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1 Degradation of diatom carbohydrates: A case study with N- and Si-  
2 stressed *Thalassiosira weissflogii*

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39 **Abstract**

40 Diatoms are a key phytoplanktonic group that affects the carbon cycle in the ocean. Although  
41 the effect of nutrient limitation on the primary productivity of diatoms is well-studied, the  
42 effect of such a limitation on the organic matter quality of diatoms and their vertical transport  
43 through the water column remains unclear. In this study, the diatom *Thalassiosira weissflogii*  
44 (TW) was grown under two different nutrient conditions, 'N-stress' and 'Si-stress', and was  
45 compared against healthy TW cells (Nutrient-replete). Biodegradation experiments of TW  
46 were performed for all of the above conditions, and the particulate fraction was monitored  
47 over time (~ one month) in terms of the organic carbon (POC), nitrogen (PON), and sugars  
48 (PCHO), including prokaryotic counting. Using these results, we estimated the degradation  
49 rate constants for POC, PON, PCHO, and the individual carbohydrate monomers  
50 (monosaccharides) for the TW cells. Our results indicated that the N- and Si-limitations  
51 increase the organic carbon content of the TW cells with a concomitant decrease in the silicon  
52 content ( $\text{bSiO}_2$ ), suggesting a modification of the TW cells. The PCHO content increased by a  
53 factor of 2.6 and 3.8 in the N-stress and Si-stress cells, respectively. At the beginning of the  
54 experiment ( $T_0$ ), the N-stress and Si-stress cells were characterized by higher amounts of  
55 glucose (5-32 mol%) and xylose (13-19 mol%), compared with the Nutrient-replete cells,  
56 which were dominated by ribose (~22 mol%), indicating differences in the physiological  
57 status of the TW cells and/or in the synthesis of storage/structural polysaccharides. The first  
58 order degradation rate constants ( $k_1$ ) for the POC were similar in all of the experiments ( $k_1 =$   
59  $0.096\text{-}0.113\text{ d}^{-1}$ ), which was not the case for the PON in which the highest values (by a factor  
60 of ~2.5) were observed for the nutrient-replete experiment. This result indicates a different  
61 behavior in the utilization and/or accessibility to prokaryotes of carbon and nitrogen during  
62 the biodegradation experiment. Moreover, ribose, glucose, and galactose exhibited the highest  
63 degradation rate constants in all of the experiments, which further reflects the differences in

64 their initial macromolecular origin (*e.g.*, storage vs structural carbohydrates) and highlights  
65 the changes in the organic matter quality during growth under nutrient-limited conditions and  
66 degradation. These results suggest that the ‘nutrient-stress’ diatoms may affect the export of  
67 carbon (particularly carbohydrates) relative to nitrogen in the ocean interior compared with  
68 the diatoms grown under optimal conditions.

69

70 Keywords :*Thalassiosira weissflogii*, biodegradation experiment, carbohydrates, N- and Si-  
71 limitation, degradation rate constants

## 72 **1. Introduction**

73           Diatoms are a key phytoplanktonic group responsible for nearly 25 to 35% of the total  
74 primary production in oceans (Aumont et al., 2003; Nelson et al., 1995; Uitz et al., 2010).  
75 Moreover, diatoms are important contributors to organic carbon export because they are often  
76 associated with the formation of large aggregates made of fresh material or phytodetritus that  
77 rapidly sink to the ocean interior, with sinking velocities averaging  $\sim 100 \text{ m d}^{-1}$  (Goutx et al.,  
78 2007; Martin et al., 2011; Sarthou et al., 2005). Finally, diatoms form most of the  
79 sedimentation fluxes along with coccolitophores (Engel et al. 2009) and large fecal pellets  
80 (Thornton, 2002; Turner, 2002); therefore, they considerably influence the functioning of the  
81 biological carbon pump (Buesseler, 1998; Volk and Hoffert, 1985).

82           The efficiency of the diatoms to export organic carbon to the ocean interior depends  
83 not only on their productivity, but also on the balance between the sinking velocity of the  
84 particles and their degradation rates, both of which are controlled by biotic and abiotic  
85 parameters. These parameters include aggregation/disaggregation rates of the particles (Logan  
86 et al., 1995), particle size/density (De La Rocha and Passow, 2007 and references therein), the  
87 prokaryotic community attached to the particles (Legendre and Rivkin, 2002), temperature  
88 (Bidle et al., 2002; Iversen and Ploug, 2013), pressure (Tamburini et al., 2006), and the  
89 presence of minerals (Engel et al., 2009; Honda and Watanabe, 2010; Moriceau et al., 2009).

90           Nutrient limitations are known to affect the production of diatoms; however,  
91 significantly less is known about their effect on diatom export. Nitrogen and silicon are the  
92 two major nutrients limiting diatom productivity in the open ocean (Moore et al., 2002).  
93 Nitrogen is considered to be the main element controlling primary marine production in the  
94 ocean, whereas silicon is only required for silicified species, such as diatoms (Falkowski,  
95 1997). Nutrient availability acts on the sinking velocities of a diatom by inducing biochemical  
96 composition changes that can modify the organic matter (OM) quality and the cell buoyancy

97 (Lynn et al., 2000; Richardson and Cullen, 1995; Shifrin and Chisholm, 1981). Nitrogen and  
98 silicon limitation generally results in a decrease in the growth rate of diatoms (Claquin et al.,  
99 2002; Martin-Jézéquel et al., 2000), the nutrient uptake (De La Rocha et al., 2010), and a  
100 decrease in the free amino acid and protein content (Martin-Jézéquel et al., 2000; Palmucci et  
101 al., 2011). The modulation of the cell cycle involving these limitations also affects the frustule  
102 silicification (Claquin et al., 2002; Martin-Jézéquel et al., 2000). Generally, frustules of  
103 nitrogen-limited diatoms are thicker than those of silicon-limited diatoms due to a longer cell  
104 cycle, which further indicates a decoupling between the silicon and nitrogen metabolisms  
105 (Claquin et al., 2002; Hildebrand, 2002).

106         Although the effect of nutrient limitation on the biochemical composition of diatoms  
107 cannot be generalized because of their different specific responses (Palmucci et al., 2011;  
108 Shifrin and Chisholm, 1981), generally the decrease in growth rate results in a decrease in the  
109 protein synthesis, which is compensated by an increase in storage compounds (e.g.,  
110 carbohydrates). For example, nitrogen starvation results in an accumulation of carbohydrates  
111 and a decrease in lipids (Shifrin and Chisholm, 1981; Suroy et al., 2014) in TW, an important  
112 diatom species that dominates phytoplankton blooms after a nutrient input in certain oceanic  
113 areas (Smetacek, 1985; Sorhannus et al., 2010). Although a significant number of studies has  
114 focused on the degradation of particles and/or diatoms by following their bulk individual  
115 components (e.g. proteins, carbohydrates, lipids, etc.; Harvey et al., 1995; Panagiotopoulos et  
116 al. 2002), very few data currently exist at the molecular level regarding the above compound  
117 classes (Harvey and Macko, 1997; Goutx et al., 2007) and especially those of carbohydrates.

118         To the best of our knowledge only the dynamics of amino acids have been explored  
119 during the decomposition of calcifying and non-calcifying *Emiliania huxleyi* cultures (Engel  
120 et al. 2009), whereas very little information exists on carbohydrates. Although carbohydrates  
121 are less abundant in diatoms (~ 5-10% of their total dry weight; Brown et al., 1997) compared

122 with amino acids, they are among the most abundant compounds in particulate (5-15%;  
123 Hernes et al., 1996; Panagiotopoulos and Sempéré, 2005a) and dissolved organic matter (2-  
124 10%; Panagiotopoulos and Sempéré, 2005b and references therein).

125 The present study aims to explore the carbohydrate changes at the molecular level of  
126 the diatom cells under variable environmental growth conditions and particularly assess the  
127 effect of nutrient stress during biotic degradation. We used *Thalassiosira weissflogii* (TW) as  
128 a model diatom and we tested the effect of two different nutrient stress conditions (nitrogen  
129 and silicon starvations) on its carbohydrates composition. Degradation of nutrient-stress algae  
130 was monitored over time (30 days), and the degradation rate constants of the individual  
131 monosaccharides were estimated. The results are compared and discussed along with the bulk  
132 degradation features of the particulate organic carbon and nitrogen.

133

## 134 **2. Materials and Methods**

### 135 **2.1 Experimental design**

136 The experimental protocol consisted of the production of TW cells which were grown  
137 in three different conditions namely: ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ followed by  
138 a degradation experiment (Fig.1). As such, the above TW cells were incubated in the darkness  
139 with the presence of a natural prokaryote community.

140 “*Nutrient-replete*” and “*Nutrient-stress*” cultures. TW was grown in Conway  
141 medium (Walne, 1966) in a large volume container culture at the Argenton Ifremer station at  
142 20 °C under a 12 h:12 h light : dark cycle and an irradiance of 241  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A  
143 volume (10 L) of culture at a cell concentration of  $4.0 \pm 0.26 \times 10^8 \text{ cell L}^{-1}$  was centrifuged  
144 and the produced pellet was stored in the freezer (-20 °C) for one week. This material  
145 corresponded to the “Nutrient-replete” cells. An additional volume of 20 L was equally  
146 divided into two aliquots (Table 1). The cells were centrifuged, rinsed with artificial seawater

147 and resuspended in two container cultures filled with f/2 medium,  $\text{NO}_3^-$  and  $\text{H}_4\text{SiO}_4$  free, for  
148 the N-stress and Si-stress experiments, respectively. Because of laboratory logistical  
149 constraints, “Nutrient-replete” and “Nutrient-stress” cells were produced in different growth  
150 media (Conway medium vs f/2 medium). Previous studies showed little difference in the  
151 diatom growth between f/2 and Conway media (Lahanan et al., 2013); therefore we may  
152 confidently consider that the growth of TW in the Conway medium is representative of our  
153 “Nutrient-replete” cells and comparable to the “Nutrient-stress” cultures.

154 The ‘N-stress’ and ‘Si-stress’ diatom container cultures were placed at 18 °C in a  
155 growth chamber under a 12 h:12 h light : dark cycle at an irradiance of 158  $\mu\text{mol photons m}^{-2}$   
156  $\text{s}^{-1}$  for one week. The silicic acid (dSi), pH, nitrates, and phosphates were measured over time  
157 to control the growth conditions of the cells (Boutorh et al *comm. pers.*). For the ‘N-stress’  
158 culture, the nitrate concentrations were below the detection limit immediately after the  
159 beginning of the starvation procedure, indicating an efficient starvation. Similarly, during the  
160 Si-starvation experiment, the dSi concentrations ranged from 0 to 3.4  $\mu\text{M}$ . The low dSi  
161 concentrations were likely due to the dissolution of a certain amount of dead cells, but the  
162 diatom still underwent Si-starvation (Sarhou et al., 2005). Moreover, during the Si-starvation,  
163 the pH concentrations increased over time and reached values  $> 9.3$  after day 6, indicating a  
164 C-limitation. This pattern was not observed in the ‘N-stress’ culture in which the dSi  
165 concentrations decreased over time to reach values  $< 3.0 \mu\text{M}$  at day 6, indicating a late Si-  
166 limitation. Similar to the above procedure, after one week of growth, the diatoms were  
167 concentrated by centrifugation and killed by freezing at -20 °C. This was confirmed by flow  
168 cytometry which showed that about 98% of the TW were killed at -20°C, while the cell  
169 integrity was not disrupted (Soler, 2010).

170 *Degradation experiments.* The TW cells (‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’)  
171 were resuspended in 10 L degradation batches, filled with 0.8  $\mu\text{m}$  filtered natural seawater



172 sampled at a 10 m depth in the Bay of Brest (4° 33' 07.19 W, 48° 21' 32.13 N) in October  
173 2011 (Fig.1). The physical and chemical hydrological characteristics of the area are provided  
174 at the following link: <http://somlit.epoc.u-bordeaux1.fr/fr/>. The seawater was filtered on 0.8-  
175 µm polycarbonate membrane (Millipore) to remove the phyto- and zooplanktonic organisms  
176 while maintaining most of the natural prokaryotic community (Duhamel et al., 2007).  
177 Epifluorescence microscopy analysis of the 0.8 µm filtrate revealed the presence of the  
178 autochthonous prokaryotic community and very few picoeucaryotes cells (results not shown)  
179 which agrees with previous studies (Duhamel et al., 2007).

180         The degradation batches were placed on an orbital shaker table at 16 °C in the dark,  
181 and punched caps were used to ensure gas exchange. The batches were sampled for the  
182 biochemical parameters (POC, PON, and PCHO), including the total count of the  
183 heterotrophic prokaryotes over 30 days. Prior to each sampling, the batches were vigorously  
184 shaken to ensure homogeneity. At the end of the degradation experiment, more than the half  
185 of the initial volume remained (~6 L) in the bottle to reduce the experimental errors related to  
186 the sample amounts. The number of replicates for each parameter (POC, PON, and PCHO) is  
187 indicated in the figure legends.

188

## 189 **2.2 POC and PON measurements**

190         Each day, 10 mL of culture was filtered on pre-combusted GF/F filters (5 h, 450 °C)  
191 for the POC and PON concentration measurements. The filters were dried overnight in an  
192 oven at 50 °C. The POC and PON levels were measured on the same filter using a Carlo Erba  
193 NA 2100 CN analyzer coupled to a Finnigan Delta S mass spectrometer (Nieuwenhuize et al.  
194 1994). The detection limit was 5 and 1 µg for POC and PON, respectively, with a standard  
195 error of 2-3%.

196

## 197 **2.3 Prokaryotic carbon and nitrogen concentration**

198         The prokaryotic cells were counted over time at each day-point of the degradation  
199 experiment to evaluate their growth. Total and free prokaryotes were counted before and after  
200 filtration on a 0.3 $\mu$ m pore size filter, respectively, in 10 mL samples stained with DAPI (4',6-  
201 diamidino-2-phenylindole). The number of attached prokaryotes was calculated by  
202 subtracting the number of free-prokaryotes in the 0.3 $\mu$ m filtrate from the total prokaryote  
203 number. It is important to note that to detach prokaryotes from diatomaceous particles a  
204 sequestering/deflocculating agent is added to the total sample (Velji and Albright, 1986).  
205 Then the sample was centrifuged at low speed to remove large particles without causing  
206 sedimentation of bacteria.

207         The amount of prokaryotic carbon during the degradation experiment was calculated  
208 using the conversion factors (20 and 50 x 10<sup>-15</sup> molC cell<sup>-1</sup> for the free and attached  
209 prokaryotes, respectively) proposed by Turley and Mackie (1994). Similarly, the amount of  
210 prokaryotic nitrogen was calculated using the N/C ratio of 0.28 proposed by Fagerbakke et al.  
211 (1996). Thus, the organic carbon from the diatoms was defined as POC - POC<sub>attached prokaryotic-C</sub>,  
212 and the organic nitrogen from the diatoms was defined as PON - PON<sub>attached prokaryotic-N</sub>.

213

## 214 **2.4 Particulate carbohydrates (PCHO) analysis**

### 215 **2.4.1 PCHO extraction and isolation**

216         Similar to the POC and PON measurements, 10 mL of culture was filtered on GF/F  
217 filters for the PCHO analysis. The filters were cut with clean scissors and transferred to 15  
218 mL glass tubes with Teflon-lined screw caps to which 6 ml of 1.2 M H<sub>2</sub>SO<sub>4</sub> was added. The  
219 samples were bubbled with N<sub>2</sub> and hydrolyzed in a sand bath for 3 h at 100 °C (Cowie and  
220 Hedges, 1984; Panagiotopoulos and Wurl, 2009). The hydrolysis was stopped by placing the  
221 tubes in an ice bath for 5–10 min. The samples were neutralized with pre-combusted CaCO<sub>3</sub>

222 (450 °C for 5 h) and centrifuged 3–4 times at 4000 rpm for 5 min. The supernatant was  
223 filtered through pre-combusted quartz wool (450 °C for 5 h) and pipetted into scintillation  
224 vials. The vials were maintained at 4 °C until the time of analysis (this time never exceeded  
225 24 h).

226

#### 227 **2.4.2 Liquid chromatography**

228 A Dionex ICS-3000 anion exchange chromatograph fitted with a pulsed amperometric  
229 detector (HPAEC-PAD) was used for all PCHO analyses. The separation of the PCHO was  
230 performed on a Dionex CarboPac PA-1 analytical column and a Dionex CarboPac PA-1 guard  
231 column. An amino trap was placed before the guard column to retain the amino acids and  
232 similar compounds, which may interfere with the PCHO analysis. The analytical column, the  
233 guard column, and the amino trap were placed into a thermal compartment set at 17 °C  
234 (Panagiotopoulos et al., 2012). Sugars were detected by an electrochemical detector (ED40-  
235 Dionex) set in the pulsed amperometric mode (standard quadruple potential).

236 Ten individual monosaccharides were detected in the hydrolysates of the particulate  
237 organic material, including deoxysugars (fucose, and rhamnose), pentoses (arabinose, ribose,  
238 and xylose), one amino sugar (glucosamine), hexoses (galactose, glucose, and mannose), and  
239 one acidic sugar (galacturonic acid). The neutral and amino sugars were separated with an  
240 isocratic 19 mM NaOH elution at 17 °C (eluent A: 20 mM NaOH; eluent B: Milli Q water).  
241 Galacturonic acid was detected in a separate analysis using a gradient of two mobile phases  
242 (eluent C: 1 M NaOH; eluent D; 0.5 M CH<sub>3</sub>COONa) according to Panagiotopoulos et al.  
243 (2012). The flow rate was set at 0.7 ml min<sup>-1</sup> for the neutral and acidic sugar analyses. The  
244 data acquisition and processing were performed using the Dionex software Chromeleon<sup>®</sup>. The  
245 analytic errors calculated by the coefficients of variation of the repeated injections of a

246 standard solution of 50 nM/sugar were 5-10% and 0.9-2.0% (n = 6) for the peak area and the  
247 retention time, respectively.

248

## 249 **2.5 Statistical analysis and kinetic parameters estimation**

250 To compare the contributions of the monosaccharides to the diatom composition in the  
251 three treatments at day 1 (triplicates), a *Kruskal-Wallis* test was performed using the R  
252 freeware (R Core Team, 2012). Our null hypothesis was that there is no difference in the  
253 monosaccharide contributions between the batches. We fixed the significance level at 5%. For  
254 each comparison showing a significant difference, a multiple comparison between the  
255 treatments was used to determine the batch that was different (Giraudoux, 2012).

256 The degradation rate constants of the bulk parameters (POC and PON) were calculated  
257 assuming two pools of organic matter (Moriceau et al., 2009). These two pools can be  
258 successively or simultaneously degraded and a 0D model can be applied to estimate the  
259 different degradation rate constants.

260 Statistically, the POC and DON data are best fitted with the model using two  
261 successive exponential decay equations, as follows:

$$262 \quad C(t) = C_0 \exp(-k_1 t), \quad 0 < t < t_s \quad (1)$$

$$263 \quad C(t) = C(t_s) \exp(-k_2(t-t_s)), \quad t > t_s \quad (2)$$

264  $C(t)$  represents the concentration at day  $t$ ,  $C_0$  is the initial concentration,  $t$  is the time in  
265 days,  $k_1$  and  $k_2$  are the two degradation rate constants, and  $t_s$  is the substitution time at which  
266 the degradation rate constant shifts from  $k_1$  to  $k_2$ .

267 Total carbohydrate (PCHO) and individual monosaccharide concentration values were  
268 best fitted into the model using only one exponential decay equation, which is in agreement  
269 with previous studies (Giroldo et al., 2003; Goutx et al., 2007; Moriceau et al., 2009),  
270 according to the following equation :

271  $C(t) = C_0 \exp(-kt)$  (3)

272 The kinetic parameters for the monosaccharide degradation were calculated with a  
273 non-linear least square model using the *nls* function in the R freeware (R Core Team, 2012).  
274 Finally, the *t-test* was used to test the null hypothesis on our measured parameters (POC,  
275 PON, and PCHO).

276

### 277 **3. Results**

#### 278 **3.1 Initial observations**

279 At the beginning of the biodegradation experiment, diatom cell concentrations were  
280  $1.80 \times 10^8$  cell L<sup>-1</sup> in the ‘Nutrient-replete’ batch,  $2.11 \times 10^8$  cell L<sup>-1</sup> in the ‘Si-stress’ batch,  
281 and  $3.02 \times 10^8$  cells L<sup>-1</sup> in the ‘N-stress’ batch (Table 1). The N- and Si-stress growth  
282 conditions induced an increase in the carbon content per cell compared with the ‘Nutrient-  
283 replete’ diatoms (8.14, 14.39, and 17.41 pmol cell<sup>-1</sup> in the ‘Nutrient-replete’, ‘N-stress’, and  
284 ‘Si-stress’ batches, respectively) (Table 1).

285 The ‘Si-stress’ and ‘N-stress’ diatoms had a lower cellular volume than the ‘Nutrient-  
286 replete’ diatoms. The ‘N-stress’ and the ‘Si-stress’ cells contained approximately two times  
287 the amount of organic carbon than the ‘Nutrient-replete’ cells (Table 1), which was even more  
288 pronounced when estimated on a per volume liter basis (12.47, 12.76, and 5.47 pmol cell<sup>-1</sup>,  
289 respectively). The organic nitrogen content did not follow the organic carbon patterns and was  
290 lower in the ‘N-stress’ cells (0.73 pmol cell<sup>-1</sup>) than in the ‘Nutrient-replete’ (1.17 pmol cell<sup>-1</sup>)  
291 and ‘Si-stress’ cells (1.51 pmol cell<sup>-1</sup>) (Table 1). Similar to previous results, these differences  
292 were observed when estimated on a per volume basis.

293 The concentration of the prokaryotic inoculums was of the same order of magnitude in  
294 all of the batches at T<sub>0</sub> indicating the same bacterial number ( $\sim 10^9$  cell L<sup>-1</sup>) at the beginning  
295 of the experiments (Table 1). The prokaryotic concentrations in the three batches showed the

296 same global trend over time, reaching a maximum at day 3 followed by a decrease until  
297 reached a steady state (Fig. 2). It is worth noting that prokaryotic concentrations reached this  
298 steady state ( $\sim 40 \times 10^9$  cell L<sup>-1</sup>) in the ‘Nutrient-replete’ batch much earlier (at day 6) than in  
299 the ‘N-stress’ and ‘Si-stress’ batches (after  $\sim 15$  days) (Fig. 2). This difference may be  
300 explained by the initially higher concentrations of the POC in the N- and Si- stressed cell  
301 batches (Table 1).

302 Our results showed an increase in the POC concentrations (approximately 7-30% of  
303 the initial POC) which was observed between T<sub>0</sub> and T<sub>1</sub> in all three batches (Fig. 2). Then  
304 POC concentrations decreased over time till the end of the experiment. The growth of the  
305 attached prokaryotes at day 1 (Fig. 2) explained only 7.5%, 0.9% and 1.2% of the increase of  
306 the POC at day 1 in the ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ experiments,  
307 respectively. This result further indicates that the increase in POC at day 1 is most likely  
308 related to a combination of biotic and abiotic processes at the beginning of the degradation  
309 experiments (e.g. agglomeration of POC due to heterogeneity, prokaryotes growth, a low TEP  
310 production by bacteria) including analytical error (Fig. 3). The increase in the POC at day 1  
311 may also be related to the absorption/desorption phenomena of the suspended particles on the  
312 bottle wall during biodegradation. These observations (increase of POC at day 1) have been  
313 previously reported in particle or diatom biodegradation experiments (Panagiotopoulos et al.,  
314 2002; Engel et al., 2009).

315 After day 1, POC decreased over time while we observed an increase in the bacterial  
316 numbers, which typically reflects the decomposition pattern of TW-POC. For all of these  
317 reasons mentioned above, we have chosen to consider the data at T<sub>1</sub> as the beginning of the  
318 degradation.

319 Similar to the POC levels, the PON concentrations also showed an increase at day 1 in  
320 all of the batches (+ 70.1, + 58.4 and + 55.6% of the initial PON concentrations in the

321 'Nutrient-replete', 'N-stress' and 'Si-stress' batches, respectively). Using conversion factors  
322 to estimate the organic nitrogen contribution from the prokaryotes (see the Materials and  
323 Methods section), the prokaryotic nitrogen accounted for 13, 10 and 8% of the total PON at  
324 day 1 in the 'Nutrient-replete', 'N-stress' and 'Si-stress' batches, respectively.

325 The C/N ratio was equal to 7.0 at the beginning of the degradation experiment in the  
326 'Nutrient-replete' batch, which is in the range of the previously reported values for TW (C/N  
327 = 5-9; Brzezinski, 1985; De La Rocha et al., 2010). This ratio was strongly influenced by the  
328 nutrient stress, with an increase of up to 11.5 in the 'Si-stress' cells and 19.7 in the 'N-stress'  
329 cells.

330

### 331 **3.2 Initial particulate carbohydrate composition (PCHO)**

332 Similar to the POC, the initial concentrations of the PCHO-C in the 'N-stress' and 'Si-  
333 stress' cells on a per volume basis were approximately two and four times higher than those in  
334 the 'Nutrient-replete' cells (0.22, 0.40, and 0.11 pmol cell<sup>-1</sup>, respectively). Note that the  
335 PCHO analyses do not discriminate between the PCHO arising from diatoms and those from  
336 prokaryotes.

337 Similar to the POC levels, the PCHO-C concentrations also peaked at day 1 and  
338 increased by 3.7%, 2.4%, and 1.1% of their initial concentrations in the 'Nutrient-replete', 'N-  
339 stress' and 'Si-stress' batches, respectively. Therefore, as observed with the POC degradation,  
340 the PCHO-C degradation rate constants were calculated from day 1. The monosaccharide  
341 composition at day 1 was dominated by ribose, galactose, and glucose in all of the batches,  
342 which accounted for more than 60 mol% of the total PCHO pool (Table 2).

343 The 'Nutrient-replete' batch was characterized by a higher ribose contribution (22  
344 mol%) compared with the 'N-stress' and 'Si-stress' batches (4 and 11 mol%, respectively) at  
345 day 0, whereas the contribution of galactose varied only slightly between the three batches (29

346 to 38 mol%; Table 2). The contribution of glucose was significantly higher in the ‘N-stress’  
347 batch compared with the ‘Nutrient-replete’ batch (Kruskal-Wallis test,  $n = 9$ ,  $p < 0.05$ ). The  
348 ribose concentrations and yields (Rib.-C/PCHO-C %) rapidly decreased over time in all of the  
349 experiments, indicating a selective extraction of this monosaccharide from the PCHO pool  
350 (Table 2). Interestingly, the ‘Nutrient-replete’ batch was particularly enriched in galacturonic  
351 acid (~8 mol%) compared with the ‘N-stress’, and ‘Si-stress’ batches ( $< 2$  mol%; Table 2).  
352 Finally, none of the other monosaccharides showed significant initial differences (at day 1)  
353 among the ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ batches.

354

### 355 **3.3 Organic matter degradation**

#### 356 **3.3.1 Bulk parameters (POC and PON)**

357 As indicated above, an increase in the POC concentrations (approximately 7-30% of  
358 the initial POC) was observed at day 1 in the three batches. Therefore, the biotic degradation  
359 kinetics were calculated beginning at day 1. The POC degradation followed similar patterns in  
360 the three batches, with a first period of degradation in which the POC degraded faster  
361 followed by a second period of degradation in which the POC was degraded more slowly  
362 (Fig. 3a). The degradation rate constants of the first period were similar in the three batches  
363 ( $0.11 \pm 0.03 \text{ d}^{-1}$ ,  $0.096 \pm 0.002 \text{ d}^{-1}$ , and  $0.11 \pm 0.01 \text{ d}^{-1}$ ; Table 3); however, this was not the  
364 case for the substitution time, which was different among the three batches ( $t_s = 5.9 \pm 0.6 \text{ d}$ ,  
365  $11.1 \pm 0.2 \text{ d}$  and  $8.5 \pm 0.2 \text{ d}$ ).

366 At the end of the rapid degradation period, ~40% of the POC from the ‘Nutrient-  
367 replete’ diatoms was degraded, whereas this amount was ~60% for the N- and Si- stressed  
368 TW during the same period. During the second period, the POC was degraded at a  
369 significantly slower rate, with degradation rate constants of  $0.040 \pm 0.001 \text{ d}^{-1}$ ,  $0.018 \pm 0.001$   
370  $\text{d}^{-1}$ , and  $0.014 \pm 0.001 \text{ d}^{-1}$  for the ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ batches,



371 respectively. Interestingly, the POC concentrations reached a “plateau” after 25 days of  
372 degradation and accounted for 27% of the initial POC in all three of the batches (Fig. 3a).

373 The degradation rate constants of the PON were consistently higher in the ‘Nutrient-  
374 replete’ batch for both of the pools ( $k_1$  and  $k_2$ ) than in the ‘N- and Si-stress’ batches (Table 3;  
375 Fig. 3b). The degradation rate constants of the PON measured in the ‘N- and Si-stress’  
376 batches only differed for the  $k_1$  constant rates (Table 3). The second pool of the PON  
377 degraded with a similar degradation rate constant in the ‘N- and Si-stress’ batches ( $k_2 \approx 0.020$   
378  $\pm 0.001$  d<sup>-1</sup>). The  $t_s$  value was one half the value in the ‘Nutrient-replete’ batch ( $5.0 \pm 0.6$  d)  
379 compared with the ‘N- and Si-stress’ batches ( $\sim 10.0 \pm 0.6$  d). Therefore, at the end of the  
380 biodegradation experiment (30 days), only 18% of the initial PON remained in the ‘Nutrient-  
381 replete’ batch, whereas 40% and 55% remained in the ‘Si-stress’ and the ‘N-stress’ batches,  
382 respectively.

383

### 384 3.3.2 Particulate carbohydrates (PCHO)

385 The degradation rate constants for the total PCHO-C were slightly lower but not  
386 significantly different in the ‘N-stress’ and ‘Si-stress’ batches ( $0.04 \pm 0.01$  d<sup>-1</sup>) than in the  
387 ‘Nutrient-replete’ batch ( $0.05 \pm 0.01$  d<sup>-1</sup>; Table 3; Fig. 4). At the end of the 30 days of  
388 degradation, 26% of the total PCHO-C remained in the ‘Nutrient-replete’ batch, whereas  
389 PCHO-C represented  $\sim 35\%$  in the ‘N-stress’ and ‘Si-stress’ batches.

390 The degradation rate constants of the individual monosaccharides showed important  
391 differences in their decay (Table 3). Ribose was the monosaccharide with the highest  
392 degradation rate constants ( $k = 0.073$ - $0.215$  d<sup>-1</sup>) followed by glucose ( $k = 0.028$ - $0.071$  d<sup>-1</sup>),  
393 and galactose ( $k = 0.018$ - $0.060$  d<sup>-1</sup>). A slight, non-significant decrease in the galactose  
394 concentrations was observed in the ‘N-stress’ batch, with a degradation rate constant three  
395 times lower than that of the ‘Nutrient-replete’ and ‘Si-stress’ batches ( $k = 0.02 \pm 0.01$  d<sup>-1</sup>)

396 versus  $0.05 \pm 0.01 \text{ d}^{-1}$  and  $0.06 \pm 0.02 \text{ d}^{-1}$ , respectively, Fig. 5a). Similarly, the ribose  
397 degradation rate constants in the ‘Nutrient-replete’ and ‘N-stress’ batches were very different  
398 and three-times lower in the latter ( $k = 0.21 \pm 0.05 \text{ d}^{-1}$  and  $0.073 \pm 0.007 \text{ d}^{-1}$  in the ‘Nutrient-  
399 replete’ and ‘N-stress’ batches, respectively, Fig. 5b). The high variability of the ribose  
400 concentrations in the ‘Si-stress’ batch did not allow an accurate estimation of the kinetic  
401 parameters ( $k = 0.07 \pm 0.04$ ,  $p > 0.1$ ). This result was also the case for glucose, which showed  
402 a non-significant degradation rate constant very close to that measured in the ‘Nutrient-  
403 replete’ batch (Table 3; Fig. 5c). In the ‘N-stress’ batch, the glucose degradation rate constant  
404 was two times higher than that of the ‘Nutrient-replete’ batch ( $k = 0.07 \pm 0.04 \text{ d}^{-1}$ ) indicating  
405 the presence of a labile pool of glucose-containing molecules.

406 The degradation rate constants of the other monosaccharides were variable, ranging  
407 from  $<0$  (arabinose) to  $0.027 \text{ d}^{-1}$  (rhamnose). As a general trend, the degradation rate  
408 constants of these individual monosaccharides were higher in the ‘Nutrient-replete’ batch  
409 compared with the ‘N and Si- stress’ batches.

## 410 **4. Discussion**

### 411 **4.1 Effect of N- and Si-starvation on the initial *Thalassiosira weissflogii* composition**

412 The effect of nutrient stress, including starvations, on the biochemical content (sugars,  
413 amino acids, and lipids) of diatoms has been addressed in previous studies for several diatom  
414 species (De La Rocha et al., 2010; Hou et al., 2007; Liu et al., 2012). These studies have  
415 shown dissimilarities in the physiological responses of the diatom, indicating a specific  
416 response against nutrient stress (Palmucci et al., 2011; Richardson and Cullen, 1995; Shifrin  
417 and Chisholm, 1981; Waite et al., 1992) and are in agreement with the present study. Our  
418 results showed that nitrogen and silicon starvations induced an increase in the carbon content  
419 per cell in TW (Table 1). Other studies have reported a decrease in the N content per cell

420 during N starvation, whereas no changes have been observed during Si starvation (De la  
421 Rocha et al. 2010). Our results showed that the N content per cell also decreased during N-  
422 starvation and slightly increased during Si- starvation (Table 1) indicating that N- and/or Si-  
423 starvations have different effects on the carbon and nitrogen content of TW.

424         The carbohydrate (PCHO) concentrations in the ‘Nutrient-replete’ cells were 2-3 times  
425 lower than those found in the ‘N-stress’ and ‘Si-stress’ cells (Table 2), suggesting an increase  
426 in the PCHO levels during starvations, which is in agreement with previous investigations  
427 performed in other diatoms species (*T. pseudonana*; Harrison et al., 1990). Other studies  
428 performed on TW employing similar nutrient starvation conditions have reported a decrease  
429 in the amino acid and lipid content (Klein Breteler et al., 2005; Martin-Jézéquel, 1992; Shifrin  
430 and Chisholm, 1981). These results suggest that nutrient starvation has different effects on the  
431 organic matter components of the diatoms (carbohydrates, lipids, and amino acids).  
432 Carbohydrates in diatoms are generally divided into cellular and extracellular polysaccharides  
433 (Myklestad, 1995). In diatoms, it is well known that the genus *Thalassiosira* releases low  
434 levels of extracellular polysaccharides, primarily composed of glucose (Urbani et al., 2005),  
435 compared with another genus, such as *Chaetoceros* (Myklestad, 1995). The amount of  
436 extracellular polysaccharides released under a nutrient stress accounts for approximately 3%  
437 of the total cellular carbohydrate in TW (Myklestad, 1995). Carbohydrates are molecules that  
438 are primarily composed of carbon atoms, and their release as extracellular polysaccharides  
439 (EPS) during stress conditions may decrease the C/N ratio of the diatom cells to their ‘initial’  
440 value (C/N value of = 5-7).

441         The changes in the carbohydrate concentration during stress conditions were also  
442 reflected in their individual sugar monomers, which have never been thoroughly examined in  
443 previous studies (Table 2). The relative abundance of most of the monosaccharides increased  
444 during Si-starvation (except for ribose and galacturonic acid), whereas the primary differences

445 for the N-starvation experiment were observed in the relative abundances of glucose, ribose,  
446 and xylose. N-starvation strongly affected the glucose content of the cells (Table 2).  
447 Therefore, the high increase in the glucose relative abundance may suggest a preferential  
448 synthesis of glucose-rich polymers such as laminarin which are well known to be storage  
449 molecules in diatoms (Størseth et al., 2005). The high relative abundance of ribose in the  
450 'Nutrient-replete' cells most likely reflects the better physiological status of these cells  
451 because ribose-containing molecules (e.g., ATP and RNA) are involved in energy  
452 metabolism. Other studies have shown a decrease in the RNA synthesis in the 'N-stress'  
453 diatoms, implying a decrease in the ribose content of the cells, which is in agreement with our  
454 results (Hockin et al., 2012; Mock et al., 2008). High amounts of ribose in marine particulate  
455 organic matter is generally attributed to fresh material (Panagiotopoulos and Sempéré, 2005a,  
456 2007), but it can also imply a good physiological status of the 'diatom' cells in the  
457 investigated sampling area.

458 The above results clearly suggest that Si-starvation may have a more pronounced  
459 effect on the cell's physiological status than on its metabolism functions. This result may  
460 indicate that silica-stress cells have lower energy requirements (no synthesis of glucose  
461 polymers) than nitrogen-stress cells. This result is in agreement with previous results on *T.*  
462 *pseudonana*, suggesting an uncoupling of Si with C metabolism (Claquin et al., 2002).

463

#### 464 **4.2 Effect of N- and Si- starvation on *Thalassiosira weissflogii* organic matter** 465 **degradation**

466 As indicated above, the kinetic calculations of the POC and PON degradation  
467 suggested the presence of two pools of organic matter with different degradation rate  
468 constants (Table 3). These results suggest that both of these pools are composed of relatively  
469 biodegradable molecules, and certain of these molecules are most likely produced during

470 starvation (extracellular sugars; Table 3 and Fig. 3a). Our results also showed that although  
471 the first order degradation rate constants ( $k_1$ ) of the ‘nutrient-replete’, ‘N-stress’, and ‘Si-  
472 stress’ for the TW-POC were nearly similar during degradation, they corresponded to  
473 different substitution  $t_s$  values. The higher  $t_s$  values were observed during the nutrient stressed  
474 TW- POC degradation ( $t_s$  values of 8.5-11.4 days) compared with the “Nutrient replete” cells  
475 ( $t_s$  value of 5.9 days; Table 3). These results clearly indicate that the TW-intracellular  
476 molecules that constitute the labile TW-POC (first order rate constants) are degraded over a  
477 longer period in which the TW cells are submitted to “stress” conditions. This result may be  
478 partially explained by the increase in the carbon content of the TW cells in which labile  
479 compounds, such as carbohydrates, are produced during stress-conditions; therefore,  
480 additional time for their degradation is required (Table 2). The degradation rate constants of  
481 the second pool ( $k_2$ ) during the TW-POC degradation were nearly similar for the “stress-cells”  
482 but significantly lower than those of the ‘Nutrient-replete’ cells ( $k_{Si-stress} \sim k_{N-stress} \ll k_{nut.-rep.}$ ;  
483 Table 3). These results indicate that the stress-conditions may increase the residence time ( $k^{-1}$ )  
484 of less labile organic molecules, which may affect their bioavailability in the environment.

485 In contrast to the POC, the first order degradation rate constants ( $k_1$ ) of PON were  
486 clearly different between each batch (Table 3, Fig. 3b). Our results indicated that TW-PON  
487 appeared to become less labile after the N- and Si- starvations. These differences were  
488 reflected in the amount of diatom PON remaining at the end of each experiment (17.4%,  
489 34.7% and 51.1% of the initial diatom PON in the ‘Nutrient-replete’, ‘Si-stress’, and ‘N-  
490 stress’ batches, respectively). These results may suggest a N-utilization from the TW  
491 intracellular N-compounds (proteins, nucleic acids, and/or ATP), which are well known to be  
492 very labile (Hockin et al., 2012). This result concurs with the low initial concentrations of  
493 ribose recorded in the “N- and Si-stress” TW-cells (Table 3), indicating that the ATP

494 molecules (ribose and N-nucleobases-rich) are most likely the first to be exhausted at the  
495 beginning of the N-starvation.

496 Although the POC/PON ratios were high at the beginning of the degradation for the  
497 ‘N- and Si-stress’ cells, they reached values close to the Redfield C/N ratio after ~ 5-11 days  
498 of degradation (Fig. 6; Sarthou et al., 2005). This result suggests a preferential prokaryotic  
499 degradation of excess C- containing compounds and may explain the high degradation rate  
500 constant of glucose (an N-free compound) obtained in the ‘N-stress’-experiment (most likely  
501 storage compounds) (Table 3; Fig. 5c). Interestingly, similar C/N ratios (6.1-6.7) have been  
502 obtained for sediment trap material collected from the Pacific Ocean (Hernes et al. 1996). The  
503 authors have interpreted these low values as a non-selective degradation of OC versus ON.  
504 Alternatively, this result may be due to a preferential selectivity that coincidentally maintains  
505 the low C/N values. Our results agree with these features, indicating a non-coincident  
506 selective degradation of excess C- and N-containing molecules.

507 The degradation rate constants of the PCHO were consistently lower than the first  
508 order degradation rate constants of the POC ( $k_1 = 0.096-0.11 \text{ d}^{-1}$ ; Table 3) but higher than the  
509 second order degradation rate constants of the POC ( $k_2 = 0.018-0.040 \text{ d}^{-1}$ ), indicating that  
510 carbohydrates are most likely the second most labile component in TW after amino acids. The  
511 PCHO degradation rate constants were higher than the individual monosaccharides, except for  
512 ribose, which exhibited the highest rates in all of the experiments (Table 3). Therefore, it  
513 appears that the PCHO rate constants reflect an average of the different degradation rates  
514 corresponding to each monosaccharide, indicating differences in their initial macromolecular  
515 origin (e.g. storage vs structural carbohydrates).

516 For example, the galactose degradation rate constants were higher in the ‘Nutrient-  
517 replete’ and ‘Si-stress’ cell degradation experiments than in the ‘N-stress’ experiment (Table  
518 3; Fig. 5a). This monosaccharide is primarily found in cell wall polysaccharides (Cowie and

519 Hedges, 1996; Hecky et al., 1973), whereas it is also reported in excreted exopolymeric  
520 substances (Giroldo et al., 2003). Based on the ectoenzymatic bacterial activities in sediments,  
521 Arnosti (2000) has suggested that the former could be less labile than the latter. Based on the  
522 above literature observations and the higher degradation rate constants observed for the  
523 ‘Nutrient-replete’ and ‘Si-stress’ experiments (Table 3), we may hypothesize that galactose  
524 would primarily originate from the excretion of the EPS (in the case of the ‘Nutrient-replete’  
525 and ‘Si-stress’ experiments), which are known to be stimulated during nutrient deficiency.  
526 Alternatively, galactose may also originate from the cell wall polysaccharides in the ‘N-stress’  
527 cell experiment based on its low degradation constant.

528         The ribose degradation rate constants showed important differences among the  
529 starvation experiments. The high degradation rate constant recorded for the ‘Nutrient-replete’  
530 experiment reflects the good physiological status of the TW cells and agrees with the elevated  
531 ribose concentrations measured at  $T_0$  (Table 3, Fig. 5b). This high rate most likely illustrates  
532 the hydrolysis of ATP polymers (rich in ribose), whereas the low rates measured in N- and Si-  
533 stress cells most likely indicate the exhaustion of the ATP molecules at the beginning of the  
534 N-starvation (see above). This result is further supported by the similarity of the first order  
535 decay constants of the PON and those of ribose for all of the biodegradation experiments  
536 (Table 3).

537         Similarly, the degradation rate constants measured for glucose may provide  
538 indications about the nature of the original polymer. Glucose may originate from laminarin  
539 (Størseth et al., 2005) or callose (Tesson and Hildebrand, 2013), which are sugar-polymers  
540 involved in energy storage (laminarin) or fibrillar structure (callose). The high degradation rate  
541 constant (indicating increased bioavailability) obtained for the glucose in the ‘N-stress’ TW  
542 most likely suggests that glucose may originate from laminarin (Table 3; Fig. 5c).

543 Our results also showed that the concentrations of some of the monosaccharides  
544 showed a small increase after day 1. This was especially the case for arabinose, galacturonic  
545 acid, and mannose (Table 2). This result may be related to the increase of the prokaryotic  
546 concentration which peaked at day 3 (Fig. 2), most likely suggesting that these  
547 monosaccharides may be *de novo* produced by prokaryotes. However, the prokaryotic carbon  
548 represented a small percentage of this increase (see above) and cannot solely explain the  
549 increase in the concentrations of these sugars.

550 The increase in the mannose and arabinose concentrations in ‘Si-stress’ batch may be  
551 related to other factors, such as a release of polysaccharides from the frustule of TW, which is  
552 dissolved slowly over time. Earlier studies on four different diatom species indicated that the  
553 polysaccharides released after the dissolution of the frustules are primarily composed of a  
554 mannose backbone bearing uronic acid residues (Chiovitti et al., 2005). In our study, the  
555 degradation rate constant of galacturonic acid in the ‘Si-stress’ batch was completely different  
556 from that of the two other batches (Table 3). This result may be explained by a modified  
557 frustule structure affected by the Si starvation (Moriceau et al., 2007; Soler, 2010). This  
558 finding strongly agrees with the high correlation between the mannose and galacturonic acid  
559 concentrations over time in the ‘Nutrient-replete’ batch ( $r = 0.88$ ).

560

#### 561 **4.3 Biogeochemical implications on the vertical transport of the nutrient-stressed TW cells**

562 In a marine environment, diatoms are often subjected to nutrient depletion depending  
563 on the environmental conditions. Although it has been suggested that diatoms may regulate  
564 their buoyancy to sink into a deeper nutrient-rich layer of the euphotic zone, the effect of the  
565 nutrient stress on their morphology is not well known (Kemp et al., 2006 and references  
566 therein). The *Thalassiosira* species, in particular, have been suggested to be very nutrient-  
567 sensitive, which significantly affects their sinking rate (Waite et al., 1992). Other studies have



568 demonstrated that nutrient-stressed TW cells increase their carbohydrate content, resulting in  
569 an increase in their density and their aggregation; therefore, they sink rapidly in the water  
570 column (Richardson and Cullen, 1995). Our results also showed an increase in the  
571 carbohydrate content of the TW cells under N- and Si-depleted conditions, which may  
572 potentially impact the behavior of TW cells (changes in sinking rates, aggregation etc) in the  
573 natural environment. Furthermore, the degradation rate constants ( $k_1$ , and  $k_2$ ) of the nutrient  
574 limited cells generally decreased compared with the nutrient-replete cells (Table 3), which  
575 further may imply a potentially higher amount of carbon for export under nutrient-stress  
576 conditions.

577         On the other hand, other studies indicated that the decrease in the cell size due to the  
578 nutrient-limitation may attenuate the sinking rates because of the dissolution of silicon, which  
579 forms the cell frustule, resulting in lighter TW cells (De la Rocha and Passow, 2007; Boutorh,  
580 2014). Our experiments also showed a decrease in the  $\text{bSiO}_2$  content in the stressed TW cells  
581 (Table 1). Overall, these results highlight the complexity of evaluating the relationship  
582 between the environmental factors (nutrient-limitation) and the sinking velocity of diatom  
583 frustules. These factors clearly influence the sequestration of organic carbon in the deep ocean  
584 at the spatial (differences in species composition) and temporal (differences in physiological  
585 status) scales and clearly have an important effect on the microbial carbon pump.

586

## 587 **Summary and concluding remarks**

588         This study explored the degradation features of the TW cells grown under nutrient-  
589 stress conditions (N- and Si- limitations), similar to those occurring in a natural environment.  
590 Our results showed that the TW stressed-cells increased their carbon content (approximately  
591 two times) and their C/N ratios (2-3 times) compared with the ‘Nutrient-replete’ cells (Table  
592 1; Fig. 6). This carbon increase was accompanied by a decrease in the  $\text{bSiO}_2$  in the nutrient-

593 stressed cells, indicating a structural modification of the TW frustule. From a chemical  
594 composition view point, this increase in the carbon was essentially reflected in the  
595 carbohydrate component of the TW cells. Hence, the carbohydrate (PCHO) concentrations in  
596 the 'Nutrient-replete' cells were 2-3 times lower than those found in the 'N-stress' and 'Si-  
597 stress' cells (Table 2) suggesting an increase in the PCHO content during starvation. The 'N-  
598 stress' and 'Si-stress' cells were characterized by higher amounts of glucose (5-32 mol%) and  
599 xylose (13-19 mol%) compared with the 'Nutrient-replete' cells, which were dominated by  
600 ribose (22 mol%), indicating differences in the physiological status of the TW cells and/or  
601 the synthesis of storage/structural polysaccharides under different nutrient growth conditions.

602         The biodegradation experiments of the 'Nutrient-replete', 'N-stress', and 'Si-stress'  
603 TW cells showed that the first order degradation rate constants for the POC were similar ( $k_1$ =  
604 0.096-0.113 d<sup>-1</sup>) but were characterized by different substitution times, indicating that  
605 intracellular and labile TW compounds require longer times to be degraded under stress  
606 conditions (Table 3). The second order degradation rate constants ( $k_2$ ) of the POC were  
607 approximately two times higher for the 'Nutrient-replete' cells than the 'N-stress' and 'Si-  
608 stress' cells, indicating that nutrient limited conditions may increase the residence time ( $k^{-1}$ ) of  
609 less labile intracellular TW compounds. The PCHO degradation rates were between the first  
610 and the second order degradation rates of the POC, suggesting that the carbohydrates are  
611 most likely the second most labile component of TW after the amino acids.

612         Ribose, glucose, and galactose exhibited the highest degradation rate constants in the  
613 various experiments which further reflects the differences in their initial macromolecular  
614 origin due to different C and N allocation (e.g. storage vs structural carbohydrates) under  
615 nutrient-limited conditions and highlights the changes in the organic matter quality during  
616 degradation.

617

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849 *Figure Legends:*

850 **Figure 1.** Schematic presentation of the experimental design and sampling of TW cells.

851 **Figure 2.** Evolution of the prokaryotic concentrations over time in the ‘Nutrient-replete’, ‘N-  
852 stress’, and ‘Si-stress’ batches during the biodegradation experiments. The prokaryotic  
853 concentrations correspond to the sum of the free and attached prokaryotes. N = 1 at each  
854 sampling point.

855 **Figure 3.** Time course responses of the (A) POC and (B) PON relative concentrations of TW  
856 in the ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ batches. The relative concentrations were  
857 calculated by dividing the concentration at day t by the concentration at day 1 for both POC  
858 and PON. The POC and PON concentrations were corrected by the C- and N- content of the  
859 attached prokaryotes (see section 2.3). The vertical lines in the graph show the substitution  
860 time for the two pools of organic matter for each experiment (Table 3). Replicates (n = 3)  
861 were performed at days 1, 5, 10, 15, 22, and 30 and were used to feed our degradation model  
862 (section 2.5).

863 **Figure 4.** Time course responses of the PCHO-C relative concentrations in the ‘Nutrient-  
864 replete’, ‘N-stress’, and ‘Si-stress’ batches. The relative concentrations were calculated by  
865 dividing the concentration at day t by the averaged concentration at day 1. The kinetics were  
866 estimated using equation (3) (section 2.5). We used the concentration measured on day 1 as  
867 the initial concentration. All of the experimental points correspond to one measurement  
868 except at day 1 and day 30 (n = 3).

869 **Figure 5.** Time course responses of (A) galactose, (B) ribose, and (C) glucose in the  
870 ‘Nutrient-replete’, ‘N-stress’, and ‘Si-stress’ batches. The kinetics were estimated using  
871 equation (3) (section 2.5) and the concentrations at day 1 as the initial concentrations. The  
872 degradation rates for galactose (N-stress batch), ribose (Si-stress batch), and glucose (Si-stress  
873 batch) were no significant at the 0.05 level (see Table 3). All of the experimental points  
874 correspond to one measurement except at day 1 and day 30 ( $n = 3$ ).

875 **Figure 6.** Time course responses of the POC/PON ratio in the ‘Nutrient-replete’, ‘N-stress’,  
876 and ‘Si-stress’ batches during the TW biodegradation. As in Figure 2, the vertical lines show  
877 the substitution  $t_s$  time (see section 2.5) for the POC (bold) and PON (not bold) parameters.

878

879 Table 1. Initial parameters measured in each batch at the beginning of the experiment. The prokaryotic inoculum  
 880 concentrations were estimated before the addition of the diatom cells.

Growth conditions	Batch volume (L)	[Diatom cells] (cell L <sup>-1</sup> )	Cellular volume <sup>1</sup> (μm <sup>3</sup> )	[POC] <sup>2</sup> /[diatom cell] (pmol cell <sup>-1</sup> )	[PON] <sup>2</sup> /[diatom cell] (pmol cell <sup>-1</sup> )	[Inoculum] (cell L <sup>-1</sup> )	[bSiO <sub>2</sub> ]/[diatom cell] (pmol cell <sup>-1</sup> )
Nutrient- replete	10	1.80 x 10 <sup>8</sup>	1488 ± 117	8.14	1.17	5.00 x10 <sup>9</sup>	1.90
N- stress	10	3.02 x 10 <sup>8</sup>	1154 ± 137	14.39	0.73	2.55x10 <sup>9</sup>	0.89
Si- stress	10	2.11 x 10 <sup>8</sup>	1364 ± 150	17.41	1.51	5.39x10 <sup>9</sup>	0.86

881 <sup>1</sup>Cellular volumes were calculated at the end of the starvation experiment.

882 <sup>2</sup>POC and PON values were corrected from the carbon and nitrogen contributions of the attached prokaryotes, respectively (see section 2.3).

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905 Table 2. Elemental carbohydrate compositions (percentage of the total sugar, mol%) and the total PCHO-C  
 906 concentrations (in  $\mu\text{M}$ ) during the 30 days of the degradation. The PCHO yields are given as the percentage  
 907 of total PCHO-C to the POC. Abbreviations: Fuc. – Fucose; Rha. – Rhamnose; Ara. – Arabinose; GlcN. –  
 908 Glucosamine; Gal. – Galactose; Glc. - Glucose; Man. – Mannose; Xyl. – Xylose; Rib. – Ribose; GalUA. –  
 909 Galacturonic Acid. The values given at day 1 and 30 are the mean of three measurements (see Fig. 3).

Experiment	Time (d)	Fuc.	Rha.	Ara.	GlcN.	Gal.	Glc.	Man.	Xyl.	Rib.	GalUA	PCHO-C	PCHO-C/POC
Nutrient - replete	0	2.73	8.10	0.32	1.03	37.53	5.51	5.53	9.34	21.97	7.95	30.01	2.03
	1	2.57	8.04	0.19	3.62	15.04	8.36	4.27	6.47	46.69	4.75	102.55	5.22
	6	4.78	15.19	1.02	4.99	15.37	9.55	9.95	13.29	16.97	8.89	85.99	8.06
	12	6.80	14.26	3.37	4.83	13.28	8.86	9.16	11.97	16.04	11.42	41.90	6.59
	25	7.07	13.59	1.86	7.06	13.11	11.78	9.49	15.72	7.21	13.11	36.74	7.66
	30	8.28	12.69	2.33	6.74	12.53	13.47	8.35	16.79	6.71	12.10	30.64	6.26
N-stress	0	5.37	7.98	0.29	1.30	28.59	32.43	6.29	13.04	3.54	1.18	77.77	1.75
	1	2.45	6.36	0.32	1.81	11.82	43.02	3.65	7.33	22.87	0.37	193.95	4.02
	6	4.16	10.91	0.36	3.52	12.76	28.53	7.57	10.52	20.13	1.56	164.45	4.97
	12	5.29	12.76	1.11	4.89	14.40	22.28	10.22	14.12	12.74	2.20	145.90	7.94
	18	5.56	12.50	1.18	5.04	15.09	24.50	10.83	13.29	9.34	2.67	108.54	6.39
	25	5.21	7.74	0.28	1.26	27.74	31.47	6.10	12.66	3.43	4.12	94.25	6.90
30	7.67	11.17	2.20	4.81	13.54	25.40	9.08	14.27	9.85	2.00	60.65	4.26	
Si-stress	0	4.10	11.38	0.30	2.01	37.62	5.20	8.13	18.65	10.56	2.04	114.93	3.05
	1	3.38	11.61	0.22	4.12	17.66	12.14	4.77	11.64	34.06	0.40	162.92	3.84
	6	3.23	12.11	0.23	5.13	8.14	19.72	5.00	9.67	36.77	0.00	219.95	6.78
	12	6.27	16.57	1.24	5.93	10.97	13.80	9.91	16.98	18.33	0.00	87.36	5.23
	18	7.15	16.22	1.57	7.41	11.89	13.53	11.82	17.91	12.50	0.00	57.51	3.54
	30	7.26	16.26	3.06	7.17	11.66	15.83	10.85	18.68	9.23	0.00	68.06	5.18

910

911 Table 3. Estimated kinetic parameters for POC, PON, PCHO, and the individual monosaccharides (see equations in section  
 912 2.5) during degradation of TW. The  $t_s$  value is the substitution time between the two pools of organic matter. The standard  
 913 error (SE) is given for each estimation. Significant estimations are underlined ( $p < 0.05$ ).

Kinetic parameters	Nutrient-replete						N-stress						Si-stress					
	$k_1$		$k_2$		$t_s$		$k_1$		$k_2$		$t_s$		$k_1$		$k_2$		$t_s$	
	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE
POC (n=34)	<u>0.111</u>	0.031	<u>0.04</u>	0.001	<u>5.908</u>	0.561	<u>0.096</u>	0.002	<u>0.018</u>	0.001	<u>11.14</u>	0.181	<u>0.113</u>	0.005	<u>0.014</u>	0.001	<u>8.544</u>	0.237
PON (n=34)	<u>0.191</u>	0.099	<u>0.054</u>	0.001	<u>4.989</u>	0.587	<u>0.044</u>	0.002	<u>0.021</u>	0.001	<u>10.54</u>	0.639	<u>0.078</u>	0.002	<u>0.019</u>	0.001	<u>10.07</u>	0.127
Kinetic parameters	$k$ (n = 9)						$k$ (n = 10)						$k$ (n = 9)					
	Value		SE				Value		SE				Value		SE			
Fuc.	0.004		0.01				0.002		0.006				0.005		0.005			
Rha.	0.027		0.011				0.017		0.01				<u>0.022</u>		0.009			
Ara.	-0.014		0.019				-0.015		0.016				<u>-0.057</u>		0.009			
GlcN.	0.022		0.01				0.01		0.013				0.014		0.01			
Gal.	<u>0.054</u>		0.01				0.018		0.009				<u>0.06</u>		0.018			
Glc.	<u>0.028</u>		0.01				0.071		0.037				0.029		0.02			
Man.	0.021		0.013				0.009		0.012				0.004		0.007			
Xyl.	0.012		0.011				0.012		0.007				0.016		0.007			
Rib.	<u>0.215</u>		0.054				<u>0.050</u>		0.007				0.073		0.043			
GalUA	0.009		0.009				-0.01		0.019				-		-			
Total PCHO	<u>0.046</u>		0.01				<u>0.036</u>		0.009				<u>0.036</u>		0.014			

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Figure

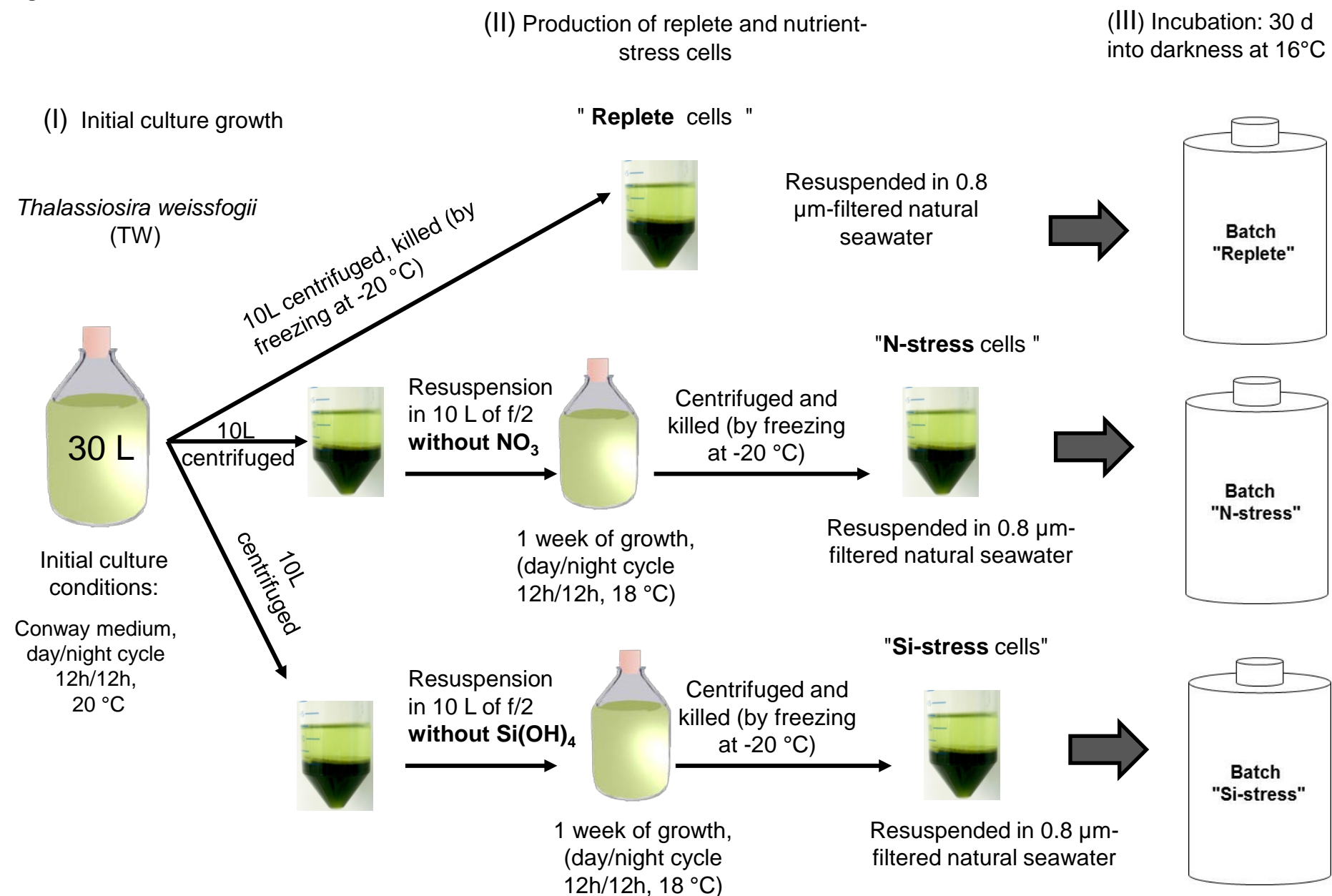


Figure 1

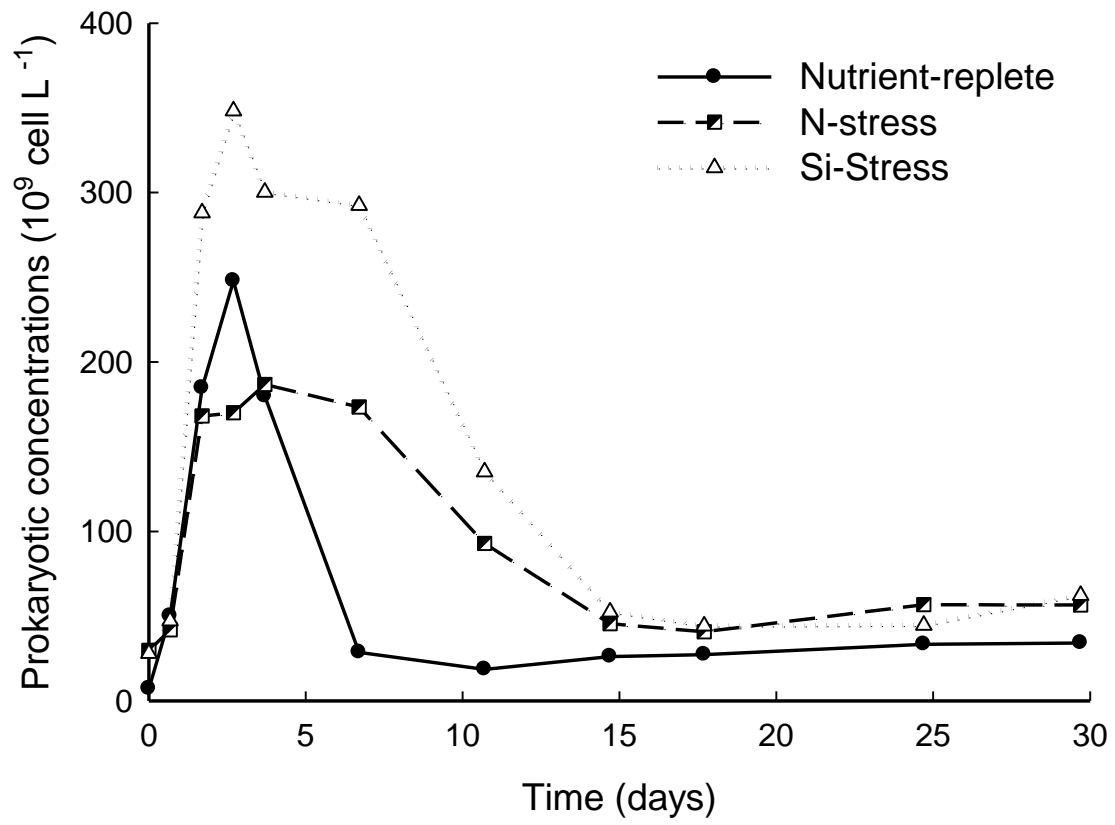


Figure 2

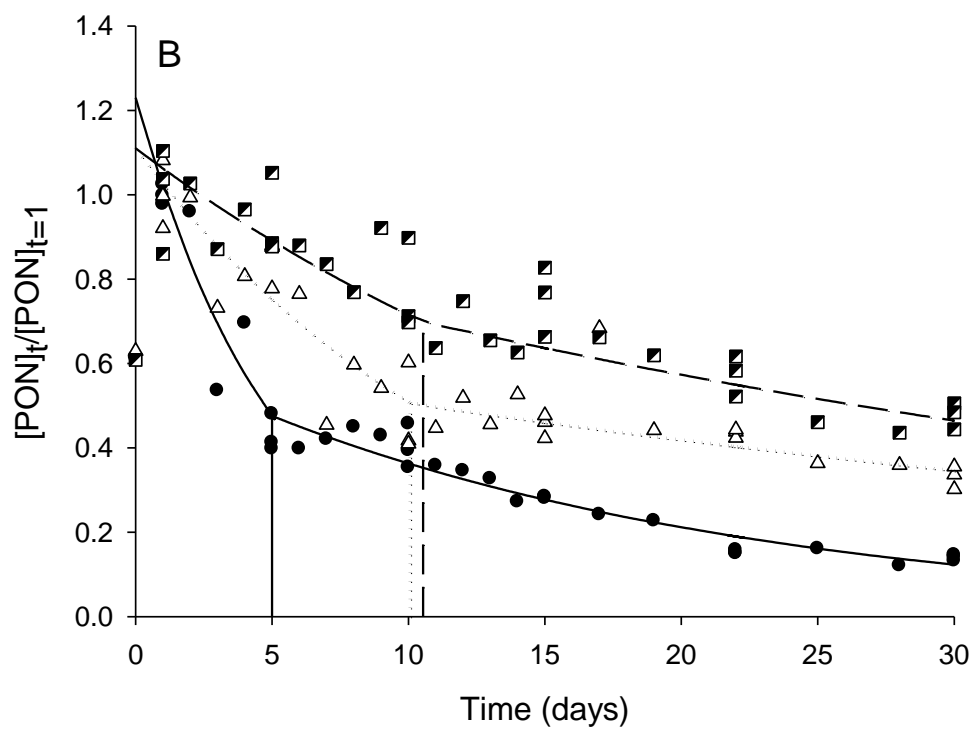
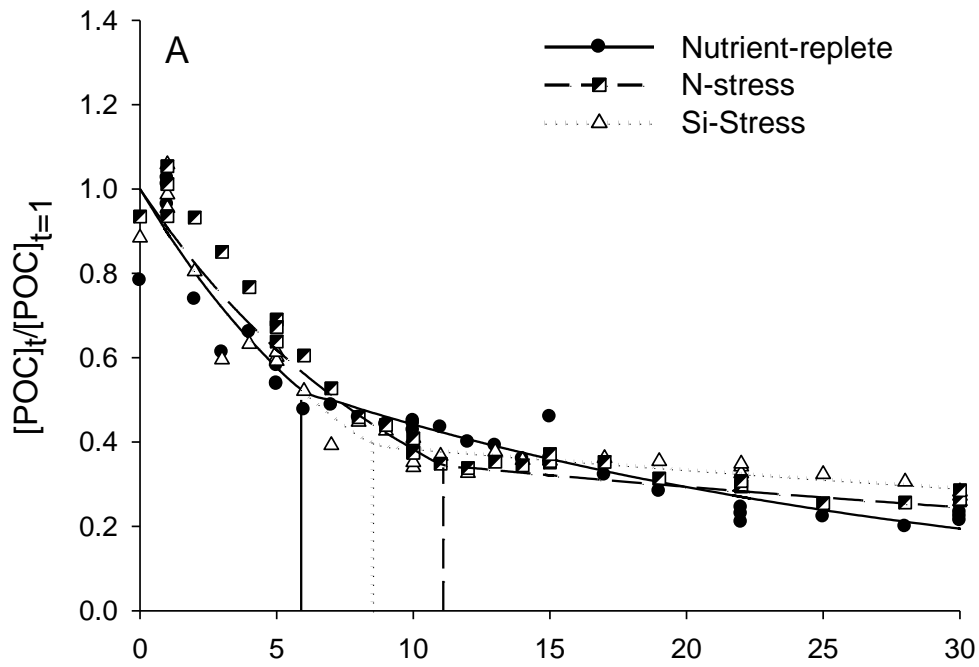


Figure 3

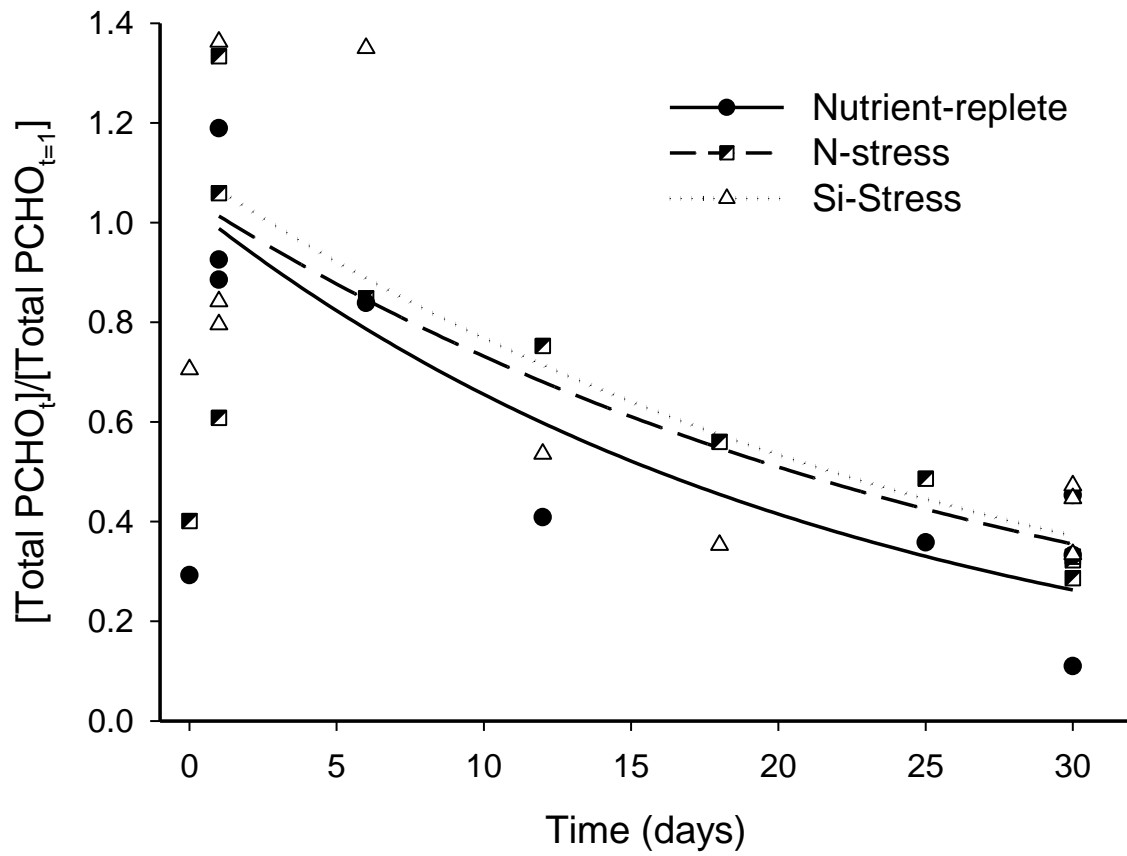


Figure 4

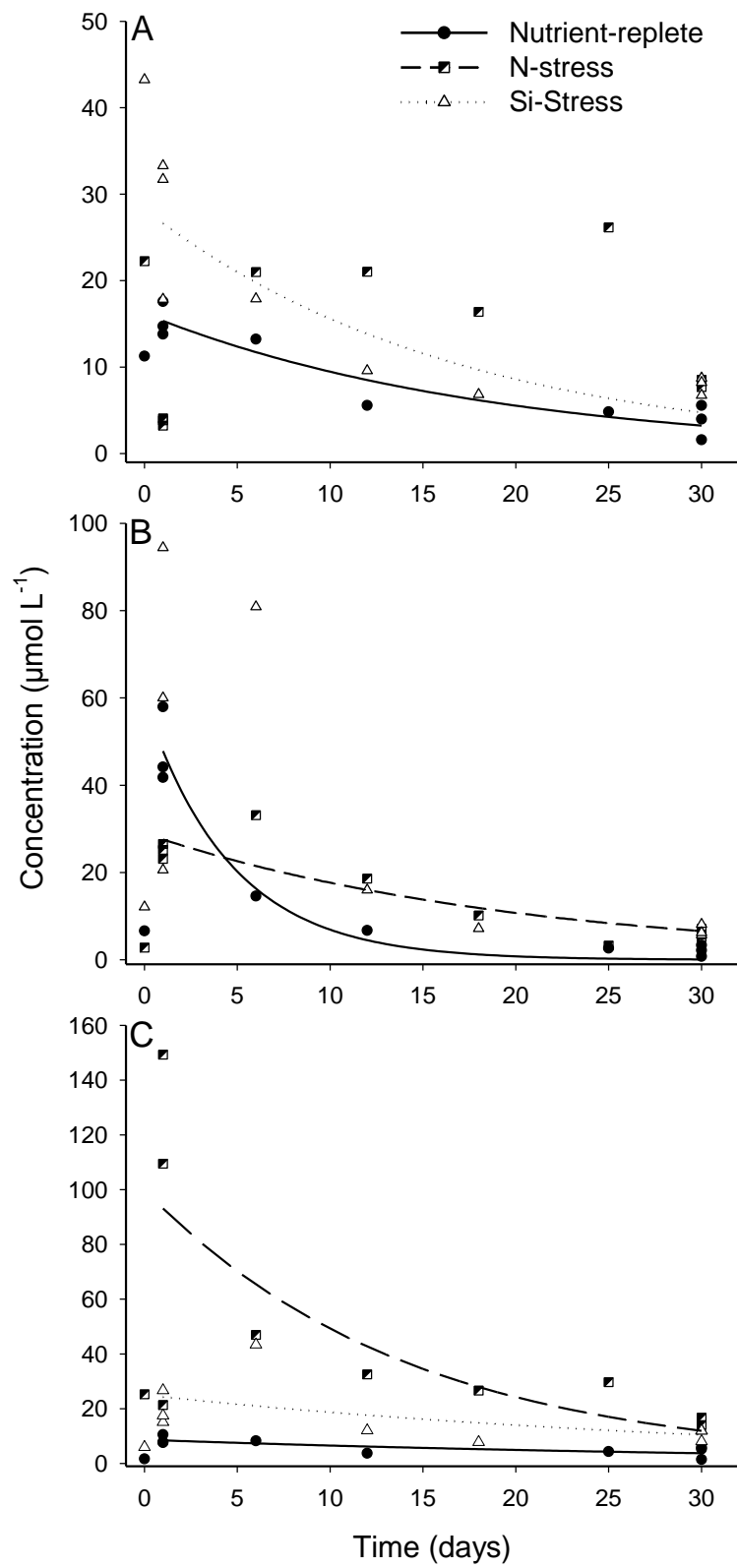


Figure 5

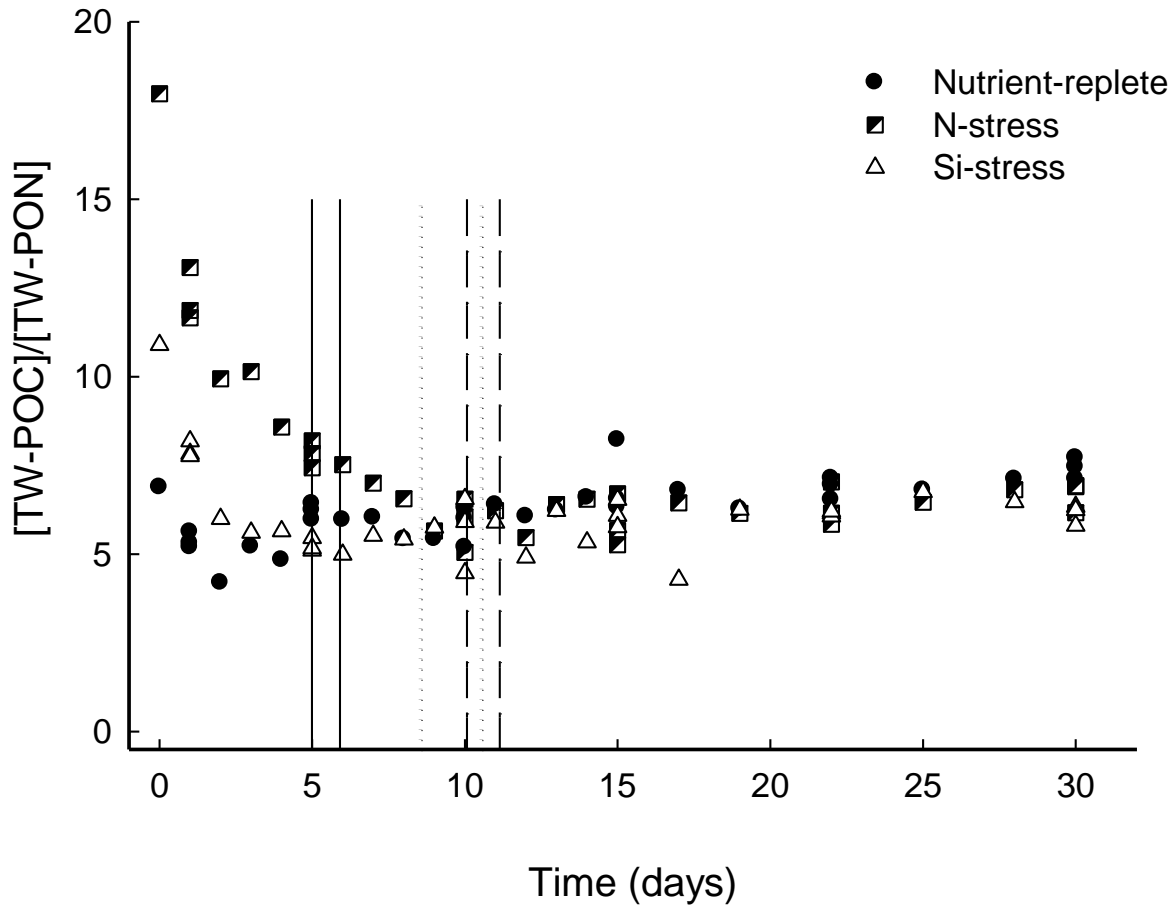


Figure 6