Si–C interactions during degradation of the diatom *Skeletonema marinoi*

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Abstract

While a relationship between ballast and carbon in sedimenting particles has been well-documented, the mechanistic basis of this interaction is still under debate. One hypothesis is that mineral ballast protects sinking organic matter from degradation. To test this idea, we undertook a laboratory experiment using the diatom *Skeletonema marinoi* to study in parallel the dissolution of one of the most common mineral ballasts, biogenic silica (bSiO₂), and the associated degradation of organic matter. Three different models were applied to our results to help elucidate the mechanisms driving bSiO₂ dissolution and organic compound degradation. Results of this modelling exercise suggest that the diatom frustule is made up of two bSiO₂ phases that dissolve simultaneously, but at different rates. In our experiments, the first phase was more soluble ($k_{bSiO_2} = 0.27 \text{ d}^{-1}$) and made up 31% of the total bSiO₂. In this phase, bSiO₂ was mainly associated with membrane lipids and the amino acids glutamic acid, tyrosine, and leucine. The second phase was more refractory ($k_{bSiO_2} = 0.016 \text{ d}^{-1}$), and contained more neutral lipid alcohols and glycine. Until it dissolved, the first bSiO₂ phase effectively protected much of the organic matter from degradation: POC degradation rate constants increased from 0.025 d^{-1} to 0.082 d^{-1} after the total dissolution of this phase, and PON degradation rate constants increased from 0.030 d^{-1} to 0.094 d^{-1} . Similar to POC and PON, the THAA degradation rate constant increased from 0.054 d^{-1} to 0.139 d^{-1} after dissolution of the first bSiO₂ phase. The higher THAA degradation rate constant is attributed to a pool of amino acids that was produced during silicification and enclosed between the two silica phases. This pool of amino acids might come from the incorporation of silica deposition vesicles into the diatom wall and might not be directly associated with bSiO₂. In contrast, most lipid degradation was not prevented by association with the more soluble bSiO₂ phase as the average lipid degradation rate constant decreased from 0.048 d^{-1} to 0.010 d^{-1} after 17 days of degradation; This suggests that most lipids were associated to rather than protected by silica, except pigments that appeared resistant

52 to degradation, independantly from silica dissolution. When the only organic compounds
53 remaining were associated with the second bSiO₂ phase, degradation rate constants decreased
54 greatly; concentrations changed only slightly after day 25.

55

Key Words: Biogenic silica, dissolution, carbon, amino acids, lipids, degradation, diatom

1. Introduction

Organic carbon produced in the ocean's surface layer by phytoplankton is conveyed to depth by particle sedimentation, and fluxes of carbon and minerals (CaCO_3 , SiO_2 and aluminosilicates) are highly correlated in the deep water column. Based on these observations, Armstrong *et al.* (2002) highlighted the importance of modeling both carbon and mineral fluxes at the same time. Mineral ballast (CaCO_3 of coccolithophorids; SiO_2 of diatoms; aluminosilicates in dust) provide excess density needed for organic matter to sink; SiO_2 and carbonate sedimentation are also linked through the ability of phytoplankton to aggregate and through grazing by zooplankton. The combination of these processes strongly increases the sedimentation rate of phytoplankton (e.g., Gehlen *et al.*, 2006; Moriceau *et al.*, 2007).

The role of mineral ballast in carbon transport is more complex than a simple impact on excess density (Lee *et al.*, 2008), but we are far from fully understanding the processes involved. Lee *et al.* (2000) and Hedges *et al.* (2001) hypothesized that mineral ballast could protect organic carbon from degradation; their hypothesis is consistent with the observation of Ingalls *et al.* (2006) that organic matter was more degraded in areas where diatoms were not the dominant bloom species when compared to sites where diatoms were the main phytoplankton group. Engel *et al.* (2008) also showed that the presence of the calcite test in coccolithophorids lowers the POC degradation rate during the recycling of these cells. Continuously increasing pressure reduced rates of SiO_2 dissolution of diatom detritus relative to rates measured under atmospheric pressure conditions (Tamburini *et al.* 2006). In parallel, naturally collected sinking particles, were also less degraded by prokaryotes when pressure was continuously increased to simulate descent from 200 to 1500 (Tamburini *et al.* 2008). Despite all these findings, few studies (e.g., Ingalls *et al.*, 2003; 2006) have investigated both organic matter degradation and biogenic mineral dissolution in natural settings. The work of Engel *et al.* (2008) investigated the

role of CaCO_3 in carbon degradation, while the present study aims to better understand the role of Si-C interactions during diatom degradation.

Diatoms are the dominant species in many ecosystems; they are responsible for up to 35% of the total primary production in oligotrophic oceans and up to 75% in coastal waters and the Southern Ocean (Nelson *et al.*, 1995; Tréguer *et al.*, 1995). Jin *et al.* (2006) estimated their global contribution to net primary production and to carbon export to be 15% and 40%, respectively. The high diatom contributions to primary production and carbon export, could potentially explain the empiric relation established by Ragueneau *et al.* (2002). This relation shows that Si/C ratios decrease with depth and follow the same pattern everywhere in the world ocean. Is there a link between bSiO_2 dissolution and POC degradation such as the one hypothesized in the work cited above?

The objective of the present study was to understand how biogenic silica influences the degradation of diatom organic carbon, and conversely the role of organic compounds in bSiO_2 dissolution. With this aim, a monospecific culture of the diatom *Skeletonema marinoi* was incubated in the presence of a natural coastal bacterial community and allowed to degrade over a 102-day period. bSiO_2 dissolution and the quantity and composition of organic compounds, including amino acids and lipids, were assessed throughout the incubation period and used to investigate Si-C interactions during decomposition. Three dissolution/degradation models were applied to the experimental data to elucidate the dissolution/degradation pattern of the components of *S. marinoi*. This modeling experiment yields a better understanding of the structure of the diatom frustule in *S. marinoi* and of the role of Si-C interactions during diatom recycling.

2. Material and methods

2.1 Biodegradation experiment

Skeletonema marinoi (CCAP 1077/3) obtained from IFREMER (Argenton station, France) was grown in f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) under 12/12 dark/light illumination. When cells reached stationary growth phase (6.5×10^6 cells ml⁻¹), they were transferred into a 4°C chamber and kept in the dark for 5 days. During this period, cells sank to the bottom of the flask, and previous tests showed that diatom viability (number of living cells versus total cells) decreased (unpublished data, method described in Garvey *et al.* 2007). The supernatant was poured off, and the overlying medium was replaced with natural seawater that had been passed through a 0.7-µm GFF filter to preserve the natural bacteria assemblage. The seawater was collected from a small inlet (Endoume) near Marseille, France, at the end of fall, when the water is naturally poor in silicic acid (dSi~2.5 µM). The mixture of *S. marinoi* and filtered sea water was then transferred to an incubation flask equipped with a magnetic stirrer and a stopper through which gas exchange could occur via a 0.2-µm Swinnex® filter. The diatoms were incubated for 102 days in the dark at 20°C. Using a peristaltic pump, samples were taken daily for the first 21 days and then at 23, 25, 46, 50, and 102 days; triplicate samples were taken at 0, 5, 11, 46, 50, and 102 days. The sampled solution was well-mixed, allowing the ratio of solid matter to solution to remain constant (Dixit *et al.*, 2001). Ten percent of the liquid volume remained at the end of the experiment. Chemical parameters measured were biogenic silica (bSiO₂), silicic acid (dSi), particulate organic carbon and nitrogen (POC, PON), dissolved organic carbon (DOC), total particulate lipids (TLip), and total hydrolyzable amino acids (THAA). In addition total bacterial abundances (diamidinophenylindole: DAPI counts) were counted. Si contamination by dissolution of glassware was measured by analyzing dSi in an incubation bottle with no cells added. We also sampled controls poisoned with 20 mg l⁻¹ HgCl₂ at 4 times (0, 2, 5, and 11 days) to verify that degradation was due to bacteria and not abiotic factors.

2.2 Analytical Methods

Biogenic Silica (bSiO₂) was determined at the beginning and end of the experiment using a variation of the method of Ragueneau and Tréguer (1994). As no lithogenic silica was present in the algal culture, the second digestion step using HF was not necessary. Ten-ml samples were filtered onto 0.2-μm polycarbonate filters. Filters were analyzed for bSiO₂ and the filtrate for dSi. For bSiO₂, filters were digested in 20 ml of 0.2N NaOH for three hours at 95°C to ensure the dissolution of all bSiO₂; dSi concentrations in the solution remained far below the solubility equilibrium of bSiO₂ at all times. After cooling, the solution was acidified with 5 ml of 1N HCl, centrifuged to remove remaining solids, and analyzed for dSi. The precision for triplicate measurements of bSiO₂ was < 5%.

Silicic acid (dSi) concentrations were determined on 10-ml filtered samples and on digested bSiO₂ samples using the molybdate blue spectrophotometric method of Mullin and Riley (1965), as adapted by Tréguer and Le Corre (1975) and modified by Gordon *et al.* (1993) for use in segmented flow colorimetry. We used a Bran and Luebbe Technicon Autoanalyzer (<1% precision).

POC and *PON* concentrations were measured using a Carlo Erba NA 2100 CN analyzer coupled to a Finnigan Delta S mass spectrometer. Five-ml samples were filtered through 0.7-μm GFF filters. The filters were desiccated overnight in an oven at 50°C and then placed in tin capsules to be introduced into the oven of the analyzer. The precision for triplicate N analyses was ± 1-6%, and for C analysis ± 1-5%.

DOC was analyzed after filtration through 0.7-μm GFF filters; 10 ml of each sample was transferred into glass ampoules and sealed after addition of H₃PO₄ as preservative. All glassware was pre-rinsed with 1N HCl and Milli-Q water before being combusted at 465°C; care was taken to minimize contamination during sampling and handling. DOC was measured by high-temperature catalytic oxidation using a Shimadzu TOC 5000 Analyzer (Sempéré *et al.*, 2003; Sohrin and Sempéré, 2005). Samples were acidified to pH 1 with 85% phosphoric acid and bubbled for 10 minutes with CO₂-free air to purge them of inorganic carbon. Three or four 100

μl replicates of each sample were injected into the 680 °C column. The precision of these replicates was $\leq 6\%$.

Total particulate lipids were analyzed after filtering 10 ml samples onto 0.7-μm GFF glass fiber filters. Filters were extracted according to Bligh and Dyer (1959). Lipid extracts were separated into classes of compounds and quantified on an Iatroscan model MK-6s (Iatron, Tokyo; H₂ flow 160 ml min⁻¹; air flow 2 l min⁻¹) as described by Goutx *et al.* (2007). The elution scheme allows reliable separation and quantification of degradation metabolites from acyl-lipid classes (Striby *et al.*, 1999). Total particulate lipids (TLip) are the sum of the separated lipid classes (Table 1). In the present work, the variability within triplicates was < 13%.

Amino acids were analyzed on 0.7-μm GFF filters after filtration of 10-ml samples. Thawed filters were treated as described in the study of Ingalls *et al.* (2003). Individual compounds were separated by HPLC using pre-column OPA derivatization after acid hydrolysis as described in Lee and Cronin (1982) and Lee *et al.* (2000). Amino acids were detected by fluorescence and identified by comparison to retention times of standards made from an amino acid mixture (Pierce, Standard H). The non-protein amino acids β-alanine and γ-aminobutyric acid (BALA and GABA) were added individually to the standard mixture. Aspartic acid (ASP) and glutamic acid (GLU) measurements include the hydrolysis products of asparagine and glutamine. THAA is the sum of the 16 characterized amino acids (Table 1). Variation among replicates was generally 15-30%. LYS replicates, however, varied more greatly at times, e.g., 50% at day 11.

Total bacterial abundances (DAPI counts): Subsamples for bacterial cell counts were fixed immediately with buffered formalin (final volume 2%). Cells were collected onto a 25-mm 0.2-μm polycarbonate Nuclepore® membrane and stained with diamidinophenylindole (DAPI; Porter and Feig, 1980). Slides were stored frozen until counting by epifluorescence microscopy (Olympus, BH2).

2.3 Kinetics

Kinetic parameters were calculated over the first 25 days where experimental data are available every 1-2 days. For each compound of interest we tested three degradation/dissolution models. Model 1 is a simple first-order rate equation as described in Greenwood *et al.* (2001) and used in many dissolution studies (e.g. Kamatani and Riley, 1979; Kamatani *et al.*, 1980; Kamatani, 1982):

$$\hat{C}(t) = C_0 \exp(-k t) , \quad (1)$$

where $\hat{C}(t)$ is the concentration (μM) estimated at time t (d), C_0 is the initial concentration, and k is the dissolution/degradation rate constant (d^{-1}).

Model 2 assumes simultaneous dissolution/degradation of two phases. The equation used is similar to that used for carbon degradation in the study of Westrich and Berner (1984):

$$\hat{C}(t) = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) \quad (2)$$

In Model 2, four parameters are estimated: C_1 and C_2 are concentrations (μM) of phase or pool 1 and 2, and k_1 and k_2 (d^{-1}) are their respective dissolution or degradation rate constants.

As it uses 2 more parameters, Model 2 always gives a better fit than Model 1 except when the initial degradation rate is slower than later rates. In this specific case Model 2 performs no better than Model 1. We therefore developed Model 3, which employs one first-order equation initially, and a second first-order equation after that. The time at which the dissolution/degradation rate constant changes is called the substitution time (t_s), and is allowed to take on any value >0 .

$$\hat{C}(t) = C_0 \exp(-k_1 t), \quad 0 < t < t_s ; \quad (3a)$$

$$\hat{C}(t) = C(t_s) \exp(-k_2 t), \quad t > t_s . \quad (3b)$$

Model 3 also contains 4 parameters.

If dissolution/degradation for a given compound is best reconstructed using Model 2, this compound is constituted by at least two phases or pools; on the other hand if Model 3 gives a better description of the dissolution/degradation pattern, either the compound studied is present in 2 phases/pools remineralizing one after the other, or a change in environmental parameters provoked a change in the dissolution/degradation rate constant at time t_s .

In addition, to allow direct comparison of the degradation/dissolution pattern among all compounds, and between this study and previous studies, an initial disappearance rate constant was calculated for each compound over the first 10 days using Model 1.

2.4 Statistics

Each fit was optimized by maximizing the likelihood statistic $\log(L)$ as described in equation 4 (Armstrong *et al.*, 2002, and references therein). Eq. (4) is based on a Gaussian distribution with a constant variance on a logarithmic scale:

$$\log(L) = -\frac{N}{2} \times \log \left(\frac{\sum (\log(\hat{C}_j) - \log(C_j))^2}{N} \right), \quad (4)$$

where N is the number of data points, C_j is a measured concentration for data point j , and the $\hat{C}_j$ is the corresponding model prediction. The difference in $\log(L)$ ($\Delta \log(L)$) between fits of two different models to the same data gives the goodness of fit of one model compared to the other. If one model gives a value for $\log(L)$ that is at least 2 points higher per added parameter than another model, it is considered to fit the data better (Hilborn and Mangel, 1997). In the present work, the simplest model (Model 1) was considered to be the best fit unless Model 2 or Model 3 yielded a $\log(L)$ more than 4 points better than that of Model 1.

3. Results

3.1 Change in biochemical composition of the diatom *Skeletonema marinoi* over time

3.1.1 General trends

Changes in bSiO₂, POC, TLip and THAA concentrations over time are shown in logarithmic scale in Fig. 1. While bSiO₂ concentrations decreased smoothly over the first 25 d, POC, PON and THAA concentrations decreased until about day 14, when the loss rate increased, especially for THAA. In contrast, TLip concentration decreased rapidly at the beginning of the experiment and reached a plateau after day 13.

Bacterial concentrations started at $0.33 \pm 0.03 \times 10^6$ cell ml⁻¹ and peaked at day 14 with a concentration of $30 \pm 2.7 \times 10^6$ cell ml⁻¹ (Fig. 2). The bacterial population increased between days 0 and 14 with a rate equal to 0.015 d^{-1} (calculated between days 2 and 14) and then generally stabilized reaching a final concentration of $20 \pm 4 \times 10^6$ cell ml⁻¹ until the end of the experiment. The bacterial growth efficiency (bacterial carbon increase divided by POC decrease) between days 0 and 14 was 2%. Bacterial carbon made up a maximum of $2.4 \pm 0.6\%$ of the POC.

TOC concentrations over time showed a pattern similar to those of POC (Fig. 3). DOC concentrations increased slightly ($5.6 \pm 0.2 \text{ mg C L}^{-1}$) but much less than POC decreased ($62 \pm 3 \text{ mg C L}^{-1}$). The bacterial carbon (C_{bact}) is so low compared to the algal organic carbon that the mineralization rate for POC algal ($\text{POC} + \text{DOC} - C_{\text{bact}}$) is the same as for the total organic carbon ($C_{\text{org}} = \text{POC} + \text{DOC}$). This rate calculated as the slope of TOC change over time (Fig. 3) was $2.68 \text{ mg C L}^{-1} \text{ d}^{-1}$ during the first 20 days of the experiment and was equivalent to 91% of the POC loss.

3.1.2 Initial biochemical composition

At the beginning of the experiment, the Si/POC ratio was 0.09 ± 0.01 , which is slightly lower than the value of 0.13 measured in fresh diatoms by Brzezinski (1985) but in the range of coastal diatoms measured by Rousseau *et al.* (2002). TLip made up $19 \pm 2\%$ of *S. marinoi*

organic carbon which is a little bit higher than Lip/POC ratios measured previously for the same species (Lavens and Sorgeloos, 1996). TLip mainly included FFA and other degradation metabolites (ALC and MG; see Table 1 for abbreviations) (Fig. 4a). Cellular membrane phospholipids (PE, DPG+PG) and chloroplast membrane glycolipids (MGDG) accounted for $10 \pm 1\%$ and $13 \pm 1\%$ of TLip, respectively. FFA were the most abundant lipid degradation metabolites ($24.8 \pm 0.5\%$) among those present. PIG included both chlorophyll *a* and its degradation products (Striby *et al.*, 1999); PIG was the largest lipid class (initially $41 \pm 5\%$; Fig. 4a). ST are involved in membrane rigidity (Parrish, 1988); they were initially minor components of *S. marinoi*.

THAA constituted a larger portion ($36 \pm 9\%$) of total organic carbon than the lipids, similar to the 45% found in *Thalassiosira weissflogii* by Cowie and Hedges (1996) and the 25% observed in diatom-rich sediments by Ingalls *et al.* (2006). THAA include the monomer constituents of protein as well as adsorbed amino acids and peptides. Sixteen amino acids were quantified. GLU, ASP and LEU (see Table 1 for abbreviations) together made up one third of the THAA, each more than $11 \pm 3\%$ (Table 2). Other amino acids were between $2.1 \pm 0.6\%$ and $9.3 \pm 2.8\%$ of the THAA except for MET and GABA, which were less than 0.8%. As GABA and MET concentrations were very low throughout the incubation, they are not described in the following paragraphs. The initial mole% amino acid compositions we found for *S. marinoi* (Table 2) were very similar in pattern to those of cultured diatoms reported by Ingalls *et al.* (2003) and Cowie and Hedges (1996), with highest mole% values for ASP, GLU, and GLY. *S. marinoi* was higher in mole% LEU than in reports of other diatoms. THAA do not include the amino acids incorporated inside the silica frustule, unless part of the frustule is dissolved during acid hydrolysis (see later discussion). We define Si-bound amino acids as Si-THAA as in Ingalls *et al.* (2003).

3.1.3 Change in biochemical composition during degradation

Si/POC decreased from 0.09 ± 0.01 mol of Si/mol of C to 0.06 ± 0.01 mol of Si/mol of C during the first 2 days of incubation and then stabilized until day 15 of the experiment. After increasing to 0.11 ± 0.01 mol of Si/mol of C between days 15 and 21, the Si/POC ratio remained constant until the end of the experiment. TLip/POC varied between 0.13 ± 0.02 mol of C/mol of C and 0.23 ± 0.03 mol of C/mol of C during the first 50 days. Then the ratio decreased to a final value of 0.06 ± 0.01 mol of C/mol of C at day 102. THAA/POC decreased regularly during the whole experiment from 0.36 ± 0.09 mol of C/mol of C initially to 0.10 ± 0.02 mol of C/mol of C after 102 days, except for a sudden increase between day 11 and 15 from 0.28 ± 0.7 mol of C/mol of C to 0.38 ± 0.10 mol of C/mol of C.

Most of the change in TLip composition (Fig. 4a) occurred between days 0 and 25. Most compounds decreased or remained the same relative to TLip (mol of C/mol of C) except for PIG, which increased from $41 \pm 5\%$ to $80 \pm 7\%$ of the TLip over the course of the experiment. Relative concentrations (mol of C/mol of C) of FFA, the second most abundant lipid class in the algae, decreased regularly from $24.8 \pm 0.5\%$ to $4.0 \pm 0.4\%$. MGDG initially made up $13 \pm 1\%$ of TLip, but totally disappeared by day 4. The MG contribution was constant ($7.0 \pm 0.3\%$) until day 14, when it was completely degraded. The contribution of membrane lipids, the glycolipid MGDG and the phospholipids (PE and DPG+PG) to TLip was low compared to results from Berge *et al.* (1995) and d'Ippolito *et al.* (2004). However, lipid composition is highly dependent on culture conditions (d'Ippolito *et al.*, 2004), and in our case the high FFA content probably masked the relative contribution from membrane lipids.

The THAA composition was relatively constant throughout the degradation experiment except between days 15 and 20 where we observed a strong peak of GLY, which increased from $13 \pm 2\%$ to $33 \pm 8\%$ (Fig. 4b). The relative concentrations of HIS (data not shown) and LYS also peaked slightly between days 15 and 20. Relative proportions of other amino acids especially ASP decreased at this time in response to the increases in HIS, GLY and LYS.

3.2 Dissolution and degradation kinetics of *S. marinoi* constituents

3.2.1 Silica kinetics

The experiment was conducted in glass bottles to eliminate carbon contamination. Controls showed that after 102 days, dSi due to leaching from the glass was a maximum of 5% of the dSi due to dissolution of diatom frustules. From an initial concentration of $680 \pm 30 \mu\text{mol L}^{-1}$, bSiO₂ decreased rapidly during the first 3 to 5 days of the experiment and then more slowly (Fig. 1). After 25 days, 52% of the initial bSiO₂ was dissolved and 76% of the initial was dissolved at the end of the experiment (102 d). The comparison between the $\log(L)$ of the three models describing bSiO₂ dissolution showed that Model 2 is 4.3×10^{16} times better than Model 1 ($\Delta\log(L) = 38.3$) and almost 4.5×10^5 times better than Model 3 ($\Delta\log(L) = 13$). bSiO₂ was the only constituent of the diatom with a dissolution pattern that was best described by Model 2 (Fig. 5a, Table 4) meaning that the frustule is most likely composed of two phases dissolving simultaneously. The first phase of bSiO₂ constituted 31% of the total bSiO₂ and was more soluble, with a dissolution rate constant of 0.27 d^{-1} . The second phase was more refractory with a dissolution rate constant of 0.016 d^{-1} (Table 4). For direct comparison with POC and PON, the initial loss rate constant of the total bSiO₂ was calculated using Model 1 over 10 days as 0.049 d^{-1} . The three dissolution constants are within the range (0.005 d^{-1} to 1.3 d^{-1}) given in the review by Van Cappellen *et al.* (2002b).

3.2.2 POC and PON kinetics

POC concentration decreased in two steps from the initial value of $7660 \pm 150 \mu\text{mol L}^{-1}$ (Fig. 1). Even with more parameters Model 2 did not improve the fit to the data obtained by Model 1 (Fig. 5b); $\log(L)$ calculations for POC loss showed that Model 3 gave the best fit ($\Delta\log(L) = 76.5$). This suggests that either two pools of POC exist and are degraded successively, or a change in some parameter not directly linked to POC chemistry provoked an

increase of the degradation rate at day 10 from 0.025 to 0.082 d⁻¹ (Table 4). PON followed the same pattern; Model 3 reproduced the data with more accuracy as shown by the $\Delta\log(L)$ of 37.3 compare to Models 1 and 2. Model 3 estimated an increase in PON degradation rate at day 12 from 0.03 to 0.094 d⁻¹ (Table 4). The first POC degradation rate constants are similar to rate constants measured in previous studies (0.036 d⁻¹ for POC, Lee and Fisher, 1992; 0.035 d⁻¹ for POC and 0.047 d⁻¹ for PON, Harvey *et al.*, 1995). For comparison with bSiO₂, we also applied Model 1 over 10 days to calculate the initial loss rate of POC and PON: 0.025 d⁻¹ for POC and 0.029 d⁻¹ for PON.

The POC degradation pattern in the Hg-poisoned controls was similar to that in unpoisoned flasks during the first 5 days but was lower between days 5 and 11. The degradation rate constant measured over the first 11 days using Model 1 was 0.021 d⁻¹.

3.2.3 Lipid kinetics

From an initial TLip concentration of 1400 ± 70 μmol C L⁻¹, almost half (42 ± 4%) degraded in 25 days; 7.0 ± 0.3 % of TLip remained after 102 days. The degradation of 7 of the 8 lipid classes is shown in Fig. 6a. TLip degradation is better described by Model 2 and 3 than by Model 1 ($\Delta\log(L)$ = 15.6 and 17 respectively). With only 1.5 point of $\log(L)$ difference between Model 2 and 3 but the same degree of complexity (4 parameters each) we chose the best fit from the best likelihood which was given by Model 3 (Table 4). The degradation rate constant for TLip was 0.048 d⁻¹ during the first 17 days of the experiment and then decreased to 0.01 d⁻¹. The initial degradation rate constant was 0.046 d⁻¹, slightly higher than the 0.023 d⁻¹ measured in the study of Harvey *et al.* (1995).

Using Model 1 we calculated that FFA, PIG, MG and PG+DPG had degradation rate constants of 0.104, 0.012, 0.045 and 0.011 d^{-1} over 25 days respectively. For MGDG, ST, PE, and ALC, Model 3 gives the best fit to the data (Table 4).

MGDG turned over slowly (0.078 d^{-1}) during the first 2 days, but then much more quickly (0.52 d^{-1} ; Table 4) and were completely gone after only 4 days. MG were also completely lost very quickly; the initial degradation rate constant of MG was 0.045 d^{-1} , but the remaining MG was gone after 14 days. This pattern does not fit any of the models used, and suggests an association of these lipids only with the first bSiO₂ phase, or no association at all.

MGDG, ST, and PE degradation rate constants increased by a factor of 8 to 10 during the experiment. They followed the same pattern as POC and PON. ALC degradation rate constant decreased after 19 days.

3.2.4 Amino acid kinetics

From the initial THAA concentration of $3020 \pm 200\text{ }\mu\text{mol C L}^{-1}$, $86 \pm 17\%$ of the THAA were degraded after 25 days; $5.0 \pm 0.5\%$ of the THAA still remained after 102 d. As for POC and PON, Model 3 was the best fit for THAA degradation ($\Delta\log(L) = 9.9$ with both Model 1 and Model 2). Concentrations of THAA decreased from day 1 to day 13 with an average degradation rate constant of 0.054 d^{-1} . After 13 d THAA turned over with a faster degradation rate constant of 0.139 d^{-1} (Table 4), before reaching a period of very low rate constants after day 25; THAA concentrations were almost constant until day 102. This last rate was not calculated by models as the modelling exercise was applied only over the first 25 days. The first degradation phase of THAA was similar to that measured on *T. weissflogii* (0.058 d^{-1}) by Harvey *et al.* (1995). Initially, the degradation rate constant of the 14 individual amino acids ranged between 0.015 and 0.070 d^{-1} , except for ASP and SER, which turned over more slowly with constants of 0.001 and 0.007 d^{-1} , respectively (Table 4). At day 11 when on average $49 \pm 10\%$ of the THAA had

degraded, all concentrations except ARG and SER suddenly increased by 6 to 58 % in 1 to 2 days (Fig. 6b). The largest releases were observed for HIS, GLY and LYS, which increased by 39 ± 13 %, 58 ± 10 % and 46 ± 12 %, respectively. For every amino acid except TYR, degradation rate constants increased after this peak. For ASP and SER the increase occurred earlier at day 5, and for TYR the degradation rate constant decreased from 0.070 to 0.012 d^{-1} at day 21. Except for TYR, amino acids turned over faster during the second degradation phase, and degradation rate constants ranged between 0.110 and 0.182 d^{-1} . The degradation rate constant of SER increased even more than the other amino acids reaching 0.897 d^{-1} (Table 4).

3.3 Relation between bSiO₂ dissolution and degradation of individual organic compounds or compound classes

3.3.1 Lipid degradation versus bSiO₂ dissolution

There was a strong linear relationship between total bSiO₂ and TLip over the whole range of bSiO₂ concentrations ($r^2 = 0.85$, $n = 26$) measured. There was no correlation between bSiO₂ and PIG, so that the relationship between bSiO₂ and TLip became even better when pigments were excluded from the other lipids ($r^2 = 0.94$, $n = 26$). FFA were well correlated with total bSiO₂ concentrations ($r^2 = 0.95$, $n = 26$); they were completely degraded during the dissolution of the second bSiO₂ phase, when bSiO₂ concentrations eventually reached $260 \mu\text{mol L}^{-1}$.

Relationships among individual lipid classes and bSiO₂ phases showed three distinct periods (Fig. 7a and b), which were related to bSiO₂ dissolution using the bSiO₂ model (Figure 5a). Period 1 (P1) is the time corresponding to dissolution of 85% of the first bSiO₂ phase and 10% of the second bSiO₂ phase; Period 2 (P2) is the time corresponding to dissolution of most of the remaining bSiO₂ from the first phase and another 10% of the second bSiO₂ phase; and Period 3 (P3) is the time when less than 1.5% of the first phase remained and 60% of the second phase dissolved. At the end of P3 20% of the bSiO₂ from the second phase remained. On average, 26-34% of the TLip degraded during Period 1; only 14-19% of TLip remained at the beginning of

Period 3. The slow decrease of concentrations observed for each lipid class except PIG, during P1 and P3 compared to P2 despite the fact that the three periods lasted the same time (~7 days, Fig. 5a), might show that most TLip except PIG degraded during P1 were associated with the first bSiO₂ phase, and most TLip except PIG degraded during P3 with the second bSiO₂ phase. These specific lipids are denoted as Si(1)-Lip and Si(2)-Lip; their composition is shown Table 3.

Of the individual classes, PE, ALC concentrations remained almost constant during P1. In contrast, MGDG was completely degraded within the first 4 days, in P1. Despite the high variability in PE and ST concentration measurements, we determined that ST and MG concentrations decreased only slightly in P1 (>27%). This trend is illustrated by their low degradation constants (0.016 d⁻¹ and 0.001 d⁻¹, respectively).

Degradation of MG, ALC, PE and ST mainly occurred in P2. PE and ST concentrations dropped in P2 and ALC degradation was even faster; Model 3 gave a higher k_1 and a longer t_s for ALC than for PE and ST. After the precipitous drop, which corresponded to the beginning of P3, 80% of ALC, FFA and PE and 40% of ST were degraded. MG were completely degraded so quickly at the beginning of P2 that the loss is more likely due to sudden release of MG adsorbed onto particles or dissolution (i.e. involving chemical mechanisms) rather than degradation (i.e. biological mechanisms).

DPG+PG were not well correlated with bSiO₂; generally there was a 36%-degradation during P1, then a fast release of DPG+PG in P2 (40-50%), possibly when the first bSiO₂ phase is completely dissolved. Due to the release in P2, 80% of the initial DPG+PG remained at the beginning of P3.

3.3.2 Amino acid degradation versus bSiO₂ dissolution

The relationship of THAA with the two bSiO₂ phases showed changes at the same times as many of the lipid classes, so we used the same three periods. During Period 1, when the first bSiO₂ phase was dissolving, only 3-22 % of the THAA degraded. For the reason described in the

previous section for lipids (3.3.1), this portion of the THAA is referred to as Si(1)-THAA. GLY and LYS lost less than 10 % of their initial concentrations; ASP and GLU lost 40 % of their initial concentrations. GLU, ASP, and LEU constituted most of the pool degrading during P1; LYS is not correlated to the first bSiO₂ phase (Table 2).

At the end of period 2, a pool of THAA was released when 95 % of the first bSiO₂ phase was dissolved (Fig. 8a and b). THAA concentrations decreased by 32 % compared to initial values. Measurement of THAA does not release Si-bound amino acids so that they would not be observed until the bSiO₂ dissolved; the THAA released (Si(2)-THAA) may have been trapped between the 2 phases. The amount of Si(2)-THAA can be calculated from the difference between the concentrations of amino acids at and before the peak maximum (Table 2). In total, THAA concentration increased by 11-16 % (in $\mu\text{mol C L}^{-1}$); the Si(2)-THAA were composed mainly of GLY ($23 \pm 8 \%$) and LYS ($23 \pm 8 \%$; see Table 2).

During P3, relative THAA concentrations declined from 34 % to 5 % of the original THAA. The THAA lost during P3 are called Si(3)-THAA, they had a composition similar to that of total THAA, except that the contribution of GLY was higher (Table 2).

4. Discussion

4.1 Importance of bacteria in *S. marinoi* degradation

The very high concentration of algae, when compared to the bacterial concentrations, and the continued degradation in the presence of HgCl₂, suggest that the loss of organic matter was due not only to biological degradation, but also to physical and chemical factors (dissolution). In the presence of HgCl₂, organic matter turned over with a low rate constant (0.02 d^{-1} , $r^2 = 0.86$, $n = 8$), which appeared to decrease even more after day 5 (0.007 d^{-1} , $n = 4$; 2 replicates). This could be due to initial dissolution of organic matter as the cell begins to fall apart; smaller rate constants after some time would then be due to the absence of bacterial degradation. However,

the lack of appropriate samples makes this observation tentative. The slower increase of DOC concentrations compared to the decrease in POC (Fig. 3) suggests that most of the POC loss may be due to bacterial degradation despite the similar degradation rate measured in HgCl₂-poisoned batches. Degradation of particulate matter in the presence of HgCl₂ has been noted before (Liu *et al.*, 2006).

It was not clear why bacteria grew so slowly after day 14 in the unpoisoned experiment. Three factors could have contributed to the decrease and stabilization of bacterial growth. First, O₂ could have been a limiting factor. We did not measure O₂ during the experiment but we calculated average TOC loss, $R_{loss} = 221.6 \mu\text{mol L}^{-1}$. Change in oxygen concentrations (C_{O_2}) with time (t) can be reconstructed from O₂ consumption rate (R_{loss}) and O₂ diffusion rate (R_{diff}) (eq. 5). The latter is given by the Whitman film model (Gladyshev 2002; eq. 6).

$$\frac{dC_{O_2}}{dt} = R_{diff} - R_{loss} \quad (5)$$

$$R_{diff} = \frac{D}{\delta} \times \frac{S}{V} \times (Cs_{O_2} - C_{O_2}) \quad (6)$$

where δ is the thickness of the diffusion layer, and is strongly dependent on stirring; D is the O₂ diffusion coefficient ($1.83 \text{ cm}^2 \text{ d}^{-1}$, Ploug, 2001); S is the surface of contact between air and water (415.5 cm^2); V is the volume of the solution, which changed progressively during sampling; and Cs_{O_2} is the saturation concentration of the O₂ in seawater ($229.9 \mu\text{M}$). Using these equations and parameters, we estimated the maximum δ above which the solution will be anoxic. Considering that the risk of consuming all O₂ is greater for a larger volume of solution (with the same surface area), we conservatively used the volume of solution at the beginning of the experiment $V=V_0$ (8100 cm^3).

We seek a value of δ_{max} , at which $\frac{dC_{O_2}}{dt}$ will always be positive when C_{O_2} approaches 0 so that the system can never go anoxic. This condition is met when

$$R_{loss} \leq R_{diff} = \frac{D}{\delta} \times \frac{S}{V} \times C_{SO_2} \quad (7)$$

or whenever

$$\delta_{max} = \frac{D}{R_{loss}} \times \frac{S}{V_0} \times C_{SO_2} \quad (8)$$

We calculated that when the volume is at its maximum in the flask (8100 cm³ at the beginning of the experiment), the depth of the diffusive layer must be less than 0.1 cm for the solution to remain oxic. The thickness of the diffusion layer is 0.27 cm with no stirring and can be as small as 0.0015 cm when the stirring is intense (Gladyshev, 2002). Since our flasks were well mixed, it is very unlikely that O₂ was a limiting factor.

A second explanation is that degradation products built up in the flask and poisoned the bacteria (Westrich and Berner, 1984; Aller and Aller, 1998). We cannot exclude this possibility, but calculating kinetic parameters over only 25 days should alleviate some of this problem. This period of time is a reasonable compromise between the need to follow the degradation of *S. marinoi* as long as possible so as to better understand reactions in the water column and the risk of accumulating inhibiting metabolites. Finally, the bacteria might have stopped growing due to viral lysis, grazing or a lack of labile substrate fuelling their growth (Fig. 2).

4.2 Importance of Si-C interactions to bSiO₂ dissolution

Previous dissolution studies have assumed that the diatom frustule is composed of a single bSiO₂ phase (see review in Van Cappellen *et al.*, 2002b). Results from our modelling exercise suggest instead that the frustule of *S. marinoi* is composed of two phases of bSiO₂ with different dissolution characteristics. This idea, previously hypothesized by Kamatani and Riley (1979) from dissolution rate measurements and by Gallinari *et al.* (2002) from solubility equilibrium measurements, is consistent with the complexity of the frustule structure. During silicification, polyamines and silaffin proteins catalyze precipitation of organo-silicon particles

of different shape and structure that determine the morphology of different diatom species (Kröger *et al.*, 2000; Hildebrand, 2003). As a result of these interactions, diatom frustules have a complex 3-D structure and are shaped like an elliptic or cylindrical box. Each half is composed of a valve and girdle bands that are built at different times in the cell cycle (Hildebrand and Wetherbee, 2003). In our study we distinguish two bSiO₂ phases and calculate their dissolution rate constants. Even though we didn't determine a direct relation between these dissolution characteristics and the structure of the frustule, we were able to determine the impact of two-phase dissolution on the organic matrix of the cells as discussed below (see section 4.3).

Diatom frustules include organic layers that consist mainly of sugars and amino acids (Hildebrand *et al.*, 2006). The major amino acids in this coating are GLY, THR, and SER, suggesting that bonding with silica occurs through SER and THR, using their OH groups (Hecky *et al.*, 1973). GLY enrichment observed during our study in the refractory (second) bSiO₂ phase might suggest a more important role of GLY. Si-C or Si-O-C interactions are thought to protect silica from dissolution until the organic matrix is removed by bacteria (Hecky *et al.*, 1973; Patrick and Holding, 1985; Bidle and Azam, 2001). The different dissolution parameters of the bSiO₂ phases may be due to different associations between silica and organic compounds in different sections of the frustule. Indeed, Abramson *et al.* (2008) observed changes in the distribution of organic compounds inside the frustule that would support this argument. The very slow dissolution rate constant for the second bSiO₂ phase could be due to protection by the organic matrix. We suggest (1) that only a part of the bSiO₂ is protected by the organic coating, and (2) that this protection lasts for a longer time than was previously thought. Low bacterial concentration could also partially explain why the protection of the second bSiO₂ phase lasted so long, while in previous studies the protection was only temporary. The linear correlation between FFA and the total bSiO₂ indicates that these compounds are associated with both phases of bSiO₂, even though all FFA were completely degraded while 31% of the bSiO₂ still remained. Few FFA were associated with the intracellular pool of lipids. Because of their amphipathic

properties due to the carboxyl group bonded to the long carbon chain, FFA probably play a role in the organization of the organic matrix involved in building the frustule (Chevallard and Guenoun, 2006). The relationship between FFA degradation and dissolution of the two bSiO₂ phases, and the fact that FFA were completely degraded before the total dissolution of the bSiO₂, might also indicate another type of interaction, possibly adsorption of FFA on the silica surface.

In the present study, lipid classes and individual amino acids showed a general correlation with bSiO₂ concentration (Figs. 7 and 8). The modelling experiment showed that bSiO₂ and carbon pool degradation followed different patterns; they are represented in our model by different sets of equations. Moreover, in our in vitro experiment, external parameters like temperature were constant and can not be responsible for the relation observed in Figures 7 and 8. Thus we can safely suppose that a causal correlation exists between bSiO₂ dissolution and the amino acids or lipids degradation. The turnover of the portion of these organic compounds that is correlated with dissolution of either the first or second bSiO₂ phase (P1 and P3) was very slow compared to the degradation of the remaining pool of these compounds. For example, for the amino acids, the turnover of Si(1)-THAA and Si(3)-THAA was slow compared to the loss of THAA in general. This correlation suggests that there may exist a direct association between each bSiO₂ phase and the corresponding organic compounds (Si(1)-THAA, Si(3)-THAA, Si(1)-Lip and Si(2)-Lip). Since the amounts of Si-THAA and Si-TLip related to each phase were similar (~10-30 %), differences between bSiO₂ dissolution rate constants stem mainly from the compositions of the pools. Some membrane lipids mainly MGDG were mostly associated with the first bSiO₂ phase. DPG+PG still had high concentrations at the end of P2 when most degradation occurred. The second bSiO₂ phase was more strongly correlated with neutral lipid alcohols (ST and ALC) but also with membrane lipids DPG+PG and PE. In the first bSiO₂ phase, GLU, ASP, and LEU constituted most of the Si(1)-THAA pool and this phase contains no LYS at all. In contrast in the second bSiO₂ phase, Si(3)-THAA had a composition similar to the diatom's intracellular THAA, except for an increase of the GLY composition.

It is not clear whether differences in organic carbon content and/or different associations between bSiO₂ and organic carbon in each of the two bSiO₂ phases explains the differences between bSiO₂ dissolution rates and also between solubility equilibria. The organic matter that makes up part of the diatom frustule helps strengthen the structure, thus increasing its resistance to mechanical forces like those from grazers (Hamm *et al.*, 2003). The role of organic compounds in silica dissolution is, however, more difficult to determine. In addition to the bonds between silica and the OH moiety of SER or THR (Hecky *et al.*, 1973), silica particles are linked to organic compounds by nitrogen bonds (Sumper and Kröger, 2004). Indeed, Gendron-Badou *et al.* (2003) noted that Si-C and Si-N bonds are present in fresh diatoms while only Si-O-Si and Si-O-R bonds are visible in fossilized diatoms. Different associations between silica and organic compounds resist degradation and dissolution differently and may explain the differences we observed between dissolution rate constants and solubility equilibria of the two bSiO₂ phases.

The different dissolution rates of the two bSiO₂ phases might also be due to different physico-chemical properties in the silica structure itself. In the presence of some sillafins, silica precipitation of porous blocks has been observed *in vitro*; in contrast, when silica precipitation is catalyzed by polyamines, spherical silica particles are formed (Sumper and Kröger, 2004). If the first silica phase is more porous due to the presence of organic matter or due to the compounds that catalyzed silica formation, the dissolution rate constant of this bSiO₂ will be higher (Van Cappellen *et al.*, 2002a). Also, when the silica structure is chemically more organized (in mineral form, as opposed to amorphous, like opal), dissolution rate and solubility are lower. Gendron-Badou *et al.* (2003) determined that the structure of bSiO₂ from fossilized diatoms from sediments is more organized than that of fresh diatom frustules due to condensation processes that continue after they are deposited. It is also possible that fresh diatoms already have a more organized phase, as suggested by the low dissolution rate (in the present study) and the low solubility equilibrium (Gallinari *et al.*, 2002) of the second bSiO₂ phase. During dissolution, the

two different rate constants will cause an increase of the ratio of the more organized phase to the amorphous phase.

The difference between dissolution rate constants and between solubility equilibria could be due to chemical bonds between silica and organic matter or to structural characteristics of the bSiO₂. In any case it may be closely linked to the presence of organic compounds either inside the frustule itself or during the silicification process. This conclusion emphasizes the need to link studies of carbon and silica production and recycling if we are to better understand both C and Si cycles. The following section will accentuate this conclusion by showing the reverse: the importance of bSiO₂ to carbon degradation.

4.3 Si-C interactions and their role on carbon degradation

bSiO₂ dissolution was best described by Model 2, while the turnover of each organic compound investigated here was best fit by Model 1 or 3 (Fig. 5). The use of Model 3 to describe compound turnover means that either (1) most organic compounds were present as two pools of matter degrading one after the other or (2) degradation rate constants increased at some point due to a change in environmental factors. In most cases the loss rate constant increased (POC, PON, THAA, PE, and MGDG) or decreased (other lipid classes) when the dissolution of the first bSiO₂ phase was almost complete, from which we conclude that the first bSiO₂ phase must have influenced loss of organic matter.

Most of the ALC, PE and ST present in the intracellular pool of organic compounds seems to have been protected by the first bSiO₂ phase, but due to the high variability of lipid class behaviour the pattern for TLip is less clear than the one for THAA. THAA degradation is consistent with the idea that Si(2)-THAA is encased within diatom frustules, and is released as soon as the first bSiO₂ phase is completely removed. While several amino acids increased in concentration between day 11 and day 20 (Fig. 6b), bacterial numbers peaked at day 13, which corresponds to the THAA maximum (Fig. 1 and Fig. 2). However, bacterial carbon accounted

for only 0.2 to 2.4 ± 0.3 % of total carbon (Fig. 3). Bacterial biomass cannot account for the increase of THAA, but the increase of bacterial number could be explained by this input of labile organic carbon. Ingalls *et al.* (2003, 2006) measured Si-THAA (THAA bound and/or within bSiO₂) obtained after complete dissolution of the bSiO₂ using successive treatments with 6N HCl and HF. They found that Si-THAA made up 0.7-7 % of the total THAA in diatoms from plankton tows and sediment trap samples. In our study, Si-THAA was a larger portion of total THAA than in the studies of Ingalls *et al.* (2003, 2006). We define three pools of THAA: Si(1)-THAA degradation is correlated to the dissolution of the first bSiO₂ phase; Si(2)-THAA is the pool of THAA that are enclosed between the two bSiO₂ phases. Both of these pools were protected by the first bSiO₂ phase. The third pool, Si(3)-THAA, is attached to the second phase of the frustule; due to the low dissolution rate constant of this phase, it was protected for longer time.

The composition of Si(2)-THAA is dominated by GLY and LYS, which are major components of silaffins (Table 2). As part of the silicification process (Hildebrand, 2003; Sumper and Kröger, 2004), these proteins are present in the silica deposition vesicles (SDV) that become part of the diatom wall at the end of frustule formation (Martin-Jézéquel *et al.*, 2000). Thus the Si(2)-THAA pool may be assembled during the silicification process, and may result from the integration of the SDV's into the frustule. Similarity between most of the substitution times *t_s* listed in Table 4 and the release time of Si(2)-THAA suggests that *k₂* represents the degradation rate constant of this portion of THAA. After being released, Si(2)-THAA turned over very fast (*k₂* in Table 4). Most of these amino acids were completely dissolved or degraded shortly after the total dissolution of the first bSiO₂ phase, which suggests that this pool of THAA is not directly bound to bSiO₂. The high loss rate constant of Si(2)-THAA may suggest that this pool of THAA was dissolved (chemical mechanism) rather than degraded (biological mechanism) as soon as it was exposed. Another possible explanation to this fast turn over rate is a change in degradation mechanism. During degradation, amino acids are released from protein

by enzymatic cleavage at the end of the proteins (exopeptidase) or in the middle of the polypeptide chain (endopeptidase). If the proteins are opened up during the silicification process, exopeptidases could act at both ends of the protein and degradation would be faster.

Due to their high turnover rate Si(2)-THAA may have been dissolved during the strong HCl treatment or degraded before analysis in the study of Ingalls *et al.* (2003; 2006). Accordingly, the quantification of Si-THAA made by Ingalls *et al.* (2003) might have only targeted THAA bound to the second bSiO₂ phase of the frustule (Si(3)-THAA). The composition of Si-THAA in Ingalls *et al.* (2003) is similar to our Si(3)-THAA except that LYS is more abundant in Si(3)-THAA and GLY slightly less abundant (Table 2). The low solubility of the second bSiO₂ phase is consistent with the increase in Si-THAA/THAA with depth in their study.

The bSiO₂ protected from degradation the organic matter that was directly associated with the frustule (Si(1)-THAA, Si(3)-THAA and Si-Lip). The first bSiO₂ phase also protected the lipids (some DPG+PG) and amino acids (Si(2)-THAA) trapped inside the frustule possibly between the 2 phases, as shown by the correlation curve (Figs. 7 and 8). Moreover, the sudden increase in POC degradation rate (Fig. 1) after release of the trapped material is not associated with an increase in bacterial concentration (Figs. 2 and 3) but with the end of the dissolution of the first bSiO₂ phase. It is hardly a coincidence that the end of dissolution of the first bSiO₂ phase occurred exactly when the degradation of the POC, PON and THAA increased. The mechanisms behind this observation are not clear yet; the dissolution of this phase probably gave bacteria better access to the internal carbon of the cell, possibly because the integrity of the frustule can not be maintained without the presence of the first bSiO₂ phase. This could happen through increasing pores size as sometimes shown by pictures of diatom frustules during dissolution, or because the box-shaped frustule opens at the end of the first bSiO₂ phase dissolution as observed after sexual phases (Crawford 1995), both triggering cell lysis. In any case we can safely conclude that the first bSiO₂ phase of the diatom frustule also protects most of

657 the intracellular carbon; at the end of the dissolution of the first bSiO₂ phase 69% of the POC
658 was still not degraded. Due to the very low dissolution rate of the second bSiO₂ phase, the
659 associated organic compounds (Si(3)-THAA and Si(2)-Lip) might be protected for a long period
660 of time; they would even be preserved in the sediment with the bSiO₂.

661 The presence of organic compounds inside the frustule and/or during silicification
662 determines the solubility of the different parts of the frustule. Moreover, intracellular carbon and
663 Si-bound organic compounds may be protected by at least some part of the frustule. These
664 reverse interactions prove that carbon and silica production and recycling must be studied in
665 parallel if we want to improve our understanding of mechanisms driving both POC and bSiO₂
666 sedimentation.

667

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859

860 Figure legends

861 Fig. 1. Changes in the relative concentrations of POC (closed black diamonds), bSiO₂
862 (open diamonds), THAA (closed grey squares) and TLip (closed grey triangles) during the
863 degradation of *S. marinoi* in the dark at 20°C during the first 25 days of the experiment. The
864 concentrations relative to initial values are on a logarithmic scale.

865 Fig. 2. Change in total bacterial concentration over time during the 102-day degradation
866 experiment.

867 Fig. 3. Change in algal TOC (open circles), DOC (open diamonds), POC (closed squares)
868 and bacterial carbon (closed circles) with time during the degradation experiment.

869 Fig. 4. Change in the concentration of (a) 6 of the 8 lipid classes in $\mu\text{mol C L}^{-1}$ relative to
870 TLip concentrations in $\mu\text{mol C L}^{-1}$ (b) 9 of the 14 individual amino acids in $\mu\text{mol AA L}^{-1}$
871 relative to THAA in units of $\mu\text{mol AA L}^{-1}$, over time during the first 25 days of the 102-days
872 degradation experiment. Note that due to the low number of C atoms in GLY, the GLY peak in
873 $\mu\text{mol AA L}^{-1}$ is more visible than if using $\mu\text{mol C L}^{-1}$.

874 Fig. 5. Model comparisons (a) Dissolution of bSiO₂. In this experiment, bSiO₂ is the only
875 compound whose loss is best represented by Model 2. $\Delta \log(L)$ between Model 2 and Model 3
876 is 13. Period 1 is the period of time corresponding to the dissolution of 85% of the first bSiO₂
877 phase and 10% of the second bSiO₂ phase. During period 2, the last 15% of the first bSiO₂
878 dissolved and 10% more of the second bSiO₂ phase dissolved. In Period 3 only the second
879 bSiO₂ phase dissolved as less than 1.5% of the first bSiO₂ phase remained. (b) The curve depicts
880 the loss of POC (or any organic compound) with a dissolution or degradation rate constant that
881 increases with the *ts*. Model 1 fits the curve using $C_0 = 8297 \mu\text{mol C L}^{-1}$ and $k = 0.047 \text{ d}^{-1}$ with a
882 likelihood $\log(L) = 98.7$. Model 2 fits the model with the same likelihood using the same
883 parameters, i.e. $C_1 + C_2 = 8297 \mu\text{mol C L}^{-1}$, $k_1 = k_2 = 0.047 \text{ d}^{-1}$. Model 3 give the best fit

($\log(L) = 174.7$) using $C_0 = 7614 \mu\text{mol C L}^{-1}$, $t_s = 10 \text{ d}$, $k_1 = 0.025 \text{ d}^{-1}$ and $k_2 = 0.082 \text{ d}^{-1}$. This example clearly shows that only Model 3 can depict accurately the loss when the rate constant increases at some point in the experiment. Moreover, Model 2 never gives a better likelihood than Model 1 under these conditions.

Fig. 6. Change in organic compound concentrations with time during the degradation of *S. marinoi*. For clarity, 7 of the 8 lipid class concentrations in $\mu\text{mol Cl}_{\text{lip}} \text{ L}^{-1}$ over time are shown (5a) and only 10 of the 14 amino acids in $\mu\text{mol AA L}^{-1}$ over time (5b). Results depicted are only for the first 25 days. Note that THAA concentrations are in $\mu\text{mol AA L}^{-1}$.

Fig. 7. Si-TLip interactions during the degradation of the *S. marinoi*. (a) Correlation between the concentrations of the dissolved bSiO_2 from the first phase relative to its initial concentration (estimated by the model) and each lipid class relative to its initial concentration. (b) Correlation between concentration of the dissolved bSiO_2 from the second phase relative to its initial concentration (estimated by the model) and each lipid class relative to its initial concentration. Period 1 is the period of time corresponding to the dissolution of 85% of the first bSiO_2 phase and 10% of the second bSiO_2 phase. During period 2, the last 15% of the first bSiO_2 dissolved, and 10% more of the second bSiO_2 phase dissolved. In Period 3 only the second bSiO_2 phase dissolved as less than 1.5% of the first bSiO_2 phase remained.

Fig. 8. Si-THAA interactions during the degradation of *S. marinoi*. (a) Correlation between the concentration of the dissolved bSiO_2 from the first phase relative to its initial concentration (estimated by the model) and individual THAA concentrations relative to their initial concentrations. (b) Correlation between concentration of the dissolved bSiO_2 from the second phase relative to its initial concentration (estimated by the model) and individual THAA concentrations relative to their initial concentrations. The periods shown are the same as in Fig. 7.

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909 Table 1: List of abbreviations used in the text to refer to organic and inorganic compounds

910 measured during the degradation experiment.

Biogenic Silica	bSiO ₂	Total Organic Carbon	TOC
Silicic acid	dSi	Particulate organic carbon	POC
Particulate organic nitrogen	PON	Dissolved Organic carbon	DOC
Total Lipid classes	TLip	Total Hydrolyzed Amino Acids	THAA
Alcohols	ALC	Alanine	ALA
Di- and monophosphatidyl glycerides	DPG+PG	Arginine	ARG
Free fatty acids	FFA	Aspartic acid	ASP
Monogalactosyldiglycerides	MGDG	Glutamic acid	GLU
Monoglycerides	MG	Glycine	GLY
Phosphatidylethanolamines	PE	Histidine	HIS
Pigments	PIG	Isoleucine	ILE
Sterols	ST	Leucine	LEU
		Lysine	LYS
		Methionine	MET
		Phenylalanine	PHE
		Serine	SER
		Threonine	THR
		Tyrosine	TYR
		Valine	VAL
		γ -Aminobutyric acid	GABA

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912 Table 2: Concentration in $\mu\text{mol C L}^{-1}$ and composition in mole% of the different pools of THAA
 913 in *S. marinoi*. The THAA row shows the initial concentration and composition of THAA before
 914 dissolution began and does not include Si-THAA. Si(1)-THAA is the pool associated with the
 915 first bSiO₂ phase, Si(2)-THAA is the pool of THAA enclosed between the bSiO₂ phases and
 916 Si(3)-THAA is the pool associated with the second bSiO₂ phase.

THAA	ALA	ARG	ASP	GLU	GLY	HIS	ILE	LEU	LYS	PHE	SER	THR	TYR	VAL	TOT
THAA	105	223	318	379	139	59	174	317	187	260	128	136	170	174	2769
% THAA / THAA _{tot}	4%	8%	11%	14%	5%	2%	6%	11%	7%	9%	5%	5%	6%	6%	
Si(1)-THAA	20	31	122	143	12	12	48	92	0	63	16	29	58	45	691
% Si(1)-THAA/ Si(1)-THAA _{tot}	3%	5%	19%	22%	2%	2%	7%	14%	0%	10%	2%	5%	9%	7%	
Si(2)-THAA	9	0	52	37	92	17	21	19	96	21	0	12	18	21	332
% Si(2)-THAA/ Si(2)-THAA _{tot}	2%	0%	13%	9%	23%	4%	5%	5%	24%	5%	0%	3%	5%	5%	
Si(3)-THAA	20	28	51	64	72	20	26	43	53	35	29	30	26	26	523
% Si(3)-THAA/ Si(3)-THAA _{tot}	4%	5%	10%	12%	14%	4%	5%	8%	10%	7%	5%	6%	5%	5%	

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918 Table 3: Concentration in $\mu\text{mol C L}^{-1}$ and composition in mole% of the different lipid class in *S.*
 919 *marinoi*. The lipid row shows the initial concentration and composition of lipid before
 920 dissolution began and does not include Si-lipid. Si(1)-Lip is the pool associated with the first
 921 bSiO₂ phase, Si(2)-Lip is the pool of THAA associated with the second bSiO₂ phase.

Lipid class	ALC	DPG+PG	FFA	MG	MGDG	PE	PIG	ST	total
Lipids	30	98	366	104	189	44	604	40	1475
lipid class/ total lipid	2%	7%	25%	7%	13%	3%	41%	3%	
Si(1)-Lip	0	0-36	180-201	15-25	0	1-19	NC	1-11	386-469
Si(1)-Lip/ tot Si(1)-Lip	0%	0-8%	43-47%	4-5%	41-49%	0-4%		0-2%	
Si(2)-Lip	0-4	40-69	3	0	0	7-13	NC	9-20	125-175
Si(2)-Lip/ tot Si(2)-Lip	0-2%	32-39%	39-55%	0%	0%	6-7%		7-11%	

922 NC: No correlation

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924 Table 4: Kinetic parameters and likelihood ($\log(L)$) calculated by the 3 models. C_0 is the initial
 925 concentration of the compound. C_1 and C_2 are the initial concentrations of the two phases (for
 926 bSiO₂) or the two pools (organic compounds). k is the degradation/dissolution rate constant
 927 calculated with Model 1. k_1 and k_2 are degradation/dissolution rate constants of C_1 and C_2 in
 928 Model 2, respectively, or used before and after the substitution time ts in Model 3, respectively.
 929 The last column indicates which model has been chosen in this study to determine the
 930 degradation/dissolution rate constant and the initial concentration of each compound. For
 931 compounds, see abbreviations table (Table 1).

	Model 1			Model 2					Model 3					Best Model fit
	C_0	k	$\log(L)$	C_1	C_2	k_1	k_2	$\log(L)$	C_0	ts	k_1	k_2	$\log(L)$	
PON	1499	0.050	98.5	26	1473	0.051	0.051	98.5	1364	12	0.030	0.094	135.6	III
POC	8297	0.047	98.7	6884	1412	0.047	0.047	98.7	7614	10	0.025	0.082	174.7	III
bSiO₂	591	0.030	70.7	209	462	0.268	0.016	109.0	658	6	0.063	0.019	97.7	II
TLip	1232	0.033	27.9	704	724	0.014	0.100	43.5	1420	17	0.048	0.010	44.9	III
ALC	44	0.080	9.2	46	5	0.122	0.001	10.1	52	19	0.099	0.001	13.5	III
FFA	297	0.104	21.8	139	207	0.068	0.214	23.1	330	15	0.125	0.077	25.0	I
MG	97	0.045	24.5	21	85	0.497	0.033	25.0	107	5	0.094	0.036	25.5	I
MGDG	199	0.145	9.0	93	107	0.145	0.145	9.0	189	2	0.078	0.520	19.6	III
PE	54	0.097	22.3	51	3	0.097	0.097	22.3	42	6	0.016	0.119	26.6	III
PG+DPG	69	0.011	17.9	55	13	0.011	0.011	17.9	65	16	0.001	0.054	19.0	I
PIG	641	0.012	29.7	32	610	0.012	0.012	29.7	627	5	0.000	0.016	30.4	I
ST	41	0.031	30.0	11	30	0.031	0.031	30.0	35	13	0.001	0.080	38.3	III
THAA	3599	0.080	32.9	1903	1696	0.080	0.080	32.9	3137	13	0.054	0.139	42.8	III
ALA	132	0.070	42.3	31	102	0.073	0.073	42.3	117	12	0.048	0.120	54.1	III
ARG	339	0.113	26.6	39	300	0.113	0.113	26.6	243	9	0.024	0.175	54.3	III
ASP	419	0.100	24.5	312	107	0.100	0.100	24.5	324	5	0.001	0.121	29.1	III
GLU	454	0.080	39.2	23	431	0.080	0.080	39.2	405	13	0.065	0.147	50.5	III
GLY	172	0.045	24.9	67	105	0.045	0.045	24.9	147	16	0.015	0.182	39.3	III
HIS	73	0.063	28.9	44	29	0.063	0.063	28.9	65	16	0.039	0.167	38.6	III
ILE	223	0.082	34.4	210	13	0.082	0.082	34.4	192	12	0.050	0.137	44.8	III
LEU	413	0.090	34.0	117	299	0.091	0.091	34.0	348	12	0.050	0.150	48.2	III
LYS	268	0.068	18.1	181	88	0.068	0.068	18.1	223	14	0.031	0.170	26.1	III
PHE	336	0.083	31.6	332	4	0.083	0.083	31.6	280	12	0.040	0.148	45.8	III
SER	162	0.075	42.6	21	140	0.075	0.075	42.6	135	5	0.007	0.090	49.7	III
THR	165	0.064	43.1	17	149	0.064	0.064	43.1	151	13	0.045	0.110	51.1	III
TYR	185	0.068	48.3	157	28	0.068	0.068	48.3	187	21	0.070	0.012	49.3	I
VAL	219	0.076	37.9	179	39	0.076	0.076	37.9	194	12	0.050	0.124	46.5	III

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Fig. 2

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