	Sediment type*	Number of denitrifiers (cells g-dry^{-1})							
		Spring (Mar–May) 2006	2007	Summer (Jun–Aug.) 2006	2007	Fall (Sep–Nov.) 2006	2007	Winter (Dec–Feb.) 2006	2007
1	Mud-sandy (1,090±1,451)**	566	60	1,779	424	425	922	246	361
2		229	436	3,171	44	1,257	607	578	587
3		205	2,008	433	7,430	407	230	214	2,061
4		591	787	3,788	1,930	132	1,838	217	918
5	Muddy sediment (50,071±17,554)**	3,762	218	4,625	347,549	999	3,014	218	1,499
6		481	1,585	11,861	532,286	1,272	974	2,601	6,241
7		2,404	49	46,278	233,809	6,240	3,158	1,294	452
8		614	364	33,976	173,807	3,526	1,546	709	509
9		1,325	948	10,460	10,103	5,724	1,205	5,438	14,728
10		1,200	4,016	9,763	620,280	10,788	2,477	4,229	1,949
11		68	11,420	7,271	544,392	3,109	1,838	1,116	2,324
12		784	5,802	11,282	477,488	702	770	903	2,720
13	Sandy sediment (580±1,142)**	172	4,720	347	119	116	226	226	246
14		394	271	511	1,307	258	15	110	237

* As the division of the tidal sediment type according to the mud content did not have a strict standard, the sediment types were classified as follows: mud content 0 %–30 %, sandy sediment; 30 %–60 %, mud-sandy sediment; 60 %–100 %, muddy sediment.

** Mean value±standard deviation of number of denitrifiers in each sediment type during the investigation period (2006 and 2007).

The number of denitrifiers tended to increase with mud content ($P>0.05$ in spring and winter, $P<0.05$ in summer and fall), because the organic matter content of sediments increased with mud content (Fig. 3) and the anaerobic environment, that was appropriate for habitat of denitrifiers, was easily formed in the muddy sediment. The number of denitrifiers increased in summer. Especially, the number of denitrifiers in muddy sediments in summer was about one order of magnitude higher than those of the other seasons. Koriyama et al. (22) investigated the seasonal variations of TOC and TN in the sediments at the sampling sites A–G (Fig. 1) from August 2007 to July 2008, and they found that TOC and TN in the sediments increased in summer and winter. Thus, it is thought that the large number of denitrifiers in summer is due to a rise in sediment temperature and the supply of organic matter, which is the carbon source of denitrifiers, to the muddy sediments in the *IAB*.

Figure 4 shows the temporal variations in number of denitrifiers in the upper layer (0–2 cm in depth), middle layer (4–6 cm in depth), and lower layer (8–10 cm in depth) of sediment at the sampling site S9 located on the muddy tidal flats. The number of denitrifiers was maximum in the upper layer in July 2006 (about 9.4×10^{4} cells g-dry^{-1}) and in the middle layer in July 2007 (about 8.5×10^{4} cells g-dry^{-1}). Mean values of the number of denitrifiers in each layer of sediment over a period of two years was about 9.2×10^{3} cells g-dry^{-1} in the upper layer, about 8.8×10^{3} cells g-dry^{-1} in the middle layer, and about 4.1×10^{3} cells g-dry^{-1} in the lower layer, respectively. We found no significant differences among the number of denitrifiers in the three layers of the muddy sediments at sampling site S9 (ANOVA, $P>0.05$).

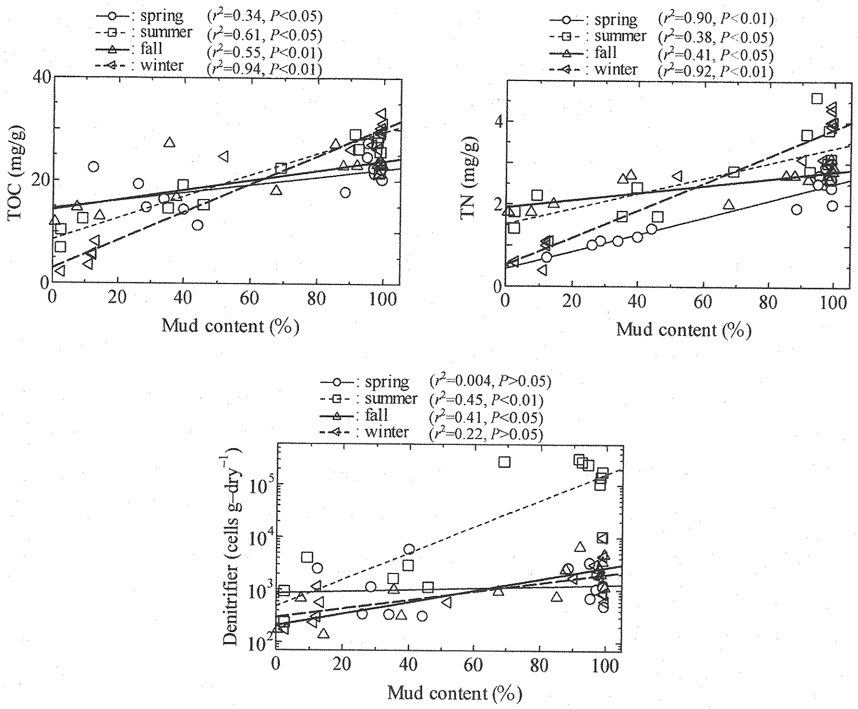

Fig. 3 Relationships between mud content, TOC, TN, and denitrifiers in the sediments

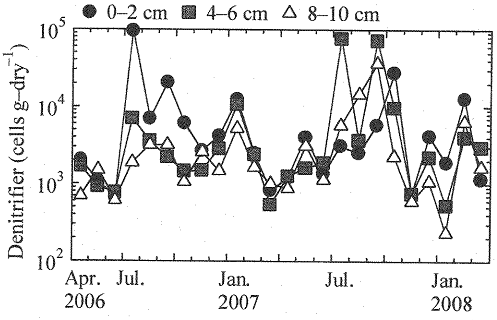

Fig. 4 Temporal variations in numbers of denitrifiers at each depth in the muddy tidal flat (sampling site S9)

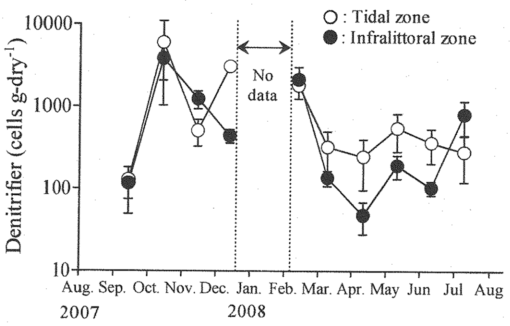

Fig. 5 Temporal variations in number of denitrifiers in the tidal zone (sampling sites A–C, n=3) and infralittoral area (sampling sites D–G, n=4).

Figure 5 shows the temporal variations of the number of denitrifiers in tidal zone (*TZ*) (sampling site A–C) and infralittoral zone (*InfZ*) (sampling site D–G) during the period from August 2007 to July 2008. The number of denitrifiers in August 2007 and January 2008 was not measured. The number of denitrifiers in both zones showed similar seasonal trends. The mean number of denitrifiers in *TZ* and *InfZ* was almost the same during summer–fall (September–November in 2007 and July in 2008); the number of denitrifiers was 1,853 cells g-dry^{-1} in *TZ* and 1,439 cells g-dry^{-1} in *InfZ*. On the other hand, the mean number of denitrifiers in *TZ* (1,065 cells g-dry^{-1}) was somewhat larger than that in *InfZ* (592 cells g-dry^{-1}), even though there was no significant difference (*t* test, $P>0.05$) during winter–early summer (December in 2007–June in 2008).

We found no significant differences in Eh or sediment temperature of the sediments (0–2 cm in depth) between *TZ* and *InfZ*, but significant differences in TOC and TN content of the sediments were found during winter–early summer (*t* test, $P<0.05$) (22). The mean values of TOC and TN content in the sediments (0–2 cm in depth) of both zones were 36.3 mg g^{-1} and 4.7 mg g^{-1} (*TZ*), and 36.0 mg g^{-1} and 4.4 mg g^{-1} (*InfZ*) during summer–fall, respectively. However, in winter–early summer, the mean values of TOC and TN content of the sediments in *TZ* (TOC: 39.7 mg g^{-1}, TN: 5.4 mg g^{-1}) were larger than in *InfZ* (TOC: 36.4 mg g^{-1}, TN: 4.2 mg g^{-1}). Thus, the difference in number of denitrifiers between both zones was thought to be related to the organic matter content in the sediments during winter–early summer.

Denitrification rate, number of denitrifiers, and inorganic nitrogen flux on the muddy tidal flat

Figures 6 and 7 show the temporal variations of number of denitrifiers and R_d in the sediment (0–10 cm in depth) on the muddy tidal flats (sampling site S9), and the relationship between them, respectively. The data of R_d during the period from November 2006 to January 2007 are not measured. The number of denitrifiers had a tendency to increase in summer (July–September) and to decrease in spring (March–May), although temporal variations in number of denitrifiers were different in each year. The temporal variations in environmental factors of sediment, mainly mud temperature, Eh, and organic matter content, were thought to have a significant impact on the number of denitrifiers. R_d had maximum values of 617.1 μmol-N m^{-2} d^{-1} in October 2006 and of 990.2 μmol-N m^{-2} d^{-1} in July 2007. The temporal variations in number of denitrifiers did not necessarily correspond to those of R_d, and the correlation between them was not statistically significant ($r^2=0.08$, $P>0.05$). The respiratory system of denitrifiers that are facultative anaerobic bacteria switches to aerobic respiration with high energy efficiency under oxic sediment conditions, even though the respiratory system is anaerobic respiration (denitrification) under anoxic or low-oxygen sediment conditions (23). We therefore concluded that this was responsible for the differences in temporal variations between the number of denitrifiers and R_d on muddy tidal flat.

Figure 8 shows the temporal variations of F_{NH4} and $F_{\mathrm{NO3+NO2}}$ at sampling site S9, and Figure 9 shows the relationship between R_d and $F_{\mathrm{NO3+NO2}}$. The positive values of F_{NH4} and $F_{\mathrm{NO3+NO2}}$ indicate the intake of NH_4^+-N and $NO_3^-+NO_2^-$-N from overlying sea water into sediment, and negative values indicate the elution of NH_4^+-N and $NO_3^-+NO_2^-$-N from sediment into overlying sea water. $F_{\mathrm{NO3+NO2}}$ was positive; that is, $NO_3^-+NO_2^-$-N was absorbed from the overlying sea water in the muddy sediment during the investigation period except January 2007. There was a statistically significant difference between $F_{\mathrm{NO3+NO2}}$ and R_d (t test, $P<0.05$). The mean value of $F_{\mathrm{NO3+NO2}}$ (495.7 μmol-N m^{-2} d^{-1}) for the investigation period was larger than that of R_d (231.4 μmol -N m^{-2} d^{-1}). Thus, it is thought that about half of the $NO_3^-+NO_2^-$-N intake from the overlying sea water into the sediment is used for denitrification and the remainder is used as a substrate for assimilation of NO_3^- reduction. Denitrification capacity has been reported to account for between 38 % and 95 % of the total NO_3^- reduction in marine sediment (24),(25). In the present study, 47 % of $NO_3^-+NO_2^-$-N intake from the overlying sea water into the sediment was consumed by denitrification. This suggests that the proportion of denitrification in total NO_3^- reduction in the muddy sediment of the Ariake Bay is roughly the same as that in marine sediment. Moreover, we found a significant positive correlation between R_d and $F_{\mathrm{NO3+NO2}}$, as shown in Fig. 9 ($r^2=0.25$, $P<0.01$). These results indicated that the denitrification activity was greatly controlled by $NO_3^-+NO_2^-$-N concentration in the overlying sea water, and the denitrification activity significantly affected the nitrogen budget in the benthic boundary layer. On the other hand, F_{NH4} was negative, or eluted from the sediment into the overlying sea water in summer except for January 2007, or in June and August 2006 and in July and September 2007 (*S*-period), and was positive, or absorbed from the overlying sea water to the sediment in other months (*W*-period). In *S*-period, the organic matter in the sediment was decomposed with a rise in mud temperature, and NH_4^+-N concentration of pore water in the sediment increased. As a result, it was thought that NH_4^+-N elusion from the sediment accelerated in *S*-period. As NH_4^+-N concentration in pore water of sediment surface layer was high (17.1 μmol l^{-1}) although the sediment temperature was low (11.0 °C), NH_4^+-N was eluted from the sediment into the overlying sea water in January 2007. However, the reason for the high NH_4^+-N concentration in sediment pore water is not clear. In *W*-period, nitrification activity in the sediment increased with the development of aerobic sediment conditions. The increase in NH_4^+-N absorption from the overlying sea water into the sediment was thought to be due to high nitrification activity.

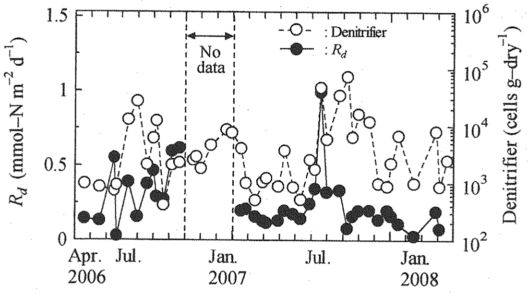

Fig. 6 Temporal variations in number of denitrifiers and denitrification rate (R_d) in the sediment in the muddy tidal flat (sampling site S9)

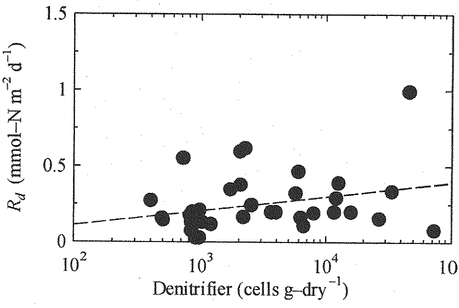

Fig. 7 Relationship between denitrification rate (R_d) and number of denitrifiers in the muddy tidal flat (sampling site S9)

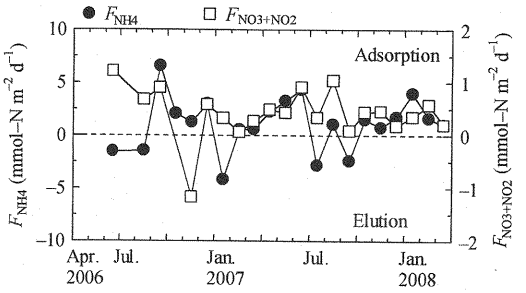

Fig. 8 Temporal variations in NH_4^+-N flux (F_{NH4}) and NO_3^-+NO_2^--N flux ($F_{NO3+NO2}$) between overlying sea water and muddy sediment (sampling site S9)

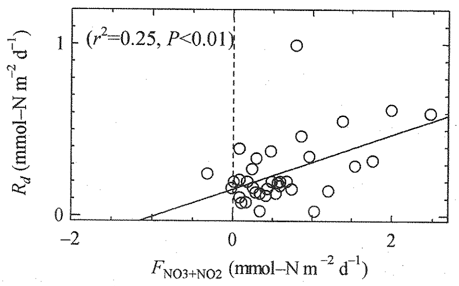

Fig. 9 Relationship between denitrification rate (R_d) and NO_3^-+NO_2^--N flux ($F_{NO3+NO2}$) in the muddy tidal flat (sampling site S9)

Vertical profiles of environmental factors related to denitrification in muddy sediment

Figure 10 shows the vertical profiles of monthly mean values in the environmental factors of sediment that are thought to greatly affect denitrification activity on the muddy tidal flats (sampling site S9). Mud temperature was almost uniform from the sediment surface to the lower layer (0–10 cm in depth). Seasonal mean values of mud temperatures were 18.4 °C in spring, 27.5 °C in summer, 23.1 °C in fall, and 10.7 °C in winter. Eh decreased from the sediment surface to the lower layers throughout the year. The vertical distributions of Eh in fall and winter shifted from negative to positive values overall as compared with summer and spring. Especially, aerobic sediment conditions were formed near the sediment surface layer in winter, because the aerobic decomposition of organic matter in the sediment was restricted and the oxygen consumption of sediment decreased in winter due to low mud temperature.

NH_4^+-N concentrations in the overlying sea water and pore water of sediment were 52.9–71.4 μmol l^{-1} and 7.9–473.6 μmol l^{-1}, respectively. NH_4^+-N concentration in pore water increased with sediment depth except in winter. NH_4^+-N concentration of pore water in the sediment surface layer was low due to the nitrification and the elution from sediment into overlying sea water; that is, NH_4^+-N tended to be released from the sediment into the overlying sea water in summer, because the decomposition of organic matter was accelerated with increasing sediment temperature and then

NH_4^+-N concentration in pore water of the sediment surface layer increased. On the other hand, in the fall and winter, Eh values increased (Eh +90.7 mV in summer to +256.3 mV in winter) and the oxidized zone prevailed in the sediment surface layer. Therefore, we concluded that NH_4^+-N concentration of pore water in the sediment surface layer decreased due to the acceleration of nitrification activity in the sediment surface layer. NH_4^+-N concentration increased with sediment depth due to regeneration and accumulation of NH_4^+-N in the sediment lower layer with decomposition of organic matter. In winter, NH_4^+-N concentration in pore water was low except at a depth of 3 cm. It is thought that one of the causes was the inactivation of NH_4^+-N regeneration with decreasing mud temperature.

NO_3^-+NO_2^--N concentrations in the overlying sea water and in pore water of sediment were 4.3–7.1 μ mol l^{-1} and 2.1–4.3 μ mol l^{-1}, respectively, and the magnitude of the latter was about two orders smaller than that of NH_4^+-N concentration in pore water. The reasons for this are as follows: the nitrification activity of sediment is low, because the anaerobic sediment environment is superior on the muddy tidal flats except in the vicinity of the sediment surface. As a result, the generation of NO_3^-+NO_2^--N is low in the muddy tidal flats because of a decrease in the nitrification activity. NO_3^-+NO_2^--N concentration was almost uniform from sediment surface to a depth of 10 cm and was comparatively low throughout the year.

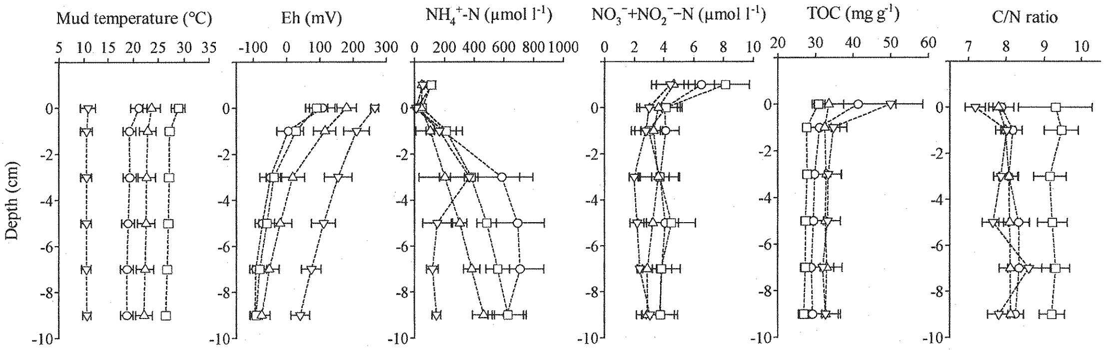

Fig. 10 Vertical distributions of seasonal mean mud temperature, Eh, NH_4^+-N, NO_3^-+ NO_2^--N, TOC, and C/N ratio in the muddy tidal flat (sampling site S9) (○: spring (n=46); □: summer (n=51); △: fall (n=54);▽: winter (n=48))

Although TOC content was relatively high in the sediment surface, it was almost uniform in the sediment with a value of about 30 mg g^{-1}, and the differences in TOC content between seasons were small. However, TOC content of the sediment surface was high in winter. We postulated that one of the causes for this is an adhesion diatom that grows thickly on the sediment surface in winter. C/N ratio showed an almost uniform profile in the sediment except in winter. The mean value of C/N ratio in sediment was highest in summer. The mean value of C/N ratio was 9.3 in summer, and ranged from 7.1 to 8.6 in other seasons. In general, the C/N ratio is significantly affected by the type and decomposition condition of organic matter (26), (27). Thus, C/N ratio was low in winter and high in summer, because organic matter was that of sea origin, such as attached algae, which is decomposed easily, in winter and of terrestrial origin, such as hydrophytes, which are not decomposed easily, in summer.

Relationships between denitrification rate and environmental factors of sediment

Table 3 shows the denitrification rates of sediments measured in both domestic and foreign coastal and tidal flat areas. The denitrification rates of tidal sediment were greater than those of coastal sediment, and the denitrification rate in sandy tidal flats was lower than in muddy tidal flats. The reason for this was thought to be because the organic matter content, which is the carbon source for denitrifiers, is higher in muddy than in sandy tidal flats (Fig. 3) and the redox potential discontinuity (RPD) layer, which is an appropriate habitat for denitrifiers, is formed more easily in muddy than in sandy sediment. The denitrification rate in the muddy tidal flat of *IAB* (sampling site S9) was of approximately the same order as those reported previously in domestic and foreign tidal flats.

Table 3 Measured denitrification rates of sediments in coastal and tidal flat areas (28)–(30)

Locations	Samples	Denitrification rates ($\text{mmol-N m}^{-2}\text{d}^{-1}$)	References
Ariake Sea (Japan)	Mud flats (0–10 cm)	0.03–0.67 (mean: 0.23)	Present study
Tokyo Bay (Japan)	Coastal sediment	0.20–0.34	Nishio T. (1982)
Hiroshima Bay (Japan)	Tidal flats	0.00–1.43	Seiki T. (2008)
	Coastal sediment	0.01–0.24	
Norsminde Fjord (Denmark)	Mud flats	0.14–0.71	Jørgensen K. S. (1988)
Delaware Inlet (N.Z.)	Mud flats (0–10.5 cm)	0.09–0.78 (mean: 0.29)	Kasper H. F. (1983)
	Sand flats (0–10.5 cm)	0.12–0.57 (mean: 0.21)	

Table 4 Correlation coefficients between seasonal mean values of denitrification rate and main environmental factors in the muddy tidal flat (sampling site S9) (**: $P<0.01$, *: $P<0.05$)

	Denitrifier number	Mud temperature	Eh	TOC	C/N ratio	$NO_3^-+NO_2^-$-N		
						Pore water	Overlying sea water	Flux
Spring	0.55	0.41	0.20	0.52	0.31	0.02	0.53	0.78^{*}
Summer	0.55	0.34	0.08	0.24	0.52	0.12	0.15	0.03
Fall	0.43	0.01	0.52	0.78^{**}	0.62	0.22	0.48	0.91^{**}
Winter	0.16	0.05	0.01	0.14	0.76^{*}	0.47	0.41	0.27

Table 5 Regression coefficients

Autonomous variable	Standardized coefficient (β)	*t* value	*P*
$F_{\text{NO3+NO2}}$	0.555	4.282	0.000
C/N ratio	–0.364	–2.812	0.007

Table 4 shows the correlation coefficients between seasonal mean values of R_d and main environmental factors of sediment at sampling site S9. The correlation coefficients were markedly different between seasons. For example, the environmental factors strongly correlated with R_d and $F_{\text{NO3+NO2}}$ in spring, $F_{\text{NO3+NO2}}$ and TOC content in fall, and C/N ratio in winter. In other words, the denitrification activity, which is a biochemical phenomenon, is related in a complex manner to various environmental factors in sediments, and the relationships between the denitrification activity and the environmental factors vary with each season. To examine the factors influencing R_d during the investigation period from April 2006 to February 2008, multiple regression analysis was carried out. The stepwise method was used as a method of selecting the autonomous variables of a multiple regression equation. The autonomous variables significantly related to R_d in the muddy tidal flat were $F_{\text{NO3+NO2}}$ and C/N ratio, and the standardized coefficients (β) were 0.56 and –0.36, respectively (Table 5). Thus, it is thought that R_d of sampling site S9 located in the muddy tidal flat is greatly controlled by the C/N ratio, which is related to the type and decomposition conditions of organic matter that is an electron donor and by $F_{\text{NO3+NO2}}$, which is related indirectly as an electron acceptor throughout the year.

CONCLUSIONS

In the present study, the relationships between denitrification activity and environmental factors of sediment in the muddy tidal flat were investigated based on in situ and laboratory data. The results of the present study are summarized as follows:

1) The seasonal mean value of the number of denitrifiers in the tidal sediment surface layer (0–4 cm in depth) of the *IAB* was highest in summer (mean value was about 1.9×10^5 cells g-dry^{-1}). The causes of this were thought to be as follows: (i) increased mud temperature; (ii) large amounts of organic matter, which is the carbon source for denitrifiers, were carried into the tidal flat of the *IAB*; (iii) the RPD layer, which is an appropriate habitat for denitrifiers, was formed easily in summer.

2) A relatively large number of denitrifiers was observed in the upper (0–2 cm in depth) and middle (2–4 cm in depth) layers of the muddy tidal sediment (sampling site S9). The annual mean numbers of denitrifiers in the upper and middle layers were approximately 9.2×10^3 cells g-dry^{-1} and 8.8×10^3 cells g-dry^{-1}, respectively.

3) There was no unique relation between monthly mean denitrification rate and the number of denitrifiers in the sediment (0–10 cm in depth) of the muddy tidal flat. This was thought to be related to the respiration system of denitrifiers, which are facultative anaerobic bacteria. We thought that there were differences in temporal variations between denitrification rate and number of denitrifiers, because the denitrifiers take aerobic respiration with high energy efficiency under the oxic sediment conditions and anaerobic respiration (denitrification) under anoxic or low-oxygen sediment conditions.

4) The monthly variation of $F_{\mathrm{NO3+NO2}}$ between overlying sea water and sediment (0–10 cm in depth) indicated that $NO_3^-+NO_2^-$-N was incorporated from the overlying sea water into the sediment of the muddy tidal flat (sampling site S9) except in January 2007. $F_{\mathrm{NO3+NO2}}$ (495.7 μmol-N m^{-2} d^{-1}) was higher than R_d (231.4 μmol-N m^{-2} d^{-1}). From this result, we concluded that about half of the $NO_3^-+NO_2^-$-N absorption from the overlying sea water into the sediment was used for denitrification and the remainder was used as a substrate for assimilation of NO_3^- reduction.

5) The correlation coefficients between monthly mean values of denitrification rate and main environmental factors in the sediment (0–10 cm in depth) of the muddy tidal flat (sampling site S9) varied with each season; that is, the denitrification activity, which is a biochemical phenomenon, was related in a complex manner to various environmental factors of sediment and the relations between them varied between seasons. However, the denitrification rate of the muddy sediment was shown by multiple regression analysis to be greatly controlled by the C/N ratio, which is related to the type and decomposition conditions of organic matter that is an electron donor, and by $F_{\mathrm{NO3+NO2}}$, which is related indirectly as an electron acceptor throughout the year.

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APPENDIX – NOTATION

The following symbols are used in this paper:

A = cross-section area of sediment core;

C = N_2O concentration of gas phase in the sediment core;

C_0 = NH_4^+-N or NO_3^-+NO_2^--N concentration in the overlying water at time 0;

C_T = NH_4^+-N or NO_3^-+NO_2^--N concentration in the overlying water at time T;

Ec = observed ORP;

F_{NH4} = NH_4^+-N flux from the sediment into the overlying sea water;

$F_{NO3+NO2}$= NO_3^-+NO_2^--N flux from the sediment into the overlying sea water;

IAB = inner area of Ariake Bay;

$InfZ$ = infralittoral zone;

ST = sediment temperature;

TZ = tidal zone;

R_d = denitrification rate;

V = volume of overlying sea water in the sediment core;

V_g = volume of gas phase in the sediment core;

V_l = volume of liquid phase in the sediment core;

α = Ostwald solubility coefficient; and

β = standardized coefficient.

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