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1 **Increase in Si:N drawdown ratio due to resting spore formation by spring bloom-forming**
2 **diatoms under Fe- and N-limited conditions in the Oyashio region**

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21 *Key Words*

22 diatom; iron; resting spore; Si:N drawdown ratio; spring bloom; Oyashio region

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24 *Running Head*

25 Fe- and N-limited resting spores

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31 **ABSTRACT:** Resting spore formation and Si:N drawdown ratios were investigated under iron (Fe)-
32 and nitrogen (N)-limited conditions using a unialgal culture of *Thalassiosira nordenskiöldii* and
33 natural phytoplankton assemblages during the spring bloom in the Oyashio region. In the unialgal
34 culture of *T. nordenskiöldii*, 20% and 100% of the cells formed resting spores under Fe- and
35 N-limited conditions, respectively. The Si:N drawdown ratios were 2- and 14-fold higher in Fe- and
36 N-limited conditions, respectively, compared to Fe- and N-sufficient conditions. At the start of the
37 natural phytoplankton incubation, 18 among 47 identified diatom species were known resting
38 spore-forming species. Approximately 15 common diatom species formed resting spores under Fe-
39 and N-limited conditions. During the natural phytoplankton incubation, the percentage of the resting
40 spores increased with time under both Fe- and N-limited conditions, reaching 25% and 40% of total
41 diatom abundance, respectively. The Si:N drawdown ratios significantly increased with an increase
42 in the contribution of resting spores in both the unialgal culture and natural phytoplankton
43 incubations. These results suggest that if the bloom dominating by neritic, resting spore-forming
44 diatom species declines by either Fe- or N-depletion, Si may be utilized preferentially to N in the
45 upper mixed layer due to the formation of heavily silicified resting spores.

46

47 **1. Introduction**

48

49 The annual spring phytoplankton bloom in temperate to polar regions is a common
50 phenomenon in which diatoms usually play a predominant role (Sarthou et al., 2005). Diatoms are a
51 major component of biological pumps and biogenic silica flux in the ocean through sedimentation of
52 unutilized phytodetritus, resting spores, and/or fecal pellets utilized by zooplanktons (Smetacek,
53 1999; Thompson et al., 2008). In the Western Subarctic Pacific (WSP) region, centric chain-forming
54 diatoms dominate the phytoplankton community during the spring bloom period, and controlling
55 macronutrient dynamics (Mochizuki et al., 2002; Liu et al., 2004). Some of these bloom-forming
56 diatoms are known to form resting spores in response to adverse environmental conditions that are
57 unfavorable for their growth (McQuoid and Hobson, 1996). Once the heavily silicified and
58 fast-sinking resting spores are formed, they sink and can sequester nutrients without significant
59 dissolution and grazing in the water column (Smetacek, 1985; McQuoid et al., 2002; Kuwata and
60 Tsuda, 2005). Resting spore formation is reported to be induced primarily by nitrogen limitation

61 (Hargraves and French, 1983). We recently demonstrated the formation of resting spores in two
62 strains of a unialgal culture of *Thalassiosira nordenskiöldii* under Fe- and N-limited conditions
63 (Sugie and Kuma, 2008), suggesting that N was directly limited by substrate depletion under the
64 N-limited condition and indirectly by intracellular Fe and N co-limitation under the Fe-limited
65 condition. However, resting spore formation of natural diatom communities under Fe-limited
66 conditions has not yet been reported.

67 Iron is one of the most important trace elements for phytoplankton growth, and it is
68 essential for several biochemical processes such as photosynthetic and respiratory electron transport,
69 and nitrogenous nutrient assimilation (Geider and La Roche, 1994). In general, one of the most
70 bioavailable iron species for phytoplankton is dissolved inorganic Fe(III) [Fe(III)'] (Anderson and
71 Morel, 1982; Morel et al., 2008). However, the thermodynamically stable oxidation state of iron in
72 oxic surface seawater is Fe(III), which has an extremely low solubility (Stumm and Morgan, 1996;
73 Waite, 2001). Furthermore, the presence of natural organic ligands such as siderophore in the surface
74 mixed layer can reduce the concentration of bioavailable Fe(III)' by complexing strongly with Fe(III)
75 in seawater (Rue and Bruland, 1995). Therefore, marine phytoplankton, especially diatoms in
76 oceanic regions situated away from iron sources, often have limited Fe (Martin, 1990; Tyrrell et al.,
77 2005). The WSP is one of the Fe-limited high-nutrient low-chlorophyll (HNLC) regions in the
78 world's oceans (Banse and English, 1999), whereas the Oyashio region and some areas at the edge of
79 the subarctic Pacific region are possible exceptions to the HNLC regime (Harrison et al., 2004;
80 Whitney et al., 2005). However, Suzuki et al. (2002) and Nishioka et al. (2003) reported that the late
81 spring-to-summer phytoplankton community in the Oyashio region was Fe-limited, with relatively
82 low ambient dissolved Fe (D-Fe) concentrations ($<0.22 \mu\text{m}$, $\sim 0.1 \text{ nmol L}^{-1}$). In addition, the surface
83 seawater in the Oyashio region during summer was a heterogeneous mixture of N-depleted and
84 HNLC-like conditions (Saito et al., 2002). Therefore, it can be assumed that the spring
85 phytoplankton bloom community in the Oyashio region would be affected and eventually regressed
86 by either Fe- or N-deficient conditions, even in regions where relatively high levels of iron are
87 supplied from Fe-rich intermediate waters and atmospheric Fe-rich dust deposition to the surface
88 mixed layer (Nishioka et al., 2007).

89 Recent studies have demonstrated that Fe influences the macronutrient consumption ratio
90 and elemental composition of diatoms, with the cellular Si:N ratio increasing under Fe-limited

91 conditions (Takeda, 1998). Other studies examining a variety of Fe-limited diatom cultures and
92 natural phytoplankton communities have suggested a reduction in cellular N content; an increase in
93 frustule silicification; and change in cell morphology, such as increased surface area-to-cell volume
94 ratio, as possible mechanisms responsible for the elevated cellular Si:N ratio (Timmermans et al.,
95 2004; Leblanc et al., 2005; Marchetti and Harrison, 2007). Similarly, the Si:N ratio is 8-fold higher in
96 N-limited resting spores of *Chaetoceros pseudocurvisetus* than in vegetative cells (Kuwata et al.,
97 1993). A few studies reported the sedimentation of resting spores in HNLC-like regions where
98 seasonal blooms and resulting Fe-depletion were observed in the southeastern edge of the Western
99 Subarctic Gyre (Onodera et al., 2002; Nishioka et al., 2003) and the Kerguelen Plateau in the
100 Southern Ocean (Armand et al., 2008a, b). Although sedimentation of resting spores has been
101 reported in HNLC-like regions that would experience Fe-depletion, the relationship between resting
102 spore formation under N- or Fe-limited conditions and macronutrient dynamics during the spring
103 bloom periods has not been closely investigated.

104 In this study, we investigated the formation of resting spores and Si:N drawdown ratios
105 under Fe- and N-limited conditions in a unialgal culture of *T. nordenskiöldii* and natural
106 phytoplankton community incubation during the spring bloom period in the Oyashio region. We
107 hypothesized that many bloom-forming neritic diatom species form resting spores under Fe- and
108 N-limited conditions. In addition, regardless of whether Fe- or N-depletion has a greater effect on the
109 spring bloom, we predict that Si is preferentially utilized to N on the basis of the formation of heavily
110 silicified resting spores.

111

112 **2. Materials and methods**

113

114 *2.1. Unialgal culture experiment*

115

116 A unialgal strain of *T. nordenskiöldii* was isolated from the surface seawater of the
117 Oyashio region (42°00'N, 145°15'E) in the northwestern Pacific Ocean side of southern Hokkaido,
118 Japan, by a capillary pipette. The unialgal strain was maintained in silicic acid-enriched [105 μmol
119 L^{-1} $\text{Si}(\text{OH})_4$] f/2 medium (Guillard and Ryther, 1962) under 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ fluorescent
120 light (QSL-100, Biospherical Instrument Inc.) in a 12-h L:12-h D cycle at 5°C. The maintenance

121 cultures were not axenic, but additional bacterial contamination was minimized by using sterile
122 techniques and serial transfers during the exponential growth.

123 All equipment used in the culture experiment were acid-cleaned, followed by rinsing with
124 Milli-Q water (Millipore), and all preparations and sampling for experiments were performed in a
125 Class 100 laminar flow cabinet to avoid inadvertent trace metal contamination. Seawater for the
126 culture experiment was collected from a coastal region near Hokkaido, in the northern Japan Sea
127 (43°23' N, 141°02' E), and was filtered through an acid-cleaned 0.22- μm GS cellulose membrane
128 filter (Millipore). The filtered seawater was autoclaved for 20 min at 121°C (108 kPa) using an
129 acid-cleaned glass Erlenmeyer flask, aged for ca. 1 week at room temperature in the flask, and
130 re-filtered through the acid-cleaned 0.22- μm membrane filter to eliminate particulate Fe(III) species.
131 The concentrations of Fe, $\text{NO}_3 + \text{NO}_2 + \text{NH}_4$ (DIN), PO_4 (P) and $\text{Si}(\text{OH})_4$ (Si) in the double-filtered
132 autoclaved seawater (base seawater) were 0.4 nmol L^{-1} , 6.2 $\mu\text{mol L}^{-1}$, 0.3 $\mu\text{mol L}^{-1}$ and
133 approximately 250 $\mu\text{mol L}^{-1}$, respectively. The Fe concentration in the base seawater was
134 determined by an automated Fe analyzer (Kimoto Electric) using a combination of an
135 8-hydroxyquinoline chelating resin concentration and luminol-hydrogen peroxide
136 chemiluminescence detection in a closed flow-through system (Obata et al., 1993). Macronutrient
137 concentrations in the base seawater were measured by a QuAatro continuous flow analyzer
138 (Bran+Luebbe). The Si:N drawdown ratios were calculated from delta Si divided by delta N during a
139 certain growth interval.

140 Diatom stock cultures were maintained in silicic acid-enriched f/2 medium with three
141 transfers (~30 doublings) during the exponential growth phase. Diatoms in the late exponential
142 growth phase were inoculated into modified f/2 medium, which was prepared without adding f/2
143 metals, EDTA and vitamins to the medium. All f/2 nutrient stock solutions were passed through
144 Chelex 100 ion-exchange resin (Bio-Rad) to remove trace metals (Morel et al., 1979). Diatoms were
145 grown in modified f/2 media to which only ferric iron and manganese stock solutions were added to
146 make final Fe and Mn concentrations of 100 and 25 nmol L^{-1} , respectively, and to obtain slightly
147 Fe-stressed cells. Previous studies have found that addition of both Mn and Fe to the modified f/2
148 medium kept the cells in physiologically good state for a suitable length of time (Peers and Price,
149 2004; Ushizaka et al., 2008); hence, we added only Fe and Mn but eliminated other trace elements in
150 the culture media in this study. Furthermore, late exponential growing cells in the modified f/2 media

151 were harvested by gravity filtration onto an acid-cleaned 0.2- μm membrane filter and immediately
152 resuspended in the base seawater to remove unused Fe in the pre-cultured media; these cells were
153 used for the following experiments.

154 In the culture experiment, macronutrient stock solutions were added to obtain the final
155 concentrations of 180 $\mu\text{mol L}^{-1}$ DIN, 15 $\mu\text{mol L}^{-1}$ P and 355 $\mu\text{mol L}^{-1}$ Si (base medium), which
156 were then determined by the approximate elemental ratio of macronutrients in the Oyashio region
157 during winter (Saito et al., 2002; Saito and Tsuda, 2003). Resuspended *T. nordenskiöldii* were
158 inoculated into base media (800 mL) in 1-L polycarbonate Erlenmeyer flasks, resulting in an initial
159 cell density of approximately 1000 cells mL^{-1} . The effect of direct Fe and Mn inputs (Fe-replete
160 treatment) was examined by adding Fe(III) and Mn(II) stock solutions to the control medium to
161 obtain the final concentrations of 100 and 25 nmol L^{-1} , respectively. Fe-limited media (Fe-limited
162 treatment) were prepared by adding only the acidic Mn(II) stock solution (final concentration of 25
163 nmol L^{-1}) to the control media. The N-limited medium (N-limited treatment) was prepared by
164 adding the f/2 metal stock solution to the modified control media without nitrate. Culture
165 experiments were conducted in triplicate. The light and temperature conditions were the same as
166 those for the stock culture described earlier. During the experiments, the number of vegetative cells,
167 resting spores and resting cells were monitored daily using unfixed cells by 6-replicate cell counts in
168 a hemacytometer with a light microscope magnified $\times 100\text{--}200$. Resting cells were identified by the
169 chlorotic, shrunken, less abundant, and asymmetrically distributed chloroplasts without stored
170 products within the cell. Resting spores have stored products such as carbohydrates within the cell
171 (Kuwata et al., 1993), thus showing specific refraction under the light microscope. Some spores had
172 both resting spore and daughter cell frustules (i.e., endogenous and semi-endogenous resting spores).
173 In contrast, vegetative cells had symmetrically distributed swelling chloroplasts. The samples for
174 nutrient analysis during cultivation (~ 20 mL) were obtained daily by filtration using a DISMIC
175 0.2- μm filter, and were measured with a QuAAtro continuous flow analyzer.

176

177 *2.2. Natural phytoplankton incubation experiment*

178

179 Experiments were conducted in the Oyashio region (42°00'N, 145°15'E) on April 20, 2007
180 as part of the Ocean Ecodynamics Comparison in the subarctic Pacific research program during the

181 KH-07-01 cruise aboard R/V Hakuho-Maru. Seawater samples for the analysis of macronutrients
182 and D-Fe were collected at depth ranging from 5 to 300 m, using acid-cleaned Teflon-coated 10-L
183 Niskin X sampling bottles (General Oceanics) attached to a CTD-carousel multi-sampling system.
184 Hydrographic data (salinity, temperature, and depth) were obtained using a CTD (Sea-Bird, Model
185 9-puls). Seawater for experiments was collected from a depth of 10 m and sieved by 100- μ m
186 acid-cleaned Teflon-mesh to eliminate large herbivorous zooplankton. The prescreened seawater
187 sample was mixed in an acid-washed 20-L polyethylene tank and then dispensed into acid-cleaned
188 320-mL polycarbonate bottles. The three treatments were carried out as follows: the unamended
189 control; Fe-limited media with addition of 15 μ mol L⁻¹ NO₃, 1.9 μ mol L⁻¹ P, 44 μ mol L⁻¹ Si and 1
190 μ mol L⁻¹ desferrioxamine B (DFB; Sigma Chem. Co. Ltd.); and N-limited media with addition of
191 1.9 μ mol L⁻¹ P, 44 μ mol L⁻¹ Si, and 5 nmol L⁻¹ Fe. DFB is a small trihydroxamate molecule that
192 complexes with inorganic Fe(III) with an extremely high conditional stability constant ($K'_{FeL, Fe(III)} =$
193 $[Fe(III)L]/[Fe(III)][L] = 10^{16.5} M^{-1}$) in seawater (Hudson et al., 1992). Thus, addition of an excess
194 concentration of the siderophore DFB relative to iron (Fe:DFB = 1:10) prevents Fe uptake in
195 phytoplankton by diminishing the concentration of bioavailable [Fe(III)] (Wells, 1999; Iwade et al.,
196 2006; Yoshida et al., 2006).

197 Triplicate and/or duplicate incubation bottles for 1-, 3-, 5-, 7- and/or 10-day cultivations for
198 each treatment were incubated at 5°C under 150 μ mol photon m⁻² s⁻¹ fluorescent light (12-h L:12-h
199 D cycle). Bottles were sacrificed at each intervals. All experimental preparations were conducted in a
200 clean room or on a clean bench (Class 100) on board. The chlorophyll *a* (chl-*a*) concentrations in the
201 samples were measured at each intervals by using the Turner Design 10-AU fluorometer
202 (Welschmeyer, 1994) after extracting the chl-*a* with *N,N*-dimethylformamide (Suzuki and Ishimaru,
203 1990). The methods for sample collection and analysis of macronutrients were the same as used in
204 the unialgal culture experiment described earlier. Growth rates were calculated from the linear
205 regression between the time and the natural log of chl-*a* concentrations. The samples (~100 mL) for
206 diatom cell densities and species compositions were collected at 0, 5 and 10 days. They were then
207 mixed with an equal volume of replicates of each treatment and fixed with formalin (1% final
208 volume) for analysis in a laboratory on land. An adequate volume of fixed seawater was poured into
209 a settling chamber (Hydro-bois) and was allowed to settle for at least 24 h before identification using
210 a phase-contrast inverted microscope (Hasle, 1978). Diatom species were identified according to

211 Hasle and Syvertsen (1997). Cell volume of the dominant diatom species was measured as described
212 by Hillebrand et al. (1999) and the cell volume was converted to carbon biomass as reported by
213 Montagnes and Franklin (2001). We could not discriminate between resting and vegetative cells
214 because the chloroplasts within the diatom cells had shrunk due to formalin fixation. Therefore, we
215 counted both cell types as vegetative cells.

216

217 **3. Results**

218

219 *3.1. Unialgal culture experiment*

220

221 *3.1.1. Vegetative cell and resting spore abundance*

222 In the Fe-replete treatment, vegetative cells increased exponentially for 5–6 days before
223 reaching the stationary growth phase. Vegetative cell density was almost constant for 4–10 days of
224 cultivation in the Fe-limited treatment, whereas a sudden decrease was observed after 4 days of
225 cultivation in the N-limited treatment (Fig. 1a). Resting cells in the Fe-limited treatment increased
226 after 4 days during the stationary growth phase of vegetative cells, corresponding to a gradual
227 increase in resting spores (Fig. 1b, c). In the N-limited treatment, there was a rapid decrease in
228 vegetative cells after day 4, and resting spores increased rapidly between 4 and 6 days, whereas the
229 resting cell density was much lower than that of the resting spores (Fig. 1b, c). The proportion of
230 resting spores in the Fe-limited treatment gradually increased to ~20% between days 4 and 11,
231 while the number of resting spores in the N-limited treatment reached >80% between 3 and 5 days
232 (Fig. 1d). There was no resting spore and cell formation in the Fe-replete treatment during the 9-day
233 experiment.

234

235 *3.1.2. Nutrient dynamics*

236 Nitrogen depletion ($<0.5 \mu\text{mol L}^{-1}$) was observed at days 3 and 9 in the N-limited and
237 Fe-replete treatments, respectively (Fig. 2a). Si utilization rates in all three treatments were almost
238 the same during 0–7 days of growth (Fig. 2b). Phosphate was not exhausted throughout the
239 experiments in all treatments (Fig. 2c). The Si:N drawdown ratio of exponentially growing *T.*
240 *nordenskiöldii* in the Fe-replete treatment was 0.59 (Table 1). Even after N depletion in the

241 N-limited treatment, Si uptake was maintained and the Si:N drawdown ratio reached ~64 during the
242 spore-forming phase (transition phase during days 3–7). The Si:N drawdown ratio in the N-limited
243 treatment reached ~8.5 by day 11, when the resting spore contribution was 100%. The Si:N
244 drawdown ratio in the Fe-limited treatment was approximately two times higher than that in the
245 Fe-replete treatment for all growth phases (Table 1).

246

247 3.2. Natural phytoplankton assemblage incubation experiment

248

249 3.2.1. Initial conditions

250 The upper mixed layer depth was 75 m, which was estimated from the first downward
251 increase in $\sigma_t \geq 0.02 \text{ m}^{-1}$ (Fig. 3a). D-Fe, DIN, P, and Si concentrations in the surface water (10 m
252 depth, 3.62°C) collected for the natural phytoplankton incubation experiment were 0.17 nmol L^{-1} ,
253 $14.8 \text{ } \mu\text{mol L}^{-1}$, $0.96 \text{ } \mu\text{mol L}^{-1}$, and $10.3 \text{ } \mu\text{mol L}^{-1}$, respectively. These micro- and macronutrient
254 concentrations were vertically homogeneous in the upper 50 m and increased gradually with depth
255 below 50 m (Fig. 3). The *in situ* Si:N ratio was ~1.1 in the upper mixed layer and increased up to ~2
256 below the 100 m stratum (Fig. 3b). Chl-*a* concentrations were also uniform in the upper 50 m (~4 μg
257 L^{-1}) and decreased with depth (Fig. 3c). Therefore, water collected for the natural assemblage
258 experiment was considered to be representative of the spring phytoplankton bloom in the Oyashio
259 region of the WSP (Saito et al., 2002).

260

261 3.2.2. Phytoplankton dynamics

262 The chl-*a*-specific growth rate from day 0 to 3 in the N-limited treatment ($0.40 \pm 0.01 \text{ d}^{-1}$;
263 $\text{avg.} \pm 1 \text{ SD}$) was significantly higher than that in the control ($0.14 \pm 0.01 \text{ d}^{-1}$; $p < 0.001$; ANOVA)
264 and Fe-limited ($0.08 \pm 0.01 \text{ d}^{-1}$; $p < 0.001$) treatments (Fig. 4a), without exhaustion of
265 macronutrients during the period (Fig. 5). Total diatom abundance was highest at day 10 with 19,850
266 cells mL^{-1} in N-limited treatment, followed by 7670 cells mL^{-1} in the Fe-limited treatment and 6230
267 cells mL^{-1} in the control (Fig. 4b). The total diatom abundances during days 5–10 were relatively
268 constant in all treatments, probably due to Si depletion in the control, N depletion in N-limited
269 culture media, and Si and/or Fe depletion in the Fe-limited culture media (Figs. 4b and 5).

270 Thirty-six centric and 11 pennate diatom species were identified in this study. Eighteen of

271 the forty-seven identified species have been reported previously to form resting spores (McQuoid
272 and Hobson, 1996; Table 2), and resting spore-forming diatom species dominated in abundance,
273 comprising up to ~85% of the diatom community at the start of the experiments (Table 3). In the
274 initial phytoplankton community, *Chaetoceros* subgenus *Hyalochaete* spp. were dominant in
275 abundance (~78%), whereas *Thalassiosira* spp. were dominant in biomass (~60%), followed by
276 *Chaetoceros* subgenus *Hyalochaete* spp. (~30%) (data not shown). In all treatments, the resting
277 spores increased with time (Fig. 4c). The relative order for the percentage of resting spores during the
278 10-day experiment was N-limited (40%) > Fe-limited (25%) >> control (5.2%) (Fig. 4d). Temporal
279 changes in the percentage of resting spores of each species are shown in Fig. 6. *Chaetoceros*
280 *compressus*, *Chaetoceros lacinosus*, *Chaetoceros similis* and *Chaetoceros socialis* were counted as
281 *Chaetoceros* spp. 1, and *Chaetoceros cinctus*, *Chaetoceros furcellatus*, *Chaetoceros radicans* and
282 *Chaetoceros tortissimus* were counted as *Chaetoceros* spp. 2, because they had indistinguishable
283 morphology in their resting spores and vegetative cells, respectively (Table 3). The initiation of
284 resting spore formation and the percentage of resting spores were remarkably different among
285 species and treatments (Fig. 6). In the N-limited treatment, *Chaetoceros debilis* and *Chaetoceros*
286 *diadema* rapidly formed resting spores during the first 5 days of the experiment (Fig. 6a, d), whereas
287 the resting spore percentages of other species increased rapidly between days 5 and 10 (Fig. 6b, c, e,
288 f). Lower resting spore percentages with lower sporulation rates were observed in the Fe-limited
289 treatment when compared to the N-limited treatment for all diatom species, except for *Chaetoceros*
290 spp. 1 and *T. nordenskiöldii* (Fig. 6b, f). The percentages of resting spores for four out of six species
291 in the Fe-limited treatment increased continuously during the 10-day period. However, resting spore
292 formation in the control (except for *Stephanopyxis nipponica*) increased only slightly throughout the
293 experiment, probably due to Si exhaustion after day 5 (Figs. 5 and 6). Resting spores were observed
294 for *C. cinctus* and *C. furcellatus*, but not for *C. radicans* (Table 3). *Leptocylindrus danicus* were
295 sporadically observed at a very low abundance, and thus its ability to form resting spores under N-
296 and/or Fe-limited conditions was not clear. *Probosira alata* were observed in all samples without
297 resting spore formation (Table 3). It was difficult to distinguish the vegetative cells of some resting
298 spore-forming species (e.g., *Thalassiosira* spp. and *Fragilariopsis* spp.) from those of
299 non-spore-forming species to calculate the specific percentage of resting spores. It is notable that the
300 diatom species that formed resting spores under Fe-limited conditions were the same as those that

301 formed resting spores under N-limited conditions (Table 3).

302

303 3.2.3. Nutrient dynamics

304 DIN was exhausted after 5 days in the N-limited treatment and after 7 days in the control.
305 However, little DIN was utilized in the Fe-limited treatment with DFB (Fig. 5a). Si was depleted
306 after 5 days in the control, linearly decreased over the 10-day period in the Fe-limited treatment, and
307 suddenly decreased after 3 days in the N-limited treatment (Fig. 5b). Phosphate was not depleted in
308 any treatment (Fig. 5c). The Si:N drawdown ratio in the control decreased from 1.17 during the first
309 3 days to ~ 0.7 over the 10-day cultivation period (Table 4). In the N-limited treatment, the Si:N ratio
310 was 0.77 before N depletion and increased to ~ 2.7 during the 10-day period. The Si:N ratio in the
311 Fe-limited treatment was relatively constant with a high value of ~ 2.4 throughout the experiment
312 (Table 4).

313

314 4. Discussion

315

316 4.1. Resting spore formation under Fe-limited condition

317

318 Several studies have found that N deficiency is an important factor in the formation of
319 resting spores in marine diatoms (Hargraves and French, 1983). This study is the first report on the
320 formation of resting spores in *Chaetoceros teres*, *Fragilariopsis oceanica*, *Porosira* sp. cf.
321 *pentaportula*, and *S. nipponica* under N-limited conditions, and in ~ 14 diatoms species under
322 Fe-limited conditions. This implies that Fe-limitation is an important trigger for the formation of
323 resting spores for many diatom species. In this study, D-Fe concentration in the surface mixed layer
324 ($0.14\text{--}0.19\text{ nmol L}^{-1}$) was similar to previous reports for the Oyashio region in late spring to summer
325 ($\sim 0.1\text{--}0.2\text{ nmol L}^{-1}$; Nishioka et al., 2003; Takata et al., 2004). Furthermore, Fe addition (N-limited
326 treatment) increased the phytoplankton growth rate during the first three days of the experiment and
327 DFB addition (Fe-limited treatment) suppressed the growth rate and nitrate drawdown rate and
328 increased the percentage of resting spores as compared to the control. These results suggest that the
329 phytoplankton community during the spring bloom in the Oyashio region was Fe-limited without
330 any intracellularly stored Fe. In addition, initial ambient macronutrient concentrations were much

331 higher than those required for vegetative growth by most diatom species (Sarhou et al., 2005).
332 Therefore, the presence of resting spores at the start of the culture experiment in sunlit surface
333 seawater was considered to be induced by Fe-limitation. We suggest that resting spore formation of
334 diatoms under Fe-limited conditions occurs often during and after the spring diatom bloom in the
335 Oyashio region, where the surface seawater during late spring to summer is sometimes in an
336 iron-limited HNLC condition (Saito et al., 2002; Suzuki et al. 2002).

337 In addition, we found a discrepancy in the timing of spore formation, which probably
338 depends on the various physiological responses among diatom species under Fe- and N-limited
339 conditions (Fig. 6). Our results suggest that *C. diadema* and *Chaetoceros* spp. 2 will predominate in
340 Fe-limited conditions, and *Chaetoceros* spp. 2 and *T. nordenskiöldii* will predominate in N-limited
341 (Fe-replete) conditions. The susceptibility of resting spore formation to Fe- and/or N-depletions for
342 each diatom species should be examined in the future. In such physically, chemically and
343 biologically complex WSP regions (Saito et al., 2002; Oguma et al., 2008), the different
344 characteristics of sporulation would affect the diatom community structure, especially after the later
345 phase of the bloom, which may be subject to Fe- and/or N-limited conditions in the Oyashio region
346 (Saito et al., 2002).

347 Resting spore formation in *Chaetoceros* subgenus *Hyalochaete* spp. and sedimentation
348 without depletion in nitrate, but possibly a HNLC condition in the surface mixed layer, were
349 observed around the Antarctic Peninsula (Bodungen et al., 1986; Leventer, 1991), the Kerguelen
350 Plateau of the Southern Ocean (Armand et al., 2008a, b), the western and central subarctic Pacific
351 Ocean, and the southern-central Bering Sea (Takahashi et al., 2002; Onodera et al., 2003; Onodera
352 and Takahashi, 2009). The study by Armand et al. (2008a, b) and this study are the only two to report
353 resting spore formation in neritic diatom-dominated blooms with a high-nitrate, low-iron, and mid-
354 to high-chlorophyll environment (this study: D-Fe 0.17 nmol L^{-1} , chl-*a* $\sim 4 \text{ } \mu\text{g L}^{-1}$; Armand et al.,
355 2008a, b: D-Fe $< 0.10 \text{ nmol L}^{-1}$, chl-*a* $> 1 \text{ } \mu\text{g L}^{-1}$). However, Armand et al. (2008a) considered
356 Si-limitation to be the trigger for sporulation, and not Fe-limitation as observed in this study. In the
357 natural phytoplankton incubation experiment, the Si concentration in the control after 5 days was
358 significantly lower than in the Si-added, Fe-limited treatment, in which could continue further
359 silicification to form the resting spores during days 5–10. Therefore, the amount of available Si
360 during and after the bloom could critically regulate the number of diatom resting spores in the

361 spore-forming species, as demonstrated by Kuwata et al. (1993) under N-depleted conditions. We
362 hypothesized that the formation of resting spores could be induced by Fe depletion in the HNLC
363 coastal boundary regions if resting spore-forming diatoms were introduced to the regions such as the
364 Oyashio region (Mochizuki et al., 2002; Liu et al., 2004).

365

366 4.2. Si:N drawdown ratio and phytoplankton dynamics

367

368 The Si:N drawdown ratios under Fe- and macronutrient-replete conditions in the unialgal
369 culture of *T. nordenskiöldii* and the natural phytoplankton incubation experiment (N-limited
370 treatment during days 0–3) were slightly lower than the Si:N ratio (~1) of vegetatively growing
371 diatoms (Brzezinski, 1985). If diatoms continue to take up Si and N at the same lower Si:N ratio
372 throughout the spring diatom bloom, Si will remain in the upper mixed layer with an increase in the
373 water Si:N ratio after the bloom, because the Si:N supply ratio is >1 during winter in the subarctic
374 Pacific regions (Harrison et al., 2004). It has been reported that the water Si:N ratio increased as the
375 spring bloom progresses in the Fe-sufficient coastal region (Kudo et al., 2000). However, the Si:N
376 drawdown ratios under N-limited conditions for both unialgal and natural phytoplankton
377 experiments increased rapidly after N depletions, whereas the ratios under Fe-limited conditions
378 were continuously higher during the cultivation periods as compared to those under Fe-replete
379 conditions (Tables 1 and 4). These results suggest that the water Si:N ratio gradually decreases as the
380 bloom progresses under Fe-limited conditions, whereas it increases under N-limited conditions.
381 Therefore, change in the water Si:N ratio in the upper mixed layer during the spring phytoplankton
382 bloom with an Si:N supply ratio of >1 during winter would be a significant indicator of whether the
383 spring bloom-forming diatom community is influenced by Fe-limitation.

384 When we combined the results of both unialgal culture and natural phytoplankton
385 incubation, the Si:N drawdown ratio increased significantly with an increase in the percentage of
386 the resting spores (Fig. 7). The exponential relationship between the Si:N drawdown ratio and resting
387 spore percentage indicates that the resting spores of *Chaetoceros* subgenus *Hyalochaete* spp. in the
388 natural phytoplankton incubations (Table 3) and the resting spores and cells under Fe-limited
389 conditions in the unialgal culture experiment (Table 4) would be lightly silicified as compared to the
390 spores of *T. nordenskiöldii* under N-limited conditions in the unialgal culture experiment. Therefore,

391 Si was preferentially utilized due to the formation of resting spores either due to Fe or N depletion.

392 The Si:N drawdown ratios from winter to summer in the subarctic Pacific regions generally
393 are always higher than the Brzezinski ratio of ~ 1 (Wong and Matear, 1999; Koike et al., 2001; Saito
394 et al., 2002; Whitney et al., 2005) and also higher than that found in the Southern Ocean (Pondaven
395 et al., 2000). The possible mechanisms for the increasing the Si:N drawdown ratio could involve an
396 increase in the cellular Si:N ratio of diatoms by Fe-limitation (Takeda, 1998), a decrease in growth
397 rate (Claquin et al., 2002; Saito and Tsuda, 2003), a high Si:N ratio of ambient water (Kudo, 2003),
398 and preferential remineralization of N over Si, i.e., the Si pump (Dugdale and Wilkerson, 1998). This
399 study demonstrates that the Si:N drawdown ratio increased with an increase in the percentage of
400 heavily silicified resting spores under Fe- and N-depleted conditions. This could be one of the
401 important mechanisms for increase in the Si:N drawdown ratio between winter and summer in which
402 the resting spore-forming diatom species dominate in the spring bloom, such as in the WSP regions
403 (Mochizuki et al., 2002; Liu et al., 2004, this study) and the Southern Ocean (Bodungen et al., 1986;
404 Leventer, 1991; Abelmann et al., 2006; Armand et al., 2008a, b).

405 The formation of heavily silicified resting spores by diatoms under Fe- and N-limited
406 conditions may be an important phenomenon in present biological and biogeochemical
407 oceanography and could serve as a proxy for paleoproductivity (Abelmann et al., 2006). This study is
408 the first to assess the role of resting spores in oceanic Si biogeochemistry under Fe- and N-limited
409 conditions; however, further studies would be required to clarify the entire mechanisms of its role in
410 coastal and oceanic regions. In addition to known heavily silicified but non-resting spore-forming
411 diatoms such as *Neodenticula seminae* in the subarctic Pacific Ocean (Takahashi et al., 2002;
412 Onodera and Takahashi, 2009), *Fragilariopsis kerguelensis* in the Southern Ocean (Abelmann et al.,
413 2006; Armand *et al.*, 2008b) and diatom aggregates (Smetacek, 1999; Michel et al., 2002), this study
414 indicated that diatom resting spores could be an important component in the transport of Si to the
415 depths in coastal and oceanic regions under the temporally and specially N-limited and HNLC-like
416 conditions, such as in the Oyashio region (Saito et al., 2002; Harrison et al., 2004). Thus, species
417 composition and physiological aspects of the diatom community may be among the most important
418 factors influencing the biogeochemical Si cycle in the ocean.

419

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429

430 **References**

431 Abelmann, A., Gersonde, R., Cortese, G., Kuhn, G., Smetacek, V., 2006. Extensive phytoplankton
432 blooms in the Atlantic sector of the glacial Southern Ocean. *Paleoceanography* 21, PA1013,
433 doi:10.1029/2005PA001199.

434 Anderson, M.A., Morel, F.M.M., 1982. The influence of aqueous iron chemistry on the uptake of
435 iron by the coastal diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 27, 789–813.

436 Armand, L.K., Cornet-Barthaux, V., Mosseri, J., Quéguiner, B., 2008a. Late summer diatom biomass
437 and community structure on and around the naturally iron-fertilised Kerguelen Plateau in the
438 Southern Ocean. *Deep-Sea Res. II* 55, 653–676.

439 Armand, L.K., Crosta, X., Quéguiner, B., Mosseri, J., Garcia, N., 2008b. Diatoms preserved in
440 surface sediments of the northeastern Kerguelen Plateau. *Deep-Sea Res. II* 55, 677–692.

441 Banse, K., English, D.C., 1999. Comparing phytoplankton seasonality in the eastern and western
442 subarctic Pacific and the western Bering Sea. *Prog. Oceanogr.* 43, 235–288.

443 Bodungen, B.V., Smetacek, V.S., Tilzer, M.M., Zeitzschel, B., 1986. Primary production and
444 sedimentation during spring in the Antarctic Peninsula region. *Deep-Sea Res.* 33, 177–194.

445 Brzezinski, M.A., 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect
446 of some environmental variables. *J. Phycol.* 21, 347–357.

447 Claquin, P., Martin-Jézéquel, V., Kromkamp, J.C., Veldhuis, M.J.W., Kraay, G.W., 2002. Uncoupling
448 of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in
449 continuous culture of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen,
450 and phosphorus control. *J. Phycol.* 38, 922–930.

- 451 Dugdale, R.C., Wilkerson, F.P., 1998. Silicate regulation of new production in the equatorial Pacific
452 upwelling. *Nature* 391, 270–273.
- 453 Geider, R.J., La Roche, J., 1994. The role of iron in phytoplankton photosynthesis, and the potential
454 for iron-limitation of primary productivity in the sea. *Photosynth. Res.* 39, 275–301.
- 455 Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana*
456 *Hustedt* and *Detonula confervacea* (Glebe) Gran. *Can. J. Microbiol.* 8, 229–239.
- 457 Hargraves, P.E., French, F.W., 1983. Diatom resting spores: significance and strategies. In: Fryxell,
458 G.A., (Ed.), *Survival strategies of the algae*. Cambridge Univ Press, New York, pp. 49–68.
- 459 Harrison, P.J., Whitney, F.A., Tsuda, A., Saito, H., Tadokoro, K., 2004. Nutrient and plankton
460 dynamics in the NE and NW Gyres of the subarctic Pacific Ocean. *J. Oceanogr.* 60, 93–117.
- 461 Hasle, G.R., 1978. Using the inverted microscope. In: Sourina, A., (Ed.), *Phytoplankton manual*.
462 UNESCO, Paris, pp. 191–196.
- 463 Hasle, G.R., Syvertsen, E.E., 1997. Marine diatoms. In: Tomas, C.R., (Ed.), *Identifying Marine*
464 *Phytoplankton*. Academic Press, London, pp. 5–385.
- 465 Hillebrand, H., Durseien, C.D., Kirschtel, D., Pollinger, U., Zohary, T., 1999. Biovolume
466 calculation for pelagic and benthic microalgae. *J. Phycol.* 35, 403–424.
- 467 Hudson, R.M.J., Covault, D.T., Morel, F.M.M., 1992. Investigations of iron coordination and redox
468 reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic
469 iron complexes. *Mar. Chem.* 38, 209–235.
- 470 Iwade, S., Kuma, K., Isoda, Y., Yoshida, M., Kudo, I., Nishioka, J., Suzuki, K., 2006. Effect of high
471 iron concentrations on iron uptake and growth of a coastal diatom *Chaetoceros sociale*.
472 *Aquat. Microb. Ecol.* 43, 177–191.
- 473 Koike, I., Ogawa, H., Nagata, T., Fukuda, R., Fukuda, H., 2001. Silicate to nitrate ratio of the upper
474 sub-arctic Pacific and the Bering Sea Basin in summer: Its implication for phytoplankton
475 dynamics. *J. Oceanogr.* 57, 253–260.
- 476 Kudo, I., 2003. Change in the uptake and cellular Si:N ratio in diatoms responding to the ambient
477 Si:N ratio and growth phase. *Mar. Biol.* 143, 39–46.
- 478 Kudo, I., Yoshimura, T., Yanada, M., Matsunaga, K., 2000. Exhaustion of nitrate terminates a
479 phytoplankton bloom in Funaka Bay, Japan: change in $\text{SiO}_4:\text{NO}_3$ consumption rate during the
480 bloom. *Mar. Ecol. Prog. Ser.* 193, 45–51.

- 481 Kuwata, A., Tsuda, A., 2005. Selection and viability after ingestion of vegetative cells, resting spores
482 and resting cells of the marine diatom, *Chaetoceros pseudocurvisetus*, by two copepods. J.
483 Exp. Mar. Biol. Ecol. 322, 143–151.
- 484 Kuwata, A., Hama, T., Takahashi, M., 1993. Ecophysiological characterization of two life forms,
485 resting spores and resting cells, of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*,
486 formed under nutrient depletion. Mar. Ecol. Prog. Ser. 102, 245–255.
- 487 Leblanc, K., Hare, C.E., Boyd, P.W., Bruland, K.W., Sohst, B., Pickmere, S., Lohan, M.C., Buck, K.,
488 Ellwood, M., Hutchins, D.A., 2005. Fe and Zn effects on the Si cycle and diatom community
489 structure in two contrasting high and low-silicate HNLC areas. Deep-Sea Res. I 52,
490 1842–1864.
- 491 Leventer, A., 1991. Sediment trap diatom assemblages from the northern Antarctic Peninsula region.
492 Deep-Sea Res. 38, 1127–1143.
- 493 Liu, H., Suzuki, K., Saito, H., 2004. Community structure and dynamics of phytoplankton in the
494 western subarctic Pacific Ocean: A synthesis. J. Oceanogr. 60, 119–137.
- 495 Marchetti, A., Harrison, P.J., 2007. Coupled changes in the cell morphology and the elemental (C, N,
496 and Si) composition of the pennate diatom *Pseudo-nitzschia* due to iron deficiency. Limnol.
497 Oceanogr. 52, 2270–2284.
- 498 Martin, J.H., 1990. Glacial–interglacial CO₂ change: The iron hypothesis. Paleoceanography 5,
499 1–13.
- 500 McQuoid, M.R., Hobson, L.A., 1996. Diatom resting stages. J. Phycol. 32, 889–902.
- 501 McQuoid, M.R., Godhe, A., Nordberg, K., 2002. Viability of phytoplankton resting stages in the
502 sediments of a coastal Swedish fjord. Eur. J. Phycol. 37, 191–201.
- 503 Michel, C., Gosselin, M., Nozais, C., 2002. Preferential sinking export of biogenic silica during the
504 spring and summer in the North Water Polynya (northern Baffin Bay): Temperature or
505 biological control? J. Geophys. Res. 107, C7, 3064, doi:10.1029/2000JC000408.
- 506 Mochizuki, M., Shiga, N., Saito, M., Imai, K., Nojiri, Y., 2002. Seasonal changes in nutrients,
507 chlorophyll a and the phytoplankton assemblage of the western subarctic gyre in the Pacific
508 Ocean. Deep-Sea Res. II 49, 5421–5439.
- 509 Montagnes, D.J.S., Franklin, D.J., 2001. Effect of temperature on diatom volume, growth rate, and
510 carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46,

- 511 2008–2018.
- 512 Morel, F.M.M., Rueter, J.G., Anderson, D.M., Guillard, R.R.L., 1979. AQUIL: A chemically defined
513 phytoplankton culture medium for trace metal studies. *J. Phycol.* 15, 135–141.
- 514 Morel, F.M.M., Kustka, A.B., Shaked, Y., 2008. The role of unchelated Fe in the iron nutrition of
515 phytoplankton. *Limnol. Oceanogr.* 53, 400–404.
- 516 Nishioka, J., Takeda, S., Kudo, I., Tsumune, D., Yoshioka, T., Kuma, K., Tsuda, A., 2003.
517 Size-fractionated iron distributions and iron-limitation processes in the subarctic NW Pacific.
518 *Geophys. Res. Lett.* 30, 1730, doi:10.1029/2002GL016853.
- 519 Nishioka, J., Ono, T., Saito, H., Nakatsuka, T., Takeda, S., Yoshimura, T., Suzuki, K., Kuma, K.,
520 Nakabayashi, S., Tsumune, D., Mitsudera, H., Johnson, W.K., Tsuda, A., 2007. Iron supply to
521 the western subarctic Pacific: Importance of iron export from the Sea of Okhotsk. *J. Geophys.*
522 *Res.* 112, C10012, doi:10.1029/2006JC004055.
- 523 Obata, H., Karatani, H., Nakayama, E., 1993. Automated determination of iron in seawater by
524 chelating resin concentration and chemiluminescence detection. *Anal. Chem.* 65, 1524–1528.
- 525 Oguma, S., Ono, T., Kusaka, A., Kasai, H., Kawasaki, Y., Azumaya, T., 2008. Isotopic tracers for
526 water masses in the coastal region of eastern Hokkaido. *J. Oceanogr.* 64, 525–539.
- 527 Onodera, J., Takahashi K., 2009. Long-term diatom fluxes in response to oceanographic conditions
528 at Station AB and SA in the central subarctic Pacific and the Bering Sea, 1990–1998.
529 *Deep-Sea Res. I* 56, 189–211.
- 530 Onodera, J., Takahashi, K., Honda, M.C., 2003. Diatom fluxes at Station KNOT in the western
531 subarctic Pacific, 1997–2000. *Bull. Plankton Soc. Jpn.* 50, 1–15 (in Japanese with English
532 abstract).
- 533 Peers, G., Price, N. M., 2004. A role for manganese in superoxide dismutases and growth of
534 iron-deficient diatoms. *Limnol. Oceanogr.* 49, 1774–1783.
- 535 Pondaven, P., Ragueneau, O., Tréguer, P., Hauvespre, A., Dezileau, L., Reyss, J.L., 2000. Resolving
536 the ‘opal paradox’ in the Southern Ocean. *Nature* 405, 168–172.
- 537 Rue, E.L., Bruland, K.W., 1995. Complexation of iron(III) by natural organic ligands in the Central
538 North Pacific as determined by a new competitive ligand equilibration / adsorptive cathodic
539 stripping voltammetric method. *Mar. Chem.* 50, 117–138.
- 540 Saito, H., Tsuda, A., 2003. Influence of light intensity on diatom physiology and nutrient dynamics in

- 541 the Oyashio region. *Prog. Oceanogr.* 57, 251–263.
- 542 Saito, H., Tsuda, A., Kasai, H., 2002. Nutrient and plankton dynamics in the Oyashio region of the
543 western subarctic Pacific Ocean. *Deep-Sea Res. II* 49, 5463–5486.
- 544 Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and fate of diatoms
545 in the ocean: a review. *J. Sea Res.* 53, 25–42.
- 546 Smetacek, V., 1985. Role of sinking diatom life-history cycles: ecological, evolutionary and
547 geological significance. *Mar. Biol.* 84, 239–251.
- 548 Smetacek, V., 1999. Diatoms and the ocean carbon cycles. *Protist* 150, 25–32.
- 549 Stumm, W., Morgan, J.J., 1996. *Aquatic Chemistry*. 3rd ed. Wiley Interscience, New York.
- 550 Sugie, K., Kuma, K., 2008. Resting spore formation in the marine diatom *Thalassiosira*
551 *nordenskiöldii* under iron- and nitrogen-limited conditions. *J. Plankton Res.* 30, 1245–1255,
552 doi:10.1093/plankt/fbn080.
- 553 Sunda, W.G., Huntsman, S.A., 1995. Iron uptake and growth limitation in oceanic and coastal
554 phytoplankton. *Mar. Chem.* 50, 189–206.
- 555 Suzuki, K., Liu, H., Saino, T., Obata, H., Takano, M., Okamura, K., Sohrin, Y., Fujishima, Y., 2002.
556 East–west gradients in the photosynthetic potential of phytoplankton and iron concentration
557 in the subarctic Pacific Ocean during early summer. *Limnol. Oceanogr.* 47, 1581–1594.
- 558 Suzuki, R., Ishimaru, T., 1990. An improved method for the determination of phytoplankton
559 chlorophyll using N, N-dimethylformamide. *J. Oceanogr. Soc. Jpn.* 46, 190–194.
- 560 Takahashi, K., Fujitani, N., Yanada, M., 2002. Long term monitoring of particulate fluxes in the
561 Bering Sea and the central subarctic Pacific Ocean, 1997–2000. *Prog. Oceanogr.* 55, 95–112.
- 562 Takata, H., Kuma, K., Iwade, S., Yamajyoh, Y., Yamaguchi, A., Takagi, S., Sakaoka, K., Yamashita,
563 Y., Tanoue, E., Midorikawa, T., Kimura, K., Nishioka, J., 2004. Spatial variability of iron in
564 the surface water of the northwestern North Pacific Ocean. *Mar. Chem.* 86, 139–157.
- 565 Takeda, S., 1998. Influence of iron availability on nutrient consumption ratio of diatoms in oceanic
566 waters. *Nature* 393, 774–777.
- 567 Thompson, R.J., Deibel, D., Redden, A.M., McKenzie, C.H., 2008. Vertical flux and fate of
568 particulate matter in a Newfoundland fjord at sub-zero water temperatures during spring. *Mar.*
569 *Ecol. Prog. Ser.* 357, 33–49.
- 570 Timmermans, K.R., van der Wagt, B., de Baar H.J.W., 2004. Growth rates, half-saturation constants,

- 571 and silicate, nitrate, and phosphate depletion in relation to iron availability of four large,
572 open-ocean diatoms from the Southern Ocean. *Limnol. Oceanogr.* 49, 2141–2151.
- 573 Tyrrell, T., Merico, A., Waniek, J.J., Wong, C.S., Metzl, N., Whitney, F., 2005. Effect of seafloor
574 depth on phytoplankton blooms in high-nitrate, low-chlorophyll (HNLC) regions. *J. Geophys.*
575 *Res.* 110, G02007, doi:10.1029/2005JG000041.
- 576 Ushizaka, S., Sugie, K., Yamada, M., Kasahara, M., Kuma, K., 2008. Significance of Mn and Fe for
577 the growth of a coastal marine diatom, *Thalassiosira weissflogii*. *Fisheries Sci.* 74,
578 1137–1145.
- 579 Waite, T.D., 2001. Thermodynamics of the iron system in seawater. In: Turner, D.R., Hunter, K.A.,
580 (Ed.) *The biogeochemistry of iron in seawater*. Wiley, New York, pp. 291–342.
- 581 Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and
582 pheopigments. *Limnol. Oceanogr.* 39, 1985–1992.
- 583 Whitney, F.A., Crawford, D.W., Yoshimura, T., 2005. The uptake and export of silicon and nitrogen
584 in HNLC waters of the NE Pacific Ocean. *Deep-Sea Res. II* 52, 1055–1067.
- 585 Wong, C.S., Mater, R.J., 1999. Sporadic silicate limitation of phytoplankton productivity in the
586 subarctic NE Pacific. *Deep-Sea Res. II* 46, 2539–2555.
- 587 Yoshida, M., Kuma, K., Iwade, S., Isoda, Y., Takata, H., Yamada, M., 2006. Effect of aging time on
588 the availability of freshly precipitated ferric hydroxide to coastal marine diatoms. *Mar. Biol.*
589 149, 379–392.
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 602 Table 1. Si:N drawdown ratio of unialgal culture experiment. Data represents means of triplicate
 603 experiments \pm 1SD. Number in parenthesis represents the cultivation days. Transition phase means
 604 the duration when the sporulation was succeeded.

605

| Treatment | Exponential | Transition | Total |
|------------|----------------------------|----------------------------|-----------------------------|
| Fe-replete | 0.59 \pm 0.00 (0–5 d) | | |
| N-limited | 2.05 \pm 0.08 (0–2 d) | 63.7 \pm 6.24 (3–7 d) | 8.46 \pm 0.30 (0–11 d) |
| Fe-limited | 0.81 \pm 0.05 (0–3 d) | 1.28 \pm 0.04 (4–9 d) | 1.17 \pm 0.07 (0–11 d) |

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632 Table 2. List of diatom species identified. Designated resting spore forming species are those with an
 633 asterisk. Genus species names are arranged alphabetically not systematically.

| 634 | Centric diatoms | Pennate diatoms |
|-----|----------------------------------|----------------------------------------------|
| 635 | <i>Asteromphalus flabellatus</i> | <i>Corethron criophilum</i> |
| 636 | <i>Astero. hookeri</i> | <i>Coscinodiscus asteromphalus</i> |
| 637 | <i>Attheya longicornis</i> | <i>Dactyliosolen fragilissimus</i> |
| 638 | <i>At. septentrionalis</i> | <i>Detonula confervacea</i> * |
| 639 | <i>Chaetoceros atlanticus</i> | <i>Eucampia groenlandica</i> |
| 640 | <i>C. cinctus</i> * | <i>Guinardia</i> sp. |
| 641 | <i>C. compressus</i> * | <i>Leptocylindrus danicus</i> * |
| 642 | <i>C. concavicornis</i> | <i>Odontella aurita</i> |
| 643 | <i>C. convoltus</i> | <i>Probosira arata</i> |
| 644 | <i>C. debilis</i> * | <i>Prosira</i> sp. cf. <i>pentaportula</i> * |
| 645 | <i>C. decipiens</i> | <i>Rhizosolenia</i> spp. |
| 646 | <i>C. diadema</i> * | <i>Stephanopyxis nipponica</i> * |
| 647 | <i>C. furcellatus</i> * | <i>Thalassiosira anguste-lineata</i> |
| 648 | <i>C. lacinosus</i> * | <i>T. antarctica</i> var. <i>borealis</i> * |
| 649 | <i>C. radicans</i> * | <i>T. nordenskiöldii</i> * |
| 650 | <i>C. similis</i> * | <i>Thalassiosira</i> spp. |
| 651 | <i>C. socialis</i> * | |
| 652 | <i>C. teres</i> * | |
| 653 | <i>C. tortissimus</i> | |
| 654 | <i>Chaetoceros</i> spp. | |
| 655 | | |
| 656 | | |
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| 660 | | |

661
 662 Table 3. Contribution (%) and abundance ($\times 10^2$ cells L^{-1}) of resting spores at initial and 10 d of
 663 cultivation. *Chaetoceros* spp. 1: *C. compressus*, *C. laciniosus*, *C. similis* and *C. socialis*. *Chaetoceros*
 664 spp. 2: *C. cinctus*, *C. furcellatus*, *C. radicans* and *C. tortissimus* (see detail in text). +: Resting spore
 665 observed; -: No resting spore detected.

| 666 | 0 day | | 10 day | | |
|-----|-------------------------------------------|-------|--------|---------|---------|
| | 667 Control N-limited Fe-limited | | | | |
| 668 | Centric diatoms | | | | |
| 669 | <i>C. debilis</i> | – | 9.8 | 85.3 | 30.0 |
| 670 | | (0) | (410) | (23500) | (1350) |
| 671 | <i>C. diadema</i> | 1.3 | 5.2 | 41.6 | 2.4 |
| 672 | | (9.6) | (187) | (9520) | (199) |
| 673 | <i>C. teres</i> | + | + | + | + |
| 674 | | (0.8) | (3) | (43) | (3) |
| 675 | <i>Chaetoceros</i> spp. 1 | 9.7 | 1.1 | 25.5 | 7.7 |
| 676 | | (169) | (2160) | (21600) | (14500) |
| 677 | <i>Chaetoceros</i> . spp. 2 ^a | 8.9 | 18.4 | 59.6 | 73.6 |
| 678 | | (391) | (429) | (24300) | (2660) |
| 679 | <i>D. confervacea</i> | – | – | +* | +* |
| 680 | | (0) | (0) | (7)* | (3)* |
| 681 | <i>P. sp. cf. pentaportula</i> | – | + | +* | +* |
| 682 | | (0) | (4) | (18)* | (5)* |
| 683 | <i>S. nipponica</i> | 2.3 | 53.3 | 100 | 78.2 |
| 684 | | (0.4) | (40) | (181) | (97) |
| 685 | <i>T. antarctica</i> var. <i>borealis</i> | + | – | + | + |
| 686 | | (0.1) | (0) | (2) | (2) |
| 687 | <i>T. nordenskiöldii</i> | – | 3.9 | 40.0 | 43.2 |
| 688 | | (0) | (5) | (423) | (140) |
| 689 | <i>L. danicus</i> | – | – | – | – |
| 690 | <i>P. arata</i> | – | – | – | – |

| | | | | | |
|-----|--------------------|-----|------|-------|-------|
| 691 | Pennete diatoms | | | | |
| 692 | <i>F. oceanica</i> | – | + | + | + |
| 693 | | (0) | (22) | (220) | (155) |

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695 ^a: Resting spores of *C. radicans* was not detected.

696 *: Data at 5 d of cultivation because of no data at 10 d of cultivation.

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722 Table 4. Si:N drawdown ratio of natural phytoplankton community incubation experiment. Data
723 represents means of triplicate experiments \pm 1SD during 0–3 d and 0–5 d cultivations, and represent
724 the ranges of duplicates for data during 0–10d cultivation period. --: No significant DIN uptake was
725 observed.

| Treatment | 0–3 d | 0–5 d | 0–10 d |
|------------|-----------------|-----------------|-----------|
| Control | 1.17 ± 0.06 | 0.81 ± 0.01 | 0.69–0.70 |
| N-limited | 0.77 ± 0.10 | 1.70 ± 0.08 | 2.55–2.80 |
| Fe-limited | -- | 2.48 ± 0.39 | 2.26–2.50 |

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752 **FIGURES CAPTIONS**

753 Fig. 1. Temporal changes in (a) vegetative cells, (b) resting cells, (c) resting spores densities and (d)
 754 resting spores percentages for unialgal culture experiment. Data represents means \pm 1 SD of
 755 triplicates.

756

757 Fig. 2. Temporal changes in (a) DIN, (b) Si(OH)₄ and (c) PO₄ for unialgal culture experiment. Data
 758 represents means \pm 1 SD of triplicates.

759

760 Fig. 3. Vertical profiles of (a) sigma-*t* and temperature, (b) DIN, Si(OH)₄ and Si:N ratio, and (c)
 761 D-Fe and chlorophyll *a*.

762

763 Fig. 4. Temporal changes in (a) chlorophyll-*a* concentrations, (b) diatom abundances, (c) resting
 764 spore densities and (d) resting spore percentages for natural phytoplankton community incubation
 765 experiment. Data for (a) represents means \pm 1 SD for triplicates and the range for duplicates.

766

767 Fig. 5. Temporal changes in (a) DIN, (b) Si(OH)₄ and (c) PO₄ for natural phytoplankton community
 768 incubation experiment. Data represents means \pm 1 SD for triplicates and the range for duplicates.

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770 Fig. 6. Temporal changes in the contribution of resting spores in (a) *C. debilis*, (b) *Chaetoceros* spp. 1,
 771 (c) *S. nipponica*, (d) *C. diadema*, (e) *Chaetoceros* spp. 2 and (f) *T. nordenskiöldii* for natural
 772 phytoplankton community incubation experiment. Note that scales of y axis were up to 100% for (a),
 773 (b) and (c), and up to 50% for (d), (e) and (f).

774

775 Fig. 7. Relationship between the Si:N drawdown ratio and the percentage of resting spores (RSP).
 776 Solid diamond represent unialgal culture experiment during 11 d cultivation period and open circle
 777 represent natural phytoplankton community incubation experiment under Fe- and N-limited
 778 treatment during 10 d cultivation period. The plotted line was obtained by least-square regression:
 779 [Si:N drawdown ratio] = $e^{0.022 \times (\text{RSP})}$ ($r^2 = 0.91$, $n = 11$, $p < 0.001$). Y intercept was set at 1 according to
 780 the value of vegetative growing diatoms (Brzezinski, 1985).

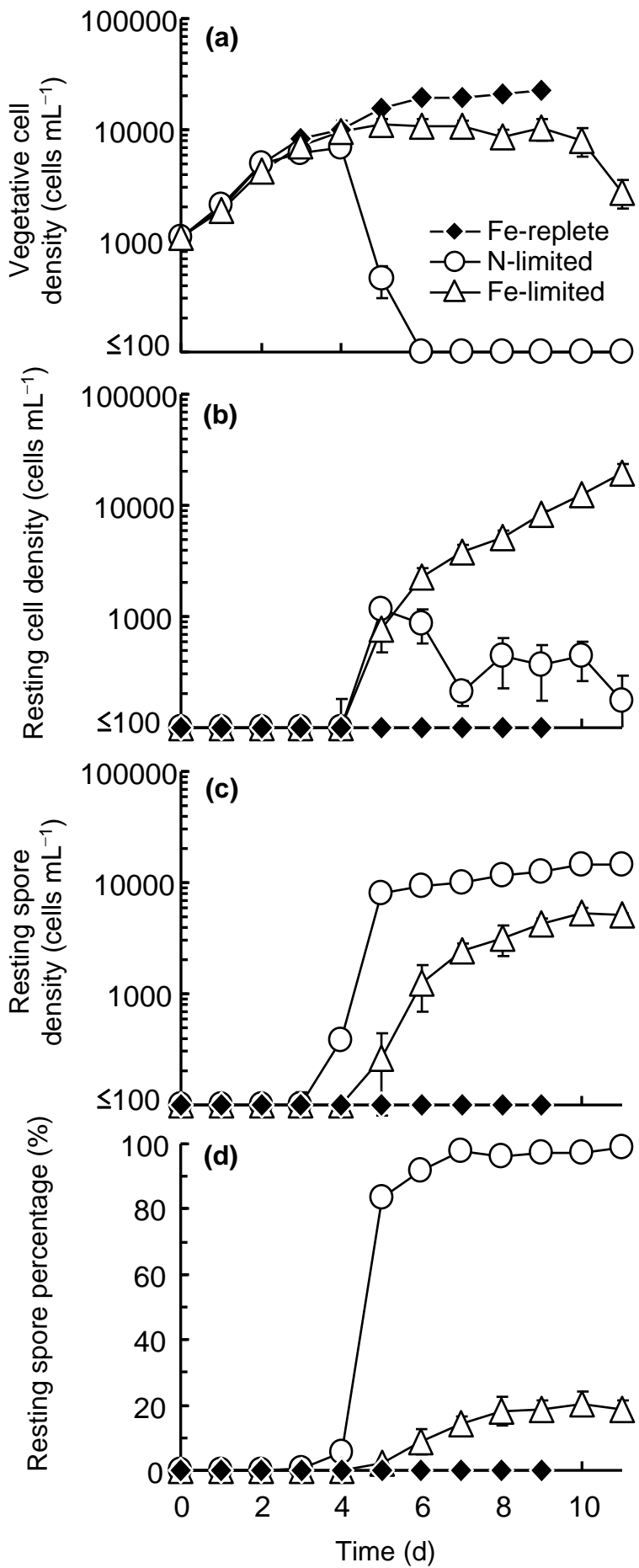


Fig. 2

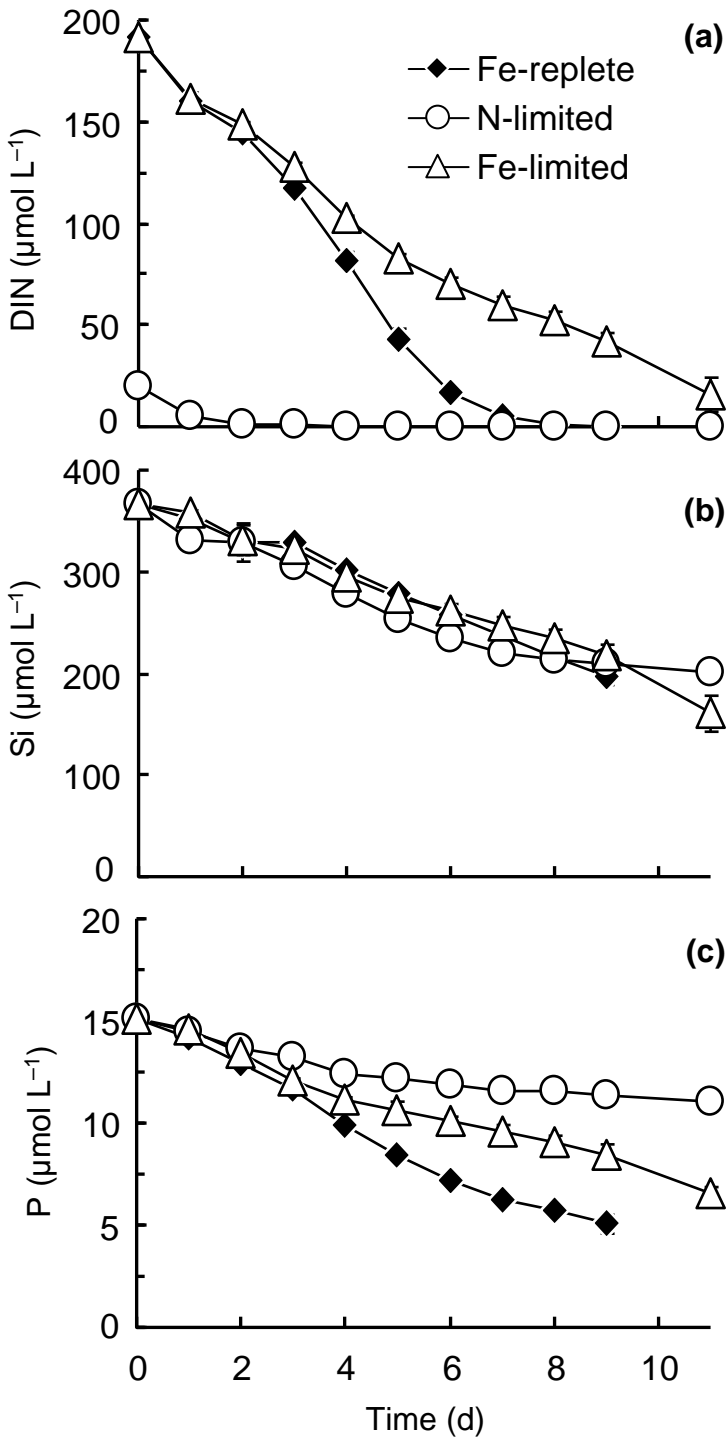


Fig. 3

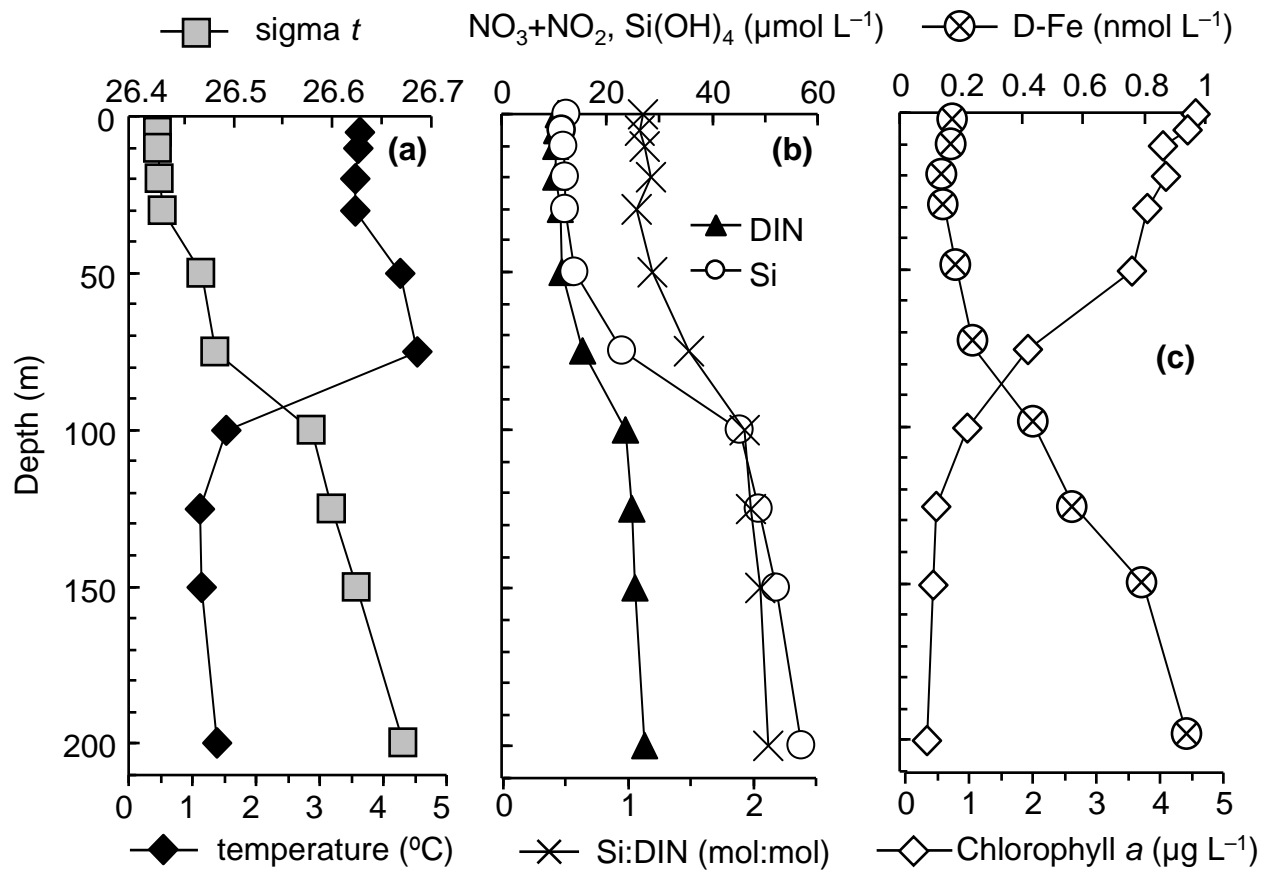


Fig. 4

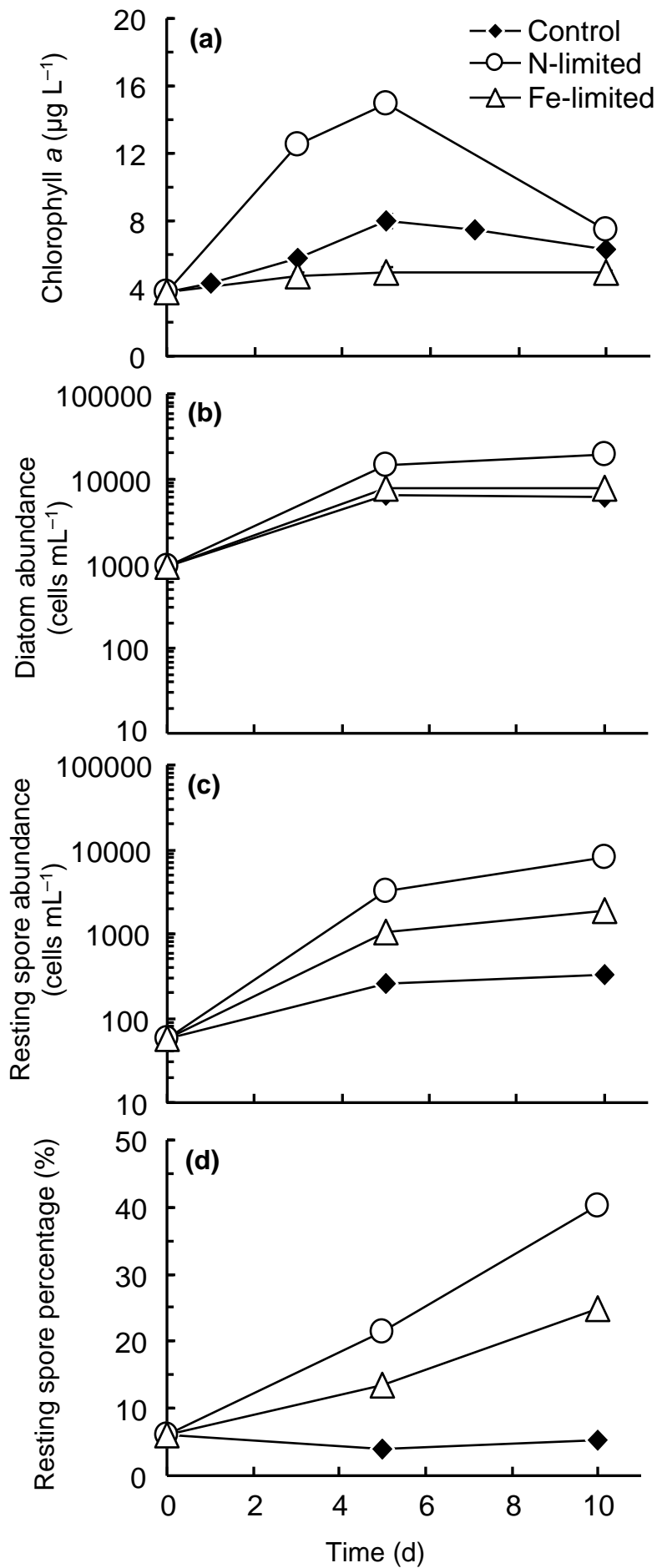


Fig. 5

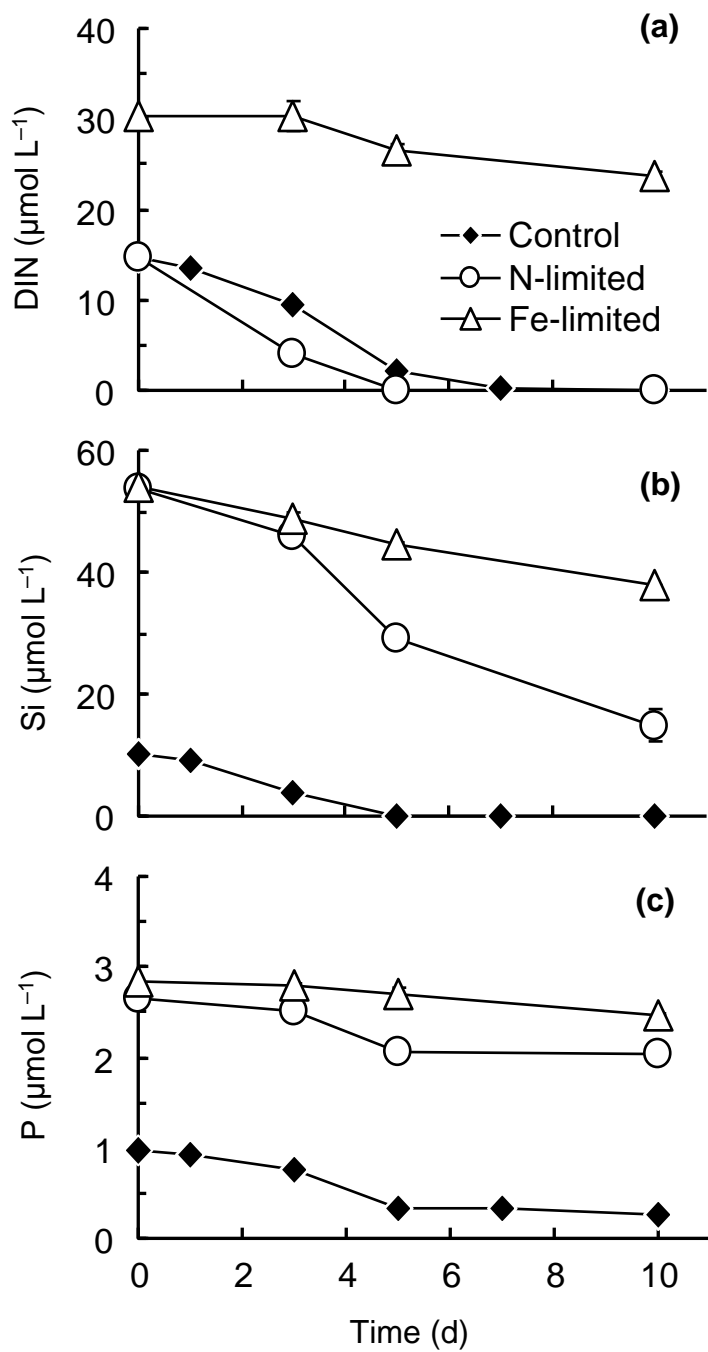


Fig. 6

