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# Climate change influences chlorophylls and bacteriochlorophylls metabolism in hypersaline microbial mat

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**Keywords:** hypersaline microbial mats, ocean acidification, mesocosms, chlorophyll derivatives, phototrophic communities

## Abstract

This study aimed to determine the effect of the climatic change on the phototrophic communities of hypersaline microbial mats. Ocean acidification and warming were simulated alone and together on microbial mats placed into mesocosms. As expected, the temperature in the warming treatments increased by 4°C from the initial temperature. Surprisingly, no significance difference was observed between the water pH of the different treatments despite of a decrease of 0.4 unit pH in the water reserves of acidification treatments. The salinity increased on the warming treatments and the dissolved oxygen concentration increased and was higher on the acidification treatments. A total of 37 pigments were

32 identified belonging to chlorophylls, carotenes and xanthophylls families. The higher  
33 abundance of unknown chlorophyll molecules called chlorophyll derivatives was observed in  
34 the acidification alone treatment with a decrease in chlorophyll *a* abundance. This change in  
35 pigmentary composition was accompanied by a higher production of bound extracellular  
36 carbohydrates but didn't affect the photosynthetic efficiency of the microbial mats. A careful  
37 analysis of the absorption properties of these molecules indicated that these chlorophyll  
38 derivatives were likely bacteriochlorophyll *c* contained in the chlorosomes of green  
39 anoxygenic phototroph bacteria. Two hypotheses can be drawn from these results: 1/ the  
40 phototrophic communities of the microbial mats were modified under acidification treatment  
41 leading to a higher relative abundance of green anoxygenic bacteria, or 2/ the highest  
42 availability of CO<sub>2</sub> in the environment has led to a shift in the metabolism of green  
43 anoxygenic bacteria being more competitive than other phototrophs.

44

45

## 46 **1. Introduction**

47

48 Since the industrial revolution, the atmospheric greenhouse gases concentration has  
49 increased sharply leading to a decrease in the pH of the surface ocean of about 0.1 pH unit  
50 (IPCC, 2014). In the coming decades, the ocean acidification will continue, associated with  
51 an ocean water warming caused by increasing levels of CO<sub>2</sub> in the atmosphere (Hutchins  
52 and Fu, 2017; IPCC, 2014). These climatic perturbations will alter the carbon and nutrients  
53 cycles on a global scale (Hutchins and Fu, 2017) and are expected to have a direct impact  
54 on all physical, chemical and biological parameters that govern marine organisms life  
55 (Hutchins and Fu, 2017). Organisms will be faced with unprecedented changes in future  
56 ocean conditions, particularly marine life will be severely affected. On France's Atlantic  
57 coasts, the Intergovernmental Panel on Climate Change (IPCC) predicts an increase in  
58 surface water temperature of 3 to 4 °C and ocean acidification of 0.4 to 0.45 pH units in its  
59 most pessimistic scenario (RCP 8.5) by the end of the century (IPCC, 2014).

60 Research on microorganisms facing climatic changes is scarce compared to that on animals  
61 and plants (Cavicchioli et al., 2019; Dutta and Dutta, 2016; Reinold et al., 2019). Microbial  
62 mats play important key-roles in marine ecosystems, such as participating in the dynamics of  
63 carbon, nitrogen et oxygen cycles. They develop at the water-sediment interface in a large  
64 variety of environments including coastal beaches (Bolhuis and Stal, 2011), salterns  
65 (Fourçans et al., 2004), hot springs (Dobretsov et al., 2011) and many other coastal/marine  
66 environments. These are complex microbial structures containing a great diversity of  
67 microorganisms coexisting at microscale according to a vertical stratification due to light and  
68 microgradients of oxygen, pH and sulphurs (Fourçans et al., 2008; Jorgensen et al., 1983;  
69 Revsbech et al., 1983; van Gemerden, 1993). Despite the stratification, microbial mats are  
70 dynamic structures where the migration of microorganisms has been described according to  
71 the diel cycle (Fourçans et al., 2008, 2006). Numerous interactions occurs in microbial mats  
72 representing thus an ecosystem on its own (Reinold et al., 2019). Microbial mats have a  
73 remarkable specific, metabolic and molecular diversity, making them highly adaptable to the  
74 changing physico-chemical conditions of the environment (Fourçans et al., 2006) as well as  
75 to contamination (Bordenave et al., 2008, 2004a, 2004b).

76 microorganisms are fundamental organisms in the functioning of microbial mats because  
77 they are primary producers.

78 Autotrophic microorganisms are fundamental organisms in the functioning of microbial mats  
79 because they are the major primary producers. They can be chemotroph such as some  
80 Bacteroidetes but the major primary producers in photosynthetic microbial mats are the  
81 phototrophic microorganisms (Sørensen et al., 2005). Among them, Cyanobacteria produce  
82 organic carbon which is then decomposed in the successive lower layers by different  
83 heterotrophs. They also secrete adhesive and protective extracellular polymeric substances  
84 (EPS), that form a matrix around the cells (Decho, 1990; Fourçans et al., 2006; Hubas, 2018;  
85 Wieland et al., 2003). This matrix is generally composed of sugars, proteins, extracellular  
86 DNA and other molecules in smaller proportions (Fourçans et al., 2006; Hubas, 2018;

87 Wieland et al., 2003). However, the composition varies depending on the physiological state  
88 of the organisms, the specific diversity, the growth stage of the mat and the physico-chemical  
89 conditions of the environment (Decho and Moriarty, 1990; Hubas, 2018; Reinold et al., 2019;  
90 Underwood et al., 2004). By binding to sedimentary particles, this matrix stabilises the  
91 microbial mat and prevents erosion phenomena (Decho, 1990), but it has many other  
92 essential roles such as nutrients supply by the sequestration and accumulation of dissolved  
93 and particulate nutrients coming from the water column that can be used by microorganisms,  
94 or it also helps microbial communication, or bring a protection against UV radiations (Decho,  
95 2000; Flemming and Wingender, 2010; Hubas, 2018). EPS are therefore a major component  
96 of microbial mats and remain indispensable for their functioning.

97 Several studies have addressed the impact of climate change on photosynthetic marine  
98 microbial communities. Some authors have shown that temperature affects the composition,  
99 the photosynthetic performance as well as the growth, biomass and physiology of  
100 microphytobenthos (Cartaxana et al., 2015; Hancke and Glud, 2004). Acidification effects are  
101 thought to occur mainly at the metabolic level, favouring the growth of some microorganisms  
102 (Baragi and Anil, 2016; Black et al., 2019; Hicks et al., 2011) but also photorespiration in  
103 diatoms and increasing the number of proton pumps for maintaining intracellular pH  
104 homeostasis and respiratory processes (Beardall et al., 2009; Black et al., 2019; Gao et al.,  
105 2012). Acidification and warming also affect the characteristics of EPS of diatoms and  
106 cyanobacteria with a modification of their composition (Li et al., 2016; Ma et al., 2019).

107 Many studies have highlighted the impact of acidification and warming water separately but it  
108 is necessary to consider them together as they can act synergistically (Baragi et al., 2015;  
109 Baragi and Anil, 2016; Li et al., 2016). The aim of this study was to simulate an acidification  
110 and a water warming on microbial mats on mesocosms according to the RCP8.5 scenario of  
111 the IPCC for 2100. Here, the phototrophic microbial communities are described and their  
112 potential composition variation will be monitored.

## 114 2. Material and methods

115

### 116 2.1 Description of the sampled sites

117

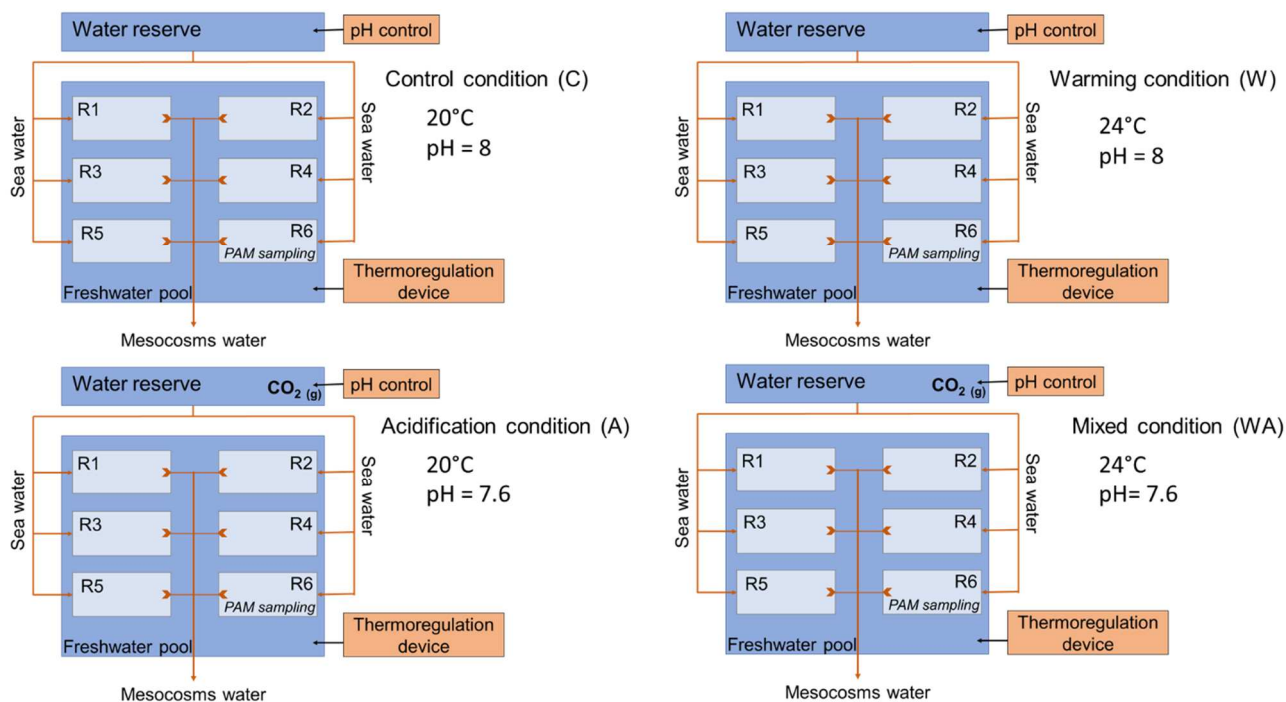
118 Microbial mats for mesocosms experiment were sampled the 30<sup>th</sup> April 2019 in salterns  
119 located in Ars-en-Ré (46°13'29.9"N 1°31'07.5"W, Ré island, France) on a non-exploited  
120 pond. The microbial mat, which had not been disturbed for at least three years, was more  
121 mature than a mat from an exploited pond. Undisturbed mats were then placed in plastic  
122 boxes and transported to laboratory at room temperature where they were put back in water  
123 within three hours following the sampling. The water height was around 3 cm as observed *in*  
124 *situ*. *In situ* physico-chemical parameters (temperature, salinity, pH) of the water were  
125 measured with a multi-parameter probe (pHenomenal® MU 6100H, VWRTM, USA) in order to  
126 apply them on the mesocosms as control values. Samples were also taken to determine  
127 nutrient salt concentrations in the pore water.

### 128 2.2 Mesocosm design

129 Microbial mats were separated into twenty-four plastic boxes (48 cm x 33 cm), each of them  
130 representing a mesocosm (Fig. 1). The mesocosms were gathered in groups of six  
131 representing a condition (five replicates and a box for pulse amplitude modulated (PAM)  
132 measures). In these six mesocosms, incoming water was from a reserve and was distributed  
133 individually with stainless steel taps. This water corresponded to seawater filtered at 80 µm,  
134 passed under UV light and whose salinity was adjusted to 60 psu with salt coming the salt  
135 marshes of the Ré island. Opposite to the water inlet, a hole allowed the water outlet in a  
136 network of PVC tubes between all the replicates. The water was not recycled. This setup  
137 permitted to maintain a stable water level at 3 cm above the mat and to renew seawater and  
138 the supplying nutrients for the development of the microbial mats. For each condition, a pool  
139 was constructed around the six plastic boxes where water was maintained at the desired  
140 temperature with a pump (EHEIM universal 600, Germany) connected to a thermoregulating

141 device (Teco®) to control the water temperature above the microbial mats. The microbial  
 142 mats were illuminated twelve hours a day following the day/night cycle observed during the  
 143 spring. To control the evaporation rate, the lights used were LED (TOP-24H company by  
 144 SYLED, France). They provided white cold light color and an intensity of  $11.8 \pm 0.9$   
 145  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  (mean  $\pm$  standard between the four treatments for a day) (HOBO  
 146 Pendant® Temperature/Light Data Logger, Onset Computer Corporation, USA) on the  
 147 surface of the microbial mats.

148 Daily monitoring was done for the physico-chemical parameters (temperature, pH, salinity  
 149 and dissolved oxygen) thanks to a multiparameter probe (pHenomena® MU 6100H, VWR™,  
 150 USA).



151

152 **Figure 1:** Schematic representation of the experimental device with the control (C) and the  
 153 acidification (A), warming (W) and mixed (WA) treatments. The replicates are represented by  
 154 the letter R, the first five are used for sampling and the sixth is dedicated to the  
 155 measurements of fluorimetry by pulse amplitude modulation (PAM).

156

### 157 2.3 Implementation of different treatments

158 A stabilisation period of five weeks was applied to acclimate the microbial mats to their new  
 159 environment (Gette-Bouvarot et al., 2015; Stauffert et al., 2013). The water temperature on

160 the mesocosms was maintained at 20°C and the salinity of the incoming water was regulated  
161 in the water reserve to maintain a salinity at 60 +/- 2 psu in the mesocosms, reproducing *in*  
162 *situ* conditions. The implementation of different treatments occurred for seven additional  
163 weeks.

164 Four treatments were applied to the microbial mats. Each pool and its six mesocosms  
165 represented a condition. The first treatment was the control treatment (C) in which the  
166 parameters were not changed. The second treatment was the warming treatment (W) in  
167 which the temperature was increased by 0.5°C every two days for 2 weeks until reaching  
168 24°C. The acidification treatment (A) represented the third condition. The water acidification  
169 was performed in the water reserve by bubbling pure CO<sub>2</sub>, allowing a drop of initial water pH  
170 of 0.1 unit every four days for 2 weeks until reaching a decrease of the initial pH of the water  
171 reserve equal to 0.4 unit, *i.e.* 7.6. The water pH in the water reserve was monitored with a  
172 continuous pH stat system (IKS aquastar, iks ComputerSysteme GmbH, Germany). Then the  
173 pH values of the pH stat system were adjusted twice a week from measurement using a pH  
174 meter (Metrohm, 826 pH mobile) with a glass electrode (Metrohm, electrode plus) calibrated  
175 on the total scale using Tris buffer solution (provided by Andrew Dickson, Scripps Institution  
176 Oceanography, San Diego). Every day, the pH values in the four treatments pools were also  
177 measured on the total scale. The fourth treatment combined warming and acidification  
178 treatments (WA). The mesocosms were then maintained under these treatments for 5 further  
179 weeks.

180 A first sampling (noted t9) was performed after the stabilisation period of the microbial mats  
181 on the mesocosms, just before changing the environmental conditions. A second sampling  
182 (t16) was performed at the middle of the period of change and a third (t23) at the end  
183 (Supplementary materials, fig. A). After this period, sampling was performed every week (t30,  
184 t37, t44, t51 and t58) (Supplementary materials, fig. A). For each sampling, ten 1 cm depth  
185 cores were collected with a 1 cm diameter cut-off syringe in each mesocosm and mixed  
186 together in order to obtain a homogeneous sample.



## 187           **2.4 Nutrient concentrations**

188   A volume of 20 mL of water from each mesocosm was filtered at 0.22  $\mu\text{m}$  in order to perform  
189   a nutrient analysis. The same was done for *in situ* samples. Half of the volume was frozen at  
190   -20°C while the other half was kept at 4°C for silicon analysis. The samples were then  
191   analyzed as described by Aminot and K  rouel (2007). Silicon, nitrate, nitrite and phosphate  
192   were measured by Segmented Continuous Flow Colorimetry (SFA) while ammonium was  
193   measured by SFA fluorimetry on an auto-analyser (SEAL AutoAnalyzer 3, SEAL analytical)  
194   (Aminot and K  rouel, 2007).

## 195           **2.5 Photosynthetic parameters**

196  
197   Chlorophyll fluorescence parameters were measured at 5 different locations of the core using  
198   a fluorometer (Monitoring Pen, MP 100-E, Photon Systems Instruments, Czech Republic)  
199   illuminated with a blue LED emitter (455 nm). The samples were placed in the dark (for 5  
200   min) before the measurement of Light Response Curves. Manufacturer predetermined LC3  
201   protocol was used following manufacturer instructions. LC3 protocol was characterised by 7  
202   steps of increasing light intensities (10, 20, 50, 100, 300, 500, 1000  $\mu\text{mol.photon.m}^{-2}.\text{s}^{-1}$ ) with  
203   an illumination duration of 60s. Minimum fluorescence level  $F_0^5$  (Jesus et al., 2006) was  
204   obtained by using a non actinic measuring light pulse (30  $\mu\text{s}$ , 900  $\mu\text{mol.photon.m}^{-2}.\text{s}^{-1}$ ), which  
205   induced the minimal chlorophyll fluorescence ( $F_0^5$ ). The samples were then subjected to a  
206   saturating light pulse (2,400  $\mu\text{mol.photon.m}^{-2}.\text{s}^{-1}$ ). This made it possible to measure the  
207   maximum fluorescence level  $F_m^5$  (Jesus et al., 2006). All measurement were done in the  
208   same conditions and at the same time.

209   The data was then downloaded from the device to a computer using FluorPen software  
210   (v1.0.6.1, Photon Systems Instruments, Czech Republic) and all calculations and analyses  
211   were performed using R.Studio software (version 4.0.3<sup>  </sup> RStudio, Inc.). From the measured  
212   parameters the effective quantum yields of photosynthesis ( $\phi(II)^5$ ) were calculated (Jesus et

213 al., 2006) (Equation 1). They indicate the community's maximum potential for photosynthetic  
214 activity.

$$\phi(II)^5 = \frac{F_m^5 - F_0^5}{F_m^5} \quad (\text{Equation 1})$$

215

216

## 217 **2.6 Pigment identification and quantification**

218

219 Lipophilic pigments were analyzed by high performance liquid chromatography (HPLC).  
220 Microbial mats were incubated with 95% methanol (buffered with 2% ammonium acetate)  
221 during 15 min, at -20°C in the dark. Extracts were then filtered with 0.2µm PTFE syringe  
222 filters and analyzed within 16h using an Agilent 1260 Infinity HPLC composed of a  
223 quaternary pump (VL 400 bar), a UV–VIS photodiode array detector (DAD 1260 VL, 190–950  
224 nm), a fluorescence detector (FLD 1260 excitation: 425 nm, emission: 655 nm), and a 100µl  
225 automatic sample injector refrigerated at 4°C in the dark. Chromatographic separation was  
226 carried out using a C18 column for reverse phase chromatography (Supelcosil, 25 cm long,  
227 4.6 mm inner diameter). The solvents used were: 0.5M ammonium acetate in methanol and  
228 water (85:15, v:v), acetonitrile and water (90:10, v:v), and 100% ethyl acetate. The solvent  
229 gradient was set according to Brotas and Plante-Cuny (2003), with a 0.5 mL min<sup>-1</sup> flow rate.  
230 Identification and calibration of the HPLC peaks were performed with  $\beta\beta$ -carotene,  
231 canthaxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyll *c2*, diatoxanthin, diadinoxanthin,  
232 fucoxanthin and pheophytin *a* standards. We identified all detected peaks by their absorption  
233 spectra and relative retention times using the Agilent OpenLab software. Quantification was  
234 performed using standard calibration curves built with repeated injections of standards over a  
235 range of dilutions. Xanthophylls, carotens and chlorophyll *b* and *c* were quantified at 470 nm,  
236 chlorophyll *a* and their derivatives as well as pheopigments were quantified at 665 nm. The  
237 relative abundance of each pigment (%) was calculated from its respective concentration in  
238 the sample (µg.mg<sup>-1</sup>).

239

## 2.7 Extracellular Polymeric Substances (EPS) characterization

240 In a 15 mL Falcon® tube, 5 mL of microbial mat was mixed with an equivalent volume of  
241 seawater obtained by mixing water from the five sampled replicate and filtered at 0.22 µm for  
242 each treatment. The tubes were subjected to mechanical agitation by vortexing and by  
243 inversion at a rate of 40 oscillations.min<sup>-1</sup> for 1 h at 4°C in the dark. Then they were  
244 centrifuged at 3500 g for 10 min at 4°C. The supernatant, containing the colloidal fraction,  
245 was recovered and stored at -20°C while the pellet, containing the bound fraction, was  
246 resuspended in 5 mL of seawater (again obtained by mixing water from the five sampled  
247 replicate and filtered at 0.22 µm for each treatment). 1 g of Dowex resin (Dowex Marathon C,  
248 Na<sup>+</sup>, Sigma-Aldrich), was prepared according to the protocol of Takahashi *et al.* (2009). The  
249 tubes were again subjected to the same protocol to obtain the supernatant containing the  
250 fraction of bound EPS was recovered and stored at -20°C.

251 The carbohydrate dosage was performed according to Dubois' colorimetric method (Dubois *et*  
252 *al.*, 1956) while the protein dosage was performed according to the BiCinchoninic acid Assay  
253 (BCA) method using the Pierce™ Protein Assay Kit (Thermoscientific). A range of glucose (L-  
254 (-)-Glucose, 98%, Sigma-Aldrich) from 0 to 3 g.L<sup>-1</sup> and a range of bovine serum albumin  
255 (BSA) from 0 to 1 g.L<sup>-1</sup> were performed in seawater from the Ré island hypersalinated at 60  
256 psu and filtered at 0.22 µm. For carbohydrates, 100 µL of EPS sample were placed in a tube,  
257 then 100 µL of 5% phenol (Solid Phenol, Sigma-Aldrich, France) and 500 µL of 98%  
258 sulphuric acid (Sulphuric Acid 98%, Carlo-Erba Reagents, France) were added. The tubes  
259 were incubated for 30 min in the dark and at room temperature. A volume of 200 µL of the  
260 standard range and each triplicate sample was deposited in a 96-well microplate (Falcon® 96-  
261 well Clear Microplat, Thermo Fisher Scientific). The absorbance of each well was measured  
262 at 490 nm with a spectrophotometer (SPECTROstar® Nano, BMG LAB). For proteins, 225 mL  
263 of reagent was prepared and stored in the dark at room temperature during the assay period.  
264 The assays were performed in 96-well microplates (Falcon® 96-well Clear Microplat, Thermo  
265 Fisher Scientific). 200 µL of reagents were placed in the wells and 25 µL standards or

266 triplicate samples were added. The microplates were incubated for 30 min at 37°C, then the  
267 absorbance of each sample was measured at 562 nm by a spectrophotometer  
268 (SPECTROstar® Nano, BMG LAB). Two calibration curves were drawn from the standard  
269 ranges and their respective abundances (corrected according to the kit indications for the  
270 BSA range), averaged over the duration of the experiment. They made it possible to  
271 determine the carbohydrate and protein concentrations. The latter were then related to the  
272 dry mass of the microbial mat.

273 To determine the dry mass of the microbial mat, a volume of 30 mL of microbial mat was  
274 collected and weighed to obtain the fresh mass ( $M_F$ ). The sample was then freeze-dried to  
275 remove water from the sample (Lyophilisateur Christ Alpha 1-4, Grosseron, France) and re-  
276 weighed to obtain the freeze-dried mass ( $M_L$ ).

## 277 **2.8 Data analysis**

278 All calculations and analyses were performed on R.Studio software (version 4.0.3® RStudio,  
279 Inc.). The mean of physico-chemical parameters, the carbohydrates and proteins  
280 concentrations of each fraction and the photosynthetic parameters of each treatment were  
281 compared with each other at each sampling time. The normality and homoscedasticity of the  
282 data were previously verified by carrying out a Shapiro test and a Bartlett test respectively. If  
283 both conditions were met, the data were subjected to an analysis of variances (ANOVA).  
284 Otherwise, a Welch ANOVA was performed in the case of non-homogeneity of variances and  
285 a Kruskal-Wallis rank sum test in the case of non-normal data. If these tests were found to be  
286 significant ( $p < 0.05$ ), then a pairwise comparison was performed using a Tukey test following  
287 the ANOVA, a Games-Howell test following a Welch ANOVA or a Nemenyi test following the  
288 Kruskal-Wallis test.

289 A Between Class Analysis (BCA) combined with hierarchical cluster analysis with a Bray  
290 dissimilarity index and ward.D2 method was done on pigments proportions.

## 291 **3. Results**

292

### 293 **3.1 Efficiency of the physico-chemical changes**

294

295 As expected, the temperature in W and WA treatments increased well by 4°C from the initial  
296 temperature, the one maintained in C and A treatments ( $23.89 \pm 0.49^\circ\text{C}$ ;  $23.82 \pm 0.48^\circ\text{C}$ ;  
297  $20.32 \pm 0.36^\circ\text{C}$ ;  $20.23 \pm 0.48^\circ\text{C}$ ; respectively) (Fig. 2, A; Supplementary materials, fig. B).  
298 There was a delay of one week before reaching this increase after the change of state  
299 because this was the time needed to heat up all the water in the pool and then the  
300 mesocosms.

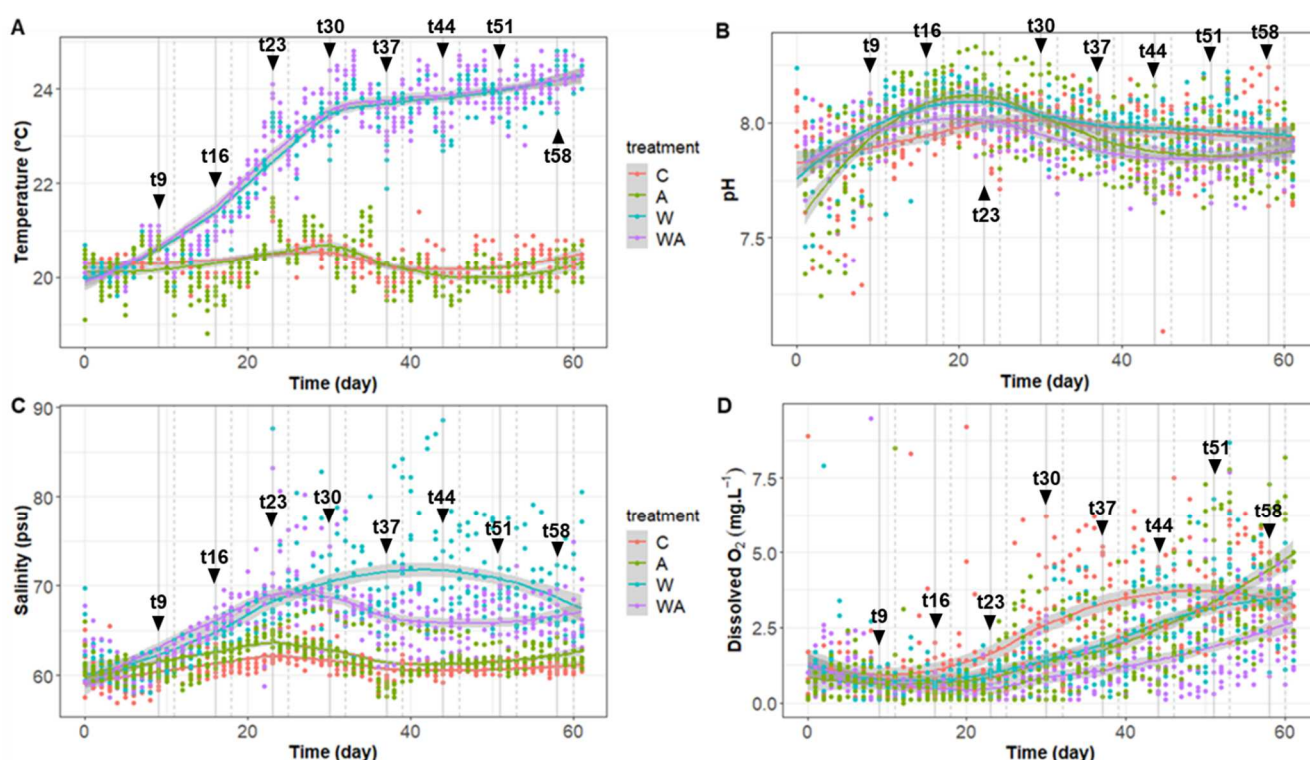
301 The pH of C and W treatments were stabilized after t37 at  $7.94 \pm 0.15$  and  $7.95 \pm 0.09$ ,  
302 respectively (Fig.2, B; Supplementary materials, fig. B). The pH of A and WA treatments  
303 decreased until t44, then remaining stable at pH  $7.87 \pm 0.13$  and  $7.86 \pm 0.10$ , respectively  
304 (Fig.2, B; Supplementary materials, fig. B). No significant difference between the four  
305 treatments was found at t9 (Kruskal,  $p > 0.05$ ).

306 The salinity (Fig. 2, C; Supplementary materials, fig. B) of C and A treatments remained  
307 stable at  $60.87 \pm 1.44$  psu and  $61.91 \pm 1.79$  psu, respectively. The salinity of the W treatment  
308 increased to a maximum mean value of  $75.87 \pm 4.84$  psu after 40 days of incubation, and  
309 then decreased again to a final value of  $67.35 \pm 4.24$  psu. The salinity of the WA treatment  
310 increased and reached its maximum value of  $71.94 \pm 6.55$  psu on day 23 of the experiment  
311 and then decreased and remained at  $66.13 \pm 2.56$  psu.

312 Dissolved oxygen remained stable between each treatment up to t16 (mean  $\pm$  standard  
313 deviation;  $0.95 \pm 1.18$  mg.L<sup>-1</sup> for control,  $0.61 \pm 1.15$  mg.L<sup>-1</sup> for pH,  $0.60 \pm 0.46$  mg.L<sup>-1</sup> for W  
314 and  $0.30 \pm 0.24$  mg.L<sup>-1</sup> for WA) (Fig. 2, D; Supplementary materials, fig. B). From t23, the  
315 dissolved oxygen of the treatments increased linearly to t58 with a slope of  $0.07$  day<sup>-1</sup> ( $R^2 =$   
316  $0.45$ , Student-test,  $p < 0.05$ ) for A,  $0.05$  day<sup>-1</sup> ( $R^2 = 0.39$ , Student-test,  $p < 0.05$ ) for W and  
317  $0.03$  day<sup>-1</sup> ( $R^2 = 0.19$ , Student-test,  $p < 0.05$ ) for WA. The A treatment had a final oxygen  
318 concentration of  $5.95 \pm 1.63$  mg.L<sup>-1</sup>, significantly higher (ANOVA,  $p < 0.05$ ) than that of the

319 other mesocosms by a factor of 1.6 for W, 2.2 for C and 2.4 for WA (mean  $\pm$  standard  
320 deviation).

321 The nutrients were analyzed thanks to the Redfield ratio calculations (Redfield, 1958). Only  
322 seven samples showed nitrogen limitation, notably four replicates of the A treatment at t30  
323 and some samples showed a limitation of silicon (Supplementary materials, fig. B and C).  
324 Most of the samples showed phosphate limitation, but such phosphate limitation was also  
325 observed *in situ* (Supplementary materials, fig. B and C).



326 **Figure 2:** Temporal variation of the temperature (°C) (A), the pH (upH) (B), the salinity (psu)  
327 (C) and the dissolved oxygen (mg.L<sup>-1</sup>) (D) of the different treatments (control (C), acidification  
328 (A), warming (W) or warming and acidification mix (WA)). The points corresponded to the  
329 values measured for each sample. The curves represented local regressions, based on the  
330 k-nearest neighbors algorithm (*geom\_smooth* function of the *ggplot2* package, loess  
331 method). The grey areas symbolised the 95% confidence intervals. The sampling days were  
332 represented by black arrows. The day 0 to day 8 corresponded to the stabilisation week.  
333

334

### 335 3.2 Pigment composition

336

337 A total of 37 pigments were identified. The analysis revealed the presence of several  
338 chlorophyll: chlorophyll *a* (Ca) and corresponding epimers and allomers, chlorophyll *b* (Cb)

339 and chlorophyll *c2* (Cc2). Bacteriochlorophyll *a* (BCa) was identified as well as echinenone,  
 340 oscillol diquinovoside, myxol quinovoside and zeaxanthin. Other pigments were identified  
 341 including lutein, alloxanthin, carotenoids and canthaxanthins and its isomers. Pigments  
 342 corresponding to alteration products were also found: pyropheophytin, pheophytin *a*,  
 343 pheophorbide *a* and chlorophyllide *a*. The presence of non-identified chlorophyll *a*-like  
 344 molecules was also observed and hereby called chlorophyll derivatives (C deriv. #1-6).

345 **Table 1:** Pigments identified by HPLC and its corresponding abbreviations.

Pigment	Notation
alpha and beta Cryptoxanthin	aCy; bCy
Alloxanthin	Al
Beta-beta caroten; beta-epsilon caroten	BB.Car; BE.Car
Unknown carotenoids	Car1; Car2
Chlorophyll <i>a</i> ; Chlorophyll <i>a</i> allomers/epimers	Ca; Ca.allo; Ca.epi
Chlorophyll derivatives	C deriv. #1-6
Bacteriochlorophyll <i>a</i>	BCa
Chlorophyll <i>b</i>	Cb
Chlorophyll <i>c2</i>	Cc2
Chlorophyllide <i>a</i>	Cda
Canthaxanthin	Ct; Ct.iso
Echinenone	Ec
Fucoxanthin and isomers	F; F.iso1
Lutein and isomers	L; L.iso1; L.iso2
Myxol quinovoside and isomers	My.iso1; My.iso2; My.iso3; My.iso4
Oscillol diquinovoside	O
Pheophorbide	Pda.1
Pheophytin	Pha
Pyropheophytin	Pya
Zeaxanthin	Z

346

### 347 **3.3 Acidification changes the pigments dynamics**

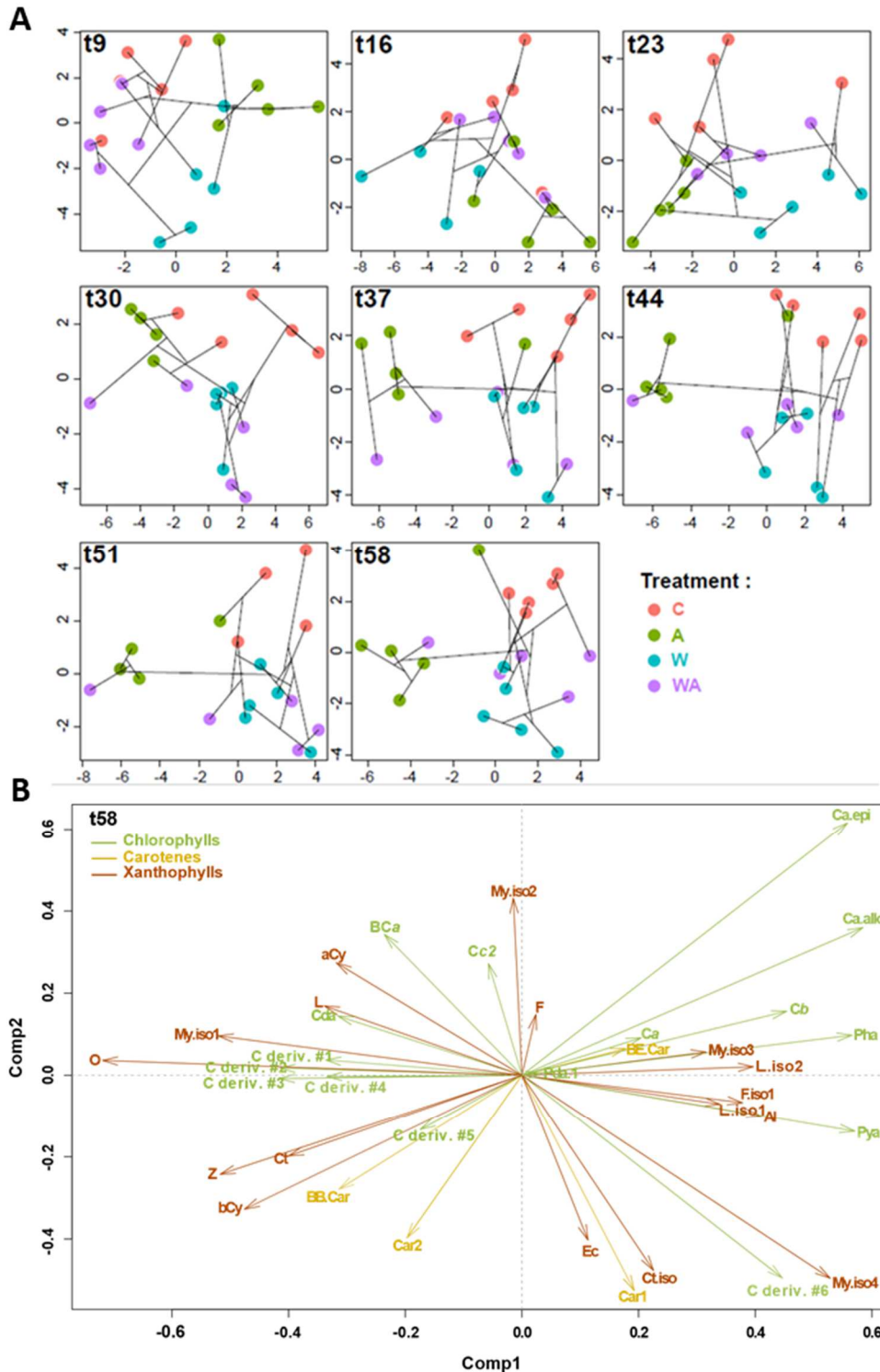
348

349 At t9, all treatments were placed under the same water temperature and pH. No difference  
 350 between pigments were observed (Fig.3) (ANOVA or Welch or Kruskal,  $p>0.05$ ).

351 The C treatment was different form the other treatments containing more myxol quinovoside  
 352 and isomers 2 and 3 than A treatment at t37 and t44 (Kruskal,  $p<0.05$ ) and than the three  
 353 other treatments at t51 (ANOVA,  $p<0.05$ ). It also contained more chlorophyll epimers at t58  
 354 than A, W and WA treatments (ANOVA,  $p<0.05$ ) (Fig.3). The W treatment was differentiated  
 355 from the A treatment with the presence of chlorophyll derivative #6 at t51 and t58 (Welch,

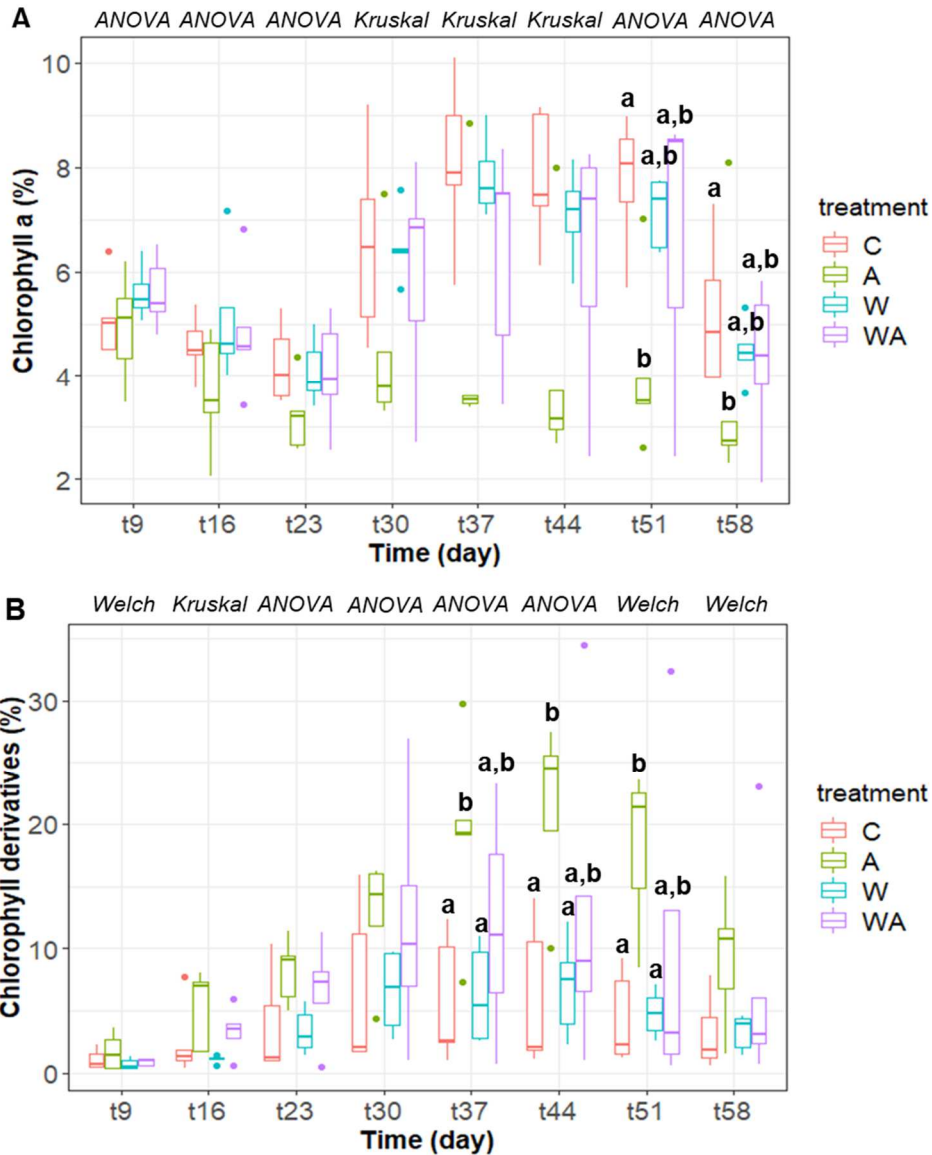
356  $p < 0.05$ ) (Fig.3). The concentration of chlorophyll *a* was around 4% at the beginning of the  
357 experiment (Fig. 4, A). It increased until t51 in all treatments except for A treatment and then  
358 decreased at t58 (Fig. 4, A). The A treatment slightly decreased at t58 and the proportion of  
359 chlorophyll *a* was lower in this treatment than in the C treatment (ANOVA,  $p < 0.05$ ) at t51  
360 and t58 (Fig. 4, A). Conversely, the proportion of chlorophyll derivatives (Fig. 4, B) was close  
361 to 0 at t9 for all treatments. It increased to t30, remained stable at t44 and then decreased  
362 again to t58 for the C, W and WA groups, while it increased to t44 before decreasing again to  
363 t58 for the A group (Fig. 4, B). The proportion of chlorophyll derivatives was significantly  
364 higher for the A treatment at t37, t44 and t51 (ANOVA and Welch,  $p < 0.05$ ) (Fig. 4, B).





365

366 **Figure 3:** Dynamics of microbial communities according to pigments composition and the  
 367 sampling time (tX) **(A)**. This figure was obtained thanks to analyze BCA combined with  
 368 hierarchical cluster analysis with a Bray dissimilarity index and ward.D2 method. A  
 369 representation more detailed with the different parameters was done at t58 **(B)**. The  
 370 correspondence between the abbreviations and the names of pigments is provided on the  
 371 **table 1**.



372

373

374 **Figure 4:** Temporal variations of **(A)** the concentration of chlorophyll a (%) and **(B)** the  
 375 concentration of chlorophyll derivatives (%). The letters t followed by a number indicates the  
 376 sampling time. The statistical test performed to show the differences between treatments at a  
 377 sampling time is indicated in italics above the figure. The letters indicated a significant  
 378 difference ( $p < 0.05$ ) found after a post-hoc Tuckey test in the case of an ANOVA or a  
 379 Games-Howell test in the case of a Welch ANOVA.

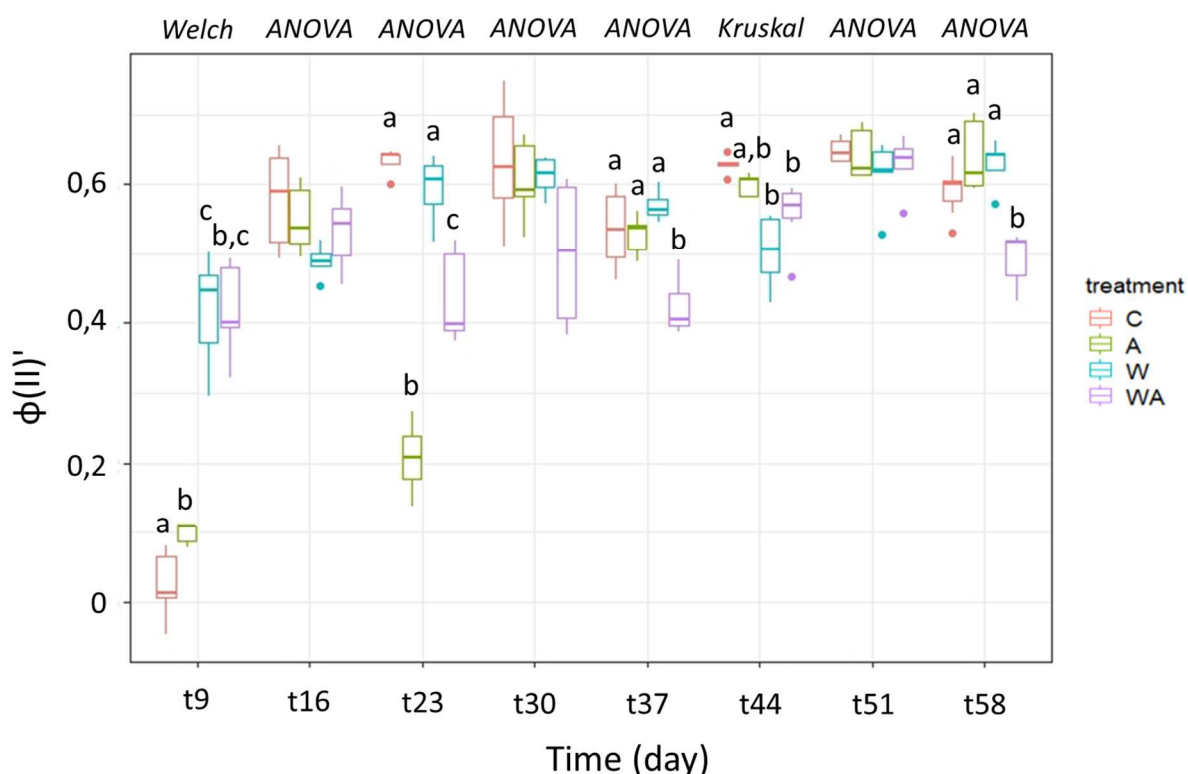
380

### 381 **3.4 Comparison of the photosynthetic yields of the microbial mat in the** 382 **different treatments**

383

384 The quantum efficiency of photosystem II ( $\phi(II)'$ ) indicated the photosynthetic efficiency of the  
 385 mat. At t9, the quantum yields of the C and A treatments were close to 0, while those of the

386 W and WA groups were 14 and 4.2 times more than C and A treatments respectively (Fig. 5).  
 387 At t16 no significant difference was observed between all the quantum yields (ANOVA,  $p >$   
 388 0.05) (Fig. 5). Other difference were observed between treatments during the experiment,  
 389 but the kinetics obtained showed no real trend between treatments (Fig. 5), only WA  
 390 treatment presented a quantum yields lower than C treatment at t37, t51 and t58 (ANOVA or  
 391 Kruskal,  $p < 0.05$ ) than W and A treatments at t37 and t58 (ANOVA,  $p < 0.05$ ).



392

393 **Figure 5:** Variation of the quantum efficiency of photosystem II ( $\phi(II)'$ ) in the different  
 394 treatment. The letters t followed by a number indicates the sampling time. Above the figure  
 395 was indicated in italics the test carried out for the comparison between treatments of the  
 396 same week. The letters indicated a significant difference ( $p < 0.05$ ) found after a post-hoc  
 397 Tukey HSD test in the case of an ANOVA, a Games-Howell test in the case of a Welch  
 398 ANOVA or a Nemenyi test in the case of a Kruskal Wallis test.

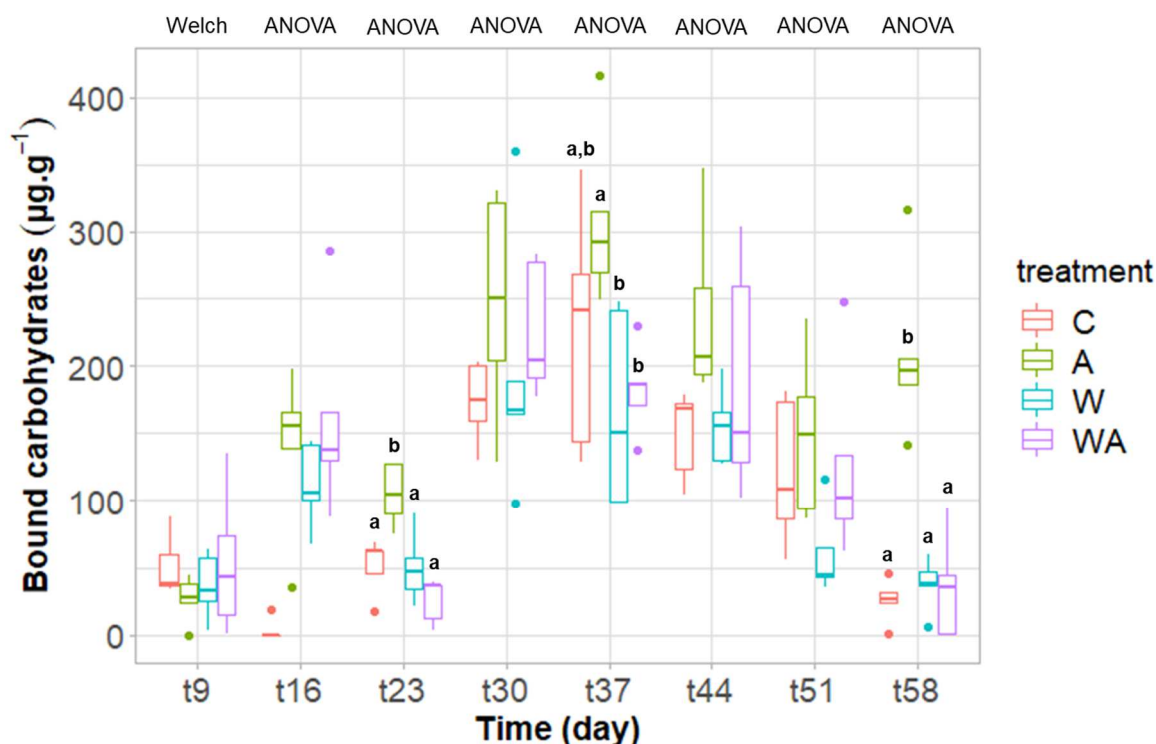
399

### 400 3.5 Acidification impacted the proportion of EPS

401

402 The A treatment was dissimilar from the other treatments, characterized by its higher  
 403 concentrations of bound carbohydrates EPS.

404 The carbohydrates bound EPS concentration was notably more important in A treatment on  
405 three times: at t23, t37 and t58 (ANOVA,  $p < 0.05$ ) (Fig. 6).



406

407 **Figure 6:** Temporal variation of the carbohydrate concentration in the bound fraction of EPS  
408 (in  $\mu\text{g.g}^{-1}$  dry mass). The letters t followed by a number indicates the sampling time. The  
409 statistical test performed to show the differences between treatments at a sampling time is  
410 indicated in italics above the figure. The letters indicate a significant difference ( $p < 0.05$ )  
411 found after a post-hoc Tukey test. Note: the ANOVA performed at t16 did not consider the C  
412 treatment because of the presence of outliers.

413

#### 414 **4. Discussion**

415

416 The aim of the study was to simulate acidification and/or warming on microbial mats. The  
417 mesocosm system has the advantage to control the physico-chemical parameters allowing to  
418 simulate future conditions, which is impossible to achieve directly in the field; but the natural  
419 environment can never be mimicked perfectly (weather, daily or even seasonal variations in  
420 physico-chemical parameters, *etc.*). Although mesocosm system will never faithfully  
421 represent reality, it is a compromise often used to conduct experimental ecology studies  
422 (Cravo-Laureau and Duran, 2014). It is therefore advisable to remain vigilant so as not to

423 extrapolate too generally the results obtained with *in situ* microbial mats. Given the diversity  
424 of structure and functioning of microbial mats throughout the world, they may act differently in  
425 their natural environment. It should also be noted that this study was a simulation of the  
426 IPCC's most pessimistic predictions (RCP8.5) for 2100. The changes simulated only  
427 concerned the decrease in water pH and the increase in water temperature, but these are  
428 not the only parameters that are expected to vary in the future. Deoxygenation of the oceans,  
429 a succession of extreme weather events, and sea level rise in some regions are also  
430 foreseeable changes that have not been tested in this study. Moreover, they have been  
431 monitored over seven weeks, whereas variations in the natural environment are supposed to  
432 occur over several decades.

433 The renewal water in the mesocosm permitted to maintain a stable water level at 3 cm above  
434 the mat and a natural supply of nutrients for the development of microbial mats. Redfield  
435 ratio showed some limitation of nitrogen and/or phosphorus in the water under some  
436 conditions but previous studies have shown that microbial mats can develop in P- (Peimbert  
437 et al., 2012) and N-limited environment (Peimbert et al., 2012) (Supplementary materials, fig.  
438 C).

439 Surprisingly, the decrease of pH under the A and WA treatments was not noticeable in the  
440 mesocosms (Fig. 2) although the pH in the respective water reserve decreased  
441 (Supplementary materials, fig. D) at the expected pH 7.6 (the initial pH was 8). This  
442 phenomenon has been observed in other studies (Crawford et al., 2011; Ma et al., 2019).  
443 The most likely hypothesis is that the rate of CO<sub>2</sub> fixation by photosynthesis was higher in the  
444 A and WA treatments, which had the effect of increasing the pH. In our experiment, dissolved  
445 oxygen increased under A treatment after the third week. Moreover, many microbial species  
446 are able to use carbon concentration mechanisms (CCMs), storing high CO<sub>2</sub> concentrations  
447 before transport into the Rubisco compartments, which minimise photorespiration. These  
448 CCMs are found in oxygenic phototrophs like cyanobacteria or most of the phytoplankton  
449 (Ma et al., 2019). The CCMs have been proposed as mechanisms to prevent photosynthetic

450 systems to directly detect ambient changes in CO<sub>2</sub> (Mackey et al., 2015). As a result,  
451 photosynthetic rates might not respond directly to ambient changes in CO<sub>2</sub> explaining the  
452 delay observed in the increase of dissolved oxygen in our experiment. Such observation is  
453 consistent with the fact that pH decreased from t23 while dissolved oxygen continued to  
454 increase (Fig. 2). Black et al. (2019) suggested that microphytobenthos use additional CO<sub>2</sub>  
455 due to acidification for photosynthesis until the maximum yield capacity has been reached.  
456 When the cells reached maximum intracellular CO<sub>2</sub> concentration through their CCMs, any  
457 additional CO<sub>2</sub> would contribute to the decrease in pH. The pH of the WA treatment followed  
458 the same dynamic as observed for the A treatment. However, the dissolved oxygen  
459 concentration under WA treatment increased less than half in comparison to the A treatment  
460 (Fig. 2, D). The CO<sub>2</sub> absorption mechanisms may have been less efficient in WA treatment  
461 because temperature decreases the solubility of CO<sub>2</sub> (Wootton et al., 2008).

462 Numerous studies have shown that photosystem II is very sensitive to environmental stress  
463 (Murata et al., 2007; Nishiyama et al., 2008; Wang et al., 2013). It is therefore important to  
464 focus on the quantum yield of photosystem II, which indicates the maximum photosynthetic  
465 activity potential of the communities. At t9, the quantum yield of photosystem II for the C and  
466 A treatments were close to 0 (Fig. 5), which indicated that the mats were not in good  
467 physiological condition when starting the experiment. Significantly lower quantum yields were  
468 observed under some treatments. At t23, under the A and the WA treatments an unexplained  
469 decrease of the quantum yield was observed (Fig. 5). The WA treatment exhibited  
470 significantly lower quantum yield than the other treatments at t37, t51 and t58 (Fig. 5),  
471 suggesting that mats have more difficulty withstanding simultaneous acidification and  
472 heating. It was not surprising that there was no difference in quantum efficiency between A  
473 and C treatments (Fig. 5). Photosynthetic organisms have a high capacity to modify pH with  
474 evidence that pH is regulated at the water/cell interface (Black et al., 2019). Our fluorometer  
475 illuminated with a blue LED emitter (455 nm). Chlorophyll *a* fluorescence per unit  
476 concentration in cyanobacteria tends to be lower than in algae when it is excited with blue

477 light. This leads to an erroneous biomass estimate of cyanobacteria. In their study, Simis *et*  
478 *al.* (2012) have sought the optimal excitation and emission pairs for the separation of  
479 cyanobacterial and algal Fv/Fm in communities. They demonstrate that the highest  
480 correlation between community and cyanobacterial variable fluorescence is obtained under  
481 orange-red excitation in the 590–650 nm range, exciting cyanobacterial phycobilipigments.  
482 No information was found about the other phototrophic communities.

483 The A treatment possessed more chlorophyll derivatives and lower chlorophyll *a* than the  
484 other treatments (Fig. 4). These chlorophyll derivatives molecules seemed to be bioindicators  
485 of a stress condition (water acidification). They were not identified by available standards.  
486 These molecules did not correspond to known metallised allomers or epimers of chlorophyll  
487 *a* (Ca), as revealed by their retention time (Supplementary materials, fig. E). Based on their  
488 absorption spectra (Fig. 7), we can also rule out the hypothesis that these molecules  
489 correspond to de-metallised derivatives of chlorophyll *a* such as pheophytin *a* or  
490 pheophorbide *a* or even to bacteriochlorophyll *a* (BCa).

491 We can assume that these molecules correspond to transmetalated Ca or BCa. Some  
492 microorganisms are known to possess bacteriochlorophyll those the central Mg ion is  
493 replaced by another metal, as observed for *Acidiphilium* where BCa possess a Zn central  
494 metal (Hiraishi and Shimada, 2001). This organism has been isolated from acidic mine ponds  
495 and it is supposed likely that pH constituted the evolutionary pressure responsible for the  
496 change of the central metal (Hiraishi and Shimada, 2001). Although this is an attractive  
497 hypothesis, it is highly unlikely that our pH treatment had the effect of promoting the  
498 synthesis of transmetalated BCa as Zn-BCa displays absorption features in the near infra-red  
499 range (Nagata *et al.*, 2003) that were not observed here.

500 Chlorophylls and porphyrin derivatives generally have two major absorption bands (*i.e.* “red”  
501 (Q-) and “blue” (Soret-) bands, Fig. 7) in the visible range, due to extended  $\pi$ -delocalization  
502 at the edge of cyclic porphyrin skeleton (Milenković *et al.*, 2012). Two main Q-bands ( $Q_{y,0-0}$   
503 and  $Q_{y,0-1}$ ) are traditionally observed in the original Ca (or Mg-Ca) (Gerola *et al.*, 2011). In our

504 case, Ca wavelengths of maximum absorption  $\lambda_{\max}$  were respectively 665 ( $Q_{y,0-0}$ ) and 615 nm  
505 ( $Q_{y,0-1}$ ).

506 Based on these findings, the unidentified derivatives do not correspond to transmetalated  
507 Ca, although those absorption characteristics are close to the Mg-Ca molecule. As shown  
508 previously by Gerola *et al.* (2011), a hypsochromic (blue) shift of both Soret and  $Q_{y,0-0}$  bands  
509 must be expected with significant decreasing of  $\lambda_{\max}$  in Zn- and Cu-Ca in comparison to Mg-  
510 Ca. Such hypsochromic shifts were not observed in the unidentified derivatives spectra.

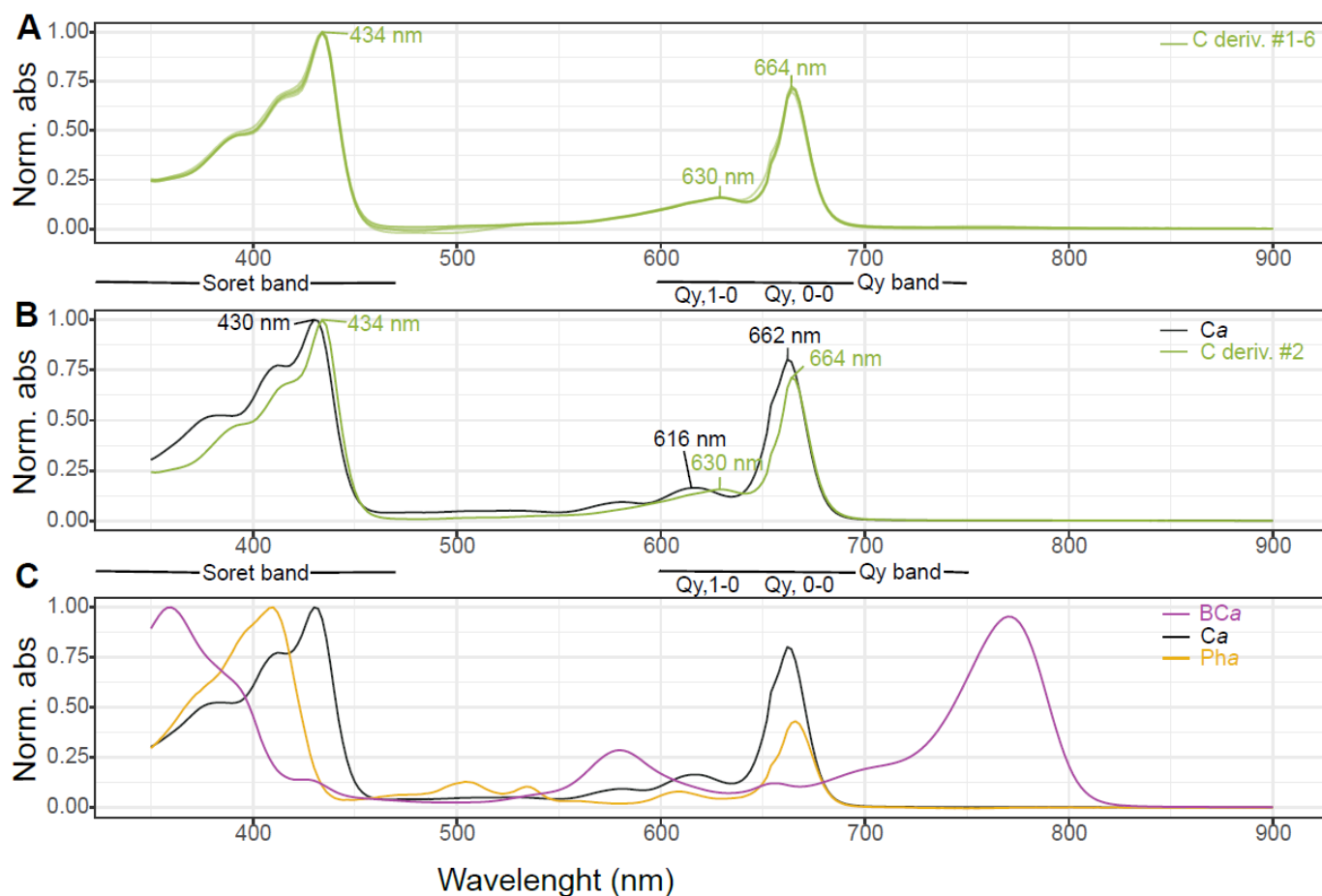
511 The only noticeable change corresponded to a decreased intensity (hypochromic effect) in  
512 the  $Q_{y,0-0}$  band in comparison with that of Mg-Ca and a bathochromic (red) shift in the  $Q_{y,0-1}$ .  
513 (Fig.7). These characteristics are similar to those of bacteriochlorophyll *c* (BC*c*) with a  
514 recorded  $\lambda_{\max}$  (630 nm) for  $Q_{y,0-1}$  close to the expected 623-629 nm (Goedheer, 1966; Oelze,  
515 1985; Pierson and Castenholz, 1974). This pigment is typically found in the chlorosomes of  
516 green anoxygenic phototroph bacteria, including the green sulfur bacteria and the green non-  
517 sulfur bacteria (Frigaard *et al.*, 2006; Scheer, 2006), which indicated that the pH conditions  
518 may have favoured the growth of these phototrophic bacteria.

519 Even moderate changes of both pH and temperature are relevant for bacterial community  
520 composition of microbial mats (Uribe-Lorío *et al.*, 2019). A decrease in chlorophyll *a* has  
521 been observed under high CO<sub>2</sub> levels suggesting that the CO<sub>2</sub> input reduce CCMs and thus  
522 save energy, particularly in pigment synthesis (Wang *et al.*, 2019; Yue *et al.*, 2019). In hot  
523 springs from Costa Rica, significant changes of microbial mat communities were observed  
524 with a significant increase of Chloroflexi (also named the green non-sulfur bacteria)  
525 abundance with decreasing pH and increasing temperature (Uribe-Lorío *et al.*, 2019).  
526 Chloroflexi have been previously reported to be more abundant at decreased pH on  
527 sediment of hydrothermal CO<sub>2</sub> seeps in Papua New Guinea (Hassenrück *et al.*, 2016) and in  
528 host-association with corals and sponges (Kandler *et al.*, 2018; Morrow *et al.*, 2015).  
529 Chlorobi, the green sulfur bacteria, have also been found to increase in abundance with  
530 acidification (Hassenrück *et al.*, 2016). On their study, Hassenrück *et al.* (2016) supposed



531 that in the sites characterized by low pH and high hydrothermal influence, including  
 532 pronounced temperature increases, the Chloroflexi and Chlorobi replaced other microbial  
 533 communities as major carbon degraders under anoxic conditions. Green anoxygenic bacteria  
 534 participate on the carbon cycling and have been shown to fix CO<sub>2</sub> (Hug et al., 2013; Sirevåg,  
 535 2004). The phototrophic communities of the microbial mats could be modified under A  
 536 treatment leading to a higher relative abundance of green anoxygenic bacteria. It is also  
 537 possible that the presence of more CO<sub>2</sub> on the environment conducted to a shift on the  
 538 metabolism of green anoxygenic phototrophic bacteria fixing the CO<sub>2</sub> and being more  
 539 competitive with the other communities.

540



541

542 **Figure 7:** Normalized absorption spectra of different pigments of the microbial mats. **A:**  
 543 superimposed absorption spectra of the 6 detected chlorophyll derivatives (C deriv.). **B:**  
 544 comparison between the absorption spectra of chlorophyll a (Ca) and C deriv. #2. **C:**

545 comparison between the absorption spectra of Ca, pheophytin a (Pha) and  
546 bacteriochlorophyll a (BCa).

547

548 The bound carbohydrate EPS production was higher under the A treatment. EPS are  
549 essential for maintaining the physical properties and proper functioning of microbial mats,  
550 and are also involved in the adaptation of communities to their environment (Dupraz and  
551 Visscher, 2005; Hubas, 2018; Prieto-Barajas et al., 2018). Therefore, EPS are an essential  
552 element, even more when microorganisms are confronted with strong variations in physico-  
553 chemical parameters. It has been observed that the degradation of polysaccharides by  
554 bacterial extracellular enzymes is accelerated at low pH (Piontek et al., 2009). It can be  
555 supposed that microbial mats could therefore synthesize more carbohydrates to compensate  
556 for this degradation. Microbial mats in the W treatment did not show any variation in  
557 concentration, suggesting that temperature has no effect on this process. Those in the WA  
558 treatment also did not change suggesting that acidification and warming have probably  
559 antagonistic effects limiting the impact of acidification on EPS. However, Li *et al.* (2016)  
560 obtained contradictory results on three freshwater microalgae as they demonstrated that  
561 acidification and warming act synergistically. The variation of bound carbohydrate EPS  
562 suggest that microbial mats subjected to acidification modified their metabolism. Tan *et al.*  
563 (2019) have shown that a polar strain of *Chlorella sp.* modulates its metabolism under  
564 acidified conditions ( $p\text{CO}_2 = 1000 \mu\text{atm}$ ), favouring its survival. They observed a decrease in  
565 the fluxes of glucose and sucrose, the main products of photosynthesis, under high  $p\text{CO}_2$ .  
566 Gong *et al.* (2020) also observed that the starch metabolism was modified by increasing the  
567 quantity of starch granules in another microalgae named *Symbiochlorum hainanensis*. These  
568 observations suggest that the organic carbon produced by photosynthesis in the microbial  
569 mats placed under the A treatment was redirected towards the synthesis of other  
570 carbohydrates which concentration had considerably increased.

571

## 572 **5. Conclusion**

573 The temperature in the warming treatments increased by 4°C from the initial temperature as  
574 expected. Despite of a decrease of 0.4 unit pH in the water reserves of acidification  
575 treatments, no significance difference was observed between the water pH of the different  
576 treatments. The salinity increased on the warming treatments because of the water  
577 evaporation. The dissolved oxygen concentration increased and was higher on the  
578 acidification treatments, certainly due to an increase of the photosynthesis because of the  
579 carbon input.

580 In the acidification treatment, our results showed that the concentration of bound  
581 carbohydrates EPS increased. This indicated that the metabolisms were modified to cope  
582 with the induced changes. In addition, the phototrophic communities of the microbial mats  
583 under the different treatments showed characteristic pigments. In particular, unknown  
584 chlorophyll derivatives were present under acidification and/or warming treatments. To the  
585 best of our knowledge the synthesis of such derivatives following an acidification or/and a  
586 warming experiment has never been reported before. These molecules were eventually  
587 identified as bacteriochlorophyll *c* and several possible isomers which suggest that  
588 experimentally induced climate change scenario may have favoured the increase of BC*c*  
589 contained on green anoxygenic phototroph bacteria.

590 The mesocosm experiment has shown that phototrophic communities of the microbial mats  
591 were able to adapt to the conditions defined by the IPCC (2014) regarding acidification and  
592 warming of surface water. The most probable explanation is that the studied microbial mats  
593 are already naturally confronted with environmental conditions (pH, light, temperature,  
594 salinity, *etc.*) that fluctuate with a great amplitude in salt marshes. They may therefore  
595 already be confronted with temperature and pH conditions predicted, for example, by the  
596 IPCC for 2100. Changes in their metabolism probably enabled them to maintain a high  
597 potential for photosynthetic activity when acidification took place. In the future, it would be  
598 interesting to observe if a difference in the phylogenetic composition of these communities  
599 occurred. This study provides new insights on the response of phototrophic communities of

600 microbial mats in a context of climatic change and permit to better understand their function  
601 in this ecosystem.

602

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604

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611

## 612 **References**

- 613 Aminot, A., Kérouel, R., 2007. Dosage automatique des nutriments dans les eaux marines., Quae. ed.  
614 Baragi, L.V., Anil, A.C., 2016. Synergistic effect of elevated temperature, pCO<sub>2</sub> and nutrients on  
615 marine biofilm. *Mar. Pollut. Bull.* 105, 102–109.  
616 <https://doi.org/10.1016/j.marpolbul.2016.02.049>
- 617 Baragi, L.V., Khandeparker, L., Anil, A.C., 2015. Influence of elevated temperature and pCO<sub>2</sub> on the  
618 marine periphytic diatom *Navicula distans* and its associated organisms in culture.  
619 *Hydrobiologia* 762, 127–142. <https://doi.org/10.1007/s10750-015-2343-9>
- 620 Beardall, J., Stojkovic, S., Larsen, S., 2009. Living in a high CO<sub>2</sub> world: Impacts of global climate  
621 change on marine phytoplankton. *Plant Ecol. Divers.* 2, 191–205.  
622 <https://doi.org/10.1080/17550870903271363>
- 623 Black, J.G., Stark, J.S., Johnstone, G.J., McMinn, A., Boyd, P., McKinlay, J., Wotherspoon, S., Runcie,  
624 J.W., 2019. In-situ behavioural and physiological responses of Antarctic microphytobenthos  
625 to ocean acidification. *Sci. Rep.* 9, 1890. <https://doi.org/10.1038/s41598-018-36233-2>
- 626 Bolhuis, H., Stal, L.J., 2011. Analysis of bacterial and archaeal diversity in coastal microbial mats using  
627 massive parallel 16S rRNA gene tag sequencing. *ISME J.* 5, 1701–1712.  
628 <https://doi.org/10.1038/ismej.2011.52>
- 629 Bordenave, S., Fourçans, A., Blanchard, S., Goñi, M.S., Caumette, P., Duran, R., 2004a. Structure and  
630 functional analyses of bacterial communities changes in microbial mats following petroleum  
631 exposure. *Ophelia* 58, 195–203. <https://doi.org/10.1080/00785236.2004.10410227>
- 632 Bordenave, S., Goñi-urriza, M., Vilette, C., Blanchard, S., Caumette, P., Duran, R., 2008. Diversity of  
633 ring-hydroxylating dioxygenases in pristine and oil contaminated microbial mats at genomic  
634 and transcriptomic levels. *Environ. Microbiol.* 10, 3201–3211.  
635 <https://doi.org/10.1111/j.1462-2920.2008.01707.x>
- 636 Bordenave, S., Jézéquel, R., Fourçans, A., Budzinski, H., Merlin, F.X., Fourel, T., Goñi-Urriza, M.,  
637 Guyoneaud, R., Grimaud, R., Caumette, P., Duran, R., 2004b. Degradation of the “Erika” oil.  
638 *Aquat. Living Resour.* 17, 261–267. <https://doi.org/10.1051/alr:2004027>

639 Brotas, V., Plante-Cuny, M.-R., 2003. The use of HPLC pigment analysis to study microphytobenthos  
640 communities. *Acta Oecologica* 24. [https://doi.org/10.1016/S1146-609X\(03\)00013-4](https://doi.org/10.1016/S1146-609X(03)00013-4)

641 Cartaxana, P., Vieira, S., Ribeiro, L., Rocha, R., Cruz, S., Calado, R., Marques da Silva, J., 2015. Effects  
642 of elevated temperature and CO<sub>2</sub> on intertidal microphytobenthos. *BMC Ecol.* 15, 10.  
643 <https://doi.org/10.1186/s12898-015-0043-y>

644 Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius,  
645 A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J.,  
646 Hutchins, D.A., Jansson, J.K., Karl, D.M., Koskella, B., Mark Welch, D.B., Martiny, J.B.H.,  
647 Moran, M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart,  
648 F.J., Sullivan, M.B., van Oppen, M.J.H., Weaver, S.C., Webb, E.A., Webster, N.S., 2019.  
649 Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* 17,  
650 569–586. <https://doi.org/10.1038/s41579-019-0222-5>

651 Cravo-Laureau, C., Duran, R., 2014. Marine coastal sediments microbial hydrocarbon degradation  
652 processes: contribution of experimental ecology in the omics'era. *Front. Microbiol.* 5.  
653 <https://doi.org/10.3389/fmicb.2014.00039>

654 Crawford, K.J., Raven, J.A., Wheeler, G.L., Baxter, E.J., Joint, I., 2011. The Response of *Thalassiosira*  
655 *pseudonana* to Long-Term Exposure to Increased CO<sub>2</sub> and Decreased pH. *PLoS ONE* 6,  
656 e26695. <https://doi.org/10.1371/journal.pone.0026695>

657 Decho, A., 2000. Microbial biofilms in intertidal systems: an overview. *Cont. Shelf Res.* 20, 1257–  
658 1273. [https://doi.org/10.1016/S0278-4343\(00\)00022-4](https://doi.org/10.1016/S0278-4343(00)00022-4)

659 Decho, A., 1990. Decho AW.. Microbial exopolymer secretions in ocean environments: their role(s) in  
660 food webs and marine processes. *Oceanogr Mar Biol Ann Rev* 28: 73-153. *Oceanogr. Mar.*  
661 *Biol. Annu. Rev.* 28, 73–154.

662 Decho, A.W., Moriarty, D.J.W., 1990. Bacterial exopolymer utilization by a harpacticoid copepod: A  
663 methodology and results. *Limnol. Oceanogr.* 35, 1039–1049.  
664 <https://doi.org/10.4319/lo.1990.35.5.1039>

665 Dobretsov, S., Abed, R.M.M., Al Maskari, S.M.S., Al Sabahi, J.N., Victor, R., 2011. Cyanobacterial mats  
666 from hot springs produce antimicrobial compounds and quorum-sensing inhibitors under  
667 natural conditions. *J. Appl. Phycol.* 23, 983–993. <https://doi.org/10.1007/s10811-010-9627-2>

668 Dubois, Michel., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, Fred., 1956. Colorimetric Method for  
669 Determination of Sugars and Related Substances. *Anal. Chem.* 28, 350–356.  
670 <https://doi.org/10.1021/ac60111a017>

671 Dupraz, C., Visscher, P.T., 2005. Microbial lithification in marine stromatolites and hypersaline mats.  
672 *Trends Microbiol.* 13, 429–438. <https://doi.org/10.1016/j.tim.2005.07.008>

673 Dutta, H., Dutta, A., 2016. The microbial aspect of climate change. *Energy Ecol. Environ.* 1, 209–232.  
674 <https://doi.org/10.1007/s40974-016-0034-7>

675 Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633.  
676 <https://doi.org/10.1038/nrmicro2415>

677 Fourçans, A., Oteyza, T.G. de, Wieland, A., Solé, A., Diestra, E., Bleijswijk, J. van, Grimalt, J.O., Kühl,  
678 M., Esteve, I., Muyzer, G., Caumette, P., Duran, R., 2004. Characterization of functional  
679 bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue,  
680 France). *FEMS Microbiol. Ecol.* 51, 55–70. <https://doi.org/10.1016/j.femsec.2004.07.012>

681 Fourçans, A., Ranchou-Peyruse, A., Caumette, P., Duran, R., 2008. Molecular Analysis of the Spatio-  
682 temporal Distribution of Sulfate-reducing Bacteria (SRB) in Camargue (France) Hypersaline  
683 Microbial Mat. *Microb. Ecol.* 56, 90–100. <https://doi.org/10.1007/s00248-007-9327-x>

684 Fourçans, A., Solé, A., Diestra, E., Ranchou-Peyruse, A., Esteve, I., Caumette, P., Duran, R., 2006.  
685 Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from  
686 Salins-de-Giraud (Camargue, France). *FEMS Microbiol. Ecol.* 57, 367–377.  
687 <https://doi.org/10.1111/j.1574-6941.2006.00124.x>

688 Frigaard, N.-U., Maqueo Chew, A.G., Maresca, J.A., Bryant, D.A., 2006. Bacteriochlorophyll  
689 Biosynthesis in Green Bacteria, in: Grimm, B., Porra, R.J., Rüdiger, W., Scheer, H. (Eds.),

690 Chlorophylls and Bacteriochlorophylls, *Advances in Photosynthesis and Respiration*. Springer  
691 Netherlands, Dordrecht, pp. 201–221. [https://doi.org/10.1007/1-4020-4516-6\\_15](https://doi.org/10.1007/1-4020-4516-6_15)

692 Gao, K., Helbling, E., Häder, D., Hutchins, D., 2012. Responses of marine primary producers to  
693 interactions between ocean acidification, solar radiation, and warming. *Mar. Ecol. Prog. Ser.*  
694 470, 167. <https://doi.org/10.3354/meps10043>

695 Gerola, A.P., Tsubone, T.M., Santana, A., de Oliveira, H.P.M., Hioka, N., Caetano, W., 2011. Properties  
696 of Chlorophyll and Derivatives in Homogeneous and Microheterogeneous Systems. *J. Phys.*  
697 *Chem. B* 115, 7364–7373. <https://doi.org/10.1021/jp201278b>

698 Gette-Bouvarot, M., Mermillod-Blondin, F., Lemoine, D., Delolme, C., Danjean, M., Etienne, L.,  
699 Volatier, L., 2015. The potential control of benthic biofilm growth by macrophytes—A  
700 mesocosm approach. *Ecol. Eng.* 75, 178–186. <https://doi.org/10.1016/j.ecoleng.2014.12.001>

701 Goedheer, J.C., 1966. Visible Absorption and Fluorescence of Chlorophyll and Its Aggregates in  
702 Solution, in: *The Chlorophylls*. Elsevier, pp. 147–184. [https://doi.org/10.1016/B978-1-4832-](https://doi.org/10.1016/B978-1-4832-3289-8.50012-6)  
703 [3289-8.50012-6](https://doi.org/10.1016/B978-1-4832-3289-8.50012-6)

704 Gong, S., Jin, X., Xiao, Y., Li, Z., 2020. Ocean Acidification and Warming Lead to Increased Growth and  
705 Altered Chloroplast Morphology in the Thermo-Tolerant Alga *Symbiodinium hainanensis*.  
706 *Front. Plant Sci.* 11. <https://doi.org/10.3389/fpls.2020.585202>

707 Hancke, K., Glud, R., 2004. Temperature effects on respiration and photosynthesis in three diatom-  
708 dominated benthic communities. *Aquat. Microb. Ecol.* 37, 265–281.  
709 <https://doi.org/10.3354/ame037265>

710 Hassenrück, C., Fink, A., Lichtschlag, A., Tegetmeyer, H.E., de Beer, D., Ramette, A., 2016.  
711 Quantification of the effects of ocean acidification on sediment microbial communities in the  
712 environment: the importance of ecosystem approaches. *FEMS Microbiol. Ecol.* 92.  
713 <https://doi.org/10.1093/femsec/fiw027>

714 Hicks, N., Bulling, M.T., Solan, M., Raffaelli, D., White, P.C., Paterson, D.M., 2011. Impact of  
715 biodiversity-climate futures on primary production and metabolism in a model benthic  
716 estuarine system. *BMC Ecol.* 11, 7. <https://doi.org/10.1186/1472-6785-11-7>

717 Hiraishi, A., Shimada, K., 2001. Aerobic anoxygenic photosynthetic bacteria with zinc-  
718 bacteriochlorophyll. *J. Gen. Appl. Microbiol.* 47, 161–180.  
719 <https://doi.org/10.2323/jgam.47.161>

720 Hubas, C., 2018. Biofilms, tapis et agrégats microbiens : vers une vision unificatrice (HDR (Habilitation  
721 à Diriger les Recherches)). Muséum National D’Histoire Naturelle.  
722 <https://doi.org/10.5281/zenodo.3784703>

723 Hug, L.A., Castelle, C.J., Wrighton, K.C., Thomas, B.C., Sharon, I., Frischkorn, K.R., Williams, K.H.,  
724 Tringe, S.G., Banfield, J.F., 2013. Community genomic analyses constrain the distribution of  
725 metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling.  
726 *Microbiome* 1, 22. <https://doi.org/10.1186/2049-2618-1-22>

727 Hutchins, D.A., Fu, F., 2017. Microorganisms and ocean global change. *Nat. Microbiol.* 2, 17058.  
728 <https://doi.org/10.1038/nmicrobiol.2017.58>

729 IPCC, 2014. *Climate Change 2014: Synthesis Report*. Contribution of Working Groups I, II and III  
730 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change 169.

731 Jesus, B., Perkins, R.G., Mendes, C.R., Brotas, V., Paterson, D.M., 2006. Chlorophyll fluorescence as a  
732 proxy for microphytobenthic biomass: alternatives to the current methodology. *Mar. Biol.*  
733 150, 17–28. <https://doi.org/10.1007/s00227-006-0324-2>

734 Jorgensen, B.B., Revsbech, N.P., Cohen, Y., 1983. Photosynthesis and structure of benthic microbial  
735 mats: Microelectrode and SEM studies of four cyanobacterial communities1. *Limnol.*  
736 *Oceanogr.* 28, 1075–1093. <https://doi.org/10.4319/lo.1983.28.6.1075>

737 Kandler, N.M., Abdul Wahab, M.A., Noonan, S.H.C., Bell, J.J., Davy, S.K., Webster, N.S., Luter, H.M.,  
738 2018. In situ responses of the sponge microbiome to ocean acidification. *FEMS Microbiol.*  
739 *Ecol.* 94. <https://doi.org/10.1093/femsec/fiy205>

740 Li, W., Xu, X., Fujibayashi, M., Niu, Q., Tanaka, N., Nishimura, O., 2016. Response of microalgae to  
741 elevated CO<sub>2</sub> and temperature: impact of climate change on freshwater ecosystems.  
742 *Environ. Sci. Pollut. Res.* 23, 19847–19860. <https://doi.org/10.1007/s11356-016-7180-5>  
743 Ma, J., Wang, P., Wang, X., Xu, Y., Paerl, H.W., 2019. Cyanobacteria in eutrophic waters benefit from  
744 rising atmospheric CO<sub>2</sub> concentrations. *Sci. Total Environ.* 691, 1144–1154.  
745 <https://doi.org/10.1016/j.scitotenv.2019.07.056>  
746 Mackey, K., Morris, J., Morel, F., Kranz, S., 2015. Response of Photosynthesis to Ocean Acidification.  
747 *Oceanography* 25, 74–91. <https://doi.org/10.5670/oceanog.2015.33>  
748 Milenković, S.M., Zvezdanović, J.B., Anđelković, T.D., Marković, D.Z., 2012. THE IDENTIFICATION OF  
749 CHLOROPHYLL AND ITS DERIVATIVES IN THE PIGMENT MIXTURES: HPLC-CHROMATOGRAPHY,  
750 VISIBLE AND MASS SPECTROSCOPY STUDIES. *Adv. Technol.* 9.  
751 Morrow, K.M., Bourne, D.G., Humphrey, C., Botté, E.S., Laffy, P., Zaneveld, J., Uthicke, S., Fabricius,  
752 K.E., Webster, N.S., 2015. Natural volcanic CO<sub>2</sub> seeps reveal future trajectories for host-  
753 microbial associations in corals and sponges. *ISME J.* 9, 894–908.  
754 <https://doi.org/10.1038/ismej.2014.188>  
755 Murata, N., Takahashi, S., Nishiyama, Y., Allakhverdiev, S.I., 2007. Photoinhibition of photosystem II  
756 under environmental stress. *Biochim. Biophys. Acta BBA - Bioenerg., Structure and Function*  
757 *of Photosystems* 1767, 414–421. <https://doi.org/10.1016/j.bbabi.2006.11.019>  
758 Nagata, M., Yoshimura, Y., Inagaki, J., Suemori, Y., Iida, K., Ohtsuka, T., Nango, M., 2003. Construction  
759 and Photocurrent of Light-harvesting Polypeptides/Zinc Bacteriochlorophyll *a* Complex in  
760 Lipid Bilayers. *Chem. Lett.* 32, 852–853. <https://doi.org/10.1246/cl.2003.852>  
761 Nishiyama, Y., Allakhverdiev, S.I., Murata, N., 2008. Regulation by Environmental Conditions of the  
762 Repair of Photosystem II in Cyanobacteria, in: Demmig-Adams, B., Adams, W.W., Mattoo,  
763 A.K. (Eds.), *Photoprotection, Photoinhibition, Gene Regulation, and Environment, Advances*  
764 *in Photosynthesis and Respiration.* Springer Netherlands, Dordrecht, pp. 193–203.  
765 [https://doi.org/10.1007/1-4020-3579-9\\_13](https://doi.org/10.1007/1-4020-3579-9_13)  
766 Oelze, J., 1985. 9 Analysis of Bacteriochlorophylls\*\*Dedicated to Prof. Dr. N. Pfennig on the occasion  
767 of his 60th birthday in recognition of his numerous contributions on the ecology and  
768 taxonomy of phototrophic bacteria., in: Bergan, T. (Ed.), *Methods in Microbiology.* Academic  
769 Press, pp. 257–284. [https://doi.org/10.1016/S0580-9517\(08\)70478-1](https://doi.org/10.1016/S0580-9517(08)70478-1)  
770 Peimbert, M., Alcaraz, L.D., Bonilla-Rosso, G., Olmedo-Alvarez, G., García-Oliva, F., Segovia, L.,  
771 Eguiarte, L.E., Souza, V., 2012. Comparative Metagenomics of Two Microbial Mats at Cuatro  
772 Ciénegas Basin I: Ancient Lessons on How to Cope with an Environment Under Severe  
773 Nutrient Stress. *Astrobiology* 12, 648–658. <https://doi.org/10.1089/ast.2011.0694>  
774 Pierson, B.K., Castenholz, R.W., 1974. A phototrophic gliding filamentous bacterium of hot springs,  
775 *Chloroflexus aurantiacus*, gen. and sp. nov. *Arch. Microbiol.* 100, 5–24.  
776 <https://doi.org/10.1007/BF00446302>  
777 Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., Engel, A., 2009. Acidification increases  
778 microbial polysaccharide degradation in the ocean. *Biogeosciences Discuss.* 7.  
779 <https://doi.org/10.5194/bg-7-1615-2010>  
780 Prieto-Barajas, C.M., Valencia-Cantero, E., Santoyo, G., 2018. Microbial mat ecosystems: Structure  
781 types, functional diversity, and biotechnological application. *Electron. J. Biotechnol.* 31, 48–  
782 56. <https://doi.org/10.1016/j.ejbt.2017.11.001>  
783 Rae, B.D., Long, B.M., Badger, M.R., Price, G.D., 2013. Functions, Compositions, and Evolution of the  
784 Two Types of Carboxysomes: Polyhedral Microcompartments That Facilitate CO<sub>2</sub> Fixation in  
785 Cyanobacteria and Some Proteobacteria. *Microbiol. Mol. Biol. Rev. MMBR* 77, 357–379.  
786 <https://doi.org/10.1128/MMBR.00061-12>  
787 Redfield, A.C., 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46, 230A–  
788 221.  
789 Reinold, Wong, MacLeod, Meltzer, Thompson, Burns, 2019. The Vulnerability of Microbial  
790 Ecosystems in A Changing Climate: Potential Impact in Shark Bay. *Life* 9, 71.  
791 <https://doi.org/10.3390/life9030071>

792 Revsbech, N.P., Jorgensen, B.B., Blackburn, T.H., Cohen, Y., 1983. Microelectrode studies of the  
793 photosynthesis and O<sub>2</sub>, H<sub>2</sub>S, and pH profiles of a microbial mat<sup>1</sup>. *Limnol. Oceanogr.* 28,  
794 1062–1074. <https://doi.org/10.4319/lo.1983.28.6.1062>

795 Scheer, H., 2006. An Overview of Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics,  
796 Functions and Applications, in: Grimm, B., Porra, R.J., Rüdiger, W., Scheer, H. (Eds.),  
797 Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications,  
798 Advances in Photosynthesis and Respiration. Springer Netherlands, Dordrecht, pp. 1–26.  
799 [https://doi.org/10.1007/1-4020-4516-6\\_1](https://doi.org/10.1007/1-4020-4516-6_1)

800 Sirevåg, R., 2004. Carbon Metabolism in Green Bacteria, in: Blankenship, R.E., Madigan, M.T., Bauer,  
801 C.E. (Eds.), Anoxygenic Photosynthetic Bacteria, Advances in Photosynthesis and Respiration.  
802 Kluwer Academic Publishers, Dordrecht, pp. 871–883. [https://doi.org/10.1007/0-306-47954-](https://doi.org/10.1007/0-306-47954-0_40)  
803 [0\\_40](https://doi.org/10.1007/0-306-47954-0_40)

804 Sørensen, K.B., Canfield, D.E., Teske, A.P., Oren, A., 2005. Community Composition of a Hypersaline  
805 Endoevaporitic Microbial Mat. *Appl. Environ. Microbiol.* 71, 7352–7365.  
806 <https://doi.org/10.1128/AEM.71.11.7352-7365.2005>

807 Stauffert, M., Cravo-Laureau, C., Jezequel, R., Barantal, S., Cuny, P., Gilbert, F., Cagnon, C., Milton, C.,  
808 Amouroux, D., Mahdaoui, F., Bouyssièrè, B., Stora, G., Merlin, F., Duran, R., 2013. Impact of  
809 Oil on Bacterial Community Structure in Bioturbated Sediments. *PloS One* 8, e65347.  
810 <https://doi.org/10.1371/journal.pone.0065347>

811 Takahashi, E., Ledauphin, J., Goux, D., Orvain, F., 2009. Optimising extraction of extracellular  
812 polymeric substances (EPS) from benthic diatoms: Comparison of the efficiency of six EPS  
813 extraction methods. *Mar. Freshw. Res.* 60, 1201–1210. <http://dx.doi.org/10.1071/MF08258>

814 Tan, Y.-H., Lim, P.-E., Beardall, J., Poong, S.-W., Phang, S.-M., 2019. A metabolomic approach to  
815 investigate effects of ocean acidification on a polar microalga *Chlorella* sp. *Aquat. Toxicol.*  
816 217, 105349. <https://doi.org/10.1016/j.aquatox.2019.105349>

817 Underwood, G.J.C., Boulcott, M., Raines, C.A., Waldron, K., 2004. Environmental Effects on  
818 Exopolymer Production by Marine Benthic Diatoms: Dynamics, Changes in Composition, and  
819 Pathways of Production<sup>1</sup>. *J. Phycol.* 40, 293–304. [https://doi.org/10.1111/j.1529-](https://doi.org/10.1111/j.1529-8817.2004.03076.x)  
820 [8817.2004.03076.x](https://doi.org/10.1111/j.1529-8817.2004.03076.x)

821 Uribe-Lorío, L., Brenes-Guillén, L., Hernández-Ascencio, W., Mora-Amador, R., González, G., Ramírez-  
822 Umaña, C.J., Díez, B., Pedrós-Alió, C., 2019. The influence of temperature and pH on bacterial  
823 community composition of microbial mats in hot springs from Costa Rica. *MicrobiologyOpen*  
824 8. <https://doi.org/10.1002/mbo3.893>

825 van Gemerden, H., 1993. Microbial mats: A joint venture. *Mar. Geol.* 113, 3–25.  
826 [https://doi.org/10.1016/0025-3227\(93\)90146-M](https://doi.org/10.1016/0025-3227(93)90146-M)

827 Wang, S., Zhang, D., Pan, X., 2013. Effects of cadmium on the activities of photosystems of *Chlorella*  
828 *pyrenoidosa* and the protective role of cyclic electron flow. *Chemosphere* 93, 230–237.  
829 <https://doi.org/10.1016/j.chemosphere.2013.04.070>

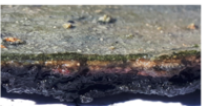
830 Wang, X., Feng, X., Zhuang, Y., Lu, J., Wang, Y., Gonçalves, R.J., Li, X., Lou, Y., Guan, W., 2019. Effects  
831 of ocean acidification and solar ultraviolet radiation on physiology and toxicity of  
832 dinoflagellate *Karenia mikimotoi*. *Harmful Algae* 81, 1–9.  
833 <https://doi.org/10.1016/j.hal.2018.11.013>

834 Wieland, A., Köhl, M., McGowan, L., Fourçans, A., Duran, R., Caumette, P., Garcia de Oteyza, T.,  
835 Grimalt, J.O., Solé, A., Diestra, E., Esteve, I., Herbert, R.A., 2003. Microbial Mats on the  
836 Orkney Islands Revisited: Microenvironment and Microbial Community Composition. *Microb.*  
837 *Ecol.* 46, 371–390. <https://doi.org/10.1007/s00248-002-0108-2>

838 Wootton, J.T., Pfister, C.A., Forester, J.D., 2008. Dynamic patterns and ecological impacts of declining  
839 ocean pH in a high-resolution multi-year dataset. *Proc. Natl. Acad. Sci.* 105, 18848–18853.  
840 <https://doi.org/10.1073/pnas.0810079105>

841 Yue, F., Gao, G., Ma, J., Wu, H., Li, X., Xu, J., 2019. Future CO<sub>2</sub>-induced seawater acidification  
842 mediates the physiological performance of a green alga *Ulva linza* in different photoperiods.  
843 *PeerJ* 7. <https://doi.org/10.7717/peerj.7048>





**Control**  
20°C – pH = 8

**Acidification treatment**  
20°C – pH =  
7.6

**Warming treatment**  
24°C – pH = 8

**Acidification +  
warming treatment**  
24°C – pH =  
7.6

Photosynthetic efficiency	=	=	=	=
Extracellular polymeric substances	=	+ (bound carbohydrates EPS)	=	=
Chlorophyll a	=	-	=	=
Chlorophyll derivatives	=	+	=	=



Change in the phototrophic communities

OR

Change in the metabolism of green anoxygenic phototroph bacteria