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► **To cite this version:**

Olivier Pringault, Marc Bouvy, Claire Carré, Eric Fouilland, Marouan Meddeb, et al.. Impacts of chemical contamination on bacterio-phytoplankton coupling. *Chemosphere*, 2020, 257, pp.127165. 10.1016/j.chemosphere.2020.127165 . hal-02792811

HAL Id: hal-02792811

<https://hal.science/hal-02792811v1>

Submitted on 10 Jun 2020

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Impacts of chemical contamination on bacterio- phytoplankton coupling

To be submitted to: CHEMOSPHERE

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1 Abstract

2 Phytoplankton and bacterioplankton are the key components of the organic matter
3 cycle in aquatic ecosystems, and their interactions can impact the transfer of carbon
4 and ecosystem functioning. The aim of this work was to assess the consequences of
5 chemical contamination on the coupling between phytoplankton and
6 bacterioplankton in two contrasting marine coastal ecosystems: lagoon waters and
7 offshore waters. Bacterial carbon demand was sustained by primary carbon
8 production in the offshore situation, suggesting a tight coupling between both
9 compartments. In contrast, in lagoon waters, due to a higher nutrient and organic
10 matter availability, bacteria could rely on allochthonous carbon sources to sustain
11 their carbon requirements, decreasing so the coupling between both compartments.
12 Exposure to chemical contaminants, pesticides and metal trace elements, resulted in
13 a significant inhibition of the metabolic activities (primary production and bacterial
14 carbon demand) involved in the carbon cycle, especially in offshore waters during
15 spring and fall, inducing a significant decrease of the coupling between primary
16 producers and heterotrophs. This coupling loss was even more evident upon
17 sediment resuspension for both ecosystems due to the important release of nutrients
18 and organic matter. Resulting enrichment alleviated the toxic effects of contaminants
19 as indicated by the stimulation of phytoplankton biomass and carbon production,
20 and modified the composition of the phytoplankton community, impacting so the
21 interactions between phytoplankton and bacterioplankton.

22 Keywords

23 pesticides; trace metallic elements; carbon cycle; primary production; bacterial
24 carbon demand

25

26

27 1 Introduction

28 Bacterioplankton and phytoplankton are the key players in the carbon cycle of
29 aquatic systems. The two microbial communities are involved in the de novo
30 production of organic matter by phytoplankton, as well as in its mineralization by
31 bacterioplankton. Since they represent the first levels of the pelagic marine food web
32 (Berglund et al., 2007), it is imperative to understand their functioning and their
33 interactions within the environment to better assess the consequences of natural or
34 anthropic perturbations for a given aquatic ecosystem. In coastal zone and shallow-
35 water environments, water column chemistry is closely related to the sediment
36 interface dynamics (Hochard et al., 2010), thus, the functioning of the
37 bacterioplankton and phytoplankton can be strongly affected during storm events
38 resulting in the release of nutrients, microorganisms and contaminants into the water
39 column (Roberts, 2012).

40 The impact of chemical contamination on the first levels of the trophic chain can be
41 observed from a structural point of view, with changes observed in the microbial
42 community, through the selection of species considered tolerant to contaminants
43 (Dorigo et al., 2010; Lekunberri et al., 2010; Pesce et al., 2009). The impact can also be
44 observed from a functional point of view, with modifications of the carbon cycle such
45 as the stimulation of heterotrophy versus autotrophy upon metal spiking (Rochelle-
46 Newall et al., 2008). Although the extent of the threat of contamination is large and
47 has been increasing due to demographic patterns and resulting anthropic pressure
48 on the coastal zone (Schwarzenbach et al., 2006; Small and Nicholls, 2003), pollution
49 remains one of the least-studied stressors in ecology to date (Johnston et al., 2015). As
50 a general rule, chemical contaminants are often considered as stressors due to their
51 toxic potential. In this context, chemical contaminants were demonstrated to induce
52 marine ecosystem functioning alterations, mainly by decreasing productivity
53 (primary production) and increasing respiration (Johnston et al., 2015). These
54 changes in ecosystem functioning by chemical contamination can be concomitant

55 with modification of the biodiversity (alpha and beta) and/or diversity losses
56 (Johnston and Roberts, 2009), with consequences on interactions between
57 bacterioplankton and phytoplankton (Goni-Urriza et al., 2018) that may, in turn,
58 impact the higher trophic levels (Hansson et al., 2013; Hulot et al., 2000).

59 The existence of phytoplankton–bacterioplankton coupling is supported by a
60 significant correlation between primary production and heterotrophic bacterial
61 production (Bouvy et al., 1998; Cole et al., 1982), but revisited more recently using a
62 larger dataset comparing dissolved primary production and bacterial carbon
63 demand in different aquatic ecosystems (Fouilland and Mostajir, 2011, 2010). In
64 coastal areas, bacteria production may or not strongly depends on phytoplankton
65 exudates according to the availability of other sources of carbon such recycled
66 trophic carbon sources (Fouilland et al., 2014) or external terrestrial sources
67 (Fouilland et al., 2018; Morán et al., 2002). In these latter cases, the bacterial carbon
68 demand can largely exceed phytoplankton production (Morán et al., 2002)The
69 coupling between phytoplankton and bacterioplankton mediates the carbon transfer
70 to the trophic web and consequently controls the ecosystem functioning.
71 Anthropogenic perturbations like nutrient loading (Prieto et al., 2015), global
72 warming (Hoppe et al., 2008) or acidification (Hornick et al., 2017) can severely alter
73 the coupling between primary producers and heterotrophic bacteria. Nevertheless,
74 the availability of labile carbon sources is often linked with chemical molecules
75 whose effects are difficult to discern on the interactions between both compartments.
76 We suggest that the release of contaminants by sediments in coastal waters might
77 affect the degree of coupling between phytoplankton and bacteria through two ways:
78 i) the inhibition of phytoplankton communities linked to the toxicity of chemical
79 compounds, and ii) the addition of external source of carbon for heterotrophic
80 bacteria.

81 In this context, the goal of this study was to assess the impact of chemical
82 contamination on the coupling between phytoplankton and bacterioplankton in two

83 contrasting ecosystems, marine offshore waters and lagoon waters. The two
84 contrasting ecosystems were sampled to test the hypothesis that the bacterial and
85 phytoplankton response to chemical contamination would be different in the two
86 sampling sites, with possible consequences for the coupling between both
87 compartments, considering the possible selective pressure and the adaptation that
88 can be triggered by the chemical environment according to the concept of pollution-
89 induced community tolerance (Blanck, 2002). For that purpose, water incubations in
90 9L microcosms were performed in different seasons, with additions of a sediment
91 elutriate or an artificial mixture of contaminants mimicking the main contaminants
92 present in sediment elutriate (pesticides and metal trace elements). The chemical
93 changes, along with the experiments with both contaminant exposures, were
94 previously analyzed (Bancon-Montigny et al., 2019), and the present study focused
95 on the changes observed for phytoplankton and bacterioplankton activities and the
96 consequences for the coupling between both compartments.

97 2 Materials and Methods

98 2.1 Study sites and sampling

99 The study was conducted in southwestern Mediterranean ecosystems, the lagoon
100 and the bay of Bizerte (Fig. S1 supplementary materials) during four distinct seasons
101 spring (April 2014), winter (February 2015), fall (November 2015) and summer (July
102 2016). Like most Mediterranean coastal lagoons (Arvanitidis et al., 2009), the Bizerte
103 lagoon (lagoon station) in the north of Tunisia is a polluted coastal ecosystem subject
104 to intense agriculture, urbanization and industrialization pressures, as well as
105 pressures from naval and commercial shipping harbors (Barhoumi et al., 2014a,
106 2014b; Yoshida et al., 2002). Consequently, sediments are strongly contaminated by a
107 wide range of pollutants, including organic contaminants (polycyclic aromatic
108 hydrocarbons, pesticides and herbicides) and metals (Barhoumi et al., 2014a, 2014b;
109 Yoshida et al., 2002). The Bizerte bay is less contaminated than the lagoon, although

110 local polycyclic aromatic hydrocarbons (PAH) contamination has been recorded in
111 the effluent from the oil refinery located on the shore of the bay (Mhadhbi et al., 2019;
112 Zrafi-Nouira et al., 2009, 2008). The sampling was carried out in an offshore station
113 (station O, 37° 16' 46.46" N 9° 53' 50.98" E) and a lagoon station (station L, 37° 12'
114 43.96" N 9° 50' 79.78" E), as shown in Fig. S1 (supplementary materials). Water
115 samples were collected from a 2 m depth; more details of the sampling procedure can
116 be found in Pringault et al. (2016). Contaminated water was obtained from the
117 resuspension of polluted sediment following the protocol described by Bonnet et al.
118 (2000); this protocol, recommended by the US Environmental Protection Agency (US
119 EPA), has been successfully used to characterize the contamination level and toxicity
120 of sediments using nonadapted species (Bonnet et al., 2000; Carr and Chapman,
121 1995). Polluted sediment was sampled in front of a cement factory in the lagoon
122 channel (station CH, 37° 15' 40.22" N 9° 51' 30.49" E) as shown in Fig. S1, using a Van
123 Veen grab. On the same day of sediment sampling, water was also sampled in the
124 same station CH (see Pringault et al., 2016); this channel zone between lagoon and
125 marine bay is among the most polluted sites in the Bizerte lagoon (Ben Said et al.,
126 2010; Yoshida et al., 2002). Just after sampling, the sediment was sieved (2 mm mesh)
127 to remove large particles and stored in a cool box until its return to the laboratory. In
128 the laboratory, sediment was mixed with channel water (1:4 w/v ratio), and sediment
129 was gently stirred for 8 h. After a 12 h settling period, the overlying solution, called
130 thereafter as "elutriate", was smoothly siphoned off and stored in the dark at 4 °C
131 until use in microcosm incubations.

132 *2.2 Incubation procedure*

133 During the four studied seasons, seawater was incubated for four days in 9 L glass
134 microcosms (22.5 cm diameter and 23 cm height), covered with a quartz lid to allow
135 full penetration of the natural sunlight, including UV radiation, according to the
136 protocol described in Pringault et al. (2016) and briefly detailed below. A series of
137 three microcosms per treatment was filled with 6 L of sample water (L and O

138 stations; control microcosms, C). Another series of three microcosms was filled with
139 4.5 L of sample water (L and O stations) completed with 1.5 L of elutriate to get a
140 final dilution of 25% (contaminated water microcosms; CW). The third series of three
141 microcosms (artificial contaminated water microcosms; ACW) was filled with 6 L (L
142 and O stations) and spiked an artificial solution of pesticides and some metabolites
143 (acetochlore, alachlore, DCPU 1-(3,4-dichlorophenyl)urea, DIA deisopropylatrazine,
144 diuron, linuron and simazine) and metals (Ni, Cu, Zn, Cd, As, Pb). Final
145 concentration of pesticides and metals were adjusted to mimic the concentrations
146 observed in elutriate as well as possible (Table S1, supplementary materials) (see
147 Bancon-Montigny et al., 2019, for more details). All microcosms, performed in
148 triplicate, were incubated outside under natural sunlight in a 3 m³ pool where
149 seawater was circulating (open system) to maintain *in situ* water temperature. Two
150 light sensors (Skye, England), measuring incident photosynthetically active radiation
151 (PAR; 400–700 nm, quantum SKP 215) and ultraviolet A and B radiation (315–380
152 nm, SKU 420 and 280–315 nm, SKU 430, respectively), monitored the light received at
153 the surface of microcosms.

154 2.3 Chemical analysis

155 Chemical analysis of the nutrients, Chlorophyll *a* (Chl*a*) dissolved organic carbon
156 (DOC), organic contaminants (pesticides and polycyclic aromatic hydrocarbons) and
157 metal trace elements (MTE) were performed using the analytical protocols described
158 in Bancon-Montigny et al. (2019) and Pringault et al. (2016).

159 2.4 Phytoplankton counts

160 Water samples were collected from each microcosm (245 mL) at the beginning and
161 the end of the microcosm incubation, then immediately fixed with buffered
162 formaldehyde at 2% final concentration. Species identification and counts were
163 realized with a BX60 Olympus microscope coupled with a Progress C3-cooled
164 Jenoptik camera. Each species was identified to the highest possible taxonomic level
165 (class, genus or species) according to Halse et al. (1997), Hoppenrath et al. (2009),

166 Kraberg et al. (2010), Viličić (2014) and the WoRMS database
167 (<http://www.marinespecies.org>). Counts were realized according to the Utermöhl
168 technique (1958) and the AFNOR standards (2006). Each taxon was counted
169 individually, except *Cyanophyceae* spp., *Chaetoceros* spp., *Pseudonitzschia* spp.,
170 *Prymnesiophyceae* spp., *Euglenoidea* spp. and many flagellates, which were grouped
171 into general taxa because of species identification difficulties. Two groups of
172 flagellates were distinguished by size class, <10 µm and 10–15 µm, without
173 distinction of the taxonomic class.

174 2.5 Metabolic activities

175 Community respiration (CR) and primary production (PP) was measured using an
176 oxygen microelectrode (Unisense, Denmark) following the protocols described by
177 Briand et al. (2004) for CR, and by Pringault et al. (2007) for PP. Water samples (1
178 mL) were placed in gas-tight glass microchambers and immersed in a water bath
179 with controlled temperature and light (*in situ* temperature and *in situ* light).
180 Dissolved O₂ concentration was measured a minimum of four times during the 8–12
181 h of incubation in the vessels. Community respiration and net production (NP) were
182 deduced from the linear regression established on measurement points in the dark
183 for CR and in the light for NP. Primary production was then computed as the sum
184 CR + NP. Bacterial respiration (BR) was expressed in µg C L⁻¹ h⁻¹ using a respiratory
185 quotient of 1 (del Giorgio and Cole, 1998) and assuming that BR represented 30% of
186 the total community respiration (Aranguren-Gassis et al., 2012). Bacterial respiration
187 was used to estimate bacterial carbon demand (BCD), which represents the sum BP +
188 BR, BP representing bacterial production measured by ³H-thymidine incorporation
189 (Smith and Azam, 1992). A detailed protocol can be found in Pringault et al. (2016).
190 Thymidine incorporation rates were converted into carbon production using the
191 conversion factors of 2.10¹⁸ cells produced per mole of thymidine incorporated and
192 20 fg C per cell (Lee and Bong, 2008).

193 2.6 Statistical analysis

194 Coupling between phytoplankton and bacterioplankton was estimated using the
195 approach proposed by Moran et al. (2002) by calculating the linear regression
196 between PP and BCD with a regression significance when $p < 0.05$. Analysis of
197 variance (ANOVA) were performed to test the significance of the differences
198 observed. Prior to analysis of variance, normality (Shapiro–Wilk test) and
199 homogeneity of variance (Levene's test) were checked. When these conditions were
200 not met, differences were evaluated using the nonparametric Kruskal–Wallis
201 ANOVA test. Posteriori paired multiple-comparisons were then performed using the
202 Tukey HSD (honestly significant difference) test. ANOVA tests and Tukey's HSD
203 tests were carried out with the level of significance set at $p < 0.05$ (Statgraphics
204 Centurion XV software).

205 3 Results

206 3.1 In situ conditions

207 The environmental conditions (Table 1) were significantly different depending on the
208 periods (seasons) and sampling sites (L and O stations). Chl a , nutrients and DOC
209 were significantly ($p < 0.05$) higher in lagoon waters. Metabolic activities exhibited
210 also differences according to season and sites. The highest rate of primary production
211 (PP) were observed during summer for offshore waters whereas community
212 respiration (CR) was maximal during fall for the lagoon system (Table 1). In the
213 lagoon ecosystem, CR was always higher than PP (PP:CR ≤ 0.3), while PP largely
214 exceeded CR (PP:CR = 1.5–1.6) in winter and summer in offshore waters. Seasonal
215 variations for environmental conditions were observed for both sites; minimum
216 values for phytoplankton biomass were observed during winter, whereas maximum
217 Chl a were observed during spring for offshore and fall for lagoon waters. Metabolic
218 activities also showed significant variations ($p < 0.05$) according to the season, with
219 maximum rates during summer and fall and minimum values during winter for both

220 sites. As observed for environmental conditions, the phytoplankton community
221 structure (expressed as relative abundance of the main phytoplankton groups) also
222 exhibited significant variations according to the season and sampling site (two
223 factors ANOVA $p < 0.05$) (Fig. 1). Lagoon and offshore waters were dominated by
224 Ochrophyta in summer, whereas dinoflagellates were the dominant group in spring
225 for lagoon, and in fall for offshore waters. Winter was characterized by the
226 dominance of Haptophyta in offshore waters and flagellates in lagoon waters.
227 Data regarding the chemical contamination of both sites by pesticides and metal trace
228 elements (Table S1, supplementary materials) are given in more details in Bancon-
229 Montigny et al. (2019).

230 *3.2 Chlorophyll a, phytoplankton groups and metabolic activities during microcosm* 231 *incubation*

232 Incubation in microcosms during 96 h provoked significant changes for biomass (Fig.
233 2 and 3) and metabolic activities (Fig. 4 and 5) in all treatments, whatever the season
234 and the sampling site. As a general rule, incubation with elutriate (CW) provoked a
235 strong increase in Chl a and bacterial biomass (Fig. 2 and 3) especially in offshore
236 waters, with values up to 5 times the concentration (Chl a) observed in controls (C) at
237 the end of the incubation period. It worth noticing that sediment elutriate (CW)
238 provoked a significant increase of bacterial biomass relative to control (Student Test,
239 $p < 0.05$), noticed at the beginning of the incubation (Fig. 3). The changes in Chl a
240 concentrations were also accompanied by modifications of the phytoplankton
241 community composition (relative abundance of the main phytoplankton groups)
242 with significant differences among treatments (one factor ANOVA $p < 0.05$) (Fig. S2
243 Supplementary materials).. Marked changes were noticed, especially during winter
244 and fall, in both ecosystems. As observed for Chl a , the main significant modifications
245 of phytoplankton composition relative to C microcosm were observed with elutriate
246 incubation (CW). During summer, offshore and lagoon waters were dominated by
247 Ochrophyta, representing up to 80% of the phytoplankton relative abundance for L

248 station; this dominance was not modified by the presence of sediment elutriate (CW).
249 In contrast, in the fall, when the dinoflagellates and flagellates (lagoon and offshore
250 sites) were the dominant phytoplankton groups, the impact of the elutriate and
251 artificial contaminant cocktail on phytoplankton structure were more pronounced
252 with significant ($p < 0.05$) modifications of the relative abundance of the main
253 phytoplankton groups, relative to the phytoplankton composition observed in the
254 controls. A similar pattern was also observed during winter when the flagellates
255 (lagoon) and the Haptophyta (offshore) were the dominant phytoplankton groups.
256 Upon sediment elutriate, Cryptophyta became the most abundant group in lagoon
257 waters (in fall and winter), whereas Ochrophyta (winter) and flagellates (winter and
258 fall) dominated in offshore waters. The large increases in Chl a concentrations in CW
259 treatments were concomitant with an important stimulation of primary production,
260 especially in offshore waters, well superior to those observed in lagoon waters (Fig. 4
261 and 5). The most pronounced effects of elutriate (CW) on Chl a and metabolic
262 activities were observed during spring and summer for offshore waters and during
263 spring for lagoon. As a general rule, changes were less marked in ACW microcosms,
264 where the artificial spiking with a cocktail of pesticides and metals provoked minor
265 changes regarding phytoplankton biomass as well as in the phytoplankton
266 composition. The most pronounced significant ($p < 0.05$) inhibition effects were
267 observed in offshore waters, with final phytoplankton biomass representing less than
268 50% and 30% of the control values for fall and winter, respectively. For lagoon
269 waters, inhibition of phytoplankton biomass by ACW treatment was even less
270 pronounced, with a significant ($p < 0.05$) inhibition (around 50%) only observed
271 during winter. Surprisingly, during the fall Chl a concentrations were higher in ACW
272 microcosms relative to control conditions.

273 Regarding the metabolic activities (PP and BCD), spiking with the artificial
274 contaminant cocktail provoked significant inhibition of PP and to a less extent of
275 BCD in offshore waters, with values representing on some occasions less than 50% of
276 the control values. As observed for Chl a concentrations, the impact of artificial

277 contamination (ACW) was significantly less pronounced in lagoon waters for both
278 PP and BCD (two factors ANOVA $p < 0.05$). The most significant ($p < 0.05$) effects on
279 both metabolic activities were observed during summer with up to 50% for PP and
280 30% for BCD of inhibition relative to control conditions.

281 3.3 Bacterio–Phytoplankton coupling

282 The metabolic activities (PP and BCD) measured during microcosm incubation were
283 used to estimate the strength of the phytoplankton–bacterioplankton coupling
284 depending on the treatments. Data measured at each season were pooled, and the
285 linear relationship between PP and BCD was estimated according to the
286 experimental conditions imposed (Fig. 6 and 7). In offshore waters, for the control
287 conditions, PP and BCD were strongly positively correlated ($r = 0.806$, $p < 0.0001$) with
288 a slope very close to 1 (1.05 ± 0.10). In contrast, for the C treatment in lagoon waters,
289 the linear relationship between PP and BCD was weaker ($r = 0.504$) with a significant
290 decrease of the slope, 0.35 ± 0.08 , relative to the value measured in offshore waters
291 for the same conditions. Incubation with elutriate (CW) and with an artificial cocktail
292 of pesticides and MTE (ACW) both provoked similar effects in offshore and lagoon
293 waters; a significant reduction of the slope and of the correlation coefficient, relative
294 to the respective values measured in C treatment (Student test, $p < 0.05$). The most
295 pronounced differences were measured for offshore waters in CW microcosms, with
296 a slope of 0.27 ± 0.07 ($r = 0.472$, $p = 0.0007$), relative to 1.05 ± 0.10 measured in C
297 microcosms. In lagoon waters, incubation with elutriate (CW) also resulted in a
298 significant reduction of the slope with a value of 0.21 ± 0.06 relative to 0.37 ± 0.08
299 measured in C microcosms. Changes were less marked in ACW microcosm for both
300 ecosystems, nevertheless, the decrease of the slope relative to the control value
301 remained significant (Student test, $p < 0.05$).

302 4 Discussion

303 The aim of the present study was to assess the consequences of chemical
304 contamination on the coupling between phytoplankton and bacterioplankton in two
305 contrasting ecosystems, an open oceanic ecosystem versus a semi-enclosed lagoon
306 ecosystem. The contaminated sediment used for sediment resuspension was sampled
307 in the lagoon channel, and according to the water circulation and the wind regime
308 (Harzallah, 2003), contaminants released by sediment resuspension following strong
309 gusts or stormy events can affect the pelagic communities in both studied
310 ecosystems. The two studied Mediterranean ecosystems exhibited contrasting
311 environmental conditions during the four seasons (Table 1) that were concomitant to
312 marked differences in chemical contamination (Table S1, supplementary materials);
313 offshore waters were significantly less contaminated than the lagoon (Bancon-
314 Montigny et al., 2019). These two contrasted environments allowed testing of the
315 hypothesis that the bacterial and phytoplankton response to chemical contamination
316 would be different in the two sampling sites, with possible consequences for the
317 coupling between both compartments, considering the possible selective pressure
318 and the adaptation that can be triggered by the chemical environment, according to
319 the concept of pollution-induced community tolerance (Blanck, 2002).

320 4.1 *In situ biomass and metabolic activities*

321 The two Mediterranean ecosystems, lagoon and offshore, were sampled during the
322 four seasons. The metabolic rates (CR: community respiration and PP: primary
323 production) and the phytoplankton biomass measured in the present study were in
324 agreement with previous studies in this region (Meddeb et al., 2018; Sakka Hlaili et
325 al., 2008) and were comparable to reports from similar ecosystems in the
326 Mediterranean basin (Ciavatta et al., 2008; Pérez-Ruzafa et al., 2005; Sfriso et al.,
327 2003), with seasonal variations for both marine and lagoon waters (Salhi et al., 2018).
328 As a general rule, phytoplankton biomass and metabolic activities involved in the C
329 cycle (PP, CR and BCD) were significantly higher in lagoon waters relative to

330 offshore waters except for PP in summer were higher rates were observed in offshore
331 waters relative to lagoon. Similarly, significant differences were observed in the
332 phytoplankton composition between the two ecosystems, confirming the particular
333 trophic status played by semi-enclosed ecosystems relative to their counterpart
334 marine adjacent waters (Newton et al., 2014). Despite a large open channel to coastal
335 sea, the watershed influence and the very long water residence time (> 200 days)
336 observed in the Bizerte lagoon (Bejaoui et al., 2017) relative to the offshore adjacent
337 waters (<20 days; (Sayol et al., 2013), allows accumulation of nutrients, DOC and
338 chemical contaminants within the lagoon. In addition, temperature and salinity
339 variations showed distinct patterns with lower temperature and salinity during
340 winter and spring in lagoon waters (compared to the offshore situation), whereas an
341 opposite situation was observed during the summer, where temperature and salinity
342 values were maximal in the lagoon system (Béjaoui et al., 2016). These distinct
343 environmental conditions favored the development of different planktonic species in
344 the lagoon environment relative to the marine adjacent waters, as confirmed by the
345 phytoplankton composition (Fig. 1) but also for the bacterioplankton structure (Goni-
346 Urriza et al., 2018).

347 Seasonal variations were observed for the phytoplankton diversity and the metabolic
348 activities involved in the C cycle, suggesting a strong impact on the organic matter
349 cycle with cascading consequences for the carbon transfer within the trophic web.
350 Using a modeling approach to estimate the flux and exchanges between the different
351 components of the pelagic marine trophic web, it was possible to assess the
352 functioning and stability of the trophic web (Sakka Hlaili et al., 2014). Meddeb et al.
353 (2019, 2018) reported food-web structure differences between the marine and lagoon
354 ecosystems in the Bizerte area, with herbivorous and multivorous dominant carbon
355 pathways in the marine and the lagoon, respectively. The ecological indices, like the
356 total system throughput (Niquil et al., 2012), used as proxies of the ecosystem
357 functioning, suggests a more efficient carbon transfer in the open marine ecosystem,
358 whereas the lagoon environment was more active, but more stressed and less

359 organized (Meddeb et al., 2018). According to the ratio between primary production
360 and community respiration (PP:CR; Table 1), a strong autotrophic activity occurred
361 in offshore waters during winter and summer seasons, while the lagoon ecosystem
362 seemed to be mainly driven by heterotrophic activity (CR>>PP); this supports the
363 assumption that an intense recycling of organic matter occurred in the lagoon waters
364 through the bacterial use of organic matter derived from the sediments and the
365 watershed depending on the bioavailability of OM which strongly affect its
366 mineralization by bacteria (Blanchet et al., 2017; Decho and Gutierrez, 2017).

367 4.2 Impact of contamination on diversity and function

368 Microbial communities from open (offshore) and lagoon waters were exposed to
369 sediment elutriate and to a cocktail of chemical contaminants (MTE and pesticides).
370 The contaminants cocktail was designed from the main compounds identified and
371 analysed in sediment elutriate (Table S1, supplementary materials) in order to assess
372 the toxic effects of these contaminants without confounding chemical factors,
373 nutrients and/or dissolved organic matter that are released during sediment
374 resuspension (Pringault et al., 2016). Nutrients and organic matter are known to
375 strongly mitigate the toxicity of pesticides and TME against microorganisms, mainly
376 attributed to a decrease of contaminant bioavailability by adsorption on dissolved
377 organic matter (Boyd et al., 2005; Coquillé et al., 2018; Lorenzo et al., 2002) and
378 changes in the nutrient status, toxicity increasing under nutrient limitation (Chia et
379 al., 2016; Miao and Wang, 2006). For offshore waters, spiking with pesticides and
380 MTE resulted in an inhibition of phytoplankton biomass as well as a reduction of
381 metabolic activities involved in the carbon cycle (PP and BCD). Previous studies have
382 observed that environmentally relevant concentrations of pesticides, within the range
383 of ng L⁻¹ similar to those observed in the artificial contamination (ACW), can severely
384 affect primary production of microalgae with a strong reduction of photosynthetic
385 efficiency up to 50% (Devilla et al., 2005). Similarly, MTE can also strongly reduce
386 phytoplankton biomass and primary production of natural phytoplankton

387 communities (Pérez et al., 2006) at $\mu\text{g L}^{-1}$ concentrations, in the same order of
388 magnitude than those used in ACW microcosm for the artificial spiking. As a general
389 rule, when assessing the impact of pollution on the marine carbon cycle and
390 ecosystem functioning, primary production is more affected by chemical
391 contamination than respiration, especially upon herbicide or metal trace
392 contamination, with a strong reduction of O_2 production or CO_2 fixation, whereas
393 respiration remains less impacted (Johnston et al., 2015). Nevertheless, the inhibition
394 of metabolic processes or biomass production observed when pelagic communities
395 are exposed to a single molecule can be alleviated when contaminants are used in
396 mixture (Johnston et al., 2015), due to the antagonist effects than pollutant can exert
397 between them (Franklin et al., 2002; Sharma et al., 1999). This hypothesis can be
398 advanced to explain the absence of clear toxic effects on phytoplankton biomass and
399 metabolic rates of the artificial mixture from pesticides and metal trace elements
400 (ACW) for the lagoon waters. In addition, as mentioned before, the lagoon ecosystem
401 is characterized by a very long water residence time (>200 days), which favors the
402 accumulation of pollutants and nutrients. This long period can promote the
403 development of a community resistant to chemical pollution according to the concept
404 of pollution induced community tolerance (Blanck, 2002) and observed for
405 meiobentic communities (Boufahja and Semprucci, 2015). Furthermore, the
406 accumulation of pollutants and nutrients in lagoon waters results in weak
407 enrichment when exposed to the artificial cocktail of contaminants (Bancon-
408 Montigny et al., 2019), reducing the potential toxicity of the added contaminants for
409 the microbial lagoon communities.

410 It is worth noting that the toxic effects observed in offshore waters upon artificial
411 spiking (ACW) were alleviated when the contaminants present in the sediment
412 elutriate were accompanied by nutrients and dissolved organic matter (CW). When
413 offshore waters were exposed to elutriate (CW), phytoplankton biomass and
414 bacterial abundances strongly increased as well as metabolic activities (primary
415 production and bacterial carbon demand). The strong stimulation of BCD in CW

416 treatment (Figs. 4) can partly be explained by the external supply of bacteria by
417 sediment resuspension, as indicated by the significant increase in bacterial abundance
418 observed at the onset of microcosm incubation in CW relative to control (Fig. 3).
419 Nevertheless, previous study showed that this increase in bacterial biomass upon
420 sediment resuspension provoked minor changes on bacterial diversity and
421 community structure (Goni-Urriza et al., 2018). Stimulation of biomass and carbon
422 production was most obvious during spring and fall when flagellates and
423 dinoflagellates dominated the phytoplankton community. In anthropogenic coastal
424 environment, sediment resuspension results in the release in the water column of a
425 trail of contaminants, nutrients and dissolved organic matter as well as benthic
426 microorganisms (Roberts, 2012), leading to enhancement of primary production and
427 phytoplankton biomass, directly by increasing the number of microalgae cells and
428 indirectly by stimulating, through nutrients supply, phytoplankton growth (Guallar
429 and Flos, 2017). In the present study, sediment elutriate was artificially mixed (1/4
430 final volume) with offshore or lagoon waters. This mixing resulted in important
431 enrichment in nutrients (especially in dissolved ammonium and silicates), dissolved
432 organic matter, metal trace elements (Mn, Fe, Mo, Zn and Ni) and pesticides
433 (simazine, alachlor, deisopropylatrazine and 1-(3,4-dichlorophenyl)urea) (Bancon-
434 Montigny et al., 2019) and exogenous bacteria by sediment resuspension (Goni-
435 Urriza et al., 2018). Enrichments in nutrients, DOM and chemical contaminants were
436 more pronounced in offshore waters relative to lagoon waters, explained by higher
437 initial concentrations of compounds in the lagoon; thus the addition of sediment
438 elutriate with their compounds does not change the chemical status of the lagoon
439 significantly (Bancon-Montigny et al., 2019). For offshore waters, the metabolic and
440 biomass changes upon elutriate were more pronounced during spring and fall with
441 *Chla* concentration and primary production up to five times more than observed in
442 the control. Spring and fall were characterized by important enrichment in nutrients,
443 iron and manganese upon elutriate exposure (CW) whereas chemical contamination
444 by Ni, Zn and pesticides were relatively low (Bancon-Montigny et al., 2019). In

445 contrast, during winter, the impact of elutriate was reduced for both phytoplankton
446 biomass and metabolic activities. This period was characterized by a strong
447 enrichment in ammonium (33 μ M relative to *in situ* 0.4 μ M before mixing)
448 concomitant with enrichment in pesticides and Ni, with twice the *in situ*
449 concentration (Bancon-Montigny et al., 2019). It is very likely that this strong
450 enrichment in ammonium and chemical contaminants did not promote favorable
451 conditions for phytoplankton growth, as indicated by the low Chl*a* concentration and
452 PP rates achieved after exposure relative to control values.

453 4.3 Bacterio–phytoplankton coupling and chemical contamination

454 Primary production (PP) and bacterial carbon demand were strongly correlated ($r =$
455 0.806, $p < 0.0001$) in control treatments for offshore waters with a slope close to 1
456 (1.046) suggesting a tight coupling between phytoplankton and bacterioplankton; the
457 carbon demand of the heterotrophic compartment could be totally sustained by
458 primary production (Morán et al., 2002). For lagoon waters, despite a significant
459 positive linear relationship, the slope was strongly lower (0.35) as well as the
460 correlation coefficient of the slope. This clearly indicated a weaker interdependency
461 between both compartments, especially during fall and winter. In these two seasons,
462 lagoon BCD strongly exceeded primary production, consequently, bacterioplankton
463 must rely on allochthonous organic carbon to sustain its carbon requirements. These
464 differences between lagoon and offshore situations in the bacterio–phytoplankton
465 coupling are in agreement with the main observations reported in marine
466 ecosystems, with tighter coupling and interdependency between bacterioplankton
467 and phytoplankton increasing from the coast to the open ocean (Fouilland et al.,
468 2018; Morán et al., 2002).

469 When lagoon or offshore waters were incubated with a mixture of contaminants
470 (pesticides and MTE, ACW), the linear relationships between both compartments
471 slightly changed, with, in both cases, a significant decrease of the slope and the
472 coefficient correlation, suggesting a decrease in the carbon dependency with respect

473 to primary production to sustain the heterotrophic metabolism. These changes on the
474 carbon dependency were more pronounced during winter and fall in offshore waters
475 with a strong reduction of primary reduction when Haptophyta or dinoflagellates
476 dominated the phytoplankton community. Inhibition of primary production were
477 concomitant to significant changes of the relative abundance of the main
478 phytoplankton groups. Interactions between phytoplankton and bacteria are species-
479 dependent (Seymour et al., 2017) and a shift in phytoplankton community structure
480 can modify bacterial production, bacterial abundance and bacterial community
481 structure (Camarena-Gómez et al., 2018) with consequently incidences on the
482 bacterio-phytoplankton coupling. In addition, under chemical contamination,
483 phytoplankton can release dissolved organic matter as extracellular polymeric
484 substances (EPS), binding out of the cell, the toxic compounds like metal trace
485 elements, or pesticides (Decho and Gutierrez, 2017; Naveed et al., 2019). Such defense
486 mechanisms were reported in phytoplankton cultures (Decho and Gutierrez, 2017;
487 Herzi et al., 2013a, 2013b) as well as in natural phytoplankton communities
488 (Rochelle-Newall et al., 2008, 2011) or peryphyton biofilm (Ivorra et al., 2000). The
489 binding of chemical compounds on the phytoplankton-derived dissolved organic
490 matter can modify its composition and its physicochemical properties (Naveed et al.,
491 2019) with incidences on its bioavailability and lability (Enya et al., 2020; Wada and
492 Suzuki, 2011), decreasing so the potential utilization by the heterotrophic
493 compartment (Seto et al., 2013), eventually loosening the coupling between both
494 compartments.

495 When the natural community from offshore and lagoon waters were incubated with
496 elutriate (CW), the slope and the correlation coefficient of the linear relationships
497 between BCD and PP strongly decreased relative to their corresponding control
498 value, especially for offshore waters, with a slope of 0.27 ($r = 0.472$) relative to 1.05 (r
499 = 0.806) in the control. For lagoon waters, the slope was even lower (0.18) relative to
500 0.35 in the control treatment. These very low values, clearly indicated that the
501 dependency between both compartments was weak when exposed to sediment

502 elutriate. Sediment resuspension released a significant amount of organic matter,
503 nutrients and bacteria, which resulted in a strong enrichment in DOM, nutrients and
504 bacterial abundance in offshore and lagoon waters, knowing the low concentrations
505 of DOM and nutrients measured *in situ* (Bancon-Montigny et al., 2019). The release of
506 DOM and nutrients consecutive to sediment resuspension is known to stimulate both
507 phytoplankton and bacterioplankton metabolisms (Roberts, 2012; Uchimiya et al.,
508 2016) with serious consequences on the biogeochemical cycles (Cotner, 2000)
509 depending on the bioavailability of OM that strongly affects its recycling (Blanchet et
510 al., 2017; Decho and Gutierrez, 2017). These conditions favor a loosening of the
511 coupling between phytoplankton and bacterioplankton; the heterotrophic
512 compartment may have access to external carbon sources, decreasing its potential
513 dependency on the phytoplankton exudates. In addition, nutrients released by
514 sediment can also modify the phytoplankton composition by alleviating nutrient
515 competition within the community and allowing the growth of microalgae taxa
516 previously nutrient-limited. A previous study has shown that sediment resuspension
517 may alter the synchrony between bacterial and phytoplanktonic compartments
518 (Goni-Urriza et al., 2018). Consequently, the tight coupling observed in offshore
519 waters between phytoplankton and bacterioplankton can be loosened upon sediment
520 resuspension by several non exclusive factors i) a modification of the phytoplankton
521 composition through the release of sediment nutrients, altering so interactions with
522 the heterotrophic bacteria, ii) the supply of an external source of labile carbon that
523 can be preferentially used by heterotrophs, iii) the supply of exogenous bacteria that
524 can enhance the overall bacterial carbon demand.

525 5 Conclusion

526 The result of the present study clearly showed that phytoplankton and
527 bacterioplankton were strongly coupled in offshore waters while the coupling was
528 less obvious in the lagoon ecosystem. We clearly showed that the presence of
529 contaminants altered the interactions between phytoplankton and bacterioplankton,

530 with a loosening of the coupling observed in offshore waters. This fact was even
531 more marked upon sediment resuspension due to the strong supplies of nutrients,
532 dissolved organic carbon and exogenous bacteria. Considering the climate scenarios
533 in the Mediterranean basin that predict an increase of storms and paroxysmic events
534 (Cramer et al., 2018), with consequently higher occurrences of sediment
535 resuspension, the functioning of coastal ecosystems will be severely impacted by
536 strong alterations of the interactions between bacterioplankton and phytoplankton
537 with non-negligible consequences for the carbon cycle and the CO₂ emissions.

538 6 Acknowledgments

539 This study was supported by the RISCO project (ANR-13-CESA-0001), funded by the
540 French National Agency for Research (ANR) and by the Joint International
541 Laboratory COSYS-Med. English grammar and syntax of the manuscript were
542 revised by Proof-Reading-Service.com.

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Figure legends

Figure 1: Main phytoplankton groups observed in Offshore and Lagoon waters as a function of the season.

Figure 2: Chlorophyll a concentration during microcosm incubation of Offshore and Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 3: Bacterial abundance during microcosm incubation of Offshore and Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 4: Temporal variations of primary production and bacterial carbon demand observed during the microcosm incubation of Offshore waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 5: Temporal variations of primary production and bacterial carbon demand observed during the microcosm incubation of Lagoon waters as a function of treatments for the four sampling seasons. Average and standard deviation (n=3).

Figure 6: Scatter plot of bacterial carbon demand vs primary production in Offshore waters for the four seasons as a function of treatments. The fitted solid lines represent the linear regression. The dotted line indicates the 1:1 relationship between the two metabolic processes.

Figure 7: Scatter plot of bacterial carbon demand vs primary production in Lagoon waters for the four seasons as a function of treatments. The fitted solid lines

represent the linear regression. The dotted line indicates the 1:1 relationship between the two metabolic processes

Figure1
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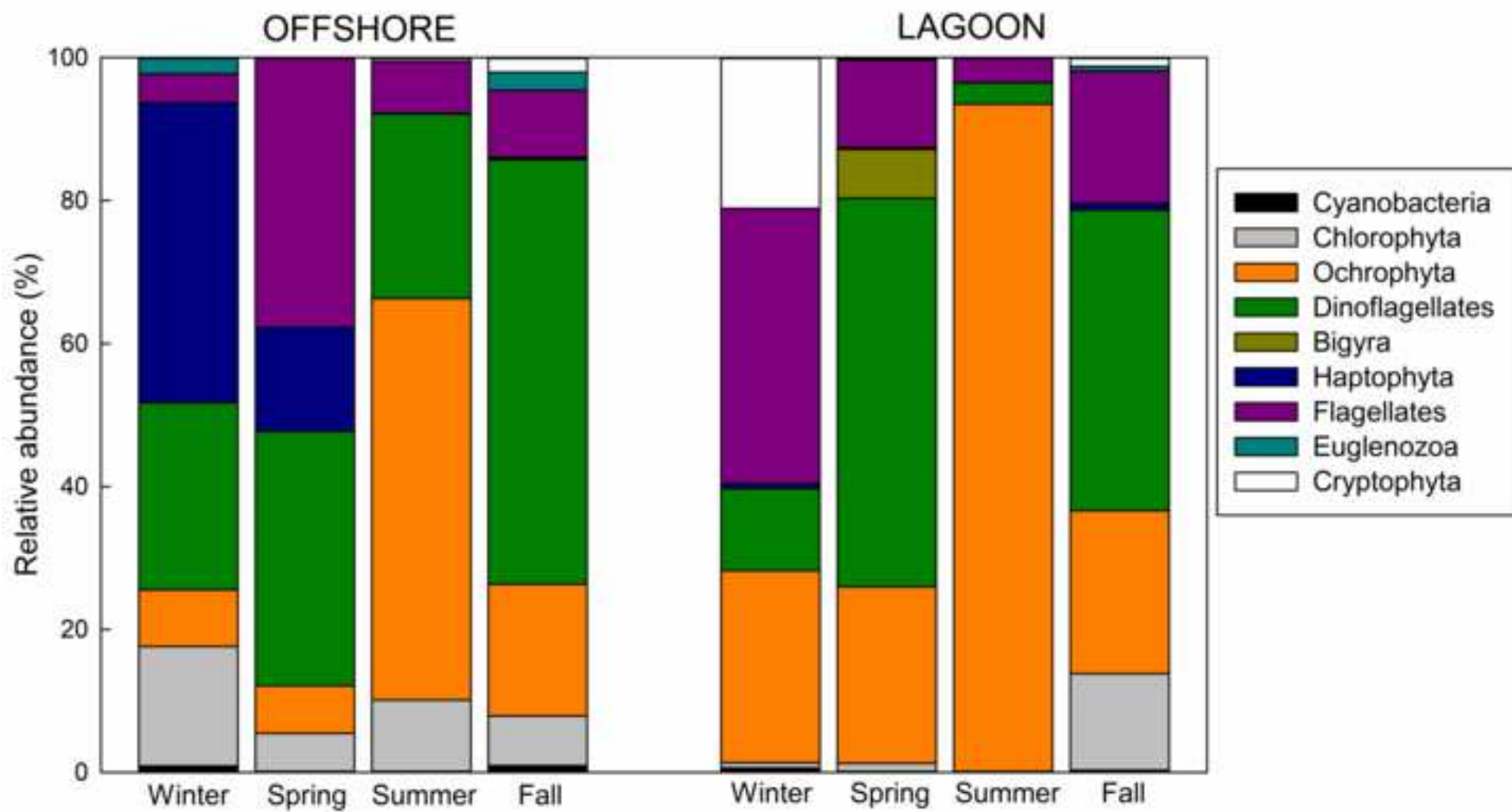


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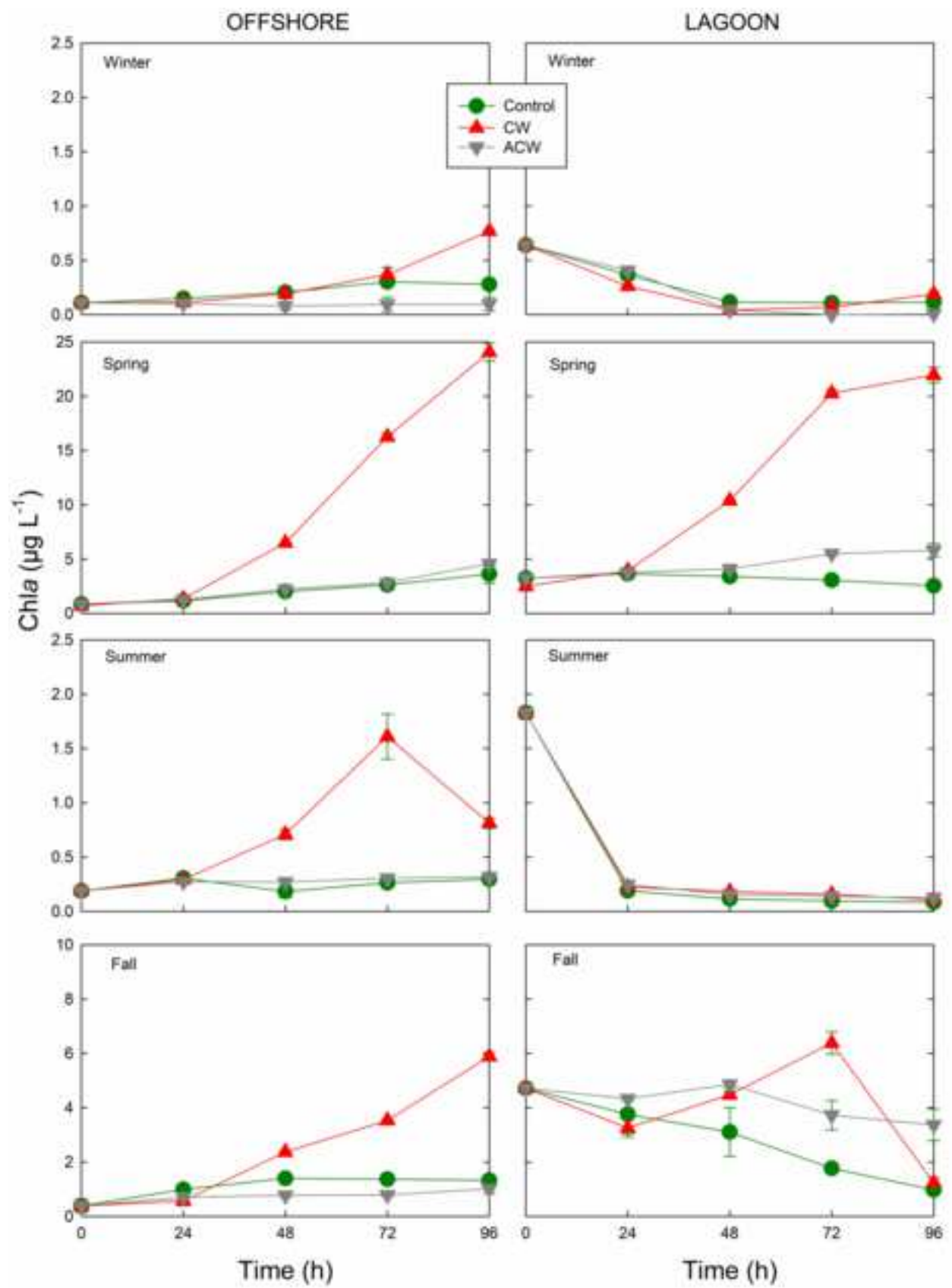


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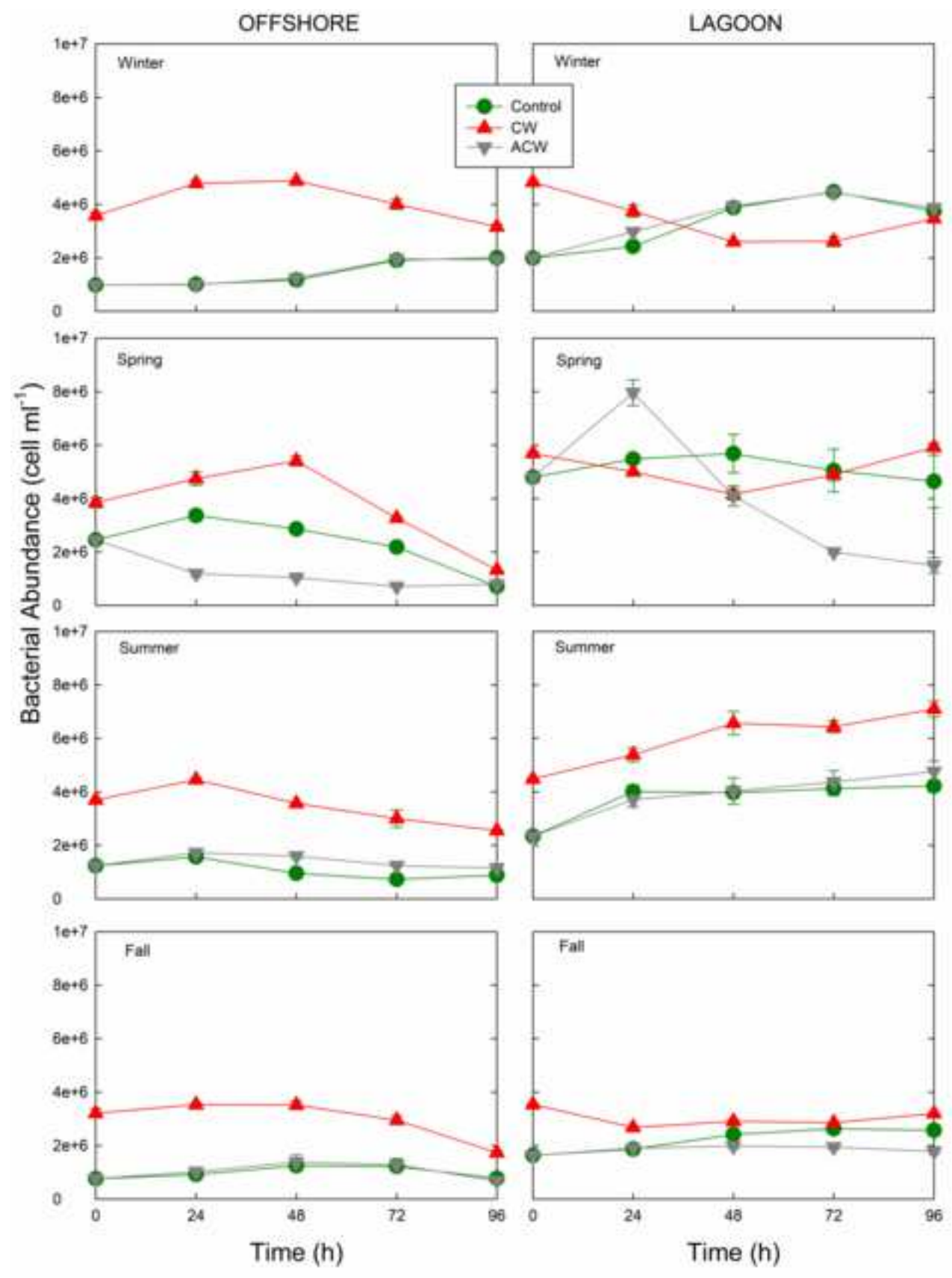


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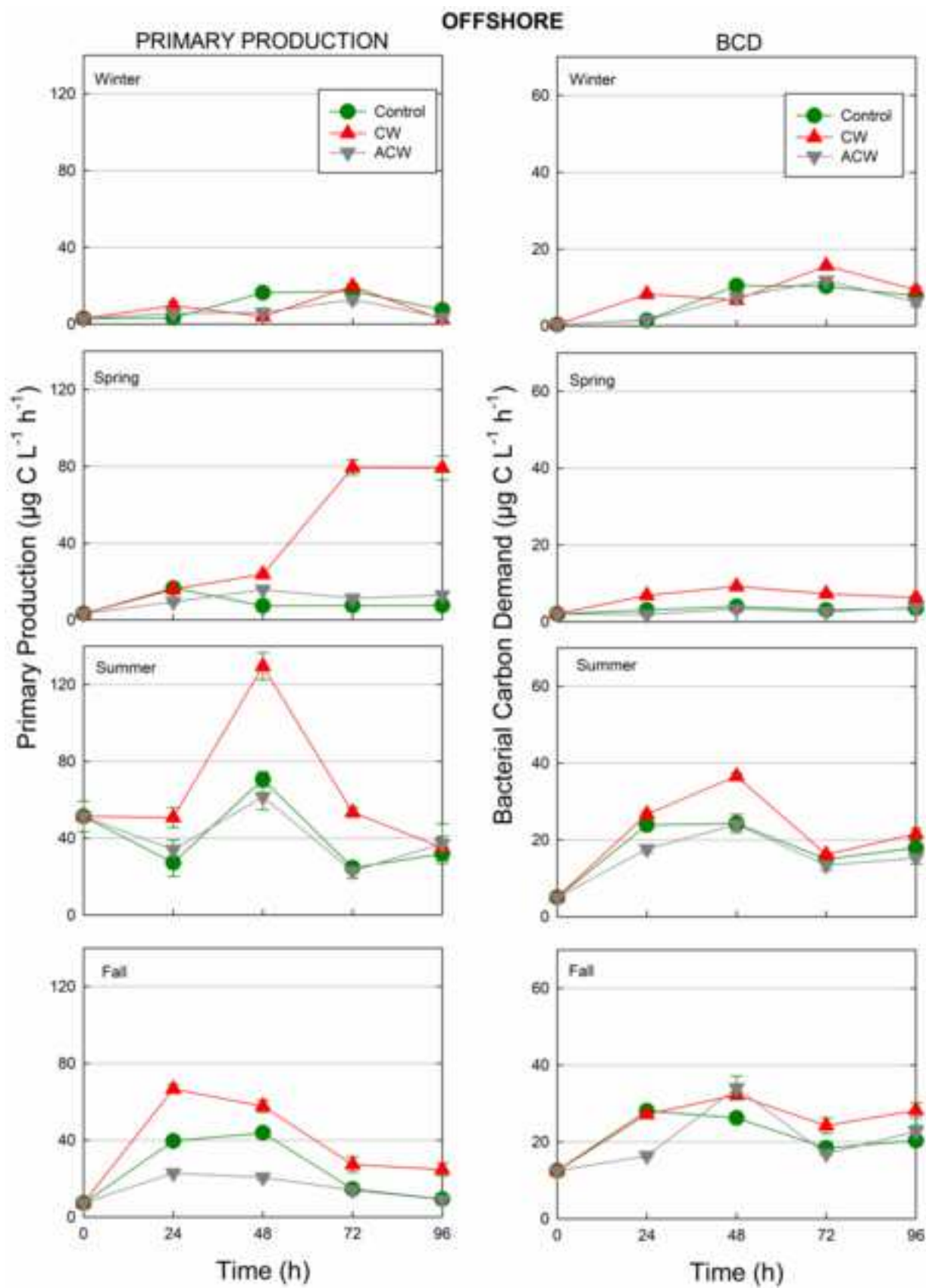


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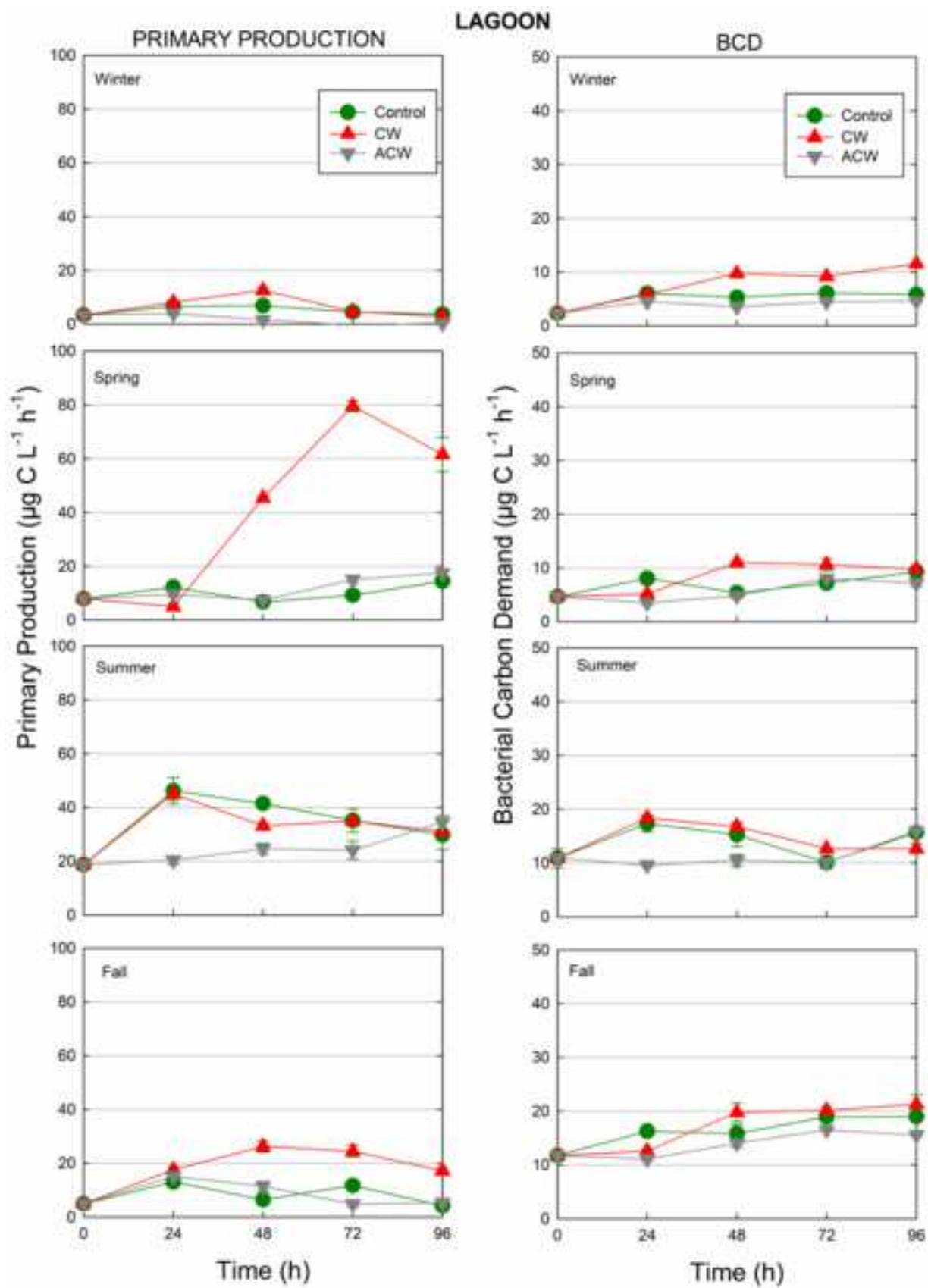


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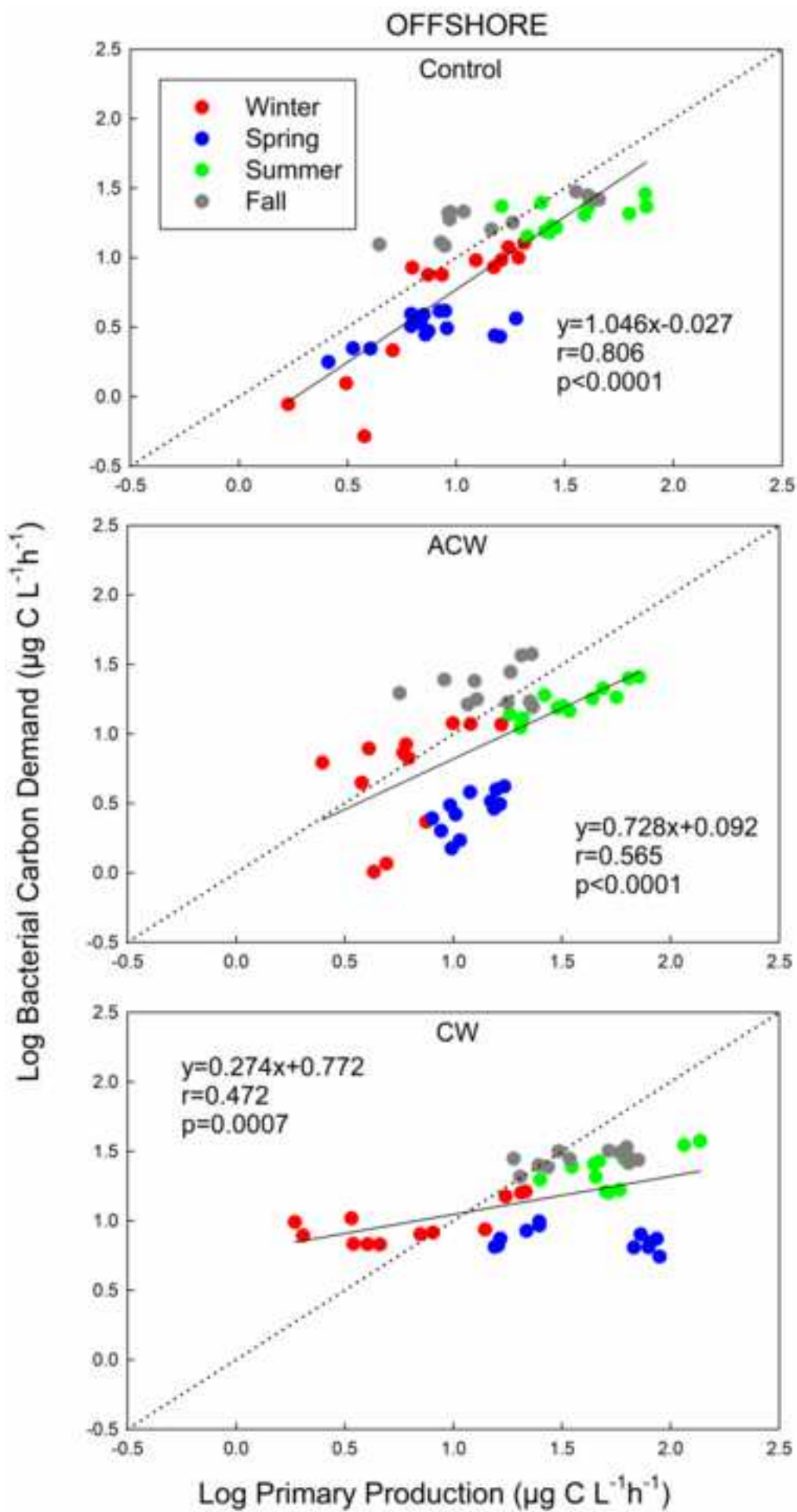
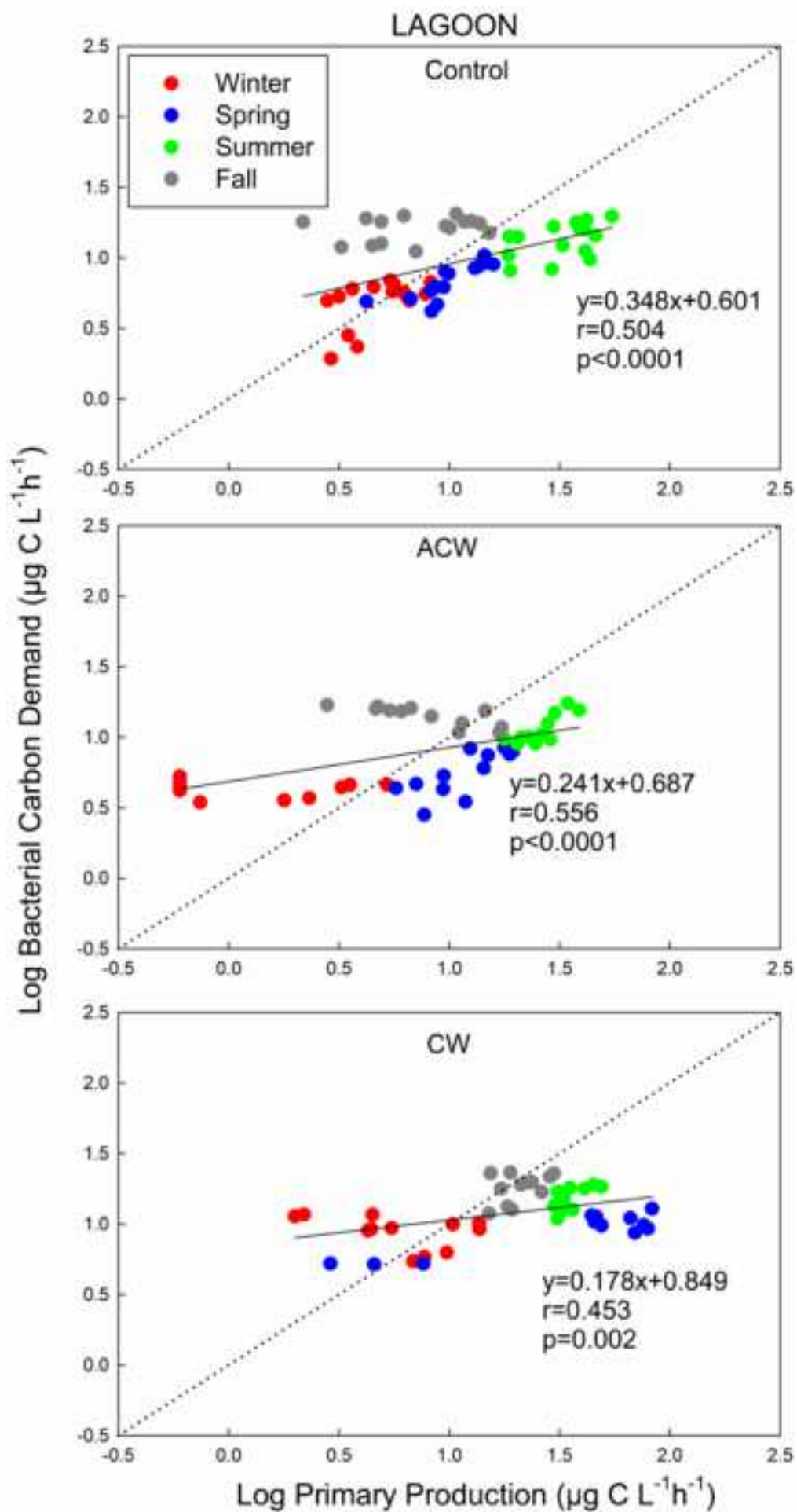


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