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Original Article

Influence of River Inflow and Microbial Activity on Distribution of Dissolved Organic Carbon in the Northern Part of Ariake Sea, Kyushu, Japan

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ABSTRACT

To identify the factors controlling the distribution of dissolved organic carbon (DOC) in the northern Ariake Sea (Japan), we measured DOC, salinity and chlorophyll *a* (Chl. *a*) along transect lines from the largest river discharging into the Ariake Sea (the Chikugo River) to the opposite shore. The DOC concentration was significantly correlated with salinity, although no correlation was found between the Chl. *a* concentration and DOC. Thus, river inflow is the primary source of DOC. However, the expected concentration of riverine DOC, represented by the y-intercept of the regression curve between salinity and DOC, was much higher than the DOC concentration of the Chikugo River, suggesting the presence of additional DOC sources to the Ariake Sea. We conducted particulate organic matter (POM) decomposition experiments and observed DOC production after incubation. Thus, microbial POM decomposition may be a source of excess DOC. This study is the first to show that river inflow and microbial decomposition of POM affect the DOC distribution in the northern Ariake Sea.

Keywords: dissolved organic carbon, river inflow, microbial transformation, Ariake Sea, Chikugo River

INTRODUCTION

Dissolved organic matter (DOM) represents an important carbon and nitrogen pool in aquatic systems [1]. DOM supplies energy and nutrients to microbes and phytoplankton, while excess DOM may contribute to coastal eutrophication and hypoxia [2,3]. Thus, understanding the factors controlling DOM supply and distribution could contribute to prediction of future environmental changes in coastal ecosystems. Dissolved organic carbon (DOC), the carbon component of DOM, is a major organic carbon pool in the ocean [4]. Fluctuations of DOC concentrations in estuaries are controlled by complex systems involving the interplay of autochthonous production, mixing and advection via river inflow and tidal currents [5]. However, few studies have analyzed the simultaneous effects of these factors on organic matter dynamics in coastal ecosystems.

The Ariake Sea is one of the most productive semi-enclosed bays in Japan, with high inputs of nutrients and organic matter from numerous inflowing rivers [6]. In addition to its high autochthonous production, the Ariake Sea also receives large amounts of particulate organic carbon (POC) from these rivers [7]. High levels of POC accumulate in the northern portion of the bay due to allochthonous organic matter transport via tidal currents and high primary productivity in this area [7,8]. Recently, severe summer hypoxia has occurred frequently in the northern Ariake Sea, resulting in severe deterioration of the ecosystem [9,10]. A potential cause of this frequent hypoxia is an increase in organic matter production [11]. A contribution of POC decomposition to

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oxygen consumption has been reported repeatedly [8,12,13]. Recently, we reported a relatively large contribution of DOC decomposition to oxygen consumption and concluded that DOC decomposition is a driver of hypoxia in the northern Ariake Sea [14]. However, no data are available on the dynamics and sources of DOC in the Ariake Sea.

The Chikugo River is the largest river discharging into the Ariake Sea. This river flows into the northern Ariake Sea at an annual average flow rate of 115.1 m^3 s^{-1} [15]. Although the Rokkaku River (annual average flow rate, 4.36 m^3 s^{-1}) [16] and the Shiota River (average flow rate prior to the rainy season calculated from dam discharges, 0.55 m^3 s^{-1}) [17,18] also discharge into the northern Ariake Sea, the flow rates of those rivers are much lower than that of the Chikugo River. We recently reported relatively high DOC concentrations in the Chikugo River [19]. The relatively high flow rate and DOC concentration of the Chikugo River led us to infer that allochthonous DOC exported from the Chikugo River contributes substantially to the DOC dynamics of the northern Ariake Sea. However, the effects of allochthonous DOC export from the Chikugo River on DOC dynamics in the Ariake Sea remain unclear due to a lack of DOC data for rivers flowing into the northern Ariake Sea.

In general, the production of autochthonous DOC from phytoplankton biomass is considered an important source of DOC in the ocean. DOC is released directly into seawater from phytoplankton via exudation, especially during senescence and decay [20]. In the northern Ariake Sea, the chlorophyll *a* (Chl. *a*) concentration, an indicator of phytoplankton biomass, was consistently high (> 10 μg L^{-1}) [14]. In addition, a considerable amount of DOC was released by the red tide-causing raphidophyte *Chattonella marina* isolated from the Ariake Sea, as reported in a previous study [21]. Thus, autochthonous production is also an important DOC source in this area.

In-situ production of DOC through allochthonous and autochthonous POC decomposition by bacteria also affects DOC dynamics [22]. High levels of POC accumulate in the northern portion of the bay due to allochthonous organic matter transport on tidal currents along with the high primary productivity in this area [7,8]. In addition, POC in this area is highly bioavailable, with 32–77% of POC being decomposed within 7 days [14]. Thus, DOC release during POC decomposition may contribute to the DOC distribution in the study area.

Here, we present data on DOC, as well as salinity and Chl. *a* concentration, which were obtained by monitoring two transects from the Chikugo River mouth to the northwestern Ariake Sea. In addition, POM decomposition experiments were conducted to test whether DOC is released during POC decomposition. Based on the field monitoring and experimental results, we discuss the factors controlling DOC distribution in the northern Ariake Sea.

MATERIALS AND METHODS

Transect monitoring

Water samples were collected at five stations located in the northern Ariake Sea from the mouth of the Chikugo River to the opposite shore (**Fig. 1**) on June 21 (before the rainy season) and October 29 (after the rainy season), 2019. Because sampling on October 29 was conducted to avoid seaweed (Japanese nori) cultivation nets, the stations sampled in October differed from those in June. Samples were collected on June 21 and October 29 from lines 1 and 2 in **Fig. 1**, respectively. These transects were selected considering the behavior of freshwater from the Chikugo River flowing toward the inner Ariake Sea [23]. Sampling was conducted during the ebb phase of a moderate tide on June 21 and a spring tide on October 29.

Salinity was measured at 0.5–1 m depth intervals from the sea surface to bottom using a multi-parameter water quality meter (WQC-24; DKK-TOA, Tokyo, Japan). Seawater samples were collected from three or four depths at each station using a 3-L Van Dorn water sampler (RIGO, Tokyo, Japan).

In October, samples for the incubation experiments were collected at each depth and poured into acid-washed 500-mL polycarbonate bottles. Samples for Chl. *a* analysis were collected at each depth and poured into 125-mL amber polyethylene bottles. The seawater samples used for DOC analysis were filtered onboard the ship using pre-combusted 0.7-μm GF/F filters (General Electric, Coventry, UK) and stored in acid-washed 125-mL high-density polyethylene bottles. Plastic gloves were worn during sample collection and processing to avoid contamination.

POM decomposition experiment

To evaluate DOC production during POM decomposition, water samples were poured into acid-washed 500-mL biological oxygen demand bottles and incubated for 5 days at the in-situ temperature in the dark. The DOC production rates were determined from the difference in DOC concentrations measured before and after incubation. Because the optical characteristics of DOM obtained from the fluorescence excitation (Ex)–emission (Em) matrix provide information on the bioavailability of DOM, humic-like and protein-like fluorescent DOM levels were also measured.

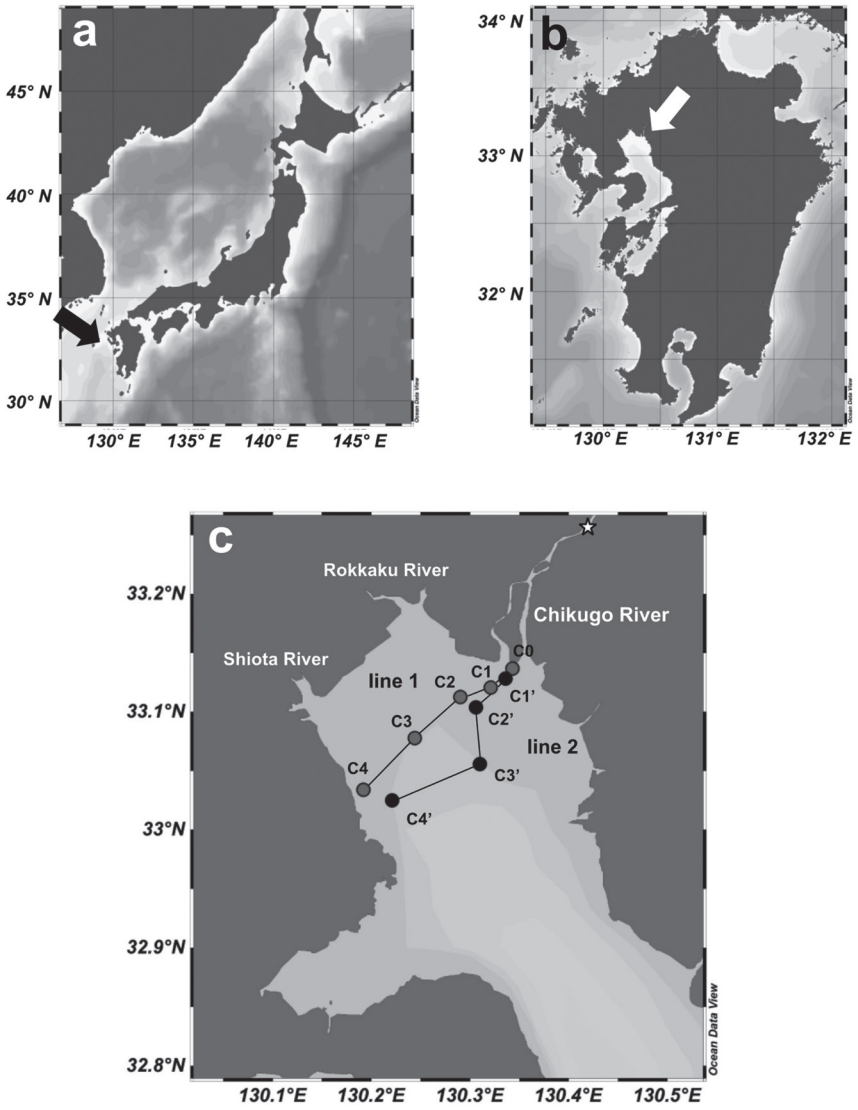

Fig. 1 Maps of the Japan island (a), the Kyushu island (b) and northern part of the Ariake Sea (c). Black and white arrows indicate the Kyushu island and northern part of the Ariake Sea, respectively. The line indicates the axial section in **Fig. 2**. Samples on June 21 and October 29 were collected from lines 1 and 2, respectively. White star indicates the monitoring station of dissolved organic carbon concentration in the Chikugo River.

Chemical analyses

To determine Chl. *a* concentrations, water samples were filtered through 0.7-μm GF/F filters (General Electric) and analyzed by fluorometry (FP-8300; JASCO, Tokyo, Japan) according to the method of Welschmeyer (1994) [24].

DOC concentrations were determined using a total carbon analyzer (TOC-V; Shimadzu, Kyoto, Japan). A calibration curve was obtained by analyzing four concentrations of standard solution prepared using potassium hydrogen phthalate. As a procedural blank, ultrapure water (Milli-Q, Direct-Q UV3; Merck Millipore, Burlington, USA) was analyzed every 10 samples, and the average pooled peak area of the Milli-Q water samples over the entire day was subtracted from all seawater sample peak areas.

Ex–Em matrices of fluorescence were determined for DOC samples using a fluorometer (FP-8300; JASCO). The measurement bandwidths were set to 5 nm for Ex and 5 nm for Em. A series of Em scans (200–500 nm) were collected over a range of Ex wavelengths of 200–500 nm in 5 nm increments. To remove the Rayleigh scattering and Raman signals, a Rayleigh- and Raman-normalized Milli-Q Ex–Em matrix was created and then subtracted from the sample

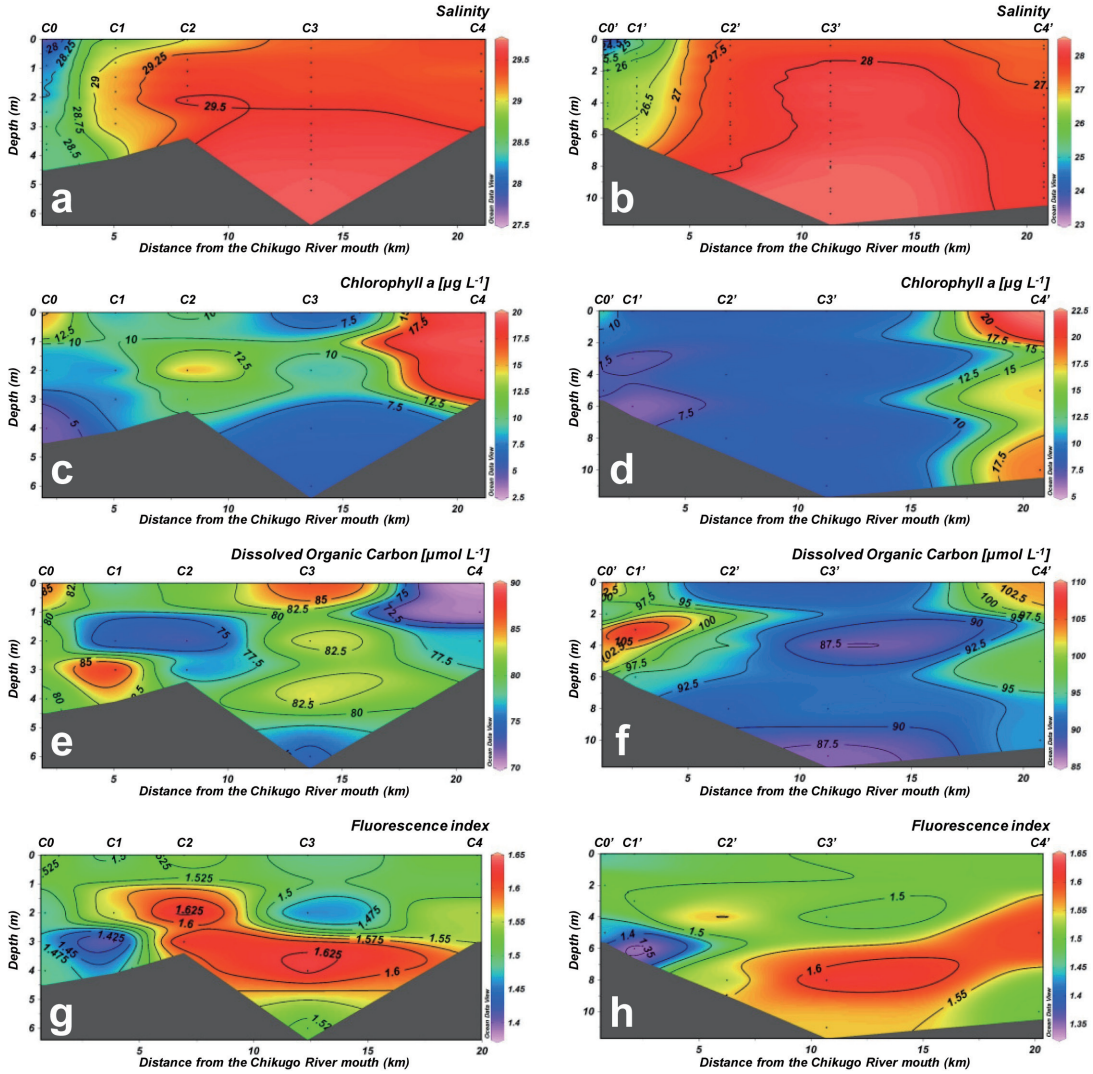

Fig. 2 Horizontal distribution of salinity in June (a) and October (b), and chlorophyll *a* concentration in June (c) and October (d), and dissolved organic carbon concentration in June (e) and October (f), and fluorescence index (FI) of dissolved organic matter in June (g) and October (h). The black dots in the graphs indicate the observation layers.

data. The fluorescence intensities were normalized to quinine sulfate units, where 1 quinine sulfate unit is equivalent to the fluorescence Em at Ex/Em = 350/450 nm wavelength of 1 μg L^{-1} quinine sulfate in 0.1 M H_2SO_4 solution. According to Mayer *et al.* (1999) [25], the peaks at Ex/Em = 220/290 nm and Ex/Em = 240/420 nm were identified as the protein-like and humic-like components, respectively. For the field samples, fluorescence index (FI), an indicator of DOM origin, was calculated as the ratio of the emission fluorescence intensities measured at wavelengths 440 and 490 nm with an excitation wavelength of 370 nm [26].

Statistical analysis

Pearson's correlation analysis was used to analyze the data. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R software (ver. 3.3.3; R Development Core Team, Vienna, Austria) [27].

RESULTS AND DISCUSSION

The salinities measured in June and October 2019 ranged from 27.7 to 29.7 (average 29.1) and 23.1 to 28.4 (average 27.0), respectively (**Figs. 2a, b**). The lowest salinity was observed in the surface water of the river mouth area (St.

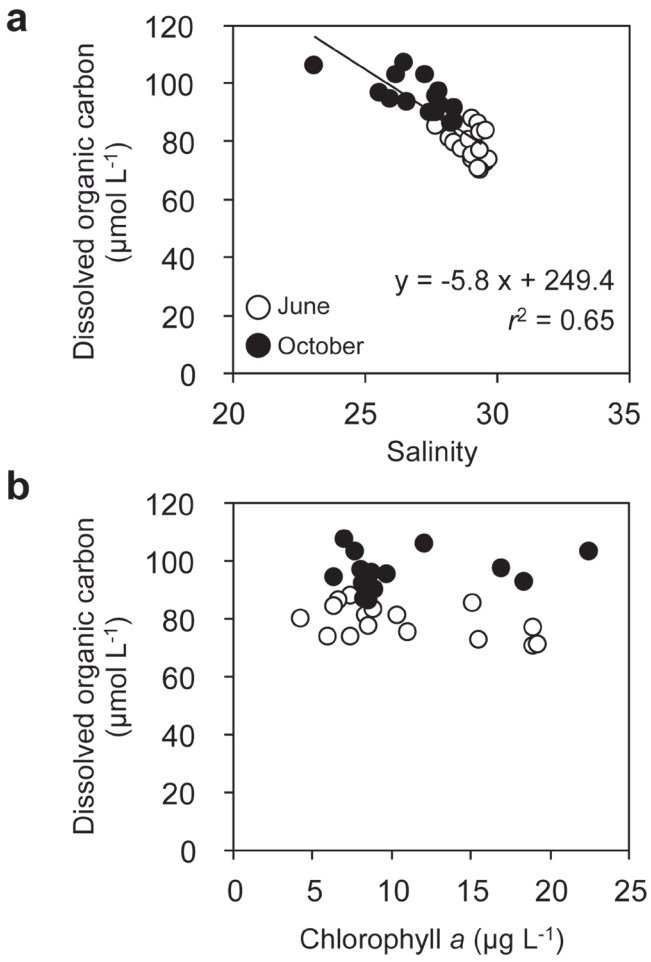

Fig. 3 Plots comparing salinity, chlorophyll *a* (Chl. *a*) concentration with DOC concentration. A line was fitted by linear regression, with the regression result given in the plots.

C0), and salinity gradually increased toward the opposite shore site (St. C4 and C4', **Figs. 2a, b**). Notably high DOC concentrations were observed, along with low salinity from the surface to the middle layer, at stations near the Chikugo River mouth (Sts. C0, C1', **Figs. 2a, b, e, f**). A negative correlation was found between salinity and DOC concentration ($r = -0.82$, $p < 0.001$, $n = 32$, **Fig. 3a**), suggesting that a major potential source of DOC is river inflow. This result is partly supported by the result of the FI. The FI has been used to identify the source of DOM originating from terrestrial about 1.4) or microbial activity (about 1.9) [26]. In this study, the value of FI in June and October ranged from 1.34 to 1.64 (average 1.47) and 1.34 to 1.63 (average 1.54), respectively (**Figs. 2g, h**). Those results indicate that terrestrial DOM distributed throughout the northern Ariake Sea. Because the study region is near the Shiota and Rokkaku Rivers, an effect of terrestrial DOC from those rivers could be expected. However, no low-salinity patches were observed at stations near the Rokkaku River (Sts. 2, 2') or Shiota River (Sts. C4, C4', **Figs. 2a, b**). These findings indicate that the Chikugo River is the primary source of DOC in the northern Ariake Sea.

However, the expected concentration of riverine DOC, represented by the y-intercept of the regression curve between salinity and DOC, was much higher than the DOC concentration of the Chikugo River, suggesting that additional DOC is produced in the salinity range of 22–30 (**Fig. 3a**). The extrapolated DOC concentration at zero salinity in this study (249.4 μmol L^{-1}) was approximately double the annual DOC concentration range in the Chikugo River (95.1–120 μmol L^{-1}, unpublished data, **Fig. 1**), indicating that DOC originating from the Chikugo River is not conservatively mixed in the northern Ariake Sea. By contrast, we previously reported that DOC shows conservative behavior in the Chikugo River estuary [19]. Thus, additional DOC may not be produced in the Chikugo River estuary but produced in the inner part of the Ariake Sea. Previous studies have reported DOM release from pore water in sediment [28]. In this study, however, DOC concentrations in bottom layers were low relative to those in upper layers (**Fig. 2e, f**). Thus, DOC from pore water likely has only a minor impact on DOC dynamics in this area. In accordance with this notion, we previously reported that sediment resuspension plays a minor role in DOC dynamics in the Chikugo River estuary [19].

The production of autochthonous DOC from phytoplankton biomass is another source of DOC. A notably high DOC concentration was found, along with a high Chl. *a* concentration, in the surface water at St. C4' in October (**Fig. 2d**), and this DOC may be of phytoplanktonic origin. However, the DOC concentration was low in the surface water at St. C4 in June (**Fig. 2e**) when the Chl. *a* concentration was high (**Fig. 2c**), and no correlation was found between the Chl. *a* concentration and DOC ($r = -0.09$, $p > 0.05$, $n = 32$, **Fig. 3b**) in this study. Thus, DOC production by phytoplankton appears not to be a major source of additional DOC.

Previous research in the East China Sea showed non-conservative behavior of DOC originating from the Changjiang River due to the presence of a DOC "pulse" [22]. They suggested that microbial decomposition of POM may be the source of the DOC pulse. In the present study, most FI values showed close to terrestrial (about 1.4), but they were a little higher than 1.4 (**Fig. 2g, h**). A little higher FI values may be associated with the microbial origins. The DOC concentrations measured in four experiments increased

Table 1 Changes in dissolved organic carbon (DOC), Protein-like and Humic-like components concentrations in incubation experiments.

Station	Depth (m)	Changes in DOM concentration		
		DOC (μmol L^{-1})	Protein-lkie (QSU)	Humic-like (QSU)
C0	0	−0.80	19.5	9.5
	2	−0.37	19.7	12.2
	5	−0.92	22.9	−11.4
C1’	0	−0.69	10.6	−1.6
	3	−1.80	5.0	−0.5
	6	−0.54	8.1	−4.1
C2’	0	0.54	12.0	0.9
	4	−0.43	2.4	2.3
	8	−0.46	−6.0	5.0
C4’	0	0.41	21.6	−5.5
	4	0.39	19.3	3.2
	8	−0.74	2.4	1.1
	11	0.06	3.7	12.6
C5′	0	−0.96	8.0	9.9
	5	−0.96	4.0	4.8
	10	−0.38	−0.8	5.8

after 5 days of seawater incubation (**Table 1**). In addition, the protein- and humic-like components of DOM increased after incubation in most experiments (**Table 1**). Those results suggest that DOC production occurred during microbial decomposition of POM. In support of this result, production of both protein- and humic-like components during microbial POM decomposition has been reported [29]. Because humic substances are one of the most refractory organic matter types in seawater [30], refractory DOC produced via POC decomposition by bacteria, which may contribute accumulation of DOC in the study area. However, no DOC production occurred in most experiments. Five days of incubation might be insufficient for detection of DOC production in most incubation experiments. In-situ DOC production via POM decomposition and consumption of the bioavailable DOC present in the original sample likely occur simultaneously during incubation experiments. In our previous study, POC was not completely decomposed during 7 days of incubation using seawater from the northern Ariake Sea [14]. On the other hand, most of the bioavailable fraction of DOC decomposed within 5 days in this study area (authors’ unpublished data), and similar results have been reported in other coastal systems [31,32]. Thus, DOC production from POM decomposition may continue to occur after 5 days of incubation, although most labile DOC is consumed within 5 days. Our finding indicates that microbial decomposition of POM may a source of the additional DOC observed in this study. Both allochthonous and autochthonous POM may be sources of excess DOC, as DOM production was observed regardless of the Chl. *a* concentration (**Fig. 2c, d, Table 1**).

CONCLUSIONS

This study is the first to provide data showing the influences of river inflow and bacterial decomposition of POM on the DOC distribution in the northern Ariake Sea. The results of this study indicate that the Chikugo River is the primary source of DOC in the northern Ariake Sea, although the DOC from this river was not conservatively mixed. Our results also indicate that microbial decomposition of POM is a source of the additional DOC produced in the study area. POM is transformed into both labile and refractory DOM via heterotrophic bacterial activities and eventually into inorganic nutrients. As a previous study reported the importance of organic matter decomposition in both red tides and hypoxia events in the northwestern Ariake Sea [14,33], future studies should evaluate the contribution of riverine DOM to organic matter decomposition in this area.

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