

Potential contribution of microalgal intracellular phosphorus to phosphorus distribution in tidal flat sediments during winter

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Abstract: Phosphorus (P) supplies from the sediment are important when evaluating the impacts of nutrient dynamics on the ecosystem. Many studies have reported P dynamics based on gradients of pore water (PW)-phosphate (PO_4^{3-}) concentrations at the sediment–water interface and iron-related abiotic reactions in response to redox conditions. However, the aim of the present study is to evaluate the contributions of intracellular (IC)-P and marine organism-mediated reactions to P-dynamics under variable environmental conditions. The focus of this study is the intertidal mudflat habitat of the innermost part of Ariake Bay, Kyushu, Japan, during winter. The vertical distribution of several forms of P fraction [i.e. PW- and IC-dissolved inorganic/organic-P (DIP/DOP), and particulate inorganic/organic-P (PIP/POP)], the number of microalgae and bacteria, and chlorophyll *a* concentrations in the sediment were measured. The results obtained revealed that the IC-P pool, especially IC-DOP, accounted for 76% of the total dissolved P in the surface sediment layer, mainly associated with the microphytobenthos. Additionally, phytoplankton, which comprised more than 60% of the surface microalgae in tidal-flat sediments, are considered to carry river-derived-P to the intertidal flats via inner bay-specific physical movements and disturbances. Incubation experiments on mud sediment under oxic/anoxic conditions suggest that dissolved P fluxes between the IC pool and PW occur actively in response to redox conditions, according to the balance between microalgal uptake and bacterial degradation. These results indicate that evaluation of the IC-P pool is indispensable to understanding P cycling in intertidal mudflats, and that microalgae could play important roles not only as food sources, but also as P reservoirs and sources of PO_4^{3-} .

Key words: Ariake Bay, intertidal mudflat, intracellular phosphorus pool, microalgae, redox condition

Introduction

The high productivity of benthic microalgae in intertidal flats supports the biomass of a variety of benthic organisms, such as filter feeders, polychaetes, and mudskippers, and therefore functions to convey fixed nutrients to higher trophic level of organisms as organic matter (Ichimi et al. 2008a, Yoshino et al. 2012). Although nitrogen (N), phosphorus (P), and silicates (Si) are indispensable elements for

planktonic and benthic algal growth, P fluxes from the sediment are very important, especially in intertidal flat areas, which are regularly subjected to an influx of N-rich river water (Yamamoto et al. 1998, Hayami et al. 2009, Koriyama et al. 2009). Generally, the biological oxidation of organic-P to phosphate (PO_4^{3-}) in pore water (PW), and its subsequent diffusion, is a dominant process releasing PO_4^{3-} to overlying water under oxic conditions. Additionally, the abiotic reaction of PO_4^{3-} isolation from its iron-bound form is reported to be important under anoxic conditions (Boström et al. 1988, Rozan et al. 2002, Koriyama et al. 2009).

In addition to the P fraction (i.e. PW- PO_4^{3-} and the iron-bound form), recent studies have shown the potential contribution of the intracellular (IC) nutrient pool to nutrient

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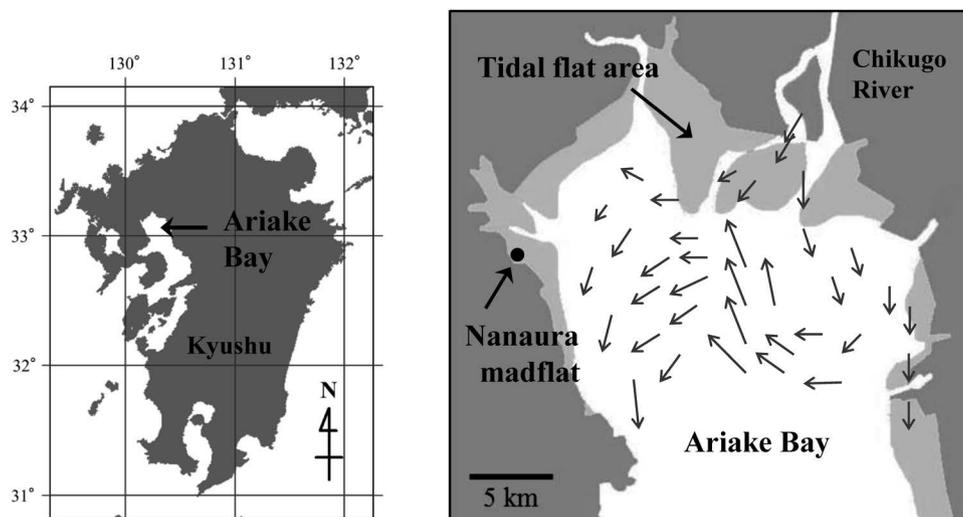


Fig. 1. Sediment sampling sites in the innermost part of the Ariake Bay. Arrows indicate predominant residual currents at spring tides (from Hamada & Kyozyuka 2006).

dynamics, mediated by a variety of organisms in marine sediments. For example, the high concentration of IC-nitrate (NO_3^-) in sulfur bacteria and Foraminifera is used as a substrate for respiration (i.e. denitrification) in anoxic environments, suggesting an important role in global N cycling (Sayama 2001, Risgaard-Petersen et al. 2006).

The IC- NO_3^- and PO_4^{3-} pools in the microphytobenthos of intertidal flats vary seasonally and potentially influence both microphytobenthic primary production and sediment–water fluxes (García-Robledo et al. 2010). Furthermore, polyphosphate accumulation in cells has been observed in marine phytoplankton (Diaz et al. 2008) and in sulfide-oxidizing bacteria in coastal upwelling regions (Goldhammer et al. 2010). However, polyphosphate-accumulating organisms (PAOs) in wastewater plants store P in the form of polyphosphate under oxic conditions, and release PO_4^{3-} to the extracellular environment under anoxic conditions (e.g. Streichan et al. 1990).

In marine environments, especially in the thin surface layers of muddy intertidal flats, similar redox conditions may occur in response to tidal oscillations because surface sediments are exposed to the air at low tide. However, at flood and ebb tide, increased physical disturbance expands the depth of the oxic layer, and during high tide, resuspended fine sediments reduce light penetration, reducing photosynthetic activity and allowing the rate of respiratory decomposition to predominate (Koh et al. 2006, Sayama 2007). However, the proportion of each P fraction [i.e. PW-P, IC-P, particulate organic P (POP), and particulate inorganic P (PIP)] in total P at the sediment–water interface and PAOs-like dynamics in response to redox conditions remain to be studied in the intertidal mudflats that broadly occupy innermost bays.

Therefore, the present study aims to investigate (1) the size of the IC-P pool compared with other P fractions, and

(2) the contribution of biological processes, through the IC-P pool, to P-dynamics in the intertidal mudflats of the innermost region of Ariake Bay, where redox conditions dramatically change due to tidal movements and the formation of hypoxic water (Hamada et al. 2008, Tokunaga et al. 2009). Both oxidized and reduced layers can exist in surface mudflat sediments, especially in winter (see Discussion), so the focus of this study is on winter mechanisms.

Materials and Methods

Study site

Ariake Bay is a semi-closed bay located on the western shore of Kyushu, Japan (Fig. 1). Due to a predominant anti-clockwise current at the surface, especially during spring tides, fine silt from the Chikugo River is carried to its western side (Yamamoto et al. 2006, Hamada & Kyozyuka 2006). Therefore a well-developed intertidal zone exists around the Chikugo River mouth and in the northwest area of the bay (e.g. Nanaura mudflat), enhanced by the very high tidal difference in Ariake Bay, which reaches a maximum of 5.5 m. The integrated monthly daylight during winter (Dec.–Feb.), averaged over 1986–2010, is about 122.7 h, two-thirds of the summer value (Japan Meteorological Agency). The mean and lowest air temperatures during winter between 1981–2010 are 5.8°C and –6.9°C, respectively.

Samples and chemical analyses

Sediment core samples for nutrients and for Chlorophyll *a* (Chl *a*) analyses were collected from the Nanaura mudflat using acrylic tubes of 88- and 55-mm diameter, respectively, during the aerial exposure period during low tides

in February 2010 and 2011. Sediment samples from the surface to a depth of 10 mm were carefully collected with a small plastic spoon at intervals of 2 mm, while the samples below 10 mm were sliced at 5–10 mm intervals as follows: 15, 20, 30, 40 and 50 mm. Outer portions of the sliced sediment disks were discarded because the sediment attached to the inner wall of the tube is generally subject to vertical contamination. Triplicate sediment samples were separately processed in 2011; whereas in 2010, each layer of triplicate core sediments was mixed, and the results of a single analysis were considered to represent a mean value.

Sediment Chl *a* was extracted with *N,N*-dimethylformamide at -20°C in the dark and analyzed using a spectral photometer (U-1100, Hitachi Ltd., Tokyo, Japan), following the method described by Porra et al. (1989). A portion of the sediment samples collected in 2010 was fixed by neutral formalin, and then microalgal cells alive at fixation (those with well formed chloroplasts) were identified and counted by Oceanic Planning Co. Ltd. (Okinawa, Japan).

Sediment samples used for bacterial cell counts were fixed in glutaraldehyde at a final concentration of 2% (v/v). Samples were subjected to ultrasonication for 5 s five times and then centrifuged at $1600\times g$ for 30 s. Bacterial cells in the supernatant were collected on a $0.2\text{-}\mu\text{m}$ pore size black Nuclepore filter (K020N047A, ADVANTEC, Tokyo, Japan), staining with 4',6'-diamidino-2-phenylindole (DAPI) and counted using epifluorescence microscopy. Since microalgal cell contents are released by freezing, IC-P was defined as the P fraction, increase after the freeze–thaw process, as previously described by Sayama (2001). The IC-P fraction was subdivided into IC- PO_4^{3-} and IC-DOP fractions (see below).

To determine each form of the P fraction in the sediment including PW, sediment samples were centrifuged at $700\times g$ for 10 min. The supernatants were filtered with $0.2\text{-}\mu\text{m}$ pore size cellulose acetate filters (25CS020AN, ADVANTEC) and frozen at -20°C until analysis for PW- PO_4^{3-} and PW-Total Dissolved P (TDP). For IC-P analysis, the remaining sediment was suspended in 3–10 mL of Milli-Q water, vortexed thoroughly, frozen at -20°C , and then thawed and immediately centrifuged. A portion of the supernatants was removed, filtered and frozen (as above) for IC- PO_4^{3-} and IC-TDP analysis. The remaining sediment was dried in an oven at 70°C to estimate dry weight.

The PO_4^{3-} concentration in both supernatants for PW- PO_4^{3-} and IC-P analysis were determined by the standard Molybdenum blue method (Koroleff 1983) using an autoanalyzer (AACS 4, BLTEC, Osaka, Japan). The total amount of IC- PO_4^{3-} was calculated from the following equation:

$$\text{IC-PO}_4^{3-} \text{ (mol-P dry-g}^{-1}\text{)} \\ = \{[\text{PO}_4^{3-} \text{ (SU)}] \times (\text{added MilliQ}_{(ml)} + \text{resid PW}_{(ml)}) \\ - [\text{PO}_4^{3-} \text{ (PW)}] \times \text{resid PW}_{(ml)}\} / \text{sediment dry weight}$$

where $[\text{PO}_4^{3-} \text{ (PW)}]$ is the PO_4^{3-} concentration in PW;

$[\text{PO}_4^{3-} \text{ (SU)}]$ is the PO_4^{3-} concentration in supernatant after thawing of frozen samples; resid $\text{PW}_{(ml)}$ is the residual PW calculated as the difference between the wet sediment weight after centrifuging and the dry weight.

For analysis of total particulate P (TPP), dried sediment samples were combusted at 550°C and TPP was extracted as PO_4^{3-} using 1N-HCl at room temperature for 24 h. PIP was defined as the fraction extracted as PO_4^{3-} from the intact dried sediment with 1N-HCl at room temperature for 24 h. The PO_4^{3-} concentration was determined using an autoanalyzer as described above, with standard samples processed in the same manner for calibration. POP was calculated as the difference between TPP and PIP.

Dissolved organic P (DOP) concentrations in the PW and IC pool were determined as the difference between PO_4^{3-} and TDP in each fraction. TDP was processed according to the persulfate oxidation method (Ridal & Moore 1990). Briefly, the PW or extracted fraction after the freeze–thaw process was autoclaved at 125°C for 4 h with the addition of potassium persulfate to convert TDP to PO_4^{3-} . Excess free chlorine was removed by the addition of ascorbic acid (following Hansen and Koroleff 1999) and then PO_4^{3-} was measured colorimetrically using an autoanalyzer as described above.

Incubation design

To evaluate P-dynamics under oxic/anoxic conditions, duplicate sediment samples collected from the surface of Nanaura mudflat in April 2011 (Fig. 1) were incubated under oxic/anoxic cycles at 12-h intervals, controlled with air or N_2 gas bubbling, respectively. Sediment (100 g) was resuspended in 1 L of filtered seawater containing final concentrations of $100\text{ }\mu\text{M}$ PO_4^{3-} and glucose 500 mgC L^{-1} and placed in continuous darkness at 20°C . At the end of each oxic/anoxic interval, samples for PW- PO_4^{3-} , POP, and PIP analyses were collected, processed, and analyzed as described above.

Visualization of polyphosphate accumulation

To characterize P compounds in the IC pool of benthic organisms, surface sediment samples were collected from Nanaura tidal flat in February 2012, fixed in glutaraldehyde (final concentration 2% v/v), and stained with DAPI. With a high concentration of DAPI, blue and yellow fluorescence under UV were used as indicators of DNA and polyphosphate, respectively (Tijssen et al. 1982). Some benthic microalgae (e.g. *Gyrosigma* sp.) and phytoplankton (e.g. *Skeletonema* sp. and *Thalassiosira* sp.), often found in Nanaura mudflat, were selected and subjected to fluorescence microscopic observation (BX51, Olympus, Tokyo, Japan) using a DAPI filter set (excitation 330–385 nm; emission $>420\text{ nm}$).

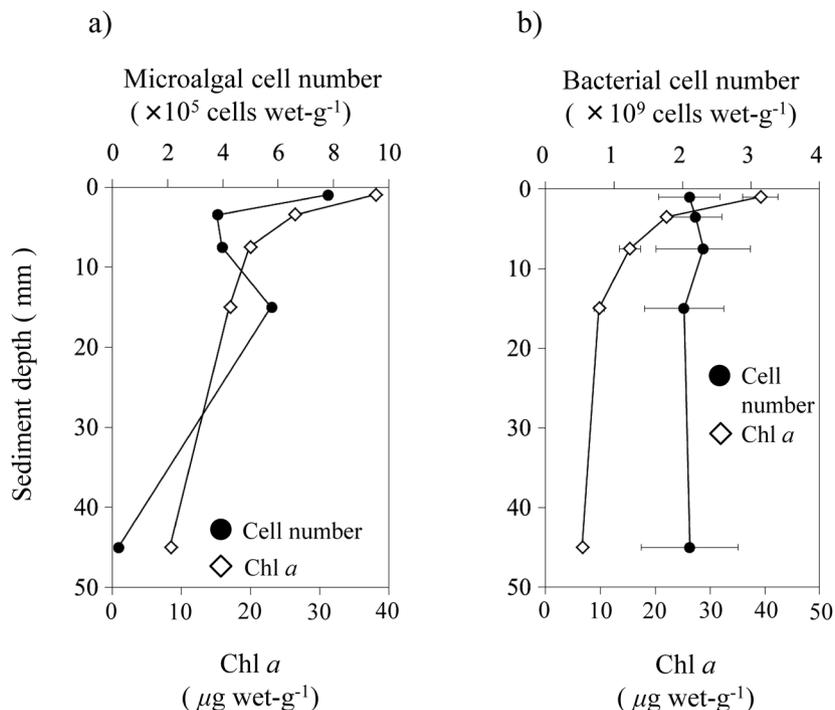


Fig. 2. Vertical profiles of the total cell number of (a) microalgae and (b) bacteria in the sediment, plotted with chlorophyll *a* concentration. For microalgae, each layer of triplicate core sediments collected in February 2010 was mixed, and the results of single analysis were considered to represent an average value. For bacteria, triplicate core sediments collected in February 2011 were separately processed, and error bars indicate the standard deviation among samples.

Results and Discussion

Contribution of benthic and planktonic microalgae to winter POP dynamics

The total number of microalgae in sediment decreased with depth (Fig. 2a), from 7.8×10^5 cells wet-g $^{-1}$ at the surface to 2.1×10^4 cells wet-g $^{-1}$ at depths of 4–5 cm. The vertical profile of Chl *a* also showed that Chl *a* decreased with depth, from 38.0 to 8.4 μ g wet-g $^{-1}$. Although a similar decreasing trend was observed for the vertical profile of Chl *a* in the following year, bacterial cell abundance was almost constant, irrespective of depth (Fig. 2b). The sediment POP concentration decreased with depth and showed a similar trend to Chl *a* (i.e. 3.4 ± 0.1 μ mol-P wet-g $^{-1}$ in the top 2 mm to 2.2 ± 0.2 μ mol-P wet-g $^{-1}$ at depths of 4–5 cm), whereas the PIP concentration increased with depth (Fig. 3). These results suggest that the high concentration of POP in surface sediments could be attributable to microalgae and/or associated bacteria. Analysis of microalgal composition in the sediment showed that more than 60% of all microalgae were composed of four major planktonic species: *Cyclotella striata*, *Skeletonema costatum*, *Thalassiosira* spp., and *Thalassionema nitzschioides* (Fig. 4). Huettel and Rusch (2000) reported that, in permeable sandy sediment with small mounds and ripples, water currents carry phytoplankton to the subsurface layer. In the Nanaura mudflat, however, planktonic microalgae seemed

to have migrated into the subsurface layer as a result of physical disturbance (i.e. resuspension and deposition). Further, Koh et al. (2006) reported that microalgae and suspended particulate matter are transported by flood-ebb tides, especially during spring tides. Since these microalgae still had healthy chloroplasts, physical disturbance and horizontal transportation may occur frequently.

Park et al. (2012) reported that more than half the microalgae in the Nanaura mudflat in summer were benthic. However, benthic microalgae (e.g. *Navicula* spp. and *Nitzschia* spp.) were minor species in the present winter study. Although river water inputs and associated nutrient fluxes are smaller in winter than in summer (Ishitani et al. 2012), blooms of major planktonic microalgae (e.g. *Skeletonema* spp.) occur in Ariake Bay even during winter (Matsubara et al. 2011). In the innermost areas of Ariake Bay, the surface residual current flows anticlockwise and carries particulate matter, including river-derived fine sediment and phytoplankton. This occurs most often during spring tides, with current speeds weakening at neap tide to deposit transported particulate matter onto the sediment in northwestern areas of the bay (Hamada & Kyojuka 2006, Yanagi & Shimomura 2006, Yamamoto et al. 2006). Phytoplankton, the growth of which depends on nutrients supplied by the Chikugo River, is used as a food source by fish larvae in the innermost areas of Ariake Bay (Suzuki et al. 2007). Moreover, the results in this study indicate that planktonic microalgae may be key agents absorbing nutri-

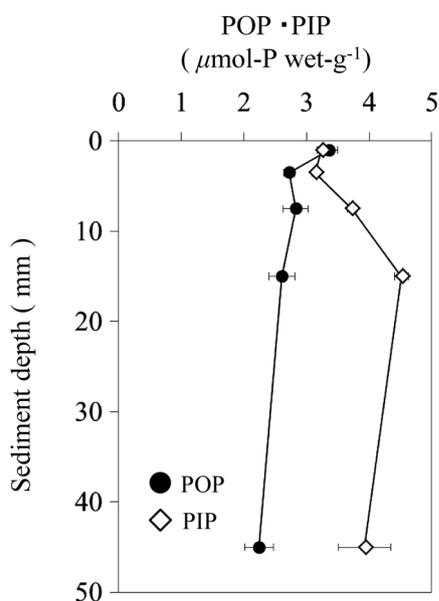


Fig. 3. Vertical profiles of particulate organic phosphorus (POP) and particulate inorganic phosphorus (PIP) in the sediment. Samples were collected and processed in the same manner as the samples for the bacterial cell number and chlorophyll *a* analyses shown in Fig. 2b.

ents around the mouth of the river, including PO_4^{3-} , and transporting them to intertidal mudflats, although the actual contribution of larger benthic algae (e.g. *Gyrosigma* sp.) to nutrient dynamics could be larger than an evaluation based on cell numbers.

Contribution of IC fractions to the dissolved-P reservoir of surface sediment

PW- PO_4^{3-} concentrations in surface sediment were low ($0.4 \pm 0.2 \mu\text{M}$), but increased up to $11.0 \pm 1.6 \mu\text{M}$ with depth (Fig. 5a); while (in the same mudflat) they are known to be high in summer ($29.4 \mu\text{M}$, Tokunaga et al. 2006; $7.1 \mu\text{M}$, A. Yamaguchi unpubl. data). A similar seasonal trend (i.e. high PW- PO_4^{3-} in summer, while low in winter) has been observed in other intertidal flats (Magni & Montani 2006). However, the concentration of PW-DOP ranged from 0.3 ± 0.5 to $2.1 \pm 1.2 \mu\text{M}$, but no significant vertical difference was noted. Both IC- PO_4^{3-} and IC-DOP concentrations decreased with depth: from $40.8 \pm 20.9 \text{ nmol-P wet-g}^{-1}$ at the sediment surface to $3.8 \pm 1.6 \text{ nmol-P wet-g}^{-1}$ at depths of 4–5 cm for IC- PO_4^{3-} ; and from 129.5 ± 41.7 to $10.6 \pm 1.9 \text{ nmol-P wet-g}^{-1}$ for IC-DOP (Fig. 5b). Since the vertical profile of IC-P concentrations followed a trend similar to that of the Chl *a* distribution and the POP profile (Figs. 2b & 3), IC-P concentrations in the sediment may be derived mainly from microalgal communities. Miyata et al. (1986) reported that *Skeletonema costatum*, grown in a chemostat system using media with various N:P ratios, preserved a high concentration of intracellular phosphorus (25–75 mM). The high concentration of IC-P in microalgal

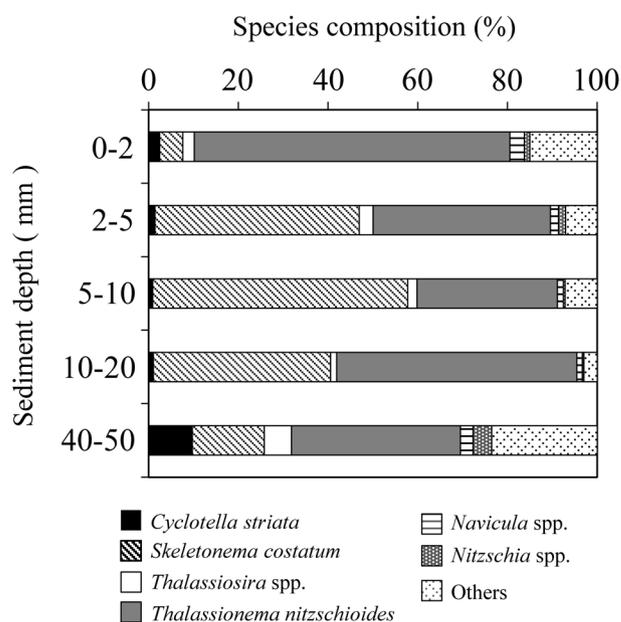


Fig. 4. Vertical distribution of diatom species and their relative abundance in the sediment. The sediment samples were partitioned from the sediment used for cell number counting (Fig. 2a). The cells with unhealthy chloroplasts (i.e. empty, shrunken, or discolored) were considered dead and eliminated from counts.

may explain the higher concentration of the P fraction categorized into IC-P observed in surface sediment in the present study (Fig. 5b). García-Robledo et al. (2010) reported a positive relationship between the microalgae of intertidal flats and intracellular nutrients in sediment, and a potential contribution of intracellular nutrients to primary production. Koh et al. (2007) reported that the highest Chl *a* concentrations and rates of Chl *a* increase in surface sediment occur in winter in the Nanaura intertidal mudflat. Seasonal fluctuation of primary production by microalgae in the present study area (Koh et al. 2007) may be partly related to the seasonal change in IC-P concentration.

To evaluate the reservoir size of each mobile P fraction, vertical profiles of PW- PO_4^{3-} and DOP concentration (Fig. 5a) were converted (nmol-P wet-g^{-1}) via multiplying by the PW volume, calculated based on water content in the sediment (i.e. 70%–80%; Fig. 6). IC-DOP was the most predominant mobile P fraction in the sediment (76% in the top 2-mm layer) and IC- PO_4^{3-} showed the second largest fraction; while PW-DOP and PW- PO_4^{3-} were only minor fractions, especially in surface sediments (Fig. 6). However, at depths of 40–50 mm, IC-DOP and IC- PO_4^{3-} accounted for 47% and 17% of the P fraction, respectively, while the PW-DOP and PW- PO_4^{3-} fractions increased up to 4% and 32%, respectively. The typical Carbon (C):Chl ratio of phytoplankton (C/Chl=15–55, reported by Sathyendranath et al. 2009) and the mean C:P ratio (106, reported by Redfield 1958) were temporarily used to confirm the validity of the IC-P fraction size estimated in the present study. The resulting potential P quota for the microalgal cells in the top

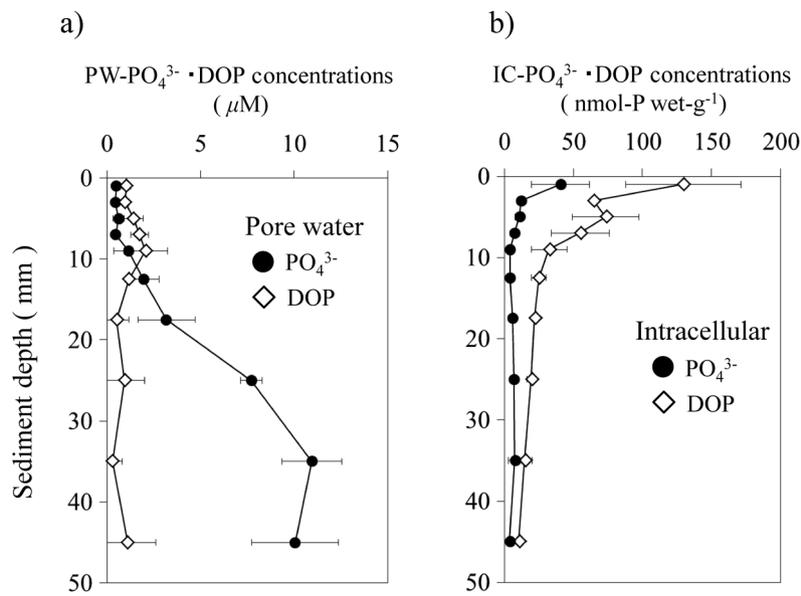


Fig. 5. Vertical profiles of (a) pore water-phosphate (PO_4^{3-}) and dissolved organic phosphorus (DOP), (b) intracellular- PO_4^{3-} , and DOP concentrations in the sediment. Samples were collected and processed in the same manner as the samples for the bacterial cell number and chlorophyll *a* analyses shown in Fig. 2b.

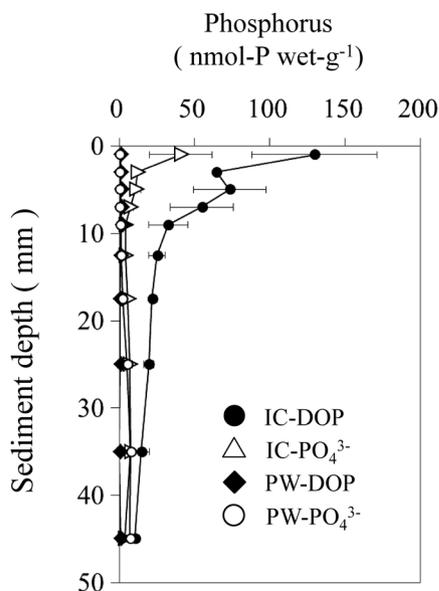


Fig. 6. Vertical profiles of intracellular-dissolved organic phosphorus (DOP) and -phosphate (PO_4^{3-}) pools, and pore water-DOP and - PO_4^{3-} pools in the sediment.

2 mm of sediment ranged from 462 to 1694 nmol-P wet-g^{-1} . Since the sum of the IC-P fraction in the same surface layer ($171.5 \text{ nmol-P wet-g}^{-1}$) accounted for about 10% or 40% of the expected P quota for microalgae, it does not contradict the suggestion proposed here that IC-P mainly originates from microalgal cells.

Fluorescent staining by DAPI further indicated that microalgae in intertidal mud flats actually accumulate polyphosphate in their cells (Fig. 7). Diaz et al. (2008) reported

that a fraction of the total P was accumulated as polyphosphate in plankton cells (7% under natural conditions and 20%–40% under P-replete incubation); thus, it is possible that polyphosphate is an important form of microalgal IC-DOP in intertidal mudflats. DOP is easily available to primary producers and bacteria possessing hydrolase enzymes such as alkaline phosphatase (Yamaguchi et al. 2005, Luo et al. 2009), unlike POP (e.g. detritus) or PIP (e.g. apatite). Therefore, IC-DOP may be an important P fraction in surface sediments. A high concentration of intracellular P and polyphosphate potentially elevates dissolved P concentrations around cells due to exudation, so it is possible that P loading in tidal mudflat sediments is reduced by the promotion of P sequestration as a form of apatite, which is not easily used by marine organisms (Diaz et al. 2008, Goldhammer et al. 2010).

Organism-mediated P fluxes between PW and the IC pool under redox conditions

Results of the incubation experiments showed clear responses to oxic/anoxic cycling. PW-PO_4^{3-} concentrations decreased under oxic conditions and increased under anoxic conditions, while POP concentrations showed the opposite trend (Fig. 8). PIP should have fluctuated with PW-PO_4^{3-} if the major reaction was abiotic (i.e. PO_4^{3-} absorbance as its iron-bound form under oxic conditions and isolation under anoxic conditions). However, PIP concentrations showed no specific trend in response to oxic/anoxic cycling, probably due to incomplete anoxic conditions. Therefore, the fluctuations of POP and PW-PO_4^{3-} in response to mild redox conditions seem to be mainly caused by biological activity, resulting from the balance

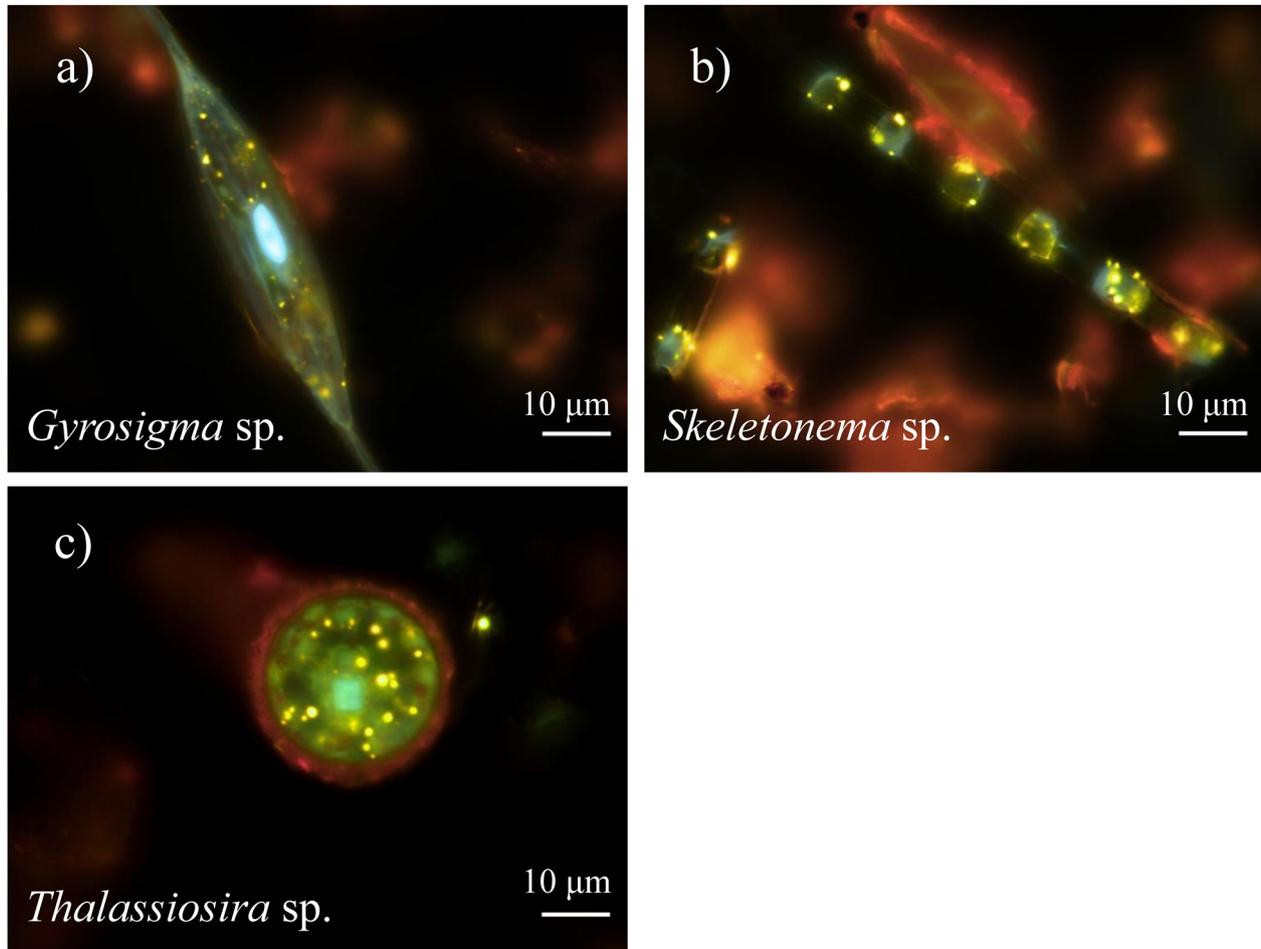


Fig. 7. Microscopic image of DAPI-stained cells of (a) *Gyrosigma* sp., (b) *Skeltonema* sp., and (c) *Thalassiosira* sp. The phytoplankton cells were picked out of the surface sediment at the intertidal mudflat. Bright yellow color indicates polyphosphate.

between biological uptake and decomposition or cell lysis. Although many studies have reported that microalgae take up nutrients even under dark conditions (e.g. Stross & Permick 1974), whether or not activity slows under anoxic conditions is uncertain. However, a variety of bacteria can accommodate their activities to either oxic or anoxic conditions (He et al. 2010, Brock & Schulz-Vogt 2011). Therefore, the decomposition of organic P and the release of IC- PO_4^{3-} to PW associated with viral cell lysis or sloppy feeding by grazers can occur under both oxic and anoxic conditions. It is therefore assumed here that microalgae and bacteria assimilated PO_4^{3-} from the water column under oxic conditions, but that the degradation rate of organic matter and cell lysis exceeded the uptake rate under anoxic conditions.

Oxygen evolution in photosynthesis can occur only in the thin surface layer because light penetration into mud sediment with a median particle diameter of $10\ \mu\text{m}$ is estimated to be limited to within 0.3 mm of the surface (Ichimi et al. 2008b, Li et al. 2009). In Nanaura intertidal mudflat, micro-level vertical profiling of dissolved oxygen (DO) concentrations in July and October reveal that the

mean depth of the oxic layer was limited to 2 or 3 mm from the surface (Sayama 2007), probably due to the limitations of photosynthetic activity and physical O_2 diffusion. Furthermore, the oxic/anoxic interface of the sediment often fluctuates due to sediment resuspension and sedimentation caused by dynamic tidal cycles (Sayama 2007). In the present study, however, the vertical profile of IC-P concentrations showed that, within 4 mm of the surface, IC- PO_4^{3-} decreased by 70% and IC-DOP by 50% (Fig. 6). Considering this, together with the results of the incubation experiments, sedimentation and subsequent migration into the subsurface layer may subject microalgae (which take up nutrients under oxic conditions) to bacterial decomposition leading to IC-P release into PW. It is not clear whether or not abiotic dynamics (e.g. nutrient absorption and/or release from iron-bound compounds, and physical disturbance) contributed to P dynamics in the present study, but oxic/anoxic conditions could also promote P uptake and release by organisms. Further, these processes may also exist around the subtidal area where hypoxia often occurs in bottom waters during summer (Tokunaga et al. 2009).

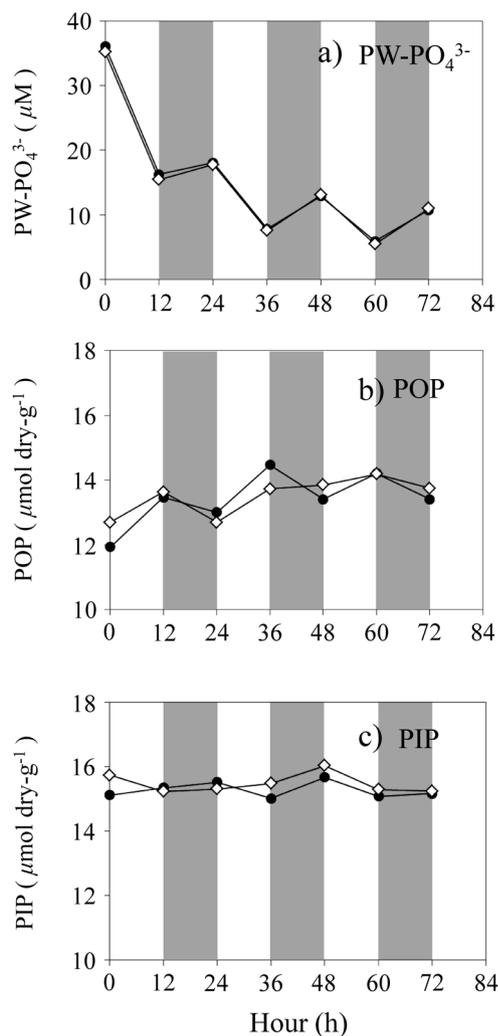


Fig. 8. Time-series variation of (a) pore water-phosphate (PO_4^{3-}), (b) particulate organic phosphorus (POP), and (c) particulate inorganic P (PIP) concentrations. Shaded regions represent the anoxic period, and open regions represent the oxic period. Although PO_4^{3-} was added to sea water to final concentrations of $100 \mu\text{M}$, some portion of PO_4^{3-} seemed to be adsorbed immediately on the sediments.

In summer, when high temperatures enhance bacterial respiratory activity and the total benthic algal productivity of surface sediments decreases (Koh et al. 2007), probably due to photoinhibition and predation by mudskippers, the degradation rate should increase compared to photosynthetic productivity. As a result, the reduced layer reaches the surface sediment and PW-PO_4^{3-} concentrations are maintained at a higher level (Koriyama et al. 2009). Therefore, in summer, the PW-PO_4^{3-} diffusion flux into overlying water probably becomes the predominant P dynamic at the sediment–water interface. However, in winter, when bacterial activity is low, the oxidized zone expands by a few centimeters from the surface (Koriyama et al. 2009), the depth of which is also affected by physical disturbances. Therefore, the biological uptake/release of dis-

solved P in response to oxic/anoxic conditions can be important in P dynamics at the sediment–water interface, especially in winter. Additionally, because the temperature of the surface sediment drops below freezing during exposure at low tide, especially at night in winter, cell disruption and the subsequent release of cytoplasmic components into the PW may play a role in P dynamics at the sediment–water interface.

Benthic microalgae in the tidal mudflats of Ariake Bay are important food sources for filter feeders (Yoshino et al. 2012). In addition to their important role as sources of food, the planktonic and benthic microalgae may also be important as P reservoirs and sources of PO_4^{3-} on the tidal flats, where excess N is generally supplied from the river.

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