Biodegradation of *Emiliana huxleyi* aggregates by a natural Mediterranean prokaryotic community under increasing hydrostatic pressure

Virginie Riou, Julien Para, Marc Garel, Catherine Guigue, Badr Al Ali, Chiara Santinelli, Dominique Lefèvre, Jean-Pierre Gattuso, Madeleine Goutx, Stephanie Jacquet, et al.

► To cite this version:

Virginie Riou, Julien Para, Marc Garel, Catherine Guigue, Badr Al Ali, et al.. Biodegradation of *Emiliana huxleyi* aggregates by a natural Mediterranean prokaryotic community under increasing hydrostatic pressure. *Progress in Oceanography*, 2018, 163, pp.271-281. 10.1016/j.pocean.2017.01.005 . hal-01975994

HAL Id: hal-01975994

<https://hal.science/hal-01975994>

Submitted on 9 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Biodegradation of *Emiliana huxleyi* Aggregates by a Natural 2 Mediterranean Prokaryotic Community under Increasing 3 Hydrostatic Pressure

4 Virginie Riou^{1,2}, Julien Para¹, Marc Garel¹, Catherine Guigue¹, Badr Al Ali^{1,3}, Chiara Santinelli⁴,

5 Dominique Lefèvre¹, Jean-Pierre Gattuso^{5,6}, Madeleine Goutx¹, Stephanie Jacquet¹, Frédéric A.C. Le

6 Moigne⁷, Kazuyo Tachikawa² and Christian Tamburini^{1,*}

7 (1) Aix Marseille Université, Université Toulon, CNRS, IRD, MIO UM 110, Mediterranean Institute of
8 Oceanography, Marseille, France

9 (2) CEREGE, UM34, Aix Marseille Université, CNRS, IRD, Aix-en-Provence Cedex, France

10 (3) Ministry of Higher Education, Tishreen University, High Institute of Marine Research, LATTAKIA, Syria

11 (4) C.N.R., Istituto di Biofisica, Pisa, Italy

12 (5) Sorbonne Universités, UPMC Univ Paris 06, CNRS-INSU, Laboratoire d'Océanographie de Villefranche, 181
13 chemin du Lazaret, F-06230 Villefranche-sur-mer, France

14 (6) Institute for Sustainable Development and International Relations, Sciences Po, 27 rue Saint Guillaume, F-
15 75007 Paris, France

16 (7) GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

17 * Corresponding author: christian.tamburini@mio.osupytheas.fr

Abstract

In the deep ocean, fluxes of particulate organic carbon (POC) and calcium carbonate are positively correlated, suggesting that CaCO_3 could increase sinking particle densities and/or protect the organic matter from degradation by prokaryotes, the so called “ballast effect”. Here, we used the Particle Sinking Simulator system to investigate the effect of increasing pressure on the biodegradation of calcifying *Emiliana huxleyi* aggregates. A ten days incubation was performed, simulating the changes in temperature and pressure experienced *in situ* in the NW Mediterranean Sea. Aggregates sinking from 200m to 1700m depth (assuming an average sinking velocity of 150 m d^{-1}) were exposed to a fresh natural mesopelagic prokaryotic community, with limited influence of culture-associated prokaryotes. *E. huxleyi* aggregates lipid composition revealed more degradation under increasing hydrostatic pressure (relative to constant atmospheric pressure), suggesting increased cell lysis. In parallel, changes in particulate inorganic carbon and total alkalinity indicated CaCO_3 dissolution, potentially accelerated under pressure. However, increased hydrostatic pressure also had a positive effect on particle aggregation, which may compensate the effect of increased cell lysis. Our results imply that in coccolithophorid-dominated sinking aggregates, the ballasting and protection effects of coccoliths may collapse throughout the water column. The increase in aggregation potential with pressure observed in controlled conditions might balance the loss of mineral ballast to a certain extent, although this needs to be confirmed *in situ*.

Keyword: biological carbon pump; mesopelagic; mineral ballast; coccolithophorid; prokaryotes; biodegradation; hydrostatic pressure

38Introduction

39The ocean's biological carbon pump transports carbon into the deep ocean in the form of organic
40matter (Eppley and Peterson, 1979). Between 1-40% of the organic carbon newly formed from CO₂
41fixation at the sea surface is transported to the deep sea (Ducklow et al., 2001), away from direct
42contact with the atmosphere. This process absorbs a large portion of the anthropogenic CO₂
43emissions in the atmosphere, limiting greenhouse CO₂ gas build-up (Le Quéré et al., 2010). However,
44many climate-sensitive factors can influence organic carbon export and remineralization by
45mesopelagic heterotrophs, such as the mineral content of sinking particles (Armstrong et al., 2001;
46Kwon et al., 2009).

47Globally, approximately 5-20 Pg C yr⁻¹ leaves the euphotic zone (20-120m depth) in the form of
48sinking particulate organic carbon (POC) (Henson et al., 2011; Siegel et al., 2014). However, most of
49this POC is potentially remineralized to CO₂ in the upper mesopelagic zone (Buesseler and Boyd,
502009; Giering et al., 2014), where the activity of particle-attached prokaryotes and zooplankton play
51an important role in POC attenuation (Cho and Azam, 1988; Giering et al., 2014; Turley and Mackie,
521994, 1995). On one hand, sinking POC collected *in situ* can be rapidly remineralized by attached
53prokaryotes, as demonstrated by incubations done at atmospheric pressure (Goutx et al., 2007;
54Panagiotopoulos et al., 2002; Sempéré et al., 2000). On the other hand, the remineralisation of
55sinking particles (diatom aggregates or faecal pellets) and dissolution of biogenic minerals (opal) by
56fresh natural surface prokaryotic assemblages were observed to slow down under increasing pressure
57(Tamburini et al., 2006, 2009). Correlation between POC and mineral fluxes (calcium carbonate, opal,
58clays) in the deep ocean has lead to the interpretation that “ballast” minerals exert an important
59control over POC export (Armstrong et al., 2001; Francois et al., 2002; Klaas and Archer, 2002).

60Oceanic provinces in which calcifying plankton dominates display lower mesopelagic remineralisation
61fluxes than systems dominated by opal-producing diatoms (Cardinal et al., 2001, 2005; Jacquet et al.,
622005). In sinking particles collected *in situ*, the presence of minerals has been suggested to preserve

63organic matter from degradation (Hedges et al., 2001; Ingalls et al., 2003). These observations suggest
64that mineral and POC dissolutions in the water column may be inter-related. To understand these
65interactions, a few biodegradation studies have been conducted using aggregates obtained from
66cultures of opal-protected diatoms, which form important seasonal blooms (Treguer and Pondaven,
672000). Biologically-mediated biogenic opal dissolution has been observed to increase with organic
68matter degradation in surface waters (Bidle and Azam, 1999, 2001; Goutx et al., 2007) and to
69diminish with temperature decrease (Bidle et al., 2002) or hydrostatic pressure increase (Tamburini et
70al., 2006). Adding another ballast mineral (CaCO_3) to diatom aggregates diminished diatom aggregate
71remineralisation by zooplankton (Le Moigne et al., 2013). Regarding CaCO_3 , biologically-mediated
72dissolution has also been suggested (Antia et al., 2008; Harris, 1994; Jansen and Wolf-Gladrow, 2001;
73Milliman et al., 1999) to explain the fact that significant CaCO_3 dissolution is occurring in the upper
741000 m of the water column above the calcite or aragonite saturation horizon (Wollast and Chou,
751998, 2001). However, there is still no or contradictory evidence that this dissolution could be
76microbiologically-mediated (Bissett et al., 2011), and the link between CaCO_3 dissolution and POC
77degradation remains unclear.

78Ocean acidification experiments using diatom aggregates supplemented with CaCO_3 ballast indicate
79that CaCO_3 dissolution and POC degradation will potentially increase with increasing pCO_2 , a
80phenomenon that is exacerbated under increasing pressure (de Jesus Mendes and Thomsen, 2012).
81However, little is known about the biodegradation of calcifiers aggregates. Among the calcifiers,
82coccolithophorids contribute to air-sea CO_2 exchange, through their combined photosynthesis (sink of
83 CO_2) and calcification (source of CO_2) activities (Poulton et al., 2007). Coccolithophorid aggregates
84formation has been reported from many recent lab studies (Biermann and Engel, 2010; Chow et al.,
852015; Engel et al., 2009a, 2009b; Iversen and Ploug, 2010), but they are considered as being more
86sensitive to fragmentation than diatom aggregates (De La Rocha and Passow, 2007).

87Laboratory biodegradation experiments using aggregates from the coccolithophore *Emiliania huxleyi*
88showed that heavily calcified strains have higher settling velocities and are better preserved over time

89than poorly calcified strains (Engel et al., 2009a, 2009b). Oxygen consumption within *E. huxleyi*
90aggregates can be 4 to 7 times larger than that of marine snow and diatom aggregates, indicating that
91they may be more prone to degradation (Ploug et al., 2008). Nevertheless, the carbon-specific
92respiration rate per meter settled was found to be lower in *E. huxleyi* aggregates than in diatom
93aggregates of similar size (Iversen and Ploug, 2010). This potentially confers coccolithophorid
94aggregates a larger potential for transporting organic carbon deeper in the ocean relative to diatom
95aggregates. It is important to note that the biodegradation experiments cited above were conducted
96in the presence of culture-associated prokaryotes or natural prokaryotic assemblages stored for a
97long time before use and the effect of fresh natural prokaryotic assemblages remains to be assessed.
98In addition, the increase in pressure experienced by aggregates sinking through the water column has
99yet to be included in such experiments.

100The objective of the present study was therefore to investigate the effect of hydrostatic pressure on
101the lipid composition (an indicator of cell degradation) and CaCO_3 dissolution within *E. huxleyi*
102aggregates in the presence of fresh free-living natural prokaryotic assemblages collected at 200 m
103depth in the NW Mediterranean Sea. Incubations were performed under controlled pressure
104conditions simulating a sinking rate of 150 m d^{-1} , in which we monitored the structure of the
105prokaryotic communities as well as the degradation of *E. huxleyi* lipids over 10 days following the
106experimental design presented in Tamburini et al. (2009). Total alkalinity, particulate inorganic and
107organic carbon (PIC and POC) and total organic carbon (TOC) concentrations were also monitored as
108indicators of CaCO_3 dissolution and organic carbon remineralization.

109**Results**

110Two sets of incubations (Exp A and Exp B) were performed at 13°C , with two pressure conditions
111applied in parallel: constant atmospheric pressure (ATM) and increasing hydrostatic pressure
112corresponding to a sinking simulation from 200 to 1700 m depth (HP). The *E. huxleyi* aggregate
113dilutions in either sterile-filtered (Exp A) or fresh GF/F-filtered (Exp B) seawater were sampled at t0

114and after 10 days in the incubation vessels (t10). Exp A was performed with sterile-filtered seawater
115to monitor the development of prokaryotes associated with the *E. huxleyi* culture, while Exp B was
116designed to observe the development on *E. huxleyi* aggregates of a natural freshly collected seawater
117free-living prokaryotic community, and its effect on the organic and inorganic carbon contents of the
118aggregates.

119***Prokaryotic development***

120The prokaryotic abundance associated with *E. huxleyi* culture aggregates (Exp A) did not change
121significantly between the start (t0: $7.7 \pm 1.8 \times 10^3$ cells mL⁻¹, average $\pm$ SD) and end of the incubation
122(ATMt10: $6.5 \pm 0.2 \times 10^3$ cells mL⁻¹; HPt10: $9.4 \pm 3.92 \times 10^3$ cells mL⁻¹). Normalizing cell concentrations by
123the total organic carbon (TOC) concentration measured in each replicate to account for potential
124variations in the initial amount of aggregates added to each bottle, no significant difference was
125observed between both pressure conditions (Fig. 1A).

126In Exp B, the total prokaryotic abundance increased by two orders of magnitude after 10 days
127(ATMt10: $2.2 \pm 0.4 \times 10^6$ cells mL⁻¹, HPt10: $2.7 \pm 0.0 \times 10^6$ cells mL⁻¹), with no significant difference
128between pressure treatments, when normalised by TOC (Fig. 1A). The growth of prokaryotes attached
129to *E. huxleyi* aggregates was stimulated under increasing pressure incubation (HP), where large
130aggregates were observed at the end (Suppl. Fig. 1). In this condition, attached prokaryotes ($> 2 \mu\text{m}$)
131were significantly more abundant (35.9 ± 14.7 % of total DAPI positive cells) than under constant
132atmospheric pressure (20.9 ± 4.7 %, Mann-Whitney-MW $p < 0.05$).

133Under both atmospheric and increasing pressure conditions, abundances of *Archaea* and *Bacteria*
134were two and three orders of magnitude higher than at t0, respectively. Although the proportion of
135*Euryarchaea* was 4.1 times lower under pressure (Fig. 1B, significant, MW $p < 0.05$), increasing
136pressure had no effect on the proportion and abundance of *Crenarchaea* (ATMt10: $2.3 \pm 0.2 \times 10^4$ cell
137mL⁻¹, HPt10: $2.6 \pm 0.4 \times 10^4$ cell mL⁻¹, MW $p = 0.51$) and *Bacteria* (ATMt10: $1.3 \pm 0.2 \times 10^6$ cell mL⁻¹, HPt10:
138 $1.4 \pm 0.2 \times 10^6$ cell mL⁻¹, MW $p = 0.83$). Among the *Bacteria*, Alpha- and Gamma-proteobacteria cell

139 concentrations increased by three orders of magnitude during the incubations, and the fraction of
 140 Alphaproteobacteria was twice as low in conditions of increasing HP than at ATM (not significant,
 141 MW $p = 0.13$). The proportion of Bacteroidetes also tended to be on average 1.9 times lower in HP
 142 than in ATM conditions (not significant, MW $p = 0.83$), but their concentrations increased only by one
 143 to two orders of magnitude.

144
 145 **Figure 1: (A)** Total prokaryotic abundance (DAPI-stained cells) normalized by sample TOC concentration in
 146 *Emiliania huxleyi* aggregates biodegradation Exp A (no natural prokaryotes added) and Exp B (GF/F-filtered
 147 natural prokaryotic community added) initially (t0) and after 10 days under constant atmospheric pressure
 148 (ATMt10) or increasing hydrostatic pressure (HPt10). White bars: free-living prokaryotes; Black bars: >2μm
 149 particle-attached prokaryotes; (B) Percent of total prokaryotes (DAPI-stained cells) at the end of Exp B,
 150 detected by catalyzed reporter deposition coupled with fluorescence *in situ* hybridization (CARD-FISH). Eub
 151 mix (Eub338, Eub-II and Eub-III): *Bacteria*; CF319a: *Bacteroidetes*; Alf968, Gam42a: alpha- and gamma-
 152 subclasses of *Proteobacteria*; Cren537: *Crenarchaea*; Eury806: *Euryarchaea*. Average ± SD. Significant differences
 153 between ATM and HP are indicated as **a** (Mann-Whitney $p < 0.05$) for particle-attached (A) or total prokaryotes
 154 (B).

155 **E. huxleyi detritus organic carbon content and lipid degradation**

156 During the Exp B, TOC and particulate organic carbon (POC) concentrations tended to decrease over
 157 the 10 days incubation under atmospheric pressure only (Fig. 2A, ATMt10, not significant, $p = 0.13$).
 158 The POC pigment content decreased significantly after 10 days of exposure to natural prokaryotic

159assemblages, with no effect of pressure (Fig. 2B). In contrast, the lipid content (excluding pigments)
 160decreased significantly in HPt10 but not in ATMt10 (Fig. 2C).

161
 162**Figure 2: Variation in organic carbon composition in Exp B: (A) total or particulate organic carbon (TOC-empty**
 163**circles, or POC-filled circles, $\mu\text{mol L}^{-1}$), (B) POC pigment fraction (%), (C) POC lipid fraction (pigments excluded,**
 164**%).** Significant differences (Mann-Whitney $p < 0.05$) are indicated as: **a** between t0 and t10. Circled data points
 165belong to the same ATM and HP replicates circled in Fig. 4.

166At the start of Exp B, the lipid composition in *E. huxleyi* aggregates was similar to the one described in
 167an earlier study with the same strain of *E. huxleyi* in exponential growth (Bell & Pond, 1996; see
 168suppl. Table 1). Overall, chloroplast lipids (including pigments and membrane monogalactosyl-
 169diacylglycerol-MGDG) and cell membrane lipids (phospholipids) dominated the lipid composition
 170(61.9-63.1 %). Sterols, hydrocarbons, neutral lipids (wax esters, triglycerides, methyl esters, ketones,
 171free fatty acids and alcohols) and metabolites (degradation lipids: mono- and di-acylglycerols)
 172accounted for 35.1-37.2 % of the particle lipids.

173Within the non-pigment lipids, a significant decrease in the proportion of chloroplast membrane and
 174whole cell membrane lipids (MGDG and PL, t0 9.8-18.2 % and 25.4-33.5 %, respectively) was
 175observed in both ATMt10 (2.5-6.8 % and 4.8-6.8 %) and HPt10 samples (0.0 % and 4.7-8.5 %).
 176Meanwhile, the proportion of hydrocarbons, methyl esters and ketones increased significantly in both

177conditions (Fig. 3). HPt10 samples experienced significant losses of membrane sterols and MGDG
 178relative to ATMt10 samples. At the same time, the relative proportion of reserve wax esters and
 179triglycerides were significantly higher in HP (HP: 5.9-12.5 %; ATM: 0.0-5.5 %), mainly due to the
 180appearance of triacylglycerides.

181
 182Figure 3: Variation in the lipid composition of *E. huxleyi* detritus in Exp B: Proportions of the different lipids
 183(pigments are excluded, % L_{tot}). MGDG: monogalactosyldiacylglycerol, PL: phospholipids, ST: sterols, HC:
 184Hydrocarbons, WE+TG: wax esters and triglycerides, ME+KET: methyl esters and ketones, ; Average $\pm$ SD.
 185Significant differences (Mann-Whitney $p < 0.05$) are indicated as: **a** between t0 and t10, **b** between ATM and HP.

186Inorganic carbon content variations

187A significant drop of particulate inorganic carbon (PIC, Fig. 4A) was observed in both ATM and HP
 188conditions after 10 d ($p < 0.05$). It was larger under HP than at ATM (not significant, $p = 0.13$). Total
 189alkalinity (A_T) increased significantly between t0 and ATMt10 (Fig. 4B, $p < 0.05$) but the increase was
 190significantly lower under HP. The increase in A_T observed between t0 and HPt10 was indeed not
 191statistically significant ($p = 0.27$).

192

193 **Figure 4: Variation in (A) particulate inorganic carbon (PIC, $\mu\text{mol L}^{-1}$), (B) total alkalinity (A_T , meq kg^{-1}), and (C)**
 194 **A_T converted to $\mu\text{mol L}^{-1}$ (using a seawater density of 1028.7 L kg^{-1}) vs PIC in Exp B. Full and dashed lines on**
 195 **panel C model the expected co-evolution of A_T and PIC during calcite dissolution. Significant differences (Mann-**
 196 **Whitney $p < 0.05$) are marked with an “a” between t0 and t10, “b” between ATM and HP. Circled data points**
 197 **point at the ATM2 and HP3 replicate incubations displaying values out of the expected range for calcite**
 198 **dissolution from the measured t0 values shown in panel C.**

199 Discussion

200 *Effect of hydrostatic pressure increase on the prokaryotic community*

201 During the present *E. huxleyi* aggregate biodegradation experiment, initial natural mesopelagic
 202 Mediterranean Sea prokaryotic concentrations increased by up to two orders after 10 days in
 203 controlled temperature and hydrostatic pressure (HP) conditions simulating particle sinking from
 204 200m to 1700m depth at a rate of 150 m d^{-1} . Such a high prokaryotic development has recently been
 205 observed with deep Mediterranean seawater natural prokaryotic communities amended with labile
 206 marine broth, both under atmospheric (ATM) and HP conditions (Wannicke et al., 2015). This might
 207 therefore indicate that *E. huxleyi* aggregates constitute a relatively labile substrate for mesopelagic
 208 Mediterranean prokaryotic communities to grow on.

209 The natural prokaryotic diversity was dominated by *Bacteria*, with a lower proportion of *Archaea* at
 210 the end of the incubation, as previously observed in similar experiments (Tamburini et al., 2006,

2112009), despite a significant increase in absolute abundance. The initial fraction of *Bacteria*,
212*Crenarchaea* and *Euryarchaea* were lower in the present experiment (30%, 7% and 4%, respectively)
213than in previous experiments with 200m depth seawater sampled in different areas of the
214Mediterranean Sea (Tamburini et al., 2006, 2009). Different sea surface mixing conditions at the time
215of the natural prokaryotic community sampling for the different experiments might have resulted in
216different initial community compositions (Pinazo, pers. Com). When focusing on the classes of
217*Bacteria* generally found associated to marine aggregates (Acinas et al., 1999; Delong et al., 1993;
218Moeseneder et al., 2001; Ploug and Grossart, 1999; Simon et al., 2002; Thiele et al., 2015), only
219Bacteroidetes and Alphaproteobacteria tended to be sensitive to increasing hydrostatic pressure
220conditions (HP). Similar trends were observed during previous particle sinking simulation experiments
221with natural faecal pellets (Tamburini et al., 2009). The latter authors proposed that the sensitivity of
222Bacteroidetes to pressure may have reduced the amount of particulate carbohydrate hydrolysed. This
223could result in a lower production of dissolved carbohydrates as substrate for Alphaproteobacteria
224(Tamburini et al., 2009). A similar mechanism might be at play in the present experiment with *E.*
225*huxleyi* detritus, which is supported by the observation of large sticky particles and aggregates under
226HP condition only, as in Tamburini et al. (2009)(Suppl. Fig. 1). These large sticky aggregates might
227indeed be the result of less degradation of transparent exopolymeric particles (TEP) under HP relative
228to ATM (Tamburini et al., 2009), associated with the fact that more dissolved carbohydrates may have
229been released under HP by *E. huxleyi*, possibly resisting prokaryotic degradation (Nanninga and
230Tyrrell, 1996) and scavenged by TEP (Engel et al., 2004).

231Interestingly, we observed that prokaryotes grew significantly more on > 2 µm aggregates under
232increasing pressure (HP) than at atmospheric pressure (ATM). This pattern was not observed in a
233previous similar controlled experiment studying the biodegradation of natural aggregates collected in
234the Mediterranean Sea (mainly faecal pellets) during 6.5 days, although the formation of visible sticky
235aggregates had also been observed under HP (Tamburini et al., 2009). The fact that the present
236simulation lasted longer and reached higher pressure levels could explain that the pattern observed

237here may not have been detected in the previous study. Finally, it also is also possible that
238coccolithophorid cells are more sensitive to pressure than faecal pellets or diatom aggregates
239(Tamburini et al., 2006, 2009). Hence, more labile intracellular organic matter would be released in
240the aggregates surroundings, favouring higher prokaryotic growth. To verify the latter hypothesis, we
241analysed the aggregate lipid contents and calculated a lipolysis index.

242***Degradation of E. huxleyi aggregates organic matter***

243Assuming a prokaryotic cellular carbon content of 12.4-53.0 fg C cell⁻¹ (Fukuda et al., 1998; Kogure
244and Koike, 1987), prokaryotes made up for 0.05-0.21% of the TOC at t₀, which increased to 1.84-
24511.44 % at ATMt₁₀ and 2.32-11.94 % at HPt₁₀. Heterotrophic growth could have been supported by
246the dissolved organic matter present at the start of the incubation, and/or by the degradation of *E.*
247*huxleyi* aggregates. A lipolysis index defined as the ratio of metabolites resulting from acyl-lipid
248degradation to the sum of acyl lipids can be used to characterize the degradation stage of labile
249organic matter (Goutx et al., 2000, 2003). Although the lipid index in *E. huxleyi* aggregates increased
250significantly between the beginning (t₀: 0.72±0.06) and the end of the experiment (ATMt₁₀:
2512.07±1.22, HPt₁₀: 1.93±0.32), there was no significant pressure effect (MW $p = 0.51$). However, the
252lipid fraction of *E. huxleyi* detritus organic carbon decreased significantly after ten days under HP,
253contrasting with previous observations using natural aggregates, mainly consisting in faecal pellets
254(Tamburini et al., 2009). In addition, compared to the lipid composition at ATM, membrane lipids
255(phospholipids, sterols, MGDG) disappeared from the particulate fraction significantly more under HP
256than at ATM, which could indicate higher *E. huxleyi* cell lysis under pressure (Baldi et al., 1997). *E.*
257*huxleyi* aggregates might therefore be more vulnerable to degradation under pressure than faecal
258pellets.

259 ***Changes in mineral content***

260 Particulate inorganic carbon (PIC) decreased more under HP than at ATM, suggesting that more
261 calcite dissolution may have occurred under increased hydrostatic pressure (Fig. 4). Enhanced PIC
262 dissolution under HP has been observed in a previous study with diatom-mineral (carbonate-
263 kaolinite-smectite) aggregates, together with a decreased POC decay rate (de Jesus Mendes and
264 Thomsen, 2012). We here show that increased CaCO_3 dissolution can occur in calcite-sheathed cell
265 aggregates sinking through mesopelagic waters in the presence of natural prokaryotic communities.

266 According to the stoichiometry of calcification, two moles of total alkalinity are produced per mole of
267 CaCO_3 dissolved (approximated as moles of PIC in Fig. 4C). However, although there was more calcite
268 dissolution under pressure, the increase in total alkalinity was larger at atmospheric pressure.
269 Nonetheless, values were within the range expected for calcite dissolution, considering the starting
270 total alkalinity and PIC conditions (Fig. 4C), except for the two outliers circled in Fig. 4 (namely ATM2
271 and HP3 incubations, also circled in Fig. 2). Processes other than CaCO_3 dissolution may explain the
272 outlying PIC- A_T pairs measured in replicates ATM2 and HP3, which respectively also displayed the
273 lowest prokaryotic abundance ($1.77 \cdot 10^6 \text{ cell mL}^{-1}$) and POC concentration ($30.7 \mu\text{mol L}^{-1}$).

274 Total alkalinity may indeed be affected by microbial aerobic and anaerobic organic matter
275 remineralization/transformation through the consumption or release of ammonium, nitrate,
276 phosphate and sulphate (Gattuso et al., 1999). The processes of respiration, nitrification and sulphate
277 reduction have adverse effects on protons release/uptake. However, the difference of $+31 \mu\text{mol L}^{-1} A_T$
278 between the value measured in ATM2 and the value expected from the triplicate t0 PIC
279 concentrations cannot be solely explained by dissimilation of ammonium by organic matter
280 respiration. This process would result in a A_T increase of 0.17 moles per mole of TOC respired (Gattuso
281 et al., 1999), when the difference in TOC between t0 replicates and ATM2 was only 5.4 to $21.6 \mu\text{mol L}^{-1}$
282¹ (which was comparable to the other 2 ATM replicates). Since the t0 replicates consisted in
283 independent splits of the initial aggregate-seawater mixture that was further split into ATM and HP
284 replicate incubations, another explanation for this outlying pair could be that the ATM2 incubation

25

285may have started with a higher PIC concentration around 65 μM (higher concentration of detached
286coccoliths).

287Similarly, the $-31 \mu\text{mol L}^{-1}$ difference in A_T between the value measured in HP3 and the expected value
288cannot be explained solely by the dissimilation of nitrate by organic matter respiration. Such a
289process would lead to a decrease in A_T of 0.13 moles per mol of CO_2 released (Gattuso et al., 1999),
290whereas the difference in TOC concentration between t0 replicates and HP3 comparable to that with
291ATM2 was observed (Fig. 2). Given the large A_T variability observed between the independent splits
292sampled at t0, it is more likely that the HP3 incubation started with a much lower A_T than the other
293HP replicates (around $2635 \mu\text{mol L}^{-1}$, or $2561 \mu\text{mol kg}^{-1}$ at $50 \mu\text{M PIC}$).

294***Implications for the carbon pump and the ballast hypothesis***

295In the Mediterranean Sea, CaCO_3 -sheathed coccolithophores can dominate the phytoplankton
296community (Ignatiades et al., 2009). They are highly diverse, including large numbers of the
297ubiquitous species *E. huxleyi* (Cros and Fortuño, 2002; Knappertsbusch, 1993). Several studies have
298stressed the need for controlled experiments to validate the hypothesis that CaCO_3 may be a more
299efficient POC “carrier” than biogenic silica (De La Rocha and Passow, 2007; Passow and De La Rocha,
3002006; Passow, 2004). Our experiment contributes to a better understanding of this issue. In contrast
301with previous experiments using natural marine snow/faecal pellets (Tamburini et al., 2009; Turley,
3021993), organo-mineral aggregates (de Jesus Mendes et al., 2007) or diatom culture aggregates (de
303Jesus Mendes and Thomsen, 2012; Tamburini et al., 2006), CaCO_3 -containing *E. huxleyi* culture
304aggregates appear more sensitive to the increase in hydrostatic pressure. Our results suggest that
305increased pressure results in higher cell lysis and CaCO_3 dissolution relative to ATM. The protecting
306effect of coccoliths may therefore not remain effective throughout the water column, since coccoliths
307might detach from cells and dissolve more readily under pressure. Calcium carbonate may therefore
308not be an efficient POC carrier to the deep ocean in the form of intact coccolithophorid cells.

309Coccolithophores are the main producers of the pelagic CaCO_3 biogenically produced in the global
310ocean ($0.8\text{--}1.4 \text{ Gt year}^{-1}$) (Feely et al., 2004) and may contribute to about 50% of pelagic CaCO_3
311deposition in sediments (Broecker and Clark, 2009; Poulton et al., 2007). Yet, the processes and rates
312of coccolith CaCO_3 dissolution in the upper layers of the ocean are poorly identified and quantified.
313Since calcification decreases the ocean CO_2 uptake capacity by reducing total alkalinity, the depth of
314calcite dissolution likely has important implications on the net gradient in pCO_2 between the ocean
315and atmosphere (Antia et al., 2001; Barrett et al., 2014).

316The dissolution of pelagic CaCO_3 particles has long been assumed to occur primarily at great depths
317below the calcite and aragonite saturation depths (Broecker, 1977). In fact, up to 80% of the CaCO_3
318that is exported out of the surface ocean might actually dissolve in the upper 1000 m, well above the
319lysocline (Chung et al., 2003; Feely et al., 2004; Iglesias-Rodriguez et al., 2002; Jansen and Wolf-
320Gladrow, 2001; Milliman et al., 1999; Wollast and Chou, 1998, 2001). Grazing by microzooplankton
321has been shown to play an important role in coccolithophorid calcite dissolution (Antia et al., 2008;
322Harris, 1994; Jansen and Wolf-Gladrow, 2001; Milliman et al., 1999). In contrast, carbonate
323dissolution on individual calcifying cells by microbes was deemed negligible (Bissett et al., 2011), but
324the presence of chemical gradients within sinking aggregates (Ploug and Bergkvist, 2015) may create
325micro-environments with lower pH and calcite saturation state that could facilitate (a)biotic
326dissolution. We show here that calcium carbonate dissolution in the presence of natural prokaryotic
327communities might be higher under increasing hydrostatic pressure. Pressure was recently suggested
328to affect dissolution kinetics over and above its influence on the saturation constants (Dong et al.,
3292016). The combined effect of pressure and microbial activities therefore remains to be assessed in a
330more mechanistic manner.

331On the other hand, we show that increasing hydrostatic pressure potentially triggers the
332development of Mediterranean mesopelagic prokaryotic communities on *E. huxleyi* aggregates (as
333opposed to diatom detritus or natural aggregates, Tamburini et al., 2006, 2009), as well as
334aggregation. The study by Tamburini et al. (2009) had already shown that particulate

335carbohydrates:POC and Transparent Exopolymer Particles (TEP):POC ratios could be significantly
336higher in conditions of increasing hydrostatic pressure. The prokaryotes themselves may promote
337aggregation, through the production of sticky extracellular muco-polysaccharides (Biddanda and
338Pomeroy, 1988; Biddanda, 1986; Turley, 1992). Moreover, coccoliths have been proposed as
339aggregation nuclei for colloidal DOM, therefore offering organic matter protection from
340solubilization/remineralization (Engel et al., 2009a). The increased aggregation observed under
341pressure in our reaction vessels could therefore compensate the loss of mineral ballast at depth, by
342increasing aggregate sizes and/or mineral scavenging potential. To what extent pressure-induced
343aggregation balances calcite dissolution *in situ* remains to be observed and assessed, by including
344model calculations, for instance.

345Methods

346*Production of Emiliana huxleyi aggregates and experimental design*

347The TW1 *E. huxleyi* strain (AlgoBank-Caen) was maintained in exponential growth phase in K/2
348medium (Keller et al., 2007) at 21°C, under a 12-12h light-dark cycle (intensity: 4300 Lux). The culture
349was supplemented with Provasoli's antibiotic concentrate (3% final concentration, Sigma) until the
350bacterial numbers reached a minimal proportion of one to two prokaryote per phytoplankton cell.
351The antibiotic solution was then removed for two culture passagings before *E. huxleyi* cells in
352exponential phase were concentrated by centrifugation (3000 rpm, 15 min) (Houdan et al., 2005;
353Sawada and Shiraiwa, 2004). Fresh detritus was obtained according to a procedure modified from
354(Tamburini et al., 2006) by exposing cell culture to 55 °C for 10 min only, which avoided cell disruption
355observed during freeze-thaw cycles. The detritus aliquots were immediately diluted in sterile-filtered
356or GF/F-filtered Mediterranean Seawater (for Exp A and Exp B, respectively, see details below) to
357reach a final concentration of about 600 µg POC L⁻¹ (cell density of 5000 cell mL⁻¹) close to natural
358coccolithophore bloom (Holligan et al., 1993). Aliquots were sampled at the starting point of the
359incubations (t₀) for the various microbiological and chemical parameters described below.

360 ***Incubation in the PArticle Sinking Simulator (PASS)***

361 The two experiments (Exp A and Exp B) were conducted in March 2007 using Mediterranean
362 seawater (13°C) collected at 200 m depth, above the Marseilles Trench (43°01'48 N-05°11'158 E,
363 600m bottom depth). In Exp A, seawater was left in the dark at 4 °C for 15 d and gently filtered
364 through 0.2 µm-porosity polycarbonate filters to subtract the prokaryotic community. In Exp B, fresh
365 natural prokaryotic assemblages <0.7 µm were recovered in the filtrate of a gentle filtration onto pre-
366 combusted GF/F filters of the freshly collected seawater sample.

367 Exp A and Exp B were incubated for 10 d at *in situ* temperature (13°C). The PASS system was used
368 following a procedure described in (Tamburini et al., 2009), except a peristaltic liquid dispenser
369 (Jencons Scientific Ltd.) was used to obtain homogeneous replicate aliquots. Aliquots were incubated
370 in 500 mL high-pressure bottles (HPBs) where pressure increased linearly using a piloted pressure
371 generator in order to reproduce the increase in pressure that occurs when particles sink.
372 Coccolithophores detritus was kept in suspension by regular half-revolutions of the HPBs in water
373 baths kept at *in situ* temperature (Tamburini et al., 2006). For Exp A, two duplicate HPBs were kept at
374 atmospheric pressure and two duplicate HPBs were exposed to increasing pressure (1.5 MPa d⁻¹
375 corresponding to a sinking rate of 150 m d⁻¹). This is applicable to mid-density particles, as discussed
376 in (Tamburini et al., 2006) and corresponds to sinking rates observed in previous *E. huxleyi* aggregates
377 studies (Engel et al., 2009b; Iversen and Ploug, 2010; Ploug et al., 2008). Exp B was performed with
378 triplicate HPBs. After 10 days of incubation (HPT10, corresponding to a depth of 1700 m), the
379 pressurised coccolithophores detritus dilutions were decompressed and sampled as described in
380 (Tamburini et al., 2009) for the parameters described below.

381 ***Microbiological analyses***

382 Samples for prokaryotic abundances (50-135 mL) were fixed using 0.2-µm filtered formaldehyde (2%
383 final concentration), kept at room temperature for 15 min, and subsequently stored at 4°C in the dark
384 for around 12h. For total (>0.2 µm) and free-living (0.2-2.0 µm) prokaryotic abundances, samples

385 were filtered onto 0.2- μm -pore-size polycarbonate filters supported by 0.45- μm -pore-size cellulose
 386 nitrate filters directly, or after filtration by gravitation onto 2.0- μm -pore-size polycarbonate filters, as
 387 in (Tamburini et al., 2009). Cells were stained with diamidinophenylindole (DAPI) according to (Porter
 388 and Feig, 1980). The fraction of “attached” or large prokaryotes $>2\ \mu\text{m}$ was calculated by subtracting
 389 values of the 0.2-2.0 μm fraction from the total fraction.

390 Samples for catalyzed reporter deposition coupled with fluorescence *in situ* hybridization (CARD-FISH)
 391 were treated as in (Tamburini et al., 2009): briefly, samples were filtered onto 0.2- μm -pore-size
 392 polycarbonate filters as for the total prokaryotic abundances. Filters were washed twice with 0.2- μm
 393 filtered MilliQ water, air-dried, and stored in scintillation tubes at -20°C until analysis. Cells were
 394 embedded in low-gelling point 0.1% agarose (Sigma), dried at 37°C for 10min, and dehydrated with
 395 95% ethanol. Bacterial cell walls were permeabilized by subsequent treatments with lysozyme (10 mg
 396 ml^{-1} , Sigma)(Amann et al., 1995) and with achromopeptidase (60 U, Sigma)(Sekar et al., 2003).
 397 Archaeal cell walls were permeabilized with proteinase K (0.2 ml ml^{-1} , Fluka)(Teira et al., 2004). Acid
 398 treatment in 0.01M HCl was performed to denature endogenous peroxylase before hybridization
 399 with the horseradishperoxylase (HRP)-labeled oligonucleotide probes listed in Table1: for a detailed
 400 protocol see (Tamburini et al., 2009). The filter portions hybridized with each probe were mounted in
 401 a Citifluor:Vectashield:DAPI mix, and DAPI and CARD-FISH-stained cells were quantified under an
 402 Olympus BX61 microscope equipped with a 100-WHg-lamp and appropriate filter sets according to
 403 (Tamburini et al., 2009).

404 **Table 1:** 16S rRNA-targeted oligonucleotide probes used in this study.

Probe	Sequence(5'-3')	Target organisms	% Formamide	References
Eub338/I	GCT GCCTCCCGTAGGAGT	Domain of bacteria	55	(Amann et al., 1990)
EubII	GCA GCCACCCGTAGGTGT	Domain of bacteria	55	(Daims et al., 1999)
EubIII	GCT GCCACCCGTAGGTGT	Domain of bacteria	55	(Daims et al., 1999)
ALF968	GGTAAGGTTCTGCGCGTT	Most of Alpha of Proteobacteria	55	(Manz et al., 1992)
GAM42a ^a	GCC TTCCACATCGTTT	Gamma-Subclass of Proteobacteria	55	(Manz et al., 1992)
CF319a	TGGTCCGTGTCTCAGTAC	Cytophaga- Flavobacter cluster	55	(Manz et al., 1996)

Cren537	TGACCACTTGAGGTGCTG	Crenarchaea	20	(Teira et al., 2004)
Eury806	CACAGCGTTTACACCTAG	Euryarchaea	20	(Teira et al., 2004)
NegControl	TAGTGACGCGCTCGA	For non-specific probe binding	55	(Karner and Fuhrman, 1997)

405^a Including an unlabeled competitor probe BET42a (5'-GCCTTCCCACTTCGTTT-3'), see (Manz et al., 1992) for
406details.

407

408**Chemical analyses**

409All glassware was pre-combusted before use. Additionally, glassware was rinsed with 1 N HCl and
410Milli-Q water after each sample. All plastic ware was rinsed with 1 N HCl and Milli-Q water and
411changed for each sample. Sample aliquots for particulate organic carbon (POC, 50-104 mL) and lipids
412(P-lips, 140-500mL) were filtered onto pre-combusted 0.7 µm GF/F filters (25 mm filter diameter)
413under a low vacuum (<50mm Hg) and preserved at -20°C or in liquid nitrogen, respectively.

414Filters for particulate total carbon (P_{tot}C) and POC were divided in two and analyzed as in Raimbault
415et al. (2008). The filter portions used for P_{tot}C were analyzed directly, while filter portions for POC
416measurement were acidified with 100 µL H₂SO₄ (0.5N) and subsequently analyzed using high
417combustion (1000°C) on a mass spectrometer (CN-Integra tracer-mass). The concentration of
418particulate inorganic carbon (PIC) was calculated from the difference between P_{tot}C and POC.

419Lipids were extracted from filters using a Bligh and Dyer (1959) protocol, and lipid classes were
420separated on chromarods and quantified on a thin layer chromatography/flame ionization detection
421(TLC/FID) Iatroscan apparatus model MK-6s (Iatron,Tokyo) coupled to a PC equipped with a Chromstar
4226.1 integration system (Bionis, Paris), as in Goutx et al. (2007). The separation of the 16 following lipid
423classes was obtained: neutral lipids including hydrocarbons (HC), sterol esters co-eluting with wax
424esters (WE), ketones (KET), triacylglycerols (TG), free fatty acids (FFA), alcohols (ALC), sterols and
425diglycerides (DG); chloroplast lipids containing pigments (Pig), glycolipids and
426monogalactosyldiacylglycerol (MGDG); monoacylglycerols (MG); and phospholipids (PL) including
427diphosphatidylglycerides co-eluting with phosphatidylglycerides (DPG+PG), phosphatidylethanol-
428amine (PE) and phosphatidylcholine (PC). A lipolysis index was calculated as in Goutx et al. (2000,

4292003), defined as the ratio of the sum of the metabolites (DG, MG, FFA, ALC) to the sum of the intact
430acyl-lipids (TG, PE, PI, DPG+PG, PC, WE, MGDG).

431TOC concentrations were obtained by adding POC to DOC values. Samples for DOC were obtained and
432preserved as in Tamburini *et al.* (2009). The DOC measurements were carried out in the laboratory at
433the Pisa section of the Institute of Biophysics (IBF), CNR, with a Shimadzu TOC-VCSN. Samples were
434acidified with 2N HCl and sparged for 3 minutes with CO₂-free ultra-high purity air in order to remove
435inorganic carbon. From 3 to 5 replicate injections were performed until the analytical precision was
436lower than 1%. The system blank was measured every day at the beginning and the end of analyses
437using low-carbon water (2-3 $\mu\text{mol L}^{-1}$ C) produced by a Milli-Q system. Measurement reliability was
438assessed twice a day by comparison of data with DOC Consensus Reference Waters (CRM; Hansell,
4392005) (measured concentration = 42.7 $\mu\text{mol L}^{-1}$, nominal concentration: 41-44 $\mu\text{mol L}^{-1}$). For further
440analytical details refer to Santinelli *et al.* (2013)

441Samples for total alkalinity (A_T) were filtered on GF/F membranes (duplicate 50 mL filtrate aliquots
442from the P_{tot}C and POC GF/F filtrations), immediately poisoned with 100 μL of a 50% saturated HgCl₂
443solution (37 g HgCl₂ in 1 L distilled water) and stored at 4°C in the dark pending analysis. A_T was
444determined on 20 mL subsamples using a home-made titration system composed of a 20 mL open
445temperature-controlled titration cell (kept at 25.0°C), an Orion 8103SC pH electrode calibrated on the
446National Bureau of Standards scale and a computer-driven Metrohm 665 Dosimat titrator,
447Switzerland. The seawater samples and the acid titrant (0.1 N HCl) were kept at a constant
448temperature of 25°C. Samples were weighted with a precision of 0.01 g before the titration to
449determine their exact volume from temperature and salinity. A_T was calculated from a Gran function
450applied to pH variations from 4.2 to 3.0 as a function of added volume of HCl. A_T measurements had a
451reproducibility of 3 $\mu\text{mol kg}^{-1}$.

452 **Statistical analyses**

453 Differences in prokaryotic abundances (N = 3 replicate incubation bottles per condition) were
454 analyzed by the non-parametric pairwise Mann-Whitney test on raw data (i.e. individual replicate
455 values), due to the low number of replicates, using the Statistica 6 software. Unless otherwise stated,
456 data are reported as averages and standard deviation (SD) and statistical difference was accepted at p
457 ≤ 0.05 .

458 **Acknowledgements**

459 Thanks are due to M.-D. Pizay for performing the determination of total alkalinity, to C. Pinazo for her
460 insights on the hydrological conditions at the sampling location, and the crew of the N/O Antedon for
461 assistance in the field. I. This work is a contribution to the Mediterranean Institute of Oceanography
462 "AT-EMBE", and to the Labex OT-Med (ANR-11-LABEX-0061, www.otmed.fr) funded by the
463 Investissements d'Avenir, French Government project of the French National Research Agency (ANR,
464 www.agence-nationale-recherche.fr) through the A*Midex project (ANR-11-IDEX-0001-02), funding
465 Virginie Riou during preparation of the manuscript.

466 **References**

467 Acinas, S. G., Antón, J. and Rodríguez-Valera, F.: Diversity of free-living and attached bacteria in
468 offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA, Appl.
469 Environ. Microbiol., 65(2), 514–22 [online] Available from:
470 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=91055&tool=pmcentrez&rendertype=abstract)
471 [artid=91055&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=91055&tool=pmcentrez&rendertype=abstract), 1999.
472 Amann, R. I., Krumholz, L. and Stahl, D.: Fluorescent-oligonucleotide probing of whole cells for
473 determinative, phylogenetic, and environmental studies in microbiology, J. Bacteriol., 172(2), 762–
474 770 [online] Available from: [http://www.pubmedcentral.nih.gov/articlerender.fcgi?](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=208504&tool=pmcentrez&rendertype=abstract)
475 [artid=208504&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=208504&tool=pmcentrez&rendertype=abstract), 1990.

476 Amann, R. I., Ludwig, W. and Schleifer, K. H.: Phylogenetic identification and in situ detection of
 477 individual microbial cells without cultivation, *Microbiol. Rev.*, 59(1), 143–169 [online] Available from:
 478 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=239358&tool=pmcentrez&rendertype=abstract)
 479 [artid=239358&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=239358&tool=pmcentrez&rendertype=abstract), 1995.

480 Antia, A. N., Koeve, W., Fischer, G., Blanz, T., Schulz-Bull, D., Scholten, J., Neuer, S., Kremling, K., Kuss,
 481 J., Peinert, R., Hebbeln, D., Bathmann, U., Conte, M., Fehner, U. and Zeitzschel, B.: Basin-wide
 482 particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric
 483 CO₂ sequestration, *Global Biogeochem. Cycles*, 15(4), 845–862, doi:10.1029/2000GB001376, 2001.

484 Antia, A. N., Suffrian, K., Holste, L., Müller, M. N., Nejstgaard, J. C., Simonelli, P., Carotenuto, Y. and
 485 Putzeys, S.: Dissolution of coccolithophorid calcite by microzooplankton and copepod grazing,
 486 *Biogeosciences Discuss.*, 5(1), 1–23 [online] Available from: [https://hal.archives-ouvertes.fr/hal-](https://hal.archives-ouvertes.fr/hal-000297955/)
 487 [000297955/](https://hal.archives-ouvertes.fr/hal-000297955/), 2008.

488 Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S. and Wakeham, S. G.: A new, mechanistic model for
 489 organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals,
 490 *Deep Sea Res. Part II Top. Stud. Oceanogr.*, 49(1-3), 219–236, doi:10.1016/S0967-0645(01)00101-1,
 491 2001.

492 Baldi, F., Minacci, A., Saliot, A., Mejanelle, L., Mozetic, P., Turk, V. and Malej, A.: Cell lysis and release
 493 of particulate polysaccharides in extensive marine mucilage assessed by lipid biomarkers and
 494 molecular probes, *Mar. Ecol. Prog. Ser.*, 153, 45–57, doi:10.3354/meps153045, 1997.

495 Barrett, P. M., Resing, J. A., Buck, N. J., Feely, R. A., Bullister, J. L., Buck, C. S. and Landing, W. M.:
 496 Calcium carbonate dissolution in the upper 1000 m of the eastern North Atlantic, *Global Biogeochem.*
 497 *Cycles*, 28(4), 386–397, doi:10.1002/2013GB004619, 2014.

498 Bell, M. V. and Pond, D.: Lipid composition during growth of motile and coccolith forms of *Emiliana*
 499 *huxleyi*, *Phytochemistry*, 41(2), 465–471, doi:10.1016/0031-9422(95)00663-X, 1996.

500 Biddanda, B. A.: Structure and function of marine microbial aggregates, *Oceanol. acta*, 9(2), 209–211,
 501 1986.

502Biddanda, B. A. and Pomeroy, L.: Microbial aggregation and degradation of phytoplankton-derived
 503detritus in seawater: 1. Microbial succession., Mar Ecol Progr Ser 42, 79–88 [online] Available from:
 504<http://www.jourlib.org/references/11414368>, 1988.

505Bidle, K. D. and Azam, F.: Accelerated dissolution of diatom silica by marine bacterial assemblages,
 506Nature, 397, 508–512, 1999.

507Bidle, K. D. and Azam, F.: Bacterial control of silicon regeneration from diatom detritus: Significance of
 508bacterial ectohydrolases and species identity, Limnol. Oceanogr., 46(7), 1606–1623,
 509doi:10.4319/lo.2001.46.7.1606, 2001.

510Bidle, K. D., Manganelli, M. and Azam, F.: Regulation of oceanic silicon and carbon preservation by
 511temperature control on bacteria, Science, 298(5600), 1980–1984, doi:10.1126/science.1076076,
 5122002.

513Biermann, A. and Engel, A.: Effect of CO₂ on the properties and sinking velocity of aggregates of the
 514coccolithophore *Emiliana huxleyi*, Biogeosciences, 7(3), 1017–1029, doi:10.5194/bg-7-1017-
 5152010, 2010.

516Bissett, A., Neu, T. R. and Beer, D. de: Dissolution of calcite in the twilight zone: bacterial control of
 517dissolution of sinking planktonic carbonates is unlikely, PLoS One, 6(11), e26404,
 518doi:10.1371/journal.pone.0026404, 2011.

519Broecker, W. and Clark, E.: Ratio of coccolith CaCO₃ to foraminifera CaCO₃ in late Holocene deep sea
 520sediments, Paleoceanography, 24(3), PA3205, doi:10.1029/2009PA001731, 2009.

521Broecker, W. S.: Recommendations of the working group on carbonate dissolution, in The fate of fossil
 522fuel CO₂ in the oceans. Proceedings of a symposium conducted by the Ocean Science and Technology
 523Division of the Office of Naval Research...held at the University of Honolulu, Hawaii, January 16-20,
 5241976, edited by N. R. Andersen and A. Malahoff, pp. 207–212, New York Plenum Press., 1977.

525Buesseler, K. O. and Boyd, P. W.: Shedding light on processes that control particle export and flux
 526attenuation in the twilight zone of the open ocean, Mar. Chem., 54(4), 1210–1232, 2009.

527Cardinal, D., Dehairs, F., Cattaldo, T. and André, L.: Geochemistry of suspended particles in the

528 Subantarctic and Polar Frontal zones south of Australia: Constraints on export and advection
 529 processes, *J. Geophys. Res. Ocean.*, 106(C12), 31637–31656, doi:10.1029/2000JC000251, 2001.

530 Cardinal, D., Savoye, N., Trull, T. W., André, L., Kopczynska, E. E. and Dehairs, F.: Variations of carbon
 531 remineralisation in the Southern Ocean illustrated by the Baxs proxy, *Deep Sea Res. Part I Oceanogr.*
 532 *Res. Pap.*, 52(2), 355–370, doi:10.1016/j.dsr.2004.10.002, 2005.

533 Cho, B. C. and Azam, F.: Major role of bacteria in biogeochemical fluxes in the ocean's interior, *Nature*,
 534 332(6163), 441–443, doi:10.1038/332441a0, 1988.

535 Chow, J. S., Lee, C. and Engel, A.: The influence of extracellular polysaccharides, growth rate, and free
 536 coccoliths on the coagulation efficiency of *Emiliana huxleyi*, *Mar. Chem.*, 175, 5–17,
 537 doi:10.1016/j.marchem.2015.04.010, 2015.

538 Chung, S.-N., Lee, K., Feely, R. A., Sabine, C. L., Millero, F. J., Wanninkhof, R., Bullister, J. L., Key, R. M.
 539 and Peng, T.-H.: Calcium carbonate budget in the Atlantic Ocean based on water column inorganic
 540 carbon chemistry, *Global Biogeochem. Cycles*, 17(4), n/a–n/a, doi:10.1029/2002GB002001, 2003.

541 Cros, L. and Fortuño, J. M.: Atlas of Northwestern Mediterranean Coccolithophores, *Sci. Mar.*, 66, 1–
 542 182, doi:10.3989/scimar.2002.66s11, 2002.

543 Daims, H., Brühl, A., Amann, R., Schleifer, K.-H. and Wagner, M.: The Domain-specific Probe EUB338 is
 544 insufficient for the Detection of all Bacteria : Development and Evaluation of a more Comprehensive
 545 Probe Set, *Syst. Appl. Microbiol.*, 22, 434–444, 1999.

546 Delong, E. F., Franks, D. G. and Alldredge, A. L.: Phylogenetic diversity of aggregate-attached vs. free-
 547 living marine bacterial assemblages, *Limnol. Oceanogr.*, 38(5), 924–934,
 548 doi:10.4319/lo.1993.38.5.0924, 1993.

549 Dong, S., Subhas, A., Berelson, W., Adkins, J. and Rollins, N.: Kinetics of Inorganic Calcite Dissolution in
 550 Seawater under Pressure, in *Ocean Sciences Meeting*, New Orleans, p. AH14A-0012. [online]
 551 Available from: <https://agu.confex.com/agu/os16/preliminaryview.cgi/Paper88301.html>, 2016.

552 Ducklow, H. W., Steinberg, D. K. and Buesseler, K. O.: Upper ocean carbon export and the biological
 553 pump, *Oceanography*, 14(4), 50–58, doi:10.5670/oceanog.2001.06, 2001.

554Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E. and Zondervan, I.: Polysaccharide aggregation
 555as a potential sink of marine dissolved organic carbon, *Nature*, 428(6986), 929–32,
 556doi:10.1038/nature02453, 2004.

557Engel, A., Abramson, L., Szlosek, J., Liu, Z., Stewart, G., Hirschberg, D. and Lee, C.: Investigating the
 558effect of ballasting by CaCO₃ in *Emiliana huxleyi*, II: Decomposition of particulate organic matter,
 559Deep Sea Res. Part II Top. Stud. Oceanogr., 56(18), 1408–1419, doi:10.1016/j.dsr2.2008.11.028,
 5602009a.

561Engel, A., Szlosek, J., Abramson, L., Liu, Z. and Lee, C.: Investigating the effect of ballasting by CaCO₃
 562in *Emiliana huxleyi*: I. Formation, settling velocities and physical properties of aggregates, Deep Sea
 563Res. Part II Top. Stud. Oceanogr., 56(18), 1396–1407, doi:10.1016/j.dsr2.2008.11.027, 2009b.

564Eppley, R. W. and Peterson, B. J.: Particulate organic matter flux and planktonic new production in the
 565deep ocean, *Nature*, 282, 677–680, doi:10.1038/282677a0, 1979.

566Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J. and Millero, F. J.: Impact of
 567anthropogenic CO₂ on the CaCO₃ system in the oceans, *Science*, 305(5682), 362–366,
 568doi:10.1126/science.1097329, 2004.

569Francois, R., Honjo, S., Krishfield, R. and Manganini, S.: Factors controlling the flux of organic carbon
 570to the bathypelagic zone of the ocean, *Glob. Biogeochem. Cycles*, 16(4), 1087,
 571doi:10.1029/2001gb001722, 2002.

572Fukuda, R., Ogawa, H., Nagata, T. and Koike, I.: Direct determination of carbon and nitrogen contents
 573of natural bacterial assemblages in marine environments, *Appl. Environ. Microbiol.*, 64(9), 3352–
 5743358, 1998.

575Gattuso, J. P., Frankignoulle, M. and Smith, S. V: Measurement of community metabolism and
 576significance in the coral reef CO₂ source-sink debate., *Proc. Natl. Acad. Sci. U. S. A.*, 96(23), 13017–
 57713022, doi:10.1073/pnas.96.23.13017, 1999.

578Giering, S. L. C., Sanders, R., Lampitt, R. S., Anderson, T. R., Tamburini, C., Boutrif, M., Zubkov, M. V,
 579Marsay, C. M., Henson, S. a, Saw, K., Cook, K. and Mayor, D. J.: Reconciliation of the carbon budget in

580the ocean's twilight zone., *Nature*, 507(7493), 480–483, doi:10.1038/nature13123, 2014.

581Goutx, M., Momzikoff, A., Striby, L., Andersen, V., Marty, J. . and Vescovali, I.: High-frequency fluxes of
582labile compounds in the central Ligurian Sea, northwestern Mediterranean, *Deep Sea Res. Part I*
583*Oceanogr. Res. Pap.*, 47(3), 533–556, doi:10.1016/S0967-0637(99)00101-6, 2000.

584Goutx, M., Guigue, C. and Striby, L.: Triacylglycerol biodegradation experiment in marine
585environmental conditions: definition of a new lipolysis index, *Org. Geochem.*, 34(10), 1465–1473,
586doi:10.1016/S0146-6380(03)00119-0, 2003.

587Goutx, M., Wakeham, S. G., Lee, C., Duflos, M., Guigue, C., Liu, Z., Moriceau, B., Sempéré, R., Tedetti,
588M. and Xue, J.: Composition and degradation of marine particles with different settling velocities in
589the northwestern Mediterranean Sea, *Limnol. Oceanogr.*, 52(4), 1645–1664,
590doi:10.4319/lo.2007.52.4.1645, 2007.

591Harris, R. P.: Zooplankton grazing on the coccolithophore *Emiliana huxleyi* and its role in inorganic
592carbon flux, *Mar. Biol.*, 119(3), 431–439, doi:10.1007/BF00347540, 1994.

593Hedges, J. I., Baldock, J. A., Gélinais, Y., Lee, C., Peterson, M. and Wakeham, S. G.: Evidence for non-
594selective preservation of organic matter in sinking marine particles, *Nature*, 409(6822), 801–4,
595doi:10.1038/35057247, 2001.

596Henson, S. A., Sanders, R., Madsen, E., Morris, P. J., Le Moigne, F. and Quartly, G. D.: A reduced
597estimate of the strength of the ocean's biological carbon pump, *Geophys. Res. Lett.*, 38(4),
598doi:10.1029/2011GL046735, 2011.

599Holligan, P. M., Fernández, E., Aiken, J., Balch, W. M., Boyd, P., Burkill, P. H., Finch, M., Groom, S. B.,
600Malin, G., Muller, K., Purdie, D. A., Robinson, C., Trees, C. C., Turner, S. M. and van der Wal, P.: A
601biogeochemical study of the coccolithophore, *Emiliana huxleyi* , in the North Atlantic, *Global*
602*Biogeochem. Cycles*, 7(4), 879–900, doi:10.1029/93GB01731, 1993.

603Houdan, A., Véron, B., Claquin, P., Lefebvre, S. and Poncet, J.-M.: Cryopreservation of the
604coccolithophore, *Emiliana huxleyi* (Haptophyta, Prymnesiophyceae), *J. Appl. Phycol.*, 17(5), 413–422,
605doi:10.1007/s10811-005-0065-5, 2005.

606Iglesias-Rodriguez, M. D., Armstrong, R., Feely, R., Hood, R., Kleypas, J., Milliman, J. D., Sabine, C. and
607Sarmiento, J.: Progress made in study of ocean's calcium carbonate budget, *Eos, Trans. Am. Geophys.*
608Union, 83(34), 365–375, doi:10.1029/2002EO000267, 2002.

609Ignatiades, L., Gotsis-Skretas, O., Pagou, K. and Krasakopoulou, E.: Diversification of phytoplankton
610community structure and related parameters along a large-scale longitudinal east-west transect of
611the Mediterranean Sea, *J. Plankton Res.*, 31(4), 411–428, doi:10.1093/plankt/fbn124, 2009.

612Ingalls, A. E., Lee, C., Wakeham, S. G. and Hedges, J. I.: The role of biominerals in the sinking flux and
613preservation of amino acids in the Southern Ocean along 170°W, *Deep Sea Res. Part II Top. Stud.*
614*Oceanogr.*, 50(3-4), 713–738, doi:10.1016/S0967-0645(02)00592-1, 2003.

615Iversen, M. H. and Ploug, H.: Ballast minerals and the sinking carbon flux in the ocean: carbon-specific
616respiration rates and sinking velocity of marine snow aggregates, *Biogeosciences*, 7(9), 2613–2624,
617doi:10.5194/bg-7-2613-2010, 2010.

618Jacquet, S. H. M., Dehairs, F., Cardinal, D., Navez, J. and Delille, B.: Barium distribution across the
619Southern Ocean frontal system in the Crozet–Kerguelen Basin, *Mar. Chem.*, 95(3-4), 149–162,
620doi:10.1016/j.marchem.2004.09.002, 2005.

621Jansen, H. and Wolf-Gladrow, D. A.: Carbonate dissolution in copepod guts: A numerical model, *Mar.*
622*Ecol. Prog. Ser.*, 221, 199–207, doi:10.3354/meps221199, 2001.

623de Jesus Mendes, P. a. and Thomsen, L.: Effects of Ocean Acidification on the Ballast of Surface
624Aggregates Sinking through the Twilight Zone, *PLoS One*, 7(12), e50865,
625doi:10.1371/journal.pone.0050865, 2012.

626de Jesus Mendes, P. A., Thomsen, L., Holscher, B., de Stigter, H. C. and Gust, G.: Pressure effects on
627the biological degradation of organo-mineral aggregates in submarine canyons, *Mar. Geol.*, 246(2-4),
628165–175, doi:10.1016/j.margeo.2007.05.012, 2007.

629Karner, M. and Fuhrman, J. A.: Determination of Active Marine Bacterioplankton: a Comparison of
630Universal 16S rRNA Probes, Autoradiography, and Nucleoid Staining, *Appl. Environ. Microbiol.*, 63(4),
6311208–13, 1997.

632Keller, M. D., Selvin, R. C., Claus, W. and Guillard, R. R. L.: Media for the culture of oceanic
633ultraphytoplankton, *J. Phycol.*, 23(4), 633–638, doi:10.1111/j.1529-8817.1987.tb04217.x, 2007.

634Klaas, C. and Archer, D. E.: Association of sinking organic matter with various types of mineral ballast
635in the deep sea: Implications for the rain ratio, *Global Biogeochem. Cycles*, 16(4), 63–1–63–14,
636doi:10.1029/2001GB001765, 2002.

637Knappertsbusch, M.: Geographic-Distribution of Living and Holocene Coccolithophores in the
638Mediterranean-Sea, *Mar. Micropaleontol.*, 21(1-3), 219–247, 1993.

639Kogure, K. and Koike, I.: Particle counter determination of bacterial biomass in seawater, *Appl.*
640*Environ. Microbiol.*, 53(2), 274–277, 1987.

641Kwon, E. Y., Primeau, F. and Sarmiento, J. L.: The impact of remineralization depth on the air–sea
642carbon balance, *Nat. Geosci.*, 2(9), 630–635, doi:10.1038/ngeo612, 2009.

643De La Rocha, C. L. and Passow, U.: Factors influencing the sinking of POC and the efficiency of the
644biological carbon pump, *Deep Sea Res. Part II Top. Stud. Oceanogr.*, 54(5-7), 639–658,
645doi:10.1016/j.dsr2.2007.01.004, 2007.

646Manz, W., Amann, R., Ludwig, W., Wagner, M. and Schleifer, K.-H.: Phylogenetic Oligodeoxynucleotide
647Probes for the Major Subclasses of Proteobacteria: Problems and Solutions, *Syst. Appl. Microbiol.*,
64815(4), 593–600, doi:10.1016/S0723-2020(11)80121-9, 1992.

649Manz, W., Amann, R., Ludwig, W., Vancanneyt, M. and Schleifer, K. H.: Application of a suite of 16S
650rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-
651flavobacter-bacteroides in the natural environment, *Microbiology*, 142(5), 1097–1106,
652doi:10.1099/13500872-142-5-1097, 1996.

653Milliman, J. D., Troy, P. J., Balch, W. M., Adams, A. K., Li, Y. H. and Mackenzie, F. T.: Biologically
654mediated dissolution of calcium carbonate above the chemical lysocline?, *Deep. Res. Part I Oceanogr.*
655*Res. Pap.*, 46(10), 1653–1669, doi:10.1016/S0967-0637(99)00034-5, 1999.

656Moeseneder, M. M., Winter, C. and Herndl, G. J.: Horizontal and vertical complexity of attached and
657free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA

658fingerprints, *Limnol. Oceanogr.*, 46(1), 95–107, doi:10.4319/lo.2001.46.1.0095, 2001.

659Le Moigne, F. A. C., Gallinari, M., Laurenceau, E. and De La Rocha, C. L.: Enhanced rates of particulate
660organic matter remineralization by microzooplankton are diminished by added ballast minerals,
661*Biogeosciences*, 10(9), 5755–5765, doi:10.5194/bg-10-5755-2013, 2013.

662Nanninga, H. and Tyrrell, T.: Importance of light for the formation of algal blooms by *Emiliania huxleyi*,
663*Mar. Ecol. Prog. Ser.*, 136, 195–203, doi:10.3354/meps136195, 1996.

664Panagiotopoulos, C., Sempéré, R., Obernosterer, I., Striby, L., Goutx, M., Van Wambeke, F., Gautier, S.
665and Lafont, R.: Bacterial degradation of large particles in the southern Indian Ocean using in vitro
666incubation experiments, *Org. Geochem.*, 33(8), 985–1000, doi:10.1016/S0146-6380(02)00057-8,
6672002.

668Passow, U.: Switching perspectives: Do mineral fluxes determine particulate organic carbon fluxes or
669vice versa?, *Geochemistry, Geophys. Geosystems*, 5(4), doi:10.1029/2003GC000670, 2004.

670Passow, U. and De La Rocha, C. L.: Accumulation of mineral ballast on organic aggregates, *Global*
671*Biogeochem. Cycles*, 20(1), doi:10.1029/2005GB002579, 2006.

672Ploug, H. and Bergkvist, J.: Oxygen diffusion limitation and ammonium production within sinking
673diatom aggregates under hypoxic and anoxic conditions, *Mar. Chem.*, 176, 142–149,
674doi:10.1016/j.marchem.2015.08.012, 2015.

675Ploug, H. and Grossart, H.: Bacterial production and respiration in suspended aggregates - a matter of
676the incubation method, *Aquat. Microb. Ecol.*, 20(1), 21–29, doi:10.3354/ame020021, 1999.

677Ploug, H., Iversen, M. H. and Fischer, G.: Ballast, sinking velocity, and apparent diffusivity within
678marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria,
679*Limnol. Oceanogr.*, 53(5), 1878–1886, doi:10.4319/lo.2008.53.5.1878, 2008.

680Porter, K. G. and Feig, Y. S.: The use of DAPI for identifying and counting aquatic microflora, *Limnol.*
681*Oceanogr.*, 25, 943–948, doi:10.4319/lo.1980.25.5.0943, 1980.

682Poulton, A. J., Adey, T. R., Balch, W. M. and Holligan, P. M.: Relating coccolithophore calcification rates
683to phytoplankton community dynamics: Regional differences and implications for carbon export,

684Deep Sea Res. Part II Top. Stud. Oceanogr., 54(5–7), 538–557, doi:10.1016/j.dsr2.2006.12.003, 2007.

685Le Quéré, C., Takahashi, T., Buitenhuis, E. T., Rödenbeck, C. and Sutherland, S. C.: Impact of climate
686change and variability on the global oceanic sink of CO₂, Global Biogeochem. Cycles, 24(4), GB4007,
687doi:10.1029/2009GB003599, 2010.

688Sawada, K. and Shiraiwa, Y.: Alkenone and alkenoic acid compositions of the membrane fractions of
689Emiliania huxleyi, Phytochemistry, 65(9), 1299–1307, doi:10.1016/j.phytochem.2004.03.015, 2004.

690Sekar, R., Pernthaler, A., Pernthaler, J., Warnecke, F., Posch, T. and Amann, R.: An Improved Protocol
691for Quantification of Freshwater Actinobacteria by Fluorescence In Situ Hybridization, Appl. Environ.
692Microbiol., 69(5), 2928–2935, doi:10.1128/AEM.69.5.2928, 2003.

693Sempéré, R., Yoro, S. O., Van Wambeke, F. and Charrière, B.: Microbial decomposition of large organic
694particles in the northwestern Mediterranean Sea: an experimental approach, Mar. Ecol. Prog. Ser.,
695198, 61–72, 2000.

696Siegel, D. A., Buesseler, K. O., Doney, S. C., Sailley, S. F., Behrenfeld, M. J. and Boyd, P. W.: Global
697assessment of ocean carbon export by combining satellite observations and food-web models, Global
698Biogeochem. Cycles, 28(3), 181–196, doi:10.1002/2013GB004743, 2014.

699Simon, M., Grossart, H., Schweitzer, B. and Ploug, H.: Microbial ecology of organic aggregates in
700aquatic ecosystems, Aquat. Microb. Ecol., 28, 175–211, doi:10.3354/ame028175, 2002.

701Tamburini, C., Garcin, J., Grégori, G., Leblanc, K., Rimmelin, P. and Kirchman, D.: Pressure effects on
702surface Mediterranean prokaryotes and biogenic silica dissolution during a diatom sinking
703experiment, Aquat. Microb. Ecol., 43(3), 267–276, doi:10.3354/ame043267, 2006.

704Tamburini, C., Goutx, M., Guigue, C., Garel, M., Lefèvre, D., Charrière, B., Sempéré, R., Pepa, S.,
705Peterson, M. L., Wakeham, S. and Lee, C.: Effects of hydrostatic pressure on microbial alteration of
706sinking fecal pellets, Deep Sea Res. Part II Top. Stud. Oceanogr., 56(18), 1533–1546,
707doi:10.1016/j.dsr2.2008.12.035, 2009.

708Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J. and Herndl, G. J.: Combining Catalyzed Reporter
709Deposition-Fluorescence In Situ Hybridization and Microautoradiography To Detect Substrate

710Utilization by Bacteria and Archaea in the Deep Ocean, *Society*, 70(7), 4411–4414,
 711doi:10.1128/AEM.70.7.4411, 2004.
 712Thiele, S., Fuchs, B. M., Amann, R. and Iversen, M. H.: Colonization in the Photic Zone and Subsequent
 713Changes during Sinking Determine Bacterial Community Composition in Marine Snow, *Appl. Environ.*
 714*Microbiol.*, 81(4), 1463–1471, doi:10.1128/AEM.02570-14, 2015.
 715Treguer, P. and Pondaven, P.: Global change. Silica control of carbon dioxide, *Nature*, 406(6794), 358–
 7169, doi:10.1038/35019236, 2000.
 717Turley, C. M.: Formation, vertical flux and remineralisation of aggregates in the ocean: a short review,
 718*Arch. fur Hydrobiol. Beih. Ergebnisse der Limnol.*, 1992.
 719Turley, C. M.: The effect of pressure on leucine and thymidine incorporation by free-living bacteria
 720and by bacteria attached to sinking oceanic particles, *Deep Sea Res. Part I Oceanogr. Res. Pap.*, 40(11-
 72112), 2193–2206, doi:10.1016/0967-0637(93)90098-N, 1993.
 722Turley, C. M. and Mackie, P. J.: Biogeochemical significance of attached and free-living bacteria and
 723the flux of particles in the NE Atlantic Ocean, *Mar. Ecol. Prog. Ser.*, 115(1-2), 191–204,
 724doi:10.3354/meps115191, 1994.
 725Turley, C. M. and Mackie, P. J.: Bacterial and cyanobacterial flux to the deep NE atlantic on
 726sedimenting particles, *Deep. Res. Part I*, 42(8), 1453–1474, doi:10.1016/0967-0637(95)00056-C, 1995.
 727Wannicke, N., Frindte, K., Gust, G., Liskow, I., Wacker, A. 769, Meyer, A. and Grossart, H.-P.: Measuring
 728bacterial activity and community composition at high hydrostatic pressure using a novel experimental
 729approach: A pilot study, *FEMS Microbiol. Ecol.*, 91(5), 1–37, 2015.
 730Wollast, R. and Chou, L.: Distribution and fluxes of calcium carbonate along the continental margin in
 731the Gulf of Biscay, *Aquat. Geochemistry*, 4(3-4), 369–393, 1998.
 732Wollast, R. and Chou, L.: The carbon cycle at the ocean margin in the northern Gulf of Biscay, *Deep.*
 733*Res. Part II Top. Stud. Oceanogr.*, 48(14-15), 3265–3293, doi:10.1016/S0967-0645(01)00040-6, 2001.
 734

735**Supplementary data**

736**Supplementary Table 1:** Lipid proportions (% Total Particulate Lipids) in the *E. huxleyi* culture at day 4
737(Bell and Pond, 1996) and during Exp B. Numbers 1, 2 and 3 stand for replicate incubations.
738Chloroplastic: pigments + monogalactosyldiacylglycerol, Phospholipids: diphosphatidylglycerides +
739phosphatidylglycerides + phosphatidylethanolamine + phosphoinositol + phosphatidylcholine, ST:
740sterols, Neutral: wax esters + methyl esters + ketones + triglycerides + Free Fatty Acids + fatty
741alcohols, Metab: Monoglycerides + DiGlycerides, HC: Hydrocarbons.

	Chloroplastic	Phospholipids	Sterols	Neutral	Metab	HC
<i>E. huxleyi</i> 4 days (Bell and Pond, 1996)	41.3 ± 0.8	21.5 ± 0.4	3.6 ± 0.4	23.3 ± 1.3	ND	9.4 ± 0.3
t0-1	39.3	22.5	2.4	23.2	4.0	7.6
t0-2	44.8	18.2	3.3	17.9	5.5	8.4
t0-3	46.5	16.6	3.8	19.2	4.4	8.1
ATMt10-1	10.8	5.0	0.0	50.3	0.0	31.2
ATMt10-2	32.5	3.4	3.1	13.6	0.0	43.6
ATMt10-3	20.7	5.8	2.5	37.7	0.0	20.6
HPt10-1	22.5	3.6	1.9	37.2	5.2	25.6
HPt10-2	19.8	6.8	0.0	43.1	0.0	21.0
HPt10-3	23.0	4.6	0.0	35.3	0.0	25.3

742**Supplementary Figure 1:** Photographs of the GF/F filters from Exp B at ATMt10 (A, B, C) and HPt10 (D,
743E, F). A-C, and B-F are the replicate incubations. Arrows point at some of the large visible aggregates
744in HPt10 incubations.

B