

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

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1	Control of the pH for marine microalgae polycultures: a key point for CO <sub>2</sub> fixation
2	improvement in intensive cultures
3	
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12	
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15	
16	Abstract
17	Recently, CO <sub>2</sub> recycling for the production of valuable microalgae has acquired
18	substantial interest. Most studies investigating CO <sub>2</sub> 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 CO <sub>2</sub> into polyalgal cultures
21	requires a careful control of $pH$ ; performance of CO <sub>2</sub> 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 CO <sub>2</sub> conversion by natural marine algal assemblage cultivated in
24	open, land-based raceways (4.5 m <sup>3</sup> , 10 m <sup>2</sup> ), working as high rate algal ponds (HRAP). Ponds
25	were enriched with nitrogen and phosphate, pure CO <sub>2</sub> 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 CO<sub>2</sub> into carbon biomass (40%) was reached at pH 7, an intermediate
- 28 level, due to the partial CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> emissions to the atmosphere (Lai et al. 36 2019; Stiles et al. 2018). Through photosynthesis, microalgae can convert CO<sub>2</sub> 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 CO<sub>2</sub> acidifies algal culture 41 medium by modifying the balance of carbonates in the water (Galloway and Krauss 1961). 42 This acidification could counterbalance CO<sub>2</sub> fixation by microalgae which alkalinises their growth environment (Galloway and Krauss 1961). A controlled injection of CO<sub>2</sub> would 43 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. achieves maximal growth rate at pH 6, maximum carbon content at pH 7 and maximal carbon 46 47 conversion efficiency at pH 8 (Vasseur et al. 2012). Other example, the optimal gowth of the 48 cvanobacteria 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 CO<sub>2</sub> 50 injection) negatively affects the densities and grazing activity of rotifers (Montemezzani et al.

2017a, b, c). If we ignore the potential effects of associated contaminants, using CO<sub>2</sub> from
industrial flue gas can combine pH control during high photosynthetic CO<sub>2</sub> fixation and
reduction of algal grazing pressure.

Large-scale industrial cultivation of marine microalgae appears to be one of the most promising approaches of sustainable sources of food (lipids and proteins) and energy (Greene et al. 2016). Narwani et al. (2016) demonstrated stabilising effects of biodiversity on biocrude production from polycultures of several assembled algal species using raceway ponds under variable environmental conditions. However, higher performance can be reached using 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 CO<sub>2</sub>. We measured algal diversity,

64 photosynthetic CO<sub>2</sub> uptake, carbon biomass accumulation, and associated development of

algal predators under three different pH values. We developed performance curves of

biological CO<sub>2</sub> conversion efficiency a function of pH. Results from this study provide a

67 comprehensive assessment of the biological CO<sub>2</sub> conversion efficiency from marine

68 microalgae cultivated in open ponds. The results from this experiment can inform engineering

69 projects for industrial CO<sub>2</sub> sequestration or mass production of marine microalgae.

70

### 71 2. Material & Methods

72 2.1. Experimental design

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

76	of 4.46 m <sup>3</sup> and surface area of 10 m <sup>2</sup> . Water mixing in the HRAPs was maintained at 0.2 m s <sup>-1</sup>
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 CO <sub>2</sub> bottle using a solenoid valve. We
83	diffused CO <sub>2</sub> 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 $CO_2$ 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 $\mu$ m 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	

- 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<sup>-1</sup>) 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 Chla measurements, between
- 118 3 and 800 ml samples (depending on sample loading) were filtered onto 13 mm GF/F
- 119 fibreglassfibre filters to determine the total Chla concentrations. For size-fractionated Chla
- 120 measurements, between 50 and 800 ml samples were filtered using 20  $\mu$ m, 5  $\mu$ m and 2  $\mu$ m
- 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
- filtered and extract absorbance was measured at 632, 649 and 665 nm (with absorbance at 750
- 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 Chla and POC measurements ( $r^2 = 0.85$ , p < 0.001, n=33) leading to a
- 134 Chla: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
- 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

- 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

taxonomic outline. Mothur was also used to identify representative sequences of operationaltaxonomic 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  $\mu$ m and 35  $\mu$ m filters to separate 35 to 100 157  $\mu$ m and > 100  $\mu$ 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  $> 100 \,\mu m$ 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<sub>2</sub> uptake

165 The photosynthetic CO<sub>2</sub> 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 (Chla) 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 photosynthetron

- 173 with a light density gradient up to 400  $\mu$ mol photons m<sup>-2</sup>s<sup>-1</sup> 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 CO<sub>2</sub> uptake rates were derived from the P-I model 187 obtained every week for each HRAP, the Chla 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 Chla biomass during a day. 191 The total gross photosynthetic CO<sub>2</sub> uptake for HRAPs was calculated as the sum of all 192 instantaneous gross CO<sub>2</sub> uptake rates obtained during the whole experiment in each HRAP. 193

194 2.3.3. Prediction of minimal CO<sub>2</sub> losses

195 We attempted to predict the minimal carbon losses through biological activity (respiration,

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

as the fraction of gross photosynthetically fixed CO<sub>2</sub> not accumulated into the C biomass over

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
- and run 2, respectively. When pooling pH measured during both experimental runs, pH
- 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 CO<sub>2</sub> 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 μm, Fig. 1), such as *Chlamydomonas* sp., favoured the
- 211 growth at pH 6, while smaller microalgae (< 2 μm, 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
- 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 CO<sub>2</sub> to control pH is a viable tool for
- 219 favouring the development of one specific microalgal species among a natural assemblage.



- 231 Although differences in microalgal diversity were observed in HRAPs, no significant
- 232 differences were found among pH treatments for the photosynthetic parameters, Pmax and Ik
- 233 (Table S1). However, photoinhibitory effect was significantly higher in HRAPs at pH 7 and 8
- 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 Ik values and lower w values in July than
- in June during the experiments (ANOVA p < 0.05, Tukey post hoc; Suppl. Table S1).



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

241

237

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

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°C) was significantly higher ( $p < 0.05$ , which
259	was determined using a t-test).
260	

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

Size fraction	Group (ind. L <sup>-1</sup> )	20th of May	22rd of June 2016 (Tf)			28th of June	26th of July 2016 (Tf)			
			pH 6	pH 7	pH 8	2016 (T0)	pH 6	pH 7	pH 8	
	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	
35-100 μm	Actinopods				416		1976	416	208	
	Nematods	10	520	936	416		104	2184		
	Rotifers								520	
	Copepods			1664	104			312	2600	
	Nematods larvae	9	104		351	5	50	513	169	
> 100	Tisbe sp.			299	156				871	
> 100 μm	Tisbe nauplius	0							104	
264	Copepod nauplii			59						

<sup>265</sup> 

266 Injection of CO<sub>2</sub> into HRAPs negatively affects zooplankton (Cladoceans, copepods, rotifers)

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 CO <sub>2</sub> was done in order to maintain a pH of 6. In addition, the final abundance
270	protozoans and metazoans >100 µm decreased as injected CO <sub>2</sub> 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 CO <sub>2</sub> replaced O <sub>2</sub> availability. In
273	contrast, the reductions in pH through the CO <sub>2</sub> 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 ( $< 50 \text{ mg L}^{-1}$ ) 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.



Figure 3. Concentration of carbon biomass (POC measurements) measured in
HRAPs (average and standard deviation of sample triplicates) during run 1 from 17

May to 22 June 2016 (A) and during run 2 from 27 June to 26 July 2016 (B).

294

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

# <u>Table 2.</u> Gross photosynthetic CO<sub>2</sub> uptake, produced carbon biomass and carbon losses (the fraction of the gross photosynthetic CO<sub>2</sub> uptake that was not accumulated into carbon biomass) measured in HRAPs at pH 6.7 and 8 during the two experimental runs

		usured in the	<sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup> <sup>1</sup>	o, / unu	o during the tw	o experime	Jinui Tui	15.		
Date	Day of experiment	pH 6			pH 7			pH 8		
		Gross photosynthetic $CO_2$ uptake (mgC L <sup>-1</sup> )	Produced C biomass (mgC L <sup>-1</sup> )	C loss (%)	Gross photosynthetic $CO_2$ uptake (mgC L <sup>-1</sup> )	Produced C biomass (mgC L <sup>-1</sup> )	C loss (%)	Gross photosynthetic $CO_2$ uptake (mgC L <sup>-1</sup> )	Produced C biomass (mgC L <sup>-1</sup> )	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 <b>31614</b>	. 29	1074	27	97	1180	40	97	1620	157	90
305	)									

288

- 306 In all HRAPs, carbon loss increased rapidly over time, reaching more than 90% of the gross
- 307 photosynthetic  $CO_2$  uptake measured throughout the experimental period (Table 2). Most  $CO_2$

308 loss is related to high microalgal respiration rate which has been reported to be 60-90% of the

309 photosynthetic fixed CO<sub>2</sub> (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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub>; 328 Fogg et al. 1965).



Figure 4. The fraction (%) of gross photosynthetically fixed  $CO_2$  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 CO<sub>2</sub> utilisation efficiency would be 56%, corresponding to lowest biological CO<sub>2</sub> loss (Fig.4) in an open raceway using natural marine inoculate. The maximal CO<sub>2</sub> 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 CO<sub>2</sub> utilisation efficiency reported for *Nannochloris* sp. cultivated in batch mode indoors in a 10 m<sup>2</sup> raceway at pH 7.5 for 14 days (Asadollahzadeh et al. 2014). Similarly, the highest CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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  $CO_2$ 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 naturalinoculate biological communities to  $CO_2$  injection in HRAPs. The use of artificial algal assemblages, well-adapted to CO<sub>2</sub> 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 CO<sub>2</sub> for microalgae production in HRAPs requires pH control. Our results show that pH 7, controlled by CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> conversion efficiency in these systems would be 60% of the available CO<sub>2</sub>. 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 CO<sub>2</sub> 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 CO<sub>2</sub> and algal species selection using pH control in HRAPs.

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### Supplementary material

<u>Figure S1.</u> 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).

