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Availability of particulate Fe to phytoplankton in the Sea of Okhotsk

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- 1 ➤ We investigated availability of particulate iron to marine phytoplankton.
- 2 ➤ Suspended particulate matter (SPM) collected from the nepheloid layer was used.
- 3 ➤ Iron in the SPM was available which provide healthy growth of phytoplankton.
- 4 ➤ Such particulate iron supports biological production around the Kuril Islands.

ABSTRACT

In a shipboard incubation study, we investigated the availability of particulate iron (Fe) to an Fe-starved phytoplankton community through the addition of suspended particulate matter (SPM; $> 1 \mu\text{m}$) collected from the nepheloid layer in the coastal region of the Sea of Okhotsk. Surface seawater incubations were also conducted at three stations around the Bussol' Strait where the SPM that possibly originated from the coastal nepheloid layer could emerge to the surface mixed layer due to strong vertical mixing around the Kuril Islands. In the SPM-added experiment, the growth rate of phytoplankton was significantly enhanced by the addition of SPM compared to the unamended control. This result clearly indicates that Fe in the SPM collected from the nepheloid layer was available to marine phytoplankton. In addition, phytoplankton particularly coastal diatoms in the nepheloid layer were viable and showed healthy growth. In the surface seawater incubation experiments, phytoplankton growth and nutrient drawdown in unamended control conditions in two of the three stations may be supported by Fe from the particulate fraction ($> 0.22 \mu\text{m}$), as estimated from stoichiometric calculation. We suggest that the bioavailable particulate Fe in SPM of the coastal region supports biological production and nutrient drawdown even after the depletion of dissolved Fe around the Kuril Islands, where strong vertical mixing occurs.

1. Introduction

Iron (Fe) is the most important micronutrient for marine phytoplankton growth, because of its key role in processes such as photosynthesis and nitrate and nitrite assimilation (Raven et al., 1999). However, the thermodynamically stable oxidation state of Fe in oxic surface seawater is predominantly Fe(III), which has an extremely low solubility (Stumm and Morgan, 1996; Kuma et al., 1996). The Fe in the North Pacific Ocean is mainly derived from continental sources, such as eolian dust, riverine input, and sedimentary Fe from the continental shelf to coastal and oceanic waters (Johnson et al., 1999, 2005; Jickells et al., 2005; Lohan and Bruland, 2006; Nishioka et al., 2007). Moore and Braucher (2008) estimated the global-scale Fe cycle using the Biogeochemical Elemental Cycling ocean model and found that the sedimentary source of Fe contributes as much as 70% of dissolved Fe (D-Fe) in pools in the surface of the northwestern North Pacific. Previous studies indicated that the high Fe water mass possibly related to the sedimentary source would be transported from the western coast of the Sea of Okhotsk to the western North Pacific region via the Kurile Straits (Nishioka et al., 2007; Misumi et al., 2011).

In the Sea of Okhotsk, dense shelf water (DSW) is produced by brine rejection due to sea ice formation during winter, which originates in the region of the Siberian and Sakhalin continental shelves (Shcherbina et al., 2004; Matsuda et al., 2009). The DSW entrains continental sediments during the formation processes and is transported southward along the Sakhalin coast at an intermediate depth (~200–500-m), forming the Okhotsk Sea intermediate water (OSIW) (Fukamachi et al., 2004). Nakatsuka et al. (2004) reported that DSW has high particulate organic matter derived from coastal sediment, which should have high particulate Fe concentrations (e.g., Landing and Bruland, 1987; Elrod et al., 2008). The OSIW flows further southeastward and outflows into the western subarctic Pacific Ocean, through the Kurile Straits with strong vertical diapycnal mixing around the Kurile Islands (Nakamura and Awaji, 2004; Ito et al., 2010, 2011). The Bussol' Strait is a key channel with respect to the exchange of seawater between the Sea of Okhotsk and the Oyashio region (e.g., Yasuda et al., 2002) because the channel is deepest at the Kuril Straits. It can be assumed that the particles in the DSW advect into the surface of the Okhotsk and Oyashio waters via strong water mixing with the OSIW. Intrusion of such bio-active particles into the surface seawater would

61 affect and play an important role in phytoplankton communities around the region. However,
62 little attention has been paid to the importance of particulate matter on the dynamics of
63 surface phytoplankton communities.

64 In general, one of the most bioavailable Fe species for photolithoautotrophic
65 phytoplankton is dissolved inorganic Fe (Fe') (Anderson and Morel, 1982; Morel et al., 2008).
66 There are apparent exceptions to this model in that organic Fe-ligand complexes could be
67 indirectly and/or directly utilized by the cells (Hutchins et al., 1999a; Maldonado and Price,
68 2001; Shaked et al., 2005; Kustka et al., 2007). In nature, almost all of the dissolved Fe(III)
69 should be bound to organic ligands, which have high and low binding affinities for Fe (Rue
70 and Bruland, 1995). A recent study demonstrated that saccharides are responsible for binding
71 Fe with low affinity in the colloidal size fraction (Hassler et al., 2011). The bioavailability of
72 Fe-saccharide complexes is higher than for Fe binding with high-affinity ligands for
73 eukaryotic phytoplankton (Hassler and Schoemann, 2009; Hassler et al., 2011). There is
74 increasing evidence that the bioavailability of D-Fe should be different depending on the
75 binding affinity of the ligand or differences in the phytoplankton groups and their habitats
76 (Hutchins et al., 1999b; Kuma et al., 2000; Hassler and Schoemann, 2009; Strzepek et al.,
77 2011).

78 The bioavailability of D-Fe species including the colloidal fraction (between < 200
79 kDa or 0.025 μm to 0.2 or 0.45 μm ; e.g., Nishioka and Takeda, 2000; Nishioka et al., 2001)
80 has been relatively well examined, although the availability of particulate Fe (P-Fe) is largely
81 unknown. According to laboratory experiments (Kuma and Matsunaga, 1995; Yoshida et al.,
82 2006), the surface reactivity or crystalline structure of solid inorganic Fe oxyhydroxide
83 controls the supply of bioavailable D-Fe species for coastal diatoms. In natural conditions,
84 Elrod et al. (2008) and Sugie et al. (2010a) reported that surface chlorophyll-*a* (Chl-*a*)
85 concentrations have significant positive correlations with total dissolvable Fe concentrations
86 (T-Fe), suggesting that particulate Fe supports a substantial portion of the ecosystem's Fe
87 requirements. Using Chl-*a* and D- and P-Fe concentrations, Nakayama et al. (2010) calculated
88 stoichiometrically that the phytoplankton biomass of intensive spring diatom blooms (> 10 μg
89 Chl-*a* L^{-1}) in the Oyashio region of the western subarctic Pacific region can be achieved by
90 the utilization of D-Fe and also Fe from the particulate phase. Furthermore, Fitzwater et al.

91 (2003) measured the T-Fe concentrations in the surface waters, which originated from the
92 benthic boundary layer of the continental shelves during upwelling events that caused a
93 substantial part of the Fe in the particulate fraction to be chemically labile (leachable by 25%
94 acetic acid for 2 h). They also suggested that a small but substantial part of labile particulate
95 Fe contributes to the bloom formation around the upwelling plumes (Fitzwater et al., 2003).
96 Although those previous studies recognize the importance of particulate Fe as a source of
97 bioavailable Fe, the availability of Fe in natural marine particles has not been examined
98 before, except for Fe coming from aeolian dust (e.g., Payten et al., 2009).

99 In this study, we conducted two types of bioassay experiments to examine the
100 availability of P-Fe for the growth of phytoplankton. First, we examined the availability of Fe
101 associated with marine suspended particulate matters for an Oyashio phytoplankton
102 community through the addition of particulate matters collected in the DSW (SPM_{DSW}).
103 Second, surface phytoplankton communities at three stations around the Bussol' Strait were
104 incubated to investigate the importance of P-Fe for the growth of phytoplankton where
105 SPM_{DSW} could appear in the surface mixed layer. The availability of the P-Fe in the surface
106 waters was evaluated by stoichiometric calculations using Chl-*a* concentration increases and
107 nutrient drawdown data with reported Fe:C and Fe:Chl-*a* values. Our results provide the first
108 direct evidence of the availability of Fe in marine suspended particulate matters for natural
109 phytoplankton communities.

110

111 2. Methods

112 2.1. SPM_{DSW} addition experiment

113 Phytoplankton incubations were performed aboard the R/V Professor Khromov
114 during August to September 2007 (Kh-07 cruise). Natural phytoplankton communities used in
115 incubation experiments for the bioavailability of Fe in the SPM_{DSW} were collected in the
116 Oyashio region (Stn Oy: 46°00'N 152°30'E; Fig. 1) using an acid-cleaned Teflon-coated 10 L
117 Niskin X sampling bottle (General Oceanics) attached to a CTD-carousel multi-sampling
118 system (Nishioka et al., 2007, 2011). Hydrographic data (salinity, temperature, and depth)
119 and transmittance were obtained using a CTD (Sea-Bird, Model 9-puls) and transmissometers
120 (Wet Labs, C-Star). Seawater for a natural phytoplankton stock was sieved with 100 µm

121 acid-cleaned Teflon-mesh to eliminate mesozooplankton and nutrients were added [$27 \mu\text{mol}$
122 $\text{L}^{-1} \text{NO}_3$, $2 \mu\text{mol L}^{-1} \text{PO}_4$ and $47 \mu\text{mol L}^{-1} \text{Si(OH)}_4$] to induce probable Fe-starved conditions
123 for the phytoplankton community before use. Nutrient stock solutions were passed through
124 Chelex-100 ion-exchange resin (Bio-rad) to remove trace metals (Morel et al., 1979). The
125 natural phytoplankton stock was incubated for 6 days in an on-deck incubator. The
126 temperature of the incubator was maintained at near-ambient sea surface temperature ($\sim 10^\circ\text{C}$)
127 by a surface seawater flow-through system. The incubator was covered with a single layer of
128 neutral density screening to achieve approximately 30% of surface irradiance.

129 The SPM_{DSW} was collected at 320 m depth east of Sakhalin (Stn DSW: $52^\circ 15' \text{N}$,
130 $144^\circ 35' \text{E}$; Fig. 1) using an *in situ* filtration system (McLane Research Laboratories Inc.). The
131 DSW mass was detected by the anomalous values in low temperature ($< \sim 0^\circ\text{C}$) with low
132 transmittance around the $\sigma_t = 26.8 \text{ kg m}^{-3}$ layer as reported previously (Fukamachi et al.,
133 2004; Shcherbina et al., 2004). Approximately 454.5 L of DSW was filtered through a $1.0 \mu\text{m}$
134 acid-washed polycarbonate filter (Nuclepore). The water on the filter was flushed by vacuum,
135 and the filter was frozen at -20°C for ~ 3 hours. Prior to the experiment, the SPM_{DSW} on the
136 filter was suspended in 500 mL of $0.2 \mu\text{m}$ filtered seawater (FSW) which was collected from
137 a depth of 200 m in the Oyashio region during the HK07-1 cruise aboard the R/V
138 Hokko-Maru (January 2007). The FSW contained $40 \mu\text{mol L}^{-1} \text{NO}_3 + \text{NO}_2$, $3.0 \mu\text{mol L}^{-1} \text{PO}_4$,
139 $63 \mu\text{mol L}^{-1} \text{Si(OH)}_4$ and $0.88 \text{ nmol L}^{-1} \text{D-Fe}$. Six treatments were prepared as follows (Table
140 1): (1) control: 270 mL of FSW plus 45 mL of Fe-starved natural phytoplankton stock culture
141 prepared above; (2) Fe-added: added inorganic Fe to the control condition to make a final
142 concentration of 5 nmol L^{-1} ; (3) DFB (desferrioxamine B): amended with $1 \mu\text{mol L}^{-1}$ DFB
143 (Sigma Chem. Co. Ltd.) to the control condition to prevent Fe uptake from the ambient
144 seawater as achieved previously (Wells, 1999; Iwade et al., 2006; Sugie et al. 2010b, 2011);
145 (4) SPM_{DSW} : 250 mL of FSW plus 45 mL of Fe-starved phytoplankton stock plus 20 mL of
146 resuspended SPM_{DSW} solution as prepared above, i.e., SPM_{DSW} was concentrated 63.5-fold vs.
147 *in situ* condition; (5) $\text{SPM}_{\text{DSW}}\text{-Phy}$: 295 mL of FSW plus 20 mL of resuspended SPM_{DSW}
148 solution without the addition of the Fe-starved phytoplankton stock; and (6) DSW_{raw} : 315 mL
149 of prescreened DSW using $100 \mu\text{m}$ acid-washed Teflon-mesh collected from 247 m at Stn
150 DSW (Table 1). The $\text{SPM}_{\text{DSW}}\text{-Phy}$ treatment was intended to examine the viability of the

phytoplankton in the SPM_{DSW} , which was once frozen. The DSW_{raw} was set to elucidate the viability of phytoplankton in the DSW mass, which mimics their appearance to the sunlit surface waters by tidally induced and strong vertical mixing, such as that observed during winter (Nakamura and Awaji, 2004).

The treatments were incubated in an on-deck incubator as described above. The treatments were prepared in 9-replicate 320 mL polycarbonate bottles in addition to the DSW_{raw} treatment, which was prepared in 4-replicate. Three bottles were sacrificed and measured after 1, 3 and 5 days for analysis of macronutrients and Chl-*a* concentrations except for the samples of the DSW_{raw} treatment, which were obtained after 3 and 5 days sacrificing two bottles for each day. The concentrations of total dissolvable Fe (unfiltered; T-Fe) and background Fe in the FSW were measured at the beginning of the experiment. The samples for diatom cell densities and species compositions were collected at 0 and 5 days of incubation. Although the abundance of the small flagellates can be expected to be high in the incubation bottles, the large number of particles in the SPM_{DSW} and SPM_{DSW} -Phy treatments obscured the counts of the flagellates. Therefore, we measured only the diatom abundance using a light microscope. The experimental advantage of counting only diatoms to examine the availability of Fe in the SPM_{DSW} is that the diatoms are photolithoautotrophs, but some mixotrophic flagellates are capable of phagotrophic acquisition of P-Fe (Tortell et al., 1996; Maranger et al., 1998). Therefore, we can examine the availability of Fe potentially dissociated from the SPM_{DSW} . The Chl-*a* specific growth rate was calculated from the linear regression between the time (day) and the natural log of the Chl-*a* concentrations. For the Chl-*a* specific growth rate calculations, the data from the beginning to the end of the incubations were used for the control, DFB, SPM_{DSW} -Phy and DSW_{raw} treatments and those from day 1 to the end of the incubations were used for the Fe-added and SPM_{DSW} treatments. The growth rate in terms of cell numbers was calculated as follows: growth rate = $\ln(N_{d5} / N_{d0}) / 5$, where N_{d5} and N_{d0} represent cell numbers at day 5 and the start of the experiment, respectively.

2.2. Surface phytoplankton incubations

180 Seawater for incubation was collected at three sampling sites located at Stn Oy, in the
181 Bussol' Strait (Stn BS: 46°30'N 151°23.2'E) and in the Sea of Okhotsk (Stn Ok: 47°30'N
182 150°20.45'E) (Fig. 1). Seawater samples were collected from 10 m depth using acid-cleaned
183 Teflon-coated 10 L Niskin X sampling bottles. Seawater for experiments was sieved with 200
184 μm acid-cleaned Teflon-mesh to eliminate large mesozooplankton such as *Neocalanus* spp.,
185 which dominate in the western subarctic Pacific region (Ikeda et al., 2008). The prescreened
186 seawater sample was homogenized in an acid-washed 20 L polycarbonate tank and then
187 dispensed into acid-cleaned 320 mL polycarbonate bottles. The three treatments were
188 conducted as follows: (1) unamended control; (2) Fe-added media with the addition of 5 nmol
189 L^{-1} Fe; and (3) Fe-limited media with the addition of 1 $\mu\text{mol L}^{-1}$ DFB. Fifteen bottles for
190 each treatment were prepared and incubated in an on-deck incubator as described above.
191 Three bottles were sacrificed at 1, 3, 5, 7 and 10 day intervals to measure the Chl-*a*
192 concentration, phytoplankton abundance and macronutrient concentrations. The samples for
193 the beginning of the experiment were taken from the residue in the 20 L tank. The Chl-*a*
194 specific growth rate was calculated as described above using the Chl-*a* data from the
195 beginning of the experiment for the control and the Fe-limited media, and from day 1 for the
196 Fe-added media to the day before the nutrient depletions. The growth rate in terms of cell
197 numbers was calculated using the same method as described in the SPM_{DSW} addition
198 experiment.

199

200 2.3. Sample analysis

201 For measuring the Chl-*a* concentrations, the samples were filtered onto GF/F filters
202 and measured with a Turner Design 10-AU fluorometer (Welschmeyer, 1994) after extraction
203 with *N,N*-dimethylformamide (Suzuki and Ishimaru, 1990). The samples for macronutrient
204 analysis during incubation were measured with a QuAAtro continuous flow analyzer
205 (Bran+Luebbe). For Fe analysis, seawater samples were acidified at pH 3.2 with 10 mol L^{-1}
206 formic acid and 2.4 mol L^{-1} ammonium formate buffer after the sample collection. The Fe
207 concentration was measured with an automated Fe analyzer (Kimoto Electric) using a
208 combination of an 8-hydroxyquinoline chelating resin concentration and luminol-hydrogen
209 peroxide chemiluminescence detection with a closed flow-through system (Obata et al., 1993).

Unfiltered acidified samples were used to measure the total dissolvable Fe (T-Fe) and the samples passed through a 0.22 μm cartridge filter (Millipore) prior to formic acid and buffer addition were used to measure the dissolved Fe (D-Fe) (detection limit: $< 0.05 \text{ nmol L}^{-1}$ in this study). In the surface phytoplankton incubations, the Fe amount in the particulate fraction (P-Fe) was defined as follows: $\text{P-Fe} = \text{T-Fe} - \text{D-Fe}$. Note that the Fe in particulate form collected using an *in situ* filtration system ($> 1.0 \mu\text{m}$ in diameter) during the SPM_{DSW} addition experiment is represented as P-Fe_{DSW} . The Fe concentrations were analyzed on-board immediately after the collection of the seawater samples at the beginning of the experiment. Our Fe measurement method was carefully assessed using SAFe (Sampling and Analysis of Fe) cruise reference standard seawater for an inter-comparison study distributed by the Moss Landing Marine Laboratory and University of California Santa Cruz. We measured $0.10 \pm 0.010 \text{ nmol L}^{-1}$ ($n = 3$) and $0.99 \pm 0.023 \text{ nmol L}^{-1}$ ($n = 3$) D-Fe by our method, respectively for concentrations of $0.094 \pm 0.008 \text{ nmol L}^{-1}$ (S) and $0.923 \pm 0.029 \text{ nmol L}^{-1}$ (D2) D-Fe (www.geotraces.org) for the reference standard seawater. For the phytoplankton cell count, seawater samples were fixed by formalin (1% final volume). An adequate volume of fixed seawater was poured into a settling chamber and allowed to settle for at least 24 hours (Hasle, 1978) for the day 0 sample, and centrifuged approximately $400\times g$ for day 5 samples (Thronksen, 1978) before identification using a phase-contrast inverted microscope and light microscope, respectively. Diatom species were identified according to Hasle and Syvertsen (1997).

230

231 3. Results

232 3.1. SPM_{DSW} addition experiment

233 3.1.1. Hydrography

At Stn DSW, cold ($< 0^\circ\text{C}$) and less saline (~ 33.3) intermediate water was detected around $\sigma\text{-}t = 26.8 \text{ kg m}^{-3}$ (Fig. 2a, b, c), which was considered as OSIW (Fukamachi et al., 2004). Anomalously low beam transmission, i.e. high abundance of suspended particulate matter was detected below 245 m at Stn DSW (Fig. 2d).

238

239 3.1.2. Phytoplankton growth

Chl-*a* concentrations increased exponentially in the controls, Fe, and SPM_{DSW} treatments over the 5-day incubation period, whereas the concentrations decreased with time in the SPM_{DSW}-Phy treatment (Fig. 3a). The order of the maximum Chl-*a* concentrations during the incubation were as follows: SPM_{DSW} ($60.9 \pm 4.5 \mu\text{g L}^{-1}$) > Fe-added ($28.3 \pm 3.8 \mu\text{g L}^{-1}$) > control ($18.9 \pm 1.0 \mu\text{g L}^{-1}$) > DFB ($5.2 \pm 0.2 \mu\text{g L}^{-1}$) > DSW_{raw} ($0.99 \pm 0.2 \mu\text{g L}^{-1}$) > SPM_{DSW}-Phy ($0.14 \pm 0.0 \mu\text{g L}^{-1}$) (mean $\pm$ 1 SD of triplicate bottles or mean $\pm$ range of duplicate bottles). The Chl-*a* data in the SPM_{DSW}-Phy treatment indicated that most of the phytoplankton on the filter collected from the DSW died during the 3 hour freezing period. We then offset the Chl-*a* data for the SPM_{DSW} treatment by subtracting the value measured at each data point in the SPM_{DSW}-Phy treatment to calculate the specific growth rate. The specific growth rate was highest in the SPM_{DSW} (0.80 ± 0.03) (mean $\pm$ 95% C.L. of regression), followed by 0.66 ± 0.03 in the Fe-added, 0.59 ± 0.13 in the DSW_{raw}, 0.58 ± 0.02 in the control, 0.30 ± 0.02 in the DFB and -0.28 ± 0.09 in the SPM_{DSW}-Phy treatment (Fig. 3b). It is notable that the growth rate was the fastest (1.0 ± 0.09) during days 3 to 5 in the DSW_{raw} treatment as compared with all treatments.

At the beginning of the experiment, pennate diatom species such as *Pseudo-nitzschia* spp. and *Cylindrotheca closterium* / *Lennoxia faveolata* complex predominated relative to the centric diatoms (we detected mainly *Chaetoceros* spp. subgenus *Phaeoceros*) in the possibly Fe-starved Oyashio phytoplankton community (Table 2). In the treatments without the addition of Oyashio phytoplankton (SPM_{DSW}-Phy and DSW_{raw}), the centric (mainly *Chaetoceros* spp. subgenus *Hyalochaete* and *Thalassiosira* spp.) and pennate diatoms (mainly *Thalassionema nitzschioides*) dominated, and they contributed equally (Table 2). In addition, *Hyalochaete* spp. resting spores were detected in the DSW_{raw} treatment at levels of 80 spores L⁻¹. It should be noted that the species in the SPM_{DSW} and DSW_{raw} treatments were considered as mainly coastal diatoms (Hasle and Syvertsen, 1997). At day 5, abundances of the pennate diatoms were three orders of magnitude higher than those of the centric species in the Oyashio phytoplankton-added treatments, except for the DFB treatment, whereas in the DSW_{raw} treatment, *Hyalochaete* spp., *Thalassiosira* spp., *T. nitzschioides*, and, notably, resting spore-forming species such as *Thalassiosira antarctica* ver. *borealis* and *Ditylum brightwellii* were observed. The total diatom abundance at the end of the incubations was the

270 following order: $SPM_{DSW} > \text{Fe-added} > \text{control} > \text{DFB} \gg SPM_{DSW}\text{-Phy} \approx DSW_{raw}$ (Table 2).
 271 This order is nearly identical to that of the maximum Chl-*a* concentrations (Fig. 3a). The net
 272 growth rates during the 5 day incubation period were similar between centric and pennate
 273 diatoms in the control, SPM_{DSW} and DSW_{raw} treatments, whereas that of the pennate diatoms
 274 was faster than that of the centric diatoms in the Fe-added treatment (Table 4). In the
 275 DFB-added treatment, the net growth rate of diatoms and flagellates was slower than that in
 276 the controls and Fe-added treatments.

277

278 3.1.3. Nutrient dynamics

279 The T-Fe concentrations in the control ($\approx$ DFB) and SPM_{DSW} ($\approx$ $SPM_{DSW}\text{-Phy}$)
 280 treatments were 0.93 and 47.7 nmol L⁻¹, respectively. The concentration of P-Fe_{DSW} (> 1.0
 281 μm) is derived as an approximation based on the measurement of the concentrated SPM in the
 282 experimental bottles. Thus, the P-Fe_{DSW} concentration was 0.83 nmol L⁻¹ in the *in situ* DSW
 283 (320 m) mass at Stn DSW. Macronutrient concentrations at the beginning of the experiment
 284 were 30.0–48.6 $\mu\text{mol L}^{-1}$ DIN ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4^+$), 2.48–3.86 $\mu\text{mol L}^{-1}$ PO₄, and 56.7–74.1
 285 $\mu\text{mol L}^{-1}$ Si(OH)₄. None of the nutrients were exhausted over the course of the experiment;
 286 for example, DIN remained at 31.7, 24.4, 40.0, 12.3, 42.1 and 28.8 $\mu\text{mol L}^{-1}$ in the control,
 287 Fe, DFB, SPM_{DSW} , $SPM_{DSW}\text{-Phy}$ and DSW_{raw} treatments, respectively. During the 5 day
 288 incubation, nutrients seemingly increased with time in the $SPM_{DSW}\text{-Phy}$ treatment probably
 289 due to the dissolution from the added SPM_{DSW} without a substantial biological uptake.
 290 Nutrient drawdown in the SPM_{DSW} treatment was offset by subtracting the value measured in
 291 the $SPM_{DSW}\text{-Phy}$ treatment (Table 3). The order of the amount of nutrient drawdown was the
 292 same as that of the increase in Chl-*a* concentrations (Fig. 3). The Si:N drawdown ratio was
 293 higher in the SPM_{DSW} compared to that of the control and Fe-added conditions (Table 3). The
 294 Si:P drawdown ratio in the SPM_{DSW} treatment was nearly the same as that of the canonical
 295 value for vegetative growing diatoms (Brzezinski, 1985), whereas the values in the control
 296 and Fe-added treatments were smaller than that in the SPM_{DSW} treatment. In the DFB
 297 treatment, no Si drawdown and low N:P drawdown ratio were measured. The nutrient
 298 drawdown in the DSW_{raw} treatment was too small to calculate the drawdown ratios correctly
 299 (Table 3).

300

301 3.2. Surface phytoplankton incubations

302 3.2.1. Hydrography

303 At the beginning of the three experiments, the upper mixed layer depth was
304 approximately 30, 30 and 20 m at Stns Oy, BS and Ok, respectively (Fig. 4), which was
305 estimated from the first downward increase in $\sigma_t \geq 0.02 \text{ m}^{-1}$. The stratifications were
306 weaker at Stns BS and Ok as compared to Stn Oy. At the Stn Oy site, the hydrographic
307 conditions were typical of the Western Subarctic Gyre (WSG) during summer, with less
308 saline waters (~ 32.6) in the surface, with dichothermal water at around 100 m depth or 26.6
309 kg m^{-3} σ_t (e.g., Yasuda et al., 2002) (Fig. 4). Hydrographic conditions at Stns BS and
310 Ok were intermediate between those of the WSG (Stn Oy) and the Okhotsk waters (Stn DSW;
311 Fig. 2). The σ_t values at Stns BS and Ok were vertically homogenous from ~ 150 down
312 to 500 m and from ~ 50 down to 700 m, respectively (data not shown), probably due to the
313 effect of intensive vertical water mixing around the Bussol' Strait. The $\text{NO}_3 + \text{NO}_2$
314 concentrations at 10 m depth used in the incubation experiment were 8.8, 18.9 and $13.8 \text{ } \mu\text{mol}$
315 L^{-1} at Stns Oy, BS and Ok, respectively. The D-Fe concentrations at 10 m were < 0.05 , < 0.05
316 and 0.10 nmol L^{-1} , and the T-Fe concentrations were < 0.05 , 0.51 and 0.18 nmol L^{-1} at Stns
317 Oy, BS and Ok, respectively.

318

319 3.2.2. Phytoplankton growth

320 At all stations, Fe addition stimulated phytoplankton growth compared to the unamended
321 controls (Fig. 5a, b, c). At the beginning of the experiment, the total diatom abundance was
322 the highest at Stn BS ($19,500 \text{ cells L}^{-1}$) followed by Stns Ok ($12,400 \text{ cells L}^{-1}$) and Oy ($8,400$
323 cells L^{-1}) (Fig. 6). The small pennate diatom *C. closterium* / *L. faveolata* complex (apical
324 axis: $10\text{--}20 \text{ } \mu\text{m}$, transapical axis: $< 1 \text{ } \mu\text{m}$, equivalent spherical diameter (ESD): $\sim 2\text{--}4 \text{ } \mu\text{m}$),
325 was predominant in the bottles at all stations. Other dominant species were also small:
326 *Fragilariopsis* spp. (ESD: $2.5\text{--}6 \text{ } \mu\text{m}$) and a narrow group of *Pseudo-nitzschia* spp. (ESD: ~ 4
327 $\text{ } \mu\text{m}$) (Fig. 6). The abundances of microscopically identifiable small flagellates (ESD: $\sim 3\text{--}10$
328 $\text{ } \mu\text{m}$) were 3 (Stn BS) to 25 (Stn Ok) times higher than those of the diatoms. During the
329 incubation experiment, small pennate diatoms, especially *C. closterium* / *L. faveolata*

330 complex and *Pseudo-nitzschia* spp., prominently increased in all treatments (Table 4).
331 Interestingly, at Stns Oy and BS, the growth rate of the centric diatoms was not enhanced by
332 the addition of Fe, whereas that of the pennate diatoms and flagellates were faster in the
333 Fe-added treatment at all stations (Table 4). The enhancement effect on the growth rates
334 through the addition of Fe, calculated from cell abundance, was smaller than that due to Chl-*a*
335 (Table 4). In the DFB-added treatment, the Chl-*a* concentration was unchanged at Stn Oy and
336 increased slightly at Stns BS and Ok. Diatom and flagellate abundance increased after the
337 addition of DFB, although the growth rates were smaller than in the controls (Table 4).

338

339 3.2.3. Nutrient dynamics

340 The NO₃+NO₂ drawdown reflected the Chl-*a* increases; the most rapid drawdown
341 was obtained with the Fe-added treatments, followed by the controls and the DFB treatments
342 (Fig. 5d, e, f). The trends in PO₄ utilization were the same as those of NO₃+NO₂ (data not
343 shown). Ammonium utilization varied among the three stations but was statistically
344 insignificant among the experimental treatments; it gradually decreased from 0.54 to ~0.2
345 μmol L⁻¹ and 0.78 to ~0.2 μmol L⁻¹ at Stn Oy and Ok, respectively, and remained constant at
346 ~0.2 μmol L⁻¹ at Stn BS. The variations in Si(OH)₄ concentrations were almost identical in
347 the controls and Fe added treatments and less utilized in the DFB treatments compared to the
348 other treatments (Fig. 5g, h, i). The Si:N drawdown ratio decreased by the addition of Fe
349 compared to what was observed in the controls at Stns Oy, BS and Ok: 0.12 ± 0.05, 0.42 ±
350 0.05 and 0.28 ± 0.01 (mean ± 1 SD of triplicate bottles) in Fe-added treatments and 0.45 ±
351 0.00, 0.78 ± 0.09 and 0.37 ± 0.10 in the controls, respectively. In the DFB treatments, the
352 Si:N ratio was the highest among three treatments ranging from 1.05 (Stn Ok) to 1.53 (Stn
353 Oy).

354

355 3.2.4. Effect of Fe manipulation

356 Four indexes were calculated to examine the Fe nutritional status of phytoplankton
357 (Fig. 7). First, the relative specific growth rate, calculated from the Chl-*a* data for the controls
358 (μ_{control}) and divided by that for the Fe-added treatment (μ_{Fe-added}), was used as an indicator of
359 apparent bioavailability of all available Fe sources in the control bottles. The μ_{control}/μ_{Fe-added}

ratio was significantly smaller at Stn Oy (0.42 ± 0.07) compared to Stn BS (0.66 ± 0.06), whereas the values were not statistically significant between Stns Oy and Ok (0.64 ± 0.12) and Stns BS and Ok (Dunnet *t*-test, $p > 0.05$; Fig. 7a). Second, we calculated $\Delta\text{Chl-}a$ by subtracting the increase in Chl-*a* in the DFB treatment from that of the controls as an index of Fe utilized from the extracellular fraction. It should be noted that phytoplankton such as diatoms acclimated in an Fe-depleted medium with amended DFB-Fe as a sole Fe source can utilize Fe from a DFB-Fe complex via cell surface metalloredutases (Maldonado and Price, 2001; Shaked et al., 2005; Strzepek et al., 2011). This suggests that the utilized extracellular Fe is underestimated due to an overestimation of the Fe in the intracellular fraction. However, the Fe uptake from the DFB-Fe complex is very small, and the Fe taken up by the cells is too small to support Chl-*a* synthesis under an extremely high DFB:Fe ratio in seawater (Wells, 1999; Yoshida et al., 2006; Sugie et al., 2011). The $\Delta\text{Chl-}a$ was smallest at Stn Oy ($6.0 \pm 0.1 \mu\text{g L}^{-1}$) followed by Stn Ok ($6.5 \pm 1.1 \mu\text{g L}^{-1}$) and Stn BS ($10.2 \pm 0.8 \mu\text{g L}^{-1}$) during a 10 day incubation period (Fig. 7b). The third and fourth indices are $\Delta\text{NO}_3+\text{NO}_2$ and ΔNH_4^+ , which were calculated by subtracting the NO_3+NO_2 and NH_4^+ drawdown in the DFB treatment from that of the controls (Fig. 7c and 7d). These indices are similar proxies to that of $\Delta\text{Chl-}a$ as stated above. The trend in $\Delta\text{NO}_3+\text{NO}_2$ was similar to the relative growth rate of $\mu_{\text{control}}/\mu_{\text{Fe-added}}$ (Fig. 7a) and $\Delta\text{Chl-}a$ (Fig. 7b); it was higher at Stn BS ($12.7 \pm 1.2 \mu\text{g L}^{-1}$) as compared to Stns Ok ($9.3 \pm 1.2 \mu\text{mol L}^{-1}$) and Oy ($8.1 \pm 0.4 \mu\text{mol L}^{-1}$; Fig. 7c). The ΔNH_4^+ was less than $0.1 \mu\text{mol L}^{-1}$ at all stations (Fig. 7d).

380

381 4. Discussion

382 4.1. Bioavailability of Fe in the SPM_{DSW}

383 The present study is the first clear demonstration that particulate matter ($> 1 \mu\text{m}$)
 384 collected in the nepheloid layer can supply bioavailable Fe that can promote the growth of
 385 phytoplankton such as diatoms. The base FSW, which was collected from a depth of 200 m in
 386 the Oyashio region, should contain ample nutrients and other trace elements except for Fe,
 387 and measurable nutrients remained in all treatments at the end of the experiment. Furthermore,
 388 NH_4^+ , which potentially promotes the growth of phytoplankton, was constant at $\sim 0.3 \mu\text{mol}$
 389 L^{-1} in the $\text{SPM}_{\text{DSW}}\text{-Phy}$ treatment throughout the experiment. This suggests that

390 remineralized elements other than Fe, such as other trace metals and nutrients, did not affect
391 the growth of phytoplankton in the SPM_{DSW}-added treatments (c.f., Coale, 1991). The higher
392 Chl-*a* concentration and diatom abundance, with slightly faster growth rates in the SPM_{DSW}
393 treatment than the Fe-added treatment, may signify that Fe from the SPM_{DSW} was supplied
394 immediately at a higher concentration than 5 nmol L⁻¹ or a higher bioavailability of Fe,
395 compared to added inorganic Fe, a large part of which is considered as solid phase inorganic
396 Fe. However, it is very difficult to compare the bioavailability of Fe in the SPM_{DSW} with that
397 in solid inorganic Fe oxyhydroxide because of the extremely different Fe concentration in the
398 media used in the present study. The rapid growth of phytoplankton in the DSW_{raw} treatment
399 during the 3 to 5 day incubation period partially supports the possibility that P-Fe_{DSW} in the
400 DSW mass was highly bioavailable. It should be noted that it is likely that the high
401 concentration of SPM_{DSW} interferes with the grazing pressure of microzooplankton (Price et
402 al., 1994), which could have enhanced the net growth rate further in the SPM_{DSW} treatment as
403 compared to the Fe-added treatment. In addition, we observed resting spores of *Hyalochaete*
404 spp. at the beginning of the DSW_{raw} treatment, which showed the fastest growth rate among
405 the treatments with a few days of lag period (Fig. 3a). Diatom resting spores are an ecological
406 strategy in order to survive under unfavorable conditions for growth (e.g., Sugie et al., 2010b),
407 and they can germinate rapidly after appearing under favorable conditions (McQuoid and
408 Hobson, 1996). We suggest that the resting spore-forming coastal diatoms contributed to the
409 fast growth of the centric species in the DSW_{raw} treatment with bioavailable Fe sources.

410 It has been well documented that the bioavailability of inorganic P-Fe for
411 photolithoautotrophs depends on the surface reactivity of the solid inorganic Fe oxyhydroxide
412 species (Kuma and Matsunaga, 1995; Chen and Wang, 2000; Yoshida et al., 2006). The
413 reactivity of solid inorganic Fe oxyhydroxide decreases with an increase in aging time and
414 temperature and depends on the crystal structure (Kuma and Matsunaga, 1995; Yoshida et al.,
415 2006). The temperature in the DSW is low (< 0°C: e.g. Fukamachi et al., 2004), and hence,
416 the bioavailability of Fe in the SPM_{DSW} may be kept high for a long time if the P-Fe_{DSW} is
417 composed of inorganic substances. Alternatively, the DSW is rich in organic detritus possibly
418 derived from intensive diatom production on the continental shelf of Sakhalin Island
419 (Nakatsuka et al., 2004). Such detritus can release Fe-binding ligands such as humic

substances during particulate remineralization in the water column by bacteria and photodissolution by irradiance in the surface layer (Mayer et al., 2009; Boyd et al., 2010). It has been reported that Fe complexes with humic type ligands are highly bioavailable, similar to inorganic Fe(III) for marine phytoplankton (Chen and Wang, 2008). In other cases, if bioactive Fe can be incorporated into or onto colloidal matrices, and then become enhanced in the particulate phase by the progressive sorption of other organic colloids with cation bridges (Wells, 2002), the Fe in the particles may become bioavailable after bacterial remineralization and/or photodissolution (Boyd et al., 2010). The effect of the short-term freezing of the SPM_{DSW} caused cell death of the phytoplankton, which may also enhance remineralization of the Fe from the SPM_{DSW} because Fe released from the dead phytoplankton potentially is of high bioavailability (Poorvin et al., 2004). In addition, a recent study reported that marine polysaccharides are important Fe-binding ligands in the colloidal fraction and that the Fe bound to polysaccharides is of high bioavailability for phytoplankton (Hasser et al., 2011). Polysaccharides are known as a precursor of transparent exopolymer particles, which are believed to be bridges to create large aggregates (Passow, 2002). These above possibilities allow us to assume that the highly bioavailable Fe could be retained in the particulate phase for a suitable length of time associated with organic matters in the DSW and OSIW. However, the bioavailability of Fe in the SPM_{DSW} may decrease with aging time during transport and with the increase in temperature when it is drawn into the surface mixed layer. Further experiments are needed to determine the accurate chemical state and bioavailability of P-Fe collected from various regions in the western subarctic Pacific with more realistic concentrations added to phytoplankton communities.

442

4.2. Phytoplankton dynamics and their available Fe sources in the surface waters

The addition of Fe enhanced phytoplankton growth in terms of Chl-*a* at all three stations indicating phytoplankton nutritional status was clearly Fe-limited in the control bottles. However, phytoplankton growth measured with cell abundances was consistently faster than that measured with Chl-*a* concentrations (Figs. 3, 5 and Tables 2, 4). The difference between the growth rate calculated from the Chl-*a* and diatom cell density was caused by the gradual decrease in the intracellular Chl-*a* concentration of the phytoplankton

450 over the incubation period (Sugie et al., 2011). The Chl-*a* cell quota was also affected due to
451 light conditions, which may differ between *in situ* and incubation conditions. It should also be
452 noted that bulk Chl-*a* concentration values reflect the whole community, while cell counts
453 target specific populations. This may also contribute to differences in growth rate determined
454 via Chl-*a* vs. cell counts.

455 The Si:N drawdown ratios of the Fe added bottles decreased compared to that of the
456 controls suggesting some contribution from diatoms, especially at Stn Oy, and their recovery
457 from iron-limited conditions, which leads to a high Si:N drawdown ratio (Takeda, 1998;
458 Sugie et al., 2010b). The growth rate of pennate diatoms was enhanced during all experiments,
459 whereas the growth of centric species was promoted in only one (Stn Ok) out of four
460 experiments (Tables 2, 4). The growth rates of centric diatoms, where the stations had a
461 dominance of oceanic *Phaeoceros* spp., were not enhanced by the addition of Fe. In contrast,
462 the phytoplankton community at Stn Ok had a relatively high abundance of coastal centric
463 diatoms such as *Hyalochaete* spp. and *Thalassiosira* spp. compared to the other stations (Fig.
464 6), which may have contributed to an increase in the growth rate of the centric diatom in
465 response to the addition of Fe. Strezepek and Harrison (2004) reported that oceanic species
466 cannot adapt quickly to rapid changes in external environments due to the trade-off between
467 adaptability vs. the capacity to survive under relatively permanent, Fe-limited oceanic
468 conditions. In addition, surge Fe uptake upon Fe addition and subsequent storage using
469 ferritin are considered to be an advantageous strategy of pennate diatom (Marchetti et al.,
470 2009). Our results support previous suggestions that pennate diatoms are better able to
471 increase growth rate with the addition of inorganic Fe in relative to oceanic centric diatoms
472 (Table 4). Ecophysiological strategies in the Fe utilization of phytoplankton appear to be one
473 of the most important factors controlling phytoplankton dynamics in oceanic Fe-limited
474 regions.

475 At the beginning of the surface water incubations (i.e., reflecting *in situ* condition),
476 the Chl-*a* concentrations were relatively high (0.85 to 1.58 $\mu\text{g L}^{-1}$) compared to oceanic
477 HNLC regions such as the central subarctic gyres and the equatorial Pacific region (e.g., Price
478 et al., 1994; Nishioka et al., 2003). The net growth rate in the unamended controls calculated
479 from the Chl-*a* data and diatom cell density ranged from 0.20 to 0.31 d^{-1} (Fig. 5) and 0.69 to

1.34 d⁻¹ (Table 4), respectively. These results indicate that the *in situ* phytoplankton communities were mildly Fe-limited, which may explain the moderately high Chl-*a* concentrations in the surface waters even under D-Fe depleted conditions. Yoshimura et al. (2010) also reported that the phytoplankton community in the Bussol' Strait in 2006, at virtually the same location as Stn BS in this study, was under a Fe-limited or Fe- and light-co-limited condition. Although D-Fe concentrations were near depletion at the beginning of the incubations, phytoplankton biomass increased in the controls, with the $\mu_{\text{control}}/\mu_{\text{Fe-added}}$ ranging from 0.42 to 0.66. In the DFB treatments, which eliminated almost all external Fe from the bioavailable fraction to phytoplankton, the net Chl-*a* increase and NO₃+NO₂ drawdown were less than those in the controls (Fig. 7). It suggests that further growth of phytoplankton in the control bottles was not only supported by Fe in the intracellular fraction but also by the extracellular fractions.

The possible bioavailable Fe source for the phytoplankton was the particulate phase under the D-Fe depleted conditions. We estimated the utilization of intra- and extracellular Fe, which can be distinguished by calculating the increase of Chl-*a* and the NO₃+NO₂ and NH₄⁺ drawdowns in the controls minus that of the DFB-added treatment (hereafter referred to as $\Delta\text{Chl-}a$, $\Delta\text{NO}_3+\text{NO}_2$ and ΔNH_4^+ , respectively; Fig. 7b, c, d). However, we cannot estimate the extracellular fraction of utilized Fe at Stn Oy due to the fact that both D-Fe and T-Fe concentrations were below the detection limit. The utilized Fe in the extracellular source was potentially underestimated because we did not take into account the Fe uptake from the DFB-Fe complex (e.g., Maldonado and Price, 1999, 2001; Strezepek et al., 2011). However as stated earlier, the Fe uptake and subsequent cell or Chl-*a* growth should be very small for an extremely high DFB:Fe ratio (1 $\mu\text{mol L}^{-1}$:< 0.05–0.5 nmol L⁻¹ (T-Fe)), as observed in previous studies (Wells, 1999; Iwade et al., 2006; Sugie et al., 2011).

First, we calculated the potential Fe requirement of the phytoplankton in the control bottles from the extracellular fraction using the $\Delta\text{Chl-}a$ values during the incubations and reported Fe:Chl-*a* values. The Fe:Chl-*a* ratio was calculated from the Fe:C ratio ($\mu\text{mol}:\text{mol}$) divided by the Chl-*a*:C ratio (g:mol) applied from reported values (Table 5). The Fe:Chl-*a* value of the Fe-limited oceanic phytoplankton ranged from 4.7 to 108 $\mu\text{mol g}^{-1}$ (Table 5). In this study, phytoplankton communities at Stns BS and Ok were Fe-limited; consequently, we

510 selected these ratios determined only under Fe-limited conditions. Because the contribution
 511 of coastal species was small (~2% at Stn BS and ~8% at Stn Ok), we used the reported values
 512 obtained from oceanic phytoplankton species. Although these values were measured through
 513 unialgal culture experiments using nano- and micro-sized phytoplankton, we had no
 514 information regarding accurate Fe:Chl-*a* values in the natural phytoplankton community and
 515 pico-sized phytoplankton. The reported Fe:C values of the oceanic phytoplankton used in the
 516 above calculation was similar to that of natural oceanic phytoplankton in Fe-limited HNLC
 517 regions (Table 5). It should be noted that the estimated Fe requirement may be potentially
 518 overestimated because the Fe requirements of pico-phytoplankton, which may contribute
 519 substantial biomass in the bottles, is believed to be small relative to that of larger
 520 phytoplankton (Timmermans et al., 2005). However, the size dependence of the Fe:C ratio is
 521 still in debate; Strzepek et al. (2011) reported that the Fe:C ratio increased with a decrease in
 522 cell volume. According to the stoichiometric calculation, the phytoplankton community at Stn
 523 BS under the D-Fe depleted ($< 0.05 \text{ nmol L}^{-1}$) and P-Fe (0.5 nmol L^{-1}) conditions required
 524 0.05 to 1.10 nmol L^{-1} Fe from extracellular sources. The D-Fe (0.10 nmol L^{-1}) at Stn Ok
 525 could support the growth of a phytoplankton community in the control bottles (required 0.03
 526 to 0.71 nmol L^{-1}) only when applied to the lowest Fe:Chl-*a* value. The presence of the coastal
 527 diatom species would tend to increase Fe demand from the extracellular fraction due to their
 528 high Fe requirements compared to the oceanic species (Table 5).

529 Second, we calculated another index for the community Fe requirement from the
 530 extracellular fraction in the controls using the data on $\Delta\text{NO}_3 + \text{NO}_2$ and ΔNH_4^+ values. The
 531 nitrogen utilizations were converted into carbon biomass using the canonical Redfield ratio
 532 (Redfield et al., 1963). The variation in C:N ratio caused by changing the availability of Fe is
 533 rather small compared to Fe:C ratios (e.g., Price, 2005; Sugie and Yoshimura, in press). The
 534 decrease in Fe requirements of phytoplankton using NH_4^+ relative to NO_3 utilization was
 535 taken into account according to the theoretical calculations of Raven (1988). The Fe:C ratios
 536 of the Fe-limited phytoplankton applied ranged from 0.4 to $\sim 10 \mu\text{mol mol}^{-1}$ (Table 5). The
 537 Fe:C ratios fall in the minimum to intermediate values of phytoplankton Fe requirements
 538 determined for unialgal culture and *in situ* phytoplankton and seston (Table 5). The
 539 phytoplankton community required 0.03 – 0.84 and 0.02 – 0.62 nmol L^{-1} Fe at Stns BS and Ok,

540 respectively, with <0.05 and 0.10 nmol L^{-1} D-Fe and ~ 0.50 and 0.08 nmol L^{-1} P-Fe
541 concentrations *in situ*, respectively.

542 These above estimations suggest that observed phytoplankton growth in control
543 experiments at both Stns BS and Ok could not have been supported by only the D-Fe phase.
544 Fitzwater et al. (2003) reported that $\sim 10\%$ of Fe in the particulate phase was labile (leachable
545 by 25% acetic acid for 2h) and that labile particulate Fe may contribute to the growth of
546 phytoplankton through Fe remineralization. In addition, a recent model study suggested that
547 the phytoplankton community in the Oyashio region of the western subarctic Pacific
548 compensates their Fe demand from remineralization and/or desorption processes via the
549 particulate phase during the summer (Shigemitsu et al., 2012). Our empirical calculations
550 support the previous measurements (Fitzwater et al., 2003) and estimations (Elrod et al.,
551 2008; Nakayama et al., 2010; Sugie et al., 2010a) that a significant portion of the Fe in the
552 particulate phase is bioavailable. Although some latent uncertainties in the stoichiometric
553 calculation remain, the Fe in the particulate phase is an important Fe source for a
554 phytoplankton community under D-Fe depleted conditions. Previous studies reported that the
555 possible bioavailability of Fe in the particulate phase would change due to the variability of
556 the source of SPM (Fitzwater et al., 2003; Elrod et al., 2008). Note that the source of SPM
557 around the Bussol' Strait most likely originates from coastal regions of the Sea of Okhotsk
558 (see below), not from the Kurile Islands due to the very narrow continental shelves with steep
559 trenches (Fig. 1). The bioavailability of Fe in the particulate fraction derived from different
560 sources and regions is an important issue to clarify in understanding the variability of Fe
561 bioavailability in coastal and oceanic waters. Furthermore, the remineralization rate for Fe in
562 the various sources of SPM is critically important to better understand the biogeochemical
563 cycle of Fe and other nutrients in the ocean.

564

565 4.3. Possible role of SPM_{DSW} around the Kuril Strait

566 The Fe in the SPM_{DSW} was apparently bioavailable for phytoplankton, as shown in
567 the present study. Previous field observations and model work indicated that a high Fe water
568 mass would be transported from the OSIW originating from coastal regions of the Sea of
569 Okhotsk to the western North Pacific region via the Bussol' Strait (Nishioka et al., 2007;

Misumi et al., 2011). In the present study, deep vertical mixing was observed at Stns BS and Ok; mixing around the Kuril Straits is a common phenomenon (e.g., Ito et al., 2010, 2011; Yoshimura et al., 2010). Therefore, a substantial portion of Fe, especially P-Fe in the surface near Stns BS and Ok, may be considered to be derived from the OSIW, the residence time of which is considered to be a few years (Yamamoto et al., 2001). The availability of P-Fe in OSIW and DSW would be substantially prolonged, possibly due to the formation of Fe-organic associations as suggested above, and the cold characteristic of the OSIW mass ($<1^{\circ}\text{C}$) may also support the bioavailability of Fe for a long time (Kuma et al., 2000). Recent studies also indicated that the contribution of lithogenic Fe to the total Fe in the SPM ($>1\ \mu\text{m}$) collected from the OSIW with the same *in situ* filtration system as the present study decreased from Sakhalin Island toward the Bussol' Strait (Shigemitsu, unpublished data). This suggests that a substantial part of the P-Fe around the Bussol' Strait is associated with organic materials, which may be available for phytoplankton as observed in the present study. These above physical and chemical factors potentially enable phytoplankton to utilize bioavailable P-Fe in the surface mixed layer even with the relatively long residence time of the OSIW. In future work, we should examine to what extent the bioavailability of P-Fe is prolonged and the importance of bioavailable P-Fe in the western subarctic Pacific regions where P-Fe contributes to relatively rich environments (Nishioka et al., 2003; Kitayama et al., 2009; Nakayama et al., 2010). In addition, it should be noted that phytoplankton in the DSW mass showed healthy growth ($\mu = 1.0\ \text{d}^{-1}$), with a lag period of a few days as observed in the DSW_{raw} treatment. The short lag time could stem from the presence of resting spore-forming diatoms such as *Hyalochaete* spp., *T. antarctica* ver. *borealis* and *D. brightwellii*, which require a few days for germination (c.f., McQuoid and Hobson, 1996). The seed population of coastal diatoms is transported around the Kuril Islands with the southeasterly flow of the OSIW. Yoshimura et al. (2010) reported that coastal diatom species such as *Thalassiosira* spp. dominated among the diatoms at the end of the incubation experiment conducted near Stn BS in August 2006, when the water column mixed down to ~800 to 1000 m without surface stratification. We also observed an increase in the coastal diatom species, with high net growth rates ranging from 0.78 to $1.1\ \text{d}^{-1}$ when using seawater collected from 50, 200, 500 and 1000 m depths at Stns Oy, BS and Ok for incubation (Sugie, unpublished data). Such

600 coastal diatom species are clearly different from the summer phytoplankton community in the
601 open western subarctic Pacific regions (Aizawa et al., 2005; Komuro et al., 2005). The
602 complex physical conditions in the Sea of Okhotsk may be able to sufficiently host
603 allochthonous coastal phytoplankton around the Kuril Straits, possibly originating from the
604 pelagic realm of the inner part of the Sea of Okhotsk. Furthermore, nutrients, especially
605 Si(OH)_4 dissolved from the SPM_{DSW} as revealed from the data in the SPM_{DSW} -Phy treatment,
606 partly enhance nutrient concentrations in the intermediate water. Nevertheless, we still need
607 to quantify the accurate remineralization rate for nutrients dissolved from the SPM_{DSW} with a
608 longer experimental period and low temperatures. If the SPM_{DSW} -like particles were
609 transported to the Kuril Straits region and subsequent diapycnal mixing occurred (Nakamura
610 and Awaji, 2004), then bioavailable P-Fe (i.e., P-Fe_{DSW} or SPM_{DSW}) and healthy coastal
611 phytoplankton could partly emerge to the sunlit surface waters associated with macronutrients
612 and bioavailable D-Fe. Such bio-active elements and allochthonous phytoplankton
613 populations supplied by mixing events seemingly support high productivity and biodiversity
614 around the Kuril Straits. In addition, the bioavailability of P-Fe for phytoplankton other than
615 diatoms is an important issue because they play a major role in the D-Fe-depleted oceanic
616 regions of the North Pacific Oceans during the summer (e.g., Suzuki et al., 2002).

617

618 Contributors

619 K.S., J.N., K.K., Y.N.V. and T.N. designed research; K.S., J.N. and T.N. performed
620 research; K.S. and J.N. analyzed data; K.S. wrote the paper and J.N., K.K. and T.N. assisted
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622

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633

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869

870 Table and figure captions

871 Table 1. Experimental design of six treatments to test the bioavailability of particulate Fe
872 collected from dense shelf water (SPM_{DSW}) for the Fe-limited phytoplankton community.
873 Symbols of plus and minus represent the factors added or not added, respectively. *: Not
874 an extra addition, but natural SPM and phytoplankton are present in the DSW_{raw}
875 treatment.

876 Table 2. Phytoplankton abundance ($\times 10^3$ cells L⁻¹) and apparent growth rate (day 0 to 5) of
877 centric and pennate diatoms during the SPM_{DSW} addition experiment.

878 Table 3. Nutrient drawdown (in $\mu\text{mol L}^{-1}$) and its ratios during 5 days of the SPM_{DSW}
879 addition experiment. Data represent mean $\pm$ 1SD of triplicate incubation bottles. *:
880 Nutrient concentrations and drawdown ratios were offset by subtracting the value
881 dissociated from the SPM_{DSW} measured in the SPM_{DSW}-Phy treatment.

882 Table 4. Phytoplankton abundance ($\times 10^3$ cells L⁻¹) and apparent growth rate (day 0 to 5) of
883 centric and pennate diatoms and flagellates during the surface water incubations.

884 Table 5. Published elemental composition of the phytoplankton and seston under Fe-limited
885 conditions. The reported values of Fe-starved phytoplankton, cultivated with strong
886 chelator such as desferrioxamine B were excluded. Values with * indicate that
887 extracellularly absorbed iron was not eliminated, whereas the other values represent
888 intracellular elemental compositions. n.d.: no data. †: Fe:Chl-a values with no Chl-a:C
889 measurement were estimated using other reported Chl-a:C values (0.08–0.38 g:mol).

890

891 Figure 1. Sampling locations around the Sea of Okhotsk. The gray contours are shown at a
892 1000 m isobath interval. Abbreviations; DSW: dense shelf water, Ok: Okhotsk, BS:
893 Bussol' Strait, and Oy: Oyashio.

894 Figure 2. Vertical profiles of (a) temperature, (b) salinity (c) sigma- t and (d) beam
895 transmission at the beginning of the SPM_{DSW} addition experiment at Stn. DSW.

896 Figure 3. (a) Temporal change in chlorophyll- a concentrations examining Fe bioavailability
897 in the suspended particulate matter collected from DSW (SPM_{DSW}) at Stn. DSW. Data
898 represent mean $\pm$ 1SD for triplicate or mean $\pm$ range for duplicate analysis. The error
899 bars are hidden when smaller than the symbols. (b) Specific growth rate for six
900 treatments. Data represent mean $\pm$ 95% C.L. of regression (see method).

901 Figure 4. Vertical profiles of (a) temperature, (b) salinity and (c) sigma- t at the beginning of
902 the surface phytoplankton incubation experiments.

903 Figure 5. Temporal changes in (a), (b) and (c) chlorophyll- a (Chl a), (d), (e) and (f)
904 NO₃+NO₂, and (g), (h) and (i) Si(OH)₄ at station Oyashio (a, d and g), Bussol' Strait (b, e
905 and h) and Okhotsk (c, f and i). Data represent mean $\pm$ 1SD for triplicate incubation
906 bottles.

907 Figure 6. Diatom species composition and cell densities at the start of the surface
908 phytoplankton incubation experiments at station Oyashio (Oy), Bussol' Strait (BS) and
909 Okhotsk (Ok).

910 Figure 7. Comparing phytoplankton response during the surface phytoplankton incubation
911 experiments in terms of (a) the relative growth rate in the unamended control to the
912 Fe-added treatment ($\mu_{\text{control}}/\mu_{\text{Fe-added}}$), (b) difference in the increased Chl- a between the
913 control and the DFB treatment ($[\Delta\text{Chl-}a]$), (c) difference in the NO₃+NO₂ drawdown
914 between the control and the DFB treatment ($[\Delta\text{NO}_3+\text{NO}_2]$), and (d) difference in ΔNH_4^+
915 drawdown between the control and the DFB treatment ($[\Delta\text{NH}_4^+]$). Data represents mean
916 $\pm$ 1SD for triplicate incubation bottles.

Table 1. Experimental design of six treatments to test the bioavailability of particulate Fe collected from dense shelf water (SPM_{DSW}) for the Fe-limited phytoplankton community. Symbols of plus and minus represent the factors added or not added, respectively. *: Not an extra addition, but natural SPM and phytoplankton are present in the DSW_{raw} treatment.

Treatment	Phytoplankton	Fe	SPM_{DSW}	DFB
Control	+	—	—	—
Fe	+	+	—	—
DFB	+	—	—	+
SPM_{DSW}	+	—	+	—
SPM_{DSW} –Phy	—	—	+	—
DSW_{raw}	—*	—	—*	—

Table 2. Phytoplankton abundance ($\times 10^3$ cells L⁻¹) and apparent growth rate (day 0 to 5) during the SPM_{DSW} addition experiment.

Treatments	Abundance at day 0		Abundance at day 5		Growth rate (d ⁻¹)	
	Centric	Pennate	Centric	Pennate	Centric	Pennate
Controls	2.6	1350	39	21300	0.54	0.55
Fe	2.6	1350	40	41400	0.54	0.68
DFB	2.6	1350	44	1700	0.56	0.05
SPM _{DSW}	28	1410	173	120000	0.84	0.90
SPM _{DSW} -Phy	28	56	84	39	0.22	-0.07
DSW _{raw}	0.32	0.30	52	28	1.0	0.90

Table 3. Nutrient drawdown (in $\mu\text{mol L}^{-1}$) and its ratios during 5 day incubation experiment. Data represent mean $\pm$ 1SD of triplicate incubation bottles.

Treatment	ΔDIN	ΔP	ΔSi	$\Delta\text{Si: } \Delta\text{N}$	$\Delta\text{N: } \Delta\text{P}$	$\Delta\text{Si: } \Delta\text{P}$
Control	14.4 ± 0.38	0.95 ± 0.16	4.00 ± 1.09	0.28 ± 0.07	15.7 ± 2.94	4.45 ± 1.68
Fe	21.7 ± 1.04	1.48 ± 0.04	7.55 ± 0.84	0.35 ± 0.02	14.7 ± 0.48	5.10 ± 0.49
DFB	6.95 ± 0.61	0.69 ± 0.09	-1.15 ± 1.20	-0.18 ± 0.18	10.1 ± 0.48	-1.89 ± 1.82
SPM _{DSW}	$38.2 \pm 1.05^*$	$1.95 \pm 0.06^*$	$28.1 \pm 1.16^*$	$0.74 \pm 0.03^*$	$19.6 \pm 0.71^*$	$14.4 \pm 0.20^*$
SPM _{DSW} –Phy	-1.92 ± 1.60	-0.17 ± 0.22	-10.9 ± 3.95	9.88 ± 6.36	3.59 ± 24.1	-93.4 ± 313
DSW _{raw}	1.18 ± 0.85	0.16 ± 0.13	1.29 ± 2.37	-0.76 ± 2.56	9.42 ± 2.37	-13.2 ± 25.9

*: Nutrient concentrations and drawdown ratios were offset by subtracting the value dissociated from the SPM_{DSW} in the SPM_{DSW}–Phy treatment.

Table 4. Phytoplankton abundance ($\times 10^3$ cells L^{-1}) and apparent growth rate (day 0 to 5) during the surface water incubations.

Station		Initial	Abundance at day 5			Growth rate (d^{-1})		
			Cont.	Fe	DFB	Cont.	Fe	DFB
Oy	Centric diatoms	0.40	24.8	23.2	6.46	0.83	0.81	0.55
	Pennate diatoms	1.67	434	814	136	1.11	1.24	0.88
	Flagellates	89.7	573	907	123	0.83	0.92	0.52
BS	Centric diatoms	1.18	119	124	25.2	0.92	0.93	0.61
	Pennate diatoms	3.90	557	661	107	0.99	1.02	0.66
	Flagellates	62.6	2926	4514	705	1.23	1.32	0.94
Ok	Centric diatoms	1.44	45.7	316	34.1	0.69	1.08	0.63
	Pennate diatoms	4.16	2080	3330	596	1.24	1.34	0.99
	Flagellates	17.1	9760	11600	358	1.27	1.30	0.61

Table 5. Published elemental composition of the phytoplankton and seston under Fe-limited conditions. The reported values of Fe-starved phytoplankton, cultivated with strong chelator such as desferrioxamine B were excluded. Values with * indicate that extracellularly absorbed iron was not eliminated, whereas the other values represent intracellular elemental compositions. n.d.: no data. †: Fe:Chl-*a* values with no Chl-*a*:C measurement were estimated using [other](#) reported Chl-*a*:C values (0.08–0.38 g:mol).

Data source	Fe:C ($\mu\text{mol}:\text{mol}$)	Chl- <i>a</i> :C (g:mol)	Fe:Chl- <i>a</i> ($\mu\text{mol}:\text{g}$)	Reference
Unialgal culture				
Diatom				
<i>Eucampia antarctica</i> (oceanic)	1.8	n.d.	4.7–23†	Strzepek et al., 2011
<i>Fragilariopsis kerguelensis</i> (oceanic)	4.2	n.d.	10–53†	Strzepek et al., 2011
<i>Proboscira inermis</i> (oceanic)	4.5	n.d.	12–56†	Strzepek et al., 2011
<i>Pseudo-nitzschia</i> spp. (oceanic)	2.8–3.7	n.d.	10–35†	Marchetti et al., 2006
<i>Pseudo-nitzschia</i> spp. (coastal)	5.2–11	n.d.	29–65†	Marchetti et al., 2006
<i>Thalassiosira weissflogii</i> (coastal)	10.6	0.08	23	Price, 2005
<i>T. weissflogii</i> (coastal)	13–22	0.11–0.31	63–115	Sunda and Huntsman, 1995
<i>Thalassiosira pseudonana</i> (coastal)	13–25	0.12–0.21	82–123	Sunda and Huntsman, 1995
<i>Thalassiosira oceanica</i> (oceanic)	2.5–7.1	0.17–0.32	11–28	Sunda and Huntsman, 1995
Others				
<i>Emiliania huxleyi</i> (oceanic)	4.1–6.0	0.16–0.38	16–29	Sunda and Huntsman, 1995
<i>Pelagomonas calceolata</i> (oceanic)	4.3–9.6	0.26–0.32	14–32	Sunda and Huntman, 1995
<i>Phaeocystis antarctica</i> (oceanic)	8.6	n.d.	23–108†	Strzepek et al., 2011
<i>Prorocentrum minimum</i> (coastal)	11–20	0.12–0.16	89–129	Sunda and Huntman, 1995
Natural particle				
Phytoplankton	6.0–8.7*	n.d.	23–75†	Twining et al., 2004
Seston	30*	n.d.	79–375†	Martin et al., 1989
Seston	3.7	n.d.	9.7–46†	Maldonado and Price, 1999

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7