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Limited impact of ocean acidification on phytoplankton community structure and carbon export in an oligotrophic environment: Results from two short-term mesocosm studies in the Mediterranean Sea

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1 **Limited impact of ocean acidification on phytoplankton**
2 **community structure and carbon export in an oligotrophic**
3 **environment: results from two short-term mesocosm studies in**
4 **the Mediterranean Sea**

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32 Highlights:

- 33 • Two large mesocosm experiments carried out in the Northwestern Mediterranean Sea
34 • Experiments conducted in the summer oligotrophic vs. winter mesotrophic periods
35 • Production limited by nutrient availability and community dominated by small species
36 • Organic matter export was not impacted by CO₂-enrichment
37 • In areas where nutrient availability exerts a strong pressure on phytoplankton growth, CO₂
38 addition will likely have very limited effects on phytoplankton diversity

39
40 Keywords:

41
42 Ocean acidification; Pelagic mesocosms; Mediterranean Sea; Oligotrophic area;
43 Phytoplankton community

44 **Abstract**

45 Modifications in the strength of the biological pump as a consequence of ocean
46 acidification, whether positive or negative, have the potential to impact atmospheric CO₂ and
47 therefore climate. So far, most plankton community perturbation studies have been performed
48 in nutrient-rich areas although there are some indications that CO₂-dependent growth could
49 differ in nutrient-replete vs. -limited regions and with different community compositions.
50 Two *in situ* mesocosm experiments were performed in the NW Mediterranean Sea during two
51 seasons with contrasted environmental conditions: summer oligotrophic stratified waters in
52 the Bay of Calvi vs. winter mesotrophic well-mixed waters in the Bay of Villefranche. Nine
53 mesocosms were deployed for 20 and 12 d, respectively, and subjected to seven CO₂ levels (3
54 controls, 6 elevated levels). Both phytoplankton assemblages were dominated by pico- and
55 nano-phytoplankton cells. Although haptophyceae and dinoflagellates benefited from short-
56 term CO₂ enrichment in summer, their response remained small with no consequences on
57 organic matter export due to strong environmental constraints (nutrient availability). In
58 winter, most of the plankton growth and associated nutrient consumption occurred during the
59 4-day acidification period (before the experimental phase). During the remaining
60 experimental period, characterized by low nutrient availability, plankton growth was minimal
61 and no clear CO₂-dependency was found for any of the tested parameters. While there is a
62 strong confidence on the absence of significant effect of short-term CO₂ addition under
63 oligotrophic conditions, more investigations are needed to assess the response of plankton
64 communities in winter when vertical mixing and weather conditions are major factors
65 controlling plankton dynamics.

66 1. Introduction

67 During the last 150 years, human activities, through the combustion of fossil fuels (oil,
68 gas and coal), have led to a dramatic release of carbon dioxide (CO₂) to the Earth's
69 atmosphere. The accumulation of CO₂ impacts the radiative forcing, thereby warming the
70 atmosphere and the ocean. The ocean acts as a climate integrator that absorbed 93% of
71 Earth's additional heat since the 1970s, offsetting much atmospheric warming but increasing
72 ocean temperature and sea level and captured 28% of anthropogenic CO₂ emissions since
73 1750 (Gattuso et al., 2015). Although providing a valuable human service by moderating the
74 rate and severity of global warming, the consequence of this oceanic CO₂ pump is the on-
75 going increase in ocean acidity (i.e. decrease in pH). Surface ocean pH has already decreased
76 by 0.1 units since the beginning of the industrial era (i.e. increased acidity of 30%; Ciais et
77 al., 2013). According to recent projections and depending on the emission scenario
78 considered, an additional decrease ranging between 0.06 and 0.32 units is expected by 2100
79 (Ciais et al., 2013).

80 The decrease in seawater pH leads to a decrease in the concentration of carbonate ions
81 (CO₃²⁻), one of the building blocks of calcium carbonate (CaCO₃), and alters the ability of
82 many calcifying organisms to precipitate CaCO₃ (e.g. Kroeker et al., 2013). In addition, a
83 decrease in seawater pH leads to an increase in dissolved CO₂ and bicarbonate (HCO₃⁻)
84 concentrations. Carbon fixation by marine photosynthetic organisms represents about 50% of
85 global Earth primary production (Field et al., 1998), and the export of part of the produced
86 organic matter from the sunlit surface layer to the deep-ocean (i.e. the biological or soft-tissue
87 pump) is responsible for ~70% of surface to deep-ocean dissolved inorganic carbon (C_T)
88 gradients (Sarmiento and Gruber, 2006). Therefore, modifications in the strength of this

89 biotically mediated carbon pump, whether positive or negative, have the potential to impact
90 atmospheric CO₂ and therefore climate (Riebesell et al., 2007).

91 CO₂ rather than the much more abundant HCO₃⁻ is the substrate used in the carbon
92 fixation step of photosynthesis and RubisCO, the enzyme catalyzing this reaction, has a low
93 affinity for CO₂ (Badger et al., 1998; Giordano et al., 2005). As such, this enzyme is
94 theoretically not saturated under current ambient CO₂ levels (Badger et al., 1998). However,
95 nearly all marine species have developed various mechanisms (carbon concentration
96 mechanisms or CCMs) to compensate for this low CO₂ availability through the energy-
97 demanding use of carbonic anhydrase enzymes or active CO₂ and/or bicarbonate transports
98 through membranes (Raven et al., 2014). There is evidence that both the RubisCO affinity for
99 CO₂ as well as the efficiency of these CCMs differ widely among taxa, species or even strains
100 (Tortell, 2000; Young et al., 2016), complicating the prediction of whether a cell's carbon
101 fixation rate will respond directly to ambient changes in CO₂ availability through increased
102 CO₂ diffusion and/or less energy expenditure needed to operate CCMs (Mangan et al., 2016;
103 Raven and Beardall, 2014). Finally, although downregulation of CCMs at elevated CO₂ has
104 been observed, the significance of this downregulation to overall cell physiology and growth
105 is not currently well constrained due to the presence of other limiting factors in the oceans
106 such as macro- or micro-nutrients and light (Hennon et al., 2015; Young and Morel, 2015).
107 All of this can partly explain the very diverse findings that have been documented on the
108 effect of increased ambient CO₂ availability on photosynthesis and growth of marine
109 phytoplankton (Dutkiewicz et al., 2015).

110 Apart from the above-mentioned variability in RubisCO affinity for CO₂ and CCMs
111 efficiency, a significant part of the observed discrepancies among available perturbation
112 studies could be explained by differences in experimental setups and environmental
113 conditions such as temperature, light conditions and nutrient availability. Phytoplankton

114 growth obviously does not only depend on carbon availability but on a combination of
115 physico-chemico-biological drivers such as macro- and micro-nutrient availability,
116 temperature, light, competition and grazing. It is therefore very likely that the response of
117 phytoplankton will differ depending on these environmental conditions (Verspagen et al.,
118 2014). Furthermore, as this is the amount of organic matter that can escapes the sunlit layer
119 that determines the capacity of the surface ocean to pump atmospheric CO₂, there is a great
120 need to evaluate the impact of CO₂, not only on phytoplankton growth but on the export of
121 this organic matter to deeper layers. The build-up of organic matter and its potential export
122 strongly depends on phytoplankton community composition (Eggers et al., 2014). Indeed,
123 large cells (e.g. diatoms) account for a large proportion of export production and ultimate
124 burial in sediments (Finkel et al., 2005). In contrast, small cells (nano- and pico-plankton) are
125 particularly important in regions with limited nutrient availability with a close coupling
126 between production and grazing through the microbial loop and a with low export capacity
127 (Riebesell and Tortell, 2011). As already mentioned, differing responses to increased CO₂
128 availability between different functional groups, size classes and species (Dutkiewicz et al.,
129 2015) have the potential to significantly alter community structure and functioning. In that
130 sense, studies focused on plankton assemblages rather than on isolated single species and
131 under very contrasted environmental conditions are very informative (Tarling et al., 2016).

132 During the last decade, there has been a noticeable increase in the number of
133 experimental assessments of the sensitivity of plankton community compositions to the on-
134 going increase in CO₂. These experiments were conducted in various areas of the world ocean
135 using different approaches, from small bottle incubations to large mesocosm deployments,
136 and over different time scales (few days to few weeks). Several of these experiments
137 highlighted significant modifications of community compositions under elevated CO₂ levels.
138 For instance, CO₂ enrichment has been shown to stimulate growth of large species such as

139 diatoms (e.g. Domingues et al., 2014; Feng et al., 2009; Reul et al., 2014; Tortell et al., 2002;
140 Tortell et al., 2008; Wu et al., 2014). Several experiments suggested stimulating effects on
141 small species (pico-phytoplankton; e.g. Newbold et al., 2012; Paulino et al., 2008; Schulz et
142 al., 2013). In contrast, Richier et al. (2014) reported significant decrease in the growth of
143 small phytoplankton species ($< 10 \mu\text{m}$) suggesting that small species are less adapted to
144 changes in their local pH while larger cells must face larger pH variations at short time scales
145 (Flynn et al., 2012). Other studies showed differential responses between species from the
146 same taxa (e.g. Endo et al., 2016; Feng et al., 2010; Kim et al., 2006; Meakin and Wyman,
147 2011) and finally among different phylotypes and phenotypes of the same species (e.g.
148 Brading et al., 2011; Rickaby et al., 2016).

149 Whether or not these modifications of community structure (e.g. increase or decrease
150 in cell size) can modify the amount of organic matter sinking to deeper layers can be
151 evaluated through the use of mesocosms. They are defined as experimental enclosures from 1
152 thousand to several thousands of litres that allow the maintenance of natural communities
153 under close-to-natural conditions and the collection of sinking organic matter (Riebesell et al.,
154 2008; Riebesell et al., 2013a). In recent years, plankton community studies performed using
155 such experimental systems have led to very contrasted outcomes in terms of community
156 composition and carbon export responses to CO_2 enrichment (see Table 1). Most of these
157 experiments have been performed in nutrient-rich areas (or following artificial nutrient
158 enrichment) dominated by large species and experiments conducted in areas limited by
159 nitrate, phosphate and/or iron are currently lacking (Paul et al., 2015a). These areas represent
160 a very large surface area of the ocean and are projected to expand in the coming decades
161 because of enhanced thermal stratification and nutrient depletion (Irwin and Oliver, 2009;
162 Polovina et al., 2008). As already mentioned, they are usually dominated by small cells
163 adapted to low-nutrient conditions and have low export capacities. Recently, and in contrast

164 to theoretical considerations (Verspagen et al., 2014), two mesocosm experiments suggested
165 that communities exposed to low nutrient concentrations may be more responsive to CO₂
166 enrichment than previously thought (Bach et al., 2016; Paul et al., 2015a). This was
167 confirmed recently by Sala et al. (2016) based on indoor experiments in a coastal site of the
168 Western Mediterranean Sea. During these experiments, effects of ocean acidification, i.e.
169 positive effect on pico- and nano-phytoplankton, were more important when nutrient
170 concentrations were low. However, it must be stressed that nutrient and chlorophyll levels
171 observed during these experiments were representative of an urbanized coastal area and much
172 higher than levels usually observed in the vast majority of the Mediterranean Sea.

173 The Mediterranean Sea is generally considered as oligotrophic but actually exhibits a
174 gradient from mesotrophic-oligotrophic in the western basin to ultra-oligotrophic in the
175 eastern basin (The Mermex group, 2011). These features are induced by the different
176 localizations of the physical (the winter mixed layer) and nutrient (the nutricline) vertical
177 interfaces, which are both determined by the large-scale circulation pattern (The Mermex
178 group, 2011). Based on satellite-derived estimates, chlorophyll *a* concentrations exhibit low
179 values (less than 0.2 µg L⁻¹) over most of the Mediterranean Sea, except for the Liguro-
180 Provençal region where large blooms can be observed in late winter-early spring (D'Ortenzio
181 and d'Alcala, 2009). Overall, phytoplankton communities are dominated by pico-
182 phytoplankton (Siokou-Frangou et al., 2010). However, because of its very diversified
183 (spatially and temporally) physical structure, localized higher nutrient availability can drive
184 more intense biological activities and transient dominance of larger species such as diatoms
185 and dinoflagellates (Bustillos-Guzmán et al., 1995). Diatoms are more abundant during the
186 transition between mixed and stratified conditions (Claustre et al., 1994). These features make
187 the Mediterranean Sea a perfect natural laboratory to study the effects of nutrient availability
188 and community composition on the response of plankton community to CO₂ enrichment.

189 In the frame of the European project ‘Mediterranean Sea Acidification under changing
190 climate’ (MedSeA; <http://medsea-project.eu>), for the first time, two short-term *in situ*
191 mesocosm experiments were performed in the Northwestern Mediterranean Sea during two
192 seasons with contrasted environmental conditions (i.e. summer oligotrophic stratified waters
193 vs. winter mesotrophic well-mixed waters) and different phytoplankton community
194 compositions (i.e. higher proportion of diatoms and lower proportion of pico-phytoplankton
195 and cyanophyceae in winter compared to summer). In this paper, we report on the response of
196 the phytoplankton community composition as well as of particulate organic matter dynamics
197 and export to CO₂-enrichment.

198 **2. Material and Methods**

199 **2.1. Study sites and experimental set-up**

200 Two mesocosm experiments were conducted in the Northwestern Mediterranean Sea:
201 the first one, in the Bay of Calvi (Corsica, France) in summer (June-July 2012), and the
202 second one in the Bay of Villefranche (France) in winter (February-March 2013). The
203 experimental set-up and mesocosm characteristics are fully described in Gazeau et al. (in
204 press, this issue). Briefly, for each experiment, nine mesocosms of ca. 50 m³ (2.3 m in
205 diameter and 12 m deep) were deployed for 20 and 12 days in the Bay of Calvi and the Bay of
206 Villefranche, respectively. Once the bottom of the mesocosms was closed, CO₂ saturated
207 seawater was added to obtain a *p*CO₂ gradient across mesocosms ranging from ambient levels
208 to 1,250 µatm, with three control mesocosms (C1, C2 and C3) and six mesocosms with
209 increasing *p*CO₂ (P1 to P6). In the Bay of Calvi, the six targeted elevated *p*CO₂ levels were
210 P1: 550, P2: 650, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. In the Bay of Villefranche,
211 the levels were P1: 450, P2: 550, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. Mesocosms
212 were grouped in clusters of 3 with each cluster containing a control, a medium and a high

213 $p\text{CO}_2$ level (cluster 1: C1, P1, P4; cluster 2: C2, P2, P5 and cluster 3: C3, P3, P6).
214 Acidification of the mesocosms was performed over four days by homogenous addition of
215 various volumes of CO_2 -saturated seawater. Once targeted $p\text{CO}_2$ levels were reached, the
216 experiment started (day 0 = 24 June 2012 and 22 February 2013 for the Bay of Calvi and the
217 Bay of Villefranche, respectively). No further CO_2 additions were performed during the
218 experiments and $p\text{CO}_2$ levels evolved in mesocosms as a consequence of air-sea fluxes,
219 temperature changes and plankton net community production. Weather permitting,
220 conductivity-temperature-depth (CTD) casts were performed on a daily basis in each
221 mesocosm and in the external environment. Surface irradiance (photosynthetically active
222 radiation; PAR) was measured continuously during the two experiments using a LI-COR LI-
223 192SA 2-Pi sensor connected to a LI-1400 data logger (Gazeau et al., in press, this issue).
224 Vertical attenuation coefficients were estimated daily in each mesocosms, based on PAR
225 profiles (0-12 m) performed using a QSP-2200 4-Pi sensor (Biospherical Instruments Inc.)
226 mounted on the CTD. Mean daily photon doses were calculated using surface PAR and
227 estimated attenuation coefficients. In the Bay of Calvi, wind speed and direction were
228 recorded with a THIES© anemometer deployed, by the University of Liège (Belgium), on top
229 of one of buildings of the at 11.8 m height at a distance of about 400 m from the mesocosms.
230 For the experiment in the Bay of Villefranche, wind speed data (daily averages) were
231 obtained from the Météo France station at the Nice-Côte d'Azur International Airport
232 (43°39'55" N, 7°12'48" E).

233 **2.2. Sampling and analytical methods**

234 Depth-integrated (0-10 m) samplings from the mesocosms and the external
235 environment (referred thereafter to as OUT) were performed daily at 8:30 (local time) during
236 both experiments. All three clusters were simultaneously sampled from a plastic platform by
237 three teams of two scientists, each using an integrating water sampler (IWS; HYDRO-

238 BIOS©). The IWS units were hanged on a Kevlar cordage and downcasts were performed
239 manually at a regular speed of 10 cm s⁻¹ after rinsing it outside the mesocosms.

240 Samples for pigment determination were taken every day at 8:30 (local time) during
241 both experiments. Two litres of sampled seawater were filtered onto GF/F. Filters were
242 directly frozen in liquid nitrogen and stored at -80 °C pending analysis at the Laboratoire
243 d'Océanographie de Villefranche (France). Filters were extracted at -20 °C in 3 mL methanol
244 (100%), disrupted by sonication and clarified one hour later by vacuum filtration through
245 GF/F filters. The extracts were rapidly analyzed (within 24 h) by high performance liquid
246 chromatography (HPLC) with a complete Agilent Technologies system. The pigments were
247 separated and quantified as described in Ras et al. (2008).

248 *Synechococcus*, *Prochlorococcus*, autotrophic pico-eukaryotes and nano-eukaryotes
249 abundances were determined by flow cytometry analysis from samples taken every 2 days at
250 4:00 and 5:00 in the Bay of Calvi and the Bay of Villefranche, respectively (local times).
251 Seawater samples (2 mL) from each mesocosm were immediately fixed with 0.2 µm pre-
252 filtered 25% glutaraldehyde (0.5% final concentration), kept at 4 °C for approximately 30
253 min, then flash frozen in liquid nitrogen and finally stored at -80 °C until further processing
254 (Troussellier et al., 1995; Vaulot et al., 1989). Single cell analysis was processed with a
255 maximum flow rate of 65 µL min⁻¹ through a Becton Dickinson, FACSCalibur flow
256 cytometer, equipped with an air-cooled Argon laser emitting at 488 nm and analyzed with the
257 Cell Quest Pro software (Becton Dickinson). The sample volume analyzed per time unit was
258 accurately defined by systematically adding to the samples fluorescent latex beads
259 suspensions of 1 µm (Polysciences Inc., Europe) at a concentration of 2.5 x 10⁵ beads mL⁻¹.
260 The abundance of autotrophic prokaryotes and pico- and nano eukaryotes was assessed from
261 unstained samples following the method described by Marie et al. (1999). In the Bay of
262 Villefranche, four groups were determined based on the optical parameters characterizing

263 each cell. *Synechococcus* (< 1.5 μm) cells were detected by their signature in a plot of orange
264 fluorescence (FL2, 565–592 nm wavelength ranges) vs. red fluorescence (FL3, > 620 nm).
265 *Prochlorococcus*, autotrophic pico-eukaryotes (< 2 μm) and nano-eukaryotes (2 – 10 μm)
266 were detected in a plot of SSC vs. red fluorescence (FL3, > 620 nm). In the Bay of Calvi, only
267 abundances of *Synechococcus* spp. and autotrophic pico-eukaryotes were assessed.

268 For particulate element concentrations, sampled seawater (0.75 - 2 L) was filtered
269 through pre-combusted glass-fiber filters. Particulate organic carbon (POC) and nitrogen
270 (PON) were determined at the Istituto Nazionale di Oceanografia e di Geofisica Sperimentale
271 (Italy) using a CHNO-S elemental analyzer (Costech ECS4010) after acidification with 1 N
272 HCl and high-temperature combustion.

273 Collection of sediment traps was performed by a diver on a daily basis in the Bay of
274 Calvi and less regularly as a consequence of bad weather conditions in the Bay of
275 Villefranche (see Gazeau et al., in press, this issue). On each occasion and for each
276 mesocosm, divers followed the same procedure: (1) hitting the cone of the mesocosms in case
277 some sinking material was retained on the walls, (2) waiting for 15 minutes, (3) closing the
278 collector, (4) collecting the 250 mL flask screwed to the trap system, (5) immediately
279 replacing the sampled flask by a new empty one and (6) opening the collector again. All
280 mesocosms were sampled within 30 min. Back in the laboratory, samples were immediately
281 preserved in a pH buffered formalin solution (5%). Swimmers (i.e. opportunistic copepods
282 and other zooplankton species that swim into the traps; Lee et al., 1988) larger than 1 mm
283 were removed (and discarded) and the remaining material was rinsed, centrifuged, freeze-
284 dried and grinded. In the Bay of Calvi, as a consequence of low amounts of material
285 especially at the end of the experiment, daily sediment traps samples were pooled as follows:
286 days 5-7, 8-10, 11-14 and 15-19. Total particulate matter was weighed for flux determination
287 and subsamples were used for POC and PON measurements performed on elemental

288 analyzers after acidification with 1N HCl. Samples from the experiment in the Bay of Calvi
289 were analyzed at NIOZ-Yerseke (The Netherlands) on a Thermo Electron Flash 1112.
290 Samples from the experiment in the Bay of Villefranche were analyzed at the Laboratoire
291 d'Océanographie de Villefranche (LOV, France) on a Elementar Vario Pyrocube.

292 **2.3. Data analysis and statistics**

293 The contribution of each phytoplankton group to total phytoplankton biomass
294 (chlorophyll *a*) was estimated by using the CHEMTAX program with input ratios from
295 Rodriguez et al. (2006) and Not et al. (2007). These pigment ratios established for open ocean
296 plankton communities were modified from the original values by comparing microscopic and
297 flow cytometry counts to HPLC analyses from samples collected in the NW Iberian coast, an
298 area dominated by pico- and nano-eukaryotes, as observed in our study.

299 All data collected during the two experiments are freely available on Pangaea, Bay of
300 Calvi: <http://doi.pangaea.de/10.1594/PANGAEA.810331> and Bay of Villefranche:
301 <http://doi.pangaea.de/10.1594/PANGAEA.835117>.

302 For these two experiments, we chose to follow a CO₂ gradient approach rather than to
303 replicate certain levels (ANOVA approach). As already done in several similar perturbation
304 experiments (Paul et al., 2015a; Riebesell et al., 2013b), stepwise multiple linear regression
305 analyses were performed to establish relationships between environmental/experimental
306 conditions including *p*CO₂ and (1) POC and PON fluxes to the sediment traps as well as their
307 ratios, (2) water column POC and PON concentrations and their ratios as well as (3)
308 chlorophyll *a*-equivalent biomass or abundances of the different identified groups. Besides
309 *p*CO₂, other environmental conditions that have been considered were temperature, salinity,
310 daily photon doses, daily averaged wind speeds and nutrient concentrations (NO_x: NO₃⁻ +
311 NO₂⁻, ammonium: NH₄⁺, phosphate: PO₄³⁻, and, only for diatoms, silicate: Si). Integrated
312 levels of temperature and salinity were acquired through the daily CTD casts performed in

313 each mesocosm. NO_x and phosphate were measured using nanomolar techniques as described
314 in Louis et al. (in press, this issue). Ammonium and silicate concentrations were determined
315 as described by Gazeau et al. (in press, this issue). As fully described in Gazeau et al. (in
316 press, this issue), daily $p\text{CO}_2$ levels in each mesocosm were determined from dissolved
317 inorganic carbon, total alkalinity, temperature and salinity using the R package seacarb
318 (Lavigne et al., 2014).

319 All analyses were performed using the R software (R Core Team, 2015) and were
320 considered significant at a probability $p < 0.01$.

321 3. Results

322 3.1. Environmental and experimental conditions during both experiments

323 Conditions in each mesocosm at the start and at the end of both experiments (days 0
324 and 20 in the Bay of Calvi and days 0 and 12 in the Bay of Villefranche) are shown in Tables
325 2 and 3. For both experiments, $p\text{CO}_2$ values in CO_2 -enriched mesocosms (P1 to P6) were
326 close to targeted levels. Ambient $p\text{CO}_2$ levels were higher in the Bay of Calvi in summer as
327 compared to the Bay of Villefranche in winter (~ 450 vs. $350 \mu\text{atm}$ respectively). While $p\text{CO}_2$
328 levels slightly decreased (pH levels slightly increased) in the Bay of Calvi during the course
329 of the experiment, especially for high CO_2 mesocosms (P5 and P6), drops in $p\text{CO}_2$ levels
330 (increases in pH levels) were much stronger in the Bay of Villefranche with mesocosms P1 to
331 P4 showing very similar levels by the end of the experiment (Fig. 1). Hydrological data
332 (temperature and salinity) are fully described in Gazeau et al. (in press, this issue). Briefly,
333 while temperature levels in the Bay of Calvi gradually increased from $\sim 22.1^\circ\text{C}$ on day 0 to
334 $\sim 24.2^\circ\text{C}$ on day 20, they were constant in the Bay of Villefranche at around $\sim 13.2^\circ\text{C}$.
335 Salinity increased roughly by 0.1-0.2 units during both experiments because of evaporation.
336 In winter in the Bay of Villefranche, surface irradiance was generally constant during the
337 entire experiment with minimal and maximal daily (sunrise to sunset) average values of 531
338 and $735 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Maximum irradiance levels (~ 1300 - $1400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)
339 were reached at around 12:00 pm and the Light:Darkness (L:D) cycle was 16.5:7.5 and 16:8,
340 respectively at the start and at the end of the experiment. In the Bay of Villefranche, minimal
341 and maximal daily (sunrise to sunset) average values of 103 and $513 \mu\text{mol photons m}^{-2} \text{s}^{-1}$
342 were recorded with a L:D regime of 11.5:12.5, and maximal irradiance levels (~ 300 - 1100
343 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) reached at 1:00 pm. Light attenuation coefficients were generally
344 constants during both experiments with higher values estimated in winter in the Bay of
345 Villefranche than in summer in the bay of Calvi ($0.19 \pm 0.07 \text{ SD}$ and $0.14 \pm 0.05 \text{ SD m}^{-1}$,

346 respectively). Higher daily averaged wind speeds were recorded during the winter experiment
347 in the Bay of Villefranche with very windy conditions experienced on day 8 that prevented
348 sampling during that day, and even winder on day 12 and the following night that irreversibly
349 damaged the bags.

350 In summer in the Bay of Calvi, NO_x concentrations initially decreased then increased
351 again after day 14 to reach similar levels than at the start of the experiment (47 ± 20 on day 0
352 vs. $60 \pm 15 \text{ nmol L}^{-1}$ on day 20; average \pm SD between the nine mesocosms). Dissolved
353 inorganic phosphate (PO_4^{3-}) quickly decreased from day 0 to day 1 and remained constant
354 during the rest of the experiment (23 ± 12 on day 0 vs. $7 \pm 2 \text{ nmol L}^{-1}$ on day 20). In winter in
355 the Bay of Villefranche, PO_4^{3-} concentrations were generally similar to the ones encountered
356 in summer in the Bay of Calvi and no strong variations could be observed in all mesocosms
357 along the course of the experiment (global average: $9 \pm 1 \text{ nM}$). NO_x levels were much higher
358 in the Bay of Villefranche than in the Bay of Calvi when bags were closed ($> 1 \mu\text{M}$).
359 However, during the acidification phase (day-4 to -1), due to favorable weather conditions
360 (low wind, high irradiance levels, data not shown), chlorophyll *a* concentrations increased,
361 consuming a large proportion of available nitrate and nitrite before the start of the
362 experimental phase (day 0). As a consequence, while $[\text{NO}_x]$ in external waters remained high
363 ($\sim 1 \mu\text{M}$), all mesocosms were depleted in NO_x with an average concentration of 129 ± 30
364 nM . NO_x to phosphate ratios were higher in the Bay of Villefranche than in the Bay of Calvi
365 (2 ± 1 and 9 ± 4 on day 0 and on day 20 in the Bay of Calvi vs. 13 ± 4 and 33 ± 18 on day 0
366 and on day 12 in the Bay of Villefranche). More details on nutrient dynamics can be found in
367 Louis et al. (in press, this issue).

368 The experiment in the Bay of Calvi was representative of summer conditions in the
369 Ligurian Sea with low nutrient concentrations, low chlorophyll *a* concentrations (see below),
370 warm waters and high irradiance levels. In the Bay of Villefranche in winter, while

371 hydrological and weather conditions were typical of winter conditions in the Northwestern
372 Mediterranean Sea (low temperature and irradiance levels), nutrients were rapidly depleted
373 inside the mesocosms before the start of the experiment, and reached levels not usually
374 encountered during this period of the year.

375 **3.2. Phytoplankton assemblages during the summer experiment in the Bay of Calvi**

376 Total chlorophyll *a* concentrations in the Bay of calvi (Fig. 2) averaged $0.07 \pm 0.01 \mu\text{g}$
377 L^{-1} in the nine mesocosms along the experiment, a value much lower than that in the
378 surrounding waters ($0.12 \pm 0.02 \mu\text{g L}^{-1}$). In mesocosms, chlorophyll *a* concentrations linearly
379 increased during the experiment (GLM, $r^2 = 0.6$, $p < 0.001$) with a maximal concentration of
380 $0.09 \pm 0.003 \mu\text{g L}^{-1}$ on day 14.

381 When pigment data for all mesocosms were pooled together (Fig. 3), the plankton
382 community in the Bay of Calvi was found to be dominated at the start of the experiment by
383 haptophyceae representing $36 \pm 5\%$ of the chlorophyll content, followed by cyanophyceae (20
384 $\pm 3\%$), chlorophyceae ($14 \pm 3\%$) and pelagophyceae ($11 \pm 2\%$). Important differences were
385 identified along the experiment between concentrations of the different species inside the
386 mesocosms and in the surrounding waters (Fig. 4). All species, except for diatoms, showed
387 lower chlorophyll *a* biomass inside mesocosms. Diatoms were virtually absent in the
388 surrounding waters, except at the end of the experiment. On day 20, while the contribution of
389 cyanophyceae, dinophyceae, diatoms, pelagophyceae and cryptophyceae did not strongly
390 change as compared to day 0, the contribution of chlorophyceae increased to $31 \pm 4\%$.

391 Based on flow cytometry measurements, *Synechococcus* abundances increased during
392 the first days of the experiment, reached maximal values on day 10 (averaged between
393 mesocosms of $29600 \pm 3000 \text{ cells mL}^{-1}$) and then decreased until the end of the experiment
394 (Fig. 5). Similar dynamics, although with more variability among mesocosms, were observed

395 for autotrophic pico-eukaryotes, with abundances one order of magnitude lower than
396 *Synechococcus*.

397 Table 4 shows total chlorophyll *a* concentrations were not correlated with $p\text{CO}_2$ but
398 showed positive trends with salinity and to a lesser extent with NH_4^+ . Chlorophyll *a*-
399 equivalent biomass of two groups of phytoplankton were significantly correlated with $p\text{CO}_2$,
400 dinophyceae and haptophyceae. For these two groups, $p\text{CO}_2$ appeared as the most important
401 contributor to the variance. Note that a maximum of 66% of the variance (i.e. for
402 chlorophyceae) observed in total chlorophyll *a* or group-specific biomasses could be
403 explained by these stepwise linear regression analyses using the tested environmental and/or
404 experimental variables.

405 3.3. Phytoplankton assemblage during the winter experiment in the Bay of Villefranche

406 In the Bay of Villefranche (Fig. 2), total chlorophyll *a* concentrations averaged $0.98 \pm$
407 $0.15 \mu\text{g L}^{-1}$ in the nine mesocosms along the 12-day experiment. Chlorophyll *a* remained
408 slightly above levels in the surrounding waters for the entire experimental period, except for
409 the last day (day 12) when concentrations increased abruptly outside the mesocosms. HPLC
410 data are available for the acidification phase of this experiment (day -4 to day -1), data show
411 that chlorophyll *a* concentrations increased during that period, consuming a large proportion
412 of available nutrients, notably nitrate and nitrite, before the start of the experimental phase
413 (see 3.1). In all mesocosms, after this initial peak, chlorophyll *a* concentrations linearly
414 decreased until the end (GLM, $r^2 = 0.8$, $p < 0.001$).

415 When pigment data for all mesocosms were pooled together (Fig. 3), the plankton
416 community in the Bay of Villefranche was dominated at the start of the experiment by
417 cryptophyceae representing $26 \pm 1\%$ of the chlorophyll *a* content and by haptophyceae at the
418 end ($32 \pm 5\%$). Following total chlorophyll *a* dynamics, almost all groups declined in terms of
419 chlorophyll *a* equivalent biomass during the 12-day experiment except for cyanophyceae

420 whose biomass almost doubled between days 0 and 12 (Fig. 6). Groups that increased during
421 the acidification phase and consumed available nitrate and nitrite belonged to cryptophyceae,
422 haptophyceae, pelagophyceae and cyanophyceae. While pelagophyceae biomass remained
423 constant throughout the experiment, cryptophyceae biomass linearly declined and
424 haptophyceae showed maximal biomass on days 2 and 4 and then slightly declined. Several
425 groups did not follow the initial chlorophyll *a* increase during the acidification phase
426 (diatoms, dinophyceae, prasinophyceae and chlorophyceae).

427 Consistently with pigment data, flow cytometry data showed that *Synechococcus*
428 abundances significantly increased during the acidification phase and reached values much
429 above environmental (external) levels (Fig. 7). After few days of stagnation (days 0 to 6),
430 abundances further increased to maximal values on day 12 (averaged between mesocosms of
431 42600 ± 3000 cells mL⁻¹). In contrast, it appears that *Prochlorococcus* took less advantage of
432 this initial acidification phase with abundances on day 0 similar to external levels. After a
433 small initial decline, abundances increased during the entire experiment with increasing
434 variability between mesocosms. While autotrophic nano-eukaryotes abundance increased
435 before the start of the experiment to levels much higher on day 0 than in the surrounding
436 waters, no difference could be observed for autotrophic pico-eukaryotes on day 0 between
437 mesocosms and the surrounding waters. Autotrophic pico-eukaryote abundance decreased
438 until day 5, with very low variability between mesocosms, and increased until the end of the
439 experiment with much larger discrepancy between mesocosms. In contrast, abundances of
440 autotrophic nano-eukaryotes decreased almost linearly between day 0 and 12 with a large
441 inter-mesocosm variability throughout the experiment.

442 Table 4 shows that the chlorophyll *a*-equivalent biomass of haptophyceae and diatoms
443 were significantly correlated with *p*CO₂, although for none of these species *p*CO₂ appeared as
444 the most important contributor to the variance. While haptophyceae appeared negatively

445 correlated to $p\text{CO}_2$, diatoms were positively correlated to this variable. Note that for these two
446 groups, less than half of the variance could be explained by these multiple regressions. For
447 most of the tested variables, salinity appeared as the most important co-variable, being either
448 positively or negatively correlated to them. As already mentioned, salinity increased gradually
449 during the experiment and these correlations most likely reflect a time effect on these
450 variables.

451 **3.4. Particulate organic matter and export**

452 In the Bay of Calvi, particulate C and N concentrations were very low and close to the
453 analyzer detection limit (respectively, 2.9 - 7.4 $\mu\text{mol C L}^{-1}$ and 0.4 - 1.3 $\mu\text{mol N L}^{-1}$). C:N
454 ratio of the particulate organic matter remained constant in the mesocosms throughout the
455 experiment (7.0 ± 1.0) and very close to ambient conditions (7.1 ± 0.7 ; Fig. 2). In the Bay of
456 Villefranche, higher POC and PON concentrations were measured (respectively, 7.9 - 20.2
457 $\mu\text{mol C L}^{-1}$ and 1.0 - 2.3 $\mu\text{mol N L}^{-1}$). As in the Bay of Calvi, C:N ratio of the particulate
458 organic matter remained constant in the mesocosms throughout the experiment (7.5 ± 0.9) and
459 lower than in the surrounding environment (12.9 ± 4.3 ; Fig. 2). During both experiments,
460 none of the measured variables (POC, PON or POC:PON) displayed any observable
461 dependence on seawater acidification (Table 4).

462 Particulate organic carbon and nitrogen export fluxes are presented in Fig. 8. During
463 both experiments, although more visible in the Bay of Calvi, organic matter export rates were
464 maximal at the start of the experiments and gradually decreased until the end of the
465 experiments. Much more variability was observed in the Bay of Villefranche with higher
466 exported quantities of organic matter. In the Bay of Calvi, exported C:N ratios were generally
467 homogeneous between mesocosms at the start of the experiment and much more variability
468 was observed towards the end. Stepwise linear regressions showed no $p\text{CO}_2$ effects on these
469 export fluxes (Table 4).

470 **4. Discussion**

471 The overall objective of our study was to evaluate the response of the phytoplankton
472 community, particulate organic matter dynamics and export to $p\text{CO}_2$ changes in the NW
473 Mediterranean Sea under contrasted physico-chemical (e.g. hydrology, nutrients and
474 irradiance) and biological conditions (assemblage composition and abundance).
475 Unfortunately, both summer and winter experiments were conducted under nutrient limiting
476 conditions on plankton communities dominated by small species.

477 The summer experiment in the Bay of Calvi was conducted under typical stratified
478 summer conditions characterized by very low nutrient and chlorophyll concentrations and
479 surface irradiance levels of $\sim 1,400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, corresponding to maximal yearly
480 values in that area (data not shown). With respect to nutrient availability, as fully discussed by
481 (Louis et al., in press, this issue), observed NO_x and phosphate depleted conditions during the
482 experiment are in the range of usually observed values in the oligotrophic Mediterranean Sea
483 in summer. At the start of the experiment, inorganic N:P ratio was 1.7 and increased up to ~ 4
484 in the mesocosms on day 20. Both a low N:P ratio and low nutrient concentrations suggest
485 that this system experienced N and P co-limitation during this period (Louis et al., in press,
486 this issue). During this experiment, the plankton community was clearly dominated by small
487 phytoplankton cells such as haptophyceae, cyanobacteria and chlorophyceae. Similar
488 conditions were reported in this area at this period of the year. Using the same mesocosm
489 setup, Giovagnetti et al. (2013) showed that the summer plankton community was dominated
490 by pico-phytoplankton, representing $\sim 70\%$ of total biomass and composed mostly of
491 haptophyceae and cyanobacteria. The same experiment showed that nano- and micro-
492 phytoplankton ($\sim 30\%$ of total biomass) were composed of haptophyceae, chlorophyceae and
493 dinoflagellates. During our experiment, phytoplankton biomass decreased during the

494 acidification phase in all mesocosms, independently of $p\text{CO}_2$ conditions, as shown by
495 fluorometric data acquired using daily CTD profiles (Gazeau et al., in press, this issue). This
496 corresponded to important organic matter sedimentation at the start of the experiment (first
497 few days) that further stabilized at low rates until the end of the experiment. No important
498 changes in the proportions of the different groups investigated were observed, at the exception
499 of chlorophyceae (see above)

500 At the end of our experiment and considering the averaged composition in all nine
501 mesocosms, dominance shifted towards chlorophyceae, in contrast to the external water
502 community which remained unchanged during the course of the experiment. This relative
503 overgrowth of chlorophyceae in all mesocosms, independently of $p\text{CO}_2$ conditions, was
504 potentially due to wall growth. Indeed, a strong wind and wave event prevented sampling on
505 day 19 (Gazeau et al., in press, this issue). On day 20 (our final sampling day), concentrations
506 in chlorophyceae (but also diatoms) increased significantly (+ 30%). This observed increase
507 was likely due to mesocosm shaking from wave actions, that released periphyton (i.e. species
508 growing on the wall of the mesocosms) in the water column. Large species such as diatoms
509 represented less than ~10% of phytoplankton biomass by the end of the experiment, although
510 biomasses were usually above those in external waters (~5 vs. < 1 ng L⁻¹). Obviously, this
511 does not appear as a surprise, as it is well known that, during the summer stratified period,
512 diatoms are outcompeted by small species, better adapted to low nutrient and high irradiance
513 levels, and usually do not represent more than 10% of the phytoplankton biomass in surface
514 waters of the Ligurian Sea (Navarro et al., 2014).

515 The winter experiment conducted in the Bay of Villefranche was carried out in order
516 to test for CO_2 enrichment effects on a Mediterranean plankton community not limited by
517 nutrient availability. However, as a consequence of very favorable weather conditions during
518 a short time window, much of the temporal dynamics observed during the experiment was

519 concentrated during the first few days before the end of the acidification process and nutrients
520 were rapidly consumed in the mesocosms. At the start of the experiment, when targeted $p\text{CO}_2$
521 levels were reached, most of the available NO_x was already consumed and irradiance
522 conditions dropped significantly (Gazeau et al., in press, this issue) precluding the formation
523 of a real bloom in the bags. Addition of nutrients would have then been necessary to activate
524 plankton dynamics in the mesocosms but this strategy was not possible as we have been
525 forced to end the experiment after 12 days as a consequence of very bad weather conditions.
526 In the Bay, as a consequence of intense vertical mixing, chlorophyll concentrations have been
527 maintained at a lower level while nutrients have been continuously replenished (Louis et al.,
528 in press, this issue). In addition to probably not reflecting properly light conditions (Gazeau et
529 al., in press, this issue), the isolation of a water mass and the reduction of mixing certainly
530 does appear as a pitfall of this mesocosm approach or of any incubation system in these
531 ecosystems which dynamics is strongly linked to physico-chemical conditions (e.g. mixing,
532 irradiance). As such, results obtained during this experiment must be taken with extreme
533 caution because of conditions inside the mesocosms not fully reflecting winter conditions in
534 this area. Nevertheless, in winter in the Bay of Villefranche, phytoplankton biomass was
535 much higher than in summer in the Bay of Calvi with values around $1 \mu\text{g L}^{-1}$ and a clear
536 dominance of small species such as haptophyceae, cryptophyceae and pelagophyceae ($> 65\%$
537 of chlorophyll *a*-equivalent biomass). Previous observations at the entrance of the Bay of
538 Villefranche have shown that the spring phytoplankton bloom usually takes place in
539 February-March and is dominated by pico-nano-phytoplankton (Thyssen et al., 2014).
540 Although not always observed, this first bloom is followed by a second one in May that is
541 dominated by diatoms and large dinoflagellates (Bustillos-Guzmán et al., 1995; Gomez and
542 Gorsky, 2003). During this year 2013, the highest annual chlorophyll *a* concentrations were
543 reached later than observed in previous years (Gazeau et al., in press, this issue). Our

544 experiment therefore coincided with pre-bloom conditions although, again, nutrients were
545 rapidly consumed in the mesocosms. Community composition did not drastically change
546 during the course of the experiment inside the mesocosms but small phytoplankton species
547 took advantage of the first few days of the experiment and are likely responsible for the strong
548 consumption of NO_x during the acidification phase (Louis et al., in press, this issue). In
549 contrast, diatoms and dinoflagellates did not take advantage of the closing of the bags and of
550 favorable weather conditions during these first few days and continuously decreased in
551 abundance until the end of the experiment. This is not a surprise since these species are
552 known to be outcompeted by smaller phytoplankton cells when nutrient limitation is
553 temporally relieved after the winter vertical mixing (Bustillos-Guzmán et al., 1995). Instead
554 of these species, autotrophic prokaryotes, especially *Synechococcus*, appeared to benefit from
555 the closing of the bags, as their abundance was 3-fold higher than the ambient levels and kept
556 increasing throughout the experiment. While autotrophic nano-eukaryotes decreased in
557 abundance after the initial chlorophyll *a* increase during the acidification phase, autotrophic
558 pico-eukaryotes benefited from the recycled nutrient pool, as a consequence of increasing
559 bacterial abundance (Celussi et al., in press, this issue), and increased in number during the
560 second part of the experiment.

561 During these two experiments, while total chlorophyll *a* concentrations appeared
562 correlated to environmental conditions (e.g. nutrients, irradiance, salinity) and/or with time,
563 no significant correlations were found with $p\text{CO}_2$. Similarly, we could not evidence any
564 relationship between $p\text{CO}_2$ and POC or PON concentrations as well as organic carbon and
565 nitrogen export to the sediment traps. When phytoplankton groups were analysed separately,
566 positive effects were found for haptophyceae and autotrophic dinoflagellates in the Bay of
567 Calvi during the oligotrophic summer period, similarly to what was found during a large *in*
568 *situ* mesocosm experiment in the Arctic (Schulz et al., 2013). In winter in the Bay of

569 Villefranche, while haptophyceae were negatively correlated with $p\text{CO}_2$, diatoms appeared
570 positively impacted, although for these two groups it must be stressed that CO_2 was not the
571 first parameter driving their variance. Such positive CO_2 -effects as observed in summer on
572 haptophyceae and autotrophic dinoflagellates are not surprising, as these species do not
573 possess very efficient CCMs (Reinfelder, 2011). Although cyanobacteria (including
574 *Synechococcus*) appeared to benefit from our experimental conditions and from the very
575 limited amount of nutrients, they were not impacted by CO_2 -enrichment. These results are
576 consistent to what was observed by Lomas et al. (2012) in the subtropical North Atlantic but
577 stand in contrast to the negative impact of ocean acidification on *Synechococcus* abundance
578 observed by Paulino et al. (2008) in a North Sea Fjord under very different trophic and
579 experimental conditions compared to our experiments (i.e. higher chlorophyll levels as well as
580 and enrichment with N and P). As suggested by Lomas et al. (2012), the response of
581 cyanobacteria might be indirect and controlled by other variables such as nutrients.

582 All in all, the short-term addition of CO_2 in our nutrient-limited systems did not
583 induce any clear effect on community composition based on pigment analysis. It must be
584 stressed that these analyses do not allow detecting potential modifications/replacements at the
585 specific or at the intra-specific level as suggested by several studies in the recent years
586 (Brading et al., 2011; Rickaby et al., 2016). Nevertheless, scanning electron microscopy
587 analyses reported by (Oviedo et al., in press, this issue) did not highlight any changes in
588 coccolithophores and siliceous phytoplankton community compositions, and especially any
589 changes in species size that could have an impact on sedimentation rates (Feng et al., 2010;
590 Tortell et al., 2008; Wu et al., 2014). During our experiments, no phylogenetic studies have
591 been conducted at the exception of diazotrophs during the summer in the Bay of Calvi (Rees
592 et al., in press, this issue). For this group, no significant changes could be evidenced. In the
593 present study, the small positive or negative effects that have been highlighted on selected

594 groups based on pigment analyses appear to be minimal and did not lead to significant
595 changes in terms of community metabolism (Maugendre et al., in press, this issue-b), bacterial
596 production (Celussi et al., in press, this issue), carbon transfer (Maugendre et al., in press, this
597 issue-a) as well as carbon and nitrogen export (this study).

598 These results clearly stand in contrast to recent experiments conducted in a coastal
599 site in the Western Mediterranean Sea, using indoor tanks (Sala et al., 2016). Similar to our
600 planned experimental protocol, two experiments were conducted under contrasting
601 conditions: winter, at the peak of the annual phytoplankton bloom, and summer, under low
602 nutrient conditions. Their results suggested microbial communities will be considerably more
603 affected by ocean acidification under oligotrophic conditions than in more productive waters.
604 It must be stressed that even during their summer low-nutrient experiment, reported nitrate
605 concentrations were almost ten times higher than concentrations observed in summer in the
606 Bay of Calvi and four times higher than observed in the Bay of Villefranche in the
607 mesocosms at the end of the acidification period. Similarly, chlorophyll concentrations during
608 our summer experiment were three times lower than observed by Sala et al. (2016) in summer
609 in the Bay of Blanes. Recently, two other experiments conducted using large *in situ*
610 mesocosms also suggested that communities in nutrient-limited areas may be more responsive
611 to changing carbonate chemistry than those having access to high inorganic nutrient
612 concentrations (Bach et al., 2016; Paul et al., 2015a). These two experiments, sharing a
613 similar experimental protocol than in the present study, were conducted over significantly
614 longer time scales (> 43 days). During both experiments, impacts of elevated CO₂ were
615 visible during the last phase when plankton communities were relying on remineralized
616 nutrients. As both our experiments did not exceed ~20 days, the build-up of remineralized
617 nutrients did not reach concentrations high enough to significantly relieve the nutrient
618 limitation. Nutrient limitation can be episodically relieved in summer through atmospheric

619 inputs (The Mermex group, 2011 and references therein) and it is now well known that pulsed
620 atmospheric nutrient inputs enhance phototrophic, heterotrophic and diazotrophic activities
621 (Guieu et al., 2014). It appears therefore of the utmost importance to target future
622 experimental efforts on the response of summer plankton communities to ocean acidification
623 in the case of a transient relieve in nutrient limitation through of a dust deposition event.

624 **5. Conclusion**

625 To conclude, for the first time, short-term *in situ* pelagic mesocosm experiments have
626 been conducted in LNLC areas of the Northwestern Mediterranean Sea to assess the response
627 of phytoplankton communities to ocean acidification. In contrast to most previous mesocosm
628 experiments, no nutrient addition took place during the experiments conducted in summer and
629 winter. The summer plankton community was dominated by pico-phytoplankton and
630 cyanobacteria and was strongly limited by NO_x and phosphate availability. Although,
631 haptophyceae and autotrophic dinoflagellates appeared to be favored by increased CO₂
632 availability during this short-term experiment, this benefit remained very minimal with no
633 impact on carbon export, as a consequence of strong environmental constraints. The winter
634 community was also dominated by small species (especially haptophyceae and
635 cryptophyceae) that reacted soon after closing the bags and during the acidification period,
636 possibly due to favorable weather conditions and irradiance levels. During this experiment, no
637 signs of short term CO₂ addition dependency were detected on plankton community structure
638 based on pigment analyses and on organic matter export. As a consequence of the very
639 dynamic nature of environmental conditions and therefore of plankton biomass and
640 composition in the Mediterranean Sea, more investigations are needed to carefully assess the
641 response of plankton communities in winter when vertical mixing and weather conditions are
642 major factors controlling plankton dynamics in this area. Future experimental protocols might

643 consider maintaining nutrient and chlorophyll levels as close as possible to ambient
644 conditions over longer time scales. Although this might be experimentally challenging, we
645 believe this is the only way to investigate these very dynamic communities. Finally, as
646 atmospheric depositions in summer have the capacity to relieve nutrient limitations and to
647 enhance plankton productions, there is a great need to perform future experiments considering
648 these pulsed nutrient additions.

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950 **Figure legends**

951 Figure 1. Carbonate chemistry conditions in the nine mesocosms and in the external
952 environment (OUT) during the experiment in the Bay of Calvi in summer 2012 (left
953 panels) and in the Bay of Villefranche in winter 2013 (right panels). Partial pressure of
954 CO_2 ($p\text{CO}_2$, upper panels) and pH on the total scale (pH_T , lower panels) were calculated
955 using seacarb, based on dissolved inorganic carbon concentrations (C_T , not shown) and
956 total alkalinity (A_T , not shown), measured daily from depth-integrated (0-10 m)
957 samples. Vertical dotted lines show the start of the respective experiments (day 0).

958 Figure 2. Depth-integrated (0-10 m) chlorophyll *a* concentrations as measured by high
959 performance liquid chromatography (HPLC; upper panels) and particulate organic
960 carbon (POC) to particulate organic nitrogen (PON) ratio (POC:PON; lower panels) in
961 the nine mesocosms and in the external environment during the experiment in the Bay
962 of Calvi in summer 2012 (left panels) and in the Bay of Villefranche in winter 2013
963 (right panels). Vertical dotted lines on the right panels show the start of the experiment
964 (day 0). No chlorophyll *a* and POC data are available before day 0 in the Bay of Calvi.

965 Figure 3. Averaged contribution (%) between all nine mesocosms of the main
966 phytoplanktonic taxonomic groups to total chlorophyll *a* concentrations at the start (day
967 0) and at the end (day 20 or 12) of the experiments in the Bay of Calvi in summer 2012
968 (left panel) and in the Bay of Villefranche in winter 2013 (right panel).

969 Figure 4. Temporal evolution of chlorophyll *a* (chl *a*) -equivalent concentrations of eight
970 taxonomic groups of phytoplankton during the experiment in the Bay of Calvi in
971 summer 2012, in the nine mesocosms and in the external environment (OUT). Prasino:
972 prasinophyceae, Dino: dinophyceae, Crypto: cryptophyceae, Hapto: haptophyceae,
973 Pelago: pelagophyceae, Chloro: chlorophyceae, Cyano: cyanophyceae.

974 Figure 5. Temporal evolution of *Synechococcus* and pico-eukaryotes abundances as measured
975 by flow cytometry, during the experiment in the Bay of Calvi in summer 2012, in the
976 nine mesocosms.

977 Figure 6. Temporal evolution of chlorophyll *a* (chl *a*) -equivalent concentrations of 8
978 taxonomic groups of phytoplankton determined from high performance liquid
979 chromatography (HPLC) measurements using modified CHEMTAX, during the
980 experiment in the Bay of Villefranche in winter 2013, in the 9 mesocosms and in the
981 external environment (OUT). Prasino: prasinophyceae, Dino: dinophyceae, Crypto:
982 cryptophyceae, Hapto: haptophyceae, Pelago: pelagophyceae, Chloro: chlorophyceae,
983 Cyano: cyanophyceae.

984 Figure 7. Temporal evolution of *Synechococcus*, *Prochlorococcus*, pico-eukaryotes and nano-
985 eukaryotes abundances as measured by flow cytometry, during the experiment in the
986 Bay of Villefranche in winter 2013, in the nine mesocosms and in the external
987 environment (OUT).

988 Figure 8. Upper panel: temporal evolution of particulate organic carbon (POC) fluxes to the
989 sediment traps during the experiment in the Bay of Calvi in summer 2012 (left panel)
990 and in the Bay of Villefranche in winter 2013 (right panel). Lower panel: particulate
991 organic carbon (POC) to particulate organic nitrogen (PON) ratio in the sediment traps
992 during the experiment in the Bay of Calvi in summer 2012. No PON data available
993 during the experiment in the Bay of Villefranche in winter 2013. Vertical dotted lines
994 show the start of the respective experiment (day 0).

995 Table 1. Summary of past mesocosm (volume between 1 and 1000 m³) ocean acidification experiments results on phytoplankton communities. ⇔, ↑ and
 996 ↓ refer to neutral, positive and negative effects on chlorophyll *a* concentrations (Chl *a*) as well as concentrations of diatoms (diat), dinophyceae (Dino),
 997 nano-eukaryotes (Nano), pico-eukaryotes (Pico) and cyanophyceae (Cyano). Impacts on carbon export are also reported when available. “√” indicates that
 998 mesocosms were enriched with nutrients (Nut: nitrate, phosphate and sometimes silicate). “-” indicates that no information is available. Cryp and Chlo
 999 refer to cryptophyceae and chlorophyceae respectively.

Reference	Study location	Season	Nut	Major group	Chl	Diat	Dino	Nano	Pico	Cyano	Export	Notes
Indoor												
Sommer et al. (2015) Paul et al. (2015b)	Kiel Bight	Fall		Diat/Dino	⇔	↓	⇔	⇔	⇔	⇔	-	
Outdoor - Floating raft												
Engel et al. (2005)	Norwegian Fjord	Spring	√	Pico/Cyano	⇔	-	-	⇔	⇔	⇔	-	Decrease of coccolithophore calcification
Kim et al. (2006)	Korean coast	Fall		Micro/Diat	-	⇔	-	-	-	-	-	
Engel et al. (2008)	Norwegian Fjord	Spring	√	Pico	⇔	⇔	-	↓	↑	-	-	
Paulino et al. (2008) Schulz et al. (2008)	Norwegian Fjord	Spring	√	-	⇔	⇔	⇔	⇔	↑	↓	-	
Hopkins et al. (2010) Meakin and Wyman (2011) Newbold et al. (2012)	Norwegian Fjord	Spring	√	Micro	↓	-	-	↑	↑	↑	-	Increase in large pico-eukaryotes
Kim et al. (2010) Kim et al. (2011) Kim et al. (2013)	Korean coast	Fall	√	Diat/Dino	⇔	↑	⇔	↑	⇔	-	-	Shift from weakly to heavily silified diatoms
Calbet et al. (2014)	Norwegian Fjord	Spring	√	Nano	⇔	↑	↓	↑	↑	-	-	
Outdoor - Free floating												

Schulz et al. (2013)				Nano	↔	↔	↔	↔	↔	↔	↔	Phase 1 before nutrient enrichment	
Brussaard et al. (2013)	Arctic Fjord	Spring	√	-	↑	↔	↑	↑	↑	↔	↔	Phase 2	
Czerny et al. (2013)				-	↓	↔	↑	↔	↑	↔	↔	↓	Phase 3
Paul et al. (2015a)				Baltic Sea	Spring	Cryp	↔	↔	-	↔	↑	↔	↔
	Chlo	↑	↔			-	↔	↑	↔	↔	↔	Phase 2	
	Chlo	↑	↓			-	↓	↑	↔	↔	↔	Phase 3	
Bach et al. (2016)	Swedish Fjord	Winter		-	-	↔	↔	↔	↔	↔	-		
		Spring		Diatoms	-	↔	↔	↔	↔	↔	↔	-	First chlorophyll build-up
		Spring		Diatoms	-	↔	↔	↔	↑	↔	↔	-	Second chlorophyll build-up on remineralized nutrients
		Spring		-	-	↔	↔	↔	↔	↔	↔	-	

1000 Table 2. Environmental and experimental conditions in the nine mesocosms and in the external environment (OUT) during the experiment in the
 1001 Bay of Calvi in summer 2012. Levels of temperature (T in °C), salinity (S), partial pressure of CO₂ (*p*CO₂ in µatm), nitrate + nitrite (NO_x in nmol
 1002 L⁻¹) and phosphate (PO₄³⁻ in nmol L⁻¹), ammonium (NH₄⁺ in nmol L⁻¹) and silicate (Si in µmol L⁻¹) at the end of the acidification period (day 0)
 1003 and at the end of the experiment (day 20) are reported. NO₃⁻ and PO₄³⁻ data from Louis et al. (in press, this issue). NH₄⁺ and Si data are from
 1004 Gazeau et al. (in press, this issue). NA: not available.

	Day 0							Day 20						
	T	S	<i>p</i> CO ₂	NO _x	PO ₄ ³⁻	NH ₄ ⁺	Si	T	S	<i>p</i> CO ₂	NO _x	PO ₄ ³⁻	NH ₄ ⁺	Si
OUT	22.2	38.0	442	50	35	150	1.9	24.3	38.2	489	NA	NA	660	1.8
C1	22.1	38.0	455	59	NA	450	NA	NA	NA	456	77	4	190	1.1
C2	22.1	38.0	447	53	25	550	NA	24.2	38.2	472	61	6	230	1.4
C3	22.1	38.0	444	69	21	210	NA	24.2	38.1	473	59	7	210	1.3
P1	22.2	38.0	583	NA	NA	330	NA	24.3	38.2	544	45	6	130	1.3
P2	22.1	38.0	698	37	23	400	NA	24.3	38.2	609	41	4	290	1.4
P3	22.1	38.0	753	36	20	225	1.7	24.2	38.2	655	42	10	100	1.3
P4	22.1	38.0	875	30	19	770	1.7	24.3	38.2	764	75	8	230	1.2
P5	22.1	38.0	1134	37	31	260	1.7	24.3	38.1	754	76	9	350	1.3
P6	22.1	38.0	1279	57	NA	130	1.7	24.2	38.2	738	61	8	180	1.4

1005 Table 3. Environmental and experimental conditions in the nine mesocosms and in the external environment (OUT) during the experiment in the Bay of
 1006 Villefranche in winter 2013. Levels of temperature (T in °C), salinity (S), partial pressure of CO₂ (*p*CO₂ in µatm), nitrate (NO₃⁻ in nmol L⁻¹), phosphate
 1007 (PO₄³⁻ in nmol L⁻¹), ammonium (NH₄⁺ in nmol L⁻¹) and silicate (Si in µmol L⁻¹) at the end of the acidification period (day 0) and at the end of the
 1008 experiment (day 12) are reported. *No data are available for day 12 therefore levels on day 11 are reported. NO₃⁻ and PO₄³⁻ data from Louis et al. (in
 1009 press, this issue). NH₄⁺ and Si data are from Gazeau et al. (in press, this issue). NA: not available.

	Day 0							Day 12						
	T	S	<i>p</i> CO ₂	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺	Si	T	S	<i>p</i> CO ₂	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺	Si
OUT	13.2	38.1	354	1166	10	62	1.3	13.2	38.2	391	1307	12	40	1.2
C1	13.2	38.1	378	167	10	79	NA	13.2	38.2	388	394	9	49	1.0
C2	13.2	38.1	347	118	12	57	1.1	13.2	38.2	354	194	11	31	1.1
C3	13.2	38.1	350	110	9	81	1.2	NA	NA	376	127	10	26	1.2
P1	13.2	38.1	494	135	10	73	NA	13.2	38.2	429	491	10	68	1.1
P2	13.2	38.1	622	133	9	64	1.2	13.2	38.2	413*	NA	10	NA	NA
P3	13.2	38.1	691	NA	9	64	1.2	NA	NA	451	236	NA	26	1.2
P4	13.2	38.1	744	72	12	80	NA	13.2	38.2	436	491	9	36	0.9
P5	13.2	38.1	932	134	15	60	1.2	13.2	38.2	497	226	10	30	1.1
P6	13.2	38.1	1250	156	8	60	1.1	NA	NA	579*	NA	NA	NA	NA

1010 Table 4. Stepwise multiple regression analysis between environmental/experimental variables (T: temperature, S: salinity, $p\text{CO}_2$: partial pressure of CO_2 ,
 1011 NO_3^- : nitrate concentrations, PO_4^{3-} : phosphate concentrations, NH_4^+ : ammonium concentrations, Si: silicate concentrations (only for diatoms), I: daily
 1012 integrated photon doses and w: daily averaged wind speeds) and total chlorophyll *a* (chl *a*) concentrations or chlorophyll *a*-equivalent concentrations of
 1013 eight taxonomic groups of phytoplankton determined from high performance liquid chromatography (HPLC) measurements using modified CHEMTAX
 1014 or *Synechococcus*, *Prochlorococcus*, pico-eukaryotes and nano-eukaryotes abundances as measured by flow cytometry, during the experiment in the Bay
 1015 of Calvi in summer 2012 and in the Bay of Villefranche in winter 2013. Note that *Prochlorococcus* and nano-eukaryotes abundances are not available for
 1016 the Bay of Calvi and that PON fluxes are not available for the Bay of Villefranche (denoted as NA). Bold text denotes significant correlations ($p < 0.01$)
 1017 between the considered variable and $p\text{CO}_2$ and the sign (+ or -) refers to the sign of the relationship between the considered variable and the
 1018 environmental/experimental parameters considered. NS: not significant.

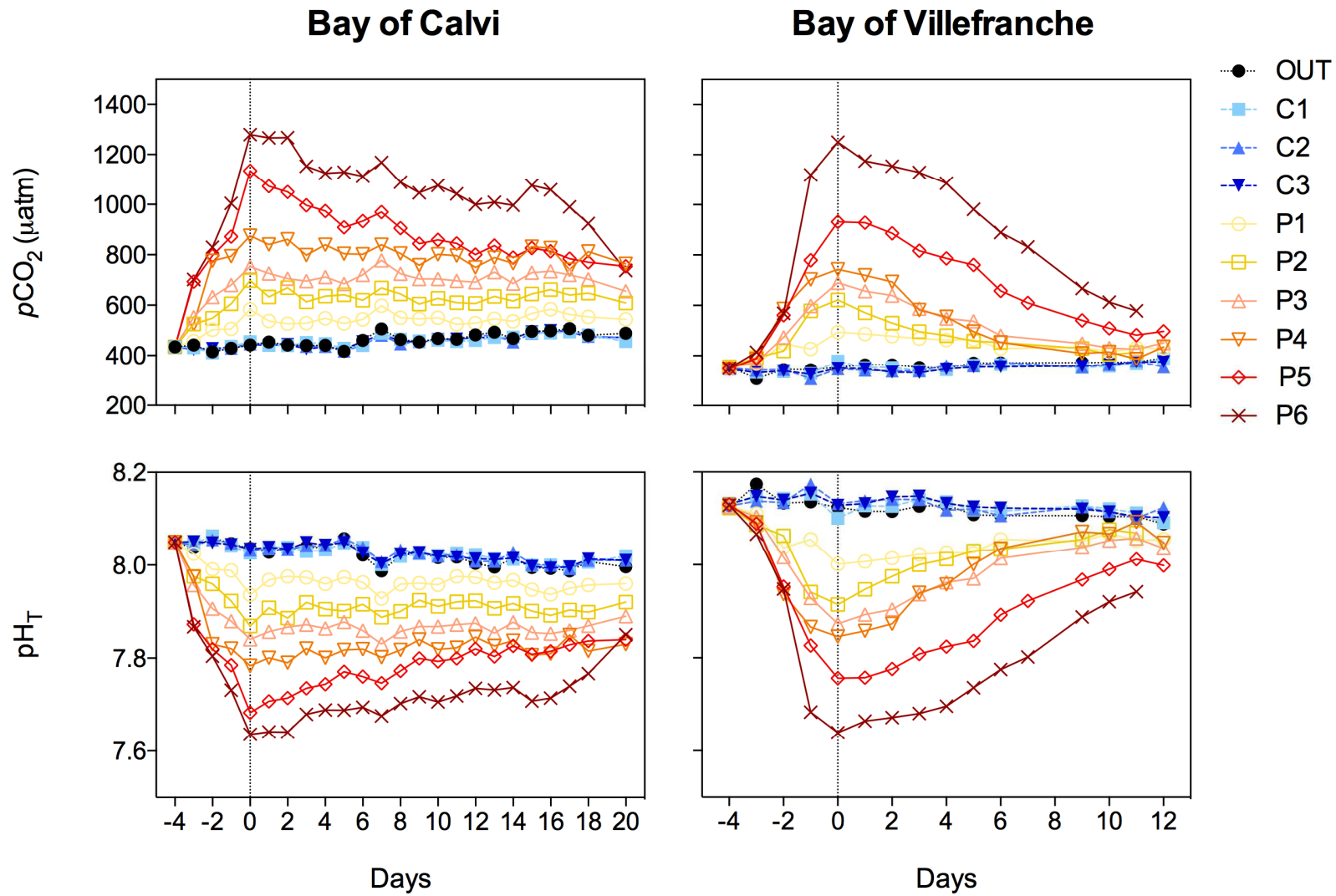
Bay of Calvi	F	Adj. r^2	df	Overall p	Variable	Sign	p	Bay of Villefranche	F	Adj. r^2	df	Overall p	Variable	Sign	p
Particulate matter								Particulate matter							
POC	NS							POC	34.5	0.57	97	< 0.001	NO_3^-	-	< 0.001
													S	-	< 0.001
PON	7.0	0.06	177		I	-	< 0.001	PON	17.3	0.32	101	< 0.001	NO_3^-	-	< 0.001
					S	-	0.003								
POC:PON	5.2	0.09	174	< 0.001	T	+	< 0.001	POC:PON	9.5	0.14	99	< 0.001	NH_4^+	+	0.006
Pigments								Pigments							

Total chl <i>a</i>	27.2	0.45	156	< 0.001	S NH ₄ ⁺	+ +	< 0.001 0.006
Prasinophyceae	13.8	0.24	157	< 0.001	NO ₃ ⁻ S I	+ + +	< 0.001 < 0.001 < 0.001
Dinophyceae	19.5	0.36	156	< 0.001	<i>p</i> CO ₂ I S NO ₃ ⁻	+ + + -	< 0.001 < 0.001 < 0.001 0.001
Cryptophyceae	32.3	0.49	156	< 0.001	NO ₃ ⁻ T I	+ - +	< 0.001 0.001 0.01
Haptophyceae	11.4	0.28	155	< 0.001	<i>p</i> CO ₂ I NO ₃ ⁻	+ + +	< 0.001 < 0.001 0.002
Pelagophyceae	19.4	0.48	153	< 0.001	S I T PO ₄ ³⁻	+ + + +	< 0.001 < 0.001 0.001 0.005
Chlorophyceae	104	0.66	158	< 0.001	S T	+ +	< 0.001 0.002

Total chl <i>a</i>	22.5	0.51	100	< 0.001	I S NO ₃ ⁻	- - -	< 0.001 < 0.001 < 0.001
Prasinophyceae	18.7	0.50	99	< 0.001	T NO ₃ ⁻ NH ₄ ⁺	+ + +	< 0.001 < 0.001 < 0.001
Dinophyceae	14.5	0.39	100	< 0.001	S w	- -	< 0.001 < 0.001
Cryptophyceae	58.5	0.77	99	< 0.001	S NO ₃ ⁻ T	- - -	< 0.001 < 0.001 < 0.001
Haptophyceae	20.3	0.48	100	< 0.001	T NO ₃ ⁻ <i>p</i> CO ₂	- - -	< 0.001 < 0.001 < 0.001
Pelagophyceae	10.6	0.27	101	< 0.001	NO ₃ ⁻	-	< 0.001
Chlorophyceae	12.2	0.39	99	< 0.001	NO ₃ ⁻ S T I	+ - + -	0.001 0.004 0.004 0.005

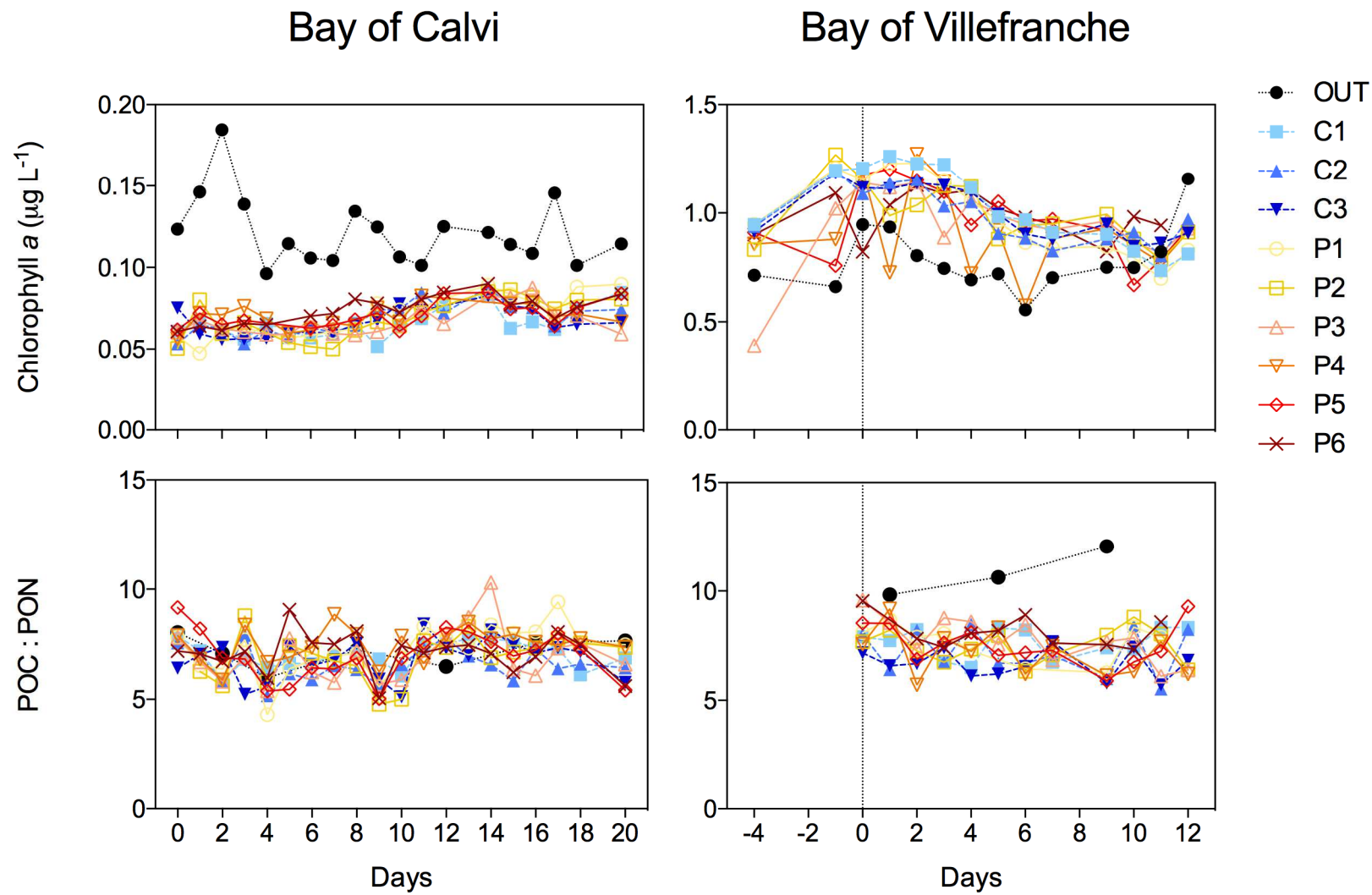
Cyanophyceae	15.6	0.27	157	< 0.001	NO ₃ ⁻	-	< 0.001	Cyanophyceae	132	0.83	101	< 0.001	S	+	< 0.001
					PO ₄ ³⁻	-	< 0.001						T	+	< 0.001
													NO ₃ ⁻	+	< 0.001
Diatoms	12	0.29	155	< 0.001	I	-	< 0.001	Diatoms	40	0.45	100	< 0.001	S	-	< 0.001
					T	-	< 0.001						pCO ₂	+	< 0.001
					PO ₄ ³⁻	-	< 0.001						NH ₄ ⁺	+	< 0.001
													I	-	0.006
Flow cytometry								Flow cytometry							
<i>Prochlorococcus</i>	NA							<i>Prochlorococcus</i>	17.8	0.49	66	< 0.001	S	+	< 0.001
													T	+	< 0.001
<i>Synechococcus</i>	18.7	0.44	85	< 0.001	NO ₃ ⁻	-	< 0.001	<i>Synechococcus</i>	98.2	0.89	64	< 0.001	S	+	< 0.001
					I	-	< 0.001						T	+	< 0.001
													NO ₃ ⁻	+	< 0.001
													NH ₄ ⁺	-	0.003
Pico-eukaryotes	12.8	0.40	84	< 0.001	NO ₃ ⁻	-	< 0.001	Pico-eukaryotes	22.8	0.61	65	< 0.001	T	+	< 0.001
													w	+	< 0.001
													S	-	< 0.001
													NO ₃ ⁻	+	< 0.001
													NH ₄ ⁺	+	0.005
Nano-eukaryotes	NA							Nano-eukaryotes	27.4	0.65	65	< 0.001	S	-	< 0.001
													NO ₃ ⁻	-	< 0.001
													w	+	< 0.001
													NH ₄ ⁺	+	0.006
Particle flux								Particle flux							

POC	33.7	0.63	74	< 0.001	T	-	< 0.001	POC	NS
PON	34.6	0.63	74	< 0.001	T NO ₃ ⁻	- +	< 0.001 0.009	PON	NA
POC:PON	22.9	0.46	75	< 0.001	S	+	< 0.001	POC:PON	NA



1019

1020 Fig. 1

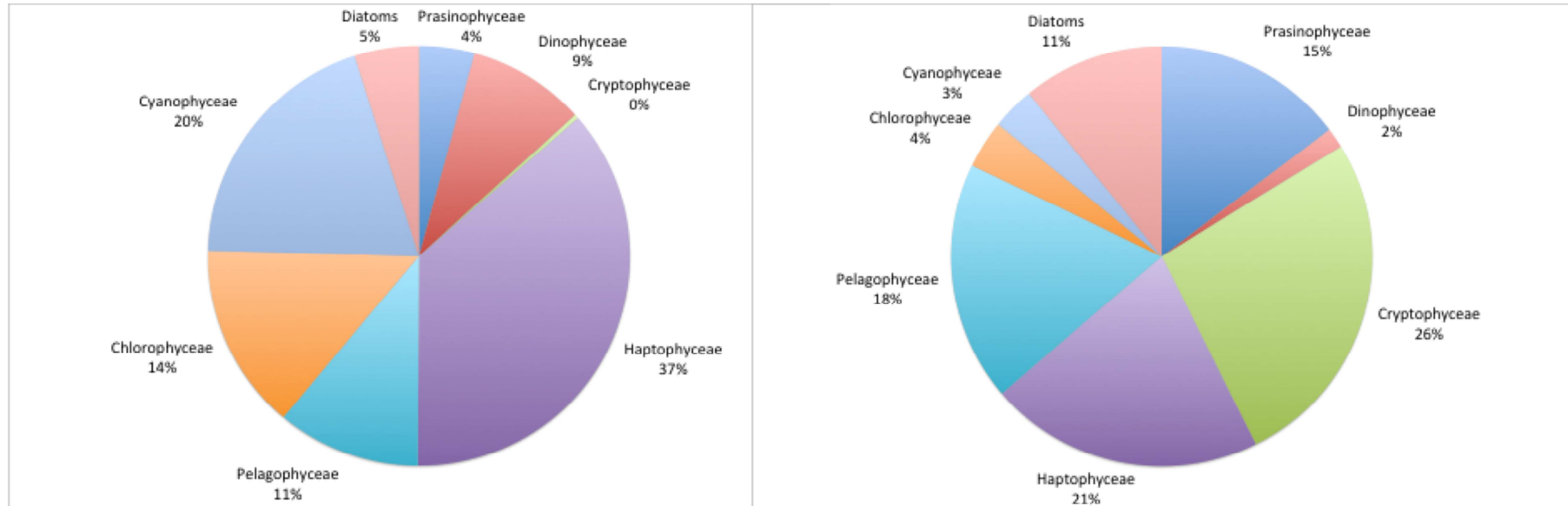


1021

1022 Fig. 2

Bay of Calvi

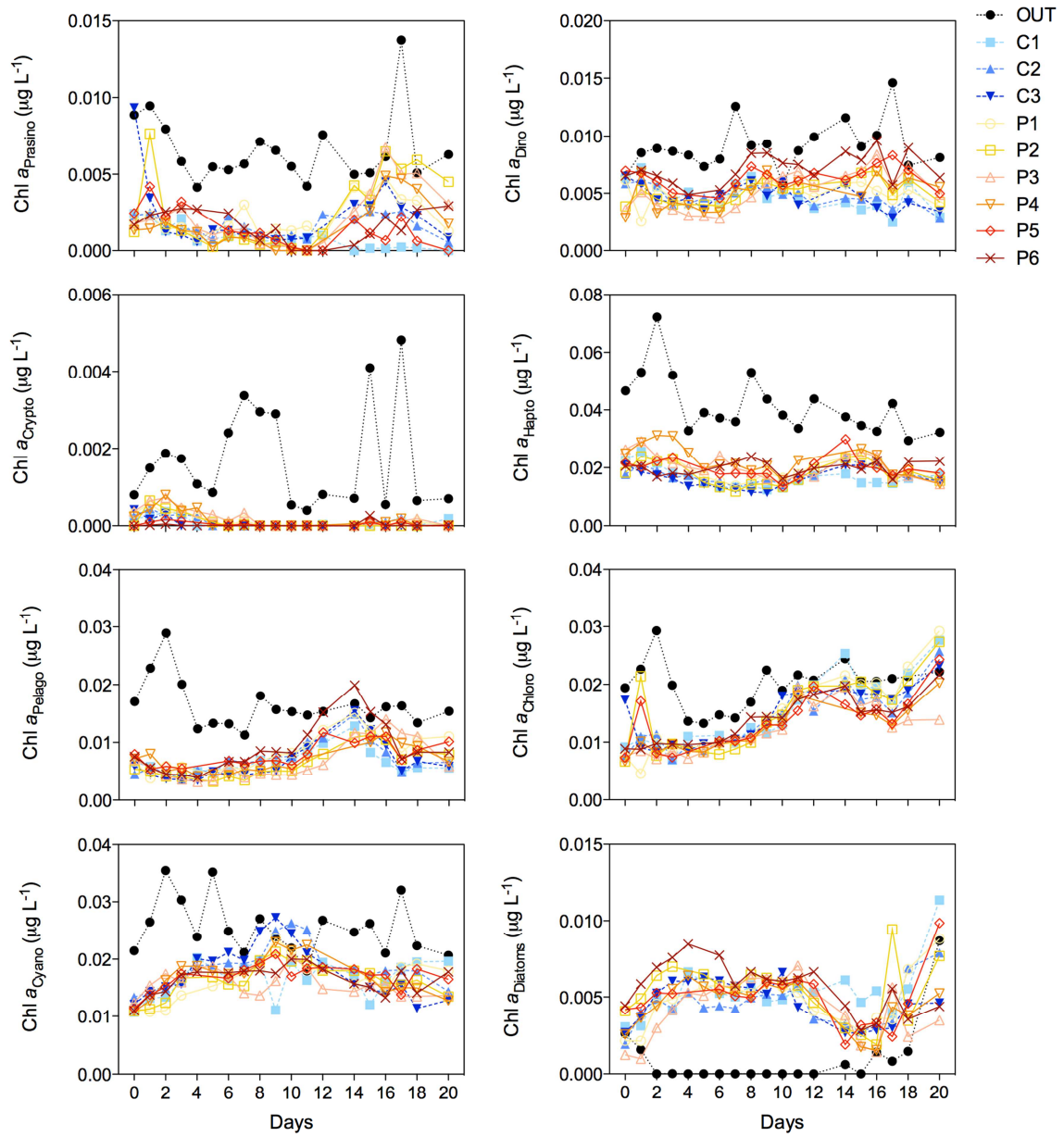
Bay of Villefranche



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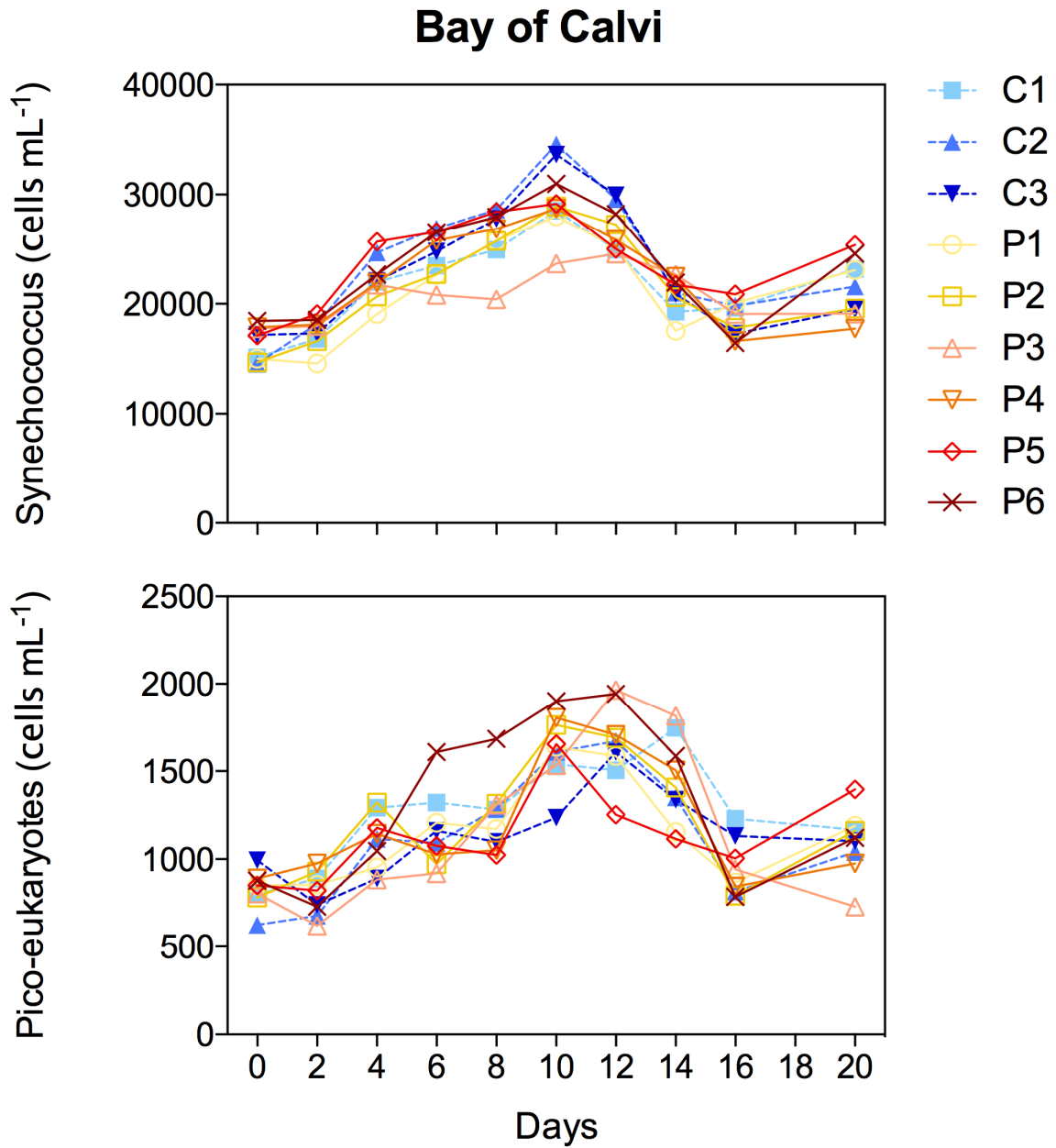
1024 Fig. 3

Bay of Calvi



1025

1026 Fig. 4

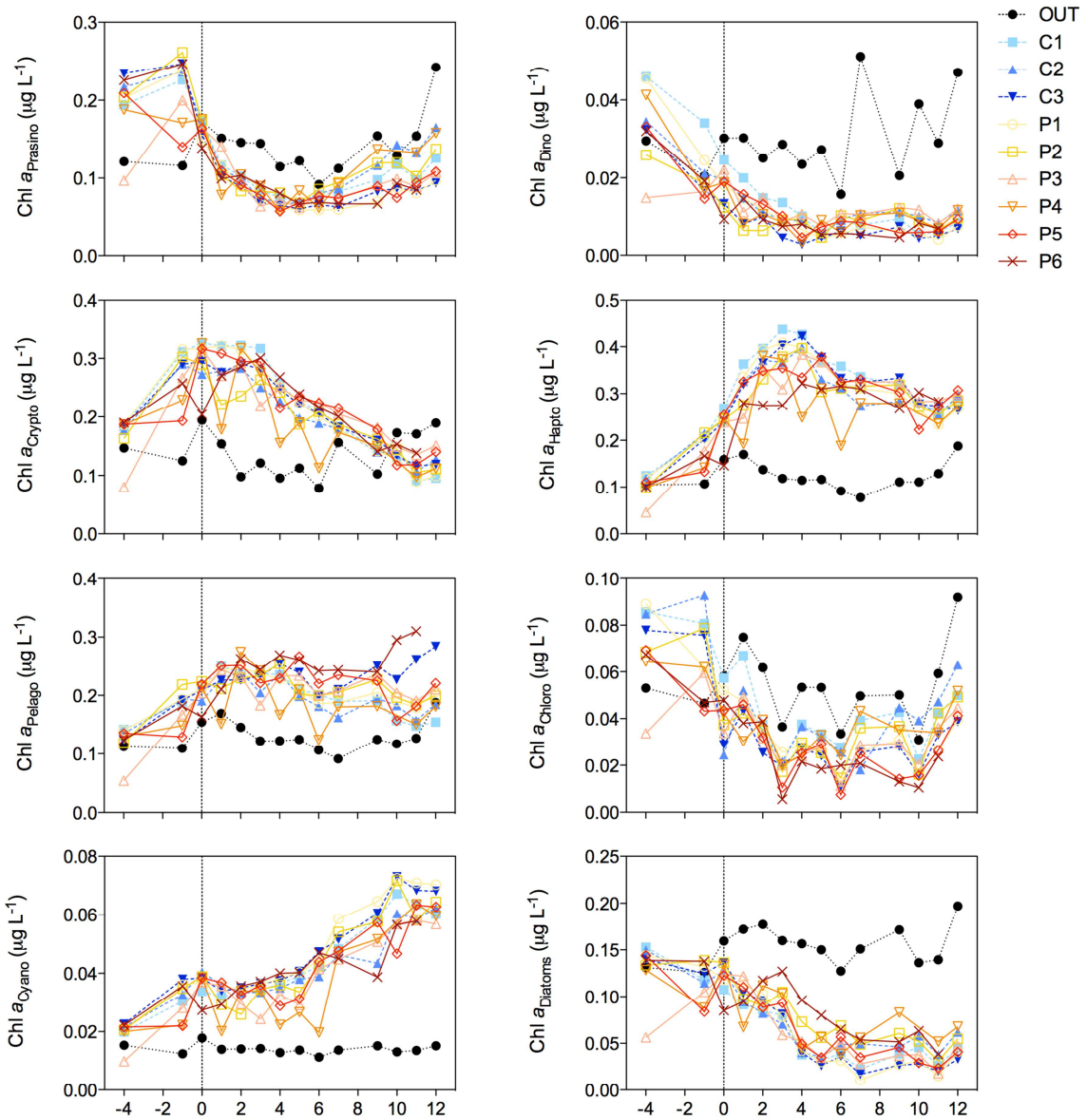


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1028 Fig. 5

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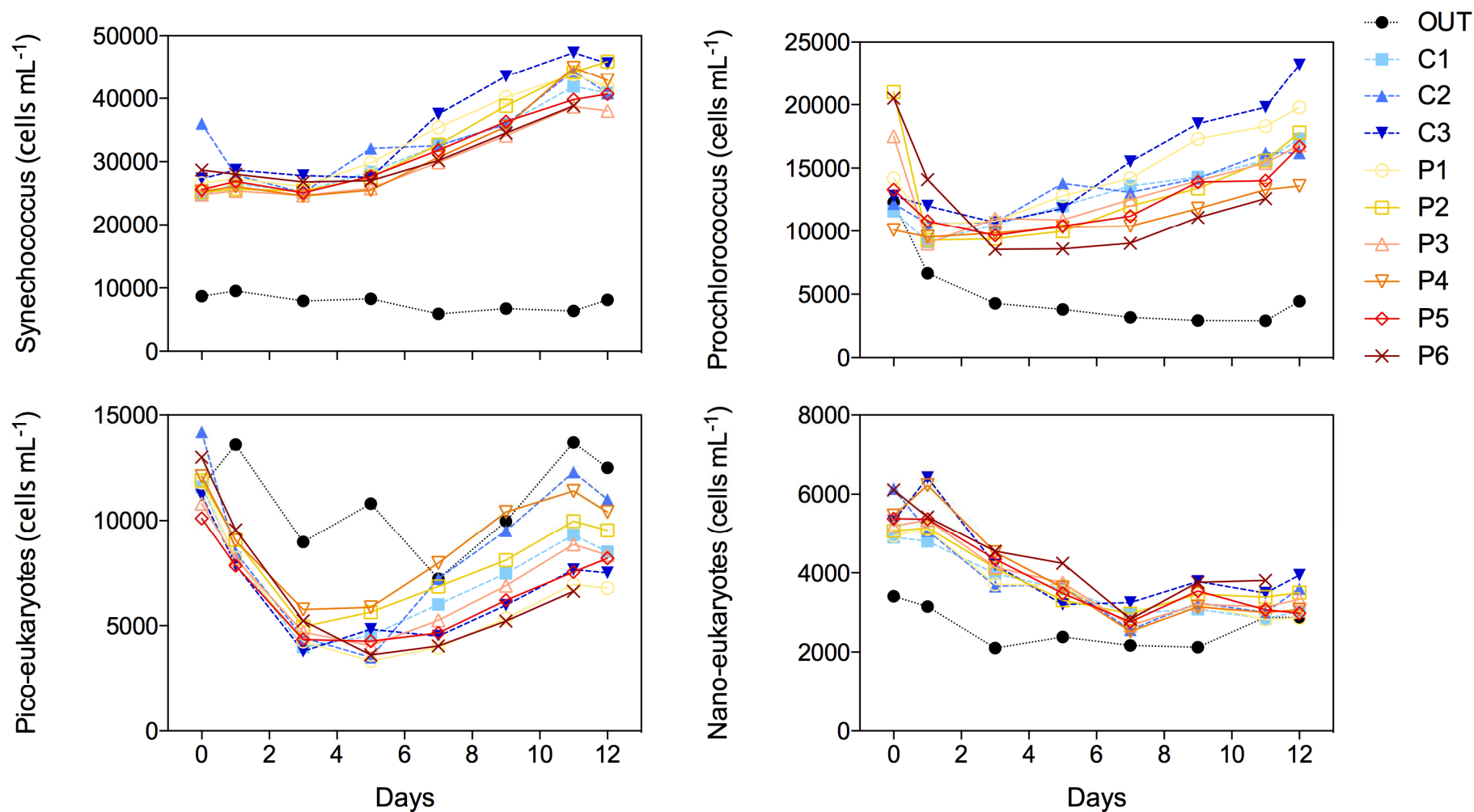
Bay of Villefranche



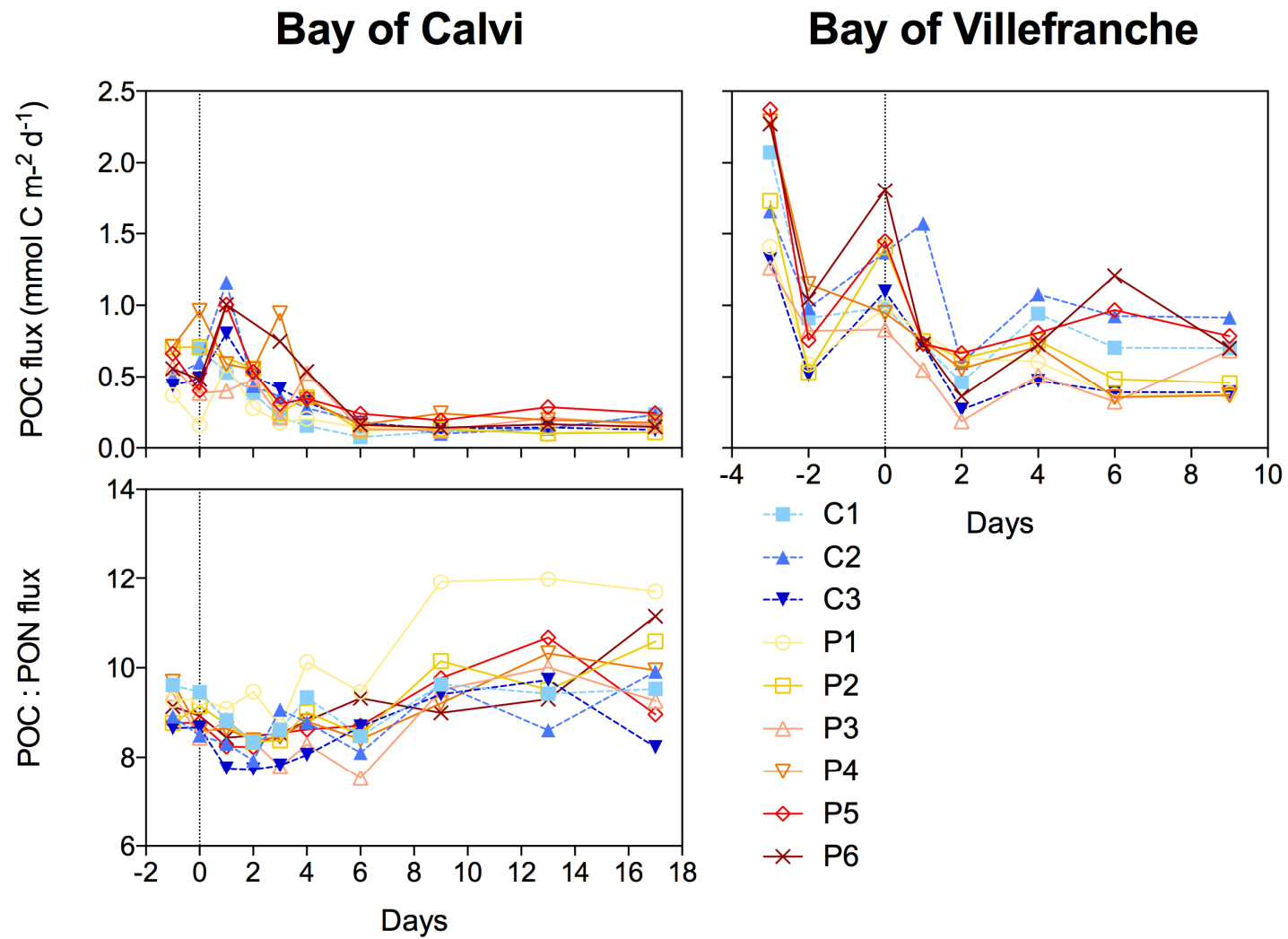
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1031 Fig. 6

Bay of Villefranche



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1033 Fig. 7



1034

1035 Fig. 8