Phytoremediation of shallow organically enriched marine sediments using benthic microalgae

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Abstract

We examined whether replantation of benthic microalgae (BMA) can remediate shallow organically enriched sediment. *Nitzschia* sp., the dominant species in the tested area (Hiroshima Bay, Japan), was isolated and mass cultured, then replanted in the same area. Changes in the condition of the sediment were monitored for five months. During the study period, we observed an increase in redox potential (ORP) and a decrease in acid-volatile sulfide (AVS) in the experimental area, indicating that the sediment condition changed from reduced to oxic. Organic matter in the sediment, represented by chemical oxygen demand (COD), ignition loss (IL) and organic nitrogen (ON), decreased significantly, while inorganic nutrients (ammonia and phosphate) increased in the interstitial water. These changes imply that oxygen produced by the replanted BMA may have enhanced aerobic bacterial activity, accelerating the decomposition of organic matter. Thus, replantation of BMA shows potential as a novel and promising "phytoremediation" method for organically enriched sediment.

Keywords: Benthic microalgae; Hiroshima Bay; *Nitzschia*; Remediation; Sediment

1. Introduction

In the Seto Inland Sea of Japan, organic matter and inorganic nutrient loads as well as the influx of toxicants have been strictly regulated since 1973 by the Special Law for Conservation of the Seto Inland Sea. As a consequence, water quality has been recovering in the entire area with the exception of only a few local areas. Yamamoto (2003) suggests that the pelagic system may be turning oligotrophic. Compared to water quality, the response of sediment quality to the measures taken to reduce the material load has been much slower because material transport in sediments is restricted by molecular diffusion. There are no remaining areas whose sediments are contaminated with toxic substances (SECA, 2004). Therefore, the final issue that should be solved in the Seto Inland Sea of Japan is how to remove organic matter from sediments accumulated in the innermost part of bays and harbors such as Osaka Bay and Hiroshima Bay etc., as reported in other developed countries (e.g., Ho et al., 2002).

The major methodologies that have been used to date to remediate organically enriched sediments have been civil engineering-oriented ones such as dredging (the excavation of contaminated sediments) and sand covering. These “surgical” methods are very direct and effective, but are not environmentally friendly; dredging removes all living things from the sediment, and sand covering may have a deadly affect on living things. Furthermore, sand covering consumes significant resources inasmuch as it requires sand imported from other areas, which may destroy the environment at the place of collection and which may also import problematic organisms together with the sand. Although the consequences of these civil engineering methods do not generally last longer than several years, they are costly (den Besten and van den Brink, 2005).

The method we propose in the present study is based on biological/ecosystem engineering and makes use of benthic microalgae (BMA); that is, it is a "phytoremediation" method (Suresh and Ravishankar, 2004) which can be thought of as a sort of herbal or internal medicine to cure the deteriorated environment. This method is much milder and more environmentally friendly for living things than the civil engineering-oriented methods mentioned above. A semi-enclosed sea such as the one examined in the present study can be considered to be a total system consisting of both a pelagic ecosystem and a benthic ecosystem; hence, the remediation of sediment quality is important because it supplies habitats for benthos which may establish a linkage between the pelagic and benthic ecosystems.

BMA generally have a higher nutrient uptake rate and effective photosynthetic ability even under low light intensity (e.g., Yamamoto et al., 2004). These characteristics may reflect their typical environmental conditions of shallow organically enriched sediments containing ample nutrients in the interstitial water and dim light at the sediment surface. While beds of sea grass or weed and tidal flats are considered to be symbolic sites which should be conserved in coastal seas, little attention has been paid to subtidal zones deeper than tidal flats and sea grass beds, which have thus been called "secret garden" (MacIntyre et al., 1996). BMA are likely to be ubiquitous phyta distributed across a wide area which receives dim light (Cahoon, 1999). Most BMA are attached diatoms which reside even on the surface of *Zostera* leaves and on the surface of tidal flats as well as on the submerged sediment surface (Barranguet et al., 1998; Heffernan and Gibson, 1983; MacIntyre et al., 1996). In *Zostera* beds, the primary organism in terms of production may be BMA rather than *Zostera*, though the biomass of BMA is lower (Moncreiff et al., 1992). Thus, BMA (that is, the attached diatoms) may be a key component which governs material cycles in shallow coastal seas.

To the best of our knowledge, the present study is the first to use BMA in the remediation of organically enriched sediments. To date, promotion of decomposition of organic matter has been attempted and achieved using a capitellid polychaete, *Capitella* sp. for the marine bottom sediment below fish net pen culture (Tsutsumi and Montani, 1993). Introduced method in the present study is similar to theirs but a little different in terms of supplying oxygen and uptake of inorganic nutrients from the sediments as mentioned below. It would be the matter to be chosen depending on the condition of the targeted area. In our study site, oxygen in the overlying water is usually depleted in mid summer. Therefore, introduction of mass cultured capitellid polychaete to the area will be no help to remediate the sediment, because most benthic animals cannot survive.

Theoretical backgrounds of our method consist of the following processes. The replanted microalgae produce oxygen by photosynthesis and cause to accelerate bacterial activities of aerobic decomposition of organic matter accumulated in the surface sediments. At the same time, the BMA take up nutrients from the interstitial water of the sediments. However, the growth of BMA means actually an increase of organic matter. Therefore, the increased organic matter should be removed by any process, for example, by grazing (through food web) and/or by increasing in the release flux of dissolved constituents and/or releasing gases into the overlying water/air (denitrification).

Thus, the three aspects that were used to evaluate the "remediation" of organically enriched sediments in this study were the followings: oxidation of reduced sediment conditions; decomposition of organic matter in the sediments as a result of aerobic decomposition; and the net removal of elements from sediments, which is achieved by biological/physical processes mentioned above. We present here the results of our field study, which, to the best of our knowledge, is the first phytoremediation trial in which BMA was applied to the natural field.

2. Materials and methods

The dominant species, *Nitzschia* sp., was isolated from surface sediment at ca. 3 m depth in the innermost area of Hiroshima Bay, Japan, and mass cultured with glass beads of 100 μm in diameter (BZ-01; As One Co., Osaka, Japan) in f/2-medium (Guillard and Ryther, 1962). *Nitzschia* sp. was selected because of its dominancy in two observations which were carried out as a preliminary step in our research in August 2001 and October 2001 (Yamamoto et al., 2004). The mass-cultured *Nitzschia* sp. was replanted to their original site where the present experimental study is carried out to prevent ecosystem disturbance by replanting to other areas, according to the concept mentioned in the treaty established in the Convention on Biological Diversity held in 1992 (http://www.biodiv.org/default.shtml). Characteristics of physiological responses of the *Nitzschia* sp. strain used in the present study to temperature, salinity and light intensity have already been published in our previous study (Yamamoto et al., 2004).

Field experiments were carried out for five months from August 20, 2003 to January 20, 2004, at the innermost area of Hiroshima Bay where the sediment is enriched with organic matter. The experimental site is illustrated in Fig. 1. About one month prior to initiating the experiments, an experimental area of 9 m^2 (3 m x 3 m) on the seafloor was marked out by poling the corners and surrounding the area with ropes, and another area of the same size was also marked out in the same manner as a control; the control area was located 13 m from the edge of the experimental area. The silt/clay fraction (< 0.075 mm in diameter of grain) occupied 90.9% in both experimental area and control area, indicating the same condition at the beginning of the experiments. Only 13 m apart each other is also meaning there may be no significant difference in terms of sedimentation of particulate matter during the experiments. Nine 1-m^2 sub-areas were prepared in each area with poles and ropes. The depth of the sites was approximately 3.0 m, with tidal range of 0.5 m at neap tide to 3.7 m at spring tide.

Mass-cultured *Nitzschia* (usually in the exponential growth phase) grown with glass beads was replanted along with the beads as evenly as possible on the sediment surface of the experimental area by a diver. We intended to carry out replantation 7 times, at 20-day intervals during the experimental period, however, one scheduled replantation (November 21, 2003) was cancelled because of an insufficient period (10 days) for the growth of cells. The temperature conditions for mass culture were roughly adjusted to the temperature *in situ* based on the information supplied by the Hiroshima Fisheries Institute.

Observation and sampling were carried out 9 times in total, including 7 times that were initially scheduled replantation times with beginning and end of the experiments at which *Nitzschia* sp. was not sowed. Sediment samples were collected by the diver during each observation and sampling session. At each sampling time, replantation was carried out after all observation and sediment samplings had been completed. Since one 1-m^2 sub-area was used each time for sediment sampling, the total remaining area for replantation was decreased every time. The amount and density of *Nitzschia* sp. are summarized in Table 1.

Six sediment cores were collected in non-disturbed condition with acrylic tubes 7.5 cm in diameter from the designated 1-m^2 sub-area of both the experimental and control sites at every sampling time. Three of the sediment cores from each site was used to identify and count BMA cells. The presence of a thin (usually less than 3 mm) brown surface layer was confirmed by visual inspection and was collected with a spatula into a small plastic container, where it was fixed with formaldehyde solution (3% in the final concentration). Later, BMA were identified and the cell number was counted under a microscope in the land laboratory.

The other 3 sediment cores were sliced every 1 cm from the surface to 3 cm and placed in small plastic containers. Temperature (data not shown), pH (data not shown) and oxidation-reduction (redox) potential (ORP; PS-112C, RM1; TOA Electronics, Ltd., Tokyo, Japan) were measured for the sample from the uppermost layer. Next, each container was capped with an air-tight lid and sealed with plastic film (Parafilm; American National Can Co., Chicago, IL, USA) to prevent oxidation. The sealed sediment samples were kept in a cool, dark container and transported to the land laboratory. The uppermost 1-cm layers of the samples were used to measure acid-volatile sulfide (AVS) using a Hedrotech-S Kit (Arakawa, 1980) and chemical oxygen demand (COD; JEMCAA, 1996) on the same day. On the day following sampling, ignition loss (IL) was determined by combusting the sediments at 600^{o}C for 3 hrs (Koyama, 1982) after determining both wet weight and dry weight (110 ^{o}C for 12 hrs). Organic nitrogen (ON) content was also determined for the dried samples with an elemental analyzer (CN-corder MT-700; Yanaco Co., Ltd., Kyoto, Japan). Total nitrogen (TN) was also determined following the method described by JEMCAA (1996).

All three layer samples were used to determine the concentrations of dissolved inorganic nutrients (ammonia and phosphate) in the interstitial water. After centrifugation (3,000 rpm, 15 min) of the sediment, the methods described by Strickland and Parsons (1972) were applied for the analyses. The average values and standard deviation among the three layers are given in the *Results* section below.

As mentioned in the *Introduction*, sediment remediation was evaluated in the present study by examining three factors. First, remediation was evaluated with respect to increases in ORP and decreases in AVS concentration. Next, we examined decreases in COD, IL and ON. Decreases in organic matter are also supported by increased dissolved inorganic nutrients in the interstitial water. Finally, we evaluated decreases in TN concentration. We selected nitrogenous compounds for evaluation primarily because nitrogen brings about denitrification as a natural purification process.

3. Results

The cell density of *Nitzschia* sp. in the experimental area was found to be increased in comparison to that in the control area, showing a peak density of ca. $4x10^4$ cells/cm^2 (Fig. 2a). In the control area, on the other hand, the cell density of *Nitzschia* sp. was fewer than $5x10^3$ cells/cm^2

during the entire experimental period. The cell density of *Nitzschia* sp. in the experimental area decreased to fewer than $1x10^4$ cells/cm^2 on 16 December, reflecting the missed replantation on 21 November. However, at the time of the next sampling, the cell number had increased to $2.5x10^4$ cells/cm^2 due to the large replantation on 16 December ($2.9x10^5$ cells/cm^2). Although the density of sowed *Nitzschia* sp. ranged from 0.52 to $2.9x10^5$ cells/cm^2 at each replantation, the cell density observed at each following sampling was ca. $0.1\text{-}0.4x10^5$ cells/cm^2. Since the total cell density of BMA including *Nitzschia* sp. ranged from 5 to $10x10^5$ cells/cm^2 (Fig. 2b), the replanted *Nitzschia* cells were approximately one order lower than the total cell number in the natural assemblage, and the cell number of *Nitzschia* sp. observed at the next sampling constituted approximately 1/5 of the natural assemblage.

The ORP showed a significant difference between the experimental and control areas at the end of the experimental period, even though no difference was observed before the end (Fig. 3a). At the end of the present experiments (20 January 2004), the ORP value had become positive in the experimental area, indicating that the sediment condition had changed from anoxic to oxic. In contrast, in the control area, the ORP value remained around zero in January.

Although AVS conditions were not identical in the experimental and control areas at the beginning of the experiment, the AVS value decreased more in the experimental area (0.7 mg/g-dw during the study period) than in the control area (0.5 mg/g-dw), as shown in Fig. 3b. Furthermore, an overall decreasing trend was observed during the study period in the experimental area, while in the control area, the AVS values remained almost constant until showing a marked decrease at the end of the study period.

COD, which was also different between the experimental and control areas at the beginning of the study, decreased in the experimental area from 26.4 to 23.6 mg/g, but increased from 21.5 to 31.1 mg/g in the control area (Fig. 4a). A clear decreasing trend was also observed in the experimental area in IL, ON and TN (Figs. 3b-3d). IL values were approximately 10% at the beginning of the study period and decreased to around 6%, while remaining almost constant in the control area at approximately 10% (Fig. 4b). The changes in ON and TN were very similar to each other, showing marked decreases in the experimental area, but only small decreases in the control area (Figs. 3c and 3d). In the experimental area, TN decreased from 2,850 to 1,770 mg/kg.

Comparing the values of ON and TN, it appears that most of the total nitrogen in the sediment consisted of organic nitrogen.

In contrast to the case of organic substances, concentrations of inorganic substances (ammonia and phosphate) in the interstitial water of the sediment clearly increased in the experimental area during the study period (Fig. 5). Except at the initial and final sampling times, ammonia levels ranged from 6 to 12 mg/l in the experimental area, while those in the control area ranged from 2 to 6 mg/l. Phosphate in the experimental area fluctuated between 1 and 2.6 mg/l in the experimental area, but remained at less than 1 mg/l in the control area. Thus, both ammonia and phosphate concentrations were almost double in the experimental area compared to those in the control area.

4. Discussion

The processes expected to occur in the surface layer of the sediment due to replanting *Nitzschia* sp. are summarized in Fig. 6, which also shows processes expected to occur without replantation. The mass cultured BMA, *Nitzschia* sp., which were in the exponential growth phase when they were sown, may have produced significant amounts of oxygen through their photosynthesis. This increased oxygen supply may have changed the reduced sediment conditions to oxic conditions, represented by increased ORP and decreased AVS values (Fig. 3). Thus, oxidation, the first stage of sediment remediation, was successfully achieved.

Oxidation of the sediment may have activated aerobic bacterial activity, resulting in an enhancement of the decomposition of organic matter indicated by the decreases in COD, IL and ON in the surface sediment (Fig. 4). These results indicate that the second stage of sediment remediation, decomposition of organic matter, was accomplished. The decomposition of organic matter by enhanced aerobic bacteria may have led to the observed increases in the ammonia and phosphate concentrations in the interstitial water, as shown in Fig. 5. Some of these increased nutrients may be taken up by BMA including *Nitzschia* sp., and the rest would be released by diffusion. It remains unclear whether the nutrient release rate was accelerated by the enhancement of organic matter decomposition, because sowed *Nitzschia* sp. are expected to take them up. Without *Nitzschia* sp. replantation, the overlying water may be anoxic/hypoxic, with a generally high release flux of nutrients. A high nutrient concentration in the interstitial water gives a higher nutrient release rate if we use the Fickian law to calculate the release rate (Yamamoto et al., 2000). It remains necessary to carry out calculations using a budgeting approach or numerical modeling to estimate the exact release flux.

Decrease in TN is only the index that was measured in the present study which may prove the net removal of elements defined as the third stage of sediment remediation. Sowed *Nitzschia* cells (including other natural BMA species) may be consumed by colonized benthic animals, which is suggested by the decrease in cell density at each observation time compared to those of the sowed numbers of cells. Colonization by benthic animals is generally supported by aerobic conditions. Under anaerobic conditions, toxic substances such as hydrogen sulfide limit the number of inhabiting species and their density, preventing colonization by most benthic animals. At the time of writing, we have not yet completed a full quantitative assessment; however, increased numbers of benthic animals might have then been consumed by fish, which may have contributed to the net decrease in biophilic elements in the sediment.

Replantation of BMA changes the pattern of material circulation, as illustrated in Fig. 6. In an ecosystem with low-density BMA, the decomposition of sedimented phytoplankton cells would lead to totally anoxic conditions, including toxic substances with time, and nutrients released from the sediments would again be utilized by phytoplankton in the water column. This creates a "negative spiral" which depletes the number of living organisms in the ecosystem. On the other hand, increased BMA due to replantation may depress the nutrient flux by taking up nutrients that are produced by the decomposition of organic matter. This may inhibit phytoplankton blooms in the water column. As mentioned earlier, an increase in nutrient release flux by replantation, would account for one possible mechanism of net removal of elements from the sediment. However, nutrient uptake activity of BMA appears to decrease the release flux from the sediments. Denitrification is also an important path of net removal of nitrogen from the system. Although quantitative evaluation was not conducted in the present study, we are now constructing a numerical model that will evaluate material flow in the system quantitatively.

Replantation of BMA and the resultant enhancement of their primary production indicate that reduced condition of organic rich sediment can be changed to oxic; this then changs the material flow in the system by creating a "positive spiral", leading to remediation of organically enriched sediments. Given the present results, we propose replantation of BMA as a novel and promising phytoremediation method of organically enriched sediments in shallow coastal seas. Furthermore, the proposed ecosystem engineering method is milder and more environmentally friendly than the civil engineering methods which are currently employed.

Acknowledgements

The authors would like to thank the Nippon Foundation for their total financial support of this study.

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Figure legends

Fig. 1. A map showing the western part of Japan and the site where the field experimental study was carried out. The experimental site is apart from the control site by 13 m and both are ca. 10 m from the coast.

Fig. 2. Temporal changes in the cell density of (a) *Nitzschia* sp. and (b) total benthic microalgae (BMA) in the surface sediments (brown oxic layer) of the experimental area (filled circles) and of the control area (open circles) during the study period (August 20, 2003 to January 20, 2004). Symbols and bars are averages and standard deviations of triplicate countings.

Fig. 3. Temporal changes in (a) oxidation reduction potential (ORP; mV) and (b) acid-volatile sulfide (AVS; mg/g) of the surface 1-cm layer sediments in the experimental area (filled circles) and in the control area (open circles) during the study period.

Fig. 4. Temporal changes in (a) chemical oxygen demand (COD; mg/g), (b) ignition loss (IL; %), (c) organic nitrogen (ON; mg/kg) and (d) total nitrogen (TN; mg/kg) of the surface 1-cm layer sediments in the experimental area (filled circles) and in the control area (open circles) during the study period.

Fig. 5. Temporal changes in (a) ammonia (NH_4; mg/l) and (b) phosphate (PO_4; mg/l) concentrations in the interstitial water of the surface sediments in the experimental area (filled circles) and the control area (open circles) during the study period. The average value (filled and open circles) and standard deviation (bar) for the three sediment layers (0-1, 1-2 and 2-3 cm) are shown.

Fig. 6. Possible processes occuring in the organically enriched surface sediment and the overlying water. (a) Natural conditions (control) without replantaion of BMA, and (b) with replantation of mass cultured BMA. The major paths and processes expected to occur due to replantation of BMA are illustrated with thick lines. BMA produce oxygen by photosynthesis, which change the sediment conditions from anoxic to oxic. This enhances the activity of aerobic bacteria, resulting an acceleration of decomposition of organic matter. Inorganic matter (nutrients) then increases in the interstitial water of the sediments, and would be partly utilized by BMA, depressing the release rate to the overlying water. Increased BMA can be

grazed by benthic animals which have colonized due to oxic sediment conditions. The benthos, in turn, would be consumed by fish.

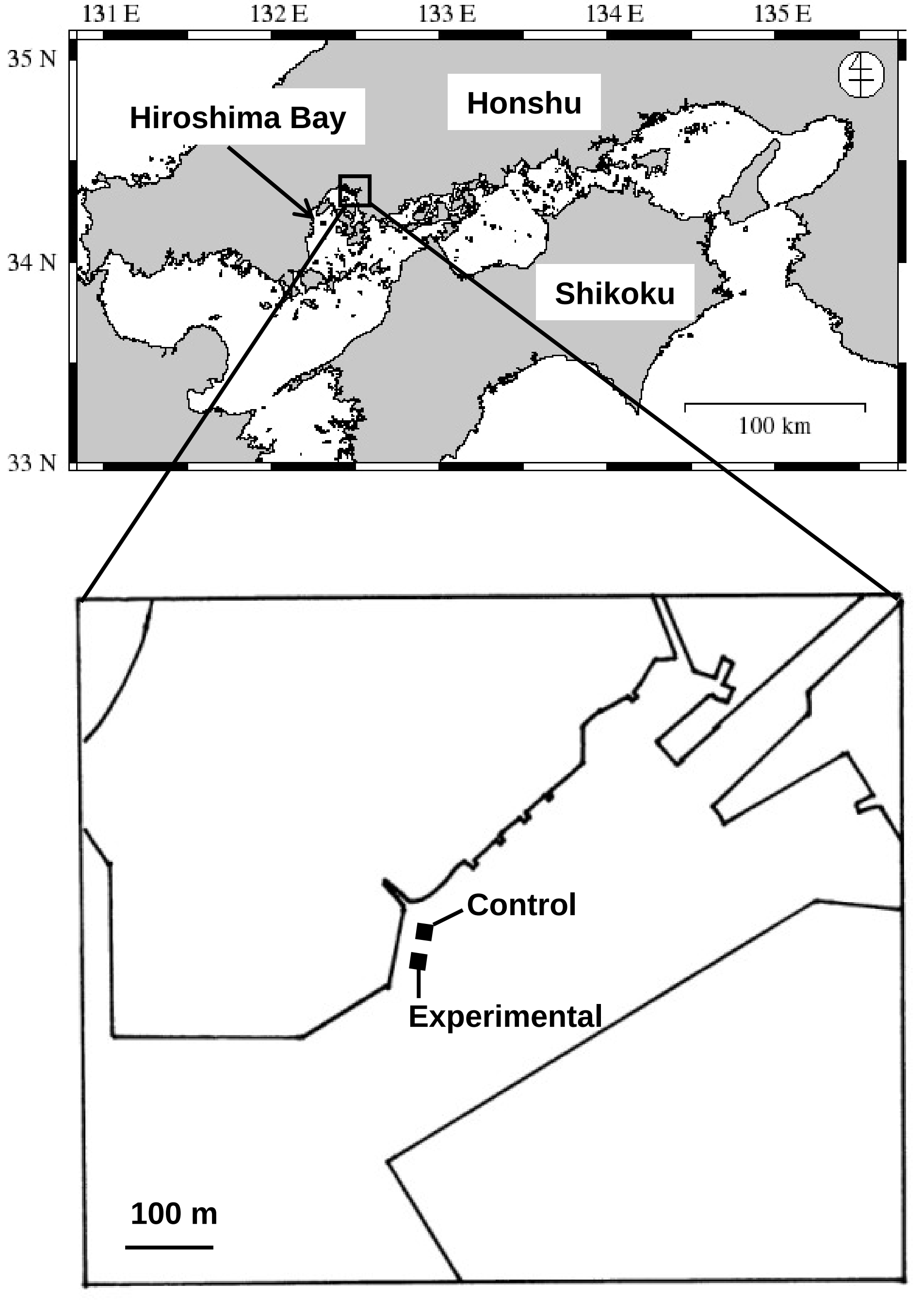

Fig. 1
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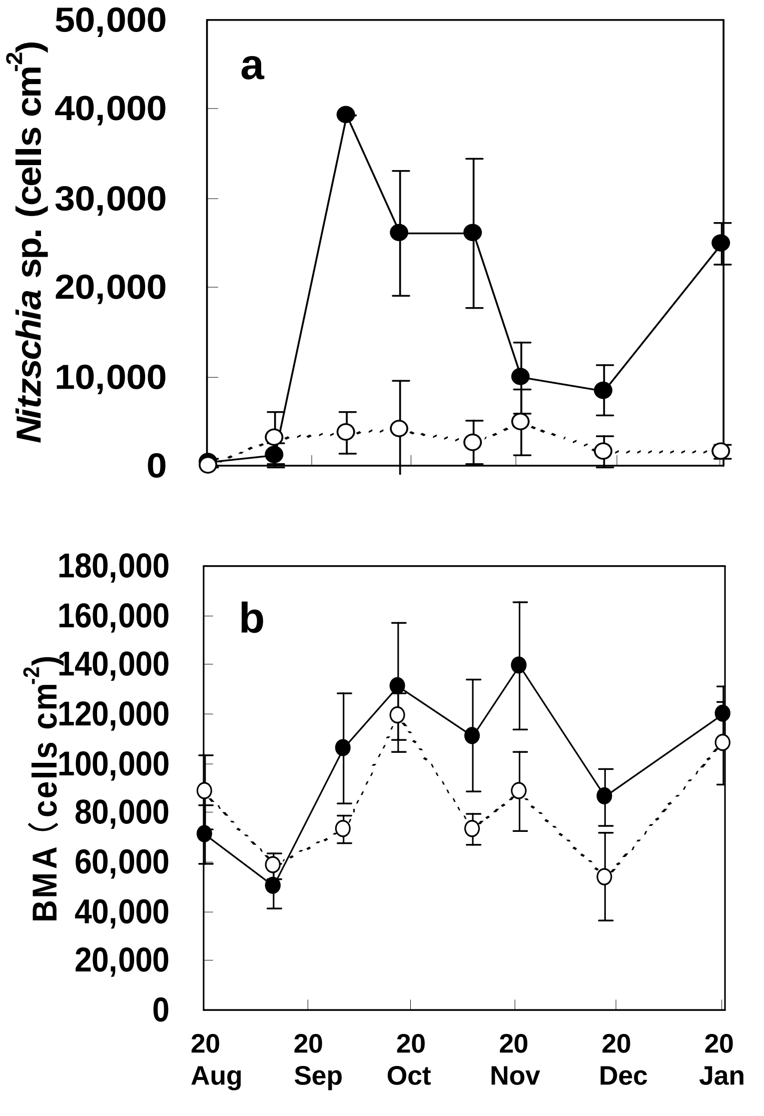

Fig. 2
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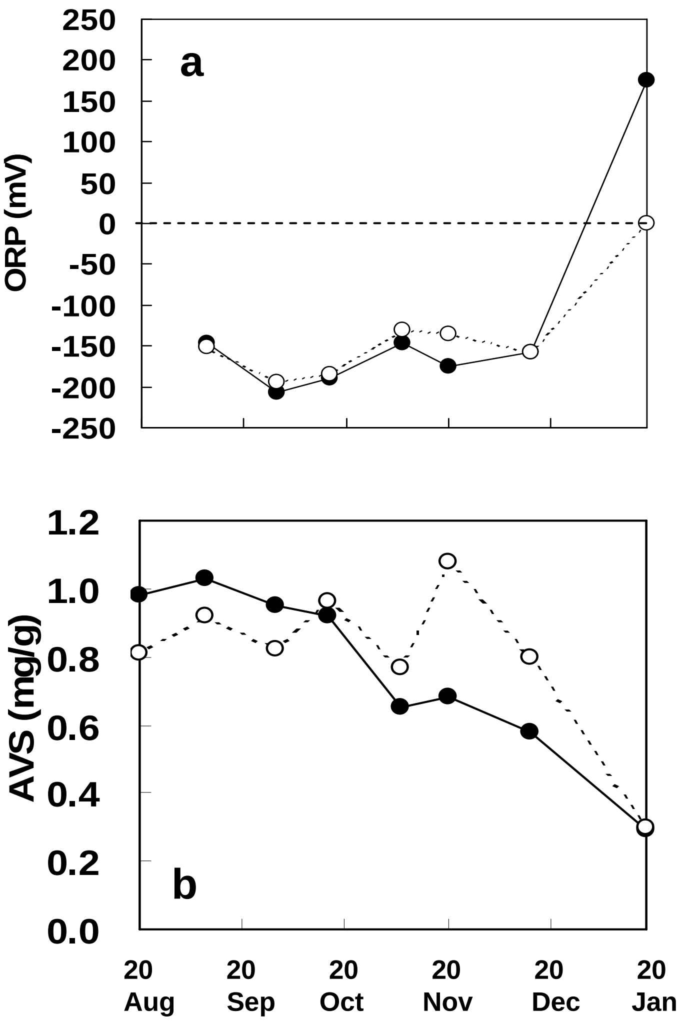

Fig. 3
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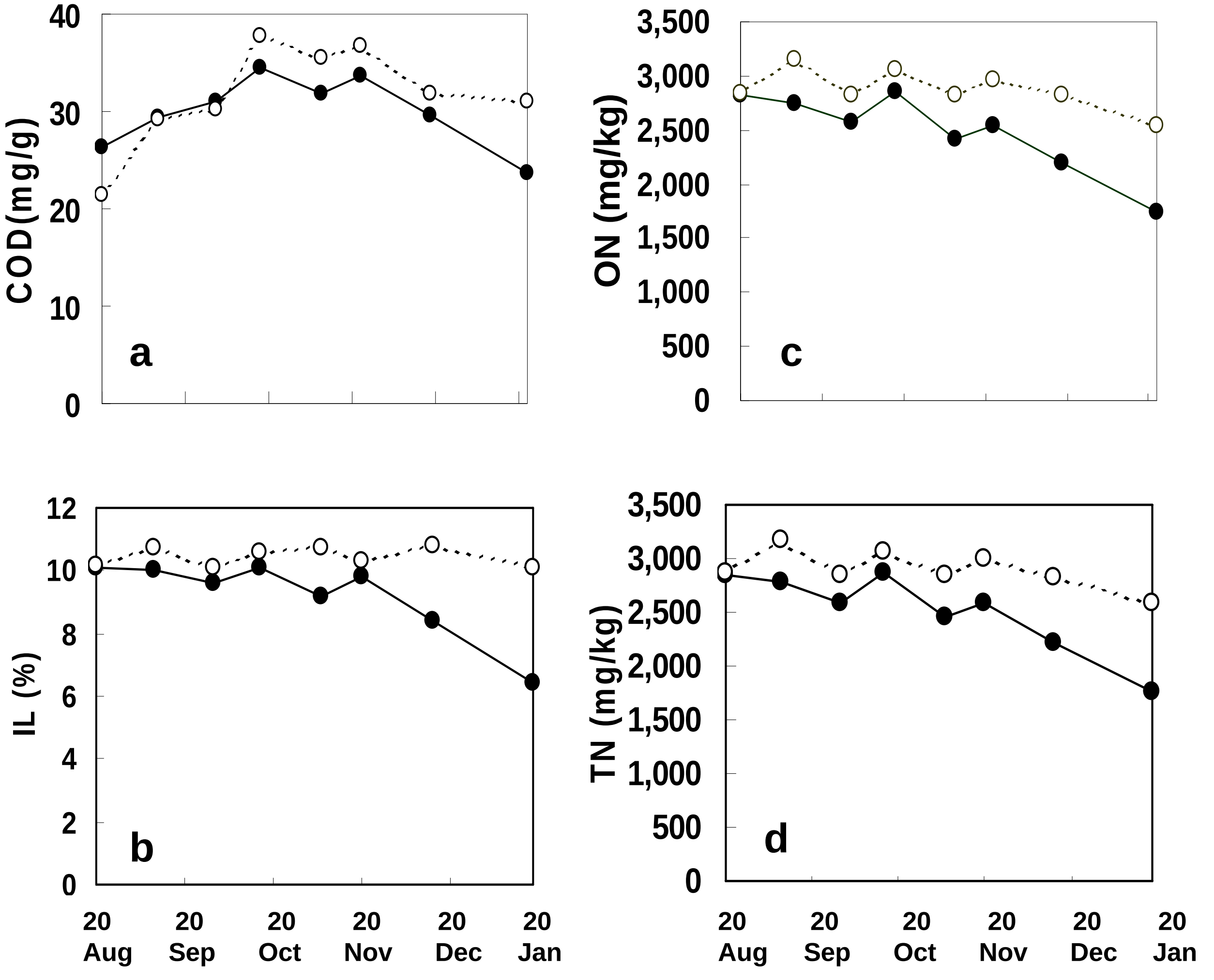

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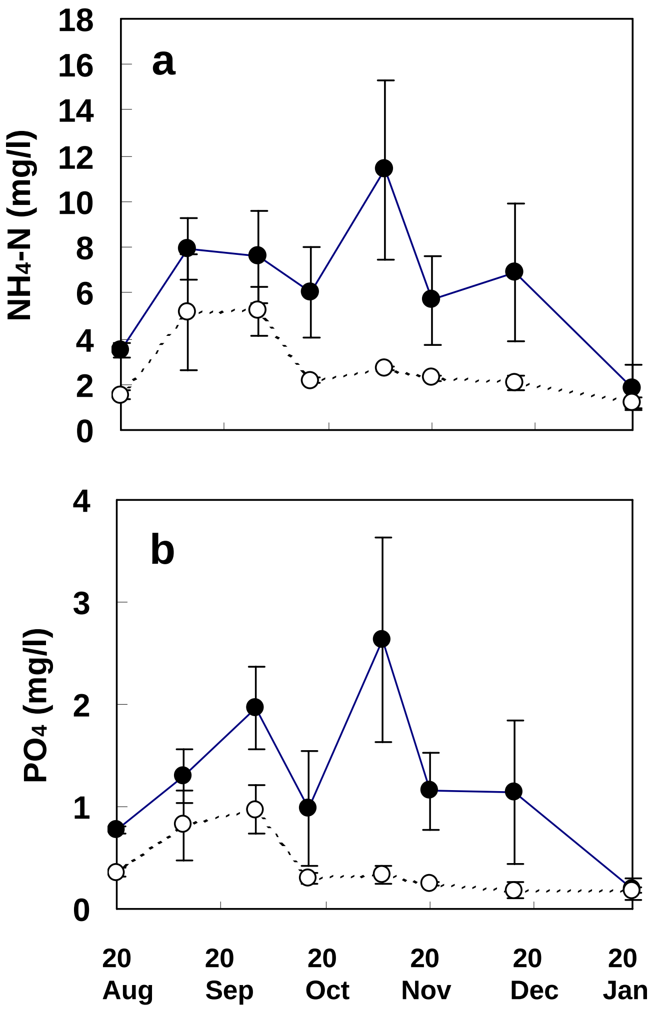

Fig. 5
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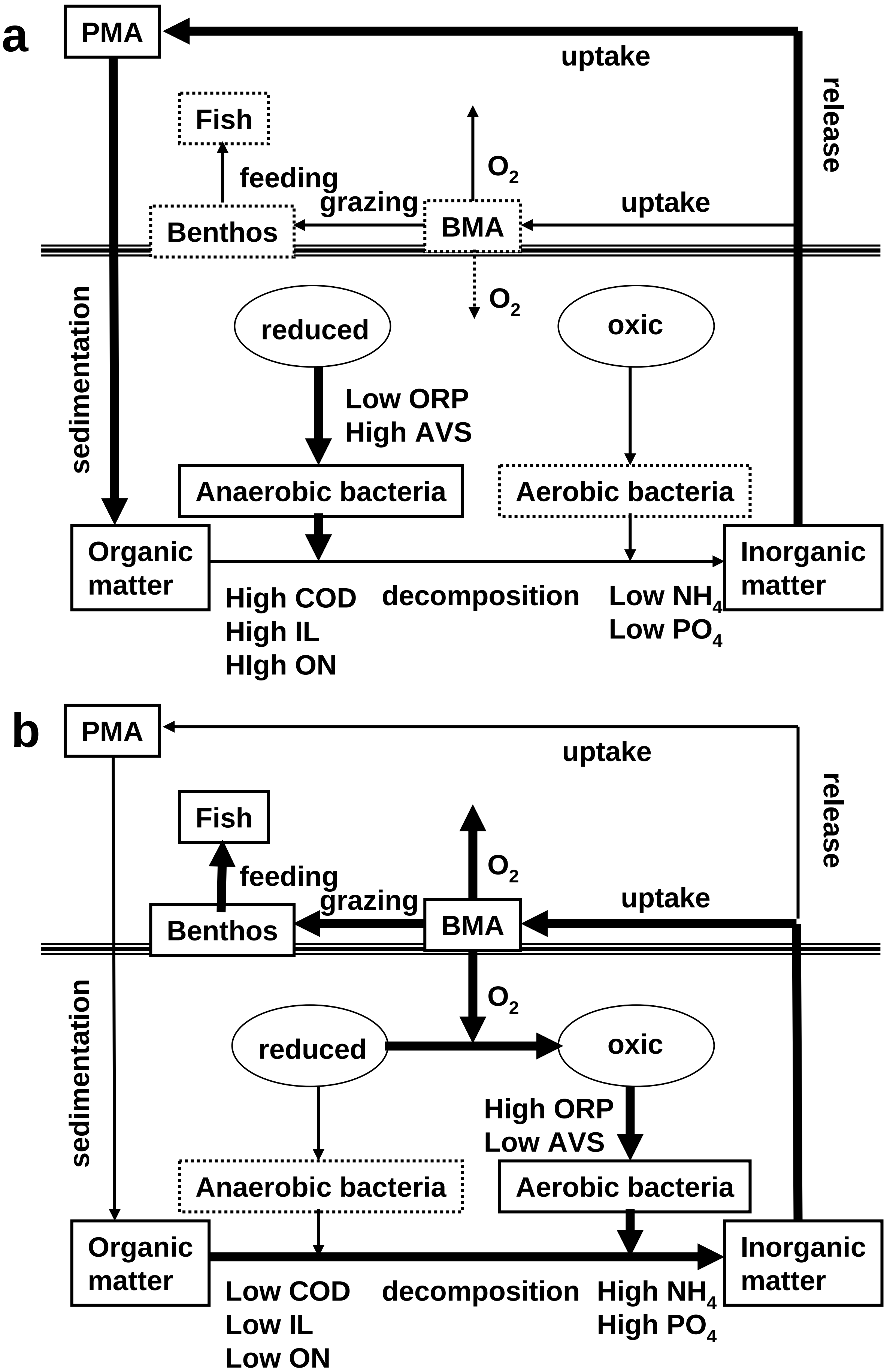

Fig. 6
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Table1. Cell number of *Nitzschia* sp. sowed, the area where *Nitzschia* sp. sowed, and calculated cell density of *Nitzschia* sp. per unit area.

Date	20-Aug	9-Sept	30-Sept	16-Oct	11-Nov	21-Nov	16-Dec
Nitzschia sp. sowed ($\times 10^9$ cells)	4.1	6.0	5.9	5.6	4.0	0.0	5.8
Area sowed (m^2)	8	7	6	5	4	0	2
Cell density of *Nitzschia* sp. ($\times 10^5$ cells/cm^2)	0.52	0.85	0.98	1.1	1.0	0.0	2.9