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1 Phosphorus limitation affects the molecular composition of
2 *Thalassiosira weissflogii* leading to increased biogenic silica
3 dissolution and high degradation rates of cellular carbohydrates

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32 **Abstract**

33 Diatoms in general, and *Thalassiosira weissflogii* (*T. weissflogii*) in particular, are
34 among the most ubiquitous phytoplanktonic species while, phosphorus (P) is an
35 essential nutrient that limits productivity in many oceanic regimes. To investigate how
36 *T. weissflogii* cultures grown under different P regimes are chemically altered before
37 and during their prokaryotic degradation, *T. weissflogii* cells were cultivated under two
38 contrasting P conditions, “P-stress” and “P-replete”. Biodegradation experiments were
39 conducted in natural seawater comprising a natural prokaryotic community. The
40 particulate fraction was monitored for 3 weeks for organic carbon (POC), nitrogen
41 (PON), biogenic silica (bSiO₂), total carbohydrates (PCHO) and individual
42 monosaccharides, including prokaryotic counting. Our results indicated that P-stress
43 induced changes in the chemical composition of the *T. weissflogii* cells, causing a
44 decrease to the Si/N (1.1 to 0.46) and Si/C (0.17 to 0.08) ratios. The “P-stress *T.*
45 *weissflogii*” cells were characterized by high amounts of galactose (23% of PCHO),
46 xylose (21%), and glucose (19%) compared to the “P-replete *T. weissflogii*” cells, which
47 were dominated by ribose (20% of PCHO), further indicating the exhaustion of ribose-
48 rich molecules (e.g., ATP) in *T. weissflogii* under “P-stress” conditions. The
49 degradation experiments showed that bSiO₂ produced under “P-stress” conditions
50 dissolved more rapidly than bSiO₂ formed under “P-replete” conditions, whereas POC
51 and PON exhibited higher degradation rate constants in the “P-replete *T. weissflogii*”
52 than in the “P-stress *T. weissflogii*” experiment. Overall, these observations show that *T.*
53 *weissflogii* submitted to P-limitation, results in changes in its initial biochemical
54 composition, increases frustule dissolution rate, and decreases the degradation of *T.*
55 *weissflogii*-organic matter by marine prokaryotes.

56 **Keywords:** Bacterial biodegradation, *Thalassiosira weissflogii*, P-stress,
57 POC, PON, carbohydrates, biogenic silica.

58

59 **1. Introduction**

60 Nitrogen (N) and phosphorus (P) are among the most important nutrients in
61 terrestrial and aquatic ecosystems. The former is mainly involved in the production of
62 plant and animal tissues (protein synthesis), whereas the latter is fundamental in many
63 biological processes as well as in structural components of cells (e.g., RNA and DNA,
64 energy transactions via ATP; Lehninger et al., 1993; Berg et al., 2002; Karl and
65 Björkman, 2015). Although N and P belong to the same group of elements on the
66 periodic table and they both contribute to eutrophication phenomena (i.e., act as
67 fertilizers; Ryther and Dunstan, 1971; Gruber and Galloway, 2008; Némery and
68 Garnier, 2016), they exhibit different oxidation states, have different uptake pathways
69 (e.g., N can be retrieved from the atmosphere by diazotrophs), and their N/P ratio may
70 span from < 16 to > 100 (Vitousek and Howarth, 1991; Downing, 1997; Paytan and
71 McLaughlin, 2007; Godwin and Cotner, 2015; Karl and Björkman, 2015). Although the
72 N and P cycles are closely linked to one another, P appears to be more critical as a
73 limiting element (Trommer et al., 2013). This is not only because of its low proportions
74 compared to N, but also because it cannot be found in the atmosphere in a gaseous state
75 (Paytan and McLaughlin, 2007; Karl and Björkman, 2015). Therefore, the availability
76 of P is critical for cell growth in marine ecosystems, especially for phytoplankton
77 (Redfield et al., 1963; Jackson and Williams, 1985; Thingstad et al., 2005; Paytan and
78 McLaughlin, 2007).

79 Despite the fact that P sources in seawater are numerous, whether in inorganic or
80 organic forms (Karl and Björkman, 2015), phytoplanktonic organisms very often cannot
81 grow and eventually turn over as a result of P-stress. P-stress conditions have been
82 observed in different parts of the ocean, including the China Sea (Harrison et al.,
83 1990a), the northwestern Mediterranean Sea (Thingstad et al., 2005), and the western
84 North Atlantic Ocean (Wu et al., 2000). Several studies have assessed the impact of P-
85 starvation stress in phytoplankton in terms of cellular growth; P-competition with other
86 species, including bacteria; the extracellular release of organic compounds; and the
87 biosynthesis of intracellular organic compounds (Urbani et al., 2005; Zohary et al.,
88 2005; Moore et al., 2008; Tanaka et al., 2011; Sebastian et al., 2016). Furthermore,
89 other studies showed that the phytoplankton in the ocean uses non-phosphorus lipids in
90 response to P scarcity stress (Van Mooy et al., 2009; Obata et al., 2013; Brembu et al.,
91 2017). All these studies show the importance of P on structuring the ocean
92 phytoplankton community and on the metabolism of microalgae cells. Despite this
93 wealth of information on P-stress on phytoplankton species, the subsequent degradation
94 of phytoplankton grown under P-stress is a research topic that has not been thoroughly
95 examined until now in the scientific literature. Thus, despite the fact that several studies
96 have focused on the degradation of phytoplankton and sinking marine particles by
97 following their bulk individual components (e.g., proteins, carbohydrates, lipids, etc.;
98 Harvey et al., 1995; Harvey and Macko, 1997; Panagiotopoulos et al., 2002; Goutx et
99 al., 2007; Engel et al., 2009) for 2-3 weeks, very little is known on the chemical
100 alteration of frustules, including the silica dissolution and decomposition rates of P-
101 stress grown diatoms that further impact the export of photosynthetic carbon. Indeed,
102 biogeochemical models predict a future increase in nutrient limitation across most of the

103 ocean (Bopp et al., 2005). Consequently, it is important to study whether P-stress grown
104 diatoms have the potential to be rapidly mineralized or accumulated within the
105 dissolved organic matter pool or marine sediments, which further determines the time
106 scale at which mineralized diatom carbon will reach the atmosphere.

107 The present study aims to examine the prokaryotic degradation and the frustule
108 dissolution, and thus, the fate of marine diatoms that are previously grown under
109 different P-conditions (“P-replete” and “P-stress”). In this study we followed the
110 changes in the chemical composition of a very well-known diatom species,
111 *Thalassiosira weissflogii*, by monitoring particulate organic carbon (POC), organic
112 nitrogen (PON), biogenic silica (bSiO₂), and total carbohydrates (PCHO) over a period
113 of 3 weeks. In contrast to previous investigations that estimated degradation rates on 3-
114 day to 1-week experiments (Goutx et al., 2007; Wetz et al., 2008; Piontek et al., 2009),
115 we chose this unusual long-term degradation experiment recognizing that dead diatoms
116 may reside for a long period of time in the ocean and especially in the bathypelagic
117 layers (Agusti et al., 2015). It is important to note that a 3-week degradation experiment
118 requires that the starting conditions must contain a high amount of diatom cells in order
119 to follow their different pools (i.e., POC, PON, PCHO, and bSiO₂), which is only
120 feasible using a dense diatom culture (Sun et al., 1993; Suroy et al., 2015). At the
121 molecular level, the effect of P-stress on the degradation rate constants of the individual
122 monosaccharides was also evaluated. The results are compared and discussed along
123 with the bulk degradation features of the POC, PON, bSiO₂ and PCHO.

124

125 **2. Materials and Methods**

126 *2.1. Experimental design*

127 The experimental protocol used in this study has been set up to follow the
128 impact of P-stress on the degradation of the different pools of organic and inorganic
129 matter (POC, PON, and bSiO₂), including the PCHO in dead diatoms, for 3 weeks. We
130 used *T. weissflogii* as a model diatom as this ubiquitous species has extensively been
131 studied in terms of transparent polymer production (Chen et al., 2015), aggregation and
132 sedimentation (Seebah et al., 2014), growth rates under N- and Si- starvation (De La
133 Rocha et al., 2010; Suroy et al., 2015), and photosynthetic efficiency under P-stress (Liu
134 et al., 2011). A schematic view of the experimental protocol employed in this study is
135 presented in Fig. 1. Briefly, *T. weissflogii* cells were grown under two different
136 conditions, namely, “P-replete *T. weissflogii*” (control condition) and “P-stress *T.*
137 *weissflogii*”. *Thalassiosira weissflogii* cells were then killed and inoculated in 0.7 μm
138 filtered seawater containing its natural prokaryotic community, to monitor the net
139 degradation for 3 weeks.

140

141 2.1.1. Preparation of “P-replete” and “P-stress” *T. weissflogii* cultures

142 The marine ubiquitous diatom *T. weissflogii* (strain CCMP 1049) was obtained
143 from the IFREMER facility (Argenton station, France), and the initial culture in f/2
144 medium was treated with a mix of antibiotics, including penicillin G, streptomycin, and
145 gentamycin, to remove bacteria (Guillard, 2005). The absence of bacteria was checked
146 by placing the inoculum on ZoBell agar plates (ZoBell, 1941). The PO₄³⁻ starvation
147 experiments were undertaken in a 10 L batch culture by switching the medium supply
148 from normal f/2 to a PO₄³⁻-free f/2 medium (Guillard and Ryther, 1962; Fig. 1). As
149 such, two 10 L culture batches were prepared: one “P-stress *T. weissflogii*” batch and
150 one “P-replete *T. weissflogii*” batch. Both axenic cultures in batches were maintained

151 for 1 week at 18 °C (± 1 °C) under a light/dark cycle (16 h: 8 h) under a photon flux
152 density of 180 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The impact of P-stress on *T. weissflogii* cells was
153 evaluated by monitoring the photosynthetic parameters using fluorescence (WATER/B
154 – PAM, Walz, Effeltrich, Germany; Schreiber et al., 1986) and Fourier-transform
155 infrared spectroscopy (Tensor 27 BRUKER; Beardall et al., 2001). The maximum
156 energy conversion efficiency or quantum efficiency of photosystem II charge separation
157 (Fv/Fm) decreased from 0.6 to 0.3 on day 7. At this time, the maximum photosynthetic
158 electron transport rate (ETR_{max}) obtained from light curves relating the ETR to the
159 irradiance decreased from 2.9 to 0.013, indicating that “P-stress *T. weissflogii*” cells
160 were not able to maintain photosynthesis after 7 days (Soler, 2010). The FTIR spectra
161 of the entire cells also showed significant changes in the *T. weissflogii* cell structure
162 under P-stress conditions (Fig. S1). The *T. weissflogii* cell numbers obtained in the 10 L
163 batches after this treatment were approximately $1.8 \times 10^8 \text{ cell L}^{-1}$ for both cultures (“P-
164 replete *T. weissflogii*” and “P-stress *T. weissflogii*”). The *T. weissflogii* cultures were
165 then concentrated by centrifugation (20 min at 2000 rpm), the supernatant was
166 discarded, and the pellets were frozen at -80 °C for 24 h to kill *T. weissflogii* without
167 breaking their cells (Soler, 2010; Fig. 1).

168

169 2.1.2. Degradation experiment

170 The pellets of the *T. weissflogii* cells grown in the two different treatments (“P-
171 replete” and “P-stress”) were resuspended in 12 L degradation batches that were filled
172 with 0.7 μm filtered natural seawater sampled at a depth of 10 m in the Bay of Brest.
173 The final *T. weissflogii* cell concentration was similar in the two degradation batches
174 (Table 1) and large enough to ensure a clear signal for all the measured parameters until

175 the end of the 3 week degradation experiment (see below). The nitrate, phosphate, and
176 silicate concentrations in seawater on the day of sampling were 0.33, 0.05, and 0.66
177 μM , respectively. The seawater temperature was 15.8 $^{\circ}\text{C}$, which was almost identical to
178 that used for the incubation experiments (see below). The seawater was filtered at 0.7
179 μm GF/F (47 mm, Whatman; baked at 450 $^{\circ}\text{C}$ for 6 h) to remove phyto- and
180 zooplanktonic organisms while maintaining most of the natural prokaryotic community
181 (Duhamel et al., 2007; Suroy et al., 2015).

182 The prokaryotic inoculum concentrations were estimated before the addition of
183 *T. weissflogii*. *Thalassiosira weissflogii* cellular concentrations were estimated on day 1,
184 considering that 24 h are necessary for *T. weissflogii* cells to adapt to the new medium
185 (prokaryotes plus dead *T. weissflogii* cells), resulting in a homogenous mixture for
186 sampling. Hence, we considered that the degradation experiment started 24 h after the
187 setup of the degradation batches, assuming that there was little variability in the POC,
188 PON, bSiO₂, and PCHO concentrations between day 0 and day 1 of the experiment.
189 Thus, the ratio of these parameters to the *T. weissflogii* cells did not change dramatically
190 in this time interval. The degradation batches were placed on an orbital shaker table at
191 16 $^{\circ}\text{C}$ in the dark, and punched caps were used to ensure gas exchange. The batches
192 were sampled daily for their biochemical parameters (POC, PON, bSiO₂, and PCHO),
193 including the total counts of heterotrophic prokaryotes over 21 days. Replicate samples
194 ($n = 2-3$) were taken throughout the degradation experiments, mainly on days 2, 6, 9,
195 12, and 19, depending on the experiment (see also figure captions). Prior to each
196 sampling, the batches were gently shaken to ensure homogeneity. At the end of the
197 experiment, approximately 40% of the initial volume remained in the bottle to minimize
198 batch effects.

199 2.2. *Prokaryotic and T. weissflogii* cell counts

200 Prokaryotes were counted on 5 mL samples at each time point by flow
201 cytometry using a FacsVerse flow cytometer (Becton Dickinson, San Jose, USA). Data
202 from the flow cytometer were processed using the BD FacSuite[™] software, according
203 to previously described methods (Troussellier et al., 1993; Marie et al., 1997; Marie and
204 Partensky, 2006). The *T. weissflogii* cell numbers were also measured by flow
205 cytometry on the first day of the biodegradation experiment to obtain information about
206 the initial *T. weissflogii* concentrations.

207

208 2.3. *POC and PON analysis*

209 Each day, a 10 mL sample from each of the degradation batches (“P-replete” and
210 “P-stress”) was collected and filtered on 0.7 µm precombusted GF/F filters (450 °C, 6
211 h) for POC and PON concentration measurements. The filters were dried overnight in
212 an oven at 50 °C. The POC and PON levels were measured on the same filter using a
213 Carlo Erba NA 2100 CN analyzer coupled with a Finnigan Delta S mass spectrometer
214 (Nieuwenhuize et al., 1994). The detection limits were 5 µg and 1 µg for POC and
215 PON, respectively, with a standard error of 2–3%.

216

217 2.4. *SiO₂ analysis*

218 To monitor the bSiO₂ dissolution, each day 10 mL samples from each of the
219 degradation batches were filtered on 0.45 µm polycarbonate filters. Filtrates were
220 directly analyzed for dissolved silica (dSi; see below), whereas filters were dried
221 overnight and used for bSiO₂ analysis using a variation of the method of Ragueneau and
222 Tréguer (1994). Following this protocol, the filters were digested in 0.2 N NaOH during

223 4 h at 95 °C, then the solutions were cooled in ice, neutralized using 1 N HCl, and
224 centrifuged to remove remaining solids. As no lithogenic silica was present in the algal
225 culture, the second digestion step using HF was omitted. The overlying water was
226 analyzed for dSi using the molybdate blue spectrophotometric method adapted by
227 Gordon et al. (1993) for use in segmented flow colorimetry. Standards were made in
228 distilled water for bSiO₂ analysis and artificial seawater for dSi. The analysis was
229 performed on a Bran and Luebbe Technicon Autoanalyzer (< 1% precision).

230

231 2.5. PCHO analysis

232 Similar to the POC, PON, and bSiO₂ measurements, a 10 mL sample from each
233 of the degradation batches was collected and filtered on 0.7 µm pre-combusted GF/F
234 filters (450 °C, 6 h) for the PCHO analysis using the procedure described by Suroy et al.
235 (2015). Briefly, filters were cut with clean scissors, transferred into glass tubes, and
236 hydrolyzed with 1.2 M H₂SO₄ for 3 h at 100 °C (Panagiotopoulos and Wurl, 2009). The
237 samples were neutralized with pre-combusted CaCO₃ (450 °C for 6 h) and were
238 pipetted into scintillation vials for liquid chromatography analysis (Suroy et al., 2015).

239 A Dionex ICS-3000 anion exchange chromatograph fitted with a pulsed
240 amperometric detector (HPAEC-PAD) was used for all PCHO analyses. Details of the
241 analytical procedure have been provided elsewhere (Panagiotopoulos et al., 2012; Suroy
242 et al., 2015). Ten individual monosaccharides were detected in the hydrolysates of the
243 particulate organic material, including deoxysugars (fucose and rhamnose), pentoses
244 (arabinose, ribose, and xylose), one amino sugar (glucosamine), hexoses (galactose,
245 glucose, and mannose), and one acidic sugar (galacturonic acid). The neutral and amino
246 sugars were separated with an isocratic 19 mM NaOH elution at 17 °C, whereas

247 galacturonic acid was detected in a separate analysis using a gradient elution
248 (Panagiotopoulos et al., 2012). The flow rate was set at 0.7 mL min⁻¹ for neutral and
249 acidic sugar analyses. The data acquisition and processing procedures were performed
250 using the software Chromeleon[®]. The analytical errors calculated by the coefficients of
251 variation of the repeated injections of a standard solution of 50 nM/sugar were 5–10%
252 and 0.9–2.0% ($n = 6$) for the peak area and retention time, respectively. The total
253 particulate carbohydrate concentrations (μM) are given in C-equivalents and the relative
254 abundances (mol%) of their monomeric constituents (e.g., monosaccharides) are given
255 in Table 2.

256

257 2.6. Statistical analysis and estimation of kinetic parameters

258 The degradation of bulk organic matter (POC and PON), bSiO₂ dissolution,
259 PCHO, and individual monosaccharides were monitored using a statistical comparison
260 of three mechanistic models.

261 Model 1 is a simple first-order exponential rate equation (1) as described by
262 Greenwood et al. (2001), that has been used in many dissolution/degradation studies
263 (e.g., Kamatani and Riley, 1979; Kamatani et al., 1980; Kamatani, 1982):

$$264 \quad \hat{C}_{(t)} = C_0 \cdot e^{(-k \cdot t)} \quad (1)$$

265 where:

266 \hat{C}_t is the concentration (μM) estimated at time t (d),

267 C_0 is the initial concentration, and

268 k is the dissolution/degradation rate constant (d⁻¹).

269 Model 2 is a second-order equation (2) that considers two pools of organic
270 matter with different reactivities. It is similar to the equation that was used by Westrich

271 and Berner (1984) for carbon degradation, and more recently it has also been employed
272 for bSiO₂ dissolution (Moriceau et al., 2009; Boutorh et al., 2016):

273
$$\hat{C}_{(t)} = C_1 \cdot e^{(-k_1 \cdot t)} + C_2 \cdot e^{(-k_2 \cdot t)} \quad (2a)$$

274 where:

275 \hat{C}_t is the concentration (μM) estimated at time t (d),

276 C₁ and C₂ are the initial concentrations (μM) of pools 1 and 2, respectively, and

277 k₁ and k₂ are their respective degradation rate constants (d⁻¹).

278

279 As equation (2) uses more parameters, Model 2 always produces a better fit than Model

280 1. It was found that for most of the examined parameters (i.e., POC, PON, and PCHO),

281 the second degradation rate constant (i.e., k₂) was equal to zero. In this case, the model

282 only has 3 variables: C₁, C₂, and k₁ (see equation 2b).

283
$$\hat{C}_{(t)} = C_1 \cdot e^{(-k_1 \cdot t)} + C_2 \quad (2b)$$

284 The best model was chosen using the Shapiro-Wilk and Constant Variance Test

285 statistical tests in Sigmaplot, which favors the goodness of fit and the lowest number of

286 parameters. Likewise, the bulk parameters (POC and PON) and PCHO were best

287 modeled using equation (2b), whereas individual monosaccharides and bSiO₂ were best

288 modeled using equations (1) and/or 2(b) (Tables 3, 4).

289

290 **3. Results**

291 *3.1. Initial observations*

292 At the beginning of the biodegradation experiment, the diatom cell

293 concentrations were 6.60 x 10⁷ cell L⁻¹ in the “P-replete *T. weissflogii*” degradation

294 batch and 6.80 x 10⁷ cells L⁻¹ in the “P-stress *T. weissflogii*” degradation batch (Table

295 1). These concentrations are similar to those observed during a simulated *T. weissflogii*
296 bloom (Diekmann et al., 2009) but an order of magnitude higher than those generally
297 observed in “natural” bloom concentrations (Grattepanche et al., 2011; Smetacek,
298 1985).

299 The results indicated that the “P-stress *T. weissflogii*” growth conditions induced
300 increases of 29% (mol %) in the carbon and 44% (mol%) in nitrogen per cell compared
301 to the “P-replete *T. weissflogii*” conditions. On the other hand, bSiO₂ showed the
302 opposite trend as it was lower in the “P-stress *T. weissflogii*” degradation batches than
303 in the “P-replete *T. weissflogii*” degradation batches (Table 1).

304 In this study, POC and PON values (Table 1) were not corrected for prokaryotic
305 carbon and nitrogen because only free and not attached prokaryotes were counted.
306 Nevertheless, the POC and PON values presented here fall within the same order of
307 magnitude as those generally reported for *T. weissflogii* species (Waite et al., 1992; De
308 La Rocha et al., 2010) and agree well with the results of a previous study of the same
309 species in which corrections for prokaryotic carbon and nitrogen were made (Suroy et
310 al., 2015).

311 Prokaryotic concentrations were measured after 24 h in both the “P-replete *T.*
312 *weissflogii*” and “P-stress *T. weissflogii*” degradation batches. The results showed that
313 the prokaryotic concentrations were similar (5.6 and 7.0×10^{10} cell L⁻¹) for the “P-stress
314 *T. weissflogii*” and “P-replete *T. weissflogii*” batches at the beginning of the
315 experiments and that they peaked on day 1 (Table 1; Fig. 2). After day 1, prokaryotic
316 concentrations slowly decreased to reach their first plateau on day 5 (2.5 and 3.1×10^{10}
317 cell L⁻¹ for the “P-stress *T. weissflogii*” and “P-replete *T. weissflogii*” batches,
318 respectively) and a second plateau on day 12 (3.3 and 3.7×10^{10} cell L⁻¹ for the “P-

319 stress *T. weissflogii*” and “P-replete *T. weissflogii*” batches, respectively) until the end
320 of the experiment (Fig. 2). Such prokaryotic features observed during biodegradation
321 experiments agree with previous results performed in sinking marine particles
322 (Panagiotopoulos et al., 2002; Panagiotopoulos and Sempéré, 2007), *Skeletonema*
323 *marinoi* (Moriceau et al., 2009), *Emiliana huxleyi* calcium carbonate frustule (Engel et
324 al., 2009), and Si/N- stress *T. weissflogii* (Suroy et al., 2015), indicating that the
325 decrease in the particulate organic matter pool is the result of prokaryotic respiration
326 and growth.

327 The results of the degradation experiment in the “P-replete *T. weissflogii*” batch
328 show increases in POC and PON concentrations (of approximately 50% and 60% for
329 POC and PON, respectively), which were observed between T₀ and T₂ (Fig. 3). A
330 similar pattern has already been observed in other studies and is probably due to re-
331 aggregation of the < 0.7µm pool related to the addition of the prokaryotic community,
332 which boosts the stickiness of the exopolymeric compounds (EPS) excreted by the *T.*
333 *weissflogii* (Gärdes et al., 2010) as well as the heterogeneity in the batch (Suroy et al.,
334 2015). In addition, PCHO, glucose, and galactose, which are known to be the main
335 constituent of the EPS copiously secreted by diatoms (Magaletti et al. 2004; Urbani et
336 al. 2005), also increased between T₀ and T₂ in the “P-replete *T. weissflogii*” batches,
337 which further reinforces the hypothesis of re-aggregation (Fig. 4b; Fig.5). After T₂, the
338 POC and PON concentrations decreased over time, which typically reflected the
339 decomposition patterns of *T. weissflogii*-POC and *T. weissflogii*-PON (Fig. 3). In
340 contrast to the “P-replete *T. weissflogii*” batch, the POC and PON concentrations in the
341 “P-stress *T. weissflogii*” batch began to decrease at T₀.

342 Contrarily, as minute or no bSiO₂ is associated with the organic matter that re-
343 aggregates in the colloidal pool, the bSiO₂ concentrations remained rather stable in the
344 “P-replete *T. weissflogii*” batch during the first days of the experiment (Fig. 4a). Similar
345 to POC and PON, bSiO₂ concentrations also began to decrease at T₀ in the “P-stress *T.*
346 *weissflogii*” batch (Fig. 4a). The degradation of all the biochemical compounds is
347 expressed in our study as its decrease relative to its highest concentration measured
348 before the degradation/dissolution started.

349 Similar to POC, the initial concentration of the PCHO–C in the “P-stress *T.*
350 *weissflogii*” degradation batch was higher than in the “P-replete *T. weissflogii*”
351 degradation experiment (169 vs. 110 μMC; Table 2). At T₀, PCHO represented 14 and
352 16% of POC for the “P-replete *T. weissflogii*” and “P-stress *T. weissflogii*” degradation
353 experiments, respectively, indicating the slight preferential allocation of photosynthetic
354 carbon into *T. weissflogii* cells under “P-stress” conditions (Table 2). As for POC and
355 PON, the PCHO–C concentrations also peaked at T₂ (Fig. 4b) for the “P-replete *T.*
356 *weissflogii*” experiment, supporting the previously mentioned hypothesis regarding
357 DOM aggregation.

358 Qualitatively, the sugar composition recorded in *T. weissflogii* comprised three
359 aldohexoses, three aldopentoses, two deoxysugars, one aminosugar, and one uronic acid
360 (see above; Table 2). Five monosaccharides generally dominated the *T. weissflogii*
361 carbohydrate composition namely, galactose, glucose, xylose, ribose, and mannose.
362 These monosaccharides accounted for 65% and 78% of PCHO in the “P-replete *T.*
363 *weissflogii*” and “P-stress *T. weissflogii*” degradation batches, respectively, at the
364 beginning of the biodegradation experiment. It is worth noting that the “P-replete” batch

365 was particularly enriched with ribose (~20%) and galacturonic acid (2%) when
366 compared to the “P-stress” batch (Table 2).

367

368 3.2. Organic matter degradation

369 3.2.1. Bulk parameters (POC, PON, and $bSiO_2$)

370 The POC and PON degradation followed similar patterns in both batches, with
371 an initial period of degradation (approximately 7–8 days from the beginning of the
372 experiment) in which the *T. weissflogii*-organic matter degraded faster, followed by a
373 second period in which the *T. weissflogii*-organic matter concentrations remained
374 constant (Fig. 3a). The best fits for the degradation of the POC and PON were obtained
375 using equation 2b.

376 The k_I degradation rate constant of POC was two times higher in the “P-replete
377 *T. weissflogii*” batch than in the “P-stress *T. weissflogii*” batch ($0.40 \pm 0.05 \text{ d}^{-1}$ vs. 0.19
378 $\pm 0.05 \text{ d}^{-1}$; Table 4). The results showed that after 7–8 days of degradation,
379 approximately 36% of the POC from the “P-replete” experiment was decomposed,
380 whereas this amount was slightly higher for the “P-stress” experiment (approximately
381 43%) over the same period (Fig. 3a). Then, POC concentrations remained stable and
382 reached a plateau accounting for approximately 60% of the initial POC in both batches
383 (Fig. 3a).

384 The degradation rate constant of PON was higher in the “P-replete” batch than in
385 the “P-stress” batch ($0.27 \pm 0.02 \text{ d}^{-1}$ vs. $0.17 \pm 0.03 \text{ d}^{-1}$; Table 3). Similar to POC, after
386 7–8 days of degradation, PON concentrations reached a plateau that accounted for
387 approximately 40% of the initial PON in both batches (Fig. 3b). The $bSiO_2$ in the “P-
388 replete” and “P-stress” batches dissolved with the exponential decrease commonly

389 observed in dissolution experiments and bSiO₂ dissolution rate constants were
390 calculated according to model 1. An opposite trend was observed when compared to
391 POC and PON degradation, with a faster bSiO₂ dissolution in the “P-stress *T.*
392 *weissflogii*” batches than in the “P-replete *T. weissflogii*” batches. Frustules from the
393 “P-stress” *T. weissflogii* batch were dissolved with a rate constant of $0.05 \pm 0.02 \text{ d}^{-1}$,
394 about twice the rate calculated in the “P-replete *T. weissflogii*” batch ($0.02 \pm 0.01 \text{ d}^{-1}$;
395 Table 3). After 3 weeks of dissolution, about 38% of the bSiO₂ has been dissolved in
396 the “P-replete *T. weissflogii*” batch while 64% of the bSiO₂ has been dissolved in the
397 “P-stress *T. weissflogii*” batch.

398

399 3.2.2. PCHO

400 In contrast to POC and PON, and alike bSiO₂, the degradation rate constant for
401 the PCHO–C was higher in the “P-stress *T. weissflogii*” batch ($0.92 \pm 0.18 \text{ d}^{-1}$) than in
402 the “P-replete *T. weissflogii*” batch ($0.15 \pm 0.08 \text{ d}^{-1}$), indicating a rapid degradation of
403 carbohydrates from *T. weissflogii* grown under P-stress conditions (Fig. 4b; Table 3). At
404 the end of 20 days of degradation, approximately 60% of the initial PCHO–C pool
405 remained in the “P-replete *T. weissflogii*” batch, whereas PCHO–C represented 30% of
406 the initial PCHO–C pool in the “P-stress *T. weissflogii*” batch.

407 The degradation rate constants showed significant differences between the
408 monosaccharides in these two experiments. In general, higher values were recorded in
409 the “P-stress” experiment than in the “P-replete” experiment, which agrees with the
410 pattern that was observed for the total PCHO pool (Table 3). Xylose, followed by
411 glucose and galactose, were the monosaccharides that exhibited the highest degradation
412 rates in the “P-stress” experiment ($k_1 = 0.80\text{--}1.52 \text{ d}^{-1}$), whereas galacturonic acid,

413 ribose, and galactose rates reached the highest values in the “P-replete” experiment (k_1
414 = 0.17–0.49 d⁻¹; Fig. 5). The degradation rate constants of the other monosaccharides
415 showed little (e.g., glucosamine and rhamnose) to no degradation (e.g., arabinose) in
416 both experiments (Table 4). The non-degradable pool (i.e., C₂) comprised 29–83% and
417 17–61% of the “P-replete” and “P-stress” *T. weissflogii* batches, respectively (Fig. 5;
418 Table 4).

419

420 **4. Discussion**

421 *4.1. Impact of P-stress on the initial biochemical composition of T. weissflogii*

422 Previous investigations that assessed the impact of P-stress on diatoms observed
423 increases in their absolute carbon (pmol C cell⁻¹), probably attributed to the biosynthesis
424 of sulfolipids and betaine lipids (Obata et al., 2013; Brembu et al., 2017), and
425 occasionally in their nitrogen (pmol N cell⁻¹) content (Lynn et al., 2000; Chauton et al.,
426 2013). The results of this study agree with the general increase of C and N content and
427 confirm that a P-stress may strongly affect the carbon and nitrogen contents per cell of
428 *T. weissflogii* (Table 1). Our results also showed a decrease in the bSiO₂ content due to
429 P-stress, which was also reflected in the Si/C and Si/N ratios (Table 1). It is generally
430 admitted that a decrease in the growth rate due to nutrient stress other than Si-limitation
431 leads to an increase of the cellular bSiO₂ content (Martin-Jézéquel et al. 2000, Claquin
432 et al. 2002). However, low Si quotas have sometimes been measured for nutrient-
433 stressed diatoms in *in situ* or laboratory studies, which may partially be explained by the
434 simultaneous decrease of the *T. weissflogii* cellular volume (Bucciarelli et al. 2010;
435 Lasbleiz et al. 2014; Suroy et al., 2015; Boutorh et al., 2016). These results are contrary
436 to what has been measured for *T. pseudonana* (Claquin et al., 2002) but agree well with

437 what has been observed for *T. weissflogii* (De La Rocha et al., 2010), emphasizing the
438 idea that diatoms of the same genus may have a different physiological response to
439 environmental stress.

440 The elemental ratios of the “P-replete” cells were similar to those reported in the
441 literature (Redfield et al., 1963; Brzezinski, 1985; Sarthou et al., 2005), with C/N of 6.7,
442 Si/N of 1.1, and Si/C of 0.17. P-stress induced a decrease of the Si/N and Si/C ratios
443 from 1.1 to 0.46, and from 0.17 to 0.08, respectively, which is similar to what has been
444 observed for N- and Si- limitations stress (Suroy et al., 2015). In our study, P-stress
445 induced a slight decrease in the C/N ratio from 6.7 to 6 (Table 1). Contrarily, most
446 previous studies observed an increase in C/N ratios under nutrient stress especially for
447 N- and Si- stress (Suroy et al., 2015; De La Rocha et al., 2010). However, a recent study
448 by Clark et al. (2014) illustrated very well the strong variability of C/N ratios under P-
449 limitation stress for *T. weissflogii*. The variability of this ratio may be explained by an
450 irregular excretion of polysaccharides to regulate the C/N ratios of diatom cells (Suroy
451 et al. 2015).

452 The PCHO concentrations in the “P-replete *T. weissflogii*” batch were lower
453 than those found in the “P-stress *T. weissflogii*” batch, accounting for 14% and 16% of
454 POC, respectively (Table 2). The carbohydrate data presented here agree with those of
455 previous investigations that assessed the impact of P-stress in *T. pseudonana* after
456 analyzing its carbohydrate and lipid components (Harrison et al., 1990b; Urbani et al.,
457 2005). Moreover, our results are consistent with the potential regulation of glycolysis
458 that has been observed in similar diatoms (e.g., *T. pseudonana*) that face strong
459 phosphate limitation (Dyhrman et al., 2012).

460 At the molecular level, the carbon allocation towards the carbohydrate
461 component under “P-stress *T. weissflogii*” conditions was mainly reflected by the
462 largest proportions of glucose, galactose, and xylose (Table 2). Galactose and xylose are
463 generally found as parts of structural, extracellular heteropolysaccharides, whereas
464 glucose is derived from both structural and storage (β -1,3 glucan; e.g., chrysolaminarin)
465 compounds in diatoms (Haug and Myklestad, 1976; Hicks et al., 1994; Chiovitti et al.,
466 2003; Størseth et al., 2005). Although the carbohydrate analysis presented here does not
467 allow for the differentiation of the origin of glucose (i.e., storage vs. structural
468 monosaccharide), the high levels of glucose, galactose, and xylose most likely reflect
469 the excretion and rapid degradation of structural polysaccharides under P-stress
470 conditions (Magaletti et al., 2004; Ai et al., 2015). It is worth noting that high glucose
471 and xylose levels have also been reported under “N-stress” conditions for *T. weissflogii*
472 (Suroy et al., 2015). The most striking result of this study is the low relative abundance
473 of ribose in the “P-stress” experiment (4.3% of PCHO) compared to the “P-replete *T.*
474 *weissflogii*” experiment (19.5% of PCHO; Table 2), which further suggests the better
475 physiological status of *T. weissflogii* cells, and the presence of ribose-containing
476 molecules (e.g., DNA and ATP) under “P-replete *T. weissflogii*” conditions. This result
477 is consistent with previous investigations that assessed N- and Si-stress in *T. weissflogii*
478 cells and showed low ribose levels (Suroy et al., 2015).

479

480 4.2. Effect of P-stress on the degradation of *T. weissflogii* organic matter, $bSiO_2$ 481 dissolution, and PCHO *T. weissflogii* dynamics

482 The kinetic calculations of the POC and PON degradation of *T. weissflogii*
483 grown under P-replete or P-stress conditions, suggest the presence of two pools of

484 organic matter: one that exhibited high degradation during the first 7–8 days, and a
485 second, less degradable pool that showed no degradation ($k_2 = 0$ for model 2b) and left
486 behind a so-called “recalcitrant” component (Table 3; Fig. 3). Such a feature has already
487 been well-documented for different types of organic matter, including fresh
488 phytoplanktonic cultures (Westrich and Benner, 1984; Harvey et al., 1995; Sempéré et
489 al., 2000; Moriceau et al., 2009). In an earlier study on *T. weissflogii* degradation under
490 Si- and N-stress conditions (Suroy et al., 2015), it was observed that the second-order
491 degradation rate constant (i.e., k_2) was almost equal to zero ($k_1 \gg k_2 \approx 0$), which agrees
492 with the data presented in Table 3. Moreover, and similarly to N-stressed *T. weissflogii*
493 (Suroy et al., 2015), the degradation rate constants for POC and PON were higher for
494 the *T. weissflogii* grown under “P-replete” conditions than those grown under “P-stress”
495 conditions, suggesting that nutrient growth conditions (herein the availability of P) also
496 impact the degradation of photosynthesized organic matter. Degradation rates of POC
497 and PON also indicated that N-containing organic molecules disappear more rapidly
498 when they are produced by *T. weissflogii* cells grown under the presence of P (Table 3).
499 Moreover, while the pool of degradable material was more rapidly utilized in the “P-
500 replete *T. weissflogii*” batch than in the “P-stress *T. weissflogii*” batch, the contribution
501 of the non-degradable pool to the global PON and POC was similar in both batches
502 (Fig. 3). Overall, the results showed that the diatom-organic matter resulting from a P-
503 stress environment might be less labile than the diatom-organic matter produced in
504 nutrient-replete surface layers. Therefore, if P limits diatom growth, diatom-organic
505 matter is less susceptible to degradation despite the fact that similar amount of total
506 PON and POC may reach the mesopelagic/bathypelagic layers after 21 days of
507 degradation (Fig. 3).

508 In contrast to with POC and PON, which degraded less rapidly under “P-stress”
509 conditions, bSiO₂ showed a higher dissolution in the “P-stress” experiment (Table 3).
510 Indeed, we measured a dissolution rate constant twice faster in the “P-stress *T.*
511 *weissflogii*” batch than in the “P-replete *T. weissflogii*” batch. The FTIR spectra of the
512 frustule cleaned from the organic coating showed that more organic matter was
513 associated to the “P-replete” than to the “P-stress” frustule (Soler, 2010; Fig. S2). This
514 result strongly suggests that when silica frustules are closely associated with a high
515 amount of organic matter, bSiO₂ dissolution rate (as shown by FTIR spectra) is slowed
516 down which agrees with a similar study performed in our lab on *Pseudonitzschia*
517 *delicatissima* (Boutorh, 2014). Indeed, the bSiO₂ dissolution involves a reaction
518 between the silanol group of the frustules and a water molecule. Therefore, a high
519 amount of organic matter associated to the frustules may reduce the access to the
520 reactive silanol groups, which further decreases the bSiO₂ dissolution rate (Loucaides et
521 al. 2010). Overall, these results suggest that bSiO₂ produced under “P-stress” contains
522 less organic matter and is less resistant to dissolution. The ballast effect of the bSiO₂
523 may, therefore be decreased initially because of the low Si/C ratios (Armstrong et al.,
524 2002; Armstrong et al., 2009; Tréguer et al., 2018; Table 1) resulted from “P-stress” and
525 secondly because bSiO₂ dissolves more rapidly in “P-stress” than in “P-replete” *T.*
526 *weissflogii* batches.

527 The rapid degradation of PCHO observed for the “P-stress *T. weissflogii*”
528 experiment (Table 3) implies an allocation of excess carbon toward highly energetic
529 molecules (i.e., carbohydrates), which can easily be assimilated after enzymatic
530 hydrolysis (Fig. 4b). At the molecular level, this finding was mainly reflected in the
531 contents of galactose, glucose, mannose, and xylose, which were among the most

532 abundant compounds (Table 2). The elevated concentrations of galactose, accompanied
533 by xylose and glucose, and mannose, in the “P-stress *T. weissflogii*” batch when
534 compared to the “P-replete *T. weissflogii*” batch most likely indicate the biosynthesis
535 and/or excretion of structural polysaccharides during diatom growth under “P-stress *T.*
536 *weissflogii*” conditions. The above monosaccharides are known to constitute the
537 building blocks of structural polysaccharides (i.e., heteropolysaccharides) and are
538 rapidly degraded by prokaryotes (Table 2; Fig. 5). Alternatively, these structural
539 polysaccharides may contribute to building up the extracellular pool of EPS (Myklestad,
540 1977; Urbani et al., 2005; Passow, 2002), rich in glucose (Waite et al. 1995), and are the
541 precursors of transparent exopolymer particles (TEP). In turn, TEP may trigger the
542 formation of large, fast-sinking aggregates that are known to increase C export (Passow,
543 2002; Moriceau et al., 2007; Seebah et al., 2014; Chen et al., 2015).

544 In contrast to the above monosaccharides, ribose exhibited higher degradation
545 rates in the “P-replete *T. weissflogii*” degradation experiment than in the “P-stress *T.*
546 *weissflogii*” degradation experiment. This result, in conjunction with the elevated
547 concentrations of ribose in the “P-replete” experiment, points toward a high lability of
548 *T. weissflogii* organic material (note that ribose may be considered as a good proxy of
549 fresh organic material as it is mostly found in metabolically active organisms), and a
550 subsequent better degradation “behavior” of prokaryotes when *T. weissflogii* cells are
551 grown under the presence of P (Table 2). Moreover, the exhaustion of ribose during the
552 growth of *T. weissflogii* in the absence of P resulted in little bioavailability of these
553 energy-rich ATP-containing molecules during degradation, as suggested by their low
554 degradation rates (Table 4). A similar observation was made for ribose under N- and Si-
555 stress conditions during the degradation of *T. weissflogii* (Suroy et al., 2015).

556 5. Conclusions

557 This study showed that “P-stress *T. weissflogii*” conditions induced an increase in the
558 contents of POC and PON in the *T. weissflogii* cells, with a concomitant decrease in
559 their silicon content (bSiO₂). The initial *T. weissflogii* chemical composition in the “P-
560 stress” cells was characterized by high abundances of glucose (19% of PCHO),
561 galactose (23%), and xylose (21%) compared to the “P-replete” cells, which were
562 dominated by ribose (20% of PCHO). These results clearly suggest that “P-stress *T.*
563 *weissflogii*” conditions cause changes in carbon allocation toward the carbohydrate
564 component (i.e., synthesis of storage/structural polysaccharides). The modeling of *T.*
565 *weissflogii* degradation in terms of bulk organic matter (POC, PON, and bSiO₂)
566 indicated that the degradation rate constants for POC and PON were always higher in
567 the “P-replete *T. weissflogii*” experiment ($k_1 = 0.27\text{--}0.40\text{ d}^{-1}$) than in the “P-stress *T.*
568 *weissflogii*” experiment ($k_1 = 0.19\text{--}0.17\text{ d}^{-1}$). Contrarily, bSiO₂ formed under “P-stress
569 *T. weissflogii*” conditions dissolved more rapidly than bSiO₂ produced under “P-replete
570 *T. weissflogii*” conditions as the latter contains more organic matter strongly associated
571 to the frustule. Overall, our study illustrates the critical role that P plays in the
572 degradation of diatom organic matter and its impact on silica frustule dissolution and
573 carbohydrate decomposition. Although further investigations are needed with different
574 types of diatoms in order to make any generalizations, it appears that P-limitation on
575 diatom growth may have important implications to the degradation of diatom-derived
576 organic matter and, consequently, to the associated export fluxes to the ocean interior.
577

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587

588 **References**

- 589 Agusti, S., Gonzalez-Gordillo, J.I., Vaqué, D., Estrada, M., Cerezo, M.I., Salazar, G.,
590 Gasol, J.M., Duarte, C.M., 2015. Ubiquitous healthy diatoms in the deep sea
591 confirm deep carbon injection by the biological pump. *Nature Commun.* 6:7608.
592 DOI: 10.1038/ncomms8608.
- 593 Ai, X.-X., Liang, J.-R., Gao, Y.-H., Lo, S.C.-L., Lee, F.W.-F., Chen, C.-P., Luo, C.-S.,
594 Du, C., 2015. MALDI-TOF MS analysis of the extracellular polysaccharides
595 released by the diatom *Thalassiosira pseudonana* under various nutrient
596 conditions. *J. Appl. Phycol.* 27, 673–684.
- 597 Armstrong, R.A., Lee, C., Hedges, J.I., Honjo, S., Wakeham S.G., 2002. A new,
598 mechanistic model for organic carbon fluxes in the ocean based on the quantitative
599 association of POC with ballast minerals. *Deep-Sea Res II* 49, 219–236.
- 600 Armstrong, R.A., Peterson, M.L., Lee, C., Wakeham, S.G., 2009. Settling velocity
601 spectra and the ballast ratio hypothesis. *Deep Sea Res. Part II* 56: 1470–1478.

602 Beardall, J., Berman, T., Heraud, P., Kadiri, M. O., Light, B. R., Patterson, G., et al.
603 2001. A comparison of methods for detection of phosphate limitation in
604 microalgae. *Aquat. Sci.* 63, 107–121.

605 Berg, J.M., Tymoczko, J.L., Stryer, L., 2002. *Biochemistry*. 5th edition, New York.

606 Brembu, T., Muhlroth, A., Alipanah, L., Bones, A. M., 2017. The effects of
607 phosphorus limitation on carbon metabolism in diatoms. *Phil. Trans. R. Soc. B*
608 372: 20160406.<http://dx.doi.org/10.1098/rstb.2016.0406>

609 Boop, L., Aumont, O., Cadule, P., et al. 2005. Response of diatoms distribution to
610 global warming and potential implications: A global model study. *Geophys. Res.*
611 *Lett.* 32. <https://doi.org/10.1029/2005GL023653>.

612 Boutorh, J., 2014. Impact des conditions nutritionnelles sur la dissolution de la silice
613 biogénique des diatomées à travers l'étude de la variabilité de la structure
614 biphasique du frustule. Ph.D. dissertation, Université de Bretagne Occidentale,
615 France, pp 14.

616 Boutorh, J., Moriceau, B., Gallinari, M., Ragueneau, O., Bucciarelli, E., 2016. Effect of
617 trace metal-limited growth on the postmortem dissolution of the marine diatom
618 *Pseudo-nitzschia delicatissima*. *Global Biochem. Cy.* 30, 57-69. DOI:
619 10.1002/2015GB005088

620 Brzezinski, M.A., 1985. The Si:C:N ratio of marine diatoms : interspecific variability
621 and the effect of some environmental variables. *J. Phycol.* 21, 347–57.

622 Bucciarelli, E., Pondaven, P., Sarthou, G., 2010. "Effects of an iron-light co-limitation
623 on the elemental composition (Si, C, N) of the marine diatoms *Thalassiosira*
624 *oceanica* and *Ditylum brightwellii*." *Biogeosciences* 7, 657–669.

625 Chauton, M.S., Olsen, Y., Vadstein, O., 2013. Biomass production from the microalga
626 *Phaeodactylum tricornutum*: Nutrient stress and chemical composition in
627 exponential fed-batch cultures. *Biomass and bioenergy* 58, 87–94.

628 Chen, J., Thornton, D.C.O., 2015. Transparent exopolymer particle production and
629 aggregation by a marine planktonic diatom (*Thalassiosira weissflogii*) at different
630 growth rates. *J. Phycol.* 51, 381-393.

631 Chiovitti, A., Higgins, M.J., Harper, R.E., Wetherbee, R., 2003. The complex
632 polysaccharides of the raphid diatom *Pinnularia viridis* (*Bacillariophyceae*). *J.*
633 *Phycol.* 39, 543–554.

634 Claquin, P., Martin-Jézéquel, V., Kromkamp, J.C., Veldhuis, M. J. W., Kraay, G. W.,
635 2002. Uncoupling of silicon compared to carbon and nitrogen metabolism, and role
636 of the cell cycle, in continuous cultures of *Thalassiosira pseudonana*
637 (*bacillariophyceae*) under light, nitrogen and phosphorus control. *J. Phycol.* 38,
638 922–930.

639 Clark, D. R., Flynn, K. J., Fabian, H., 2014. Variation in elemental stoichiometry of the
640 marine diatom *Thalassiosira weissflogii* (*Bacillariophyceae*) in response to
641 combined nutrient stress and changes in carbonate chemistry. *J. Phycol.* 50, 640–
642 651. doi:10.1111/jpy.12208.

643 De La Rocha, C.L., Terbrüggen, A., Völker, C., Hohn, S., 2010. Response to and
644 recovery from nitrogen and silicon starvation in *Thalassiosira weissflogii*: growth
645 rates, nutrient uptake and C, Si and N content per cell. *Mar. Ecol. Prog. Ser.* 412,
646 57–68.

647 Diekmann, A.B.S., Peck, M.A., Holste, L., St John, M.A., Campbell, R.W., 2009.
648 Variation in diatom biochemical composition during a simulated bloom and its
649 effect on copepod production. *J. Plankton Res.* 31, 1391–1405.

650 Downing, J.A., 1997. Marine nitrogen: Phosphorus stoichiometry and the global N:P
651 cycle. *Biogeochemistry* 37, 237–252.

652 Duhamel, S., Moutin, T., Van Wambeke, F., Van Mooy, B., Raimbault, P., Chaustre,
653 H., 2007. Growth and specific P-uptake rates of bacterial and phytoplanktonic
654 communities in the Southeast Pacific (BIOSOPE cruise). *Biogeosciences* 4, 941–
655 956.

656 Dyhrman, S.T., Jenkins, B.D., Rynearson, T.A., Saito, M.A., Mercier, M.L., Alexander,
657 H., Whitney, L.P., Drzewianowski, A., Bulygin, V.V., Bertrand, E.M., Wu, Z.,
658 Benitez-Nelson, C., Heithoff, A., 2012. The transcriptome and proteome of the
659 diatom *Thalassiosira pseudonana* reveal a diverse phosphorus stress response.
660 *PloS one*, 7, e33768. <https://doi.org/10.1371/journal.pone.0033768>.

661 Engel, A., Abramson, L., Szlosek, J., Liu, Z., Stewart, G., Hirschberg, D., Lee, C.,
662 2009. Investigating the effect of ballasting by CaCO₃ in *Emiliana huxleyi*, II:
663 Decomposition of particulate organic matter. *Deep-Sea Res. II* 56, 1408–1419.
664 DOI:10.1016/j.dsr2.2008.11.028

665 Gärdes, A., Iversen, M. H., Grossart, H. P., Passow, U., Ullrich, M. S., 2010. Diatom-
666 associated bacteria are required for aggregation of *Thalassiosira weissflogii*. *ISME*
667 *J.* 5, 436–445.

668 Godwin, C.M., Cotner, J.B., 2015 Aquatic heterotrophic bacteria have highly flexible
669 phosphorus content and biomass stoichiometry. *ISME J.*
670 <https://doi:10.1038/ismej.2015.34>

671 Gordon, L.I., Jennings, J.C., Ross, A.A., Krest, J.M., 1993. A suggested protocol for
672 continuous flow automated analysis of seawater nutrients. In Group, C. o. [Ed.]
673 OSU, College of Oc. Descriptive. Chem. Oc. Grp. Tech. Rpt. OSU College of
674 Oceanography Descriptive, Corvallis, pp. 1–55.

675 Goutx, M., Wakeham, S.G., Lee, C., Duflos, M., Guigue, C., Liu, Z., Moriceau, B.,
676 Sempéré, R., Tedetti, M., Xue, J., 2007. Composition and degradation of marine
677 particles with different settling velocities in the northwestern Mediterranean Sea.
678 *Limnol. Oceanogr.* 52, 1645–64.

679 Grattepanche, J-D., Vincent, D., Breton, E., Christaki, U., 2011. Microzooplankton
680 herbivory during the diatom–Phaeocystis spring succession in the eastern English
681 Channel. *J. Exp. Mar. Biol. Ecol.* 404, 87–97.

682 Greenwood, J.E., Truesdale, V.W., Rendell, A.R., 2001. Biogenic silica dissolution in
683 seawater - in vitro chemical kinetics. *Prog. Oceanogr.* 48, 1–23.

684 Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen
685 cycle. *Nature*, 451, 293-96. <https://doi:10.1038/nature06592>.

686 Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella*
687 *nana* hustedt and *Detonella confervacea* (cleve) gran. *Can. J. Microbiol.* 8, 229–
688 239.

689 Guillard, R.R.L., 2005. Purification methods for microalgae, in: Andersen, R.A (Ed.)
690 *Algal Culturing Techniques*. Elsevier Academic Press, Burlington, MA, USA, pp.
691 117–132.

692 Harrison, P.J., Hu, M.H., Yang, Y.P., Lu, X., 1990a. Phosphate limitation in estuarine
693 and coastal waters of China. *J. Exp. Mar. Biol. Ecol.* 140, 79-87. DOI:
694 10.1016/0022-0981(90)90083-O.

695 Harrison, P.J., Thompson, P.A., Calderwood, G.S., 1990b. Effects of nutrient and light
696 limitation on the biochemical composition of phytoplankton. *J. Appl. Phycol.* 2,
697 45–56.

698 Harvey, H.R., Tuttle, J.H., Bell, J.T., 1995. Kinetics of phytoplankton decay during
699 simulated sedimentation: Changes in biochemical composition and microbial
700 activity under oxic and anoxic conditions. *Geochim. Cosmochim. Acta* 59, 3367–
701 77.

702 Harvey, H.R., Macko, S.A., 1997. Kinetics of phytoplankton decay during simulated
703 sedimentation: changes in lipids under oxic and anoxic conditions. *Org. Geochem.*
704 129–140.

705 Haug, A., Myklestad, S.M., 1976. Polysaccharides of marine diatoms with special
706 reference to *Chaetoceros* species. *Mar. Biol.* 34, 217–22.

707 Hicks, R.A., Owen, C.J., Aas, P., 1994. Deposition, resuspension, and decomposition of
708 particulate organic matter in sediments of lake Itasca, Minnesota, USA.
709 *Hydrobiologia* 284,79–91.

710 Jackson, G.A., Williams, P.M., 1985. Importance of dissolved organic nitrogen and
711 phosphorus to biological nutrient cycling. *Deep-Sea Res* 32, 223–35.

712 Kamatani, A., Riley, J.P., 1979. Rate of dissolution of diatom silica walls in seawater.
713 *Mar. Biol.* 55, 29–35.

714 Kamatani, A., Riley, J.P., Skirrow, G., 1980. The dissolution of opaline silica of
715 diatom tests in seawater. *J. Oceanogr. Soc. Jpn* 36, 201–8.

716 Kamatani, A., 1982. Dissolution rates of silica from diatoms decomposing at various
717 temperature. *Mar. Biol.* 68, 91–96.

718 Karl, D.M., Björkman, K.M., 2015. Dynamics of dissolved organic phosphorus, in:
719 Hansell, D.A., Carlson, C.A., (Eds.) *Biogeochemistry of marine dissolved organic*
720 *matter*. Academic Press, New York. pp. 233–318.

721 Lasbleiz, M., Leblanc, K., Blain, S., Ras, J., Cornet-Barthaux, V., Hélias Nunige,
722 S., Quéguiner, B., 2014. Pigments, elemental composition (C, N, P, Si) and
723 stoichiometry of particulate matter, in the naturally iron fertilized region of
724 Kerguelen in the Southern Ocean, *Biogeosciences* 11, 5931–5955,
725 doi.org/10.5194/bg-11-5931-2014.

726 Lehninger, A.L., Nelson, D.L., Cox, M.M., 1993. *Principles of Biochemistry*, 2nd ed.
727 Worth Publishers, New York.

728 Liu, S., Guo, Z.L., Li, T., Huang, H., Lin, S.J., 2011. Photosynthetic efficiency, cell
729 volume, and elemental stoichiometric ratios in *Thalassiosira weissflogii* under
730 phosphorus limitation. *Chinese J. Oceanol. Limnol.* 29, 1048–56.

731 Loucaides, S., Behrends, T., Van Cappellen, P., 2010. Reactivity of biogenic silica:
732 surface vs bulk charge density. *Geochim. Cosmochim. Acta* 74, 517–530.

733 Lynn, S.G., Kilham, S.S., Kreeger, D.A., Interlandi, S.J., 2000. Effect of nutrient
734 availability on the biochemical and elemental stoichiometry in the freshwater
735 diatom *Stephanodiscus minutulus* (*Bacillariophyceae*). *J. Phycol.* 36, 510–22.

736 Magaletti, E., Urbani, R., Sist, P., Ferrari, C.R., Cicero, A.M., 2004. Abundance and
737 chemical characterization of extracellular carbohydrates released by the marine
738 diatom *Cylindrotheca fusiformis* under N- and P- limitation. *Eur. J. Phycol.* 39,
739 133–42.

740 Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle
741 analysis of natural populations of marine picoplankton by flow cytometry using the
742 nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.* 63, 186–93.

743 Marie, D., Partensky, F., 2006. Analyse des micro-organismes marin. In *La cytométrie*
744 *en flux*, Lavoisier, Edition Tec and Doc; Ronot, R., Grunwald, D., Mayol, J.-F.,
745 Boutonnat, J., coordonateurs, p. 210–33.

746 Moore, C.M., Mills, M., Langlois, R., Milne, A., Achterberg, E.P., La Roche, J., Geider,
747 R.J., 2008. Relative influence of nitrogen and phosphorus availability on
748 phytoplankton physiology and productivity in the oligotrophic sub-tropical North
749 Atlantic Ocean. *Limnol. Oceanogr.* 53, 291–305.

750 Moore, C.M., Mills, M.M., Arrigo, K.R., Berman-Frank, I., Bopp, L., Boyd, P.W.,
751 Galbraith, E.D., Geider, R.J., Guieu, C., Jaccard, S.L., Jickells, T.D., La Roche, J.,
752 Lenton, T.M., Mahowald, N.M., Maranon, E., Marinov, I., Moore, J.K.,
753 Nakatsuka, T., Oschlies, A., Saito, M.A., Thingstad, T.F., Tsuda, A., Ulloa O.,
754 2013. Processes and patterns of oceanic nutrient limitation. *Nat. Geosci.* 6: 701-
755 710. doi: 10.1038/NGEO1765.

756 Moriceau, B., Gallinari, M., Soetaert, K., Ragueneau, O., 2007. Importance of particle
757 formation to reconstructed water column biogenic silica fluxes. *Glob. Biogeochem.*
758 *Cycles* 21, GB3012. doi:10.1029/2006GB002814.

759 Moriceau, B., Goutx, M., Guigue, C., Lee, C., Armstrong, R.A., Duflos, M., Tamburini,
760 C., Charrière, B., Ragueneau, O., 2009. Si–C interactions during degradation of the
761 diatom *Skeletonema marinoi*. *Deep-Sea Res. II* 56,1381–95.

762 Myklestad, S.M., 1977. Production of carbohydrates by marine planktonic diatoms. 2.
763 Influence of N/P ratio in growth medium on assimilation ratio, growth rate, and

764 production of cellular and extracellular carbohydrates by *Chaetoceros affinis* var
765 Willei (Gran) Hustedt and Skele. J. Exp. Mar. Biol. Ecol. 29:161–79.

766 Némery, J., Garnier, J., 2016. The fate of phosphorus. Nature Geosci. 9, 343–344.

767 Nieuwenhuize, J., Maas, Y.E.M., Middelburg, J.J., 1994. Rapid analysis of organic
768 carbon and nitrogen in particulate materials. Mar. Chem. 45, 217–24.

769 Obata, T., Fernie, A.I., Nunes-Nesi, A., 2013. The Central Carbon and Energy
770 Metabolism of Marine Diatoms. Metabolites, 3, 325–346;
771 doi:10.3390/metabo3020325.

772 Palmucci, M., Ratti, S., Giordano, M., 2011. Ecological and evolutionary implications
773 of carbon allocation in marine phytoplankton as a function of nitrogen availability:
774 a fourier transform infrared spectroscopy approach. J. Phycol. 47, 313–323.

775 Panagiotopoulos, C., Sempéré, R., Obernosterer, I., Striby, L., Goutx, M., Van
776 Wambeke, F., Gautier, S., Lafont, R., 2002. Bacterial degradation of large particles
777 in the southern Indian Ocean using in vitro incubation experiments. Org. Geochem.
778 33, 985–1000.

779 Panagiotopoulos, C., Sempéré, R., 2007. Sugar dynamics in large particles during in
780 vitro incubation experiments. Mar. Ecol. Prog. Ser. 330, 67–74.

781 Panagiotopoulos, C., Sempéré, R., Para, J., Raimbault, P., Rabouille, C., Charrière, B.,
782 2012. The composition and flux of particulate and dissolved carbohydrates from
783 the Rhone River into the Mediterranean Sea. Biogeosciences 9, 1827–1844.

784 Panagiotopoulos, C., Wurl, O., 2009. Spectrophotometric and chromatographic analysis
785 of carbohydrates in marine samples, in: Wurl, O., (Ed.) Practical Guidelines for the
786 Analysis of Seawater. Taylor and Francis, Boca Raton, FL, pp. 49–65.

787 Passow, U., 2002. Production of transparent exopolymer particles (TEP) by phyto- and
788 bacterioplankton, *Mar. Ecol. Prog. Ser.* 236, 1–12.

789 Piontek, J., Handel, N., Langer, G., Wohlers, J., Riebesell, U., Engel, A., 2009. Effects
790 of rising temperature on the formation and microbial degradation of marine diatom
791 aggregates. *Aquat. Microb. Ecol.* 54, 305-318. DOI: 10.3354/ame01273.

792 Paytan, A., McLaughlin, K., 2007. The oceanic phosphorus cycle. *Chem. Rev.* 107,
793 563–76.

794 Ragueneau, O., Treguer, P., 1994. Determination of biogenic silica in coastal
795 waters: Applicability and limits of alkaline digestion method. *Mar. Chem.* 45, 43–
796 51.

797 Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the
798 composition of sea water, in: Hill, M.N., (Ed.) *The sea: ideas and observations on*
799 *progress in the study of the seas.* Interscience, New York, pp 26–77.

800 Ryther, J.H., Dunstan, W.H., 1971. Nitrogen, phosphorus and eutrophication in the
801 coastal marine environment. *Science* 171:1008–13.

802 Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and
803 fate of diatoms in the ocean: a review. *J. Sea Res.* 53, 25–42. DOI:
804 10.1016/j.seares.2004.01.007.

805 Schreiber, U., Schliwa, U., Bilger, W., 1986. Continuous recording of photochemical
806 and nonphotochemical chlorophyll fluorescence quenching with a new type of
807 modulation fluorometer. *Photosynth. Res.* 10, 51–62.

808 Sebastian, M., Smith, A.F., Gonzalez, J.M., Fredricks, H.F., et al. 2016. Lipid
809 remodeling is a widespread strategy in marine heterotrophic bacteria upon

810 phosphorus deficiency. ISME journal. 10, 968-78. <https://doi:>
811 10.1038/ismej.2015.172

812 Seebah, S., Fairfield, C., Ullrich, M.S., Passow, U., 2014. Aggregation and
813 Sedimentation of *Thalassiosira weissflogii* (diatom) in a Warmer and More
814 Acidified Future Ocean. Plos One 9. [https:// doi: 10.1371/journal.pone.0112379](https://doi:10.1371/journal.pone.0112379).

815 Sempéré, R., Yoro, S-C., Van Wambeke, F., Charrière, B., 2000. Microbial
816 decomposition of large organic particles in the northwestern Mediterranean Sea: an
817 experimental approach. Mar. Ecol. Prog. Ser. 198, 61–72.

818 Shifrin, N.S., Chisholm, S.W., 1981. Phytoplankton lipids interspecific differences and
819 effects of nitrate, silicate and light-dark cycles. J. Phycol. 17, 374-84.

820 Smetacek, V., 1985. Role of sinking in diatom life-history cycles: ecological,
821 evolutionary and geological significance. Mar. Biol. 84, 239–251.

822 Soler, C., 2010. Impact des conditions de croissance sur le métabolisme et les
823 interactions Si-OC des diatomées– conséquences sur la vitesse de reminéralisation
824 de la silice biogène et de la matière organique. Ph.D. dissertation, Université de
825 Bretagne Occidentale, France, pp. 207.

826 Størseth, T.R., Hansen, K., Reitan, K.I., Skjermo, J., 2005. Structural characterization of
827 β -D-(1→3)-glucans from different growth phases of the marine diatoms
828 *Chaetoceros mülleri* and *Thalassiosira weissflogii*. Carbohydr. Res. 340:1159–64.

829 Sun, M.Y., Lee, C., Aller, R.C., 1993. Anoxic and oxic degradation of C-14-labeled
830 chloropigments and a C-14-labeled diatom in long-island sound sediments.
831 Limnol. Ocenogr. 38, 1438–1451.

832 Suroy, M., Panagiotopoulos, C., Boutorh, J., Goutx, M., Moriceau, B., 2015.
833 Degradation of diatom carbohydrates: A case study with N- and Si- stressed
834 *Thalassiosira weissflogii*. J. Exp. Mar. Biol. Ecol. 470, 1–11.

835 Tanaka, T., Thingstad, T.F., Christaki, U., Colombet, J., et al. 2011. Lack of P-
836 limitation of phytoplankton and heterotrophic prokaryotes in surface waters of
837 three anticyclonic eddies in the stratified Mediterranean Sea. Biogeosciences 8,
838 525-38. DOI: 10.5194/bg-8-525-2011.

839 Thingstad, T.F., Krom, M.D., Mantoura, R.F.C., Flaten, G.A.F, Groom, S., et al. 2005.
840 Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean.
841 Science 309:1068–71.

842 Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, et al. 2018. Influence of diatom
843 diversity on the ocean biological carbon pump. Nature Geoscience 1: 27–37. DOI:
844 10.1038/s41561-017-0028-x.

845 Trommer, G., Leynaert, A., Klein, C., Naegelen, A., Beker, B., 2013. Phytoplankton
846 phosphorus limitation in a North Atlantic coastal ecosystem not predicted by
847 nutrient load. J. Plankton Res. 35:1207-1219. doi:10.1093/plankt/fbt070.

848 Troussellier, M., Courties, C., Vaquer, A., 1993. Recent applications of flow cytometry
849 in aquatic microbial ecology. Biol. Cell 78, 111–21.

850 Urbani, R., Magaletti, E., Sist, P., Cicero, A.M., 2005. Extracellular carbohydrates
851 released by the marine diatoms *Cylindrotheca closterium*, *Thalassiosira*
852 *pseudonana* and *Skeletonema costatum*: effect of P-depletion and growth status.
853 Sci. Total Env. 353, 300–6.

854 Van Mooy, B.A.S., Fredricks, H.F., Pedler, B.E., Dyrman, S.T., et al (2009)
855 Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus
856 scarcity. *Nature* 458: 69–72.

857 Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: How
858 can it occur? *Biogeochemistry* 13, 87–115.

859 Waite, A.M., Bienfang, P., Harrison, P.J., 1992. Spring bloom sedimentation in a sub-
860 arctic ecosystem.1. Nutrient sensitivity. *Mar. Biol.* 114, 119–29.

861 Westrich, J., Berner, R., 1984. The role of sedimentary organic matter in bacterial
862 sulphate reduction: the G model tested. *Limnol. Oceanogr.* 29, 236–49.

863 Wetz, M.S., Hales, B., Wheeler, P.A., 2008. Degradation of phytoplankton-derived
864 organic matter: implications for carbon and nitrogen biogeochemistry in coastal
865 ecosystems. *Estuar. Coast. Shelf Sci.* 77: 422-432. DOI:
866 10.1016/j.ecss.2007.10.002.

867 Wu, J., Sunda, W., Boyle, E.A., Karl, D.M., 2000. Phosphate depletion in the western
868 North Atlantic Ocean. *Science* 289, 759–62.

869 ZoBell, C.E., 1941. Studies on marine bacteria. I. The cultural requirement of
870 heterotrophic aerobes. *J. Mar. Res.* 4, 42–75.

871 Zohary, T., Herut, B., Krom, M.D., Mantoura, R.F., et al 2005. P-limited bacteria but N
872 and P co-limited phytoplankton in the Eastern Mediterranean – a microcosm
873 experiment. *Deep Sea Res. II* 52, 3011–2.

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878 *Table Captions:*

879 **Table 1.** Initial parameters measured in each batch at the beginning (T_0) of the
880 experiment. The previous assumption also considers that there is little variability in the
881 POC, PON, and $bSiO_2$ concentrations between day 0 and day 1 of the experiment. Thus,
882 the ratio of these parameters to the diatom cell does not change drastically between day
883 0 and day 1.

884

885 **Table 2.** Elemental carbohydrate compositions (as percentages of total sugar, mol%)
886 and the total PCHO–C concentrations (μM) during the 21 days of degradation. The
887 PCHO yields are given as a percentage of the total PCHO–C relative to the POC.
888 Abbreviations: Fuc. – Fucose; Rha. – Rhamnose; Ara. – Arabinose; GlcN. –
889 Glucosamine; Gal. – Galactose; Glc. – Glucose; Man. – Mannose; Xyl. – Xylose; Rib. –
890 Ribose; GalUA. – Galacturonic Acid. The number of replicates is given in the caption
891 of Fig. 4.

892

893 **Table 3.** Estimated kinetic parameters for POC, PON, $bSiO_2$ and PCHO during the
894 degradation of *T. weissflogii*. The standard error (SE) is given for each estimate.
895 Extremely significant estimates are underlined ($p < 0.0001$). Kinetic parameters (k_1 , C_2)
896 were calculated according to models 1 and 2 using equations (1) and (2b) (see statistical
897 analysis and estimation of kinetic parameters section). k_1 and C_2 units are given in d^{-1}
898 and $\mu mol/\mu mol$, respectively.

899

900 **Table 4.** Estimated kinetic parameters for individual monosaccharides during the biotic
901 degradation of *T. weissflogii*. The standard error (SE) is given for each estimate.

902 Extremely significant estimates are underlined ($p < 0.0001$). Abbreviations are the same
903 as in Tables 2 and 3. Kinetic parameters (k_1 in d^{-1} and C_2 in $\mu\text{mol}/\mu\text{mol}$) were
904 calculated according to model 1 and/or 2 using equations (1) and/or (2b).

905

906 *Figure Captions:*

907

908 **Figure 1.** Schematic presentation of the experimental design of sampling, growth and
909 prokaryotic degradation of *T. weissflogii* cells (Fig. adapted from Suroy et al 2015).

910

911 **Figure 2.** Evolution of prokaryotic concentrations over time in the “P-replete *T.*
912 *weissflogii*” and “P-stress *T. weissflogii*” batches during the biodegradation
913 experiments. The prokaryotic concentrations correspond only to the free prokaryotes
914 that were measured in the batches. At each sampling point $n = 2$.

915

916 **Figure 3.** Time course responses of the (A) POC and (B) PON relative concentrations
917 of *T. weissflogii* in the “P-replete *T. weissflogii*” and “P-stress *T. weissflogii*” batches.
918 The relative concentrations were calculated by dividing the concentration on day t by
919 the concentration on days 0 and 2 for “P-stress *T. weissflogii*” and “P-replete *T.*
920 *weissflogii*” batches, respectively, for both POC and PON. The kinetics were estimated
921 using equation (2b) (section 2.6). Replicate subsamples ($n = 3$) for “P-replete” and “P-
922 stress” batches were performed on days 2, 6, 9, 12, and 19, and were used to feed the
923 degradation model (section 2.5). In the “P-stress” batch, only an $n = 2$ was obtained at
924 day 12 for both POC and PON.

925

926 **Figure 4.** Time course responses of the relative concentrations of (A) bSiO₂ and (B)
927 PCHO in the “P-replete *T. weissflogii*” and “P-stress *T. weissflogii*” batches. The
928 relative concentrations were calculated by dividing the concentration on day t by the
929 concentration on days 0 and 2 for the “P-stress *T. weissflogii*” and “P-replete *T.*
930 *weissflogii*” batches, respectively, for both bSiO₂ and PCHO. The kinetics were
931 estimated using equations (1) and (2b). For bSiO₂ degradation, replicate subsamples (*n*
932 = 3) were performed on days 2, 6, 9, and 19; and 2, 6, 9, 12, and 19 for the “P-replete *T.*
933 *weissflogii*” and “P-stress *T. weissflogii*” experiments, respectively. On day 12, only an
934 *n* = 2 was obtained for the “P-replete *T. weissflogii*” experiment. For PCHO
935 degradation, replicate subsamples (*n* = 3) were performed on days 6, 12, and 19 and 2,
936 6, and 19 for the “P-replete *T. weissflogii*” and “P-stress *T. weissflogii*” experiments,
937 respectively. On days 9 and 12, only an *n* = 2 was obtained for the “P-stress *T.*
938 *weissflogii*” experiment.

939

940 **Figure 5.** Time course responses of (A) galactose, (B) glucose, (C) xylose and (D)
941 ribose in the “P-replete *T. weissflogii*” and “P-stress *T. weissflogii*” batches. The relative
942 concentrations were calculated by dividing the concentration on day t by the
943 concentrations on day 0 and 2 for the “P-stress” and “P-replete” batches, respectively,
944 for individual monosaccharides. The kinetics were estimated using equations (1) and
945 (2b). The degradation rates, including experimental points, are given in Table 5.

946 Table 1.

Growth conditions	Batch volume (L)	[<i>T. weissflogii</i> cells] (cell L ⁻¹)	[POC] / [<i>T. weissflogii</i> cell] (pmol cell ⁻¹)	[PON] / [<i>T. weissflogii</i> cell] (pmol cell ⁻¹)	[Prokaryotic inoculum] (cell L ⁻¹)	[bSiO ₂] / [diatom cell] (pmol cell ⁻¹)
P-replete <i>T. weissflogii</i>	12	6.60 x 10 ⁷	12.0	1.8	7.02 x 10 ¹⁰	2.0
P-stress <i>T. weissflogii</i>	12	6.80 x 10 ⁷	15.5	2.6	5.64 x 10 ¹⁰	1.2

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969 Table 2.
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Experiment	Time (d)	Fuc.	Rha.	Ara.	GlcN.	Gal.	Glc.	Man.	Xyl.	Rib.	GalUA	PCHO–C	PCHO–C/POC	
«P-replete <i>T. weissflogii</i> »	0	10.1	6.29	1.03	2.66	18.4	12.4	13.4	14.2	19.5	2.07	110	13.9	
	1	8.72	5.73	0.72	4.87	16.7	16.6	15.1	12.1	16.8	2.69	112	7.23	
	2	10.0	6.61	1.14	4.16	18.5	14.5	11.6	13.1	18.6	1.80	104	6.12	
	4	9.29	5.11	2.06	5.14	18.6	19.5	13.6	13.4	12.5	0.93	113	8.19	
	5	9.34	6.44	2.95	9.11	17.2	15.4	10.7	15.7	12.0	1.33	107	8.11	
	6	11.3	7.86	2.69	5.39	16.3	18.2	10.4	14.9	12.0	0.94	83.9	7.06	
	7	11.7	9.49	2.09	5.95	13.5	17.4	10.4	16.1	12.7	0.67	80.0	7.01	
	10	10.8	10.5	2.28	8.32	13.1	17.5	14.0	13.9	8.85	0.82	61.3	5.78	
	11	9.77	7.41	1.78	8.38	14.3	18.5	16.8	14.7	7.75	0.58	83.6	7.82	
	12	7.75	9.30	1.74	8.47	14.9	18.5	18.5	13.7	6.53	0.56	89.3	9.04	
	14	9.12	9.00	2.18	7.72	15.0	20.0	12.0	15.3	9.35	0.45	68.4	5.74	
	16	8.92	10.1	2.86	6.37	12.1	21.5	10.1	15.4	11.4	1.31	46.1	4.32	
	17	9.41	10.0	2.37	6.96	13.9	20.0	11.1	15.0	9.61	1.66	62.4	5.99	
	18	8.00	8.71	3.15	5.18	14.5	21.0	13.8	16.0	8.40	1.20	80.1	7.29	
	19	10.0	9.99	4.03	5.71	12.2	19.4	9.47	16.8	11.2	1.25	54.2	4.97	
	20	7.64	8.42	13.2	6.39	12.2	19.0	9.74	13.3	8.73	1.35	70.6	6.47	
	21	8.17	8.92	6.95	6.68	12.3	19.3	11.6	16.1	8.56	1.42	65.8	6.01	
	«P-stress <i>T. weissflogii</i> »	0	6.11	2.90	6.56	1.40	23.0	18.6	15.4	21.4	4.32	0.34	169	16.0
		1	8.05	4.87	3.58	1.57	20.4	24.8	16.7	13.0	6.37	0.69	97.2	9.57
		2	12.3	6.76	4.61	2.05	21.2	13.8	15.5	15.0	8.36	0.58	68.5	7.32
		5	11.7	6.06	7.11	3.44	17.3	14.8	14.0	14.1	11.1	0.39	59.3	7.42
6		13.2	6.24	9.80	1.78	15.0	16.4	7.82	14.8	14.3	0.62	46.2	5.67	
7		11.2	6.40	5.79	3.15	18.1	20.4	9.66	14.8	10.2	0.35	73.4	9.43	
8		12.5	6.31	8.81	2.29	15.4	21.1	9.05	14.0	10.1	0.48	44.3	5.96	

9	16.0	6.58	10.8	1.39	11.1	17.3	6.97	15.8	13.5	0.61	34.9	5.76
10	13.0	8.09	6.74	3.08	21.1	12.5	9.62	16.2	9.29	0.41	72.9	12.1
11	16.5	8.43	9.22	1.52	14.3	12.8	8.09	17.7	10.2	1.34	40.5	6.74
12	14.8	7.99	8.61	1.91	14.9	15.5	9.74	16.2	8.84	1.55	49.4	9.16
14	13.3	8.06	9.97	1.30	11.6	18.3	7.64	15.7	10.6	3.53	44.1	6.36
15	18.2	7.34	8.22	1.78	13.3	14.4	10.2	15.5	7.93	3.15	53.7	9.00
16	21.3	7.43	9.33	0.68	12.4	13.8	7.33	15.4	10.7	1.50	42.1	7.23
17	18.0	6.50	7.84	1.13	14.0	14.3	11.3	18.4	7.34	1.22	56.3	7.45
18	15.0	7.36	9.50	2.77	16.2	15.6	12.5	15.8	4.07	1.17	68.2	7.99
19	17.4	5.93	13.1	1.92	14.2	17.8	7.61	14.7	5.60	1.72	43.2	7.07
20	11.1	5.41	5.83	6.43	22.1	14.2	11.2	15.6	7.02	1.21	73.2	11.6
21	14.6	4.69	10.3	3.49	15.7	16.4	8.45	15.7	8.81	1.86	32.7	5.00

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Table 3.

Kinetic parameters	«P-replete <i>T. weissflogii</i> »						«P-stress <i>T. weissflogii</i> »					
	k_1		C_2		R^2	n	k_1		C_2		R^2	n
	Value	SE	Value	SE			Value	SE	Value	SE		
POC	0.40	<u>0.05</u>	0.62	<u>0.01</u>	0.94	$n = 27$	0.19	0.05*	0.54	<u>0.03</u>	0.87	$n = 25$
PON	0.27	<u>0.02</u>	0.34	<u>0.01</u>	0.98	$n = 27$	0.17	<u>0.03</u>	0.29	<u>0.04</u>	0.91	$n = 24$
bSiO ₂	0.02	<u>0.01</u>	-	-	0.94	$n = 29$	0.05	<u>0.02</u>	-	-	0.95	$n = 32$
PCHO	0.15	0.08	0.55	<u>0.08</u>	0.66	$n = 22$	0.92	<u>0.18</u>	0.29	<u>0.02</u>	0.81	$n = 27$

987 * $p < 0.001$ (very significant at the 99.9% level).

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Table 4

Kinetic parameters	« P-replete <i>T. weissflogii</i> »						« P-stress <i>T. weissflogii</i> »					
	k_1		C_2		R^2	n	k_1		C_2		R^2	n
Value	SE	Value	SE	Value			SE	Value	SE	Value		
PCHO	0.08	0.03*	0.31	0.13*	0.93	$n = 21$	0	0	-	-	0	$n = 27$
Fuc.	0.01	0.01	-	-	0.08	$n = 22$	0.26	0.16	0.61	0.06	0.45	$n = 24$
Rha.	0	0	-	-	0	$n = 22$	0	0	-	-	0	$n = 27$
Ara.	0.02	0.01	-	-	0.15	$n = 22$	0.21	0.22	0.38	0.11*	0.20	$n = 26$
GlcN.	0.17	0.06*	0.37	0.08*	0.77	$n = 22$	0.80	<u>0.16</u>	0.20	<u>0.02</u>	0.80	$n = 27$
Gal.	0.10	0.13	0.83	0.32	0.36	$n = 22$	0.90	<u>0.18</u>	0.25	<u>0.02</u>	0.79	$n = 27$
Glc.	0.03	0.01*	-	-	0.25	$n = 22$	0.62	<u>0.10</u>	0.17	<u>0.02</u>	0.87	$n = 27$
Man.	0.10	0.08	0.56	0.14*	0.64	$n = 22$	1.52	<u>0.29</u>	0.22	<u>0.01</u>	0.88	$n = 27$
Xyl.	0.32	<u>0.02</u>	0.27	<u>0.01</u>	0.98	$n = 22$	0.05	<u>0.01</u>	-	-	0.73	$n = 26$
Rib.	0.49	0.19*	0.29	<u>0.03</u>	0.66	$n = 22$	0	0	-	-	0.00	$n = 27$
GalUA												

996 * $p < 0.05$ (significant at the 95% level)

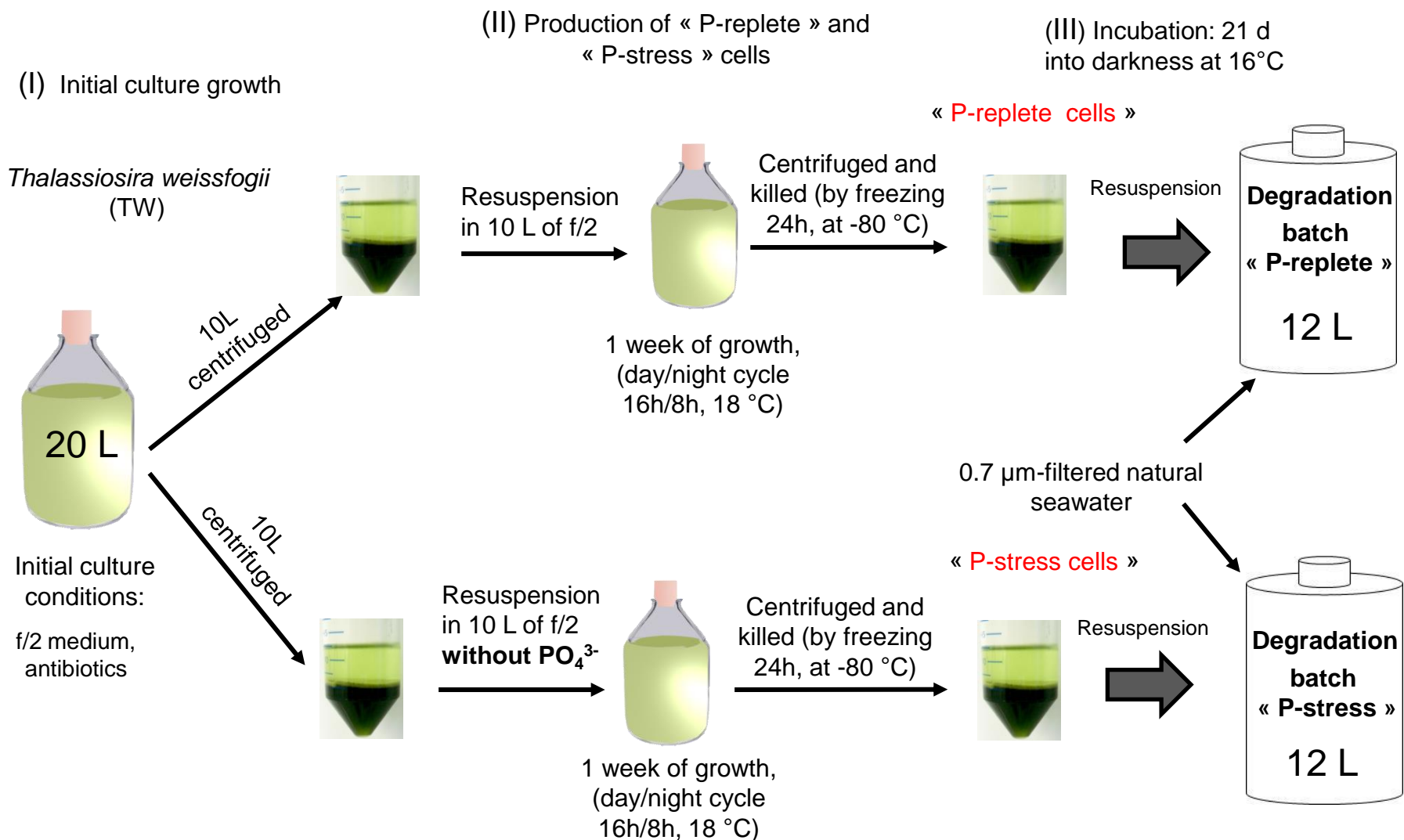


Figure 1

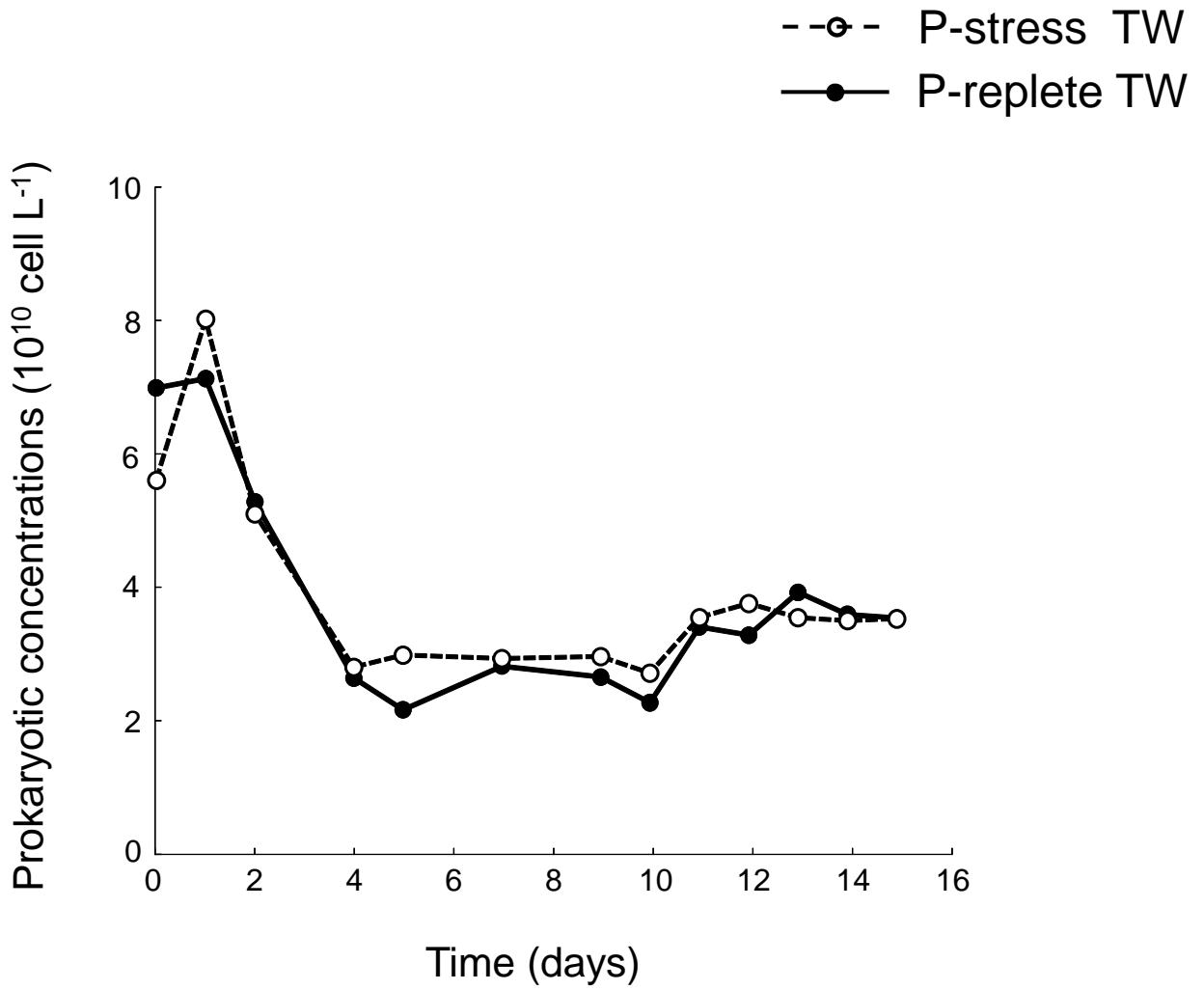


Figure 2

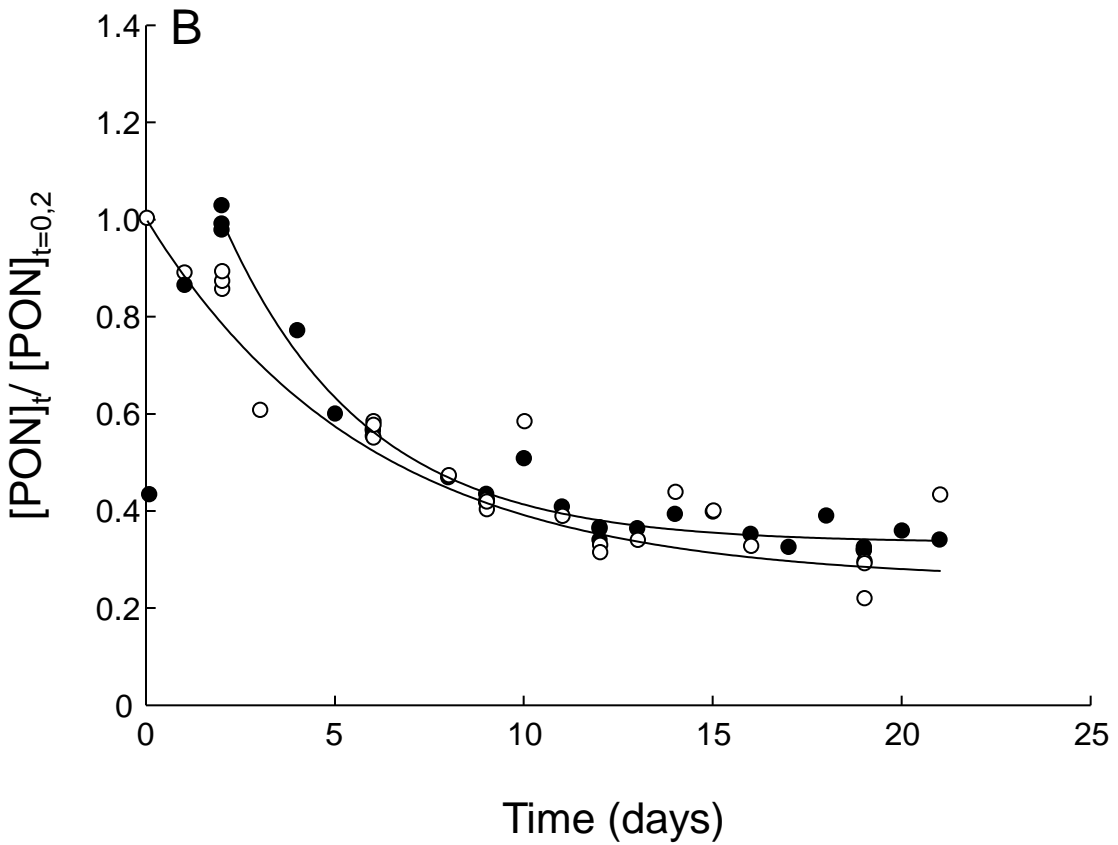
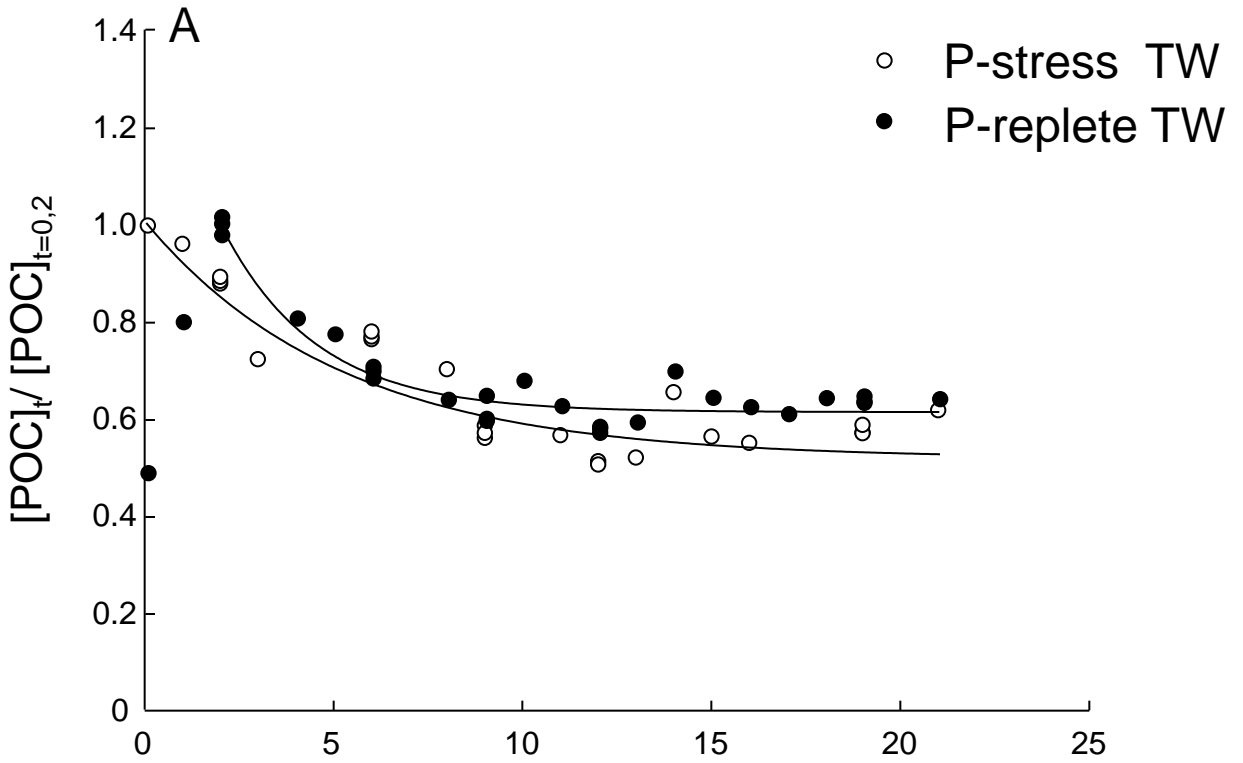
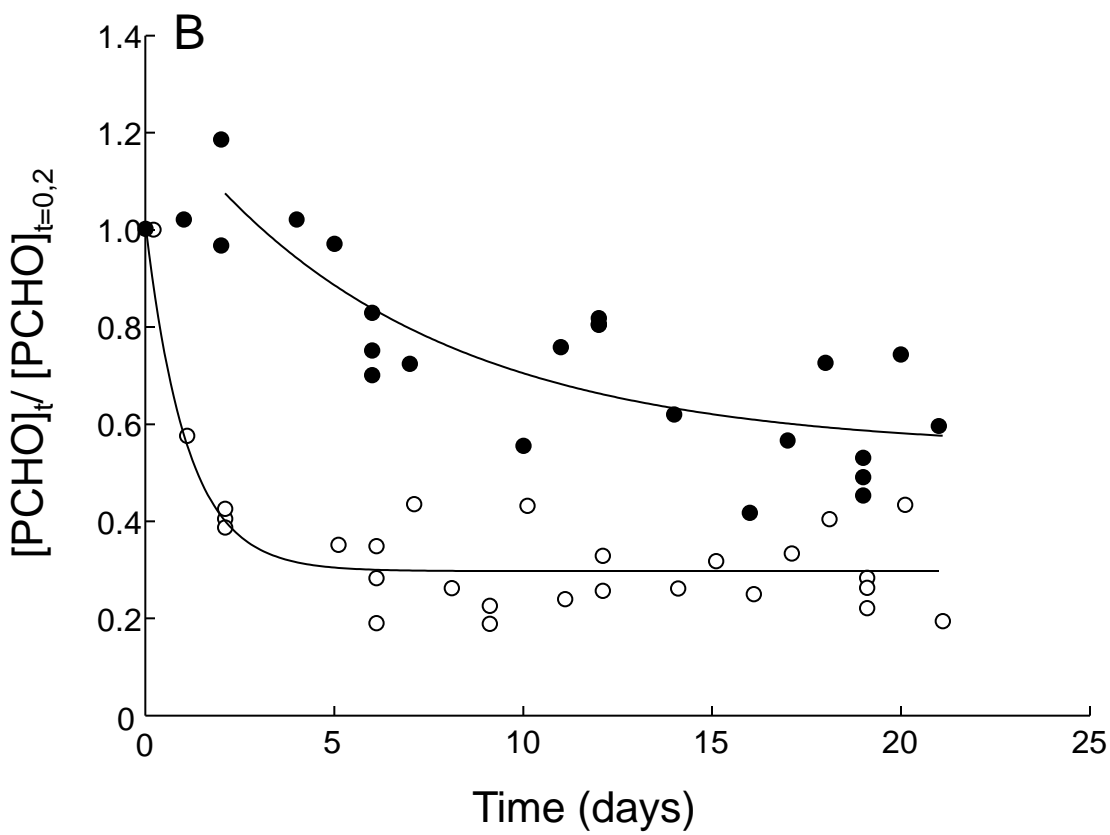
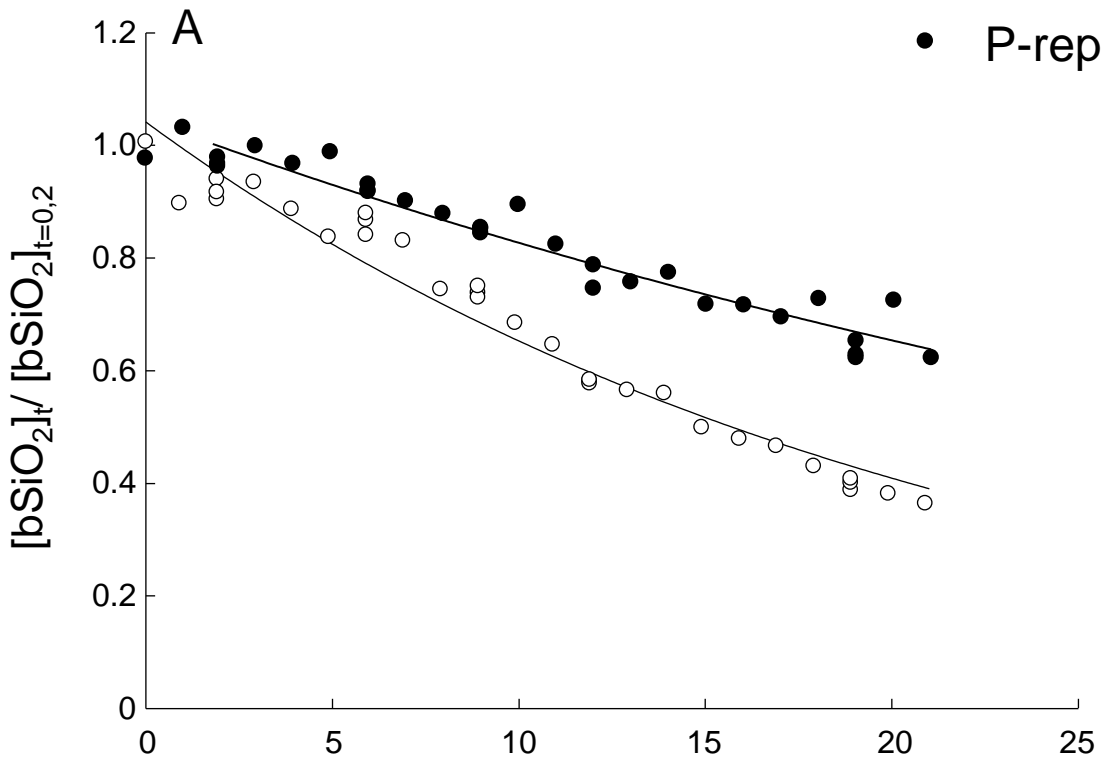


Figure 3

- P-stress TW
- P-replete TW



Time (days)

Figure 4

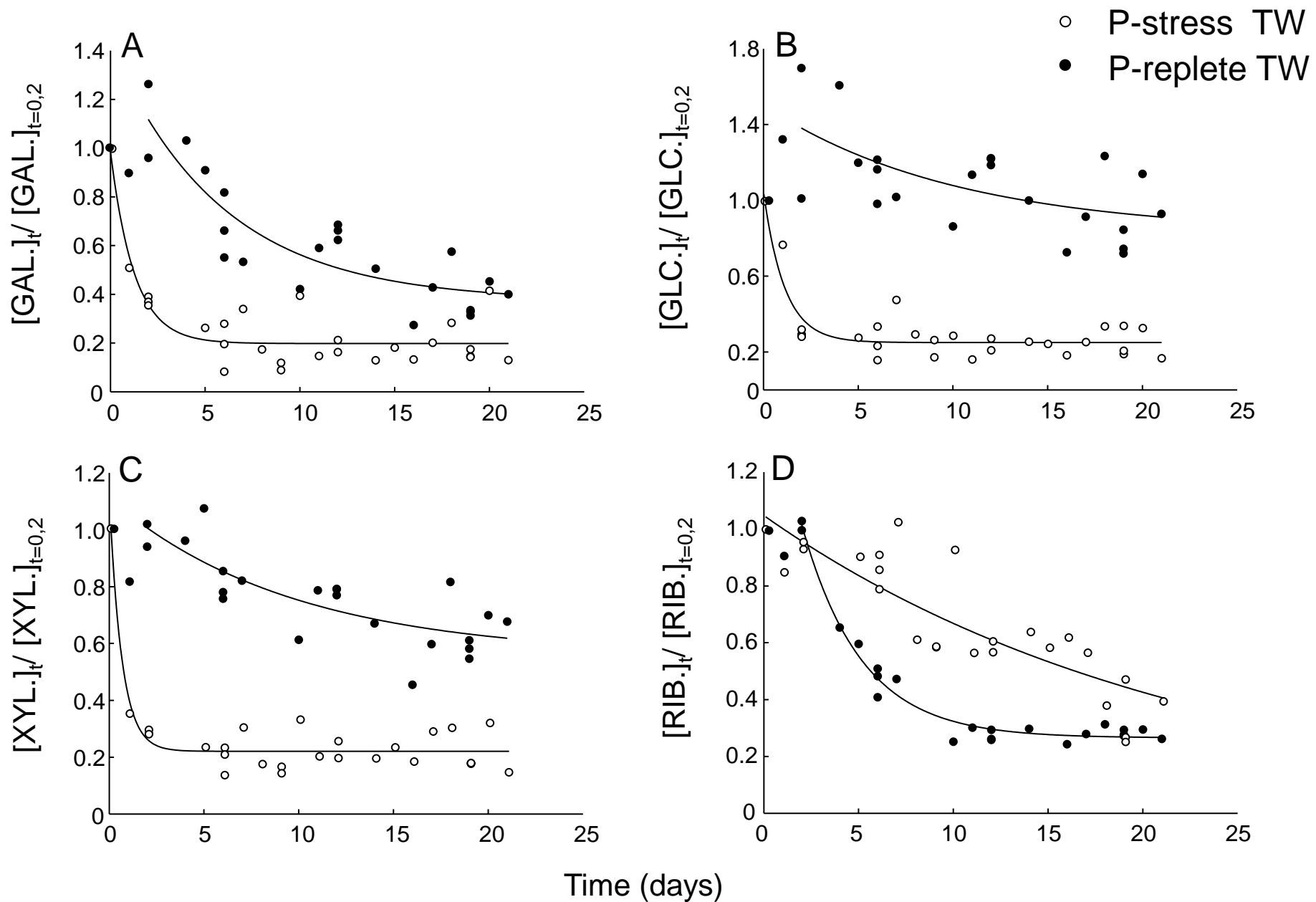


Figure 5

Supporting information for

Phosphorus limitation affects the molecular composition of *Thalassiosira weissflogii* leading to increased biogenic silica dissolution and high degradation rates of cellular carbohydrates

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Figure legends:

Fig. S1: FTIR spectra of the “P-replete *T. weissflogii*” (black line) and “P-stress *T. weissflogii*” cultures (dash line). Cells analyzed by FTIR come from the preliminary culture experiment that allowed us to establish the culture protocol ensuring a physiological response of *T. weissflogii* cells to P-stress. Cultures preparation: *T. weissflogii* were cultured in duplicate in f/2 and f/2 -free PO₄³⁻ media for 1 week at 18 °C (± 1 °C) under a light/dark cycle (16 h: 8 h) and a photon flux density of 180 μmol photons m⁻² s⁻¹. Sample preparation: Aliquots of the *T. weissflogii* cultures were centrifuged, frozen and freeze dried. The powder obtained was mixed with potassium bromide (KBr) and milled to obtain a fine powder. The powder was compressed into a thin pellet before being placed in a desiccator (Schott) under 1 bar pressure for 30 minutes. FTIR analysis: spectra were collected on an FTIR spectrometer equipped with a KBr beam splitter and fitted with a deuterated tri-glycinesulfate (DTGS) detector. The figure show spectra between 2000 and 600 cm⁻¹ but absorbance spectra were collected between 3600 and 600 at a spectral resolution of 4 cm⁻¹ with 10 scans added and averaged. Band assignments: band 1 and 2 are characteristic of amide I (~1650 cm⁻¹) and amide II (~1550 cm⁻¹) functions, respectively, bands 3 to amine, bands 4 are mostly attributed to carbohydrate (Giordano et al. 2001) while the bands noted under 5 in the figure represented the Si–O bounds of the bSiO₂ (Coates, 2000; Giordano et al., 2001).

Fig. S2: FTIR spectra of the frustule in the “P-replete *T. weissflogii*” (red line) and in the “P-stress *T. weissflogii*” batch (blue line) at day 2 (T₂) of degradation. Briefly, samples were collected after 2 days of degradation and frustules were extracted according Suroy et al.

(2014). The sample preparation and FTIR analysis were carried out as previously described in the legend of Fig. S1. Band assignments: band 1 (centered around 2850 cm^{-1}) was attributed to lipids (Coates 2000), the bands noted 2 on the spectrum are characteristic of amide I ($\sim 1650\text{ cm}^{-1}$) and amide II ($\sim 1550\text{ cm}^{-1}$) functions (Giordano et al. 2001, Coates 2000), bands 3 are mostly attributed to carbohydrate (Giordano et al. 2001) while the 3 bands noted under 4 in the figure represented the Si–O bounds of the bSiO_2 (Coates, 2000; Giordano et al., 2001).

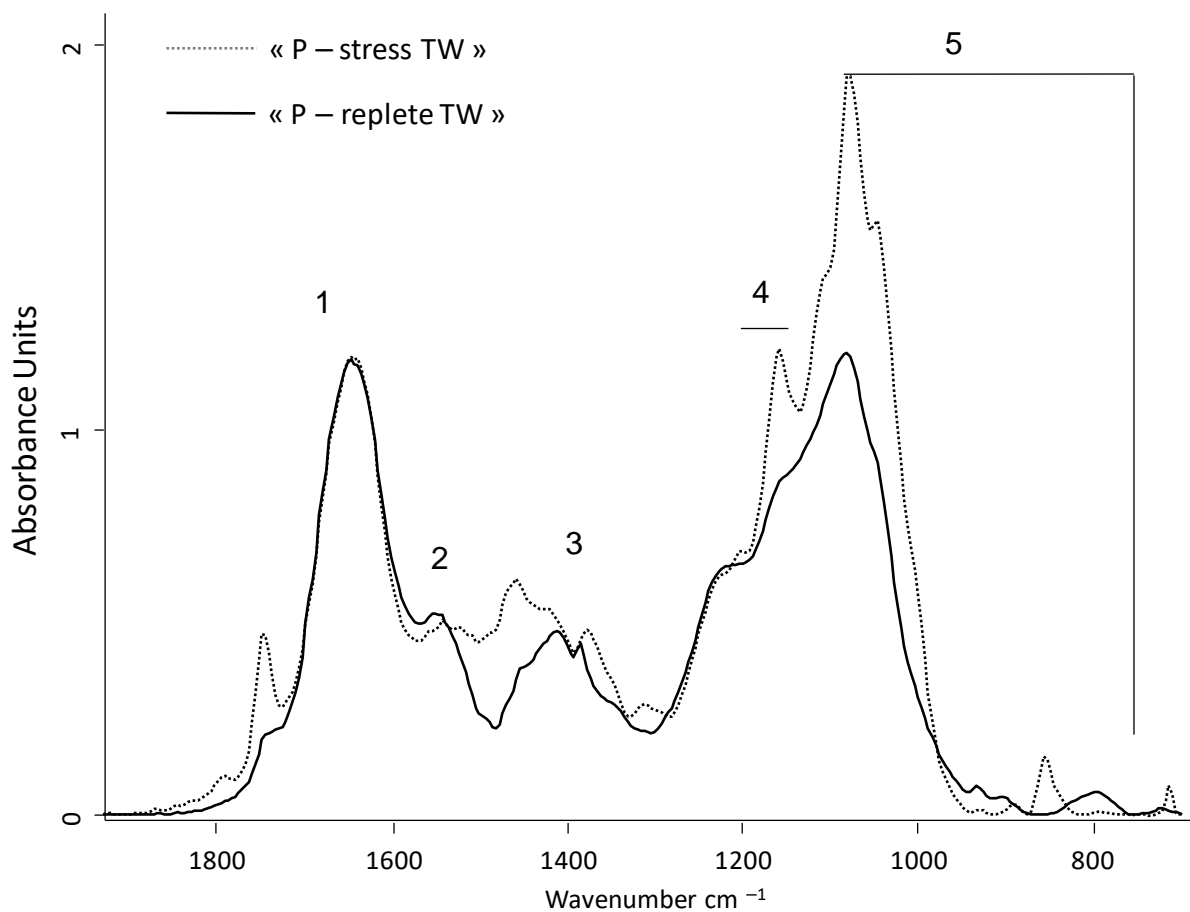


Figure S1

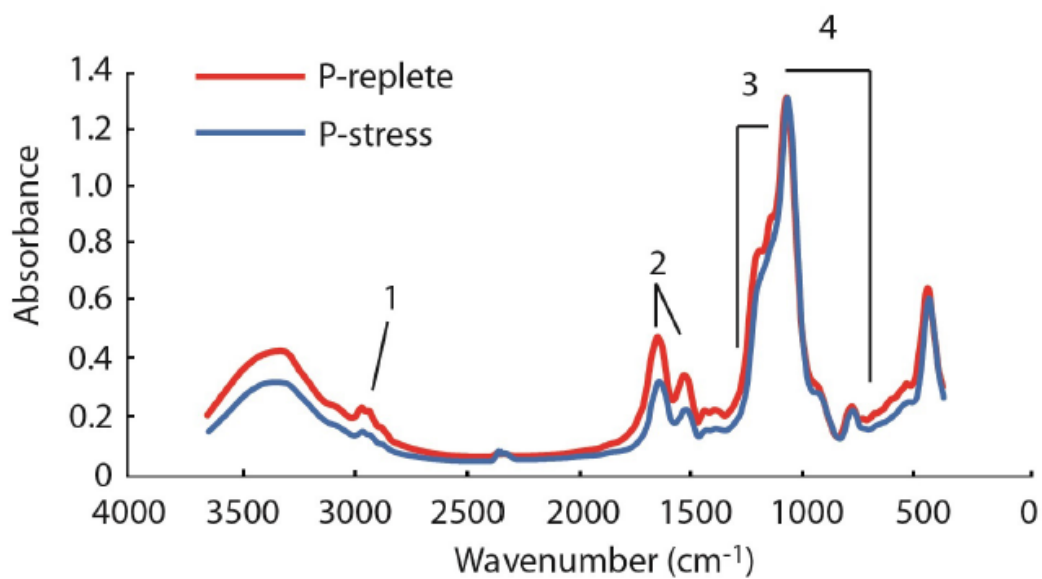


Figure S2

References

- Coates, J. (2000). Interpretation of infrared spectra, a practical approach. *Encycl. Anal. Chem.*
- Giordano, M., Kansiz, M., Heraud, P., Beardall, J., Wood, B., and McNaughton, D. (2001). Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (Bacillariophyceae). *J. Phycol.* 37, 271–279.
- Suroy, M., Boutorh, J., Moriceau, B. & Goutx, M. 2014. Fatty acids associated to frustule of diatoms and their fate during degradation – a case study in *Thalassiosira weissflogii*. *Deep-Sea Res.* I 86: 21-31.