

Tidal time-scale variation in nutrient flux across the sediment–water interface of an estuarine tidal flat

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Abstract

We determined the range of the tidal variations in nutrient flux across the sediment–water interface and elucidated mechanisms of the flux variation in two estuarine intertidal flats (one sand, one mud) in northeastern Japan. Nutrient flux was measured using in situ light and dark chambers, which were incubated for 2 h, 2–6 times per day. Results showed that nutrient concentration in overlying water varied by tide and was also affected by sewage-treated water inflow. The nutrient fluxes responded quickly to the tidal variation in overlying water chemistry and the range of the variation in flux was as large as the seasonal-scale variation reported in previous studies. In the sand flat, salinity increase likely enhanced benthos respiration and led to increases in both O₂ consumption and PO₄³⁻ regeneration under low illumination, while benthic microalgae were likely to actively generate O₂, uptake PO₄³⁻ and suppress PO₄³⁻ release under high illumination (>900 μmol photons m⁻² s⁻¹). Also in the mud flat, PO₄³⁻ flux was related with O₂ flux, although the range of temporal variation in PO₄³⁻ flux was small. In both the flats, NH₄⁺ flux was always governed by NH₄⁺ concentration in the overlying water; either an increase in NH₄⁺ uptake or a decrease in NH₄⁺ release was observed as the NH₄⁺ concentration rose due to inflow of river water or input of sewage-treated water. Although NO₃⁻ tended to be released in both tidal flats when low NO₃⁻ concentration seawater dominated, their relationship was likely to be weakened under conditions of low oxygen consumption and suppressed denitrification. It is likely that tidal variation in nutrient flux is governed more by the nutrient concentration than other factors, such as benthic biological processes, particularly in the case where nutrient concentration in the overlying water is relatively high and with wide amplitude.

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1. Introduction

The nutrient exchange across the sediment–water interface of tidal flats is an important pathway for nutrient cycles in shallow coastal ecosystems. The evaluation of the exchange flux is indispensable to identifying the effects of tidal flats on the coastal environment and for effective planning and management of tidal flat ecosystems (Kurihara, 1988; Reay et al., 1995; Sanders et al., 1997; Yin and Harrison, 2000). However, there are a number of factors which influence the

flux, and which vary spatially and temporally, making an accurate flux determination difficult. An accurate calculation of the flux is problematic, particularly in estuarine tidal flats, because of the dramatic temporal changes in environmental conditions due to the alternating replacement of overlying water with sea and river waters. Unless short-term fluctuations are taken into account in areas strongly affected by the tide, like estuaries, estimating a typical flux of nutrients is all but impossible.

Tidal variations in the flux are still a very obscure phenomenon, which is due to there having been insufficient observations focusing on tidal variations in the flux and environmental conditions. In most previous studies, nutrient flux measurement has been conducted only once, or once a month or season, using sediment core samples or in situ chambers (e.g. Reay et al., 1995; Rysgaard et al., 1995; Forja and Gómez-Parra, 1998). There is

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a possibility that short-term fluctuations in the flux have been missed in those investigations. Hence, the range of flux variations and their sensitivity to short-term changes in environmental factors have yet to be clarified.

Various physical and chemical factors affect the nutrient exchange process at the sediment–water interface. The nutrient exchange flux and the vertical concentration profile in sediment have been shown to be influenced by changes in nutrient concentrations of overlying water (Asmus, 1986; Kristensen, 1993; Magalhães et al., 2002), water temperature (Klump and Martens, 1989; Forja et al., 1994; Rysgaard et al., 1995; Sundbäck et al., 2000), hydrodynamic condition (Rutgers van der Loeff, 1981; Vidal and Morguí, 1995; Oldham and Lavery, 1999), and sediment condition (Blackburn and Henriksen, 1983; Jensen et al., 1990; Enoksson, 1993; Sloth et al., 1995). Various biological processes, which can greatly modify the nutrient flux, have also been reported, including the mineralization of organic nutrients (Hopkinson, 1987; Reay et al., 1995; Yin and Harrison, 2000), oxygen production and nutrient uptake by benthic microalgae (Sundbäck and Graneli, 1988; Reay et al., 1995; Nishimura et al., 1996; Kuwae et al., 1998; Wilson and Brennan, 2004), nitrification and denitrification (Kemp et al., 1990; Risgaard-Petersen et al., 1994; Rysgaard et al., 1994, 1995; Sundbäck et al., 2000), and various macrofaunal activities, for example, irrigation, excretion, respiration, and physical disturbance of sediment (Clavero et al., 1991; Forja and Gómez-Parra, 1998; Mortimer et al., 1999; Webb and Eyre, 2004).

Previous studies have frequently reported on the seasonal variations in nutrient flux in response to variations in physical and chemical factors (e.g. water quality, temperature, sediment condition). With regard to time scales of less than a season, diurnal variations in nutrient flux have been attributed to the response of microphytobenthos to changes in illumination (Sundbäck and Graneli, 1988; Reay et al., 1995; Kuwae et al., 1998; Sundbäck et al., 2000). In estuaries, the water column chemistry and particularly the solute concentrations may change with the relative contribution of seawater and fresh water. These variations may modulate sediment–water fluxes on a tidal time scale. Tidal variations in the flux, however, have rarely been reported (Cabrita et al., 1999; Yin and Harrison, 2000).

The purposes of this study were to determine the range and sensitivity of nutrient flux responding to tidal variations in water column chemistry caused by tidal replacement of river water and seawater and to elucidate the mechanism of tidal flux variation in an estuarine tidal flat. This study presents several flux measurements per tidal cycle, obtained by repeated operations of the in situ chambers at sandy and muddy intertidal flats in a single estuary. An understanding of the short-term variations contributes to a determination of the typical flux of nutrients in the tidal flat.

2. Methods

2.1. Study site

We studied a sand and a mud intertidal flat on the east coast of Honshu Island, Japan (Fig. 1). Those tidal flats are located

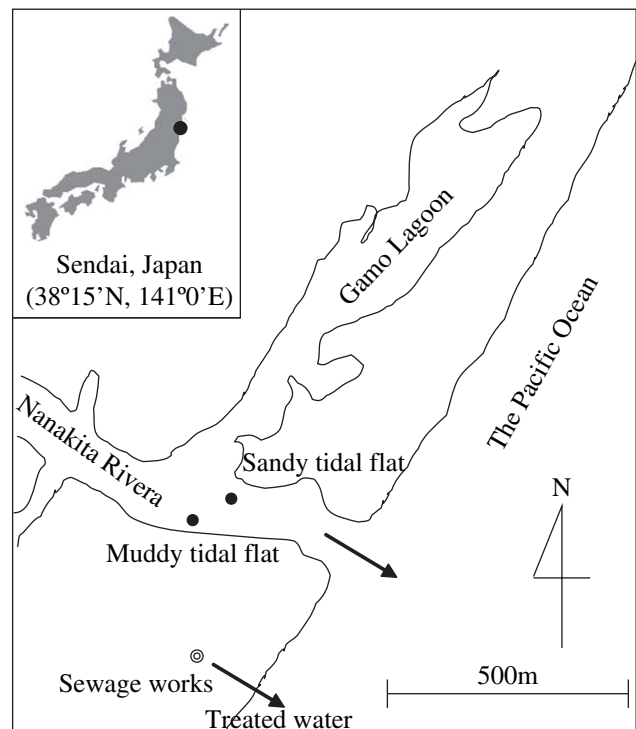


Fig. 1. Map of the study sites in the Nanakita estuary, Japan.

at the north and south banks of the Nanakita estuary, respectively. Each of the tidal flats has an area of about 20,000 m². The Nanakita River discharges to the Pacific Ocean ordinarily at 860,000–1,300,000 m³ day⁻¹. Sewage-treated water is discharged to the Pacific Ocean at a rate of 500,000 m³ day⁻¹ from 500 m south of the river mouth. The tidal amplitudes ranged from about 0.4 to 1.4 m in this estuary. In both the sand and mud flats, the bed elevation of the sampling point was 0.3 m below mean water level of this estuary. Investigations were conducted three times in the sand flat and five times in the mud flat in the summer of 1999. All investigations were carried out under similar fair weather conditions.

2.2. Repeating nutrient flux measurements

A transparent acrylic (light) chamber and an opaque vinyl chloride (dark) chamber were used to measure the short-term exchange flux across the sediment–water interface for dissolved oxygen (O₂) and inorganic nutrients. Each cylindrical chamber (33 cm inner diameter and 30 cm height) has two symmetrically facing side-ports of 10 cm diameter with rubber stoppers at the upper edge of the side-wall to allow for water exchange with the surroundings. The chambers were furnished with a sampling tube and an internal balloon for pressure compensation during sampling. Before the in situ measurements were taken, a salt tracer confirmed the water tightness of the chambers.

In the field, the chambers were inserted into the tidal flat sediment during low tide. The height and volume of the water column in the chamber were standardized to 10 cm and 8.5 l, respectively, maintaining the side-ports just above

the sediment surface. The chambers were operated as follows: (1) during chamber submersion, the side-ports were left open for at least 30 min to allow for the exchange of chamber water; (2) the side-ports were closed and a water sample was taken by syringe for initial O₂ and nutrient analysis; (3) chambers were incubated for 2 h; (4) a final sample was taken for solute analysis and side-ports were opened to allow water exchange for the next incubation cycle. This cycle was repeated 2–6 times per day of investigation, excluding the period of emersion or those times when water level was less than 10 cm from the sediment surface. In addition to the chamber operation, light and dark acrylic 3-l bottles were placed on the sediment near the chambers. To correct for the effect of biotic activities in the water column in the nutrient flux determination, the bottle water was exchanged before incubations and the samples were collected at the beginning and end of each 2-h incubation concurrently with samplings of the chamber.

The 30-min period of open side-ports ensured the complete exchange of chamber water, which was tested by making sure the higher salinity in the chambers was reduced to ambient. Chambers were manually operated, and we walked slowly and carefully to the chambers so as not to stir up the sediment. On each day of the investigation, the chambers and bottles were set at the site at the start of the day and taken away after the last flux measurement of the day. The points where chambers and bottles were set were marked with stakes and excluded from later measurements to avoid any potential influence of sediment disturbance on the flux measurement. The chambers and bottles were always set within an area of about 50 m² in which the sediment properties were thought to be approximately homogeneous. To minimize disturbing sediments of the study sites and ensure the 2-h cycle operation of chambers and bottles, spatial replicates were not set in this study.

Once the water samples were collected from the chambers and bottles, the temperature, pH and salinity were measured in the field with portable meters (TOA, HM-12P; SINAR MEDICAL, NS-3P). Salinity was measured using the Practical Salinity Scale. Part of the water sample was filtered through a 0.45- μ m membrane filter and concentrations of ammonium (NH₄⁺), nitrate (NO₃⁻) and phosphate (PO₄³⁻) were determined by auto-analyzers (BRAN+LUEBBE, TRAACS800) in the laboratory. The remaining part of the water sample was immediately treated with manganous sulfate and alkali azide reagents in a 300-ml bottle in the field and the concentration of dissolved oxygen was determined by titration (modified Winkler technique). Water depth and illumination (Apogee Instruments, QMSS-SUN) were measured concurrently with the chamber and bottle operation.

The fluxes of NH₄⁺, NO₃⁻, PO₄³⁻ and O₂ across the sediment–water interface were calculated for every cycle using the following formula:

$$F = \frac{[(C_E - C_S) - (C'_E - C'_S)]V}{TA}$$

where F is the solute flux across the sediment–water interface ($\mu\text{mol m}^{-2} \text{h}^{-1}$), C_S and C_E are the solute concentrations in the

chamber at start and end, respectively ($\mu\text{mol l}^{-1}$), C'_S and C'_E are the solute concentrations in the bottle at start and end, respectively ($\mu\text{mol l}^{-1}$), V is the water volume in the chamber (8.5 l), T is the incubation time (2 h), and A is the sediment surface area in the chamber (0.085 m²). Positive and negative fluxes signify release from sediment and uptake by sediment, respectively.

2.3. Pore water, sediment and macrobenthos sampling

In order to broadly inspect biological metabolism and diffusive transport of nutrients in sediment, we sampled pore water. Sediment pore water was collected with porous ceramic tips (5 cm length and 1 cm diameter) (SANKEI Co.) which were inserted at 5, 10 and 20 cm sediment depths near the chambers. Once in each cycle of the chamber and bottle operation, 20 ml pore water samples were collected with vacuum glass bottles from a tube connected to the porous ceramic tips on August 17–18 and 26 at the sand and the mud flats, respectively. The pore water samples were rapidly filtered and analyzed for NH₄⁺, NO₃⁻ and PO₄³⁻ using the abovementioned methods.

Four sediment cores (7 cm diameter) were sampled in each of the sand and mud flats. The three cores were sliced at 0–2, 4–6, and 14–16 cm depths, respectively. The sediment organic content was determined by weight loss of the dried sediment sample following 2 h of ignition at 600 °C. The grain size distribution was determined by sifting the remaining core sample.

To collect macrobenthos samples, sediment to the depth of 30 cm was collected from the chambers by shovel and sifted by 1 mm sieve after the flux measurements were taken. Both the number of individuals and dry weights of benthos on the sieve were measured. The dry weight of bivalves was measured after the shells were removed.

2.4. Regression analysis

Stepwise multiple regression analysis was conducted to infer significant factors which affect nutrient fluxes on the short-time scale of less than a day. Explanatory variables for each nutrient flux were water temperature, illumination, salinity, and oxygen and each nutrient concentration in the overlying water. For each tidal flat, the flux measurement results of light and dark chambers and from all the sampling days are included within the same statistical analysis. The illumination at the dark chamber was regarded as zero for all the flux measurement cycles. SAS version 8.02 was used for this statistical analysis. Statistical significance of regression analysis was judged by a criterion of $p < 0.05$.

3. Results

3.1. Properties of the study site

Table 1 shows sediment characteristics and the density of individuals and dry weight of macrobenthos in the study sites.

Table 1

Sediment organic content (%), mean sediment grain size (μm), silt-clay ($<74\ \mu\text{m}$) content (%), and dry weight (g m^{-2}) and individuals (N m^{-2}) of macrobenthos in the study sites (mean \pm S.E., sand flat: $n = 4$, mud flat: $n = 6$). Weight of Mollusca is without shell. The densities of individuals are in parentheses

	Sand flat	Mud flat
Organic content	1.3 ± 0.1	4.8 ± 0.9
Mean grain size	339 ± 13	214 ± 40
Silt-clay content	0.19 ± 0.04	28.8 ± 4.1
Macrobenthos		
Mollusca	75.4 ± 16.6 (389 \pm 80)	0.1 ± 0.04 (2 \pm 2)
Polychaeta	2.9 ± 0.3 (135 \pm 78)	20.1 ± 3.1 (1666 \pm 170)
Crustacea	0.0 ± 0.0 (0.0 \pm 0.0)	6.9 ± 4.5 (59 \pm 25)

Biota differed between the tidal flats, with the predominant macrobenthos being bivalves (*Nuttallia olivacea*) in the sand flat and polychaetes (*Neanthes japonica*) in the mud flat.

As for nutrient concentration in the interstitial water, PO_4^{3-} and NH_4^+ concentrations in the sand flat and NH_4^+ concentration in the mud flat were variable following the change in the concentration of overlying water (Fig. 2). PO_4^{3-} and NO_3^- concentrations in interstitial water were generally lower than overlying water in both the flats. NH_4^+ concentration in interstitial water was slightly lower in the 0–5 cm section than in the deeper section. NH_4^+ concentration in the deeper section was higher than that in the overlying water, particularly in the mud flat.

3.2. Tidal variation in nutrient concentration in the overlying water

Overlying water quality varied noticeably within a day and between days (Fig. 3). The range of nutrient concentration in overlying water throughout this study was about one order of magnitude greater than nutrient concentration variations

observed in the chambers during 2-h incubations. Although relationship between PO_4^{3-} concentration and salinity was unclear, low PO_4^{3-} concentration ($<1.2\ \mu\text{mol l}^{-1}$) was not observed under low salinity (Fig. 4a). Markedly higher NH_4^+ concentrations ($>25\ \mu\text{mol l}^{-1}$) and wide range of the concentration were observed at high salinity levels (>25), while the concentrations were within narrow range at low salinity (<10) (Fig. 4b). NO_3^- concentration showed a negative linear relationship with salinity ($r^2 = 0.83$, $p < 0.0001$) (Fig. 4c).

Based on a single preliminary analysis of the sewage-treated water from the sewage plant near the site (Fig. 1), PO_4^{3-} , NH_4^+ and NO_3^- concentrations in the treated water were 4.6, 1406 and $1.4\ \mu\text{mol l}^{-1}$, respectively (unpublished data), while nutrient concentration in offshore seawater around the study area was generally very low (water quality investigation by Miyagi prefecture). The lowest PO_4^{3-} and NH_4^+ concentrations observed at high salinity (ca. 28) were much lower than concentrations in the sewage-treated water. Hence, the PO_4^{3-} and NH_4^+ increases observed at high salinity in the study site were probably due to inflows of seawater contaminated with sewage-treated water, which often occurs at flood tide probably in response to the capricious wind and wave direction. NO_3^- concentration was not likely to be influenced by the sewage-treated water inflow, because NO_3^- concentrations in seawater and sewage-treated water were not significantly different. Meanwhile, it was obvious that PO_4^{3-} , NH_4^+ and NO_3^- concentrations of river water were higher than those in pure seawater.

3.3. Nutrient flux across the sediment–water interface

The nutrient flux at the sediment–overlying water interface varied widely from release to uptake within a day and among days in both the sand and mud flats (Fig. 3). The result of stepwise multiple regression analysis suggested that either nutrient concentration or salinity was the possible factor dominating

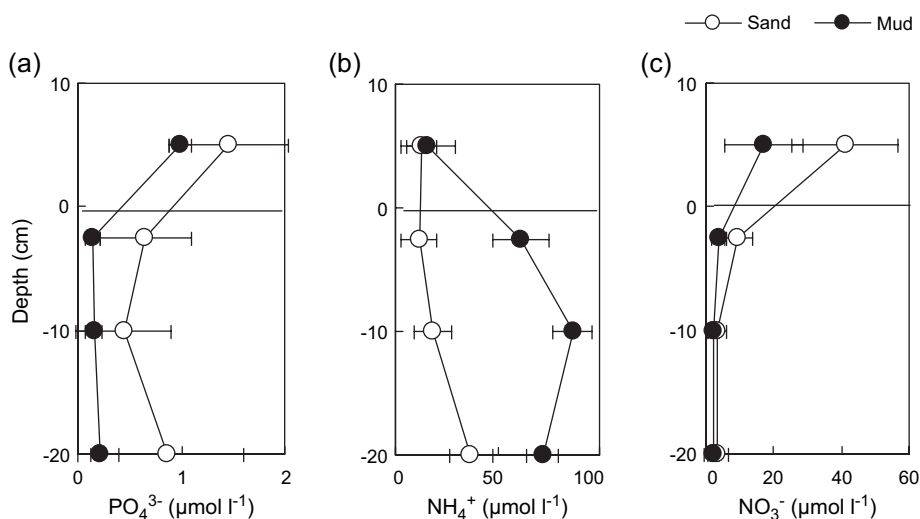


Fig. 2. Vertical profiles of (a) phosphate, (b) ammonium and (c) nitrate concentrations of interstitial water in the tidal flats sediment. These profiles were measured at eight samplings in August 16–17 in the sand flat and at four samplings in August 26 in the mud flat. Plot and error bar indicate mean value and S.D. of those repeated measurements.

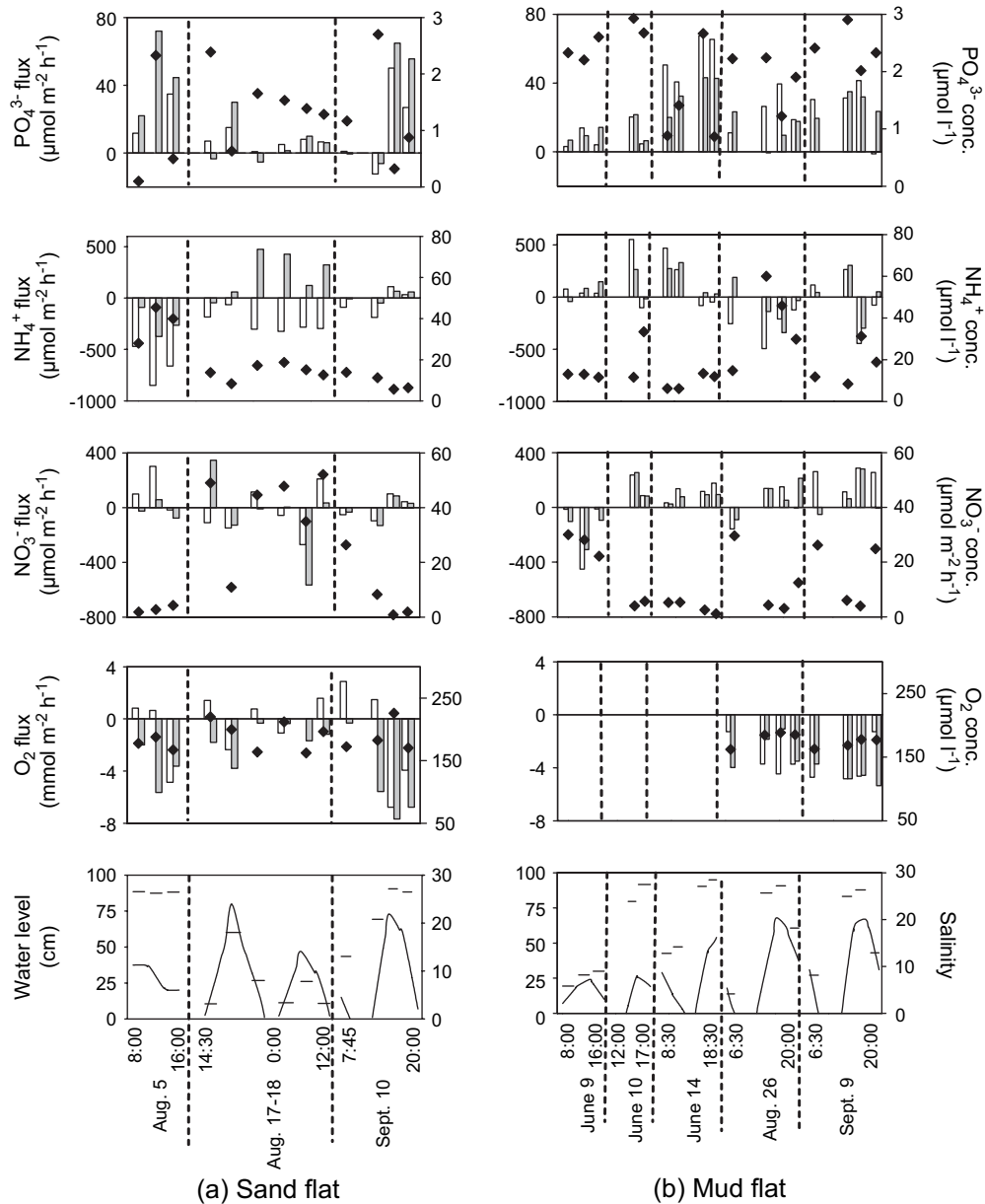


Fig. 3. Variation in overlying water concentration and flux at sediment–water interface of dissolved oxygen, phosphate, ammonium and nitrate. White and grey bars show fluxes in light and dark chambers, respectively, and black diamond plots indicate the initial concentration of nutrient and oxygen in the chamber operation cycles. Temporal variation in salinity (short thick bar) and water level (curved line) are shown in the lowest graphs. Dissolved oxygen concentration and flux were not measured in the mud flat during the cycles in June.

each material flux in the sand flat except NO_3^- flux (Table 2). Even in cases where two parameters were selected as explanatory factors for flux variation, such as NH_4^+ and O_2 fluxes, nutrient concentration or salinity had relatively high partial correlation coefficients and low p values. In the mud flat, NH_4^+ concentration was likely to dominate NH_4^+ flux, while no significant factor was detected for the other materials' flux variation.

3.4. PO_4^{3-} flux variation

The short-term fluxes of O_2 and PO_4^{3-} had a greater range in the sand flat than in the mud flat (Fig. 5). PO_4^{3-} flux was

negatively and linearly related to O_2 flux in both tidal flats, while PO_4^{3-} flux was related with PO_4^{3-} concentration in the overlying water in the sand flat as well (Table 2). The slopes of the relationships were almost equivalent in the sand and mud flats and the molar ratio of O_2 consumption to PO_4^{3-} release from sediment was 112 to 1 ($r^2 = 0.81$, $p < 0.0001$), calculated from all plots combined for light level and sediment type.

The relationships between salinity and O_2 flux in both tidal flats are shown in Fig. 6, in which illumination levels were classified into light ($<900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ or $>900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ only for the sand flat) and dark ($0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The inflow of sewage-treated water was judged by salinity of >25 and NH_4^+ concentration of

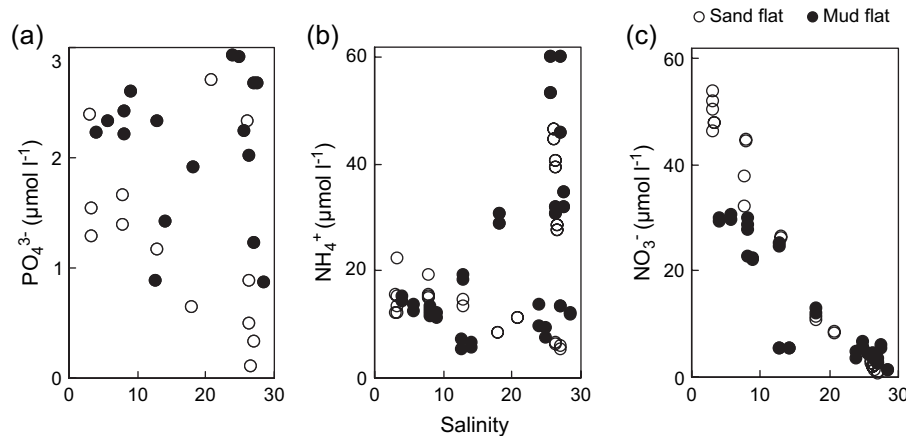


Fig. 4. Relationships of (a) phosphate, (b) ammonium and (c) nitrate concentration with salinity in overlying water.

$>25 \mu\text{mol l}^{-1}$, based on the fact that NH_4^+ concentration was ordinarily much lower (about $<15 \mu\text{mol l}^{-1}$) in seawater in this area. In the sand flat, the relationships between salinity and O_2 flux depended strongly on illumination: when dark and $<900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, they had negative linear relationships ($r^2 = 0.75$, $p < 0.0001$) except for when there was a small O_2 consumption accompanying the inflow of sewage-treated water; and, when $>900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, O_2 flux was usually positive, not responding to either salinity or the inflow of sewage-treated water. In the mud flat, O_2 flux usually had a negative value and did not respond to illumination, salinity, and sewage-treated water. Meanwhile, we found chlorophyll *a* density on the sediment surface of the mud flat was almost equivalent to that of the sand flat in our supplementary investigation (Ogawa and Sakamaki, unpublished data).

3.5. NH_4^+ flux variation

In the sand flat, NH_4^+ uptakes by sediment were greater under light than under dark conditions (Fig. 7). The NH_4^+ flux

was related linearly and negatively with NH_4^+ concentration in the overlying water both under light and dark conditions (light: $r^2 = 0.92$, $p < 0.0001$; dark: $r^2 = 0.38$, $p = 0.0254$), indicating that the flux was governed by the concentration. In the mud flat, the light condition did not significantly affect NH_4^+ flux and range. The flux had a negative linear relationship with the concentrations (light: $r^2 = 0.58$, $p < 0.0001$; dark: $r^2 = 0.57$, $p < 0.0001$), as observed in the sand flat.

3.6. NO_3^- flux variation

In the sand flat, NO_3^- flux was weakly related to water temperature (Table 2). As for the relationship to NO_3^- concentration of the overlying water, NO_3^- flux was within a relatively narrow range when $<15 \mu\text{mol l}^{-1}$, while NO_3^- flux ranged widely from uptake to release when $>15 \mu\text{mol l}^{-1}$, in both the flats (Fig. 8). In the sand flat, when $<15 \mu\text{mol l}^{-1}$, NO_3^- flux was negatively related with NO_3^- concentration ($r^2 = 0.55$, $p = 0.0038$). In the mud flat, NO_3^- flux was negatively related with NO_3^- concentration (light: $r^2 = 0.28$, $p = 0.0287$;

Table 2

Results of stepwise multiple regression analysis for each inorganic nutrient flux with environmental variables which are subject to change in a day (nutrient concentration and salinity in the overlying water, water temperature and irradiance). The case of no significant factor detected is shown as nd

Flux	Predictor	Squared partial correlation coefficient	Pr > t
Sandy			
PO_4^{3-}	PO_4^{3-} concentration	0.23	0.0191
NH_4^+	Irradiance	0.29	0.0036
	NH_4^+ concentration	0.47	0.0003
NO_3^-	Water temperature	0.32	0.0036
O_2	Irradiance	0.31	0.0025
	Salinity	0.44	0.0006
Muddy			
PO_4^{3-}	nd		
NH_4^+	Water temperature	0.004	0.0490
	NH_4^+ concentration	0.70	0.0003
NO_3^-	nd		
O_2	nd		

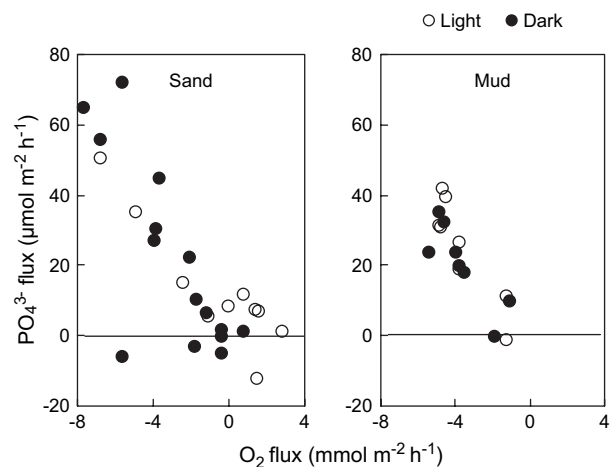


Fig. 5. Relationship between phosphate exchange flux and dissolved oxygen exchange flux in the tidal flats.

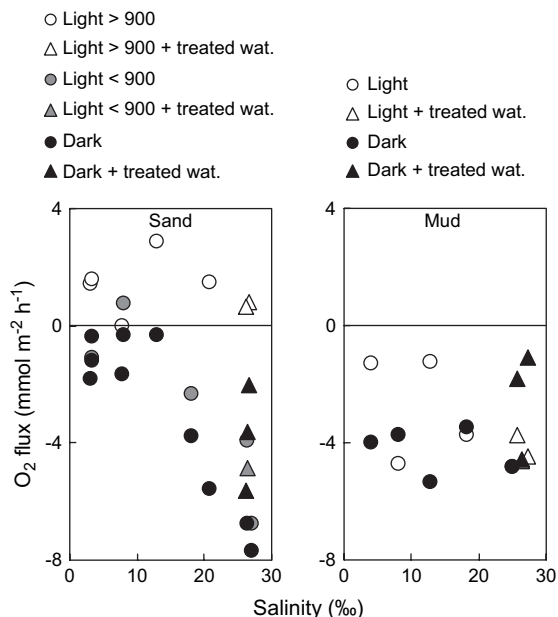


Fig. 6. Relationship between salinity and dissolved oxygen exchange flux in the tidal flats. In these graphs, illumination levels were classified into light (<900 μmol photons m⁻² s⁻¹ or >900 μmol photons m⁻² s⁻¹ only for the sand flat) and dark (0 μ photons m⁻² s⁻¹). Inflows of sewage-treated water were expected when both ammonium >25 μmol l⁻¹ and salinity >25 were observed.

dark: $r^2 = 0.57, p = 0.0005$). NO₃⁻ flux did not differ substantially under light and dark conditions in either tidal flat.

4. Discussion

4.1. Mechanism of tidal variation in PO₄³⁻ flux

The theoretical biological oxidation of organic matter proceeds according to the following stoichiometric equation (Richards et al., 1965): $(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 106O_2 = 106CO_2 + 16NH_3 + H_3PO_4 + 106H_2O$, which means that the

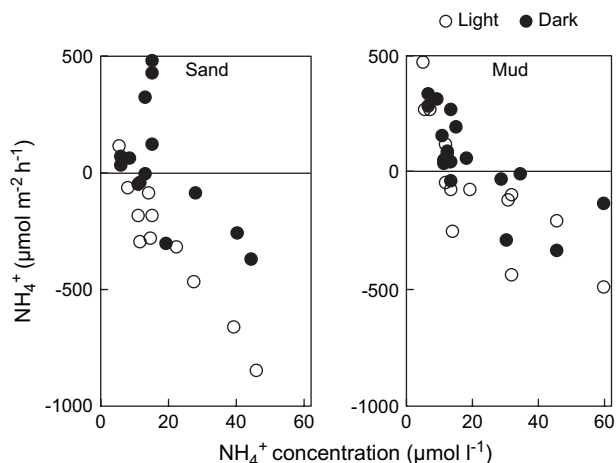


Fig. 7. Relationship between ammonium concentrations in overlying water at the start of chamber incubation and ammonium exchange flux.

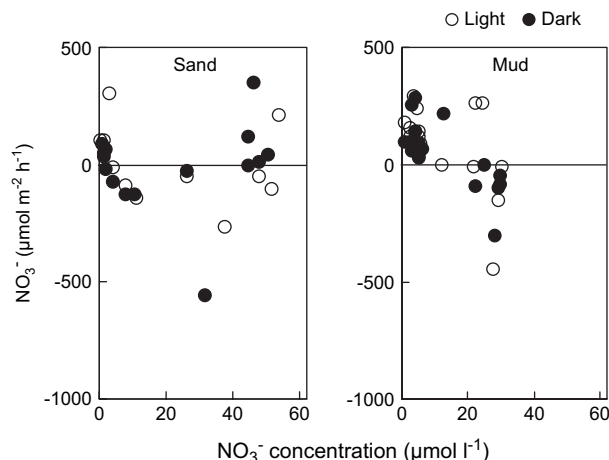


Fig. 8. Relationship between nitrate concentrations in overlying water at the start of chamber incubation and nitrate exchange flux.

molar ratio of consumed oxygen to mineralized phosphorus is 106 to 1 under complete oxidation. The ratio calculated in the present study, 112:1, corresponded approximately to the theoretical value. This correspondence suggests that the PO₄³⁻ release rate was largely governed by aerobic phosphorus mineralization in sediment and was practically equivalent to the mineralization rate. The linear relationships between O₂ consumption and PO₄³⁻ release were similar to those observed in several previous studies (Seiki et al., 1989; Reay et al., 1995) and a ratio of 156:1 was obtained from muddy sediment (Reay et al., 1995).

The combination of PO₄³⁻ release and mineralization such as in this study, however, is unlikely to be observed everywhere and factors governing PO₄³⁻ flux are changeable, with many other sites not producing the linear relationship between O₂ and PO₄³⁻ fluxes (Hopkinson, 1987; Reay et al., 1995; Rysgaard et al., 1995; Kohata, 2000). Magalhães et al. (2002) found significant relationship of PO₄³⁻ flux to PO₄³⁻ concentration in the overlying water in their estuarine study site where PO₄³⁻ concentration in the overlying and interstitial waters was much higher (<30 μmol l⁻¹) than that of our site. Although PO₄³⁻ flux was associated with PO₄³⁻ concentration in the overlying water in the sandy flat studied, it was less significant than the relationship with O₂ consumption. Relatively low PO₄³⁻ concentrations in interstitial water and overlying water may have caused the distinct and sensitive effect of biological mineralization in our study site.

The negative linear relationship of O₂ consumption with salinity in the sand flat under dark conditions and <900 μmol photons m⁻² s⁻¹ (Fig. 6a) is probably attributable to variation in respiration of the bivalve, *Nuttallia olivacea* which occurred at high density there (Table 1). Sano (1999) estimated respiration rate of this bivalve in the laboratory as 17.2 μmol h⁻¹ g DW⁻¹ at a salinity of 28 and found that their filtration rate declined with salinity decrease in the range of less than 20. This physiological characteristic of the bivalve suggests its possible contribution to the temporal variation in O₂ flux observed in the sand flat. Inhibition of respiration under low salinity has been reported for various marine and

estuarine invertebrates (Shumway and Koehn, 1982; Williams, 1984; Stanzel and Finelli, 2004), supporting the physiological mechanism of the decrease in O_2 consumption at low salinity in the sand flat.

Even at high salinity, O_2 consumption declined occasionally when inflow of sewage-treated water was detected, suggesting possible inhibition of benthos respiration by water chemistry other than salinity. Acute and chronic toxicity of contaminants in sewage-treated water such as ammonia and chlorine have been reported for various aquatic animals (e.g. Epifanio and Srna, 1975; Brungs, 1976; Ward and DeGraeve, 1978, 1980; Colt and Armstrong, 1981; Ruyet et al., 1995). Although we cannot confirm that such contaminants rapidly lower benthos respiration, the coincidence of the decline in oxygen consumption with the inflow of the treated water suggests the treated water reduced oxygen consumption.

On the other hand, when illumination was $>900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the positive and steady flux of O_2 in the sand flat (Fig. 6a) was probably caused by the increase of O_2 concentration at the sediment interface due to active benthic algal photosynthesis. This boost of O_2 flux by algae likely overcame the effects of salinity variation and the inflow of sewage-treated water on O_2 flux. In this case PO_4^{3-} release was relatively small, which might be attributed to the algal uptake or to the absorption of PO_4^{3-} (Sundbäck and Graneli, 1988). Therefore, in the sand flat the tidal variation in PO_4^{3-} flux during low algal production is thought to be mainly caused by the benthos reaction to tidal salinity changes and occasional intrusions of sewage-treated water.

In sediments with high organic content, bacteria consume O_2 and restrict aerobic respiration (Rasmussen and Jørgensen, 1992; Lee et al., 1997). Under such conditions, the oxygen supplied by benthic microalgae is rapidly used up at the sediment surface, which maintains a deficit of O_2 at the sediment surface and eliminates the O_2 release from sediment (Epping and Jørgensen, 1996). O_2 concentration in the overlying water was stable near saturation level in our study sites, so that the differences in O_2 concentrations in the overlying water and in the sediment were thought to be steady. This may have led to the comparatively consistent O_2 flux, the net aerobic respiration and the net PO_4^{3-} regeneration in the mud flat.

4.2. Mechanism of tidal variation in NH_4^+ flux

It has been reported that the NH_4^+ flux is governed by biological processes such as mineralization and nutrient uptake by microphytobenthos, as suggested by strong proportional relationships between the O_2 , PO_4^{3-} and NH_4^+ fluxes (Hopkinson, 1987; Reay et al., 1995; Sundbäck et al., 2000). In the sand flat in our study, the fact that there were differences in NH_4^+ flux for the light and dark chambers suggested that NH_4^+ uptakes by benthic microalgae significantly affected the NH_4^+ flux (Fig. 7) (e.g. Reay et al., 1995; Nishimura et al., 1996; Kuwae et al., 1998). In both the tidal flats in this study, however, the NH_4^+ flux was linearly and significantly related with NH_4^+ concentration in the overlying water (Fig. 7), while NH_4^+ flux was unrelated to O_2 and PO_4^{3-} fluxes.

This suggests that the wide range of variation in NH_4^+ concentration in the overlying water predominantly governs the NH_4^+ flux at the sediment–water interface rather than biological processes at our sites.

In the mud flat, NH_4^+ of deeper section probably diffused constantly to the surface layer in which NH_4^+ concentration was much lower (Fig. 2b). This potentially operates to negate the NH_4^+ concentration decrease at the sediment subsurface by biological effects and diffusive release, which may be a reason for the minor difference of the NH_4^+ fluxes in the light and dark chambers in the mud flat. NH_4^+ diffusion from high concentration sources in deep sediment sections likely negates biological influence as well as the wide NH_4^+ concentration variation in overlying water.

In both the studied tidal flats, the relationships between salinity, NH_4^+ concentration and NH_4^+ flux indicated that three patterns exist in the tidal variations in NH_4^+ flux in response to the NH_4^+ concentrations in overlying water: (1) the suppressed uptake by sediment or activated release from sediment under low NH_4^+ concentrations during the inflow of pure seawater at the flood tide; (2) the activated uptake by sediment or suppressed release from sediment under high NH_4^+ concentrations during inflow of river water at the ebb tide; and (3) the highly activated uptake by sediment under markedly high NH_4^+ concentrations during the inflow of seawater contaminated with sewage-treated water at the flood tide.

4.3. Mechanism of tidal variation in NO_3^- flux

In the case where NO_3^- concentration of interstitial water was kept low by inactivated nitrification and activated denitrification, NO_3^- concentration in the overlying water governs denitrification and NO_3^- flux (Rysgaard et al., 1994, 1995). Some previous studies showed a clear negative relationship between NO_3^- flux and NO_3^- concentration in overlying water (Asmus, 1986; Ogilvie et al., 1997; Cabrita and Brotas, 2000; Magalhães et al., 2002). Similarly, in our study site, the negative relationship of NO_3^- flux with NO_3^- concentration and predominant NO_3^- release under relatively low NO_3^- concentration of the overlying water suggested that diffusive flux at the sediment interface depended on the concentration in overlying water. In addition, low NO_3^- concentration of interstitial water (Fig. 2c) is probably attributed to denitrification and supports the linkage of NO_3^- flux and denitrification in our study site.

Under relatively high NO_3^- concentration, however, the negative relationship was unclear and flux widely ranged from uptake to release, particularly in the sand flat studied. The abovementioned linear relationships between NO_3^- concentration, salinity and O_2 flux (Figs. 4c and 6a) suggest that O_2 consumption is relatively small under the high NO_3^- concentration in the sand flat. Aerobic conditions in sediment are advantageous for nitrification and disadvantageous for the denitrification of NO_3^- transported from the overlying water (Risgaard-Petersen et al., 1994; Rysgaard et al., 1994, 1995). In this case, nitrification potentially increases NO_3^- concentration at the sediment subsurface and affects denitrification and diffusive NO_3^- transport at sediment–water

interface. In addition, nitrification is also significantly affected by NH_4^+ concentration in overlying water and benthic microalgal nitrogen uptake and O_2 production (Risgaard-Petersen et al., 1994; Rysgaard et al., 1995; An and Joye, 2001). Those processes may have complicated processes governing the NO_3^- flux fluctuation at the sediment–water interface and weakened the linkage of NO_3^- flux with NO_3^- concentration under relatively high NO_3^- concentration. Although those processes are potentially affected by tidal variation in NH_4^+ and NO_3^- concentrations in overlying water and benthic O_2 consumption in our study site, they defy simple explanation.

4.4. Range of tidal flux variation

A few previous studies in which nutrient concentration in the overlying water varied much more widely showed a much larger range of flux variation than our results (Table 3). For instance, in muddy sites of Cabrita and Brotas (2000), the range of NO_3^- concentration in the overlying water was about $700 \mu\text{mol l}^{-1}$, resulting in a markedly large range of NO_3^- flux variation and a distinct linkage of the flux with the concentration. Also in our study, NH_4^+ flux ranged more widely than in other studies and was significantly related to the NH_4^+ concentration, which was attributed to wide variation in NH_4^+ concentration caused by sewage-treated water inflow. These results suggest that at the site that receives high concentration nutrients, the overlying water concentration and nutrient flux are subject to a wide range and are linearly related. In contrast, in cases where nutrient concentration in the overlying water is low and temporally stable, other factors such as

various biological processes may govern the flux within a relatively narrow flux range (Table 3). For instance, in our study in which NO_3^- concentration ranged no more than $60 \mu\text{mol l}^{-1}$, NO_3^- flux variation was much smaller than in the Cabrita and Brotas's (2000) study and there was no clearly associated factor.

Although we did not take spatial replicate measurements to detect variation from spatial patchiness of sediment or benthos, our regression analyses of the temporal data clearly showed that the overlying water chemistry significantly contributed to the flux variation particularly for PO_4^{3-} and NH_4^+ . This was probably because tidal variation in overlying water chemistry resulted in wide range of the flux despite spatial patchiness in flux caused by other factors, such as benthos density and interstitial water profile. These suggest that the relative importance in factors controlling the flux should be carefully considered to properly design field flux measurements; the relative importance of spatial or temporal replicates depends on how widely overlying water chemistry temporally varies.

Nutrient and O_2 flux rapidly and dramatically responded to tidal variation in overlying water chemistry in our study sites. The ranges of the tidal time-scale variation in nutrient flux in this study were in general no less than the seasonal-scale temporal and the meter-to-kilometer-scale spatial variations which were defined as the effects of variation in benthos density, temperature and sediment condition (i.e. organic matter content and interstitial water concentration profile) in previous studies (Table 3). Our results indicate that unless tidal variations in environmental conditions are taken into consideration

Table 3
Ranges (Min./Max.) of nutrient flux and significant factors for its variation reported in previous studies

	Flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)				Spatial or temporal variation/significant factors causing flux variation
	O_2	PO_4^{3-}	NH_4^+	NO_3^-	
Forja and Gómez-Parra, 1998		104/263	629/833		Spatial/macrobenthos
Callender and Hammond, 1982	–3500/–163	–25/167	–200/1083	–300/221	Spatial/macrobenthos
Clavero et al., 1991		9/20			Seasonal/macrobenthos
Reay et al., 1995					
Sand	–2161/188	–2/10	–13/103	–23/12	Seasonal/conc.
Mud	–4657/507	0/21	–4/377	–20/5	Seasonal/mineralization
Jensen et al., 1990			7/63	–33/15	Seasonal/sediment condition
Rysgaard et al., 1995 ^a	–6000/1000	–25/30	–10/750	–140/30	Seasonal/macrobenthos, temp.
Ogilvie et al., 1997 ^{a,c}					
Mud			–800/1800	–3000/1000	Seasonal/conc.
Cabrita and Brotas, 2000 ^{a,c}			–116/150	–14,900/5300	Seasonal/conc.
Asmus, 1986 ^a			–290/371	–354/173	Seasonal/conc.
Magalhães et al., 2002	–2000/19,313	–152/0	–978/106	–810/–40	Seasonal/conc.
Kristensen, 1993	–3542/3750		–13/50	–25/17	Seasonal/conc., photic condition
Sundbäck et al., 2000 ^a					
Sand			–17/45	–60/10 ^b	Seasonal temp., photic condition
Mud			–20/70	–40/50 ^b	Seasonal/sediment condition
Hopkinson et al., 2001	–1254/–258	–1/21	–1/168	–8/28	Seasonal/temp., sediment condition
Sakamaki (this study)					
Sand	–7678/2870	–75/72	–850/475	–565/346	Tidal/conc. (NH_4^+ , salinity (PO_4^{3-}))
Mud	–5345/–1066	–22/67	–493/552	–452/287	Tidal/conc. (NH_4^+ , NO_3^-)

^a Data derived from figures.

^b $\text{NO}_3^- + \text{NO}_2^-$.

^c Data arranged by Magalhães et al. (2002).

in sites where water chemistry is significantly affected by the tide, typical flux may not be correctly determined. In particular at such sites, it would be necessary to conduct repeated flux measurements on a shorter time scale. In addition, in any case of nutrient flux measurement, temporal variations in environmental factors should be more clearly demonstrated in reports and its effects on flux should be better described to determine accurate fluxes and elucidate the significant factors causing flux variation.

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