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Oceanic nutrient supply and uptake by microphytobenthos of the Hichirippu Lagoon, Hokkaido, Japan

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ABSTRACT: We conducted field surveys to determine spatio-temporal variations in the water quality (including inorganic nutrient concentration) of a coastal lagoon, Hichirippu Lagoon (3.56 km^2), in Hokkaido, Japan. During a typical supply period (winter), a 24 h continuous survey was conducted to evaluate nutrient supply from the open ocean and examine nutrient utilization by microphytobenthos (MPB) based on the mass balance of a biophilic element. During February 2008, the majority of NO_3+NO_2-N was supplied from the open ocean, and the dissolved inorganic nitrogen (DIN) budget was 3.25 kmol N d^{-1}. In March 2008, the total amount of organic nitrogen was 39.0 to 588 kmol N for MPB in the surface sediment (depth, 0.5 cm). Assuming that MPB in the surface sediment used all NO_3+NO_2-N input (3.93 kmol N d^{-1}), their temporal division rate (NO_3+NO_2-N uptake/total amount of MPB) would be 0.01 to 0.10 d^{-1}. Here the annual DIN budget was estimated to be 258 kmol N yr^{-1}, which mainly depended on MPB-fixed oceanic DIN during winter.

KEY WORDS: Microphytobenthos · Coastal ecosystem · Nutrient cycles · Dissolved inorganic nutrients · Mass balance

INTRODUCTION

Coastal lagoons occupy 13% of the world's coastline and are among the most common coastal environments (Kjerfve 1986). Their productivity makes them major fishing areas. A coastal lagoon system is mainly considered a river-dominated system that is easily affected by anthropogenic eutrophication (Knoppers et al. 1991, Nedwell et al. 1999, Kemp et al. 2005). The coastal lagoon system of Ohuira Lagoon, northwestern Mexico, changed repeatedly between estuarine–marine and estuarine–terrestrial environments because of human activity (Ruiz-Fernández et al. 2007), and that of Nakaumi Lagoon, southwestern Japan, was eutrophicated by several factors, including increased nutrient loading, herbicide usage, and dike building (Katsuki et al. 2008). Freshwater systems are considered the main nutrient source for coastal lagoons (Flores-Verdugo et al. 1988, Forès et al. 1994, Boynton et al. 1996, Paerl 1997); however, oceanic nutrients (especially dissolved inorganic forms) also play an important role in

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determining the nature of the lagoon system. To date, relatively few studies have quantified oceanic nutrient sources to lagoons. In those studies, the impact of the oceanic source was masked by anthropogenic impact, except for one study of an undisturbed environment (Suga et al. 2011). By performing network analysis of nutrient cycling data for Tancada Lagoon, northeast Spain, Forès et al. (1994) suggested the relative importance of the increase in oceanic dissolved inorganic nitrogen (DIN) as a nitrogen source during seawater-dominated periods; however, they found that primary producers mainly depended on internal nitrogen recycling of terrestrial particulate matter from a rice field. Souza et al. (2003) described the heavily eutrophicated coastal lagoon system along the Brazilian coast acts as an effective sink for marine and terrestrial phosphorus and as a DIN source for coastal waters. Conversely, the mass balance study of Suga et al. (2011) suggested that nitrate supplied from the open ocean (Pacific Ocean) through the tidal inlet of Hichirippu Lagoon, northern Japan, was necessary to maintain the rich natural environment of the lagoon ecosystem during winter.

Recently, microphytobenthos (MPB) have been identified, along with phytoplankton, as important primary producers in coastal lagoons because of solar radiation reaching the sea floor (Anderson et al. 2003, Tyler et al. 2003), suspension of this microalgal community in the water column (de Jonge & Beusekom 1992, de Jonge 1995), primary production that is sometimes higher than the local phytoplankton community (Montani et al. 2003, Ichimi et al. 2008), and adaptation to various nutrient concentrations from low (water column) to high (sediment pore water) conditions (Leynaert et al. 2009). The idea that the MPB community can function as a filter controlling dissolved nutrient flux at the sediment/water interface was presented 30 yr ago (Henriksen et al. 1980, Sundbäck et al. 1991, Cahoon & Cooke 1992, Cahoon 1999, Sundbäck et al. 2000, Sandwell et al. 2009, Komorita et al. 2010). Moreover, MPB activity may remove additional nutrients from the overlying water (Nilsson et al. 1991, Sundbäck et al. 2004, Sakamaki et al. 2006, Engelsen et al. 2008). Nedwell et al. (1999) hypothesized that benthic primary production may account for a higher proportion of nutrient uptake in oligotrophic estuaries with lower nutrient loads; however, this remains to be elucidated (Forster & Kromkamp 2006).

Our study was conducted as part of a long-term project that aimed to quantify ecosystem dynamics from lower to higher trophic levels (including migratory birds) of a coastal lagoon (Hichirippu Lagoon). This lagoon has a rich natural environment and is designated as a wetland under the Ramsar Convention, Hokkaido, Japan. In this lagoon, MPB biomass (depth, 0.5 cm) was approx. 80 times higher than phytoplankton biomass in the water column (depth, 1 m) (Kajihara et al. 2010). In the tidal flats of this lagoon, excretions of the short-necked clam *Tapes* (*Ruditapes*) *philippinarum* may sustain primary production by MPB during summer, and nutrient supply may not be solely based on riverine inputs (Komorita et al. 2010). Nutrients supplied from outer regions of the lagoon are required to sustain production in the lagoon. Suga et al. (2011) suggested that nitrate imports from the Pacific Ocean through the tidal inlet are necessary to maintain the lagoon ecosystem during winter; however, there is no evidence of nutrient flow or a possible main sink in this lagoon. We assumed that dense MPB patches would take up these nutrients during winter and thereby sustain the lower trophic levels of the ecosystem.

We aimed to evaluate the pre-disturbance condition of the coastal lagoon ecosystem on water quality and to quantify nutrient flux and MPB. Semi-enclosed coastal lagoons allow ecological functions to be precisely evaluated by comparing these functions with the total nutrient input and nutrient dispersal by tidal exchange with the outer ocean (Hung & Kuo 2002, Kohata et al. 2003, Komorita et al. 2010). Hichirippu Lagoon is connected with the Pacific Ocean by a narrow channel. We were able to measure the tidal exchange of water and nutrients by observing the flow rate and nutrient concentration in the channel. We conducted several field surveys to investigate spatio-temporal variations in the water quality of the lagoon, including inorganic nutrient concentrations. Because nitrogen (the deficient element) supplied through the tidal inlet was mainly NO_3+NO_2-N (Suga et al. 2011), we divided the seasonal variation period into supply and mineralization periods based on the NO_3+NO_2-N to DIN concentration ratio at the inlet. During the typical supply period (winter), a 24 h continuous survey was conducted to quantify nutrient flux from the open ocean and examine the possibility that MPB utilized the nutrients based on the standing stock. The annual nutrient budget was estimated based on the summer budget, determined in a recent study (Komorita et al. 2010). In the present study, we discuss the dynamics of nutrients and MPB (i.e. primary producers) in Hichirippu Lagoon, the importance of oceanic nutrient sources relative to other nutrient sources, and nutrient uptake by MPB in this lagoon.

MATERIALS AND METHODS

Study area

Hichirippu Lagoon is shallow (mean water depth, ca. 1 m) with brackish water and has an area of approx. 3.56 km^2. It borders the Pacific Ocean along the eastern shore of Hokkaido Island, Japan (43° 05′ N, 145° 02′ E; Fig. 1). Several small creeks empty into the north end of the lagoon, although they have a relatively small effect on water budget, ranging 0.9 to 8.0 % of the total volume (Suga et al. 2011). During spring tide, the maximum tidal flow (predominant direction, northwest to southeast) reaches approx. 40 cm s^{-1} at the tidal inlet (Komorita et al. 2010) and ensures strong vertical mixing of the lagoon water (Suga et al. 2011). During extreme low tide, the exposed tidal flat area is approx. 0.19 km^2 or 5.3 % the lagoon area. In 1998 to 2004, 30 to 92 metric tons of *Tapes* (*Ruditapes*) *philippinarum* were harvested annually from these tidal flats (Chirippu Fishery Cooperative Union, unpubl. data). This study was conducted at 8 subtidal stations along the tidal flow center (Fig. 1).

Sampling procedure

From April 2004 to March 2008, long-term monitoring (monthly or bi-monthly) of seawater was performed during spring tide at 8 subtidal stations located along a transect line (Fig. 1) from the tidal inlet (Stn 0) to the innermost station (Stn 15) (Fig. 1). During seawater sampling, we monitored temperature, salinity, nutrient concentrations (NH_4-N, NO_3+NO_2-N, PO_4-P, and $Si(OH)_4$-Si), and chlorophyll *a* (chl *a*) concentrations in the floodwater at these stations. Hydrological measurements were performed vertically at 10 cm intervals using a conductivity, temperature, and depth profiler (YSI 556; YSI Hydrodata). Seawater samples (2 l) were collected from the surface as well as 10 cm above the sea floor (samples at Stn 0 were obtained from the surface only) using a motor pump (YPM-12; flow rate, 70 l min^{-1}; intake diameter, 105 mm; Meiwa).

During the continuous survey conducted at Stn 0 (cross-sectional area, 240 m^2; width, 80 m; depth, 3 m; Fig. 1), we obtained 15 surface seawater samples and vertical profiles of water temperature and salinity at 20 cm intervals once every hour before and after slack water as well as once every 2 h from 10:00 on 13 February to 10:00 on 14 February. At Stn T (Fig. 1), the tidal height was monitored using a tide indicator (RMD-5225A; Rigo) every 10 min before the continuous survey (from 7:00 on February 13).

Moreover, on 12 March 2008, we conducted a parallel field investigation of chl *a* concentrations in the surface sediments at 7 subtidal stations (excluding Stn 0). At each sampling station, sediment samples for chl *a* analysis were carefully collected using an Ekman–Birge grab sampler, which sampled a 20 × 20 cm area to a depth of 20 cm. The topmost 0.5 cm of

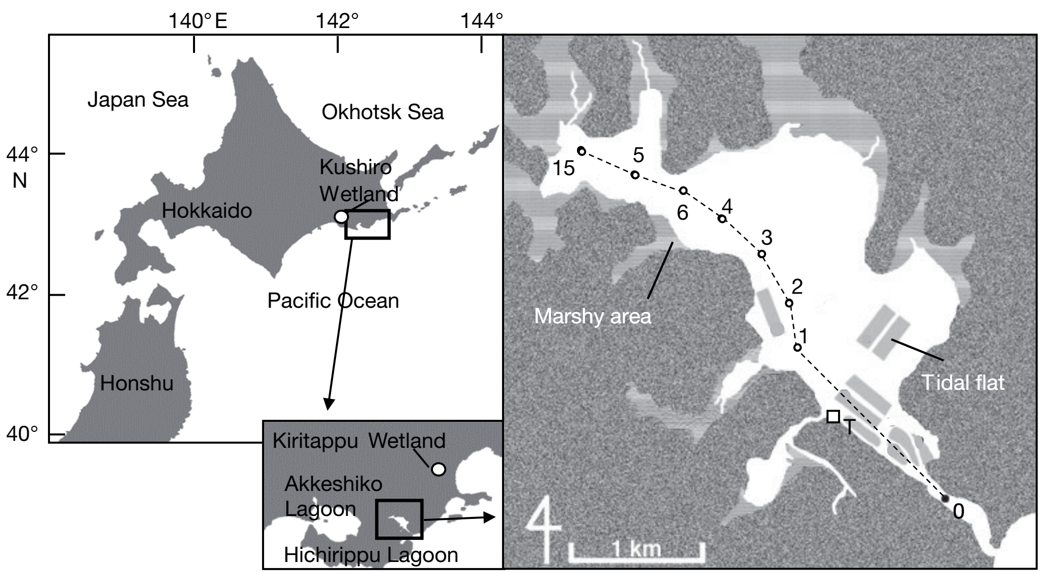


Fig. 1. Study area and location of sampling stations in a series of insets (following the arrows). In the map to the right, numbers and the letter 'T' represent the sampling stations from the subtidal area and tidal level monitoring, respectively. Dashed line indicates the transect line. Open circles represent the sediment sampling stations

the sediment was collected from 10 samples using an acrylic core tube (diameter, 3 cm); the 10 samples were collected using the grab sampler. The top layer of the sediment cores was often brownish in color; thus, the disturbance effect of the sediment in the cores seemed to be negligible for subsequent measurements.

Sample processing

In the laboratory, seawater samples were filtered through glass fiber filters (GF/F; Whatman) into a Teflon beaker and then transferred to polystyrene test tubes. The filtrates were stored at –20°C to prepare for nutrient analysis using an autoanalyser (QuAAtro; BL TEC; Strickland & Parsons 1972). After filtration, chl *a* was extracted from the seawater samples using 90% acetone. Furthermore, chl *a* in wet sediment samples was extracted from duplicate subsamples (ca. 0.5 g) obtained using 90% acetone. After 24 h incubation in the dark at –20°C, the samples were sonicated for 5 min. Chl *a* concentrations (µg chl *a* l^{-1}) in the supernatants obtained before and after acidification with 1 N HCl according to Lorenzen's (1967) method were analyzed using a fluorophotometer (Turner 10-AU-5, Turner Designs; Parsons et al. 1984). The dry weight (DW) of the sediment sample was substituted for the volume of pore water. Values thus expressed were corrected for porosity as measured by the water content in µg chl *a* g^{-1} DW. This water content was determined after drying a sediment sample (ca. 1 g) at 60°C for 24 h. In addition, the measurement of chl *a* concentration in the surface sediment (depth, 0.5 cm) was expressed in relation to area (mg chl *a* m^{-2}) by considering the bulk density of the sediment particles as 2.5 g cm^{-3} and spatio-temporal variations in pore water content (Montani et al. 2003).

Data analysis

The carbon contents (C/chl) of phytoplankton (Eppley 1968) and MPB (de Jonge 1980) were approx. 20 to –50 and 10 to 150, respectively. Their elemental compositions (C/N) were 6.6 (Redfield et al. 1963) and 7.5 (Montani et al. 2003), respectively. The standing stock of nitrogen in the microalgae was obtained by multiplying these elemental ratios by the microalgal standing stock.

The current speed (V; cm s^{-1}) was estimated every 10 min at the tidal inlet (Stn 0) according to $V(m) = [TH(m) - TH\ (m + 10)] \times (A)/S/(m \times 60\ \text{s})$, where V = current speed (cm s^{-1}) at time 'm' (every 10 min), TH = tidal height, A = extent of the impact of the open ocean (half the lagoon area, 1.78 km^2; Komorita et al. 2010), and S = cross-sectional area (240 m^2).

The current speed was shown as a 1 h running mean and converted from cm s^{-1} to m h^{-1} by multiplying with 100 cm, 60 s, and 60 min. Hourly nutrient flux (F; mmol m^{-2} h^{-1}) was expressed using $F(h) = C(h) \times (h)$, where C = nutrient concentration (mmol m^{-3}) converted from µmol l^{-1} based on a continuous survey and $V(h)$ = 1 h running mean of the current velocity at every hour.

The freshwater input (FW; × 10^3 m^3 d^{-1}) was estimated daily according to $FW(d) = RF(d) \times CA$, where RF = mean daily rainfall during 30 d before the continuous survey (data obtained from the Sakaki Meteorological Agency Station, which is located approx. 10 km northwest of Hichirippu Lagoon), and CA = water catchment area of 20.64 km^2 (Hokkaido Institute of Environmental Sciences 2005).

Statistical analysis

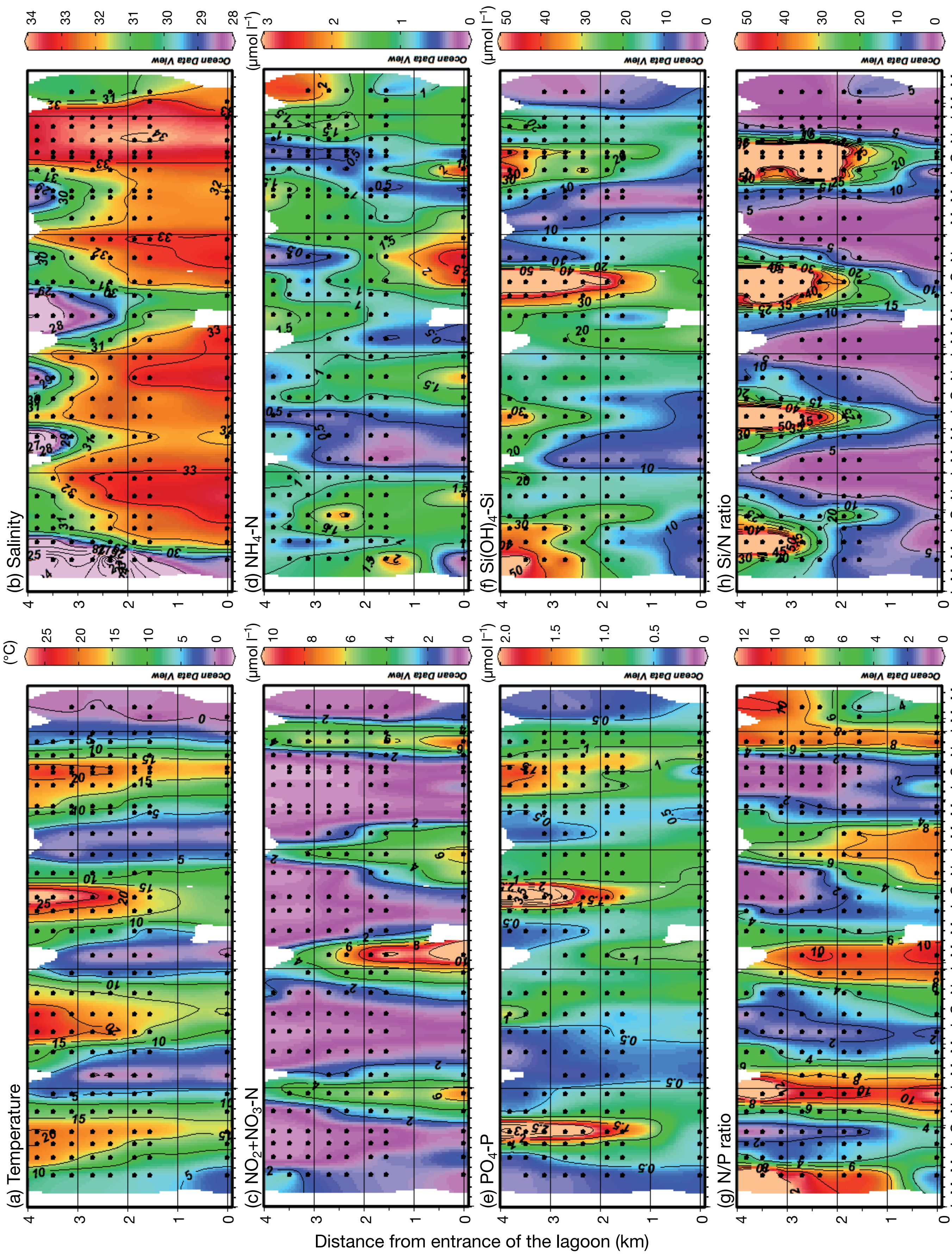
Regression analysis was used to test the relationship between water temperature and the NO_3+NO_2-N to DIN concentration ratio at the tidal inlet throughout the sampling period ($p < 0.05$) using StatView (Hulinks). To identify the primary factors influencing variations in environmental parameters during the continuous survey (tidal height, current speed, water temperature, salinity, and nutrient concentration), we performed principal component analysis (PCA) of these data (n = 15) using SPSS 11.5.

RESULTS

Seasonal variations in seawater

Fig. 2 shows seasonal variations in sea surface temperature, salinity, and nutrient concentrations along the transect line from April 2004 to March 2008. Because the water column was strongly mixed vertically by the flood tide, the collected surface water samples were representative of the water column. On August 8, 2006, the water temperature peaked at 29.2°C at the innermost station (Stn 15) at a distance of

Fig. 2. Seasonal variation in (a) water temperature, (b) salinity, (c,d,e,f) nutrient concentrations, and (g,h) stoichiometric nutrient ratios in the surface seawater


(a) Temperature
(°C)
(b) Salinity
(c) NO_2+NO_3-N
(d) NH_4-N
(e) PO_4-P
(f) $Si(OH)_4$-Si
(g) N/P ratio
(h) Si/N ratio
(µmol l^{-1})
Ocean Data View
J M M J S N J M M J S N J M M J S N J M M J S N J M M
2004 2005 2006 2007 2008
Distance from entrance of the lagoon (km)

3.8 km from the tidal inlet. From August to September each year, this temperature rose above 20°C at a distance of 2 to 4 km from the tidal inlet (Fig. 2a). Smaller spatial temperature gradients were observed in winter (February or March) than in summer, and the lowest temperature was between −1.3°C and 0.3°C each year. Conversely, salinity did not demonstrate a clear seasonal pattern and fluctuated between 27.5 and 34.1 throughout the sampling period (Fig. 2b).

NO_3+NO_2-N concentration around the tidal inlet showed a clear seasonal pattern, with low concentrations (0.1 to 3.9 µmol l^{-1}) in April to September and high concentrations (6.3 to 11.6 µmol l^{-1}) in December to March (Fig. 2c). Conversely, NH_4–N concentration increased during summer (August to October) within a distance of 1.5 km, and it reached 2.0 to 4.9 µmol l^{-1} during summer every year (Fig. 2d). During winter, the ocean appeared to be the main DIN source, which is in the form of NO_3+NO_2-N.

PO_4-P concentration exceeded 1 µmol l^{-1} from July to August and reached its highest value of 4.0 µmol l^{-1} at the innermost station on 19 August 2004 (Fig. 2e). The concentration fluctuated between 0.1 and 1.0 µmol l^{-1} on other occasions. Spatio-temporal variations in $Si(OH)_4$-Si concentration were similar to those in PO_4-P concentration; the highest value of 117 µmol l^{-1} was recorded at the innermost station on 8 August 2006 (Fig. 2f). During summer, a riverine-derived source appeared to produce the majority of phosphate and silica.

DIN concentration increased sharply around the tidal inlet during winter (December to March; Fig. 2c). The molar ratios of N, P, and Si were distinctly different between winter (December to March: N/P, 9.1–12.5; Si/N, 1.4–3.9) near the tidal inlet and other seasons (April to November: N/P, 0.1–6.8; Si/N, 4.8–295) around the innermost part of the lagoon (Fig. 2g,h).

Fig. 3a shows seasonal variations in the NO_3+NO_2-N to DIN concentration ratio at the tidal inlet. This ratio dropped below the mean value of 0.5 throughout the sampling period during summer (July to September). A significant correlation was observed between temperature and the concentration ratio at the tidal inlet (Fig. 3b; $r^2 = 0.56$; $p < 0.0001$; $n = 37$).

Continuous survey

During the continuous survey between 13 and 14 February 2008, there were 2 tidal cycles (low and high tides; Fig. 4a). The tidal level varied from 45.4 cm (15:00 on 13 February) to 147.9 cm (7:40 on 14 February) (Fig. 4a). The current speed reached its highest outflow (−35.2 cm s^{-1}) at 11:10 on 13 February and inflow (31.1 cm s^{-1}) at 5:40 on 14 February. Temperature varied from −1.9°C to −1.1°C and salinity from 31.8 to 33.6 (Fig. 4b). Temporal variations in NO_3+NO_2-N, PO_4-P, and $Si(OH)_4$-Si concentrations occurred with such variations in tidal height and peaked at 7.7, 0.9, and 15.8 µmol l^{-1}, respectively, during the high tide at 21:00 on 13 February (Fig. 4c,d). Conversely, temporal variations in NH_4-N concentration showed a reverse pattern and ranged from 0.3 to 1.2 µmol l^{-1} (Fig. 4c).

In the forward selection procedure of PCA, the rank next to the second PCA component was excluded from the eigenvalues and degrees of freedom. The variable explained 56.7% of the variance of the first PCA component (Table 1). This component seemed to reflect water moving with variations in tidal height, which showed the highest factor load-

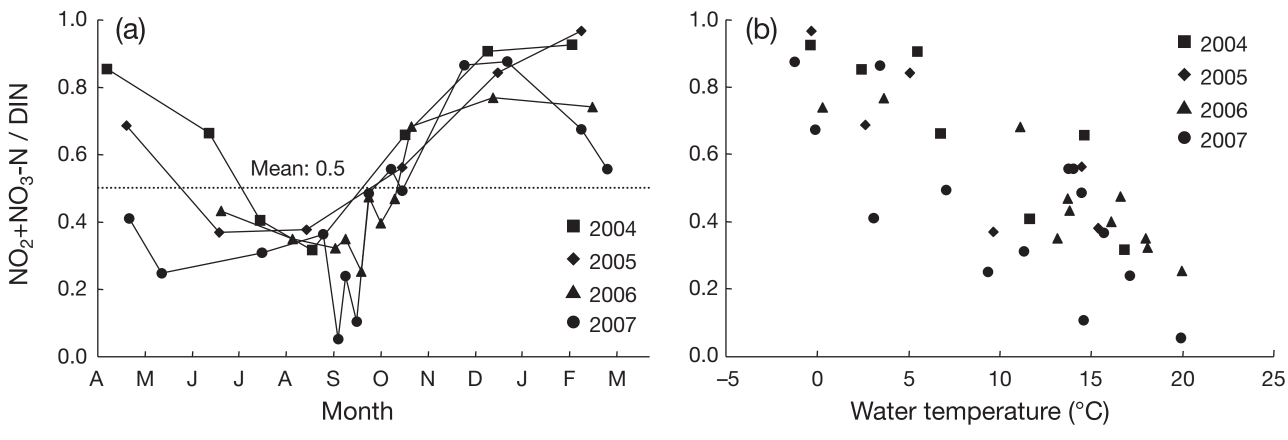


Fig. 3. (a) Seasonal variation in the concentration ratio of NO_3+NO_2-N to dissolved inorganic nitrogen (DIN) at Stn 0. (b) Relationship between water temperature and the concentration ratio of NO_3+NO_2-N/DIN

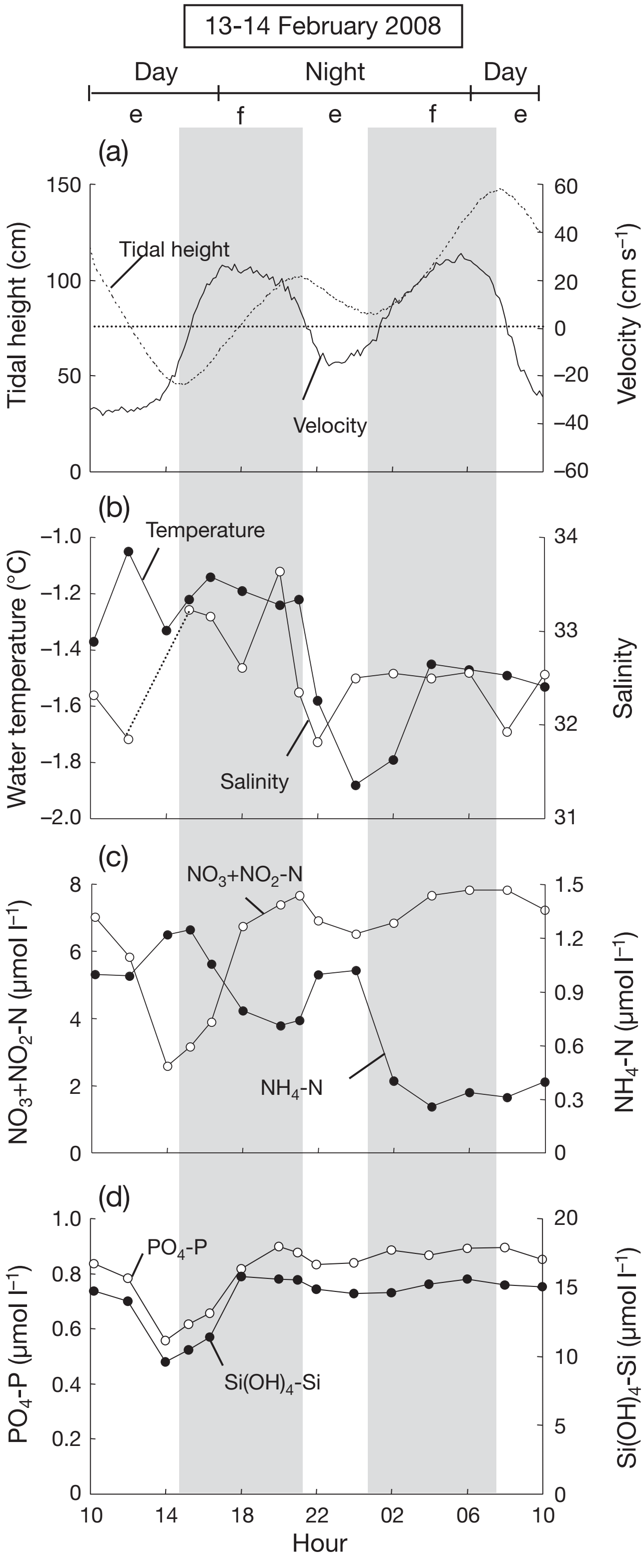


Fig. 4. (a) Tidal height and velocity, (b) temperature and salinity, and (c,d) nutrient concentrations in seawater at the tidal inlet of the lagoon during a 24 h period on 13 and 14 February 2008. Grey and white areas represent flood ('f') and ebb ('e') tides, respectively

Table 1. Summary of results of principal component analysis using 8 variables. Significant correlation between each environmental variable and the 1st principal component (PC) are indicated with **bold** text: $p < 0.01$

	1st PC
Eigenvalue	4.53
% Variance	56.7
Factor loading of variables	
Tidal height	**0.868**
Velocity	0.153
Water temperature	–0.479
Salinity	–0.453
NH_4-N	**–0.777**
NO_2+NO_3-N	**0.977**
PO_4-P	**0.961**
$Si(OH)_4$-Si	**0.917**

ings ($r = 0.868$, $p < 0.01$) of the physical components. This component strongly affected NO_3+NO_2-N ($r = 0.977$, $p < 0.01$), PO_4-P ($r = 0.961$, $p < 0.01$), and $Si(OH)_4$-Si concentrations ($r = 0.917$ $p < 0.01$). Conversely, the factor NH_4-N loading was negative (NH_4-N: $r = –0.777$, $p < 0.01$).

Nutrient budget

Table 2 shows the daily freshwater, seawater, and nutrient budgets in the continuous survey conducted at the tidal inlet during the supply period. Freshwater input was 0.16 % of seawater influx through the tidal inlet. While the daily seawater budget showed a slight excess of exports over imports ($–78.3 \times 10^3$ m^3 d^{-1}), the NO_3+NO_2-N, PO_4-P, and $Si(OH)_4$-Si budgets were 3.93 kmol N d^{-1}, 0.32 kmol P d^{-1}, and 5.78 kmol Si d^{-1}, respectively, and were higher than the outflow (Table 2; Fig. 4a,c,d). The molar budget ratio was N:P:Si = 10.2:1:18.3 (Table 2).

Seasonal variation in the NO_3+NO_2-N to DIN concentration ratio at the tidal inlet depended on water temperature (Fig. 3b). Here this ratio was used as an index of supply from the open ocean because NO_3-N concentration in the surface layer depended on vertical mixing during winter (Kasai 2000; Fishery Research Agency 2008). We defined the months where the ratio dropped below the mean value of 0.5 at least once during the sampling period as the 'mineralization period' (April to October: 245 d) and the other months as the 'supply period' (November to March: 120 d).

Table 3 shows the integrated nutrient budget during the mineralization period (calculated on the basis of the results of Komorita et al. 2010), supply period,

Table 2. Water and nutrient budgets at the entrance of the lagoon on 13 and 14 February 2008 and the elemental ratio of the budget. Negative values indicate export from the lagoon

	Water ($\times 10^3$ m^3 d^{-1})	NO_3+NO_2-N ($kmol\ d^{-1}$)	NH_4-N ($kmol\ d^{-1}$)	PO_4-P ($kmol\ d^{-1}$)	$Si(OH)_4$-Si ($kmol\ d^{-1}$)
Freshwater	3.44				
Influx	2129	13.3	0.98	1.58	28.2
Efflux	–2207	–9.33	–1.67	–1.26	–22.4
Budget	–78.3	3.93	–0.68	0.32	5.78
N:P:Si			10.2:1:18.3		

Table 3. Integrated nutrient budget over each period (see details in 'Results: Nutrient budget'). Nutrient budget during mineralization period was calculated based on the value of Komorita et al. (2010). Negative values indicate export from the lagoon

Period	Duration (d)	Nutrient budget (kmol per period) NO_3+NO_2-N	NH_4-N	PO_4-P	$Si(OH)_4$-Si
Mineralization	245	35	–166	14	–4350
Supply	120	471	–82	38	694
Annual	365	506	–248	52	–3660

and 1 yr. The integrated DIN, PO_4-P, and $Si(OH)_4$-Si budgets during the supply period were positive values, i.e. 389 kmol N 120 d^{-1} (= 471 kmol (NO_3+NO_2-N) 120 d^{-1} plus –82.1 kmol (NH_4-N) 120 d^{-1}; Table 3), 38.0 kmol P 120 d^{-1}, and 694 kmol Si 120 d^{-1}, respectively. The integrated DIN and $Si(OH)_4$-Si budgets during the mineralization period were negative values, i.e. –131 kmol N 240 d^{-1} and –4350 kmol Si 240 d^{-1}, respectively, in contrast to the integrated PO_4-P (14 kmol P 240 d^{-1}) budget. The annual DIN and PO_4-P budgets were positive values, i.e. 258 kmol N yr^{-1} and 52 kmol P yr^{-1}, respectively, whereas the annual $Si(OH)_4$-Si budget was a significantly negative value of –3650 kmol Si yr^{-1}.

Microalgal standing stocks

In March 2008, the mean biomass per unit area of MPB in the surface sediment (depth, 0.5 cm; 96.7 ± 39.8 mg chl *a* m^{-2}) was approx. 100 times higher than that of phytoplankton in the water column (depth, 1 m; 0.9 ± 0.1 mg chl *a* m^{-2}). The nitrogen-based biomass of MPB and phytoplankton was 10.8 to 161 mmol N m^{-2} and 0.227 to 0.569 mmol N m^{-2}, respectively. The total amount of nitrogen in the lagoon was 38.3 to 574 kmol N for MPB and 0.81 to 2.02 kmol N for phytoplankton, multiplied by the lagoon area (3.56 km^2).

DISCUSSION

External nutrient supply

Comparison of the N/P and Si/N ratios of long-term samples with the Redfield ratio (N/P = 16; Si/N = 1; Redfield et al. 1963) clearly suggested nitrogen deficiency in this lagoon (Fig. 2g,h) (Komorita et al. 2010, Suga et al. 2011). During winter, a high NO_3+NO_2-N concentration (6.3 to 11.6 µmol l^{-1}) near the tidal inlet (Fig. 2c) and input through this inlet (Table 2) is important for the lagoon ecosystem. At the sampling station approx. 10 km offshore from this lagoon (42° 50′ N, 144° 50′ E), NO_3-N concentration in the surface layer increased between 15 and 20 µmol l^{-1} because of strong vertical mixing throughout the approx. 200 m depth during winter (January to March). The concentration dropped below measurable limits after a spring phytoplankton bloom in April (Kasai 2000; data from a-line database[1]). Thus, the open ocean appeared to be the major NO_3+NO_2-N source in this study area during winter, and the annual DIN budget from the tidal inlet was estimated to be 258 kmol N yr^{-1} (NO_3 + NO_2-N plus NH_4-N, Table 3).

Other nutrient sources for coastal lagoon systems include riverine (Nedwell et al. 1999, Mann 2000), groundwater (Paerl 1997, Nedwell et al. 1999), and atmospheric nutrient deposition (Paerl 1997). In this study area, freshwater input from riverine systems was estimated to be 3.44 × 10^3 m^3 d^{-1} during the supply period (Table 2) and accounted for 0.16 % of seawater inflow. These results, which conformed to a recent study, showed that freshwater has a relatively small effect on the water budget, ranging from 0.9 to 8.0 % of the total volume in this lagoon (Suga et al. 2011) and suggested that the riverine input of DIN was a minor contributor to the ecosystem only during summer (Komorita et al. 2010). Because high NO_3-N concentration in the groundwater declines sharply as a result of denitrification before discharge (Onodera et al. 2007), it is highly unlikely that groundwater was the primary nitrogen source in this lagoon.

In terms of atmospheric deposition, Nedwell et al. (1999) indicated that direct inputs to an estuary are

[1]http://fra.affrc.go.jp/a-line; Fishery Research Agency, accessed 5 December 2008

small compared with the fluvial component. Global atmospheric deposition of nitrogen in 1890 (non-polluted area alone) and 1990 (non-polluted and polluted areas) was estimated to be 17 and 27 Tg N yr^{-1}, respectively (Galloway & Cowling 2002). If we assume these values to be atmospheric nitrogen deposition, the average flux was 6.5 and 10.4 µmol N m^{-2} yr^{-1} divided by the surface area of the planet (ca. 5.1×10^{14} m^2). Based on the values obtained by Galloway and Cowling (2002), atmospheric nitrogen deposition flux in this lagoon, isolated from the highly populated areas, was estimated to be between 0.02 kmol N (3.56 km^{-2}) d^{-1} and 0.04 kmol N (3.56 km^{-2}) d^{-1}. In the present study, NO_3+NO_2-N input through the tidal inlet was 3.93 kmol N d^{-1} (Table 2). Thus, the contribution of atmospheric nitrogen deposition is less than 2% of the supply from the open ocean during winter in this study area.

In addition to the supply of dissolved inorganic nutrients, several studies suggested that the coastal area receives a considerable amount of particulate organic matter (POM) from outside the area (Cadée 1980, Forès et al. 1994, Camacho-Ibar et al. 2003, Kohata et al. 2003, Barros et al. 2010). The present study indicated that the impact of nutrient flow from the open ocean is somewhat underestimated because we neglected the effect of POM supplied from the neighboring ocean.

Nutrient budget and nutrient uptake by MPB during winter

Microalgal growth rates are positively affected by water temperature (Goldman & Carpenter 1974, Rose & Caron 2007). However, the growth rate of planktonic diatom species (*Thalassiosira nordenskioeldii* and *Detonula confervacea*) isolated from low temperature environments (Saroma Lagoon, Hokkaido, Japan) reaches 0.3 to 0.4 d^{-1} at –1.8°C (Suzuki & Takahashi 1995), similar to that during the supply period of this study (–1.9°C to –1.1°C; Fig. 4b). Thus, biophilic elemental budgets (Table 2; Fig. 4a,c,d) during the continuous survey were a result of nutrient uptake by microalgae that are adapted to the low temperature.

Based on this interpretation, we estimated the amount of organic nitrogen represented by MPB and phytoplankton in this lagoon. Assuming that MPB in the surface sediment (depth, 0.5 cm) used all NO_3+NO_2-N input (3.93 kmol N d^{-1}), the temporal division rate (NO_3+NO_2-N uptake/total amount of MPB) of MPB would be 0.01 to 0.10 d^{-1}. MPB appears to be able to achieve this rate, which is consistent as per report by Suzuki and Takahashi (1995). Conversely, assuming that phytoplankton in the water column used all NO_3+NO_2-N input, the temporal division rate would be 1.94 to 4.85 d^{-1}. The reported phytoplankton division rates under low temperatures (–1.8°C) are at least one-seventh of the abovementioned range (0.3 to 0.4 d^{-1}); thus, phytoplankton alone may not be responsible for the observed levels of NO_3+NO_2-N uptake in this lagoon.

As observed in this lagoon during winter, denitrification may be performed by many heterotrophic anaerobic bacteria under relatively high NO_3+NO_2-N concentrations (Nielsen et al. 1995, Ogilvie et al. 1997, Trimmer et al. 1998, Sundbäck et al. 2000). NO_3-N concentration is identified as one of the main factors controlling denitrification (Seitzinger 1988, Rysgaard et al. 1995), in addition to water temperature (Trimmer et al. 1998). According to Deek et al. (2011), the denitrification rate appeared to increase linearly with nitrate (denitrification [µmol m^{-2} h^{-1}] = 1.01 or 0.39 × nitrate [µmol l^{-1}] + 1.07 or 0.01, in fine or coarse sand stations, respectively) availability, which was relatively low (<30 µmol l^{-1}) and restricted to winter (water temperature was 1 to 8°C). If we calculate the denitrification rate using these equations and mean NO_3+NO_2-N concentration of 6.4 µmol l^{-1} obtained during the continuous survey, the denitrification rate in this study area was 0.06 to 0.181 mmol N m^{-2} d^{-1}, and the total denitrification rate was estimated to be 0.21 to 0.64 kmol N d^{-1} multiplied by the lagoon area (3.56 km^2). The contribution of denitrification would be 16% of the supply from the open ocean (3.93 kmol N d^{-1}; Table 2) during winter in this study area. However, other nutrients were also supplied through the tidal inlet during the continuous survey (Table 2), and the molar ratio of the nutrient budget during the supply period (N:P:Si = 10.2:1:18.3; Table 2) approached that of MPB (10.1:1:17.8; Montani et al. 2003). Thus, MPB appears to be the major nutrient sink during winter, and we assumed that denitrification contributed to the nutrient budget next to the microalgal communities.

CONCLUSION

During winter, the ocean was the main DIN source, a deficient nutrient, in this lagoon. It appears that MPB fixed the oceanic nutrient during the supply period, and the main nutrient sink seemed to be MPB. Although further studies of the nutrient budget from several sources and fine time scales (monthly or bi-weekly), in addition to those of POM, are needed to determine the precision budgets in this lagoon, the annual nitrogen budget of this study was estimated to be 258 kmol N yr^{-1}, which mainly depended on oceanic DIN fixed by MPB during winter.

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