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1 Control of the pH for marine microalgae polycultures: a key point for CO₂ fixation
2 improvement in intensive cultures

3

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12

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15

16 **Abstract**

17 Recently, CO₂ recycling for the production of valuable microalgae has acquired
18 substantial interest. Most studies investigating CO₂ conversion efficiency in algal cultures
19 were based on single species, although a stabilising effect of algal diversity on biomass
20 production was recently highlighted. However, addition of CO₂ into polyalgal cultures
21 requires a careful control of pH; performance of CO₂ conversion, growth and carbon biomass
22 production are affected by pH differently, depending on the species of microalgae. This study
23 investigates the efficiency of CO₂ conversion by natural marine algal assemblage cultivated in
24 open, land-based raceways (4.5 m³, 10 m²), working as high rate algal ponds (HRAP). Ponds
25 were enriched with nitrogen and phosphate, pure CO₂ was added and algal cultures were

26 grown under three different fixed pH levels: pH 6, 7 and 8. The highest conversion of
27 photosynthetically fixed CO₂ into carbon biomass (40%) was reached at pH 7, an intermediate
28 level, due to the partial CO₂ asphyxiation of algal predators (copepods, ciliates), while being
29 under the suboptimal conditions for the development of marine amoebae. Under this pH, the
30 theoretical maximal biological conversion of available CO₂ into carbon biomass was
31 estimated to be 60% in naturally inoculated open ponds.

32

33 1. Introduction

34 Algae has become a focal point in the circular economy to valorise industrial waste in order to
35 continually use resources and reduce industrial CO₂ emissions to the atmosphere (Lai et al.
36 2019; Stiles et al. 2018). Through photosynthesis, microalgae can convert CO₂ from industrial
37 flue gas into biomass that can potentially be used for the production of biofuel, biochemical
38 products or green chemistry (Bhola et al. 2014; Brune et al. 2009; Wang et al. 2008).
39 Industrial flue gas contains potentially toxic contaminants which can lead to reductions in the
40 production of biomass (Hess et al. 2017). Additionally, injection of CO₂ acidifies algal culture
41 medium by modifying the balance of carbonates in the water (Galloway and Krauss 1961).
42 This acidification could counterbalance CO₂ fixation by microalgae which alkalises their
43 growth environment (Galloway and Krauss 1961). A controlled injection of CO₂ would
44 maintain an optimal pH level that could be adjusted to optimise for metabolic activity of algal
45 species (Galloway and Krauss 1961). For example, the marine microalgae *Nannochloris* sp.
46 achieves maximal growth rate at pH 6, maximum carbon content at pH 7 and maximal carbon
47 conversion efficiency at pH 8 (Vasseur et al. 2012). Other example, the optimal growth of the
48 cyanobacteria *Spirulina platensis* and the green algae *Chlorococcum littorale* are 9 and 4,
49 respectively (Zeng et al. 2011). In microalgal predators, lower pH (associated with CO₂
50 injection) negatively affects the densities and grazing activity of rotifers (Montemezzani et al.

51 2017a, b, c). If we ignore the potential effects of associated contaminants, using CO₂ from
52 industrial flue gas can combine pH control during high photosynthetic CO₂ fixation and
53 reduction of algal grazing pressure.

54 Large-scale industrial cultivation of marine microalgae appears to be one of the most
55 promising approaches of sustainable sources of food (lipids and proteins) and energy (Greene
56 et al. 2016). Narwani et al. (2016) demonstrated stabilising effects of biodiversity on biocrude
57 production from polycultures of several assembled algal species using raceway ponds under
58 variable environmental conditions. However, higher performance can be reached using
59 natural algal polyculture than assembled polyculture (Thomas et al. 2019).

60 We suggest that an optimal pH can be reached for maximising carbon conversion
61 efficiency of a natural marine polyculture of microalgae while reducing grazers development.
62 Natural marine polycultures were cultivated in High Rate Algal Ponds (HRAP) in a
63 Mediterranean climate with different levels of pure CO₂. We measured algal diversity,
64 photosynthetic CO₂ uptake, carbon biomass accumulation, and associated development of
65 algal predators under three different pH values. We developed performance curves of
66 biological CO₂ conversion efficiency a function of pH. Results from this study provide a
67 comprehensive assessment of the biological CO₂ conversion efficiency from marine
68 microalgae cultivated in open ponds. **The results from this experiment can inform engineering
69 projects for industrial CO₂ sequestration or mass production of marine microalgae.**

70

71 **2. Material & Methods**

72 2.1. Experimental design

73 An intensive culture of natural microalgae from Mediterranean seawater was
74 performed outdoors in high rate algal ponds (HRAP) as described in Metaxa et al. (2006).
75 HRAPs are oval-shaped ponds (6 m long, 2 m wide and 0.6 m deep) with a working volume

76 of 4.46 m³ and surface area of 10 m². Water mixing in the HRAPs was maintained at 0.2 m s⁻¹
77 using a vacuum airlift column developed and patented by COLDEP® (Barrut et al. 2013).
78 Briefly, the column was connected to the HRAP and was composed of a central tube, the top
79 of which was hermetically closed and connected to a vacuum pump. Water was raised to top
80 of the central tube with a vacuum and was allowed to flow over the central tube so that it
81 could be returned to the HRAP. We maintained pH using an automatic JBL pH-controller
82 system (JBL ProFlora m1003) connected to a pure CO₂ bottle using a solenoid valve. We
83 diffused CO₂ through a ceramic gas diffuser in the HRAP. The JBL ProFlora system used a
84 combined Ag/AgCl KCl-electrolyte pH electrode at operational IUPAC scale calibrated
85 monthly using commercial NIST buffers with precision of 0.05 pH units with an additional
86 systematic error of about 0.15 pH units relative to seawater scale (Riebesell et al. 2011).

87 Experiments were conducted at the Ifremer Station of Palavas-les-Flots, Hérault,
88 France during spring and early summer 2016 to coincide with the climatic period of
89 favourable algal growth. The first run of experiment (run 1) occurred from 17 May to 22 June
90 2016 (36 days); run 2 occurred from 27 June to 26 July 2016 (29 days). For each experimental
91 run, three HRAPs were monitored simultaneously on batch feeding mode and maintained at
92 pH of 6, 7 and 8, respectively. We assessed cumulated photosynthetic CO₂ fixation,
93 production of carbon biomass, microalgal diversity and predator identification.

94 For all experiments, seawater was pumped from the Mediterranean Sea, inoculated
95 using non-specific microalgae inoculant and filtered through a 100 µm sand filter before
96 entering HRAPs. All HRAPs were supplemented with phosphorus to have final
97 concentrations of 5 mg L⁻¹ of phosphate and nitrogen to achieve 80 mg L⁻¹ of nitrate and
98 ammonium.

99

100 2.2 Physical and chemical variables

101 2.2.1 Meteorological variables

102 Global, direct and diffuse solar radiation were recorded every hour at the Météo-
103 France station at Fréjorgues airport, 7 km from the experimental site.

104

105 2.2.2 Water pH, salinity, light and temperature measurements

106 Water temperature (accuracy of 0.5°C) in HRAPs were recorded every 10 minutes and
107 salinity (accuracy of 0.1 PSU) in HRAPs was recorded once a day using a PONSEL ODEON
108 X meter. In addition to JBL ProFlora system, water pH was measured every 10 minutes in one
109 point of the well-mixed HRAPs (water circulation of 0.2 m.s⁻¹) using a combined Ag/AgCl
110 KCl electrolyte ODEON electrode with a resolution of 0.01 pH units and an accuracy of 0.1
111 pH units. The underwater light intensity in HRAPs was recorded every 15 min at three depths
112 (sub-surface, 20 and 40 cm) using waterproof light data loggers (UA-002-64 HOBO, Onset).

113

114 2.3 Biological variables

115 2.3.1 Microalgae biomass and diversity

116 The algal biomass was assessed daily when possible for Chlorophyll *a* (Chl*a*) and
117 weekly for particulate organic carbon (POC) measurements. For Chl*a* measurements, between
118 3 and 800 ml samples (depending on sample loading) were filtered onto 13 mm GF/F
119 fibreglass fibre filters to determine the total Chl*a* concentrations. For size-fractionated Chl*a*
120 measurements, between 50 and 800 ml samples were filtered using 20 µm, 5 µm and 2 µm
121 membrane filters and GF/F fibreglass (0.7 µm nominal pore size). Filters were stored frozen
122 at -20°C until extraction with 5 ml of 100% ethanol. Extractions were performed after
123 ultrasonication in an ice bath followed by overnight extraction at 4°C. Samples were then
124 filtered and extract absorbance was measured at 632, 649 and 665 nm (with absorbance at 750
125 nm used for a baseline correction) using a Shimadzu UV1800 zeroed with 100% ethanol.

126 Chlorophyll concentration was calculated according to Ritchie (2008). For the POC
127 measurements, between 5 and 100 mL samples were filtered onto precombusted Whatman
128 GF/F filters and stored at -80°C for analysis. The filters were then dried at 60°C for 24 h,
129 pelleted and analysed using an ANCA mass spectrometer (Europa Scientific). Because of the
130 small volume of samples (<100 ml), we assumed that microalgae were mainly collected and
131 retained onto the filters (nominal pore size of 0.7 µm) and the POC measurement was
132 therefore representative of microalgae carbon biomass. This is supported by positive linear
133 correlation between Chl a and POC measurements ($r^2= 0.85$, $p < 0.001$, $n=33$) leading to a
134 Chl a :POC ratio of 55, similar to the value measured for marine green algae and
135 Prymnesiophyceae in the Mediterranean Sea (Latasa 2005).

136 Microalgal community structure was determined in all HRAPs at the beginning (Day 0) and at
137 the end of each experimental run (Day 36 and Day 29) using 18S rRNA gene analysis. For
138 each experimental run, 10 mL samples were filtered onto 0.2 µm membranes (PALL ALL
139 Supor® 200 PES) and stored at -20°C for subsequent DNA extractions. The DNA was
140 extracted using DNeasy PowerWater Kit (Qiagen) according to the manufacturer's
141 instructions. The V4 region of the 18S rRNA gene was amplified over 30 amplification cycles
142 at an annealing temperature of 65°C, with forward and reverse primers (5'-
143 CTTTCCCTAACGACGCTCTTCCGATCTGCGGTAATTCCAGCTCCAA-3' and 5'-
144 GGAGTTCAGACGTGTGCTCTTCCGATCTTTGGCAAATGCTTTCGC-3', respectively) with
145 their associated linkers. The resulting products were purified and loaded onto an Illumina
146 MiSeq cartridge for sequencing, paired with 300 bp reads following manufacturer's
147 instructions (v3 chemistry). Sequencing and library preparation steps were carried out at the
148 Genotoul Lifescience Network Genome and Transcriptome Core Facility in Toulouse, France
149 (get.genotoul.fr). A modified version of the standard operation procedure for MiSeq data
150 (Kozich 2013) in Mothur version 1.35.0 (Schloss 2009) was used for alignment and

151 taxonomic outline. Mothur was also used to identify representative sequences of operational
152 taxonomic units (OTUs).

153

154 2.3.2 Algal predator diversity

155 To characterise the presence and abundance of protozoan and metazoan organisms, 2 L water
156 samples were filtered sequentially through a 100 μm and 35 μm filters to separate 35 to 100
157 μm and $> 100 \mu\text{m}$ size fractions, which were then stored in neutralised formalin (4% final
158 concentration). Protozoan and metazoan taxa from a subsample of the 35 to 100 μm fraction
159 were identified, sized and enumerated using an Olympus AX70 dissecting microscope with a
160 Nageotte counting chamber. Protozoan and metazoan taxa from a subsample of the $> 100 \mu\text{m}$
161 fraction were identified and enumerated using an Olympus Stereomicroscope SZX7 with a
162 Bogorov counting chamber.

163

164 2.3.3. HRAP photosynthetic CO_2 uptake

165 The photosynthetic CO_2 uptake by microalgae in HRAPs was computed from the relationship
166 between biomass-specific gross photosynthetic oxygen production and the light intensity
167 (photosynthesis-irradiance P-I model). The weekly photosynthetic oxygen production was
168 measured at the laboratory as detailed below. **Algal biomass (Chl a) was measured every day**
169 **when possible in the HRAPs.** Average light intensity received by microalgae was measured
170 every 15 min at mid-depth (0.2 m) in each HRAP.

171 Samples were collected weekly from HRAPs, distributed to air-tight 30 mL
172 polycarbonate flasks and placed in a custom-made, controlled-temperature photosynthetic
173 with a light density gradient up to 400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ using an array of 4 W white
174 LEDs. Photosynthetically active radiation (PAR) was measured using a US-SQS/L spherical
175 quantum microprobe (Walz, Effeltrich, Germany). The temperature was kept constant within

176 the HRAPs by heating or cooling. Dissolved oxygen (to determine net photosynthesis) was
177 measured using a FireSting optode system (Pyro-Science, Aachen, Germany) every 20 min
178 for 6 h of incubation in all illuminated flasks. Net photosynthetic rate was calculated at all
179 light intensities. Samples were also incubated in the dark at the same temperature to
180 determine respiration rate (oxygen consumption). Gross photosynthetic rates for all light
181 intensities were calculated as the sum of net photosynthetic rates and respiration rates. Gross
182 photosynthetic rates were expressed in carbon units using a photosynthetic quotient of 1
183 (Falkowski and Raven 1997) and in algal biomass units using Chlorophyll *a* measurements.
184 These were plotted against irradiance using the P-I model described by Eilers and Peters
185 (1988) using the phytotools R package (<https://cran.r-project.org/package=phytotools>). The
186 instantaneous gross photosynthetic CO₂ uptake rates were derived from the P-I model
187 obtained every week for each HRAP, the Chl*a* biomass measurement measured every day and
188 the light measurements taken every 15 min at mid-depth during P-I model assessment. For the
189 calculation of instantaneous photosynthetic rates, we assumed there was no significant change
190 of P-I parameter values during a week and the Chl*a* biomass during a day.

191 The total gross photosynthetic CO₂ uptake for HRAPs was calculated as the sum of all
192 instantaneous gross CO₂ uptake rates obtained during the whole experiment in each HRAP.

193

194 2.3.3. Prediction of minimal CO₂ losses

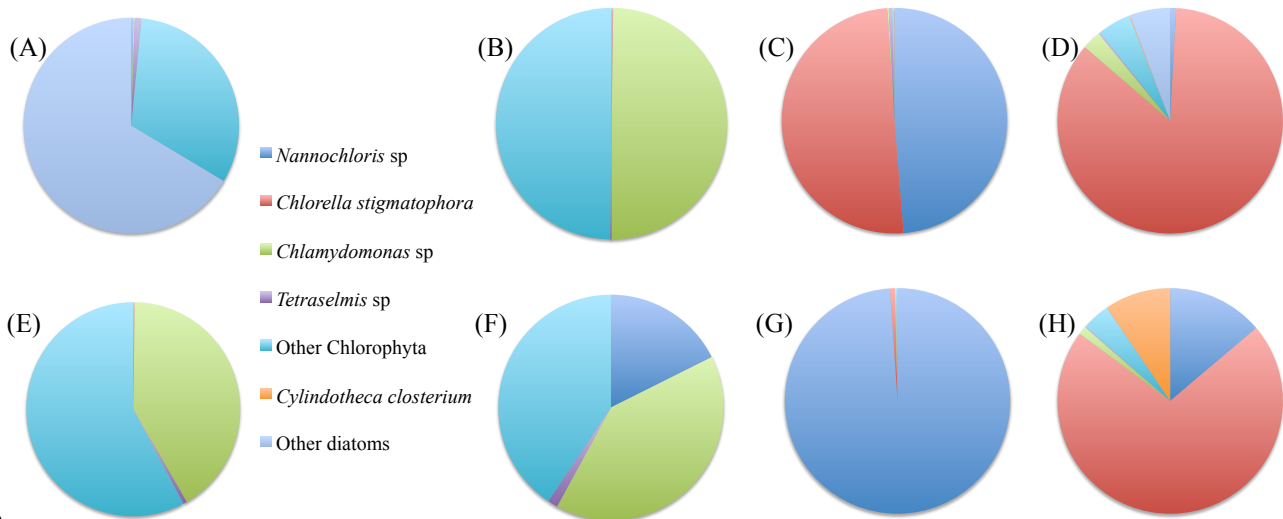
195 We attempted to predict the minimal carbon losses through biological activity (respiration,
196 exudation, predation) using a generalised additive model (GAM) in R (R Core Team, 2019)
197 with the mgcv package (Wood, 2017). Carbon lost through biological activity was calculated
198 as the fraction of gross photosynthetically fixed CO₂ not accumulated into the C biomass over
199 time series from HRAPs at pH 7.

200

201 3. Results & Discussion

202 3.1. Influence of pH on algal diversity and predation

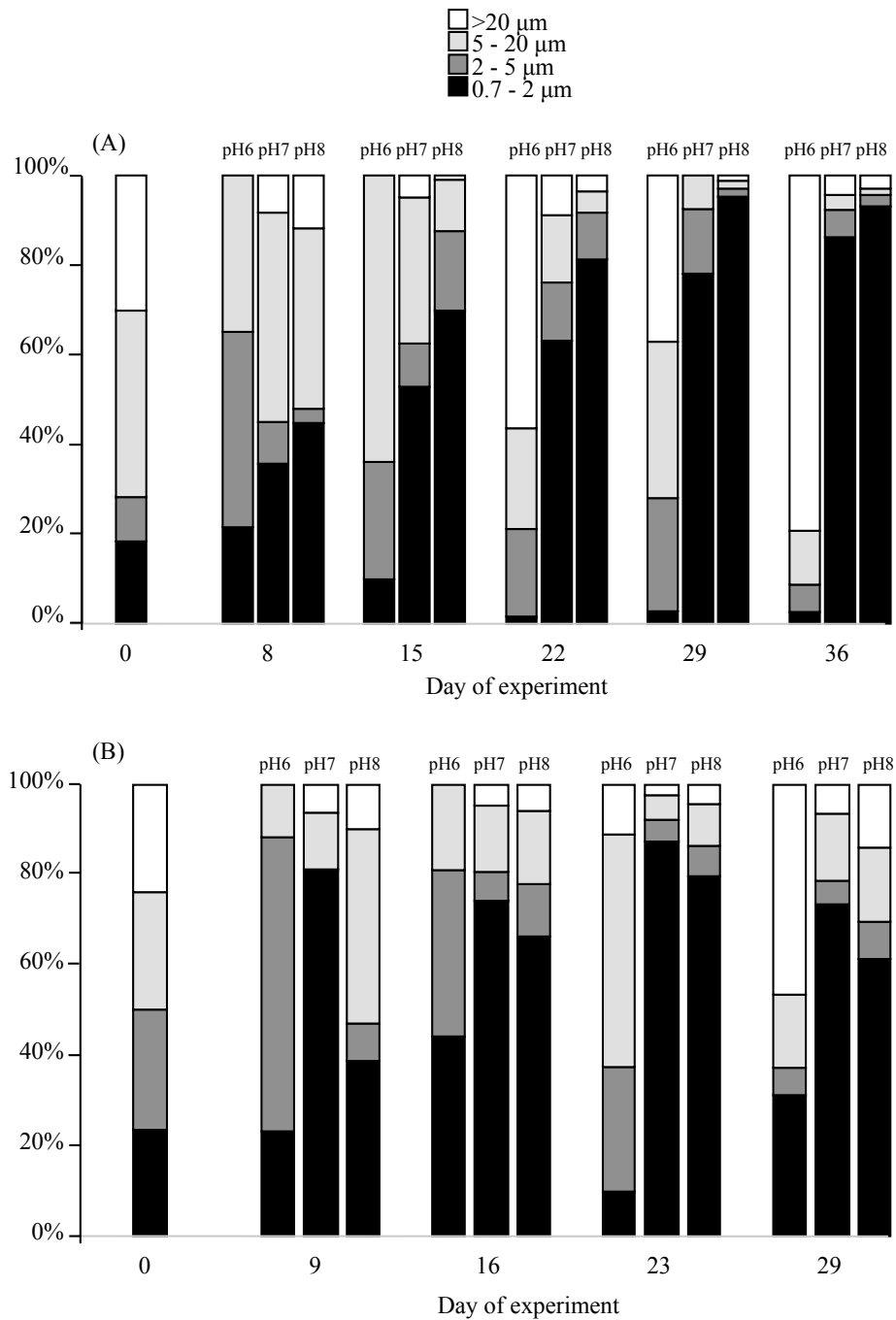
203 In the pH-6 HRAPs, pH averaged 6.2 ± 0.2 (Figure S1). Lower pH variability was
204 observed in the pH-7 HRAPs (Figure S1), where pH averaged 6.7 ± 0.1 and 7.0 ± 0.1 during run
205 1 and run 2, respectively. In the pH-8 HRAPs, pH averaged 8.2 ± 0.1 and 7.9 ± 0.1 during run 1
206 and run 2, respectively. When pooling pH measured during both experimental runs, pH
207 averaged 6.2 ± 0.2 , 6.9 ± 0.2 , and 8.1 ± 0.2 in HRAPs with pH targeted at 6, 7 and 8 respectively.
208 The addition of CO₂ acidifies seawaters and impacts the growth of marine planktonic
209 organisms found in naturally buffered seawater at pH 8.07 in the Mediterranean Sea (Hassoun
210 et al 2015). Larger microalgae ($> 5 \mu\text{m}$, Fig. 1), such as *Chlamydomonas* sp., favoured the
211 growth at pH 6, while smaller microalgae ($< 2 \mu\text{m}$, Fig. 1), such as *Nannochloris* sp. and
212 *Chlorella stigmatophora*, attained maximal growth rates at pH 7 and 8, respectively (Fig. 2).
213 Those two species have been reported to be highly resilient to environmental variability,
214 rapidly growing, and living in marine polluted waters (Butcher 1952, Fabregas 1986, Cho et
215 al. 2007). Of economic interests for industries, *Nannochloris*-like species (Henley et al. 2004)
216 show a high potential for removing nitrogen and phosphorus from nutrient-polluted waters
217 whilst maintaining a stable biochemical profile under environmental changes (von
218 Alvensleben et al. 2013). Our results suggest that adding CO₂ to control pH is a viable tool for
219 favouring the development of one specific microalgal species among a natural assemblage.



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Figure 1. The diversity of microalgae was determined using 18S rRNA gene analysis. The first experiment ran from 17 May to 22 June 2016 (A-D). Organisms identified in the beginning of the experiment (A) and those identified at the end of the experiment in HRAPs at pH 6 (B), pH 7 (C) and pH 8 (D). The second experiment ran from 27 June 27 to 26 July 2016 (E-H). Organisms identified at the beginning of the experiment (E) and at the end of the experiment in HRAPs at pH 6 (F), pH 7 (G) and pH 8 (H).

231 Although differences in microalgal diversity were observed in HRAPs, no significant
 232 differences were found among pH treatments for the photosynthetic parameters, P_{max} and I_k
 233 (Table S1). However, photoinhibitory effect was significantly higher in HRAPs at pH 7 and 8
 234 than in HRAPs at pH 6 (ANOVA $p < 0.05$, Tukey post hoc; Table S1). As expected,
 235 microalgae in all HRAPs were acclimated to higher I_k values and lower w values in July than
 236 in June during the experiments (ANOVA $p < 0.05$, Tukey post hoc; Suppl. Table S1).



237

238 Figure 2. Size fractions of algal biomass measured in HRAPs at pH 6, 7
 239 and 8 during the first experimental run 1 (A) and the second experimental
 240 run 2 (B).
 241

242 Interestingly, rhizopod development in amoebae was observed in HRAPs at pH 6 (Table 1).

243 Rhizopod amoebae are known to feed on organic matter, bacteria, and small algal cells

244 (Carney and Lane 2014; Day et al. 2017); they also have higher growth rates at pH lower than

245 seawater (Rodriguez-Zaragoza 1994). The rapid development of amoebae would prevent the

246 abundance of small algal cells observed under the other pH conditions. In contrast, ciliates
 247 (both attached and free) grew rapidly at pH 8 and dominated the community of predators in
 248 HRAPs. Copepods (adults and nauplii) were also observed in HRAPs at pH 7 and 8 but were
 249 not observed at pH 6. The pH, therefore, seems to have a direct impact on the presence of
 250 predators of algae. Low pH has a negative effect on ciliates and copepods but a positive effect
 251 on the presence of amoebae. As a consequence, low levels of microalgal accumulation were
 252 observed at pH 6. The development of the typical marine trophic food web (ciliates,
 253 copepods) was observed in the HRAP at pH 8, leading to the dominance of very small algal
 254 cells. At intermediate pH of 7, the abundance of predators was the lowest (Table 1) and the
 255 accumulation of microalgal biomass was affected by the initial community structure of
 256 predators and temperature. Indeed, a lower level of algal biomass accumulation was observed
 257 in the HRAP at pH 7 during run 2 when the initial presence of rhizopod amoebae (Table 1)
 258 and water temperature (average level was $> 25^{\circ}\text{C}$) was significantly higher ($p < 0.05$, which
 259 was determined using a t-test).

260

261 Table 1: Abundance of all the main groups of protozoans and metazoans identified by
 262 microscopy in the HRAP at pH 6, 7 and 8 at the beginning and at the end of two experimental
 263 runs of the study.

Size fraction	Group (ind. L ⁻¹)	20th of May 2016 (T0)	22rd of June 2016 (Tf)			28th of June 2016 (T0)	26th of July 2016 (Tf)		
			pH 6	pH 7	pH 8		pH 6	pH 7	pH 8
35-100 μm	Free ciliates		208	312	3952	103	3328	1664	34320
	Attached ciliates	52	3432	832	120016			1040	43888
	Rhizopods		18408	624	1040	11	57928	8008	1352
	Actinopods				416		1976	416	208
	Nematods	10	520	936	416		104	2184	
	Rotifers								520
	Copepods			1664	104			312	2600
> 100 μm	Nematods larvae	9	104		351	5	50	513	169
	<i>Tisbe</i> sp.			299	156				871
	<i>Tisbe</i> nauplius	0							104
	Copepod nauplii			59					

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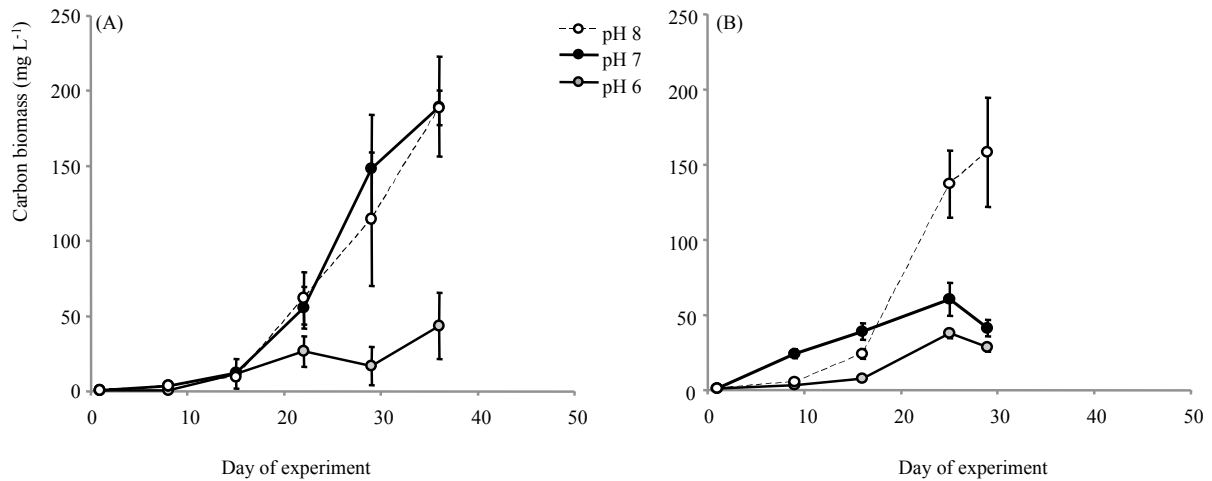
266 Injection of CO₂ into HRAPs negatively affects zooplankton (Cladocceans, copepods, rotifers)
 267 through asphyxiation, allowing an increase in algal productivity and biomass (Montemezzani

268 et al. 2017a, b). Similarly, in this study, no copepods grew in HRAPs where a continuous
269 injection of CO₂ was done in order to maintain a pH of 6. In addition, the final abundance
270 protozoans and metazoans >100 µm decreased as injected CO₂ accumulated (lower pH) in the
271 HRAPs (Table 1). This highlights the difficulty that large-sized aquatic organisms had
272 maintaining a costly mode of respiration in media where CO₂ replaced O₂ availability. In
273 contrast, the reductions in pH through the CO₂ injection favoured the development of
274 rhizopods (mainly amoebae) that strongly limited algal biomass accumulation in the HRAPs.
275 This is the first report of this observation in the literature, and it may have dramatic
276 consequences on marine microalgae cultures in large-scale facilities.

277

278 3.2. Influence of pH on algal biomass accumulation

279 In this study, the highest accumulation of carbon as algal biomass (> 150 mg L⁻¹) was
280 observed in HRAPs at pH 8 and the lowest values (< 50 mg L⁻¹) were observed in HRAPs at
281 pH 6 (Fig. 3). Interestingly, algal biomass in HRAPs at the intermediate pH 7 had more
282 variability and was influenced by seasonality, greater for run 1 than run 2 (Fig. 3). In all
283 HRAPs, lower algal biomass was observed during run 2. Initial algal diversity was different
284 between runs with abundance of *Nannochloris* sp. observed in June relatively to July. Water
285 temperature was higher in July (average of 25.3°C) than in June (average of 21.8°C) and
286 influenced predator development (Table 1), leading to higher predatory pressure on
287 microalgae.



289

290 Figure 3. Concentration of carbon biomass (POC measurements) measured in
 291 HRAPs (average and standard deviation of sample triplicates) during run 1 from 17
 292 May to 22 June 2016 (A) and during run 2 from 27 June to 26 July 2016 (B).
 293

294

295 The loss of carbon that was photosynthetically fixed but not ultimately accumulated
 296 into algal carbon biomass was estimated from the difference between the total photosynthetic
 297 CO₂ uptake and the algal carbon biomass accumulated over time (Table 2). When differences
 298 were larger, algal biomass lost greater amounts of carbon through algal CO₂ respiration, algal
 299 carbon exudation and algal mortality by predation or viral lysis.

300

301 Table 2. Gross photosynthetic CO₂ uptake, produced carbon biomass and carbon losses (the
 302 fraction of the gross photosynthetic CO₂ uptake that was not accumulated into carbon biomass)
 303 measured in HRAPs at pH 6, 7 and 8 during the two experimental runs.

Date	Day of experiment	pH 6			pH 7			pH 8		
		Gross photosynthetic CO ₂ uptake (mgC L ⁻¹)	Produced C biomass (mgC L ⁻¹)	C loss (%)	Gross photosynthetic CO ₂ uptake (mgC L ⁻¹)	Produced C biomass (mgC L ⁻¹)	C loss (%)	Gross photosynthetic CO ₂ uptake (mgC L ⁻¹)	Produced C biomass (mgC L ⁻¹)	C loss (%)
20/05/16	3	0	0.0		0	0		0	0	
25/05/16	8	10	0.1	99	10	3	68	19	3	85
01/06/16	15	600	11	98	159	12	93	147	9	94
08/06/16	22	2270	26	99	767	55	93	1079	61	94
15/06/16	29	2328	16	99	1431	148	90	1985	114	94
22/06/16	36	2541	43	98	2602	189	93	3760	188	95
28/06/16	1	0	0.0		0	0		0	0	
06/07/16	9	22	2.1	91	57	23	60	15	4	73
13/07/16	16	371	6.2	98	386	38	90	203	23	89
22/07/16	25	877	36	96	875	59	93	1246	136	89
26/07/16	29	1074	27	97	1180	40	97	1620	157	90

304
305

306 In all HRAPs, carbon loss increased rapidly over time, reaching more than 90% of the gross
307 photosynthetic CO₂ uptake measured throughout the experimental period (Table 2). Most CO₂
308 loss is related to high microalgal respiration rate which has been reported to be 60-90% of the
309 photosynthetic fixed CO₂ (Herzig and Falkowski 1989). Carbon loss through respiration is
310 combined with the carbon loss through algal exudation and predation increasing with
311 substrate limitation and time, respectively. Interestingly, the lowest carbon loss (60 and 68%)
312 was observed within the first week of the experiments at pH 7 (Table 2). This supports our
313 observations that intermediate pH reduces grazing on marine microalgal growth as a result of
314 partial asphyxiation of ciliates and copepods, but maintains optimal conditions for
315 development of amoebae that prefer more acidic waters.

316

317 3.3 Predictions of maximal biological CO₂ conversion efficiency in HRAP

318 We predicted the theoretical level of minimal carbon loss through biological activity
319 (respiration, exudation, predation) using a GAM model with a smooth function of time and
320 four degrees of freedom. Biological activity explained 77% of deviance ($R^2 = 0.64$) for
321 biological carbon loss. Using this model, a new time series and confidence interval were
322 predicted for time points that varied from 0 to 44 days using a step of 1 day (Fig. 3). The
323 minimal fraction of gross photosynthetically fixed CO₂ that was not accumulated into biomass
324 was predicted using the value reached at day 0 (Figure 4). This fraction ($44 \pm 25\%$) is
325 considered the minimal loss of carbon due to CO₂ respiration required for algal metabolism
326 (typically 20–30% of the maximal algal growth rate; Geider and Osborne 1989) and algal
327 carbon exudation, or natural phytoplankton (7–50% of total photosynthetically fixed CO₂;
328 Fogg et al. 1965).

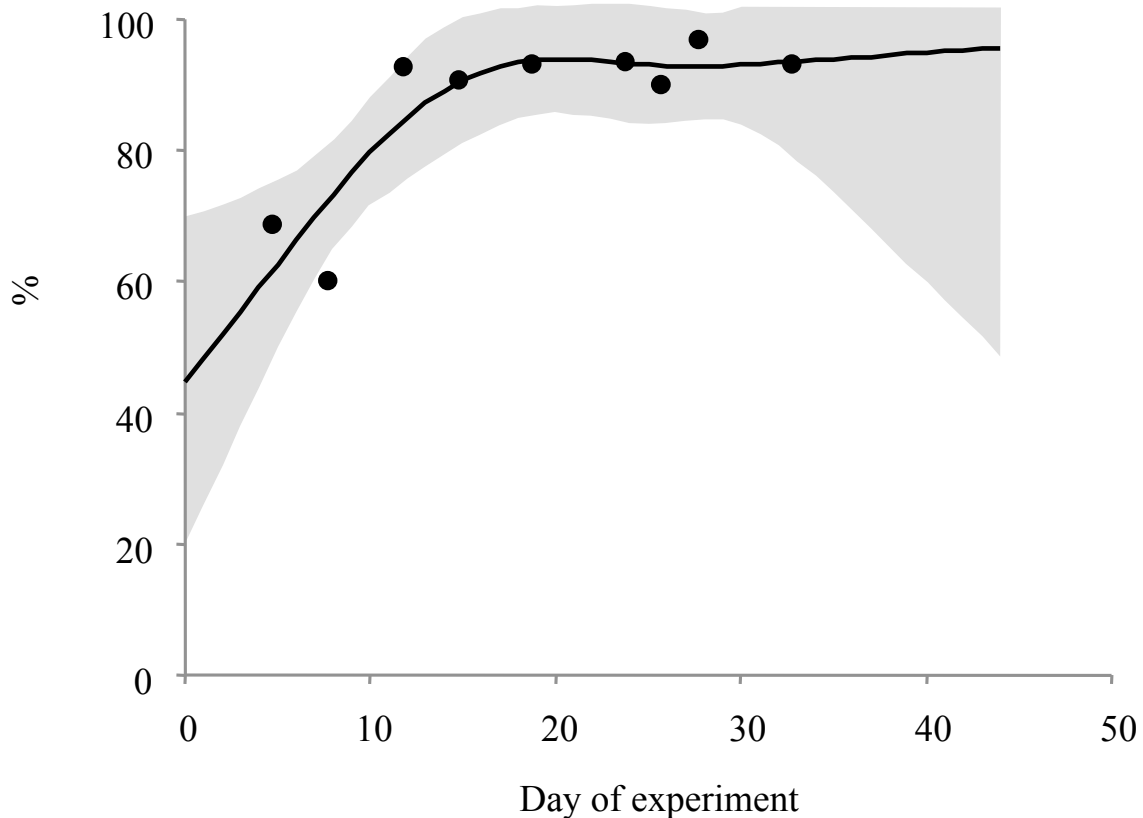


Figure 4. The fraction (%) of gross photosynthetically fixed CO₂ that was not accumulated into the C biomass (biological losses) measured in HRAPs at pH 7 during run 1 and run 2. The time series was modelled using a generalised additive model (GAM) and was plotted (full lines) along with their confidence intervals (grey areas) at time points 0 to 44 days using 1-day intervals.

Therefore, the maximal theoretical CO₂ utilisation efficiency would be 56%, corresponding to lowest biological CO₂ loss (Fig.4) in an open raceway using natural marine inoculate. The maximal CO₂ utilisation efficiency reported during this study (40%) was measured one week after the beginning of the experiment. This value corresponds to values (31–49%) determined for CO₂ utilisation efficiency reported for *Nannochloris* sp. cultivated in batch mode indoors in a 10 m² raceway at pH 7.5 for 14 days (Asadollahzadeh et al. 2014). Similarly, the highest CO₂ utilisation efficiency observed by Asadollahzadeh et al. (2014) occurred one week after the beginning of the experiment when algal productivity was at its highest level. From our results, we show a positive correlation between water resident time and loss of fixed CO₂ through biological activity, especially through predation. We suggest

that microalgal cultivation time should be sufficiently short to avoid the substantial development of predators while maintaining high algal productivity. The present experiment was run under batch culture mode where microorganisms are inoculated to a fixed volume of medium and they gradually consumed nutrients until exhaustion. A continuous culture mode seems more adapted than batch culture mode, because fresh medium is continuously added to the culture while used medium and cells are harvested at the same time. For continuous culture mode, the dilution rate (inverse of the resident time of growing cells) can be adjusted according to climatic variations for maintaining algal cells at high productivity rates and for removing slow-growth predators. Our results also show that CO₂ injection can be used for fixing pH at requested values to control the development of algal predators and to favour rapid-growth stress-tolerant microalgae species such as *Nannochloris* sp. (Henley et al. 2002, Vasseur et al. 2012). This supports previous observations reported in studies with CO₂ addition in HRAPs and performed in various geographical areas (New Zealand, Southern France, Southern Spain) using natural biological inoculates (de Godos et al. 2016; Mehrabadi et al. 2017; Uggetti et al. 2018). This suggests a common response of different natural-inoculate biological communities to CO₂ injection in HRAPs. The use of artificial algal assemblages, well-adapted to CO₂ capture under low pH (Piiparen et al. 2018), may facilitate experimental procedures and industrialisation. However, a very recent study shows that a natural algal polyculture was more productive and stress resilient than an optimised artificially assembled polyculture when cultivated with anaerobic digester effluent and with or not grazer additions (Thomas et al. 2019). Therefore, assembled polycultures may not be as resilient as natural assemblages to cope with grazing pressure, variable growth conditions and potential contaminants when using industrial flue-gas and/or wastewater

4. Conclusion

Injection of CO₂ for microalgae production in HRAPs requires pH control. Our results show that pH 7, controlled by CO₂ addition with a variability of 0.2 pH unit, favoured the growth of *Nannochloris* sp., reduced populations of marine algal predators and photosynthetically fixed accumulated CO₂ at 40 % efficiency into carbon biomass. More acidic pH values led to the development of amoebae and restrict algal biomass accumulation. By extrapolating our experimental results, we predict that the maximal CO₂ conversion efficiency in these systems would be 60% of the available CO₂. Such value may be achievable if running HRAPs in a continuous mode, with a water resident time required for maintaining a rapid algal growth whilst reducing the development of algal predators. When using an industrial CO₂ source, pH regulation using injection of industrial fumes could be an effective tool for driving an algal polyculture toward more desired species. Further investigations should assess the impact of flue gas-associated contaminants on microalgal diversity, biomass production and resilience, as well as the effects of seasonal variability throughout the year on annual capability of fixing CO₂ and algal species selection using pH control in HRAPs.

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Supplementary material

Figure S1. Values of pH measured in the HRAPs with pH targeted at 6, 7 and 8 during run 1 (from 17 May to 22 June 2016) and run 2 (from 27 June to 26 July 2016).

